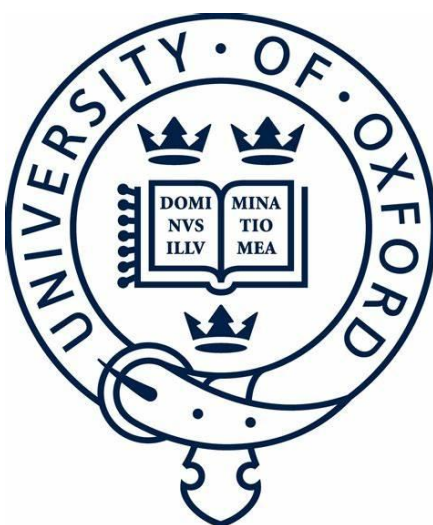


Exploring the impact of patient-derived NMDAR NR1-specific IgG antibodies on the developing mouse striatum



Anežka D.B. Macey-Dare
Department of Pharmacology

Trinity Term 2024

Supervisors:

Prof. Dr Tommas Ellender^{1,2}, Prof. Daniel Anthony¹, Prof. Liliana Minichiello¹

1. Department of Pharmacology, Oxford 2. Department of Biomedical Sciences, Antwerp

**A thesis submitted to the University of Oxford in partial fulfilment of the requirements for
the degree of Doctor of Philosophy.**

Abstract

Maternal antibodies targeting neuronal proteins, such as the NMDA receptor (NMDAR) and its NR1 subunit, can cross the foetal blood-brain barrier and disrupt neurodevelopment. Previous studies suggest that these NR1-targeting antibodies may be elevated in mothers of children with neurodevelopmental disorders, and animal models have shown that exposure to them during gestation leads to developmental abnormalities that persist into adulthood. The central hypothesis of this thesis is that early exposure to maternal NR1-IgG antibodies disrupts the development of the striatum, leading to long-term structural and functional alterations in this critical brain region, which may underlie behavioural deficits. However, the specific mechanisms by which these antibodies impact the developing brain, particularly striatal circuits, remain poorly understood.

To address this, a novel *in utero* model was developed to deliver patient-derived NR1-IgG antibodies directly into the mouse brain via intraventricular injections. Immunohistochemical analyses revealed that the embryonic lateral ganglionic eminence (LGE), a key progenitor region for striatal development, exhibits high expression of the NMDAR NR1 subunit. Both polyclonal and monoclonal NR1-IgG antibodies were shown to bind to the LGE following *in utero* delivery, establishing a robust model for further investigation. Using this model, the impact of transient NR1-IgG exposure during the embryonic neurogenic period was examined. Analysis demonstrated reduced size of the primordial striatum, decreased proliferation of progenitor cells in the LGE, impaired neuronal migration, and an increase in microglial density and altered microglial morphology. In postnatal studies to examine the impact of transient NR1-IgG exposure, whole-cell patch-clamp electrophysiology and morphological reconstructions of striatal spiny projection neurons (SPNs) revealed immature intrinsic properties, simplified neuronal morphology, and altered synaptic function, including a reduced NMDA/AMPA ratio. Postnatal MRI scans corroborated these findings, showing reduced striatal volume. Additionally, an imbalance in the D1/D2-SPN ratio was observed, with fewer D2-SPNs. Increased microglial density

and altered microglial morphology persisted postnatally, further implicating microglial involvement in the observed changes. Behaviourally, early motor reflexes were delayed and affected animals exhibited reduced anxiety-like behaviour in open-field tests and increased time on the accelerating rotarod, suggesting motor- and anxiety-related impairments. Treatment with the AMPAkin CX516 during the first postnatal week partially rescued the behavioural phenotype, indicating that enhancing glutamatergic signalling via alternative routes may mitigate some of the effects of NR1-IgG exposure.

Overall, this thesis presents a novel experimental model to study the effects of maternal NR1-IgG antibody exposure on the developing striatum. The findings demonstrate that early exposure leads to significant embryonic and postnatal alterations in striatal development, which manifest as behavioural deficits. Importantly, the partial rescue of these phenotypes through AMPA receptor modulation highlights potential therapeutic avenues for addressing neurodevelopmental disorders associated with maternal antibodies.

Acknowledgements

If it takes a village to raise a child, it has taken a large city to complete this DPhil. I am deeply thankful to all those who have contributed to an Oxford kid's childhood dream, which has been both the ultimate privilege and ultimate challenge.

My first enormous thank you must go to Prof. Dr Tommas Ellender. I will be forever grateful to Tommas for taking a chance on me as the first ever member of his newly opened Oxford lab almost a decade(!) ago now; that gap year spent meticulously counting and reconstructing D1- and D2-SPNs whilst listening to Avril Lavigne on loop really kickstarted this whole DPhil adventure. Tommas, thank you for the unending support and invaluable guidance, scientific and beyond, throughout this DPhil and for so many years beforehand (no more 2am emails, I promise!). I truly lucked out with the most patient and positive supervisor I could ever have wished for, and I am so thankful to have been a part of the ever-growing Ellender Lab right from the start.

My immense thanks also go to Prof. Daniel Anthony for his kind and diligent supervision in Oxford following the Ellender Lab move to Belgium. Daniel, I am particularly grateful for your knowledgeable input on shaping the project and final thesis, and to both you and the whole Anthony Lab for being such a welcoming and fun group to join. Further, many thanks to Prof. Liliana Minichiello for your supervision and helpful guidance.

A big thank you also to Prof. Peter Magill and Dr Natalie Doig at the Oxford MRC BNDU, who have both been pivotal in this whole journey. Pete and Nat, thanks for the inspiring talk at my sixth form all those years ago with all those curious animal brains in jars, for hosting me on an eye-opening summer week, and for recommending me to Tommas in the first place, who knew this is where it would all lead!

I was lucky enough to join several exciting lab projects prior to my DPhil which fuelled my love of wet-lab research. A particular thank you to Prof. Walid Khaled, Kyren, Sara, Mark and the rest of the Khaled

Lab for two wonderful summers spent in the Cambridge Department of Pharmacology, and to Prof. Massimo Avoli, Maxime, Siyan, Cristen, Debbie and the rest of the Avoli Lab for the enjoyable months spent at McGill University and the Montreal Neurological Institute, Canada. I would also like to thank my tutors at Green Templeton College including Alison Franklin, Dr Alison Stenton and Prof. Sara Shaw, and the staff at Imperial who supported my DPhil application and transition back to medical school, especially Prof. Mary Morrell, Prof. Alison McGregor, Nicole Barnes and Dr Pooja Gulati. More widely, I would like to thank my encouraging and supportive school and undergraduate teachers who made learning so enjoyable during earlier years.

Next, I would like to thank those behind the scenes who kept the DPhil ship sailing seamlessly. Thank you to the administrative teams at the Department of Pharmacology, MRC DTP and Green Templeton College, the animal facility teams, and the Antwerp student housing team. Special thanks also to Hicham and Mike for their wizardry aiding all things technical, to security guard James for being a friendly face at the Pharmacology door on my frequent midnight/weekend lab excursions, and to the kind staff and security at St Mary's Hospital Library for providing a tranquil space to polish off this thesis as I naïvely juggled two demanding degrees simultaneously.

Many thanks also to the Medical Research Council, Green Templeton College and Santander for funding this DPhil.

Science is always a team effort and my collaborators have made this project possible. Many thanks to Dr Sukhvir Wright, Prof. Sarosh Irani and Prof. Dr Norbert Goebels for providing the human antibodies and for their expert clinical and scientific insights throughout, and to everyone who donated their antibodies for research. Thank you also to Dawn Shepherd for assistance with qPCR, Dr Khadija Guerti and Karin Cotthem for help with the HEK-cell assays, Johan Audekerke for support with postnatal MRI scanning and Elke Calus for patiently teaching me and assisting with behavioural experiments, as well

as to the Akerman, Magill, Mancuso, Platt, UZA Hospital and Antwerp Bioimaging Labs for selflessly sharing equipment, reagents and advice. I would also like to extend my thanks to my transfer and confirmation examiners: Prof. Angela Vincent, Prof. Simon Butt and Prof. Zoltán Molnár for their useful discussions and comments, and to Prof. Ed Mann and Dr Ester Coutinho for examining this final thesis.

Past and present members of the Ellender Lab deserve a huge thank you for making the rollercoaster that is wet-lab research such an enjoyable ride: Jack G, Ricardo, Fran VH, Farid, Rebecca, Monzilur, Miles, Sungwon, Elliot, Luke, Seb, Fé, Helena, Yana, Yasmin, Stephanie, Noor, Brenna and all the other wonderful faces over the years. Extra special thanks must go to Ricardo, Jack, Yana and Fran. Ricardo, thank you for teaching me everything from micro-dissecting embryonic brains to custom-designing primers and for being there to help out at all hours, all whilst humouring my constant chat about Colin the Caterpillar (sorry, he is cool though!). Jack, a big thanks for assisting with anything from troubleshooting temperamental experiments and managing misbehaving mice to smuggling cold boxes onto the Eurostar and answering my many questions about how to actually write up a DPhil, always with a big smile on your face; you have been such a joyful presence in the lab, and it has been a pleasure doing our DPhils in parallel together. Yana, thank you for helping to behaviour test my mice and for being a lovely friend in Antwerp. And Fran, part of the OG Ellender crew, thank you for your friendship and guidance both in and outside of the lab over so many years. Thank you also to Sophie A, Emma, Sophie N, Sarah, Kashif, Colin and the rest of next door's Akerman Lab for taking me under your wing when I first joined the department as a visiting student, and for the many enjoyable lunchtime conversations together.

A shout out also to all the clubs that sponsored my sports addiction during my time at Oxford, including the Oxford University Korfball, Handball, Athletics, Netball and Basketball Clubs, and Green Templeton College Netball and Boat Clubs for so many highlights on and off the court/field/water. A special thank you to my wonderful korfball pals who became a second family during my time here, I hope to be back

on court taking more “defended” shots with you all very soon (bring on Alumni Varsity!). I would also like to thank everyone at ELK Korfball Club for making the last year in London a lot of fun, including Flo, James, Reece, Drew, Kelly, Junal, Chiara, and especially Matthew for ensuring this final write-up period was sufficiently fuelled by Nandos.

A big thank you to all the friends in Oxford who kept me sane during my DPhil. Jack W, Lizzie J and Maggie, my fellow DPhil korfers, thank you for the many laughs (read: cries) together over botched experiments and lamentable PhD memes and all the fun on the court too, of korf! An extra special thank you to Jack for always being there for a much-needed coffee, walk or catch up over the phone and also for reading through many a chapter draft. Ella (bro!), thank you for being such a constant positive and encouraging presence, and Kara, thank you for all the chats over coffee, Najar’s and Bbuona pizza; you have both kept me going more than you know. Jaymin, thanks for providing support as amazing as your trick shots, keep cutting shapes on and off the court, and Alex, thank you for all the legendary ALTS nights (when the rink wasn’t melting!). Thank you also to Flora, Dhanush, Hannah J, Fran T, Chris, Matt, Joe, Alejandro, Saskia, Sarah, Amadea, Patricia, Rob, Michelle, Chimdi and all the other wonderful people who have made my DPhil time so special.

Thank you also to all my friends beyond the DPhil for being there from before this journey started, including my oldest school friends: Sif, Izzy, Natalie and Emily; original Ox friends: Ally, Tom and Shelley; and the OG Imperial crew: Hannah, Elsa, Ralph, Szymon, Olivia, Rory and Loui. A special mention to my closest friends at medical school: Lizzie R, my real-life fairy godmother, thank you for so much guidance on all things PhD, medicine, halls and questionable life decisions; and Daim, my neuro BSc buddy, thank you for being in my corner, for a constant supply of unhinged memes, and for picking me up on the cloudiest of days, I could never have finished this without you.

I want to thank my family from the bottom of my heart. I am blessed to be the child of two superstar parents: Rupert and Beatrix (who met doing their own DPhils; my mum was in fact pregnant with me in her own viva!) and have three wonderful(ly weird) younger siblings: Claudia, Teresa and Robert. Mami, Daddy, Claudi, Baby S and Rod, I am so happy to have been able to live at home again during the DPhil years with the constant dad jokes, sing-a-longs and dance workouts, long COVID walks, meaningful evening debriefs and simply someone to hug at the end of a long day. Thank you for your unwavering love and support and thank you for always motivating me whilst never letting me take myself too seriously. I love you all endlessly. Thank you also to Kathy and Sandra for all your support and kindness over these past few years, you have become my second family. Moreover, thank you to my wider family, and especially my grandparents: Grandma, Grandpa, Babka and Laci, who were a constant source of advice and encouragement, all of whom I sadly lost during the DPhil, and also to my wonderfully supportive great aunt Nessa. My Grandma's favourite quote - "If at first you don't succeed, try, try and try again" – has proven great advice over these years, not only for many a tedious experiment, but for wider life too. I hope I have made you all proud.

And finally, to Karim, thank you for being around for almost all of the ride, no one got to see this journey more intimately than you did. A wise man once told me "It's always darkest before dawn". Cheers to sunshine time. Rooting for you always.

"It's a great day to have a great day."



Declaration of Authorship

I, Anežka D.B. Macey-Dare, declare that the work presented within this thesis is wholly my own, other than some experiments which were completed in collaboration with others and some explanatory diagrams taken from published sources. I acknowledge contributions from others below:

1. General Introduction

Figure 1.6 Figure from Pröbstel and Zamvil (2019) [replaced by my own drawing in ORA online thesis]

2. General Methods

Figure 2.3 Dissection method designed by Dr Ricardo Márquez-Gómez (Department Pharmacology, Oxford)

Figure 2.8 TITREPLANE protocol diagram from EUROIMMUN [redacted in ORA online thesis]

3. Experimental Chapter A

Figure 3.4 NR1-expressing HEK-cell binding assay was performed by Dr Khadija Guerti, Karin Van Cotthem and colleagues (UZA Hospital Lab, Antwerp)

4. Experimental Chapter B

Figure 4.5 IUE experiments and data analysis was a collaborative effort with others in the Ellender lab including Dr Fran Van Heusden, Dr Jack Gordon, Rohan Krajeski, Prof. Andrew Sharott and Prof. Dr Tommas Ellender. The work in this figure was published as part of a manuscript which I joint-first co-authored:

Van Heusden, F.*, **Macey-Dare, A.***, Gordon, J., Krajeski, R., Sharott, A., and Ellender, T. (2021).

Diversity in striatal synaptic circuits arises from distinct embryonic progenitor pools in the ventral telencephalon. *Cell Reports*. 35, 109041. * joint-first author

Figure 4.7 FlashTag inset in methods panel A from Govindan et al. (2018)

Figure 4.8 TUNEL inset in methods panel A from Thermo Fisher Click it Plus TUNEL Assay Manual (2021)

[N.B. both Figure 4.7 and 4.8 insets above are replaced by my own drawings in ORA online thesis]

5. Experimental Chapter C

Figure 5.9 Methods panel A was and published in a manuscript I co-authored:

Krajeski, R.N., **Macey-Dare, A.**, Van Heusden, F., Ebrahimjee, F. and Ellender, T.J. (2019).
Dynamic postnatal development of the cellular and circuit properties of striatal D1 and D2
spiny projection neurons. *Journal of Physiology*. 597(21): 5265-5293.

Figure 5.12 Volumetric MRI scans were performed by Johan Van Audekerke and colleagues (BioImaging
Lab, Antwerp)

6. Experimental Chapter D

Figure 6.2 and 6.3 A small number of non-rescue open-field and rotarod experiments were performed
by Elke Calus (Department of Biomedical Sciences, Antwerp)

Figures 6.4, 6.5 and 6.6 Rescue injections were administered, and reflex testing was performed by Prof.
Dr Tommas Ellender and Yana Van Der Poll (both Department of Biomedical Sciences, Antwerp). Rescue
open-field and rotarod experiments were performed by Elke Calus (Department of Biomedical Sciences,
Antwerp)

The following figures were made using BioRender: **1.1, 1.2, 1.5, 1.7, 1.10, 1.11, 2.17, 2.18, 3.2 A, 3.3 A,
3.6 A, 4.2 A, 4.7 A, 4.8 A, 4.10 A, 5.1 A, 6.1 A, 6.1 B, 6.2 A, 6.3 A, 6.4 A, 7.1**

Abbreviations

µg Microgram	bHLH Basic Helix-Loop-Helix
µl Microlitre	BNST Bed nucleus of the stria terminalis
µm Micrometre	BrdU Bromodeoxyuridine
µM Micromole	BSA Bovine serum albumin
°C Degrees Celsius	CA Cell area
1° / 2° / 3° Primary / secondary / tertiary	CAAR-T Chimeric autoantibody receptor T-cells
A / GluA AMPAR subunit	CAMKII Calcium-calmodulin dependent protein kinase II
aCSF Artificial cerebrospinal fluid	CASPR2 Contactin-associated protein 2
ADHD Attention deficit hyperactivity disorder	cDNA Complementary DNA
ADP Adenosine diphosphate	CHA Convex hull area
aIP Apical intermediate progenitors	CNQX 6-cyano-7-nitroquinoxaline-2,3-dione
AMPA α-amino-3-hydroxy-5-methyl-isoxazolepropionic acid	CNS Central nervous system
AMPA AMPA receptor	CP Undifferentiated cortical plate
ASD Autism spectrum disorders	CREB Cyclic adenosine monophosphate response element-binding protein
ATP Adenosine triphosphate	CS Corticostriatal
BCA Bicinchoninic acid	
BDNF Brain-derived neurotrophic factor	

CSF Cerebrospinal fluid

CTIP2 Chicken ovalbumin promoter
transcription factor interacting protein 2

CTX Cortex

CX3CR1 C-X3-C chemokine receptor 1

Cβa Chicken beta actin

D1 Dopamine receptor 1

D2 Dopamine receptor 2

DAB 3,3'-diaminobenzidine

DAP5 D-(-)-2-amino-5-phosphonopentanoic
acid

DAPI 4', 6-diamino-2-phenylindole

DARPP32 Dopamine and cyclic AMP-regulated
neuronal phosphoprotein

ddH₂O Double distilled water

DLS Dorsolateral striatum

DMS Dorsomedial striatum

DMSO Dimethyl sulfoxide

DNA Deoxyribonucleic acid

E Embryonic day

EAAT Excitatory amino acid transporter

EC External capsule

EdUTP 5-ethynyl-2'-deoxyuridine triphosphate

EEG Electroencephalogram

EphB2 Ephrin type-B receptor 2

EPSC Excitatory postsynaptic current

EPSP Excitatory postsynaptic potential

ErbB4 Epidermal growth factor receptor B4

ERK Extracellular signal-related kinase

FcRn Neonatal fragment crystallisable
receptor

FG Fast Green dye

FLEX Flip-excision plasmid

fMRI Functional magnetic resonance imaging

FOXO Forkhead box O protein

FT FlashTag

GABA Gamma-aminobutyric acid

GABA_AR GABA type A receptor

GFP Green fluorescent protein

GPI / e Globus pallidus interna / externa

GRIN Glutamate ionotropic receptor NMDAR
subunit

GTP Guanosine triphosphate

HD Huntington's disease

HEK Human embryonic kidney

HEPES 4-(2-hydroxyethyl)piperazine-1-
ethanesulfonic acid

Hes Hairy and enhancer of split

HSVE Herpes simplex virus encephalitis

Htt Huntingtin protein

Hz Hertz

IBA1 Ionised calcium-binding adapter
molecule 1

IgG Immunoglobulin G

IL Interleukin

IPs Intermediate progenitor

IP Intraperitoneal

IR Input resistance

ISI Interspike interval

IUE *In utero* electroporation

IV Intravenous

IVIG Intravenous immunoglobulin

K / GluK KAR subunit

K+ Potassium

KA Kainate

KAR Kainate receptor

kDa Kilodalton

KGluc Potassium gluconate

LGE Lateral ganglionic eminence

LGI1 Leucine-rich, glioma inactivated 1

LTD Long-term depression

LTP Long-term potentiation

Mash1 Mammalian achaete-scute homolog 1

Math Meprin and tumour necrosis factor
receptor-associated factor homolog

mEPSC Miniature EPSC

mEPSP Miniature EPSP

Met Metacam

mg Milligram

MGE Medial ganglionic eminence

mGluR Metabotropic glutamate receptor

MGV Mean grey value

MK801 Dizocilpine

ml Millilitre

mM Millimole

MM Mood's median

mOsmol/L Milliosmoles/litre

mPFC Medial prefrontal cortex

MRI Magnetic resonance imaging

mRNA Messenger ribonucleic acid

ms Millisecond

MTC Membrane time constant

MWM Morris water maze

MWU Mann-Whitney U

MZ Marginal zone

MΩ Megaohm

n Number

NBQX 2,3-dihydroxy-6-nitro-7-sulfamoyl-
benzo[f]quinoxaline

NDS Normal donkey serum

NFAT Nuclear factor of activated T-cells

ng Nanogram

NMDA N-methyl-D-aspartate

NMDAR NMDA receptor

NMDARE NMDAR antibody-mediated
encephalitis

NMOSD Neuromyelitis optica spectrum
disorder

NOS Nitric oxide synthase

NR / GluN NMDAR subunit

NRG1 Neuregulin 1

OCD Obsessive compulsive disorder

OF Open-field

OFC Orbitofrontal cortex

OPs Other progenitors

p Postnatal day

P2Y₁₂ Purinergic G-protein coupled receptor
12

pA Picoamps

PAX6 Paired box 6

PBS Phosphate-buffered saline

PBS-Tx Phosphate buffered saline + tween 20

PEAQX Phosphonic acid

PFA Paraformaldehyde

PH3 Phosphohistone 3

PI3K-Akt Phosphatidylinositol 3-kinase

PPDA 1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid

PPE Pre-proenkephalin

PPR Paired-pulse ratio

PS Primordial striatum

PSD95 Postsynaptic density protein 95

PTSD Post-traumatic stress disorder

PVDF Polyvinylidene fluoride

RASGRF1 Ras protein-specific guanine
nucleotide-releasing factor 1

RFP Red fluorescent protein

RFU Relative fluorescence unit

RG Radial glia

RIPA Radio-immunoprecipitation assay

RM ANOVA Repeated measures analysis of
variance

RMP Resting membrane potential

RPM Revolutions per minute

RT-qPCR Reverse transcriptase quantitative
polymerase chain reaction

Runx-1 Runt-related transcription factor 1

SD Standard deviation

SEM Standard error of the mean

sEPSC Spontaneous EPSC

SIN Striatal interneuron

SNc / r Substantia nigra pars compacta /
reticulata

SP Cortical subplate

VZ Ventricular zone

SPN Striatal projection neuron

w Week

STN Sub thalamic nucleus

WSR Wilcoxon signed rank

SVZ Sub-ventricular zone

T Trimester

TAE Tris-acetate-EDTA

TBS Tris-buffered saline

TdT Terminal deoxynucleotidyl transferase

TGF- β 1 Transforming growth factor beta 1

TS Tail striatum

TTX Tetrodotoxin

TUNEL Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling

T α 1 Tubulin alpha 1

V Volt

VCS Visual classification scale

Vet Vetergesic

VGLUT Vesicular glutamate transporter

VS Ventral striatum

Figures & Tables

1: General Introduction

1.1 Simple overview of neurogenesis

1.2 Timescale overview of basic neurodevelopmental processes in humans and mice

1.3 Membrane structure and conformations of the NMDAR

1.4 Temporal and regional expression of NMDAR subunits

1.5 Key roles of NMDARs in neuronal and synaptic development

1.6 Transfer of NR1-IgG antibodies from mother to foetus during pregnancy

1.7 Types of gestational passive transfer model

1.8 Simplified overview of striatal anatomy

1.9 Basal ganglia connectivity

1.10 Neurogenesis in the cortex and LGE

1.11 Microglial states

1.12 Summary of thesis aims

2: General Methods

2.1 Polyclonal and monoclonal patient-derived NR1-IgG and healthy control-IgG antibodies

2.2 Additional polyclonal patient-derived NR1-IgG and healthy control-IgG antibodies used in a small subset of experiments

2.3 Steps to dissect the cortex from the LGE in embryonic E16.5 brains

2.4 Sequences of custom-designed primers for RT-qPCR reactions

2.5 'Dawn Powerup Protocol2' protocol for RT-qPCR

2.6 PCR gel of custom-designed primers

2.7 Western blot serial protein dilutions and representative standard curve

2.8 Graphical summary of the TITERPLANE protocol

2.9 Different substances injected in utero

2.10 Agar embedding embryonic brains

2.11 E16.5 rostral, medial and caudal slices

2.12 Table of all immunohistochemistry experiments performed

2.13 Table of all primary antibodies used

2.14 Table of all secondary antibodies used

2.15 Whole cell patch-clamp recording capacitive current changes

2.16 Microglial morphological parameters analysed with FracLac analysis

2.17 Early postnatal reflexes

2.18 Post-weaning behavioural tests

3. Experimental Chapter A

3.1 Vesicular glutamate transporters in the wild-type embryonic cortex and LGE

3.2 Transcript-level glutamate receptor expression in the wild-type cortex and LGE

3.3 Protein-level NR1 expression in the wild-type embryonic cortex and LGE

3.4 NR1-expressing HEK cell-based assay of patient-derived polyclonal and monoclonal NR1-IgG

3.5 Binding of patient-derived NR1-IgG antibodies in fixed wild-type coronal embryonic slices

3.6 Dynamics of patient-derived NR1-IgG antibodies when injected in utero

4. Experimental Chapter B

4.1 Embryonic immunohistochemistry analysis methods

4.2 Altered morphology in the LGE following brief in utero NR1-IgG exposure

4.3 Decreased progenitor proliferation in the LGE following brief in utero NR1-IgG exposure

4.4 Greatest decreases in progenitor proliferation in the medial LGE and close to the ventricle

4.5 Characteristics of two populations of IUE labelled progenitors in the healthy LGE

4.6 Characteristics of two populations of IUE labelled LGE progenitors co-injected with NR1-IgG

4.7 Decreased cell migration in the LGE following brief in utero NR1-IgG exposure

4.8 No change in cell death in the LGE following brief in utero NR1-IgG exposure

4.9 Other NR1-IgG antibodies also impair cell migration in the LGE after brief in utero exposure

4.10 Increased microglial density in the LGE after brief in utero NR1-IgG exposure

4.11 Altered microglial morphology in the LGE after brief in utero NR1-IgG exposure

5: Experimental Chapter C

5.1 Altered intrinsic properties of early postnatal SPNs following brief in utero NR1-IgG exposure

5.2 Similar effects of in utero exposure to polyclonal and monoclonal control-IgG antibodies on early postnatal SPN intrinsic properties

5.3 Similar effects of in utero exposure to polyclonal and monoclonal NR1-IgG antibodies on early postnatal SPN intrinsic properties

5.4 Effects of in utero NR1-IgG exposure on SPN intrinsic properties in specific two-day time windows

5.5 No change in dendritic arborisation of early postnatal SPNs following in utero NR1-IgG exposure

5.6 Reduced spine density on secondary dendrites of early postnatal SPNs following in utero NR1-IgG exposure

5.7 Greatest differences in SPN morphology putatively occur at the end of the second postnatal week

5.8 Reduced sEPSC amplitude in early postnatal SPN excitatory synapses following in utero NR1-IgG exposure

5.9 Altered cortical excitatory synaptic properties in early postnatal SPNs following in utero NR1-IgG exposure

5.10 Reduced NMDA/AMPA ratio in early postnatal SPNs following in utero NR1-IgG exposure

5.11 In utero NR1-IgG exposure predominantly affects pre-synaptic properties of early postnatal SPNs in specific short time windows

5.12 Alterations in striatal size, cell composition and density following in utero NR1-IgG exposure

5.13 Increased striatal microglial density following in utero NR1-IgG exposure

5.14 Morphological alterations in striatal microglia following in utero NR1-IgG exposure

6. Experimental Chapter D

6.1 Altered early postnatal reflexes following in utero NR1-IgG exposure

6.2 Altered anxiety-related open-field behaviours following in utero NR1-IgG exposure

6.3 Increased time on accelerating rotarod following in utero NR1-IgG exposure

6.4 'Rescue' of early postnatal reflex delays in NR1-IgG in utero exposed animals using AMPAkine CX516

6.5 Partial 'rescue' of alterations in open-field behaviour in NR1-IgG in utero exposed animals using AMPAkine CX516

6.6 'Rescue' of alterations in rotarod behaviour in NR1-IgG in utero exposed animals using AMPAkine CX516

7. General Discussion

7.1 Graphical summary of key thesis findings

Supplementary Figures

S2.1 Supporting validation data and controls for custom-designed primers

S3.1 Personal correspondence with Dr Sukhvir Wright detailing experimental end point titrations for the NR1-IgG and control-IgG polyclonal antibodies tested on NR1-expressing HEK cells in 2018

S4.1 Supporting control data for morphology-related IHC in Figure 4.2

S4.2 No significant difference in the areas that PH3+ cells (Figure 4.3) were counted in

S4.3 No significant correlations between area that PH3+ cells (Figure 4.3) were counted in, and number of PH3+ cells

S4.4 Supporting sham control data for progenitor proliferation-related IHC results (Figure 4.3)

S4.5 Supporting sham control data for FlashTag and TUNEL assay results (Figures 4.7 and 4.8)

S4.6 Embryonic microglial morphological parameters (Figure 4.11)

S5.1 Intrinsic properties of SPNs recorded from the p7-14 striatum (Figure 5.1)

S5.2 Intrinsic properties of SPNs recorded from the [A] p7-8, [B] p9-10, [C] p11-12 and [D] p13-14 striatum (Figure 5.4)

S5.3 Correlations of key intrinsic properties and morphological features

S5.4 Key correlation graphs between intrinsic properties and morphological features

S5.5 Corticostriatal excitatory input properties of SPNs recorded from the p7-14 striatum (Figures 5.9 and 5.10)

S5.6 Corticostriatal excitatory input properties of SPNs recorded from the [A] p7-8, [B] p9-10, [C] p11-12 and [D] p13-14 striatum (Figure 5.11)

S5.7 Impacts on early postnatal SPN corticostriatal connections within specific two-day time windows (Figure 5.11)

S5.8 Correlations of NMDA/AMPA ratio and key electrophysiological (intrinsic and synaptic) properties

S5.9 Key correlation graphs between NMDA/AMPA ratio and electrophysiological properties

S6.1 Day, group x day and group RM ANOVA statistics on p21-23 behaviour data (Figures 6.2 and 6.3)

S6.2 Data from testing of postnatal reflexes [A] righting reflex, [B] negative geotaxis and [C] cliff avoidance in NR1-IgG and control-IgG in utero injected animals, tested daily from p7-14 (Figure 6.1)

S6.3 Data from testing of postnatal reflexes [A] righting reflex, [B] negative geotaxis and [C] cliff avoidance in NR1-IgG and control-IgG in utero injected animals, tested daily from p7-p10, following administration of 'rescue' injections of AMPAkine CX516 (Figure 6.4)

S6.4 Day, Group x Day and Group RM ANOVA statistics on p21-23 behaviour data for [A] rescue NR1-IgG vs non-rescue NR1-IgG, [B] rescue NR1-IgG vs non-rescue control-IgG, [C] rescue NR1IgG vs rescue control-IgG, and [D] rescue control-IgG vs non-rescue control-IgG datasets (Figures 6.5 and 6.6)

Contents

Abstract.....	2
Acknowledgements.....	4
Declaration of Authorship	9
Abbreviations	11
Figures & Tables.....	17
Contents	24
1. General Introduction	31
1.1 NMDARs and their importance in healthy neurodevelopment.....	31
1.1.1 Neurogenesis in a nutshell.....	31
1.1.2 Glutamate: an important early neuromodulator.....	35
1.1.3 Glutamate receptors: NMDARs	37
1.1.4 NMDAR subunits and subtypes.....	42
1.1.5 Roles of NMDARs in neuronal and synaptic development.....	47
1.1.6 Brief overview of other ionotropic glutamate receptors: AMPARs and KARs.....	55
1.2 NMDAR antibodies and implications for neurodevelopment	59
1.2.1 NMDAR antibody-mediated encephalitis (NMDARE)	59
1.2.2 Current understanding of NMDAR antibody pathogenesis.....	61
1.2.3 NMDARE in pregnancy and impact on neurodevelopment.....	63
1.2.4 Studying pathogenic impacts of NMDAR antibodies using <i>in vivo</i> models.....	67
1.2.5 Rescue models	71
1.3 The striatum as an important, unexplored neurodevelopmental target of NMDAR antibodies	72
1.3.1 Striatal networks and functions.....	73
1.3.2 Striatal cellular development.....	77
1.3.3 Striatal circuit development.....	79
1.3.4 A brief overview of cortical development in the context of the striatum.....	83
1.3.5. Why is the striatum an interesting potential target for NMDAR antibodies?	85
1.4 A focused introduction to microglia	88
1.4.1 Microglia functions and states.....	88
1.4.2 Roles of microglia in neurodevelopment.....	90

1.4.3 Microglia, NMDARs and NMDARE antibodies	91
1.5 Thesis Aims.....	93
2. General Methods	95
2.1 Mouse model background	95
2.1.1 Mouse model background and ethics.....	95
2.1.2 Generation of pregnant dams with dated plugs.....	95
2.2 Patient-derived antibodies	97
2.2.1 Background information of patient-derived antibodies	97
2.2.2 Ethical approval for patient-derived antibodies	99
2.3 Transcript and protein quantification methods.....	100
2.3.1 RNA extraction	100
2.3.2 cDNA synthesis.....	101
2.3.3. RT-qPCR.....	101
2.3.4 Gel electrophoresis of amplification products.....	103
2.3.5 Western blot	104
2.4 Cell-based assay methods.....	107
2.4.1 NMDAR antibody cell-based assay	107
2.5 <i>In utero</i> surgical methods	109
2.5.1 Pre-operative procedures	109
2.5.2 Intra-operative procedures.....	112
2.5.3 Post-operative procedures	113
2.6 Antibody immunohistochemistry methods	114
2.6.1 Fixation and sectioning of brain tissue	114
2.6.2 Experiments using fixed slices	115
2.7 Electrophysiology methods.....	126
2.7.1 Slice preparation	126
2.7.2 Recording conditions	126
2.7.3 Stimulation and recording protocols	127
2.8 Morphological reconstruction methods.....	131
2.8.1 Embryonic brain weight	131
2.8.2 Analysis of striatal volume using structural MRI.....	131
2.8.3 SPN reconstructions using DAB labelling.....	131
2.8.4 Microglial reconstructions	133

2.9 Behavioural methods	134
2.9.1 Pre-weaning tests	134
2.9.2 Early adolescent behavioural tests	136
2.10 Rescue model generation	138
2.11 Statistical methods.....	139
3. Experimental Chapter A: Defining a novel <i>in utero</i> intraventricular model of NR1-IgG antibody delivery to the embryonic mouse brain	140
3.1 Chapter Introduction	140
3.2 Chapter Brief Background	141
3.2.1 The striatum and glutamate	141
3.2.2 Expression of glutamate transporters	142
3.2.3 Dynamic expression of glutamate receptors	142
3.2.4 NMDARE, NR1-IgG antibodies and the striatum.....	144
3.2.5 Key NR1-IgG and other antibody models.....	146
3.3 Chapter Aims.....	148
3.4 Chapter Methods	149
3.4.1 qPCR methods.....	149
3.4.2 Western blot methods	150
3.4.3 Cell-based assay methods.....	150
3.4.4 Immunohistochemical methods	151
3.4.5 <i>In utero</i> surgical methods	152
3.4.6 Statistical methods.....	153
3.5 Chapter Results	154
3.5.1 Exploring expression of early glutamate transporters in the embryonic brain	154
3.5.2 Transcript- and protein-level NR1 target expression in the embryonic brain	156
3.5.3 <i>In vitro</i> binding of patient-derived NR1-IgG antibodies.....	160
3.5.4 <i>Ex vivo</i> dynamics of patient-derived NR1-IgG antibodies	163
3.6 Chapter Discussion.....	168
3.6.1. Vesicular glutamate transporters VGLUT1 and VGLUT2 are both present in the embryonic cortex and LGE, suggesting that glutamate loaded vesicles are present;	168
3.6.2. Transcript- and protein-level expression of NR1 are both higher in the LGE than cortex suggesting that the NR1 subunit is highly expressed and potentially functional;.....	171
3.6.3. Both polyclonal and monoclonal patient-derived NR1-IgGs bind NR1 <i>in vitro</i> in a cell-based assay;.....	173

3.6.4. Both NR1-IgG antibodies bind the mouse LGE <i>in vitro</i> and <i>ex vivo</i> , with the monoclonal antibody exhibiting stronger binding;.....	174
3.6.5. Antibodies exhibit a similar pattern of distribution in the LGE <i>ex vivo</i> , and show higher binding at 24 hours than 48 hours post-injection;	175
3.7 Chapter Summary	177
4. Experimental Chapter B: How does brief <i>in utero</i> exposure to NR1-IgG antibodies affect neurogenesis and early striatal formation embryonically?	178
4.1 Chapter Introduction	178
4.2 Chapter Brief Background	179
4.2.1 Early striatal (and cortical) development.....	179
4.2.2 Roles of NMDARs in neurogenesis and early neurodevelopment.....	180
4.2.3 Disruption of NMDARs during neurodevelopment by NR1-IgG antibodies	183
4.2.4 The striatum as an interesting, unexplored target of developmental NR1-IgG impacts.....	184
4.3 Chapter Aims.....	186
4.4 Chapter Methods	187
4.4.1 <i>In utero</i> surgical methods	187
4.4.2 Immunohistochemical methods	188
4.4.3 Statistical methods.....	192
4.5 Chapter Results	193
4.5.1 Embryonic impacts of NR1-IgG on gross and regional morphology	193
4.5.2 Embryonic impacts of NR1-IgG on progenitor proliferation.....	195
4.5.3 Embryonic impacts of NR1-IgG on specific LGE progenitor populations.....	198
4.5.4 Embryonic impacts of NR1-IgG on cell migration and cell death	202
4.5.5 Validating effects using four further patient-derived polyclonal NR1-IgG antibodies	206
4.5.6 Embryonic impacts of NR1-IgG on microglia	207
4.6 Chapter Discussion.....	212
4.6.1 Gross LGE morphology: Significant decrease in the area of the primordial striatum, and significant increase in the width of the LGE proliferative zone;	213
4.6.2 Overall progenitor proliferation: Significant decrease in PH3+ progenitor proliferation in the LGE, with the greatest decreases in medial sections and in the proliferative zone;	214
4.6.3 Specific progenitor populations: No significant difference in the percentage of labelled Tα1-expressing progenitors;	216
4.6.4 Cell migration and cell death: Significant decrease in cell migration but no significant differences in cell death in the LGE;	217

4.6.5 Other patient-derived NR1-IgG antibodies;.....	219
4.6.6. Microglia: Increase in activated microglial density in the LGE and alterations in morphology suggestive of a more activated state and/or maturational differences;.....	220
4.7 Chapter Summary	223
5. Experimental Chapter C: How does brief <i>in utero</i> exposure to patient-derived NR1-IgG affect striatal cellular and circuit development postnatally?	224
5.1 Chapter Introduction	224
5.2 Chapter Brief Background	225
5.2.1 Cellular development of SPNs in the early postnatal period	225
5.2.2 SPNs and the development of striatal circuits	226
5.2.3 Influence of microglia on shaping early postnatal circuits	228
5.2.4 The importance of NMDARs in early postnatal cellular and circuit development	228
5.2.5 Focusing on the impact of NR1-IgG antibodies on the early postnatal striatum	230
5.3 Chapter Aims.....	231
5.4 Chapter Methods	232
5.4.1 <i>In utero</i> surgical methods	232
5.4.2 Electrophysiological methods	232
5.4.3 Morphological methods.....	235
5.4.4 Statistical methods.....	238
5.5 Chapter Results	239
5.5.1 Postnatal impact of NR1-IgG antibodies on the intrinsic properties of SPNs.....	239
5.5.2 Postnatal impact of NR1-IgG antibodies on SPN morphology	249
5.5.3 Postnatal impact of NR1-IgG antibodies on excitatory synaptic inputs and corticostriatal connections.....	254
5.5.4 Postnatal impact of NR1-IgG antibodies on striatal morphology and cell composition.....	263
5.5.5 Postnatal impact of NR1-IgG antibodies on microglia in the striatum	266
5.6 Chapter Discussion.....	269
5.6.1 SPN intrinsic properties: Differences in early postnatal (p7-14) SPN intrinsic properties, suggestive of relative electrical immaturity; and.....	270
5.6.2 SPN morphology: Subtle differences in early postnatal SPN morphology, including a significant decrease in spines on secondary dendrites;.....	270
5.6.3 SPN excitatory synaptic inputs: Reduced sEPSC amplitude but no changes in sEPSC frequency, suggestive of a post-synaptic effect;	274

5.6.4 NMDA/AMPA ratio: Significantly reduced NMDA/AMPA ratio, with greatest difference at p13-14;	275
5.6.5 Corticostriatal connections: Changes in pre-synaptic properties and short-term plasticity, but no differences in post-synaptic properties analysed;.....	276
5.6.6 Regional morphology and cell composition: Decreased striatal volume, a trend towards decreased SPN cell density and a significantly shifted D1/D2 ratio (fewer D2-SPNs) in the fourth postnatal week;.....	279
5.6.7 Microglia: Increase in activated microglia density and alterations in morphology suggestive of a more activated state in the fourth postnatal week striatum;	280
5.7 Chapter Summary	283
6. Experimental Chapter D: How does brief <i>in utero</i> exposure to patient-derived NR1-IgG impact postnatal behaviour and can the behavioural phenotype be rescued using an AMPAkine?	284
6.1 Chapter Introduction	284
6.2 Chapter Brief Background	285
6.2.1 NMDAR antibodies and behaviour	285
6.2.2 Behavioural testing in antibody models	286
6.2.3 Rescuing deficits following early NMDAR disruption	287
6.3 Chapter Aims.....	289
6.4 Chapter Methods	290
6.4.1 <i>In utero</i> surgical methods	290
6.4.2 Behavioural methods	290
6.4.3 Rescue methods.....	292
6.4.4 Statistical methods.....	293
6.5 Chapter Results	294
6.5.1 Postnatal impact of NR1-IgG antibodies on early reflex behaviour	294
6.5.2 Postnatal impact of NR1-IgG antibodies on early adolescent open-field behaviour	296
6.5.3 Postnatal impact of NR1-IgG antibodies on early adolescent accelerating rotarod behaviour	300
6.5.4 Effects of boosting glutamatergic transmission with AMPAkine CX516 on behaviour in NR1-IgG <i>in utero</i> exposed mice	301
6.6 Chapter Discussion.....	313
6.6.1 Significant delays in the development of certain early postnatal reflexes (surface righting, negative geotaxis and cliff avoidance) in NR1-IgG <i>in utero</i> exposed animals;	314
6.6.2 Alterations in anxiety-related aspects of open-field behaviour suggestive of reduced anxiety;	315

6.6.3 Minimal differences in motor-related aspects of open-field behaviour;	317
6.6.4 A surprising significant increase in time spent on the accelerating rotarod;	319
6.6.5 Rescue of early postnatal reflex delays through boosting glutamatergic transmission with CX516 AMPAkinic injections;	321
6.6.6 Minimal rescue of open-field behaviour following CX516 rescue treatment; and	321
6.6.7 Normalisation of the time spent on the rotarod following CX516 rescue treatment;	321
6.7 Chapter Summary	324
7. General Discussion	325
7.1. Summary of Findings.....	325
7.2. Implication of Findings.....	329
7.2.1. The <i>in utero</i> intraventricular model for studying maternal antibody impacts	329
7.2.2. <i>In utero</i> NMDAR antibody exposure and the developing brain.....	333
7.2.3. Management, rescue and reversibility of NMDAR antibody impacts	337
7.3 Future Directions	344
7.4 Conclusion.....	347
8. Bibliography.....	348
9. Appendix	403

1. General Introduction

1.1 NMDARs and their importance in healthy neurodevelopment

1.1.1 Neurogenesis in a nutshell

Most neurons in the mature adult brain are generated embryonically¹. Though the specific processes vary by region, neurogenesis (i.e. the generation of neurons) (**Figure 1.1**) in all brain regions begins with an initial pool of multipotent neural stem cells. These can proliferate to expand their pool through symmetrical expansion or asymmetrically divide to produce new neuronal (or other) progenitor cell types that are committed to producing neurons or glia following subsequent differentiation and division steps (**Guan et al. 2001, Götz 2005, Oliviera et al. 2018**). Neuronal progenitors follow the same pattern, either dividing symmetrically to expand their own pools, or dividing asymmetrically to produce other progenitors or neuroblasts, which mature into mature neurons. Neurogenesis also involves other steps including cell migration, survival and apoptosis. The processes underlying neurogenesis are driven by a combination of both extrinsic cues and regional and temporal intrinsic programs (**Paridaen and Huttner 2014**).

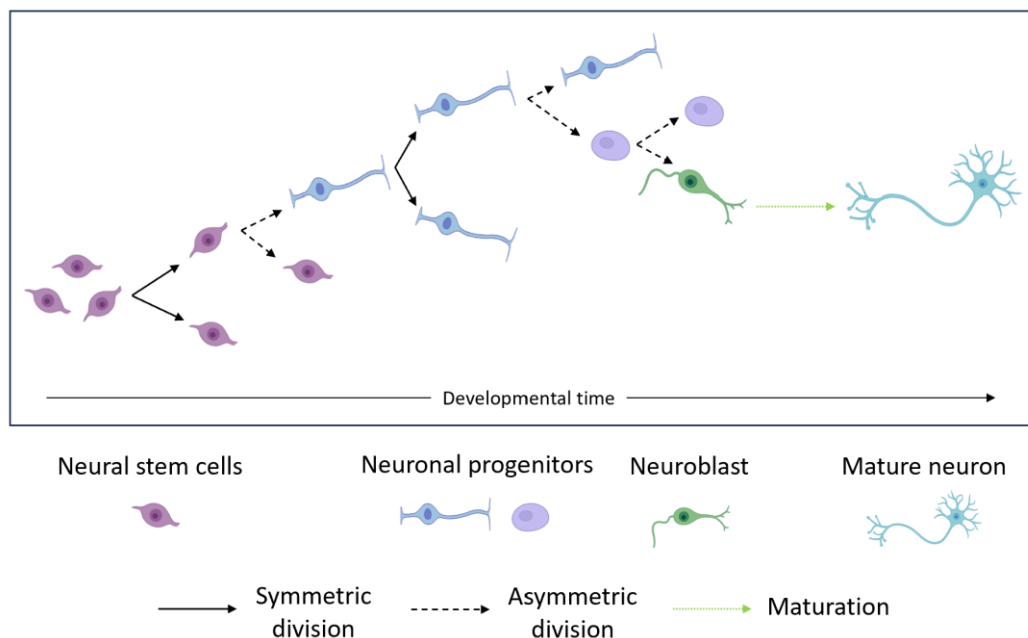


Figure 1.1. Simple overview of neurogenesis. Figure based on Oliviera et al. 2018.

¹ Please note, the processes described in this introduction refer to the mouse/rodent brain unless stated.

Once neurons in the brain are born, they must connect with one another to communicate. Healthy neural circuitry requires precise transfer of information at junctions called synapses, which are either excitatory or inhibitory depending on the neurotransmitters they release. Synaptic connectivity results from a careful balance between synaptic formation ('synaptogenesis') and subsequent synaptic pruning between neurons, a process essential for the correct functioning of the brain (**Märting et al. 2000, Maffei et al. 2004, Cline 2005, Tao and Poo 2005, Åkerman and Cline 2006, Cline and Haas 2008, Dorn et al. 2010, Gambrill and Barria 2011, Gu et al. 2016**).

A detailed explanation of neurogenesis and synaptogenesis in the focal region of this thesis, the striatum, (and in the relevant comparator region, the cortex) is given in **Section 1.3. The striatum as an important, unexplored neurodevelopmental target of NMDAR antibodies**.

A timescale summary of the relevant basic neurodevelopmental processes for human and rodent is given below (**Figure 1.2**):

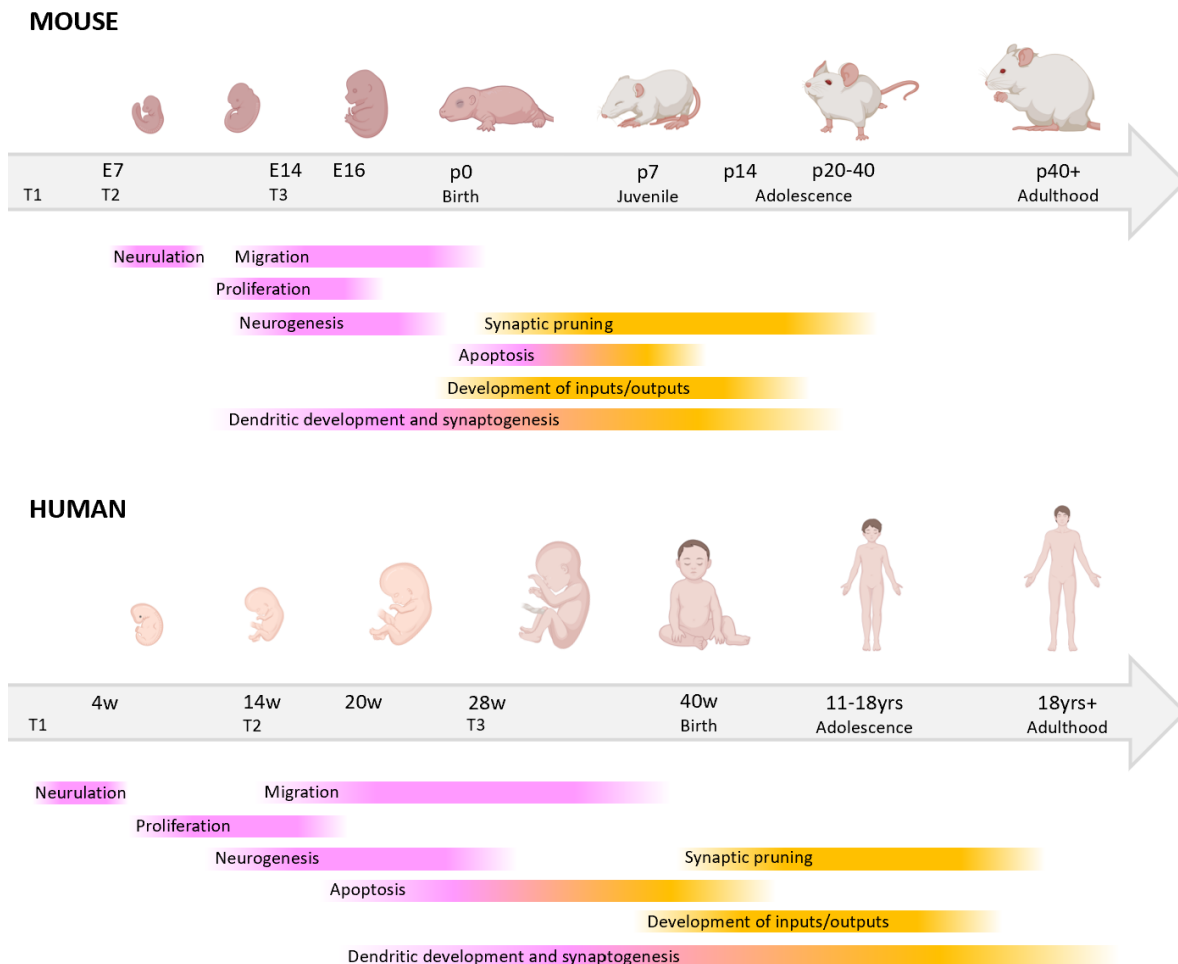


Figure 1.2. Timescale overview of basic neurodevelopmental processes in humans and mice. Whilst overall development is similar in both species, individual processes happen at different points and over different relative timescales. Mice are born relatively premature developmentally compared to humans and generally undergo less protracted developmental processes. In both mice and humans, earlier processes are more reliant on molecular cues whilst later processes depend more on emerging electrical activity. Processes occurring embryonically and postnatally are coloured in pink and orange respectively. Key: T: trimester, E: embryonic day, p: postnatal day, w: weeks. Figure based on **Chini and Hanganu-Opatz 2020**.

To study neurodevelopment, many studies, including those of this thesis, utilise mouse (or other animal) models, since it is highly challenging to use human brains directly, owing to the practical and ethical

constraints on using embryonic and early postnatal human tissue. Mouse models are useful for several reasons including their breeding rate, amenability to genetic alterations, and ease of animal care (Houdebine and Hedrich 2004, Kabakci et al. 2004, Dietrich et al. 2014, Ellenbroek and Youn 2016, Beauchamp et al. 2022). There are similarities between human and mouse brains including substantial homology in basic neuronal types, ion channels, synaptic receptors, transcriptional programs, and other key molecular components (Shi et al. 2021, Loomba et al. 2022). Studies also indicate evolutionary conservation of many circuits including several in the cortex and striatum, such as cortical inhibitory circuit motifs (Kim et al. 2023) and corticostriatal motor circuits (Balsters et al. 2020). There are also notable differences. Beyond the clear discrepancy in size, the human brain is a much more complex structure, with an ~1000 times increase in neuron numbers and numerous complex neocortical folds. There are many additional specialised regions with no mouse homologues (Preuss 1995, Wong et al. 2023), as well circuits that are not conserved across species (Balsters et al. 2020). There are also cellular differences, including more progenitor and neuronal subtypes and longer progenitor cell cycle times in humans (Florio and Huttner 2014, Mason and Price 2016, Marshall and Mason 2019).

Animal models and clinical studies have facilitated key insights into healthy neurodevelopment and developmental origins and potential mechanisms underpinning neurodevelopmental disorders (Del Pino et al. 2018). This thesis aims to use a mouse model to explore how antibodies against the glutamatergic NMDAR, which can be passed from mother to foetus during pregnancy, impact development of a brain region called the striatum, which is important in many motor processes and cognitive functions. In this **General Introduction**, firstly the roles of the important early neuromodulator glutamate and key glutamate receptors, particularly the NMDA receptor (NMDAR), in neurodevelopment will be introduced. Next, current knowledge on antibodies against the NMDAR and the implications for neurodevelopment will be detailed. Lastly, the striatum as an important, unexplored neurodevelopmental target for NMDAR antibodies will be introduced.

1.1.2 Glutamate: an important early neuromodulator

Embryonic neurogenesis relies on a combination of both extrinsic cues and intrinsic programs within the developing brain (**Paridaen and Huttner 2014, Mira and Morante 2020**). One important extrinsic cue is the neurotransmitter glutamate, the principal excitatory neurotransmitter released by neurons in the CNS. The neuron-generating ('neurogenic') zones of the embryonic brain, such the zones bordering developing ventricles (the 'ventricular zones'), contain high concentrations of glutamate (**Behar et al. 1999, Haydar et al. 2000, Jansson et al. 2014**). In the mature brain when synaptic innervation is completely developed, glutamate synthesis and transport is facilitated by astrocytes. Glutamate levels are maintained by the glutamate-glutamine cycle, whereby astrocytes take up glutamate and convert it to glutamine, whilst neurons take up glutamine and convert it to glutamate, which is then packaged into synaptic vesicles by vesicular glutamate transporters (**Hediger 1999, Daikhin and Yudkoff 2000, Meldrum 2000, Maragakis and Rothstein 2004**). Conversely, in the immature brain, glutamate regulation may be dominated by neurons, since gliogenesis occurs later than neurogenesis, and does not begin until the end of the embryonic period (~embryonic day (E)18 in mice) (**Temple 2001, Alvarez-Buylla et al. 2001**). Indeed, the relatively high levels of glutamate in the embryonic brain may be due to lack of uptake by glial cells, or alternatively the consequence of an immature blood-brain barrier, which is also mediated in part by glial cells, and which in the mature brain promotes the removal of glutamate and maintenance of low extracellular fluid glutamate concentrations via excitatory amino acid transporters (EAATs) (**Engelhardt 2003, Hawkins 2009, Zhao et al. 2021**). Both neuronal precursors and neurons may provide early glutamate sources, with both bypassing axons and ingrowing growth cones shown to release neurotransmitters embryonically (**McConnell et al. 1989, Kim et al. 1991, Gao and Van den Pol 2000, Métin et al. 2000**). There are also potential early non-neuronal sources of glutamate (beyond glial cells). For example, glutamic acid (i.e. non-ionised glutamate) is present at high concentrations in female reproductive fluid and can act as a signalling molecule in pre-implantation embryos via glutamate

receptors (**Špírková et al. 2022**) whilst undifferentiated embryonic stem cells have been shown to promote proliferation *in vitro* through secreting glutamate (**Teng et al. 2023**).

The level of glutamate in the brain for healthy physiological functioning and proper development requires tight regulation, which is done through the action of glutamate transporters: three vesicular glutamate transporters (VGLUT1-3) and five structurally distinct EAATs (1-5) (**McConnell et al. 1989, Kim et al. 1991, Gao and Van den Pol 2000, Métin et al. 2000, Héja et al. 2006**). These transporters can directly regulate the extracellular glutamate concentration seen by local progenitors and neurons, via uptake or non-vesicular release (**Allen et al. 2004**), thereby playing an important role in neurogenesis and neuronal development. They also show distinct temporal and regional distributions, enabling them to closely regulate glutamate over early neurodevelopment.

Regarding VGLUTs, VGLUT1-3 are primarily responsible for transporting glutamate into synaptic vesicles, but also show expression prior to formation of synapses, suggesting wider functions during neurogenesis and development (**Boulland et al. 2004**). VGLUTs may have precise but not yet clearly established specific neurodevelopmental roles, with previous work demonstrating both complementary expression patterns in different subsets of glutamatergic cell populations (**Herzog et al. 2001, Varoqui et al. 2002, Freneau et al. 2004**), and dynamic changes over development. Temporally, VGLUT1 is present at low levels embryonically, with VGLUT2 being the predominant transporter in late embryonic and early postnatal stages, and VGLUT3 only occurring postnatally (**Boulland et al. 2004**). Regionally, VGLUT1 is expressed most highly in the hippocampus, whereas VGLUT2 is expressed most highly in the brainstem, midbrain, thalamus and hypothalamus. Both exhibit low level cortical and striatal expression. Pertinent to this thesis, precise embryonic expression patterns in the striatum are not known but in the early postnatal striatum it has been shown that VGLUT1 and VGLUT2 are largely confined to the two key compartments of the of the striatum (the striatal patches and the striatal matrix, detailed in **Section 1.3. The striatum as an**

important, unexplored neurodevelopmental target of NMDAR antibodies) respectively, with this difference disappearing by adulthood (**Boulland et al. 2004**), suggesting a possible distinct roles in early striatal development.

EAATs are beyond the scope of this thesis, but briefly for completeness: EAAT3 is the main embryonic neuronal transporter, present in many neuronal subtypes (**Fairman et al. 1995, Furuta et al. 1997b**), whilst EAAT1 and EAAT2 are expressed embryonically on early progenitor or later neuronal subtypes respectively (**Sutherland et al. 1996, Furuta et al. 1997a, Hartfuss et al. 2001, Namba et al. 2005**). EAAT4 and EAAT5 are only found postnatally and restricted primarily to cerebellar/forebrain neurons and retinal cells respectively (**Arriza et al. 1997, Furuta et al. 1997b, Dehnes et al. 1998, Rauen et al. 2000, Massie et al. 2001**).

1.1.3 Glutamate receptors: NMDARs

Glutamate exerts its effects both in the developing and mature brain via glutamate receptors. The glutamate receptors include two classes: the ionotropic receptors (NMDARs, AMPA receptors (AMPA) and KA receptors (KARs)), and the metabotropic receptors (1-8). The ionotropic receptors are ligand-gated cation-permeable channels, divided based on their activating antagonists and pharmacological properties (**Hansen et al. 2017**). This thesis focuses on NMDARs and their role in neurodevelopment.

NMDARs mediate a large component of excitatory synaptic transmission. They are found throughout the brain across development and are key players in normal brain function, including synaptic plasticity and neuronal maturation (**Paoletti et al. 2013**). NMDAR dysregulation is also involved in an array of neurological and psychiatric diseases (**Lau et al. 2007, Mony et al. 2009, Traynelis et al. 2010**).

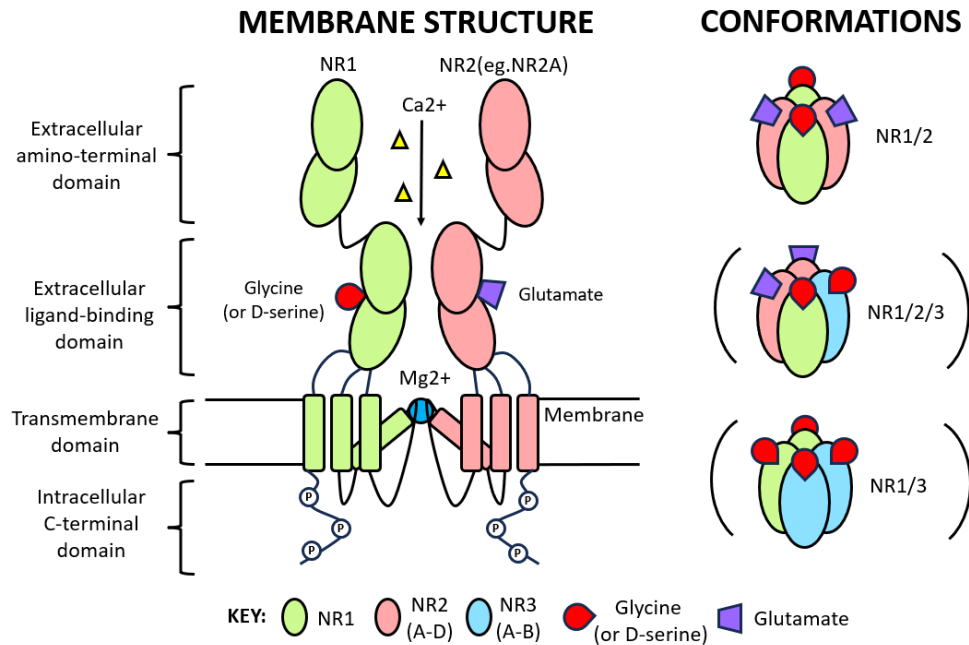


Figure 1.3. Membrane structure and conformations of the NMDAR. The NMDAR is a heterotetrametric channel composed of four homologous subunits: two obligatory NR1 subunits and two NR2/3 subunits. Subunits all have a similar structure, consisting of an extracellular amino-terminal domain, and extracellular ligand-binding domain (where either glutamate or glycine (or D-serine) binds), a transmembrane domain, and an intracellular C-terminal domain. Different combinations of subunits produce different NMDAR subtypes which have distinct biological, pharmacological and signalling properties. Based on Kehoe et al. 2013 and Gallo et al. 2023.

Structurally, NMDARs, are heterotetrametric channels composed of four homologous subunits (Traynelis et al. 2010, Mayer 2011, Paoletti 2011) (Figure 1.3). There are seven subunits grouped into three families: the NR1 subunit, four NR2 subunits (NR2A-D), and two NR3 subunits (NR3A-B). Each receptor consists of two obligatory NR1 subunits, coupled with two NR2/3 subunits (Schüler et al. 2008, Karakas and Furukawa 2014). Subunits have distinctive and varied properties, with give NMDARs a rich span of pharmacological and biological properties (Schüler et al. 2008, Karakas and Furukawa 2014).

Similarly to other ionotropic glutamate receptors, each subunit of the NMDAR consists of four distinct structural regions. Firstly, there is the extracellular amino-terminal binding domain, which is involved in allosteric regulation and assembly of subunits. Secondly, there is the extracellular ligand-binding domain,

formed by the S1 and S2 discontinuous segments, which bind glycine/D-serine in NR1 and NR3 subunits, and glutamate in NR2 subunits. The amino-terminal binding domain and the ligand binding domain form dimers (**Furukawa et al. 2005, Karakas et al. 2011**). Thirdly, there is the transmembrane domain, composed of three transmembrane helices and an m2 pore loop which surrounds the ion filter. Lastly, there is the intracellular C-terminal domain which binds signalling molecules and governs receptor trafficking and anchoring (**Traynelis et al. 2010, Mayer 2011, Paoletti 2011**). Within the structure, the receptor operates as a pair of dimers. The most common type of receptor is composed of two NR1 and two NR2 subunits, which typically arrange themselves in an NR1-NR2-NR1-NR2 conformation (**Salussolia et al. 2011, Riou et al. 2012**).

As an important point, it has been suggested that assembly of subunits into functional NMDARs requires a certain level of maturation and proper intracellular trafficking, and there is divided literature on whether they exist on dividing stem cells and neuronal precursors. Several calcium imaging and electrophysiological studies show that although many subunits are detected embryonically (**Akazawa et al. 1994, Monyer et al. 1994, Petralia et al. 1994a, Petralia et al. 1994b, Jiang et al. 2004**), NMDARs are only present in young, post-mitotic neurons during or just before the start of migration (**Lo Turco et al. 1991, Rossi et al. 1993, Lo Turco et al. 1995, Behar et al. 1999, Maric et al. 2000, Ye et al. 2000, Hirasawa et al. 2003**), with a similar delay between subunit expression and functional receptor assembly suggested by *in vitro* experiments with progenitor cell lines (**Ray and Gottlieb 1993, Sucher et al. 1993, Turetsky et al. 1993, Morley et al. 1995, Strübing et al. 1995, Casado et al. 1996, Asahi et al. 1998, Varji et al. 2001, Jelitai et al. 2002**). However, there are also experiments indicating presence of functional NMDARs on certain precursor cells and progenitors in the hippocampus (**López et al. 1997**). This discrepancy could potentially be explained by differences in experimental techniques, functional NMDARs only being present on subpopulations of progenitors, or differences in embryonic vs adult neurogenic processes,

since the latter study was performed *in vitro* using adult rather than embryonic hippocampal progenitor cells.

Location-wise, NMDARs are found at multiple subcellular locations. Within the synapse, they are classically thought of as post-synaptic membrane receptors, but they are also found on the pre-synaptic membrane in several regions (**Braisser and Feldman 2008, Huang et al. 2011, Buchanan et al. 2012, Banerjee et al. 2014, Larsen et al. 2014**), including on corticostriatal pre-synaptic membranes (**Park et al. 2014**). The literature has started to define functionally distinct roles of pre- and post-synaptic NMDARs, including differences in NMDAR subunit composition and downstream signalling cascades (**Banerjee et al. 2016**). Specific developmental roles have also been identified in a recent study of visual circuit development; post-synaptic NMDARs were shown to contribute to synaptic maturation, and regulation of pre-synaptic release probability, as well as morphological development of pre- and post-synaptic neurons, whilst pre-synaptic NMDARs were shown to influence retinotectal synaptic inputs and influence morphological dynamics of post-synaptic neurons in an opposite manner to their pre-synaptic counterparts (**Kesner et al. 2020**). NMDARs are also found extrasynaptically, and it has been estimated that more than 65% of NMDARs can shuttle between synaptic and extrasynaptic sites (**Tovar and Westbrook 2002**). Cellular location of NMDARs has been shown to impact cellular responses; activation of synaptic NMDARs promotes neuronal health and resistance to oxidative insults, promoting cell survival, whilst activation of extrasynaptic NMDARs has been linked to several cell death pathways, including inactivation of pro-survival factors such as CREB, and promotion of pro-death factors including FOXO (**Chrivia et al. 1993, Kwok et al. 1994, Papadia et al. 2008, Al-Mubarak et al. 2009, Lau and Bading 2009, Hardingham and Bading 2010**). It is thought that a shift in the balance from synaptic to extrasynaptic NMDAR signalling may be important in neurological diseases including Huntington's disease, where synaptic NMDAR activity promotes formation of non-toxic inclusions, but extrasynaptic NMDAR activity

promotes the toxicity of mutant Htt protein (**Hardingham and Bading 2010, Milnerwood et al. 2010, Tu et al. 2010**).

Mechanistically, NMDARs have several properties that make them distinct from other types of ionotropic glutamate receptor (**Cull-Candy and Leszkiewicz 2004, Traynelis et al. 2010, Paoletti 2011**). One key difference is that the ion channel has a voltage-dependent magnesium block. NMDAR activation therefore mediates slower transmission than AMPAR/KARs since AMPAR/KARs can gate whenever glutamate is present whereas NMDARs require additional post-synaptic depolarisation (**Mayer et al. 1984, Nowak et al. 1984**). Their unusually slow kinetics are also driven by a slow glutamate unbinding following activation. Additionally, NMDARs are also highly permeable to calcium. This, coupled with their extended time course, means they enable high intracellular calcium influx on activation, triggering many intracellular cascades (**Lau and Zukin 2007, Traynelis et al. 2010, Zorumski and Izumi 2012, Paoletti et al. 2013, Volianskis et al. 2015**) such as NRG1/ErbB4 pathways involved in promoting cellular migration (**Pregno et al. 2011**) and NFAT pathways involved in regulating cell survival (**Vashishta et al. 2009**). They also have extensive C-terminal domains which enable multiple intracellular interactions. NMDAR activation also requires co-agonist binding which provides an additional regulatory mechanism in regions where glycine/D-serine are not present at concentrations which fully saturate co-agonist binding sites (**Bergeron et al. 1998, Billups and Atwell 2003**). Under these conditions, receptor activation itself is controlled by synaptic glutamate release, whilst the magnitude of the activation can be modulated by the glycine/D-serine concentration. NMDARs are also equipped with multiple modulatory sites which give them a high degree of sensitivity to the extracellular environment (**Zhu and Paoletti 2015**).

1.1.4 NMDAR subunits and subtypes

The existence of multiple subtypes of NMDARs was suggested over three decades ago with early *in vitro* electrophysiological and binding studies (**Cull-Candy and Leszkiewicz 2004, Lau and Zukin 2007, Mony et al. 2009, Traynelis et al. 2010, Paoletti 2011**). Subsequent cloning studies revealed NMDARs that differ in their subunit composition, with the identification of the seven subunits (NR1, NR2A-D, NR3A-B). Although this thesis explores impact of antibodies against the NR1 subunit which is an obligatory subunit found in all NMDARs, a brief introduction to receptor subtypes and other subunits is provided here to aid understanding of how specific NMDARs, which have variable functions and properties, and dynamic developmental and regional expression (**Figure 1.4**), may be impacted at different developmental timepoints.

The NR1 subunit is the obligatory subunit and therefore found in all receptors. It has eight distinct isoforms resulting from alternative splicing, which impact the gating, pharmacological and trafficking properties of the receptor (**Rumbaugh et al. 2000, Horak and Wenthold 2009, Vance et al. 2012**). In line with the widespread distribution of NMDARs, transcriptional studies suggest NR1 subunits are ubiquitously expressed throughout the CNS from E14 onwards (**Watanabe et al. 1992, Akazawa et al. 1994, Monyer et al. 1994**). NR1 subunit expression peaks during the third postnatal week before declining slightly into adulthood (**Laurie and Seeburg 1994, Paupard et al. 1997**). It is the most highly expressed subunit throughout development, with highest expression in the cortex and hippocampus, and lower expression in the striatum, thalamus, cerebellum and colliculi (**Laurie and Seeburg 1994, Monyer et al. 1994, Paupard et al. 1997**).

The NR2 subunits, which are major dictators of the functional heterogeneity of NMDARs, show marked temporal and regional variation (**Monyer et al. 1994, Ewald and Cline 2009**). In the embryo, only NR2B and NR2D are expressed with NR2A and NR2C expression not starting until around birth (**Cull-Candy and**

Leskiewicz 2004). NR2A, NR2B and NR2C expression peaks around the third postnatal week, whereas NR2D peaks earlier in around the first postnatal week. Regionally, in the early postnatal period NR2A is mostly restricted to the hippocampus and cortex initially but is found more widely as the brain matures. NR2B is expressed predominantly in the cortex, thalamus, hypothalamus and spinal cord embryonically. In the early postnatal period, it shows strongest expression in the cortex and hippocampus, and lower expression in the striatum, thalamic nuclei, and cerebellum. NR2C is expressed in the early postnatal period at low levels in the thalamic nuclei and cerebellum and shows very strong expression in the in the cerebellum from the second postnatal week onwards. NR2D is expressed embryonically in the spinal cord and midbrain structures, but notably not the telencephalon. During the early postnatal period it is expressed predominantly in midbrain structures and at lower levels in the cortex, hippocampus, and cerebellum (**Monyer et al. 1994, Cull-Candy and Leskiewicz 2004, Ewald and Cline 2009**).

NR3 subunit expression also shows variability (**Henson et al. 2010, Pachernegg et al. 2012**). Studies have demonstrated that NR3A is expressed embryonically and peaks at the end of the first postnatal week, before sharply decreasing by the end of the third postnatal week to adult levels (**Ciabarra et al. 1995, Sucher et al. 1995, Wong et al. 2002**). NR3B has been detected from birth and peaks at the end of the second postnatal week, remaining elevated into adulthood (**Matsuda et al. 2002**). Regionally, NR3A is expressed embryonically in the thalamus, hypothalamus, brainstem and spinal cord, and early postnatally also in the hippocampus, amygdala and cortex (**Ciabarra et al. 1995, Sucher et al. 1995, Wong et al. 2002**). Conversely, NR3B expression is restricted to the motor neurons found in the brain stem and spinal cord (**Nishi et al. 2001, Chatterton et al. 2002**).

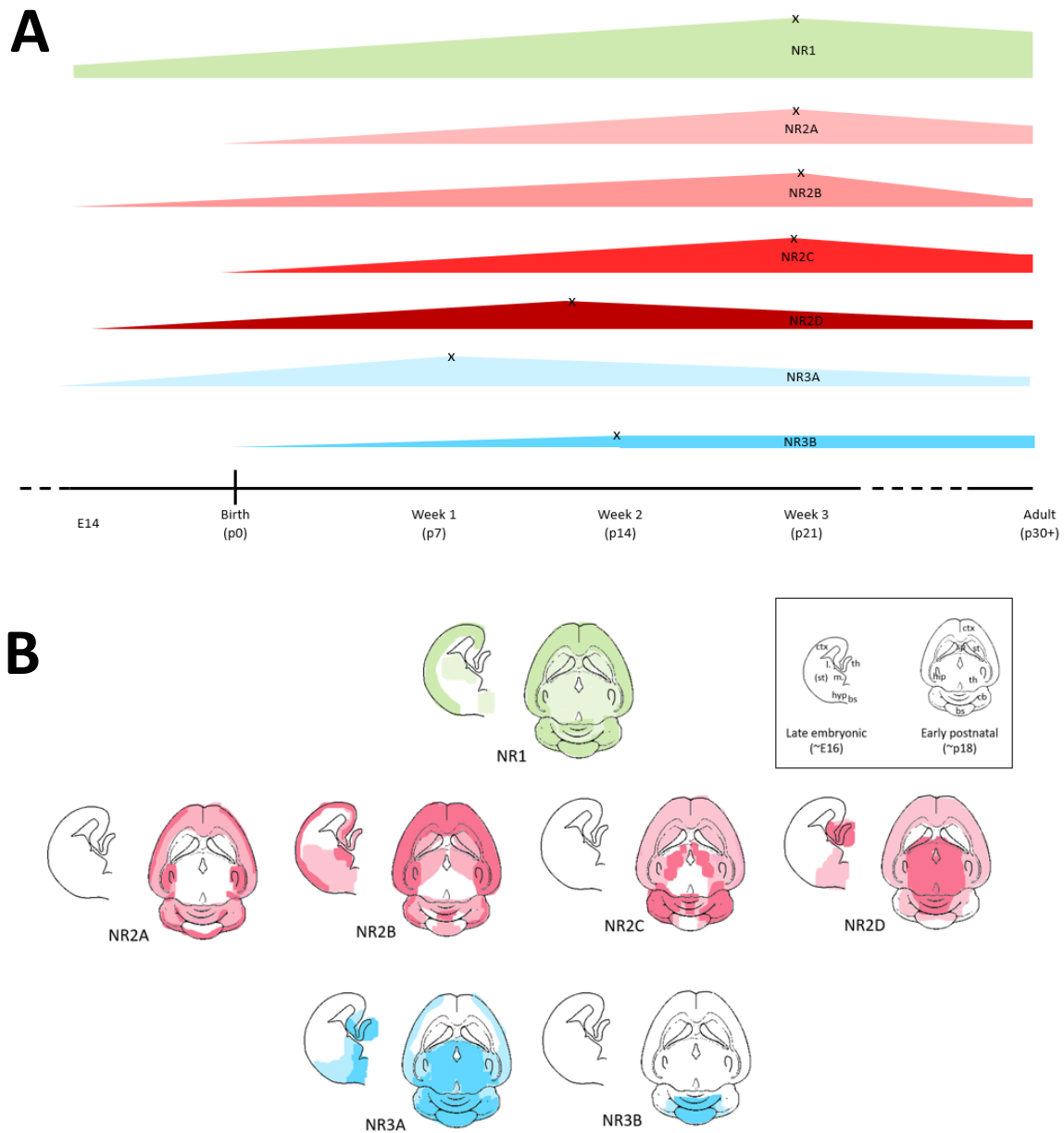


Figure 1.4. Temporal and regional expression of the NMDAR subunits. **[A]** Graphical summary of the temporal expression of the different NMDAR subunits. Separate colours represent separate families of subunits (NR1 in green, NR2 in red, NR3 in blue). The height of each box represents relative level of subunit mRNA expression. The time of peak expression is marked with an x. Scaling is approximate. **[B]** Graphical summary of macro regional expression of the different NMDAR subunits in the developing brain during the late embryonic (E16+) and relatively early postnatal (~p18) periods. Separate colours represent separate families of subunits (NR1 in green, NR2 in red, NR3 in blue) with higher expression in darker tones. Key: bs: brain stem, cb: cerebellum, ctx: cortex, hip: hippocampus, hyp: hypothalamus, l.: LGE, m.: MGE, sp: septum, st: striatum, th: thalamus, p: postnatal day. Scaling and distribution are approximate. Figures based on data from *Monyer et al. 1994, Chatterton et al. 2002, Matsuda et al. 2002, Wong et al. 2002, Ewald and Cline 2009.*

Having many subunits with overlapping regional expression profiles alludes to multiple different NMDAR subtypes. Indeed, more than ten functionally distinct receptors have been reported in the literature (**Cull-Candy and Liszkiewicz 2004, Paoletti 2011**), but there may be many more than this. Each of the two 'non-NR1' subunits in a receptor can either be from the NR2 or NR3 subunit families, meaning both di-heteromeric (i.e. composed of two different subunit types) and tri-heteromeric receptors (i.e. composed of three different subunit types) exist. In terms of di-heteromeric receptors, NR1/2B and NR1/2A convey an important portion of juvenile and adult NMDARs respectively. NR1/NR3 receptors exist and can produce glycine-induced excitatory currents (**Chatterton et al. 2002, Piña-Crespo et al. 2010**), but NR3 subunits are generally thought to form NR1/2/3 complexes *in vivo* (**Henson et al. 2010, Pachernegg et al. 2012**). For the tri-heteromeric receptor, NR1/2A/2B receptors are found abundantly in the mature brain, especially in the cortex and hippocampus (**Al-Hallaq et al. 2007, Gray et al. 2011, Rauner and Köhr 2011**). NR1/2A/2C and NR1/2B/2D are also reported, but these are far less common.

NMDAR subtype expression also varies between both neuronal populations and subcellular location. Some populations such as layer V cortical neurons express multiple subtypes which are preferentially upregulated in response to specific inputs (**Kuman and Huguenard 2003, Cull-Candy and Leszkiewicz 2004**), and others have very limited expression of certain subtypes, for example there is little expression of NR2D in indirect pathway striatal projection neurons (SPNs) (**Landwehrmeyer et al. 1995**). Subcellular location-wise, no subtype is confined to any specific subcellular location (**López de Armentia and Sah 2003, Harris and Pettit 2007, Petralia et al. 2010**), but broadly speaking post-synaptic membranes most typically contain NR2A-containing subtypes (**Hardingham and Bading 2010, Gladding and Raymond 2011**), with pre-synaptic membranes displaying greater subtype diversity based on cell type and region, and NR1/NR2A, NR1/NR2B and NR1/NR2/NR3A assemblies all previously identified (**Woodhall et al. 2001, Jourdain et al. 2007, Brasier and Feldman 2008, Larsen et al. 2011**). It is important to note that NMDARs are mobile, especially those containing NR2B, and they are thought to laterally diffuse between synaptic

and extrasynaptic locations, enabling dynamic site-specific regulation of receptors (**Tovar and Westbrook 2002, Groc et al. 2006**). The most common extrasynaptic NMDAR subtypes are also those containing NR2B (**Hardingham and Bading 2010, Gladding and Raymond 2011**). Additionally, there are non-neuronal pools of NMDARs with atypical conformations, including on glial cells, further increasing the heterogeneity of NMDARs within the CNS (**Burzomato et al. 2010, Henson et al. 2010**).

The assembly of receptor subtypes with differing subunit composition provides marked variability in their pharmacological, biological and signalling properties (**Sprengel et al. 1998, Martel et al. 2012, McKay et al. 2012**). Properties of the NR2 subunit-containing configurations fall on a spectrum, with NR2A-containing receptors exhibiting the highest conductance, magnesium block, calcium permeability, open probability, lowest glutamate sensitivity and fastest (glutamate governed) excitatory post-synaptic decay times (**Vicini et al. 1998, Cull-Candy and Leszkiewicz 2004, Traynelis et al. 2010, Paoletti 2011**), and NR2D-containing receptors at the other end of the spectrum for these properties (with NR2B- and 2C-containing receptors in the middle respectively). Variable properties allow precise tuning of synaptic responses and enable diversity in the physiological function of NMDARs at synaptic vs extrasynaptic sites, within different regions and neuronal subtypes, and during neurodevelopment (**Cull-Candy and Leszkiewicz 2004, Traynelis et al. 2010, Paoletti 2011, Hansen et al. 2017**).

Modulation of synaptic function and information processing is also reliant on trafficking of NMDARs to and from synapses (**Yang et al. 2022**). Surface trafficking of NMDARs is particularly important in a developmental context because it also orchestrates trafficking of the other key ionotropic glutamate receptor, AMPARs, which is fundamental for synaptic maturation (**Yang et al. 2022**). Trafficking, localisation and signalling varies by subunit composition (**Sprengel et al. 1998, Martel et al. 2012, Sanz-Clemente et al. 2012**), modulated by phosphorylation of specific NR2 subunit regions (**Salter and Kalia 2004, Sanz-Clemente et al. 2012**). Developmentally important interactions include CaMKII, RASGRF1 and

the PSD95-NOS complex, which all preferentially interact with NR2B-containing NMDARs. CaMKII governs synaptic maturation and plasticity through induction of LTP (**Gambrill and Barria 2011, Wang et al. 2011, Lisman et al. 2012**), whilst RASGRF1 and PSD95-NOS have fundamental roles in modulating neuronal survival and plasticity (**Hardingham and Bading 2010**).

Pharmacologically, several antagonists are useful for investigating contributions of subunit composition experimentally, including PEAQX, which blocks NR2A-containing receptors, ifenprodil which blocks NR2B-containing receptors (**Williams 1993**) and PPDA, which blocks NR2C/D-containing receptors (**Allyson et al. 2010, Krajewski et al. 2019**). The contributions of NMDARs as a whole can also be studied through antagonism of the whole receptor by antagonists such as DAP5 and MK801 (**Hansen et al. 2017, Krajewski et al. 2019**).

1.1.5 Roles of NMDARs in neuronal and synaptic development

A large body of literature demonstrates the importance of glutamate and its receptors, particularly the NMDAR, in the development of neurons and synapses throughout the brain (**Manent et al. 2006, Yozu et al. 2008, Bortone and Polleux 2009, De Marco García et al. 2011, Le Magueresse and Monyer 2013, Kelsch et al. 2014, García et al. 2015, Akgül and McBain 2016, Chittajallu et al. 2017, Akgül et al. 2019, Hanson et al. 2019, Akgül and McBain 2020**) (*Figure 1.5*).

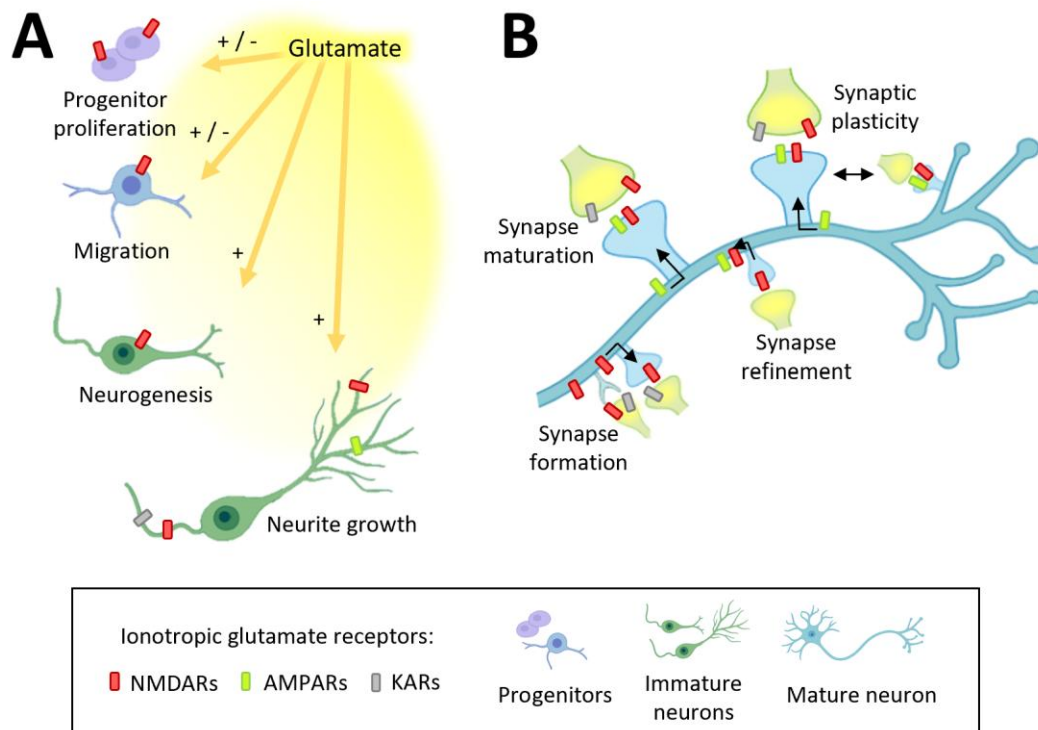


Figure 1.5. Key roles of NMDARs in neuronal and synaptic development. The different ionotropic glutamate receptors, NMDARs, AMPARs and KARs, contribute to neuronal and synaptic development from the embryonic stages [A] and into the mature neuron [B]. [A] NMDARs are the key ionotropic glutamate receptors governing early neuronal development, regulating processes including progenitor proliferation, migration, and wider neurogenesis. NMDARs also regulate dendritic growth along with AMPARs, and axon development and arborisation alongside KARs. [B] NMDARs are also involved in many processes surrounding synaptic development including synapse formation, maturation, refinement, and plasticity. In synaptic maturation AMPARs are also recruited to the synapse, whilst in synaptic refinement they are removed. In synaptic plasticity, NMDARs are involved in both pre- and post-synaptic plasticity mechanisms. Figure based on **Hansen et al. 2021**.

During neurogenesis, an initial population of neuroepithelial cells differentiate into radial glia, which, through a series of subsequent intermediate progenitors (IPs), subsequently give rise to all neurons and glial cells (**García et al. 2017**). NMDAR-mediated mechanisms have been shown to regulate the rate of proliferation of neural progenitor cells, and impact differentiation. *In vitro* experiments have shown that neural progenitor proliferation and differentiation are influenced by Notch and bHLH gene pathways, with

Notch activation upregulating anti-neural bHLH genes such as *Hes*, maintaining neural precursors in a proliferative state thus impeding differentiation, whilst pro-neural bHLH genes like *Mash1* and *Math* enforce differentiation to post-mitotic neurons (**Ohtsuka et al. 2001, Cau et al. 2002, Kageyama et al. 2005, Yoon et al. 2005**). Inhibition of NMDARs has been shown to increase *Hes* expression, increasing progenitor proliferation and non-committal (**Hirasawa et al. 2003**), whilst the converse is true on activation of NMDARs, leading to increased expression of pro-neural genes and differentiation (**Deisseroth et al. 2004**). Thus, activation of NMDARs directs embryonic neural progenitors to exit the cell cycle and undergo post-mitotic neuronal differentiation (**LoTurco et al. 1995, Sadikot et al. 1998, Haydar et al. 2000, Bai et al. 2003, Luk et al. 2003, Suzuki et al. 2006, Li et al. 2011a**). Regional or cell-type specific differences in the role of NMDARs on embryonic neuronal progenitor proliferation have also been identified, with *in vivo* studies demonstrating that *in utero* exposure to NMDAR antagonists resulted in reduced proliferation in the striatal precursor region, the lateral ganglionic eminence (LGE), but had little impact on cortical progenitors in a mouse model (**Luk et al. 2003**).

Further, early studies indicate that NMDARs play a role in regulating early cell survival, with *in vivo* antagonism of NMDARs in the late embryonic and early postnatal period shown to trigger widespread neuronal apoptosis across the developing brain (**Ikonomidou et al. 1999**). Their specific role in cell survival is not well characterised in the very early brain but in the later adolescent and mature brain, their role appears to be convoluted, since they are well established to both promote neuronal survival and apoptosis (**Hardingham and Bading 2010**). Which role they carry out here has been suggested to depend on a range of factors such as receptor activity level, subunit composition and cellular location. In terms of activity level, recent literature suggests that normal NMDAR activity promotes cell survival through several genetic pathways including PI3K-Akt, ERK and CREB, whilst both hyper- and hypoactivation of NMDARs is linked to neuronal death (**Mao et al. 2022**). In embryonic striatal cultures, it has also been shown that low concentrations of NMDA promote proliferation whilst high concentrations are toxic (**Luk**

et al. 2003). Regarding subunit composition, studies using *in vitro* and *in vivo* stroke models suggest NMDARs containing NR2A and 2B subunits promote neuronal survival and death respectively (**Sattler et al. 1999, Aarts et al. 2002, Lai et al. 2011, Martel et al. 2012, Wu and Tymianski 2018**). However, this may be over-simplistic as it does not consider the roles of the other NMDAR non-obligate subunits, which are also suggested to be important (**Nakanishi et al. 2009, Baron et al. 2010, Chung et al. 2016**). Lastly, regarding cellular location, studies have demonstrated that synaptic NMDAR activity promotes cell survival through inducing both a protective multi-step genetic program that protects against apoptotic and excitotoxic insults, and also transcriptional suppression of intrinsic apoptotic pathways, whilst extrasynaptic NMDAR activity promotes cell death through mechanisms such as suppression of pro-survival CREB pathways (**Hardingham and Bading 2010**). Important to this thesis here, experiments on *in vitro* corticostriatal cultures show that striatal spiny projection neurons (SPNs; the main neuronal type of the striatum and the focus of this thesis), co-cultured with fewer cortical inputs, show reduced pro-survival signalling via CREB pathways and increased susceptibility to NMDA-induced apoptosis, with a greater proportion of extrasynaptic NMDARs present (**Buren et al. 2016**), suggesting that cortical input can also regulate SPN cell survival through NMDAR-mediated pathways.

NMDARs are also shown to have an impact on neuronal migration, with activation of NMDARs shown to promote migration of cortical excitatory neurons and cerebellar granule cells *in vitro* (**Komuro and Rakic 1993, Behar et al. 1999, Hirai et al. 1999, Hirasawa et al. 2003, Mancini and Atchison 2007**). The story is however more convoluted than it first appears, since several studies have also shown that deletion of NR1 had no significant impact on cortical cytology (**Messersmith et al. 1997, Iwasato et al. 2000**), although mutations in the NR1-encoding *GRIN1* gene in clinical studies are linked to abnormal cortical formation in some patients (**Fry et al. 2018**). The reason for this discrepancy is not fully clear but may be due to additional factors such as genetics or gestational conditions. Alternatively, it could be that the deletions are dominant negative, so in the total absence of certain NMDARs other glutamatergic receptors are able

to compensate, whereas *de novo* mutations give rise to the presence of incorrectly functioning NMDARs which could cause dysregulation of processes. Roles in migration are also likely to be cell-type or regional specific, since activation of NMDARs has been shown to have no impact on hippocampal interneuron migration, which is instead likely regulated by AMPAR-mediated mechanisms (**Manent et al. 2006, Mortone and Polleux 2009**).

NMDARs also play an important and complex role in dendrite and axon formation, with roles in both promoting and preventing neurite growth, and spontaneous glutamate release from developing axons thought to stimulate dendritic growth prior to formation of axo-dendritic contacts (**Andreae and Burrone 2015**). The reason for promotion or prevention of growth may be neuron-type specific, with NMDAR activation shown to promote dendritic growth in *Xenopus* tectal and rodent spinal motor neurons (**Kalb 1994, Rajan and Cline 1998, Rajan et al. 1999**), but NMDAR antagonism shown to increase dendrite length in cortical neurons (**Rocha and Sur 1995, McAllister et al. 1996**). Alternatively, there may be a role of subunit-specific differences; CaMKII and GTPase RhoA pathways have been shown to promote dendritic stabilisation and growth respectively *in vitro*, but interaction with specific NMDAR subunits has also been shown to contextually modulate their effects, with NR2B-CaMKII preventing dendritic growth in postnatal day (p)5 glutamatergic neurons *in vitro*, but promoting growth at p12, and upregulation of NR2A-CaMKII also facilitating *in vivo* dendritic lengthening (**Bustos et al. 2017**). NR2D has also been shown to be crucial for proper arborisation of GABAergic interneurons (**Chittajallu et al. 2017, Pla et al. 2018, Hanson et al. 2019**). Further, whether NMDARs promote or prevent neurite growth may be context-specific, with experiments using *in vitro* embryonic corticostriatal cultures showing that SPNs with a reduced number of cortical inputs have significantly shorter dendritic length and reduced complexity, likely mediated by NMDARs (**Buren et al. 2016**). Additionally, NMDARs have been shown dictate axonal arborisation and correct path finding of axons (**Cline 1987, Cline and Constantine-Paton 1989, Simon et al. 1992, Iwasato et al. 1997, Rajan et al. 1999, Ramoa et al. 2001, Ruthazer et al. 2003, Lee et al. 2005, Yamasaki et al.**

2014, Van Horn et al. 2017). Mechanistically, axonal development is regulated by axonal NMDARs through control of axonal dynamics, as well as through assembly of active zone proteins and synaptic vesicles to allow maturation of pre-synaptic sites (**Corlew et al. 2007, Corlew et al. 2008, Sceniak et al. 2012, Gill et al. 2015, Gao et al. 2018b**).

Once neurons are born, they connect through either inhibitory or excitatory synapses to form neural circuits. Glutamate is the major excitatory neurotransmitter, acting mainly through NMDARs and AMPARs, and GABA is the major inhibitory neurotransmitter, acting mainly through GABA_A receptors (GABA_ARs) (**Ben-Ari et al. 2007**). Synaptic connectivity involves synaptogenesis and subsequent pruning which also establishes the excitatory-inhibitory balance within the brain, essential for healthy physiological functioning (**Märtin et al. 2000, Maffei et al. 2004, Cline 2005, Tao and Poo 2005, Åkerman and Cline 2006, Cline and Haas 2008, Dorrn et al. 2010**). In the early postnatal rodent brain, large numbers of synapses are initially formed. NMDARs are expressed on immature neurons prior to synaptogenesis (**Ben-Ari et al. 2007**). They play a role in both excitatory and inhibitory synapse formation and have a well characterised role in synaptic refinement through controlling developmental synaptic pruning and strengthening (**Rabacchi et al. 1992, Zhang et al. 2000, Ruthazer et al. 2003, Colonnese and Constantine-Paton 2006, Zhang et al. 2013, Personius et al. 2016**).

They are particularly important in the early postnatal brain, since glutamatergic synapses contain very few AMPARs and therefore early transmission is primarily NMDAR-mediated (**Isaac et al. 1995, Liao et al. 1995, Isaac et al. 1997**). Additionally, they regulate the developmental increase of AMPARs at the synapse (**Liao et al. 1999, Adesnik et al. 2008, Zhang et al. 2013**), a key process in synaptic and circuit maturation. During early development and up to three weeks postnatally, excitatory glutamatergic synapses preferentially express NMDARs (**Li et al. 2017**). Formation and stabilisation of dendritic spines (protrusions along dendrites that form major synaptic sites) has been shown to be mediated by NMDARs through

clustering at nascent glutamatergic synapses in some studies (**Engert and Bonhoeffer 1999, Maletic-Savitic et al. 1999, Cottrell et al. 2000, Washbourne et al. 2002, Alvarez et al. 2007, McKinney et al. 2010, Kwon and Sabatini 2011, Yasuda et al. 2011**) but not others, suggesting this function may be cell-type or context specific. NMDARs are thought to play an important role in the development of corticostriatal synapses specifically, with my lab and others showing that the early postnatal weeks are highly dynamic in terms of NMDAR subtype expression at these synapses (**Monyer et al. 1994, Dehorter et al. 2011, Krajewski et al. 2019**). In mice, loss of specific NMDAR-mediated corticostriatal inputs takes place alongside a reduction in an immature pattern of striatal activity at the start of the second postnatal week, correlated to the onset of locomotion (**Dehorter et al. 2011**), suggesting an important role in corticostriatal synaptic maturation, whilst genetic deletion of NR1 has also been shown to decrease spine density in cortical neurons, indicating a function in synaptic maintenance (**Ultanir et al. 2007**).

NMDARs are also important for inhibitory (i.e. GABAergic) synapse formation and development. There is strong localisation of NMDARs at developing inhibitory synapses in the immature brain, suggesting a key role in inhibitory synapse formation (**Gundersen et al. 2004, Szabadits et al. 2011, Cserép et al. 2012**). Studies also show that loss of NMDARs results in impaired assembly of components in both the pre- and post-synaptic membrane, indicating a function in facilitating inhibitory synapse assembly (**Gu et al. 2016**). Furthermore, both *in vitro* and *in vivo* enhancement of NMDAR activity in embryonic hippocampal neurons boosts transmission across inhibitory synapses, whilst deletion of NMDARs strongly limits their development (**Gu et al. 2016**). Mechanistically, experimental evidence suggests that this process requires calcium/calmodulin binding to the C0 domain of the NR1 subunit of the NMDAR, with the initial NMDAR activation suggested to be facilitated by GABA_ARs (**Gu et al. 2016**), since GABA is excitatory rather than inhibitory in embryonic and early developing neurons (**Cherubini et al. 1991, Ben-Ari et al. 2007**). It has also been demonstrated that NMDARs modulate maturation of inhibitory synapses through several other pathways including calcineurin, CaMKII, NOS and astrocytic release of TGF- β 1 (**Nugent et al. 2007, Bannai**

et al. 2009, Marsden et al. 2010, Muir et al. 2010, Diniz et al. 2014, Petrini et al. Flores et al. 2015).

Studies also indicate that subunit composition may impact inhibitory synapse development, with antagonism of NR2A but not NR2B resulting in increased spine head width on striatal SPNs (Vastagh et al. 2012).

Synaptogenesis and refinement also coincide with a developmental switch of NR2B to NR2A subunits in NMDARs (Sheng et al. 1994) which results in altered receptor temporal integration properties and a clear acceleration of NMDAR-mediated EPSCs (Flint et al. 1997, Stocca and Vicini 1998, Barth and Malenka 2001). NR2B-containing receptors are found in most glutamatergic synapses, whereas NR2A-containing receptors are trafficked to synapses in an activity-dependent manner (Barria and Malinow 2002, Aizenman and Cline 2007, Storey et al. 2011). NR2B- and NR2A-containing receptors are thought to exert opposite signalling effects, slowing or promoting synaptic maturation and stabilisation respectively (Ohno et al. 2010, Gray et al. 2011, Wang et al. 2011a, Henle et al. 2012, Kelsch et al. 2014, Wang et al. 2021).

NMDARs are also key in synaptic plasticity; the ability of experience-linked neural activity to modify neural circuit function for extended periods (Citri and Malenka 2007, Hunt and Castillo 2012, Morris 2013). In short term plasticity, which typically results from short bursts of activity causing transient increase of pre-synaptic calcium and altered neurotransmitter release probability, NMDARs play a modest role (Chamberlain et al. 2008). However, in long-term plasticity, whereby activity patterns induce long-lasting (hours to days) increase or decrease in synaptic strength, NMDARs have a much greater role (Hansen et al. 2021). At excitatory synapses, NMDARs increase or decrease AMPAR synaptic localisation, thus modulating excitatory transmission and synaptic plasticity through a variety of signalling pathways (Malenka and Bear 2004, Lüscher and Malenka 2012, Diering and Huganir 2018). In summarised terms, in NMDAR-mediated long-term potentiation (LTP; the long-term increased activity at a synapse), NMDAR activity increases dendritic spine calcium and activates intracellular signalling cascades, promoting

inclusion of additional AMPARs into the post-synaptic membrane as well as increasing synaptic AMPAR conductance (**Citri and Malenka 2007**). Parallel changes in synaptic structure and enhanced local dendritic protein synthesis providing critical proteins for maintenance of synaptic strength results in a 'long-term' enlarged synapse.

In long-term depression (LTD; the long-term decreased activity at a synapse), modest activation of NMDARs results in a modest post-synaptic increase in calcium and activation of protein phosphatases amongst other signalling proteins, resulting in uncoupling of AMPARs from the post-synaptic membrane, accompanied by spine shrinkage likely due to loss of receptors (**Nägerl et al. 2004, Zhou et al. 2004, Hsieh et al. 2006**). Expression of NMDARs varies amongst synapses influencing their capacity for long-term potentiation or depression; corticostriatal synapses have a greater expression of NMDARs relative to thalamocortical synapses, which makes LTP more inducible (**Calabresi et al. 1992a, Ding et al. 2008**). Subunit composition can also influence long-term synaptic plasticity, with studies in the cortex showing that long-term depression of synapses requires activation of NR2B-containing NMDARs, whilst long-term potentiation requires NR2A-containing NMDARs (**Massey et al. 2004, Erreger et al. 2005**).

1.1.6 Brief overview of other ionotropic glutamate receptors: AMPARs and KARs

Though NMDARs are the primary focus of this thesis, a very short contextual summary of the two other major ionotropic glutamate receptors, AMPARs and KARs, is provided below.

AMPAs are the other major type of ionotropic glutamate receptor and primarily mediate fast glutamatergic neurotransmission. They are tetrameric complexes formed from four homo- or heteromeric subunits (GluA1-4, also referred to as A1-4) (**Traynelis et al. 2010, Gan et al. 2015**). Similarly to NMDARs, the subunit composition of AMPARs confers distinct biophysical and molecular properties to the receptor. They also vary across region and through development (**Henley and Wilkinson 2016**).

Early studies show GluA1 is expressed embryonically from ~E16 onwards and increases during late embryonic and early postnatal development, peaking at the end of the fourth postnatal week (**Durand et al. 1993, Märtin et al. 1998**). GluA2, A3 and A4 are expressed later in embryonic development and show a similar subsequent whole brain temporal expression pattern (**Durand et al. 1993, Jakowec et al. 1998, Märtin et al. 1998**). In whole brain, GluA3 and A4 are expressed at much lower levels than A1 and A2.

In terms of embryonic regional expression of individual AMPAR subunits embryonically, there are few studies available but important early work has shown that GluA1 and GluA2 are both expressed highly during E15-19, peaking close to birth before decreasing to adult levels postnatally, whereas GluA3 slowly increases from the embryonic period onwards and peaks in adulthood, and GluA4 demonstrates constant levels throughout development (**Lilliu et al. 2001**). The same study identified that in the mesencephalon GluA1-3 are expressed embryonically, peaking at E15-17, before plateauing to adult values close to birth, whereas GluA4 increases throughout development peaking in adulthood. Early postnatal studies have also shown that shortly after birth, GluA1 is expressed most highly in the hippocampus and nucleus accumbens, and at moderate levels in the striatum, cortex, thalamus and cerebellum (**Jakowec et al. 1998**). As the brain matures over the first three postnatal weeks, expression in the striatum and nucleus accumbens decreases (**Jakowec et al. 1998**) but remain at similar levels in the hippocampus and cerebellum (**Jakowec et al. 1998**) and increases in the cortex (**Märtin et al. 1998**). GluA2 shows high expression in the hippocampus, with lower expression in the striatum, nucleus accumbens, cortex, thalamus and cerebellum at birth. Over early postnatal development, expression in the striatum, thalamus and cerebellum decreases, whilst remaining high in the hippocampus and moderate in the cortex. GluA3 shows moderate early (p2) expression in the hippocampus and cerebellum, and very low expression in the striatum, nucleus accumbens, thalamus and cortex (**Jakowec et al. 1998**). It is expressed predominantly in the hippocampus and cortex during the first three postnatal weeks (**Durand et al. 1993**).

GluA4 shows low early expression in the hippocampus and cortex, and very low expression in the striatum, thalamus and cerebellum, with little change through early development (**Jakowec et al. 1998**).

In terms of functions in neurodevelopment, AMPARs are expressed during embryonic stages, especially proliferative phases (**Mi et al. 2018**), and *in vitro* experiments suggest they have roles in neurogenic processes including regulating cell migration and neurite extension (**Métin et al. 2000, Poluch et al. 2001, Poluch and König 2002, Harlow et al. 2015**), with expression of AMPARs and KARs (as well as NMDARs) seen on immature neurons prior to functional synapse formation (**Soria and Valdeomillos 2002, Luján et al. 2005, Manent et al. 2006**). Studies have also shown that correct early expression of AMPARs is crucial for proper anatomical and physiological development of emerging cortical microcircuits (**Tashiro et al. 2006, Adesnik et al. 2008, Gray et al. 2011, Lu et al. 2011, Zhang et al. 2013**). AMPARs are primarily involved in postnatal synaptic maturation and plasticity (**Henley and Wilkinson 2016**), and it has been shown by my lab and others that during the early postnatal period both corticostriatal and thalamostriatal synapses undergo a decrease in NMDA/AMPA ratio and a concomitant increase in AMPAR/KAR expression, with the increase more gradual in corticostriatal synapses (**Krajeski et al. 2019**). NMDARs are crucial for proper recruitment of AMPARs to synapses, with embryonic ablation of NMDARs shown to impair AMPAR expression and synaptic development in neocortical and hippocampal circuits (**Akgül and McBain 2016, Chittajallu et al. 2017**). In the striatum, AMPARs have a function in signalling on both sides of the synapse (**Petralia and Wenthold 1992, Fujiyama et al. 2004, Fiszman et al. 2007, Polgár et al. 2008**). They also play a key role in NMDAR-mediated synaptic plasticity, generating the post-synaptic depolarisation required to remove the NMDAR magnesium block, and act as the target for intracellular protein trafficking machinery and enzymes following calcium release (**Lee et al. 2003, Huganir and Nicoll 2013, Henley and Wilkinson 2016**).

KARs are the third type of ionotropic glutamate receptor. They are also functional tetrameric channels made up of GluK subunits which can be grouped into low affinity (GluK1-3) and high affinity (GluK4-5) subtypes (**Lauri et al. 2021**). Similarly to AMPARs, they also contribute to fast-excitatory neurotransmission and have key functions on both the pre- and post-synaptic membranes in specific neuronal populations. Developmentally, KAR mRNA is detectable in the embryonic rodent brain and most subunits peak in the late embryonic or early postnatal periods, coinciding with a period of high synaptogenesis (**Bahn et al. 1994, Lilliu et al. 2002, Ritter et al. 2002, Ryazantseva et al. 2020**). In the mature brain, GluK2 and K5 are widely expressed, whereas GluK1, K3 and K4 are restricted to specific cell subtypes or developmental timepoints (**Wisden and Seeburg 1993, Bahn et al. 1994, Huettnner 2003, Hadzik et al. 2017**). Studies suggest that KARs have multiple developmental functions, including facilitation of post-synaptic depolarisation enabling synaptic NMDAR activation (when AMPAR currents are labile), thus enabling synaptic plasticity (**Kidd and Isaac 1999, Van Zundert et al. 2010**), and tonic inhibition of facilitation of glutamate release probability, depending on synapse subtype (**Lauri et al. 2005, Lauri et al. 2006, Ryazantseva et al. 2020**). KARs and their functions are largely beyond the scope of this thesis but are reviewed in detail in **Lauri et al. (2021)**.

*

1.2 NMDAR antibodies and implications for neurodevelopment

1.2.1 NMDAR antibody-mediated encephalitis (NMDARE)

Increasing literature from the last two decades suggests antibodies can be raised against distinct neuronal proteins and receptors ('anti-neuronal antibodies'), causing a category of neurological disorders known as autoimmune encephalitis (**Giannoccaro et al. 2020**). More than 25 antibody types have been described in patients, including antibodies raised against NMDARs (**Dalmau et al. 2019**), AMPARs (**Haselmann et al. 2018**), GABARs (**Lancaster et al. 2010, Petit-Pedrol et al. 2014**), LGI1 (**Petit-Pedrol et al. 2018, Kornau et al. 2020, Fukata et al. 2021**) and CASPR2 (**Dawes et al. 2018**). Many of these have been discovered to exhibit direct pathogenicity, therefore representing novel clinical entities (**Prüss et al. 2021**). The discovery of such antibodies has notably altered clinical practice; these patients often present with challenging psychiatric and neurological symptoms and historically were susceptible to misdiagnosis and ill-informed treatment options, but awareness of antibodies as a potential root cause for such presentations has enabled earlier diagnostic testing and access to immediate immunotherapy for antibody removal (**Graus et al. 2016, Pollak et al. 2019**).

One of the most common types of autoimmune encephalitis is NMDAR antibody-mediated encephalitis (NMDARE) where anti-neuronal antibodies most commonly target the NR1 subunit of the glutamatergic NMDAR. NMDARE was first reported nearly 20 years ago in four patients (**Vitaliani et al. 2005**), and shortly after, it was shown to be associated with antibodies against the NMDAR (**Dalmau et al. 2007**). Epidemiologically, it is relatively rare, with an estimated incidence of 1.5/million per year (**Dalmau and Graus 2018**). It predominantly affects young females (median age 21 years, male: female ratio 2:8), with around 1 in 5 of these cases being associated with ovarian teratomas (**Dalmau et al. 2007, Dalmau et al. 2008, Florance et al. 2009, Irani et al. 2010, Titulaer et al. 2013, Viacoz et al. 2014**). It may also follow

herpes simplex virus encephalitis (HSVE), particularly in young children, likely a secondary response to the virus-induced neuronal damage (**Armangue et al. 2018**).

In adults, it typically presents with a multi-stage clinical syndrome, starting with an initial prodromal phase, subsequent prominent psychiatric symptoms and progression onto cognitive impairment, movement disorders, seizures, and reduced consciousness and respiration in extreme cases (**Dalmau et al. 2008**). The literature suggests a difference between adults and children; children more commonly present with 'neurological' symptoms including seizures, abnormal movements, irritability and insomnia, and show more speech or movement disorders and focal deficits (eg. paresis or ataxia) over the disease course, whereas adults often exhibit a more 'psychiatric' presentation including psychosis and abnormal behaviours, and more frequently progress to show memory impairments and hypoventilation (**Titulaer et al. 2013, Dalmau et al. 2019**).

Formal diagnosis of NMDARE requires a rapid (< 3 month) onset of one, or more, of the six major symptom categories (1. Abnormal psychiatric behaviour or cognitive function, 2. Speech dysfunction 3. Seizures 4. Movement disorders, 5. Decreased consciousness, 6. Autonomic dysfunction or central hypoventilation) coupled with detection of NR1-IgG antibodies within the CSF (along with exclusion of a recent history of HSVE or Japanese B encephalitis since they may re-present with relapsing symptoms) (**Titulaer et al. 2013, Graus et al. 2016**). On investigation, MRI is often non-specific, but CSF abnormalities are seen in almost 80% of patients, and EEG is abnormal in more than 90% of patients (**Armangue, et al. 2013, Titulaer et al. 2013**). EEG often show encephalopathic patterns, with or without ictal discharges, and a unique 'extreme delta brush' pattern is also seen in up to ~30% of cases (**Schmitt et al. 2012**).

Treatment of NMDARE aims to suppress the immune system (steroids, intravenous immunoglobulins (IVIG)) and remove circulating antibodies (plasma exchange) as a first-line approach, transitioning to second-line therapies (immunomodulatory agents eg. rituximab or cyclophosphamide) if required

(Titulaer et al. 2013, Nosadini et al. 2015). Electroconvulsive therapy has also been used in some patients with severe catatonia (Warren et al. 2019). Tumour removal in paraneoplastic cases is also essential and correlates with improved outcomes (Dalmau et al. 2008). Patients usually respond to immunotherapy, particularly if initiated early, with a significant neurological improvement seen in up to 80% of patients (Titulaer et al. 2013). However, relapses or residual symptoms are reported in nearly a quarter (23%) of patients, and about 5% of cases are fatal (Irani et al. 2010, Titulaer et al. 2013).

1.2.2 Current understanding of NMDAR antibody pathogenesis

Multiple studies have explored the pathogenic mechanisms underlying NMDAR antibodies. Tumours (mostly ovarian teratomas) and viruses are two established immunological triggers of NMDARE, but in up to half of patients the immunological trigger is unknown.

Several experiments (Leypoldt et al. 2015, Chen et al. 2018, Ding et al. 2018, Kunchok et al. 2019) have implicated a systemic synthesis of NMDAR antibodies by antibody-producing cells, with the cervical lymph nodes and ovarian teratomas recently established as two key germinal centres (Al-Diwani et al. 2022). Following exposure to the NMDAR antigen, it is thought that native B-cells become activated and can cross the blood-brain barrier. Once inside the brain they can undergo further stimulation, antigen-led maturation, and clonal expansion before differentiating into NMDAR antibody producing plasma cells (Dalmau 2016). In cases of gestational transfer, they would cross the blood brain barrier when it is permeable, which is from around the start of the second trimester (~week 13) onwards (Huang et al. 2020).

NMDAR antibodies have been shown to bind typically to the extracellular domains of NMDARs (most commonly NR1-IgG antibodies, which bind to the NR1 subunit of the receptor) (Huang et al. 2020), resulting in altered surface dynamics and impaired associations with other proteins, including disrupted

surface cross-talk with the EphB2 receptor, a key receptor for stabilising the NMDAR at the cell surface (Hughes et al. 2010, Mikasová et al. 2012, Planagumà et al. 2016, Castillo-Gómez et al. 2017, Ladépêche 2018, Pan et al. 2018). This subsequently leads to receptor internalisation, with antibody-bound NMDARs trafficked through recycling endosomes and lysosomes (Moscato et al. 2014). The resulting decreased surface density and synaptic localisation of NMDARs (Dalmau et al. 2008, Hughes et al. 2010, Mikasová et al. 2012) has serious downstream impacts on NMDAR network and synaptic functioning (Hughes et al. 2010, Mikasová et al. 2012, Moscato et al. 2014, Rosch et al. 2018).

Additionally, alongside internalisation, it has recently been shown that binding of NMDAR antibodies leads to a longer NMDAR channel opening time prior to internalisation (Lynch et al. 2018). It has been hypothesised that the antibodies may preferentially bind to open synaptic NMDARs (typically NR2A-containing) at active excitatory synapses, and extrasynaptic NMDARs (typically NR2B-containing) at active inhibitory synapses. This hypothesis would fit the clinical paradox of increased seizures despite NMDAR hypofunction and would be supported by literature using model-based estimates showing that NMDARE patients have a selective deficit in NMDAR signalling (Symmonds et al. 2018) and work showing that NMDAR antibodies can change neuronal population dynamics and predispose the brain to paroxysmal abnormalities (Rosch et al. 2018). Moreover, it would fit with experiments showing NMDAR antibodies induce dispersal of NR2A-containing NMDARs, through blockade of the NR1/2 subunit and EphB2 stabilising protein interactions at synapses (Mikasová et al. 2012).

Additionally, recent work using super-resolution microscopy exploring the events prior to internalisation of NMDARs has demonstrated that NMDAR antibodies cause a subunit-specific nanoscale redistribution of NMDARs. In cultured neurons, it was shown that NMDARs form nanosized objects both synaptically and extrasynaptically, and that exposure to NMDAR antibodies resulted in an increase of synaptic and extrasynaptic receptors inside the nano-objects. The clustering was subunit specific and predominantly

affected NR2B-containing NMDARs. Following receptor internalisation, non-internalised receptors were preferentially retained at the synapse and returned to a control level of clustering (**Ladépêche et al. 2018**). This is a point of interest in the context of NMDAR subunit composition demonstrating variability both regionally and developmentally.

1.2.3 NMDARE in pregnancy and impact on neurodevelopment

During pregnancy, maternal antibodies cross the placenta to help with the establishment of the foetal immune system (**Simister 2003**). The only antibody isotype transferred is the immunoglobulin G (IgG) isotype (**Brambell and Hemmings 1949, Brambell and Halliday 1956, Morphis and Gitlin 1970**), and transport occurs placentally via the neonatal Fc receptor. In humans, IgG transfer occurs exclusively *in utero*, primarily during the second and third trimesters (**Palmeira et al. 2012**). In mice, transfer also occurs at a similar time *in utero*, (from ~E12, which equates approximately to the second and third human trimesters), but conversely, there is also significant transfer postnatally through breast milk (**Jacobson et al. 1999, Van de Perre 2003**). There is also the potential for pathological anti-neuronal antibodies harboured by the mother to be transferred across the blood-brain barrier into the foetal brain during gestation (as well as in the early postnatal period in mice), such as pathogenic NR1-IgG (**Jurek et al. 2019, Pröbstel and Zamvil 2019**) (**Figure 1.6**).

Regarding transfer of these antibodies into the brain, there has been a historical notion that the blood-brain barrier *in utero* is poorly formed, thus allowing antibodies and other substances to easily diffuse into the brain (**Goasdoué et al. 2017**). However, this theory has been challenged by work demonstrating the presence of tight junctions as early as the first trimester (**Saunders et al. 2012, Goasdoué et al. 2017**), as well as the presence of a CSF-brain barrier in early life, formed by strap junctions between neuroependymal cells (**Whish et al. 2015**), suggesting the brain is much less passively accessible *in utero* than previously thought. Additionally, there appears to be an incongruence in the timings of when the

blood-brain barrier is most permeable (the first trimester), and when IgG placental transfer is highest (the second and third trimesters). This suggests a role for active transport facilitating antibody entry to the brain, which is supported by work in guinea pigs showing a specific transport mechanism for IgG via an as yet uncharacterised carrier (**Zlokovic et al. 1990**). Further, in mice, microglial invasion into the brain also begins at a similar time to the accumulation of IgG (~E10.5), with both originating from the same source (the yolk sac), suggesting a possible role for microglia in mediating IgG entry (**Ginhoux et al. 2010, Mohanty et al. 2010**).

There has been increasing literature in recent years suggesting that transfer of maternal anti-neuronal antibodies, such as those against NMDAR, can lead to neurodevelopmental diseases, which present neonatally or at later postnatal timepoints (**Giannoccaro et al. 2020**). Indeed, studies suggests such antibodies play roles in disorders including autism spectrum disorders (ASD), learning disabilities, and schizophrenia amongst others (**Singer et al. 2009, Braunschweig et al. 2012, Brimberg et al. 2016, Coutinho et al. 2017a, Jurek et al. 2019**).

Antibodies against NMDAR are particularly interesting in this context. Firstly, NMDARE, is the most common autoimmune encephalitis (**Dalmau et al. 2019**), and NR1-IgG is also thought to be among the most common anti-neuronal antibodies in asymptomatic individuals, with an estimated seroprevalence of 1% amongst the general population (**Dahm et al. 2014, Lang and Prüss 2017**). Secondly, 60-80% of patients are women of child-bearing age (**Irani et al. 2010, Titulaer et al. 2013, Viaccoz et al. 2014**) and NMDARE can occur during pregnancy, with a several studies reporting a high frequency (>50%) of adverse outcomes including pre-term labour and foetal death (**Kumar et al. 2010, Mathis et al. 2015, Joubert et al. 2020**).

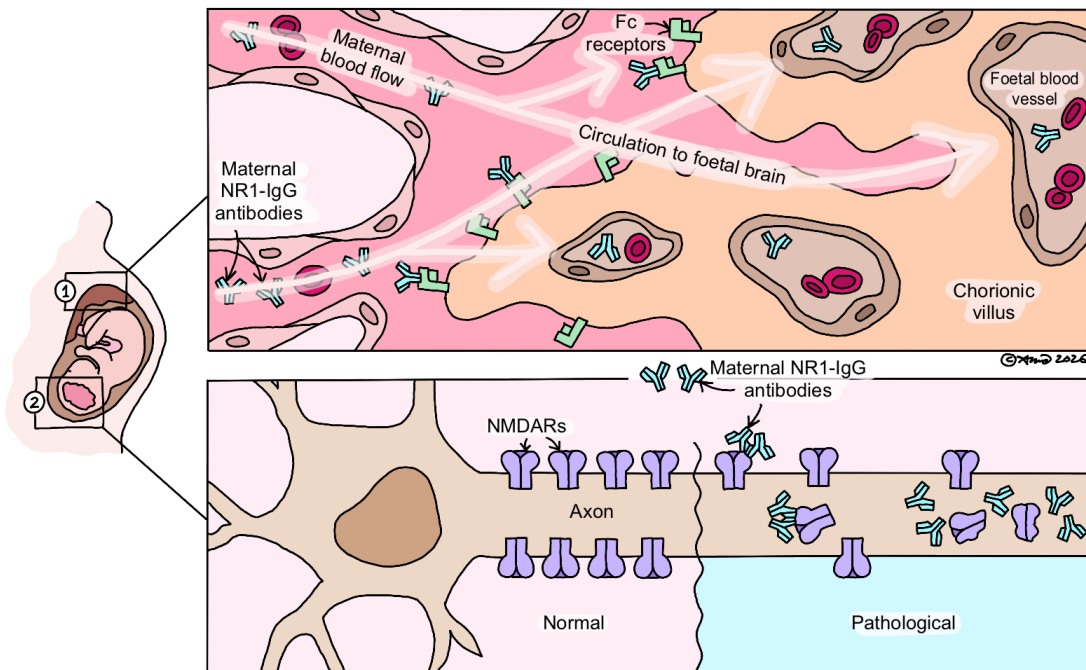


Figure 1.6. Transfer of NR1-IgG antibodies from mother to foetus during pregnancy.

Maternal NR1-IgG antibodies can be transferred placentally via the neonatal Fc receptor during the second and third trimesters. These antibodies can cross the blood-brain barrier and bind to the NR1-subunit of the NMDARs in the developing foetal brain, resulting in NMDAR internalisation and hypofunction. Transfer of such antibodies is linked to a host of neurodevelopmental disorders including ASD, learning disabilities and schizophrenia, amongst others. Diagram adapted from **Pröbstel and Zamvil 2019**.

Clinical literature supports the link between NMDAR antibodies and potential neurodevelopmental impacts (reviewed in **Scorrano et al. 2024**). **Coutinho et al. (2017a)** describes a relatively high incidence of NMDAR antibodies (5.8%) during pregnancy and found that NMDAR antibodies trended towards being more frequent in mothers of children with neurodevelopmental disorders, (7.7% vs 4.6%), though the difference did not reach significance. **Jurek et al. (2019)** also found a marginal increase in NMDAR antibody titres in mothers of children with a range of neuropsychiatric disorders relative to mothers with healthy children. Mutations in genes encoding subunits of the NMDAR have also been found in children with a range of neurodevelopmental and epileptic disorders (**Burnashev and Szepetowski 2015**). Clinical case

reports suggest that in pregnant NMDARE patients, foetal exposure to NMDAR antibodies is associated with neurological deficits in some neonates, though most are born healthy (**Jagota et al. 2014, Hilderink et al. 2015, Chourasia et al. 2017, Shi et al. 2017, Kalam et al. 2019, Joubert et al. 2020, Scorrano et al. 2024**). In a recent case series review of their own cases by **Joubert et al. (2020)**, it was found that from eleven pregnancies, six (55%) were premature. Ten (91%) babies were born healthy and one (9%) was born with transient respiratory distress. At follow up between 7-96 months, all showed normal neurological development. The same group also analysed 21 cases in the literature between 2010 and 2019. From these, they found sixteen (76%) babies were delivered, with 9 (56%) being born prematurely. Thirteen (81%) babies were healthy at birth, with two (13%) showing transient neurological or respiratory symptoms, and one (6%) dying of brain oedema. Follow up between 6-36 months for eight of the babies showed normal development in seven (88%), and cortical dysplasia in one (13%). Many of these patients were diagnosed at an early stage of pregnancy (within the first trimester) and treated with intensive immunotherapy (**Shi et al. 2017, Kalam et al. 2019, Joubert et al. 2020**). In all cases where neurological sequelae were seen, NMDAR antibodies were detected either in the serum or foetal cord at the time of birth. Interestingly, in one case reported, NMDAR antibodies were reported at birth but the baby was healthy. Antibody pathogenicity is likely to be restricted to cases in which high antibody titres coincide with the period in which antibodies can cross the foetal blood brain-barrier more readily, which is typically from the second trimester onwards, and hence early treatment likely contributed to the good outcomes seen in many of the cases reported here (**Kumar et al. 2010**). It should be noted that it is not completely clear whether complications seen are due to the NMDAR antibodies or the severity of the mother's condition and/or treatments given during gestation (**Giannocco et al. 2020**). It is also important to consider that the short follow-up of these children and lack of systematic neurological assessments may have potentially missed neurological alterations.

1.2.4 Studying pathogenic impacts of NMDAR antibodies using *in vivo* models

In the past three decades, rodent models have been developed to study the effects of antibodies in the adult context, and more recently in the context of gestational transfer and neurodevelopment (Giannoccaro et al. 2020). Most rodent models are induced models, whereby the disease is artificially instigated, with either passive transfer or, less commonly, active immunisation. Active immunisation involves exposure to an antigen to generate an immune response whereas passive transfer involves reproducing disease by transferring serum, immunoglobulins, antibodies or antibody-producing cells, typically derived from patients with the disease eg. NMDARE. Antibodies may be either polyclonal (from several different immune cells with affinity for the same antigen but variable epitopes) or monoclonal antibodies (from identical immune cells that are clones of a specific parent) (Lipman et al. 2005). Most NMDARE models are passive transfer models that involve either stereotaxic injection or continuous infusion of NMDARE antibodies (or NMDAR antibody-containing material eg. patient CSF) intraventricularly into mice (reviewed in Giannoccaro et al. 2020). Typically, they utilise NMDARE patient cerebrospinal fluid or NMDARE patient-derived polyclonal or monoclonal NR1-IgG antibodies.

More recently, there has been increasing interest in using *in vivo* models to study antibodies in the context of gestational transfer and impacts on neurodevelopment, and several types of passive transfer model have been developed for this purpose (Figure 1.7). These typically involve injecting the pregnant dam at a point when antibodies can cross the blood brain barrier more readily (~E12+). For NMDAR antibodies specifically, this has been successfully utilised using both maternal intraperitoneal and tail-vein injections (Martínez-Cerdeño et al. 2016, Jurek et al. 2019, Giannoccaro et al. 2020, García-Serra et al. 2020) and subsequently establishing crossing of antibodies into the embryonic brains using various immunohistochemical methods. Models are also starting to be developed whereby embryonic brains are directly injected *in utero*, thereby bypassing the need for maternal to foetal transfer. The benefit of this

approach is that it enables simple and direct impacts to be studied, removes the potential confounding factor that variable volumes of antibody may be transferred from mother to foetus, and also practically requires a much smaller antibody volume. This method, using a midline laparotomy and direct intraventricular injections *in utero*, has been used for autism specific maternal antibodies (Camacho et al. 2014, Martínez-Cerdeño et al. 2016, Ariza et al. 2017), but to our knowledge has not been used or characterised for NR1-IgG antibodies.

In this thesis, we develop and explore a gestational *in utero* passive transfer model using NR1-IgG antibodies from NMDARE patients. To contextualise this in the current literature briefly, several other postnatal and gestational passive transfer models of NMDARE have been developed, which offer insights into functional impacts and potential disease mechanisms.

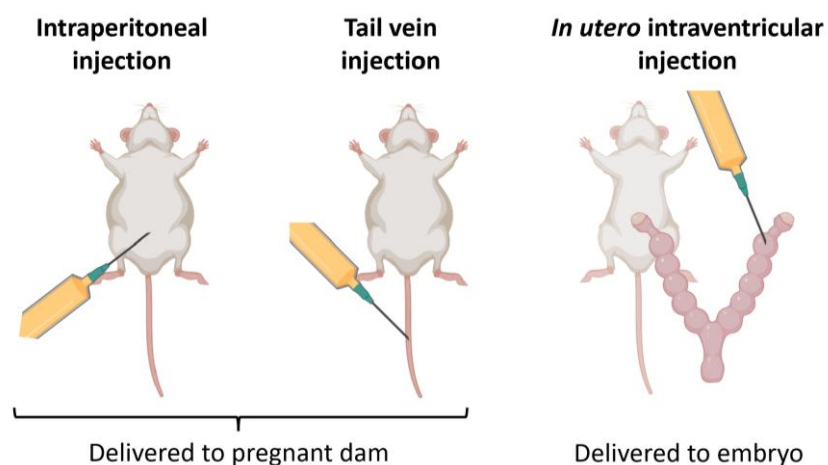


Figure 1.7. Types of gestational passive transfer model. Most passive transfer models of NMDAR antibody transfer involve injecting the pregnant dam with the antibodies at a point where the embryonic blood-brain barrier is permeable, either through intraperitoneal (IP) or tail-vein injections. There is also the possibility to deliver the antibodies directly to the embryonic brain via *in utero* intraventricular injection, which has been used for other antibodies but not for NMDAR antibodies. This approach has benefits such as removing issues with variable maternal-foetal transfer and requiring smaller volumes of antibody.

The key postnatal passive transfer models developed are reviewed in detail in Giannoccaro et al. (2020). Models vary between using either IgG antibodies or CSF from NMDARE patients, and also between using

either a single exposure or longer (typically 2 week) infusions. Antibodies in all cases are delivered directly to the brain. For studies using either single injection or 2-week infusion of IgG antibodies, there are variable results. In one study using single injection of antibodies delivered in mice at 8 weeks, there was increased seizure susceptibility with concordant IgG binding, but no significant NMDAR loss (**Wright et al. 2015b**). In further studies using 2-week IgG infusions delivered at approximately the same time point, some (**Malviya et al. 2017, Sharma et al. 2018**), but not all (**Taraschenko et al. 2019**) showed pathological behavioural changes including increases in spontaneous locomotor activity (**Sharma et al. 2018**), and decreased memory in the novel-object recognition test, along with decreased NMDARs in the hippocampus (**Malviya et al. 2017**). In 8-week-old rats, increased glutamate within the extracellular space was also observed following single IgG injection (**Manto et al. 2010**).

Experiments using CSF instead of IgG antibodies has also produced somewhat similar results. Single CSF injections in 8-week-old rats revealed a decrease in memory (using the Morris Water Maze (MWM) test) (**Würdemann et al. 2016**), and increases in glutamate (**Manto et al. 2010**). 2-week infusions of CSF into 8-week-old mice also revealed decreases in cognition and memory and increased depressive behaviour (**Li et al. 2015, Planagumà et al. 2015, Planagumà et al. 2016**), along with decreases in NMDAR. In one study, these effects could be prevented using EphB2, which stabilises the NMDAR (**Planagumà et al. 2016**).

There are far fewer gestational passive transfer models that have been developed so far; however, the existing ones typically report behavioural deficits in the offspring during the early postnatal period that can persist into adulthood. These include **Jurek et al. (2019)**, who used intraperitoneal injection of NR1-IgG at E13 and E17 and found *in utero* exposure to NR1-IgG resulted in impaired neurodevelopmental reflexes, hyperactivity, reduced anxiety and altered sensorimotor gating in adolescence and adulthood, as well as **García-Serra et al. (2020)** who used maternal tail-vein injections between E14-16 and found delayed innate reflexes, poor motor coordination, and deficits in memory and nest-building.

Mechanistically, **Jurek et al. (2019)** found significant reduction in density of NMDARs, as well as altered electrophysiological properties including decreased sEPSC amplitudes in young neonates, and reduced brain volumes in adult mice, whilst **García-Serra et al. (2020)** identified reduced cell surface and synaptic NMDARs, decreased spine density and increased dendritic arborisation of cortical cells, cellular compaction of the cortical layers, and increased activation of microglia. Further, a recent study by **Kuchling et al. (2023)** found using intraperitoneal injection of NR1-IgG at E13 and E17 resulted in selective impairment of hippocampal functional connectivity at 8 weeks and 10 months old.

More widely, **Martínez-Cerdeño et al. (2016)** found that *in utero* injection of IgG antibodies from mothers of children with ASD at E14 and E16 resulted in increased stem cell proliferation, adult brain size and weight, and size of adult cortical neurons. Several studies using embryonic injection of similar antibodies identified translations into the behavioural phenotype; **Camacho et al. (2014)** found adult offspring displayed autistic like-stereotypical behaviours in spontaneous grooming and marble burying tasks, and **Singer et al. (2009)** found increased activity in the open-field test in adolescence, as well as increased anxiety and alterations in sociability during adulthood. They also observed increased cytokine and glial activation.

There is variability in the outcomes seen from NMDAR passive transfer models. This is likely due to model differences including different agents (eg. antibodies, CSF etc.) used, different stages, duration and target brain regions of exposure, different methods of delivery, and different species of rodent used. Differences may also be due to the use of monoclonal vs polyclonal antibodies, since monoclonal antibodies have a much greater specificity for the antigenic target and may therefore induce greater effects with a smaller titre of antibody present.

1.2.5 Rescue models

A recent focus has been investigating methods through which impairments to the NMDAR may be rescued. Current methods which have been explored in rodent models and show promise include using NMDAR co-agonists (glycine or D-serine) to rescue impairments in NMDAR-dependent synaptic plasticity in a Fragile X syndrome model (**Bostrom et al. 2015**); using EphB2 (an NMDAR stabilising ligand disrupted by NR1-IgG antibodies (**Mikasová et al. 2012**)) to stabilise the NMDAR and reduce behavioural deficits (**Planagumà et al. 2016**); using postnatal tamoxifen-inducible Cre recombinase injections to rescue consequence of early genetically induced NMDAR deficiency (**Mielnik et al. 2020**); and using blockade of the neonatal FcRn receptor prior to injection of NR1-IgG to prevent placental transfer and subsequent foetal pathogenic effects (**García-Serra et al. 2021**).

Additionally, there has been early investigation into rescue of the Huntington's disease phenotype and disrupted glutamatergic signalling, through boosting AMPAR-mediated transmission with AMPAkinase CX516 (**Braz et al. 2022**). Relevant to the striatal focus of this thesis, Huntington's disease involves a large component of striatal specific pathology, with evidence to suggest alterations in NMDARs and NMDAR-mediated transmission, including early loss of SPNs with high levels of NMDARs, and impaired function of NMDARs at corticostriatal synapses (reviewed in **Fan and Raymond 2007**). Therefore, such rescue might also be relevant in the case of striatal dysfunction caused by NR1-IgG antibodies.

*

1.3 The striatum as an important, unexplored neurodevelopmental target of NMDAR antibodies

The presence of seizures in NMDARE has driven a largely hippocampal focus in literature so far (Li et al. 2015, Wright et al. 2015b, Würdemann et al. 2016, Malviya et al. 2017, Kersten et al. 2019, Jurek et al. 2019, Taraschenko et al. 2019, Giannoccaro et al. 2020, Wright et al. 2021), alongside some investigation into cortical changes (Rosch et al. 2018, García-Serra et al. 2020). However, evidence suggests the striatum, and its precursor, the LGE, may be of particular importance and interest regarding the effects of NMDAR antibodies during development.

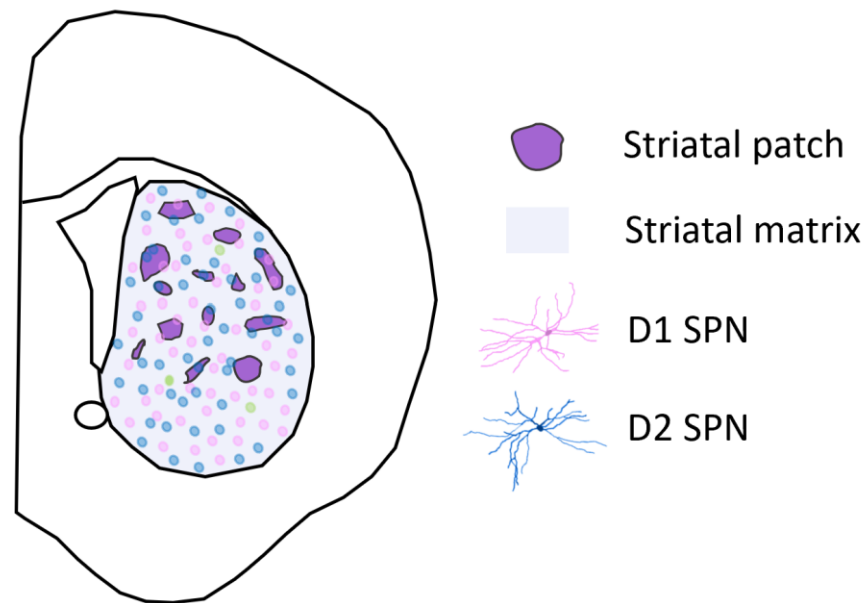


Figure 1.8. Simplified overview of striatal anatomy. A simplified diagram showing half a coronal section of the mature mouse striatum, demonstrating key basic anatomical features including the patch-matrix structure and the intermingled distribution of D1- and D2-SPNs, which together compose ~95% striatal neurons. Pink cells = D1-SPNs, blue cells = D2-SPNs, green cells = SInS.

For overview, the striatum is the main input nucleus of the basal ganglia, a collection of subcortical structures which together are involved in a range of functions including motor control, executive function, reward, cognition, and reinforcement learning (Graybiel et al. 1994, Grillner et al. 2005, Yin and

Knowlton 2006, Nestler et al. 2009, Hidaka et al. 2010, Kravitz et al. 2012, Lee et al. 2012, Cui et al. 2013, Graybiel et al. 2015, Fieblinger et al. 2021). Anatomically, the mature striatum is composed of a patch-matrix structure, and consists primarily of spiny projections neurons (SPNs, ~95%, two subtypes: D1- and D2-SPNs) as well as striatal interneurons (SINs, ~5%) which are intermingled within the striatum (Albin et al. 1989, Gerfen et al. 1990, Märtin et al. 2019) (*Figure 1.8*).

1.3.1 Striatal networks and functions

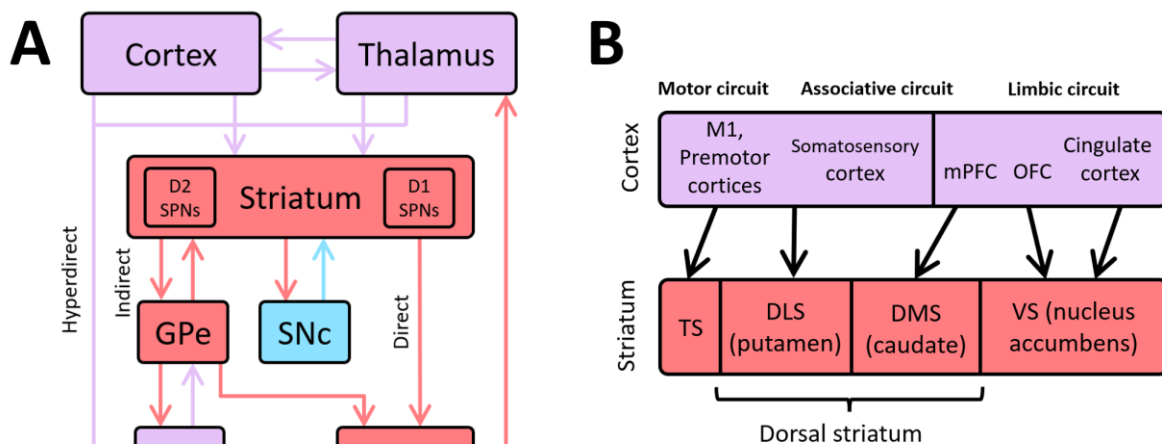


Figure 1.9. Basal ganglia connectivity. **[A]** A simplified diagram of the major pathways between the basal ganglia nuclei, cortex and thalamus. Processed information leaves the striatum either via the direct pathway composed of D1-SPNs, that targets the basal ganglia output nuclei GPi and SNr, or via the indirect pathway composed of D2-SPNs, that targets the SNr and GPi via the GPe and STN. Pathways and nuclei are colour coded as: purple = excitatory (glutamatergic), red = inhibitory (GABAergic) and blue represents dopaminergic. Abbreviations: GPe = globus pallidus externa, SNc = substantia nigra pars compacta, STN = sub thalamic nucleus, GPi = globus pallidus interna, SNr = substantia nigra pars reticulata. **Adapted from Bolam 2009.** **[B]** A simplified diagram of the topographical organisation of corticostriatal circuits onto specific striatal subdomains. mPFC = medial prefrontal cortex, OFC = orbitofrontal cortex, TS = tail striatum, DLS = dorsolateral striatum, DMS = dorsomedial striatum, VS = ventral striatum. Based on **Peixoto et al. 2016, Cataldi et al. 2022.**

The striatum receives input from three other major brain regions: glutamatergic inputs from the cortex and the thalamus, as well as dopaminergic inputs from the midbrain. The cortico-striatal-thalamic-cortical

circuits are arranged topographically in functionally separated and integrated loops along a dorsal-ventral striatal gradient (**Alexander et al. 1986, Haber and Knutson 2010**), and as such the striatum can be divided into the dorsal, ventral and tail regions (**Cataldi et al. 2022**). In rodents, the dorsal striatum receives inputs from the pre-frontal, associative and somatosensory cortices, and can be further segregated into the dorsolateral (DLS) and dorsomedial (DMS) regions based on cortical inputs (**Graybiel et al. 2008, Hunnicutt et al. 2016**). The ventral striatum receives input from the limbic cortices and amygdala whilst the tail striatum primarily receives inputs from the sensory cortices (**Cataldi et al. 2022**) (**Figure 1.9 A, B**). The striatum is also associated with two key output pathways: the direct pathway which monosynaptically projects to the GPi and SNr and is associated with D1-SPNs, and the indirect pathway which projects to the GPe and is associated with D2-SPNs (**Figure 1.9 A**).

The striatum is involved in many important behaviours largely linking motivation and motor movement, including performance of simple motor tasks, and complex cognitive tasks such as decision making, reward processing and social interaction (**Graybiel and Grafton 2015, Lorenzetti and Cousijn 2016**). Striatal functions are highly broad and complex, so below is a summary of the key functions most relevant to this thesis.

The dorsal striatum is mostly thought to be involved in motor control, particularly automated fine skills and micromovements (**Yin et al. 2009, Thorn et al. 2010**). More specifically, studies in mice suggest that the DMS partakes in motor skill acquisition, whilst the DLS is more involved in mastered motor tasks (**Yin and Knowlton 2004, Yin et al. 2009, Kupferschmidt et al. 2017**). Dorsal striatal lesions result in impaired motor function, the specifics of which vary based on exactly where the lesion occurs (**Mink 1996**). DLS lesions have been shown to result in impairments including slowed movement, difficulty starting movement, inability to conduct two concurrent movements, impaired movements in the context of visual stimuli, and impaired posture (**Denny-Brown and Yanagisawa 1976, Miyachi et al. 1997**) whilst DMS

lesions have been shown to result in reduced limb movement and impaired movements in the context of auditory stimuli (**Condé et al. 1981, Alexander and Crutcher 1990**).

The striatum is also involved in emotional processing, with the ventral striatum primarily regulating this function via inputs received from the ventral pre-frontal cortex (**Lago et al. 2017**). It has been shown that stress results in a shift in memory processing from flexible cognitive processes to more rigid habit-based memory, which is mediated by the striatum (**Vogel et al. 2016**), and there is also emerging literature for the role of this striatal region in anxiety-related pathways (**Groenwegen et al. 1987, Fudge et al. 2002, Liljeholm and O'Doherty 2012, Avery et al. 2014, Calhoon and Tye 2015, Torrisi et al. 2015, Lago et al. 2017**). The bed nucleus of the stria terminalis (BNST), which has a similar developmental origin to the striatum, also has an established key role in stress and anxiety circuits and is implicated in several anxiety-related disorders including PTSD and generalised anxiety disorder (**Ahearn et al. 2007, Buff et al. 2017, Clauss et al. 2019, Feola et al. 2023**). Further, it has been shown that the striatum plays an important role in social interaction, with fMRI studies showing that computations carried out in the striatum are important for successful social interactions (**Báez-Mendoza and Schultz 2013**).

The striatum has also been shown to play a key role in reward learning and reward-based decision making (**Cox and Witten 2019**). Within this, studies demonstrate that different parts of the striatum have different roles; the DMS is involved in regulating goal-directed behaviours reliant on response-outcome associations (**Yin et al. 2005, Balleine et al. 2007, Yin et al. 2009, Balleine and O'Doherty 2010, Redgrave et al. 2010, Thorn et al. 2010, Liljeholm and O'Doherty 2012, Dolan and Dayan 2013**), with both the DMS and DLS active during goal-directed learning (**Balleine et al. 2007, Donahue et al. 2018**), whereas the nucleus accumbens (a major part of the ventral striatum), is responsible for evaluating outcomes and motivation (**Setlow et al. 2003, Roitman et al. 2005, Balleine and O'Doherty 2010, Liljeholm and O'Doherty 2012**) and also for controlling dopamine release in the striatum through projections to

midbrain dopaminergic neurons (**Groenewegen et al. 1999, Watabe-Uchida et al. 2012**). The tail striatum has also been demonstrated to play a role in goal-directed action (**Valjent and Gangarossa 2021**).

The striatum also plays a key role in development of habits. Multiple studies have demonstrated that the striatum is required for habit formation, and that striatal lesions can blunt or block pre-formed habitual behaviour (**Balleine and Dickinson 1998, Yin and Knowlton 2006, Belin et al. 2009**). Learning of initial motivated behaviours that may subsequently become habits is governed by the ventral striatum, whilst the dorsal striatum is required for habit maintenance (**Atallah et al. 2014**). This pattern is reflected in studies exploring development of addictive behaviours, where development of initial addictive behaviours largely involves influence of the midbrain dopaminergic system acting on its ventral striatal target, with the dorsal striatum becoming more involved as the addictive behaviours progress (**Everitt and Robbins 2013**).

The direct and indirect pathways are key for modulation of striatal-related behaviours. In the classical view of striatal circuitry, these pathways were posited to exert opposite effects, with activation of the direct or indirect pathways respectively thought to promote or inhibit striatal behaviours and actions (**Krawitz et al. 2010, Bariselli et al. 2019**). However, more recent work suggests that this model does not reflect the full complexity of how these pathways underpin striatal-related behaviours, with multiple studies demonstrating that both pathways are concurrently activated during action initiation, response and reinforcement (**Cui et al. 2013, Tecuapelta et al. 2014, Tecuapelta et al. 2016, Vicente et al. 2016, Barbera et al. 2016, Klaus et al. 2017, Markowitz et al. 2018, Parker et al. 2018, Bariselli et al. 2019**). These studies suggest that the role of indirect pathway neurons is more precise than broad inhibition and is likely to have a context or action-specific inhibitory function, with precise underlying mechanisms still not fully understood (**Zalocusky et al. 2016, Parker et al. 2018, Bariselli et al. 2019**). What is clearer is that balance of these pathways appears to be crucial for healthy neurological function. Imbalance has

been linked to neurological disease across the lifetime spectrum spanning early neurodevelopmental disorders including Tourette's (McNaught and Mink 2011, Albin et al. 2018), OCD (Graybiel and Rauch 2000, Langen et al. 2011), ADHD (Del Campo et al. 2011) and ASD (Shepherd 2013) up to late-onset neurodegenerative diseases including Parkinson's disease (Taverna et al. 2008) and Huntington's disease (Cepeda et al. 2013).

1.3.2 Striatal cellular development

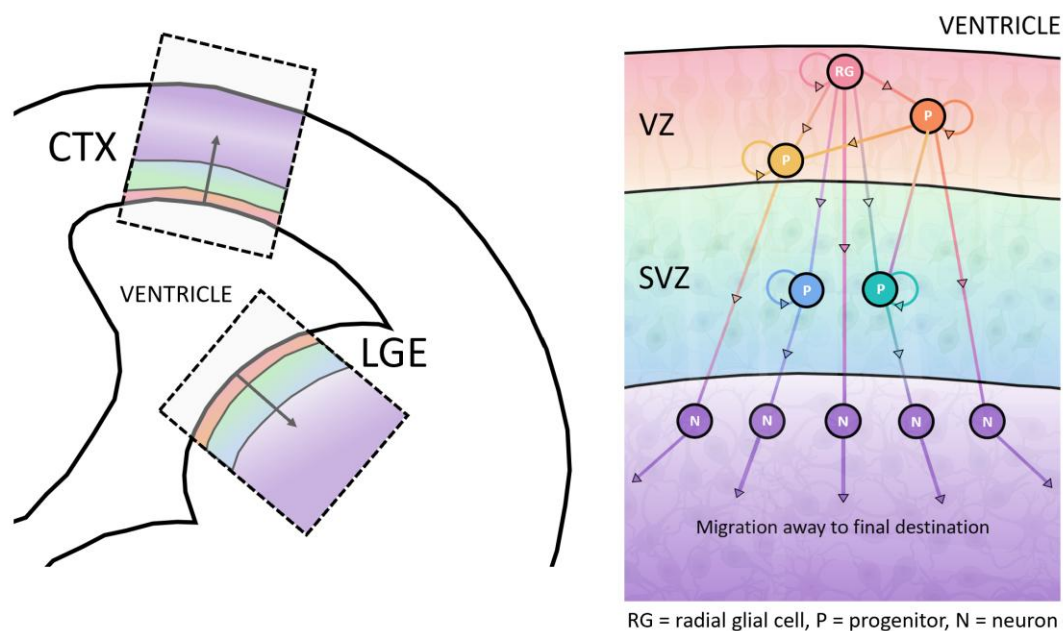


Figure 1.10. Neurogenesis in the cortex and LGE. In general terms, for both the cortex, and the LGE, radial glia (RG) initially line the ventricle and give rise to progenitors (P) in the major proliferative regions: initially the ventricular zone (VZ, red/orange) and later the sub-ventricular zone (SVZ, green/blue). The radial glia and progenitors in these zones proliferate and differentiate, thereby directly or indirectly producing post-mitotic neurons (purple) (N). The neurons subsequently migrate away to their final positions in the developing cortex / striatum. Figure based on **Turrero García and Harwell 2017**.

The SPNs that compose the majority of the adult striatum originate from the LGE, one of four main regions in the ventral telencephalon. Here, during early neurodevelopment, an initial neuroepithelial population

differentiates into radial glia and other embryonic progenitors. These subsequently give rise to the neurons (neurogenesis) and other glia that compose the striatum (**Marín and Müller 2014**) (**Figure 1.10**).

Anatomically, radial glia and other apical progenitors initially line the LGE ventricle (the 'ventricular zone' (VZ)). As development progresses, some cells start dividing at basal locations, resulting in the formation of a new basal area known as the 'subventricular zone' (SVZ) (**Smart 1976**). This zone progressively expands to become the main proliferative region, and this expansion gives rise to a diverse population of intermediate progenitors (IPs) (**Smart 1976, Fentress et al. 1981, Pilz et al. 2013**). Specific progenitor pools in the LGE can be identified and lineage traced into the mature striatum through expression of markers such as Tα1 (**Van Heusden, Macey-Dare et al. 2021**). It has been shown that there are two waves of expansion in the LGE that give rise to neurons in the two morphological compartments of the mature striatum; the first, from ~E10-13.5 gives rise almost exclusively to the neurons that form the striatal 'patches' (otherwise known as striasomes), and the second, from ~E12.5-17.5 predominantly gives rise to the neurons of the striatal matrix (**Kelly et al. 2018**). Neurogenesis in the LGE peaks at ~E15 (**Arlotta et al. 2008**). As these progenitors further divide, the post-mitotic neurons follow a well characterised sequence of migration from birthplace to final destination (**Villar-Cerviño et al. 2015**) and concurrent progressive differentiation towards final identity. At ~E18, there is a developmental switch from neurogenesis to gliogenesis (**Anthony and Heintz 2008, Minocha et al. 2017, Turrero García and Harwell 2017**).

From the diverse pool of embryonic progenitors in the LGE, most of the post-mitotic neurons become GABAergic SPNs. A small portion (~5%) of cells in the mature striatum are striatal interneurons (SINs), which mostly arise from the neighbouring MGE (a structure medial to the LGE in the embryonic brain) (**Wichterle et al. 1999, Corbin et al. 2001, Wichterle et al. 2001, Stenman et al. 2003**). SPNs are medium-sized GABAergic neurons consisting of a cell body with densely packed spines along their dendrites (**Chronister et al. 1976, Wilson & Groves 1980, Wilson et al. 1983**). SPNs can be subdivided into two

approximately equal-sized populations; 'D1-SPNs', which express dopamine D1 receptors, substance P and dynorphin, and 'D2-SPNs' which express dopamine D2 receptors and enkephalin (**Gerfen et al. 1990, Yung et al. 1996**). The two SPN populations are involved in different basal ganglia circuits and as such differentially regulate motor and cognitive behaviour (**Graybiel et al. 1994, Grillner et al. 2005, Yin and Knowlton 2006, Kravitz et al. 2010, Tecuapetla et al. 2016**). SINs modulate the activity of SPNs and can be split into two main classes: GABAergic interneurons, and cholinergic interneurons. The GABAergic SIN population may be subdivided based on morphology, electrical properties and molecular marker expression (**Kawaguchi et al. 1993, Muñoz-Manchado et al. 2018**; reviewed in detail in **Silberberg and Bolam 2015** and **Tepper et al. 2018**).

In general, the cell types of the striatum are intermingled throughout the structure rather than being individually contained in defined regions (**Knowles et al. 2021**). From developmental studies it has been shown that both D1- and D2-SPNs are produced in both waves of expansion (**Kelly et al. 2018**), although D1-SPNs exhibit a slightly earlier birthdate (**Marchand and Lajoie 1986, Van der Kooy and Fishell 1987, Kelly et al. 2018**). Following production in the LGE SVZ, both D1- and D2-SPNs migrate towards just beyond the sub-ventricular zone, where they actively intermix before integrating into the compartment under formation at that time (either patch or matrix). This intermixing produces the mosaic cell architecture that is critical to proper striatal function (**Gangarossa et al. 2013, Knowles et al. 2021**).

1.3.3 Striatal circuit development

Following proliferation and migration, the early postnatal phase sees the maturation of SPN electrophysiological and morphological properties, alongside circuit integration. *In vivo* and *ex vivo* recordings of early postnatal SPNs have demonstrated that they are electrically immature shortly after birth, characterised by hyperexcitability and high input resistance, as well generation of only small, immature action potentials (**Tepper and Trent 1993, Tepper et al. 1998, Belleau and Warren 2000,**

Dehorter et al. 2011, Peixoto et al. 2016, Krajeski et al. 2019). Increased neuronal intrinsic excitability has been shown to be fundamental for developmental processes including neuronal growth and synaptogenesis (**Spitzer 2006**). In the first postnatal week, SPNs are mainly linked via gap junctions (**Tepper et al. 1998, Venance et al. 2004, Yu et al. 2012, Krajeski et al. 2019**) which are thought to aid development of synaptic connections (**Yu et al. 2012**), and help synchronise SPN activity (**Venance 2004, Hestrin and Galarreta 2005**). At this early stage, SPNs exhibit a reticular morphology, with thin varicose dendrites and very few dendritic spines (the main sites of glutamatergic synapses onto SPNs) (**Sharpe and Tepper 1998, Krajeski 2019, Sharpe and Tepper 1998**).

Over the following weeks, intrinsic excitability decreases due to resting membrane potential hyperpolarisation secondary to increased numbers of inward rectifying potassium (K⁺) channel currents, which is mediated by dopamine signalling (**Liebermann et al. 2018**). This is coupled with the ability to produce larger action potentials at greater firing frequencies (**Peixoto et al. 2016, Krajeski et al. 2019**). During the second postnatal week, there is also a shift in the NMDA/AMPA ratio towards a bias in AMPAR recruitment, linked to maturation of synapses (**Petralia et al. 1999, Peixoto 2016, Krajeski et al. 2019**). In the same period, gap junctions are also replaced by inhibitory synaptic connections which are detected between both SPN types by the second postnatal week (**Krajeski et al. 2019**). Over this period, the SPNs also mature morphologically, with a shift towards radial morphology and substantial increase in dendritic arbour length and complexity, as well as a protracted increase in spine density, which peaks around the second postnatal week, corresponding to the emergence of complex, coordinated motor behaviours (**Tepper et al. 1998, Gertler et al. 2008, Dehorter et al. 2011, Peixoto et al. 2016, Krajeski et al. 2019**).

By the end of the fourth postnatal week, SPNs have an electrophysiological and morphological appearance closely reminiscent of adult SPNs, including the emergence of membrane potential bistability in the shape of up and down states, which is critical for the ability of SPNs to integrate and process various inputs and

generate mature output action potentials (**Carter and Sabatini 2004, Peixoto et al. 2016, Krajeski et al. 2019**).

Importantly, several differences have been identified in the electrical and morphological development of D1- and D2-SPNs. D1-SPNs are thought to be born at an earlier timepoint (**Marchand and Lajoie 1986, Van der Kooy and Fishell 1987, Kelly et al. 2018**) and various groups including my lab have demonstrated them to be electrically more mature during the initial postnatal period, with lower input resistance and relatively earlier ability to produce large action potentials compared to their D2-expressing counterparts (**Peixoto et al. 2016, Krajeski et al. 2019**). Additionally, it has been shown that inhibitory synaptic connections between SPNs are detected only in D1-SPNs in the first postnatal week, (but both types by the second postnatal week), likely reflective of the earlier birthdate of D1-SPNs (**Marchand and Lajoie 1986, Van der Kooy and Fishell 1987, Kelly et al. 2018**). Morphologically, whilst my lab found both SPN types develop mostly in parallel (**Krajeski et al. 2019**), others have shown morphological discrepancies during development including a larger dendritic arbour and greater number of primary dendrites in D1-SPNs (**Gertler et al. 2008, Benthall et al. 2018**).

The early postnatal period also sees the concurrent development of inputs onto and outputs from SPNs, and formation of striatal circuits. Patch SPNs start to project towards their output targets during the late embryonic period (E17+), and matrix SPN projections develop mostly during the first postnatal week (**Fishell and Van der Kooy 1987, Fishell and Van der Kooy 1989**). At a similar timepoint, the developing striatum also undergoes a period of physiological cell death, where ~30% SPNs die, in a manner unbiased towards location (**Fishell and Van der Kooy 1991**).

Glutamatergic innervation into the striatum occurs primarily via the cortex and thalamus. Cortical projections originate predominantly in cortical layers V and II/III and begin to develop during the mid-embryonic period (~E11-16) (**Sur and Leamey 2001, Sohur et al. 2014**). They are separated into

intertelencephalic (i.e. corticostriatal) neurons which project to other cortical and striatal regions, and pyramidal neurons which project to subcortical regions such as the thalamus (**Shepherd 2013**). In rodents, corticostriatal projections enter the striatum during the first postnatal week, around p3. The earliest projections primarily emanate from the limbic cortex and converge with dopaminergic fibres (predominantly onto striatal patches), positing that the limbic corticostriatal pathways may be the earliest to develop (**Eblen and Graybiel 1995, Peixoto et al. 2016**). Conversely, projections from motor and associative cortices (targeting striatal matrix) develop later and undergo development mostly during the second postnatal week (**Christensen et al. 1999**), coinciding with a key period of motor program refinement (**Dehorter et al. 2011, Kalueff et al. 2015**). Synapse driven depolarisation in the striatum occurs between p5-7, tied to increased oscillatory pattern activity (**Khazipov et al. 2004, Dupont et al. 2006, Yang et al. 2009, Klavinskis-Whiting et al. 2023**), but cortical innervation is not detected in all SPNs until ~p9, suggestive of ongoing corticostriatal synaptogenesis. Strengthening of corticostriatal synapses continues up to at least p18, reflected in the progressive increase of post-synaptic current amplitude in SPNs on cortical stimulation (**Hurst et al. 2001, Peixoto et al. 2016, Krajeski et al. 2019**). Interestingly, it has also recently been shown by my lab that SPN embryonic origin can influence synapse specificity of corticostriatal afferents (**Van Heusden, Macey-Dare et al. 2021**). These first postnatal weeks see excessive corticostriatal projection growth and subsequent refinement, with numbers of cortical neurons innervating the striatum decreasing during this period (**Innocenti and Price 2005, Sohur et al. 2014**).

Thalamostriatal projections and dopaminergic striatal inputs are not a focus of this thesis but are included briefly below for completion:

Like the cortex (**Figure 1.9 B**), the thalamus innervates the striatum in a topographical manner, with multiple thalamic nuclei providing striatal innervation and each nucleus projecting widely (**Smith et al. 2014**). Development of thalamic projections is thought to begin at an earlier timepoint, and they are already detectable in all areas of the striatum by birth (**Vercelli et al. 2003, Nakamura et al. 2005**).

Strengthening of thalamostriatal synapses occurs over a longer timeframe, with amplitudes of SPN post-synaptic currents following thalamic stimulation progressively increasing until at least the end of the fourth postnatal week (**Krajeski et al. 2019**).

Dopaminergic inputs are the first inputs to develop, with mesencephalic dopaminergic neurons extending into the developing striatum embryonically as early as E12.5 and key enzymes for dopamine synthesis expressed by E14 (**Olson and Seiger 1972, Seiger and Olson 1973**). Nigrostriatal dopaminergic axons enter the developing striatum around E14, initially innervating the lateral regions before stretching across the striatum to innervate medial regions by E18, and functional dopamine release is present by birth (**Specht et al. 1981, Voorn et al. 1988, Ferrari et al. 2012**), with dopamine particularly important for modulating striatal circuit maturation in late embryonic and early postnatal phases (**Ferrari et al. 2012**). Of relevance, dopamine is important for D1-SPN maturation and decreased nigrostriatal dopaminergic modulation halts the developmental decrease in D1-SPN excitability (**Lieberman et al. 2018**). In the first postnatal weeks, dopaminergic projections undergo extensive maturation with activity becoming ‘adultlike’ from ~p15 (**Grace and Onn 1989, Seutin 1998, Cui 2004, Guzman et al. 2009, Dufour et al. 2014**).

1.3.4 A brief overview of cortical development in the context of the striatum

Although this thesis primarily focuses on the striatum, there is some exploration of the impact of antibodies against the NMDAR on cortical progenitors and neurons *in utero*, as well as on corticostriatal connections, and therefore it is important to briefly introduce cortical development within this context.

The cerebral cortex is responsible for higher cognitive functioning. It has a six-layer structure, with the upper layers (I-IV) connecting and processing information locally within the cortex, and the deep layers (V-VI) projecting over longer distances to the subcortical targets, including the striatum (**Broadmann 1909, Toyama et al. 1974, Gilbert and Wiesel 1979**). Unlike the striatum, the cortex predominantly arises from the dorsal telencephalon, which gives rise to the glutamatergic excitatory (pyramidal/principal)

neurons which compose ~80% of cortical cells (**Lodato and Arlotta 2015**). The other 20% consist of GABAergic interneurons which are produced in the ventral telencephalon and migrate into the developing cortex (**Marín 2013, Bandler et al. 2017, Laclef and Métin 2018**).

Cortical neurogenesis begins slightly before striatal neurogenesis, at ~E10-12 (**Götz and Huttner 2005, Matsuzaki and Shitamukai 2015**), but the processes transitioning from apical radial glial cells to a wider pool of neurogenic progenitors and onto post-mitotic neurons is largely similar to striatal development (**Uzquiano et al. 2018**). Neurons produced during early neurogenesis (E11.5-14.5) form the deep layers of the cortex, whilst those produced during late neurogenesis (E14.5-17.5) migrate past the early born neurons to form the superficial layers (**Luskin 1988, McConell and Kaznowski 1991, Walsh and Cepko 1993, Frantz and McConnell 1996, Desai and McConnell 2000, Shen et al. 2006, Gao et al. 2014, Kaplan et al. 2017**). Gliogenesis subsequently follows neurogenesis (**Molnár et al. 2019**).

Development of corticostriatal circuits is described above in **Section 1.3.3. Striatal circuit development**. Corticostriatal connections have been shown to play a key role in correct physiological development of the striatum and striatal circuits, highlighted by *in vitro* embryonic corticostriatal culture experiments demonstrating that reduced cortical inputs impaired both morphological and electrophysiological maturation of SPNs including a reduced dendritic complexity and total dendritic length, and concurrent decreased membrane capacitance, reduced numbers of excitatory synapses, and a trend towards a decrease in mEPSC frequency (**Buren et al. 2016**). Important in the context of this thesis, the study also identified a shift of NMDARs from synaptic to extrasynaptic locations, positing that differences observed may be underpinned by NMDAR-mediated mechanisms.

1.3.5. Why is the striatum an interesting potential target for NMDAR antibodies?

A body of pre-clinical and clinical literature points towards the striatum as an important but unexplored neurodevelopmental target of NMDAR antibodies, much of which is captured earlier in this **General Introduction**. To summarise:

Considering progenitors and early striatal development, literature suggests regional differences in expression and importance of glutamatergic receptors in the neurodevelopmental context, with NMDARs particularly important for striatal neurogenesis (**Luk et al. 2003, Jansson and Åkerman 2014, Krajieski et al. 2019**). Glutamate is a key modulator of progenitor proliferation, migration, differentiation, and survival (**Jansson and Åkerman 2014**). It has been shown *in vitro* that proliferation of striatal neuroblasts are sensitive to NMDAR antagonist MK801, and similarly, *in utero* exposure to antagonists during the neurogenic period results in a significant decrease in progenitor proliferation, DNA synthesis and striatal neurons numbers (**Luk et al. 2003**).

Considering striatal cellular and circuit development and maturation, it has been shown *in vitro* that NMDAR composition modulates SPN dendritic and spine morphology (**Vastagh et al. 2012**) and that they are also important in the development of cellular intrinsic properties (**Hou and Zhang 2017**). NMDARs are also key for development and plasticity of glutamatergic synapses (**Malinow and Malenka 2002, Song and Hyganir 2002**) and are particularly important for early postnatal synaptic transmission, since early glutamatergic synapses contain very few AMPARs (**Isaac et al. 1995, Liao et al. 1995, Isaac et al. 1997**). Recent work by my lab and others has shown that the early postnatal weeks are highly dynamic in terms of NMDAR expression within the cortical striatal circuitry, with pharmacological studies revealing a progressive decrease in NR2C/D subunit containing NMDARs (**Monyer et al. 1994, Dehorter et al. 2011, Krajieski et al. 2019**), as well as a transient significant increase in NR2A/B subunit containing NMDARs specifically in the second postnatal week (**Krajieski et al. 2019**). The second postnatal week also sees D1-

SPNs exhibiting larger NMDAR-mediated cortical inputs, which are posited to result from transient changes in receptor expression or differential input maturation (**Krajeski et al. 2019**).

Considering behaviour, *in vivo* studies have demonstrated that exposure to NMDAR antibodies can result in behavioural deficits consistent with impaired striatal and wider function, which emerge in the early postnatal period and can persist into adulthood. These include delayed innate reflexes, hyperactivity, reduced anxiety, impaired motor coordination and deficits in nest building (**Jurek et al. 2019, García-Serra et al. 2020**). Recent loss-of-function NR1 mouse studies have shown that adult genetic rescue of NMDARs fails to reverse many striatal-seated behaviours such as hyperactivity and stereotypy, likely due to the limited increase in striatal NMDAR function (**Mielnik et al. 2020**).

The NMDARE clinical picture also points to the striatum as a key target. The latter symptoms of NMDARE include dyskinesia, dystonia (**Florance et al. 2009**) and movement disorders (**Irani et al. 2010**), which implicate motor circuit dysfunction including those of the basal ganglia and striatum (**Lanciego et al. 2012**). It has also been shown in numerous studies that autoimmune diseases are more prevalent in mothers of children with a range of neurodevelopmental and neuropsychiatric disorders, which exhibit an array of behavioural pathology (**McAllister et al. 1997, Comi et al. 1999, Ross et al. 2003, Sweeten et al. 2003, Croen et al. 2005, Urowitz et al. 2008, Atladóttir et al. 2009, Mostafa et al. 2010, Ghassabian et al. 2012, Mostafa et al. 2014, Dalsgaard et al. 2015, Vinet et al. 2015, Jurek et al. 2019**). Further, children with NMDARE often experience a more ‘neurological’ presentation, with the above striatal-linked symptoms, rather than the classical psychiatric presentation observed in adulthood onset of the disease (**Armangue et al. 2013, Wright et al. 2015a**).

Whilst this thesis focuses on the striatum, the cortex is also used as a comparator region for exploring impacts of these antibodies and therefore it is helpful to briefly summarise the key existing knowledge in this area. Seminal early studies suggest that corticogenesis is primarily AMPAR/KAR-mediated rather than

NMDAR-mediated, with agonist activation of AMPARs/KARs in embryonic cortical explants and organotypic slices found to regulate cortical DNA synthesis and proliferation (**LoTurco et al. 1995, Haydar et al. 2000**), and *in utero* exposure to NMDAR antagonists having little impact on development of embryonic cortical progenitors in a mouse model (**Luk et al. 2003**). Despite studies suggesting that corticogenesis is less NMDAR-mediated, studies do suggest an impact of NMDAR antibodies on the developing cortex. The most relevant study used placental transfer of NMDAR antibodies via maternal tail-vein injection from E14-16 of pregnancy (**García-Serra et al. 2020**) and identified cortical changes both embryonically and in the developing postnatal brain. Cortical plate thinning was observed at both E17-18 and p21. In the p21 cortex, other changes were also observed including decreased glutamatergic synapse density and cortical neuronal morphological alterations including increased dendritic arborisation and decreased numbers of spines. Interestingly, many of these changes in the cortex were transient, resolving by p70. Further work using computational modelling and local field potential recordings in an NMDARE mouse model identified that NMDAR antibodies cause alterations in cortical circuit microcircuit dynamics including a specific shift in cortical circuit excitatory coupling (**Rosch et al. 2018**).

*

1.4 A focused introduction to microglia

Microglia serve as the immune cells of the CNS and are a first line of defence for the brain (**Jurga et al. 2020**). They play key roles in neurodevelopment and processes needed for correct brain functioning and maturation, with alterations in microglia implicated in several neurodevelopmental disorders including ASD, Rett Syndrome and schizophrenia (**Morgan et al. 2010, Tetreault et al. 2012, Cronk et al. 2015, Horiuchi et al. 2017, Velmeshev et al. 2019**). Additionally, NMDARs and microglia are closely linked, and literature also suggests roles for microglia in NMDARE pathology (**Dalmau et al. 2007, Tüzün et al. 2009, Camdessanché et al. 2011, Chang et al. 2023, Rahman et al. 2023**).

1.4.1 Microglia functions and states

The general function of microglia is to monitor the environment and induce a response to any potential danger signals. Once danger is recognised, microglia shift to an 'activated' state which includes a dynamic switch in their function and morphology, and release of cytokines, alerting nearby cells to the threat and altering their function, triggering an inflammatory state (**Du et al. 2017, Jurga et al. 2020**). Microglia can also induce cell death in malfunctioning neurons (**Wake et al. 2009, Hornik et al. 2016**) and have important protective capacity, stopping post-response inflammation through emitting anti-inflammatory cytokines, and aiding repair of the local environment (**Gomes-Leal 2012**).

Microglia undergo morphological and functional changes both during development, and during exposure to immunogenic stimuli and pathological conditions (**Zusso et al. 2012, Prinz and Priller 2014**). They can be broadly classified on a spectrum from 'unactivated' to 'activated' (**Figure 1.11**). Unactivated microglia are the most common microglial type in the developed brain (**Lawson et al. 1990, Soltys et al. 2005, Karperien et al. 2013, Torres-Platas et al. 2014**). They predominantly exhibit a ramified morphology, with a small, symmetrical soma and elongated branched processes (**Soltys et al. 2005, Nimmerjahn et al. 2005**). In this state, the microglia are continuously scanning their surroundings through extending and

retracting their processes (**Nimmerjahn et al. 2005, Olah et al. 2011**). A variety of immunogenic stimuli can lead to rapid microglial activation, which involves dramatic functional and morphological alterations, and a stepwise de-ramification (**Brockhaus et al. 1996, Heppner et al. 1998, Dailey and Waite 1999, Stence et al. 2001, Wierzbza-Bobrowicz 2002, Arcuri et al. 2017, Fernández-Arjona et al. 2017, Gutmann and Kettenmann 2019**). Activated microglia are typically amoeboid in shape with few, thick processes, or no processes at all, and a large, irregular soma (**Torres-Platas et al. 2014**). This microglial type is also the most frequent type in embryonic brains, before a shift to a more ramified morphology during postnatal development (**Zusso et al. 2012**).

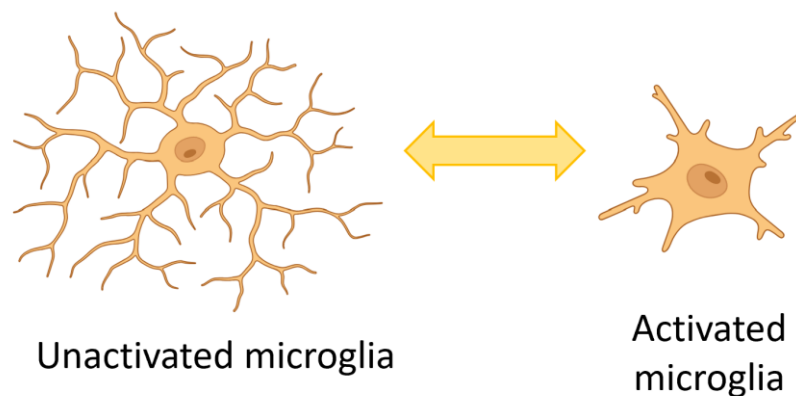


Figure 1.11. Microglial states. Microglia undergo morphological and functional changes both during development and during exposure to immunogenic stimuli and pathological conditions, moving on a spectrum between an ‘unactivated’ and ‘activated’ state. There are also many heterogeneous subtypes that exist between these two states.

Microglia exhibit a broad spectrum of intrinsic heterogeneity and have been classified into many subtypes, representative of transitional points between unactivated and activated states (**Stratoulis et al. 2019, Tan et al. 2020**). These varying phenotypes reflect functional differences and are therefore important to identify. The most recent literature utilises advanced statistical procedures to identify a vast range of microglial subclasses under different physiological and pathological conditions based on morphology and gene expression (**Fernández-Arjona et al. 2017, Heindl et al. 2018, Young and Morrison 2018, Leyh et al. 2021**).

1.4.2 Roles of microglia in neurodevelopment

In mice, microglia are derived from primitive myeloid progenitor cells within the yolk sac and subsequently invade the brain at ~E9.5 (**Ginhoux et al. 2010**). Once inside, they mature, exhibiting dynamic morphological activity including a shift from an early amoeboid shape to increasingly ramified structure (**Arnoux et al. 2013, Hanamsagar et al. 2017**). From E14-16, numbers within the brain grow rapidly, due to a second wave of migration into the brain from the periphery alongside local self-sustaining proliferation and renewal (**Ajami et al. 2007, Ajami et al. 2011, Swinnen et al. 2013**).

The early invasion of microglia, including their presence in the neuroepithelium where early progenitors proliferate, implies a functional role in early neurodevelopment. A bi-directional role for microglia in neurogenesis is hypothesised; both supporting cell generation and removing unrequired cells (**Ueno and Yamashita 2014**). *In vitro*, cultured embryonic cortical cells from mice genetically lacking microglia have shown reductions in proliferation (**Antony et al. 2011**), whilst *in vivo*, reduced microglia have been shown to lead to decreased cellular differentiation (**Erblich et al. 2011**) or restricted numbers of neural progenitor cells (**Cunningham et al. 2013**), with the latter study finding that microglia regulated progenitor numbers predominantly through phagocytosis of non-dying progenitors. Microglia are believed to have an important role in regulating cell survival, with several studies involving pharmacogenetic and pharmacological elimination of microglia *in vivo* demonstrating loss of microglia can result in widespread cell death in cortical and other neuronal populations (**Ueno et al. 2013, Arnoux et al. 2014, Strahan et al. 2017**). Microglia have also been shown to be involved in promoting cell death through secretion of cytotoxic molecules (**Frade and Barde 1998, Sedel et al. 2004**) and other phagocytic processes (**Marín-Teva et al. 2004, Wakkseman et al. 2008, Cunningham et al. 2013**).

Further, recent evidence suggests a role for microglial promotion of early synapse formation, with pharmacogenetic microglial ablation studies in adolescent mice revealing both impaired synapse

formation and elimination (**Parkhurst et al. 2013**). They are also thought to support axon growth and guidance (**Streit 2001, Kaur et al. 2007**). Additionally, *in vivo* imaging studies have demonstrated formation and stabilisation of dendritic spines post microglial contact on the dendritic shaft (**Miyamoto et al. 2016**), along with other microglial contributions to synapse formation, both during a short neurodevelopmental time-period (**Reshef et al. 2017**). There is also a large body of work supporting the role of microglia in removal of excess synapses (**Lukens and Eyo 2021**).

1.4.3 Microglia, NMDARs and NMDARE antibodies

Within a functional context, NMDARs and microglia are closely linked. They are important in many neuronal-microglial interactions, with studies showing for example that stimulation of NMDARs on active neurons induces ATP/ADP release, which binds Gi-coupled P2Y₁₂ receptors on microglia, promoting extension of their processes towards the neurons (**Dissing-Olesen et al. 2014, Eyo et al. 2014, Eyo et al. 2015, Kato et al. 2016, Pfeiffer et al. 2016, Sipe et al. 2016, Bernier et al. 2019, Cserép et al. 2019**). More recently it has been shown that the NR2A subunit specifically regulates these interactions (**Eyo et al. 2018**). Neuronal-microglial interactions are important in a host of neurological processes including circuit refinement, microglia-mediated synaptic pruning and neuroplasticity (**Ueno and Yamashita 2014, Jurga et al. 2020**).

Further, impairing microglial function through removal of BDNF (a key plasticity mediator) has been shown to reduce neuronal expression of glutamate receptors including the NMDAR NR2B subunit, and result in synaptic deficits including impaired NMDAR-mediated mEPSCs, which also correlate to impaired rotarod behaviour and neuronal spine growth (**Parkhurst et al. 2013**). It has also been shown that selective activation of microglial CX3CR1 receptors induces microglial release of IL-1 β , which enhances pre-synaptic glutamate release through modulation of NMDA signalling on post-synaptic neurons. This is an important alternative mechanism of synaptic facilitation without the requirement of enhanced neuronal activity (**Clark et al. 2015**).

There is also literature detailing the links between microglia and NMDARE antibodies. Clinical evidence and autopsy data indicate that NMDARE patients in the acute phase of illness experience infiltration of immune cells, with recent work showing elevated blood and CSF biomarkers of microglial activation in NMDARE patients (**Chang et al. 2023**) and marked microglia activation and microgliosis seen in NMDARE patient post-mortem examinations (**Dalmau et al. 2007, Tüzün et al. 2009, Camdessanché et al. 2011**). Maternal transfer studies of NMDARE antibodies and other antibodies such as CASPR2 have also begun to unpick their role in autoimmune encephalitis. Embryonic brains from an NMDAR gestational transfer model indicated microglial activation alongside decreased NMDAR clusters and synaptic density, and behavioural deficits (**García-Serra et al. 2020**), whilst a gestational transfer model of CASPR2 antibodies showed long-term microglial activation and concurrent reduced synapses and behavioural impairments (**Coutinho et al. 2017b**). It has also been shown very recently *in vitro* that microglia proactively remove NMDARs bound by NR1-IgG antibodies, as well as their associated post-synaptic proteins, thereby contributing to the aetiology of NMDARE, though mechanisms by which antibody-receptor complexes induce microglial activation remain unexplored (**Rahman et al. 2023**).

*

1.5 Thesis Aims

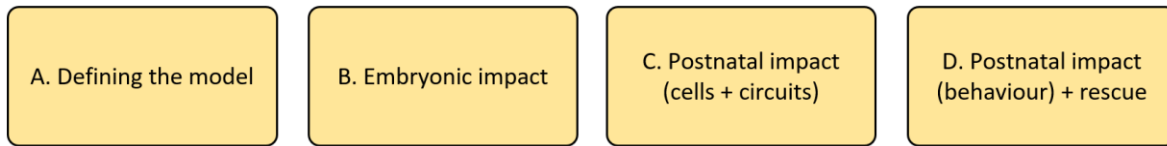


Figure 1.12. Summary of thesis aims.

The aim of this DPhil was to explore the impact of patient-derived antibodies against the NR1 subunit of the NMDAR on the development of the striatum in a gestational mouse model. **Experimental Chapter A** aimed to establish and characterise a novel *in utero* intraventricular model of patient-derived NR1-IgG antibody delivery. This involved delivering polyclonal and monoclonal NMDARE patient-derived NR1-IgG or healthy control-IgG antibodies intraventricularly *in utero* at E14.5. Within the model definition, the main sub-questions to be investigated included quantifying expression of the NR1 subunit target (and other glutamatergic subunits) in the wild-type brain and studying the dynamics of the antibodies within the model context such as quantifying antibody binding to the NR1 subunit *in vitro* and *in vivo*, and investigating antibody distribution and removal in the LGE. The purpose and benefit of establishing such a model was that it enabled effects to subsequently be explored using only a very small quantity of antibody (~0.5µl/mouse) and also allowed for simple and direct effects to be studied in an early developmental context but with fewer maternal factors at play.

Experimental Chapter B aimed to use the *in utero* model to explore how brief exposure to NR1-IgG patient-derived antibodies during the embryonic neurogenic period affects neurogenesis and striatal formation embryonically. This involved injection of the antibodies at E14.5 and collection of tissue at E16.5. This chapter aimed to quantify the embryonic impact on parameters including gross brain morphology such as brain weight, primordial striatum size, and width of proliferative zones; progenitor proliferation including proliferating cell numbers, cell distribution and impacts on specific populations;

cell migration and death; and microglial parameters including density, location and morphology. Experiments utilised a variety of immunohistochemical methods to accomplish this. Additionally, it aimed to compare multiple NR1-IgG antibodies via the most robust parameter.

Experimental Chapter C aimed to explore how brief *in utero* exposure to NR1-IgG patient-derived antibodies during the neurogenic period affects striatal cellular and circuit development postnatally. This involved injecting the antibody at E14.5 but studying animals during early postnatal phases, including electrophysiology experiments to investigate intrinsic properties, corticostriatal connections and effects on the NMDA/AMPA ratio; and immunohistochemical and volumetric study of gross and cellular morphology and cell composition including volumetric analysis of the striatum, the D1/D2 ratio and cellular reconstructions; as well as microglial density and morphology.

Finally, **Experimental Chapter D** aimed to explore how brief exposure to NR1 patient-derived antibodies affected (striatal-related) behaviour postnatally, including early reflex behaviours such as righting reflex, cliff avoidance and negative geotaxis, and more complex behaviours during early adolescence including the open-field test and the accelerating rotarod. It also aimed to explore whether the early phenotype observed following *in utero* exposure to NR1-IgG antibodies might be able to be ‘rescued’ through boosting glutamatergic signalling via AMPARs by injecting AMPAkine CX516 over the first postnatal week, utilising the protocol in **Braz et al. (2022)** established for a Huntington’s disease model with similar phenotypic deficits.

2. General Methods

To explore the thesis aims, a novel *in utero* intraventricular model of NR1-IgG antibody delivery was established. For ease of reference, the methods below follow the general thesis structure. Relevant methods are also summarised within each **Experimental Chapter** and this chapter acts as a fully detailed reference of those methods.

2.1 Mouse model background

This DPhil project used mice as a model organism, whereby the embryos of pregnant dams were intraventricularly injected at embryonic day (E)14.5 with patient-derived NR1-IgG antibodies (or matched human control-IgG antibodies).

2.1.1 Mouse model background and ethics

All experiments were performed on C57Bl/6 mice (Charles River), housed in individually ventilated cages with a 12:12h light-dark cycle at 22°C ($\pm 1^\circ\text{C}$), and *ad libitum* access to food and water. The research in this project was conducted in the UK and Belgium. In the UK, animal care and procedures were performed according to the UK Animals (Scientific Procedures) Act 1986, with prior approval from the local University of Oxford Ethical Review committee, and the project was carried out under PPL P62F223E9 and PIL I65599636. In Belgium, animal care and procedures were performed according to the Declaration of Helsinki guidelines with approval by the University of Antwerp Ethical Committee for Animal Experimentation, and the project was carried out under licence ECD 20/2026 and FELASA certificate 030/10/2020-100.

2.1.2 Generation of pregnant dams with dated plugs

The experimental model in this study used pregnant dams with dated plugs to ensure antibodies were delivered at the desired point in the pregnancy. To produce each accurately dated pregnant dam, a single

female mouse was placed in a cage with a male mouse on Monday morning each week. From Tuesday onwards, the female was checked at the same time daily for the presence of a vaginal plug, and midday of the day a plug was found was considered as E0.5. Once a plug was found, the male was removed from the cage and returned to his home cage. Females were also 'whittened' on the Friday before pairing by adding a small amount of male bedding, to increase the chances of becoming pregnant (**Whitten 1956**). If no plug was present by the Friday plug check, the male was returned to his home cage anyway, and the female monitored for ~21 days to ensure a pregnancy had not been missed.

2.2 Patient-derived antibodies

2.2.1 Background information of patient-derived antibodies

This study set out to explore the effects of NR1-IgG antibodies on the developing mouse striatum. To do this, experiments used NR1-IgG and matched control-IgG antibodies derived from NMDAR antibody-mediated encephalitis (NMDARE) patients and healthy controls respectively. Two main NR1-IgG antibodies and their matched control-IgG antibodies were used. One was a polyclonal NR1-IgG antibody ('poly NR1') with a polyclonal matched control-IgG antibody ('poly control'), whilst the other was a monoclonal NR1-IgG antibody ('mono NR1') with a monoclonal matched control-IgG antibody ('mono control'). All these antibodies have been used in previous studies and all NR1-IgG antibodies have been demonstrated to have pathogenic effects (**Kreye et al. 2016, Malviya et al. 2017, Wright et al. 2021**). Antibodies were kindly donated by Dr Sukhvir Wright (Aston Institute of Health and Neurodevelopment, Birmingham) and Prof. Dr Norbert Goebels (Department of Neurology, Düsseldorf) and arrived pre-prepared ready for experimental use. Methods detailing derivation of polyclonal antibodies are found in **Wright et al. (2021)**. Methods detailing derivation of monoclonal antibodies are found in **Malviya et al. (2017)**. Briefly, in general terms, polyclonal antibodies are made through purification using Protein G beads, capturing multiple epitopes. Conversely, monoclonal antibodies are made through cloning a single epitope and are therefore far more target-specific. The only additional step performed was dilution of antibodies where necessary with sterile PBS to ensure NR1-IgG and control-IgG were matched in terms of concentration.

Table 2.1 below summarises the details of the polyclonal and monoclonal NR1-IgG and control-IgG antibodies used:

Antibody name	Type	Derived from	Initial conc. provided	~ Conc. injected	~ Dilution in embryo	Reference
Poly NR1	Polyclonal NR1-IgG	11y F NMDAR encephalitis patient	37mg/ml	30mg/ml	1:20	Wright et al. 2021
Poly control	Polyclonal control-IgG	35y M healthy control	30mg/ml	30mg/ml	1:20	Wright et al. 2021
Mono NR1	Monoclonal NR1-IgG (SSM5)	4 NMDAR encephalitis patients' clonally expanded CSF plasma cells	200µg/ml	140µg/ml	1:20	Malviya et al. 2017
Mono control	Monoclonal control-IgG (12D7)	Isotype healthy controls, irrelevant antigen specificity (NY-ESO-1)	139µg/ml	139µg/ml	1:20	Malviya et al. 2017

Table 2.1. Polyclonal and monoclonal patient-derived NR1-IgG and healthy control-IgG antibodies. Kindly donated by Dr Sukhvir Wright (Aston Institute of Health and Neurodevelopment, Birmingham) and Prof. Dr Norbert Goebels (Department of Neurology, Düsseldorf).

In **Experimental Chapter A**, the polyclonal and monoclonal antibodies were compared using both *in vitro* and *ex vivo* methods (**Figures 3.4, 3.5, 3.6**). It was demonstrated that these antibodies have similar effects, and in subsequent **Experimental Chapters B, C and D**, both antibodies were interchangeably used for injections at E14.5 to generate the study animals. The similarity of the effects was also further validated in **Experimental Chapter C**, through comparing the effects of each antibody on the striatal spiny projection neuron (SPN) intrinsic properties (**Tables 5.2, 5.3**).

Aside from these antibodies, one experiment in **Experimental Chapter B** compared the effects of four additional small polyclonal NR1-IgG samples and matched controls (**Figure 4.9**). These were derived from NMDARE patients at the John Radcliffe (JR) Hospital in Oxford by Prof. Sarosh Irani and colleagues using the same method as **Wright et al. (2021)**. **Table 2.2** below summarises these antibodies:

Antibody name	Type	Derived from	Initial conc. provided	~ Conc. injected	~ Dilution in embryo	Reference
JR040735	Polyclonal NR1-IgG	NMDARE patients	16.9mg/ml	16.9mg/ml	1:20	Unpublished
JR023732			16.8mg/ml	16.8mg/ml	1:20	
JR080007			13.4mg/ml	13.4mg/ml	1:20	
JR053741			25.3mg/ml	25.3mg/ml	1:20	
JR1553.1046	Polyclonal Control-IgG	Healthy controls	22.1mg/ml	22.1mg/ml	1:20	Unpublished
JR1552.1045			25.8mg/ml	25.8mg/ml	1:20	
JR1620.1026			14.1mg/ml	14.1mg/ml	1:20	
JR1542.1010			42.1mg/ml	42.1mg/ml	1:20	

Table 2.2. Additional polyclonal patient-derived NR1-IgG and healthy control-IgG antibodies used for a small subset of experiments to compare the effects of antibodies from different patients. Kindly donated by Prof. Sarosh Irani (Nuffield Department of Clinical Neurosciences, Oxford).

The estimate for the approximate dilution of the antibodies in the embryo was based on work by Zappaterra et al. (2013), who established the volume of CSF in an E14 mouse embryo is ~15µl.

2.2.2 Ethical approval for patient-derived antibodies

Antibodies were kindly donated by Dr Sukhvir Wright (polyclonal NR1-IgG and polyclonal control-IgG), Prof. Dr Norbert Goebels (monoclonal NR1-IgG and monoclonal control-IgG) and Prof. Sarosh Irani (all 'JR' polyclonal NR1-IgG and control-IgG antibodies). All participants gave written informed consent for collection and study of their antibodies. Ethical approval was given by the Oxfordshire Regional Ethical Committee A for antibodies provided by Dr Sukhvir Wright and Prof. Sarosh Irani, and by the local ethics committee of the Medical Faculty of the University of Münster, Germany for antibodies provided by Prof. Dr Norbert Goebels.

2.3 Transcript and protein quantification methods

2.3.1 RNA extraction

For transcript-level quantification of NR1 and other glutamatergic subunits at E16.5 (**Experimental Chapter A, Figure 3.2**), pregnant dams were deeply anaesthetised using isoflurane, and decapitated. Embryonic heads were collected and stored in an RNase-free tube containing ~1000µl of RNAlater (Invitrogen, #AM7020) at 4°C for one week. Brains were then transferred to an RNase-free petri dish containing sterile cutting aCSF containing (mM): 65 sucrose, 85 NaCl, 1.25 NaH₂PO₄, 2.5 KCl, 7 MgCl₂, 25 NaHCO₃, 10 glucose. Brains were carefully dissected under a microscope into the cortex and the LGE, paying close reference to the embryonic brain atlas (**Figure 2.3**).

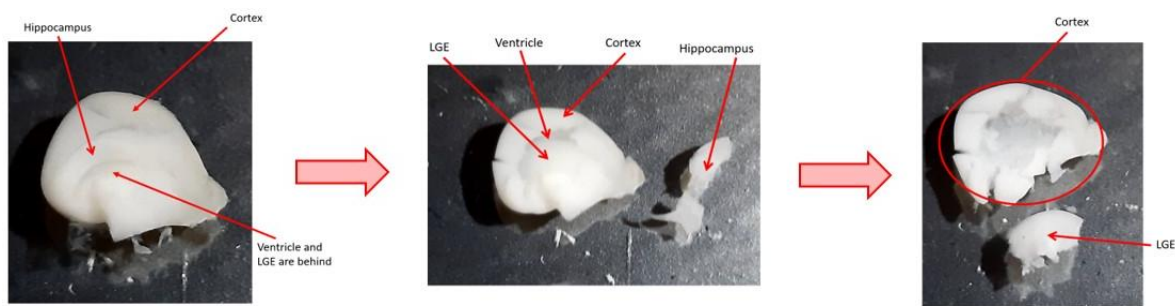


Figure 2.3. Steps to dissect the cortex from the LGE in embryonic E16.5 brains. Left: The brain is first cut along the midline, then, working with one half, central midline structures are carefully removed using sterile tweezers. Middle: This exposes the hippocampus, which is then gently peeled back and removed, exposing the LGE beneath. Right: The LGE can then be ‘scooped out’ using a small spatula, to leave just the cortex behind. Method and photo credit: **Dr Ricardo Márquez-Gómez, Ellender Lab, Oxford.**

All tools were cleaned with RNAaseZAP (Sigma Millipore, #R2020) and surfaces thoroughly wiped with RNase Wipes (Thermo Fisher, #AM9786) prior to dissection. mRNA was then extracted using a QIAGEN kit as per the manufacturer’s protocol: the dissected striatal and cortical samples were transferred to individual QIAshredder columns (QIAGEN, #7905A) on ice, which were filled with 307µl of PureLink lysis buffer (Invitrogen, #12183018A) and 4µl of 2-mercaptoethanol to dissociate the sample. The columns were subsequently centrifuged at 20,000 x g (Eppendorf Centrifuge 5424) before the addition of 1 volume

(310µl) of 70% ethanol and vortexing to get rid of any precipitate. The entire volume of each sample was then transferred to a PureLink spin cartridge and centrifuged for a further 30s at 13,000 x g. The samples were further washed several times using PureLink wash buffers I and II as per the manufacturer's protocol. Cartridges were then spun dry for 1 minute and placed in a recovery 1.5ml Eppendorf tube. 50µl of nuclease free water was added to each tube membrane and the samples incubated at room temperature for 1 minute before centrifuging at 20,000 x g for 2 minutes to elute the mRNA. Quality and concentration were assessed using a Nanodrop ND-1000 Spectrophotometer. The Nanodrop was re-calibrated between each sample. A 260/280 absorbance ratio of ~2.0 was considered pure, and a concentration of >30ng deemed a cut off for continuation to cDNA generation.

2.3.2 cDNA synthesis

cDNA was generated from 1µg mRNA using a SuperScript IV-VILO kit (Thermo Fisher Scientific, #11766050) as per the manufacturer's protocol. First, to perform genomic DNA digestion, 6µl of 10x ezDNase buffer, and 6µl of ezDNase enzyme were added to the remaining 48µl of each mRNA sample from above. Each sample was then incubated for 2 minutes at 37°C in a water bath before being placed on ice. The sample was split equally across six 50µl PCR tubes (i.e. 10µl per tube) before 4µl of either the reaction mix (SuperScript IV VILO Master Mix), or the control mix (SuperScript IV VILO Master Mix noRT) was added. Each tube was topped up with 6µl nuclease free water and placed in a PxE 0.2 thermocycler (Thermo Electron Corporation), before undergoing the following reaction: 1. 25°C for 10 minutes (anneal primers), 2. 50°C for 10 minutes (reverse transcription), 3. 85°C for 5 minutes (inactivate samples), 4. 4°C, infinity. Samples were stored at -20°C until use.

2.3.3. RT-qPCR

RT-qPCR was performed as previously described (**Han et al. 2020**): primers targeting the exon-intron region of the NMDAR subunits (NR1, 2A-D, 3A-B), AMPAR subunits (A1-4), as well as CTIP2 (primarily a

striatal marker), PAX6 (primarily a cortical marker), and β -actin (a house keeping gene) were custom-designed using the NCBI primer blast web server (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) and confirmed using Beacon Designer (PRIMER Biosoft International). CTIP2 and PAX6 were included to confirm accurate dissection, and β -actin to normalise total protein and check sample quality. Three primers were designed for each receptor, and they were first tested for specificity using postnatal cDNA, with the most specific single pair per receptor used for further experiments (**Table 2.4**). Postnatal cDNA was extracted from whole mouse brain cortical or striatal samples using the methods described under the **Sections 2.3.1 RNA extraction** and **2.3.2 cDNA synthesis** above.

mRNA	Sequence (5' to 3')	
	Forward primer	Reverse primer
NR1	GTGTCCCTGTCCATACTCAAGTCC	ATTGCTGCGGGAGTCACATTC
NR2A	ATGACTATTCTCCGCCTTTCCG	AAGCGTCCAACCTCCCAGTTTTC
NR2B	AATGAAGAACGGTCAGAGGTG	CACTGAATGGCTCTAAGAAGGCAG
NR2C	CTTAGTGACAAGAAGTTTCAGCGC	AGCGCTGGTTGAACTTGACC
NR2D	TGCTGCGAGACTATGGCTTC	GTGTCATCCACCGGCACTTC
A1	GGTCCGCCCTGAGAAATCC	TCAGAGCACTGGTCTTGTC
A2	GGGAAGTAAGGAAAAGACCAGT	GTGCATTCTTTGCCACCTTCA
A3	GGTGACTCCAAGGACAAGAC	TTTGGACTCTGCCCCTGATT
A4	AAGGCTATGGTGTAGCGACG	TCCTTGGGTCCACATTCACC
PAX6	GGAGCCTTGACAACGACGAA	CACTCCGCTGTGACTGTTCT
CTIP2	TACTGTCACCCACGAAAGGC	AAGGCTCATCTTTACACTGGCA
β-ACTIN	CCAGCCTTCCTTCTTGGGTATC	CTTTACGGATGTCAACGTCACAC

Table 2.4. Sequences of custom-designed primers for RT-qPCR reactions. Primer verification in Figure 2.6 and Supplementary Figure S2.1.

Before the assay, the cDNA samples were diluted to ~400 μ l using nuclease free water. Primers were also diluted with nuclease free water to yield a concentration of 100 μ M. A dilution of 1:2 was prepared per primer, for a final dilution of 50 μ M respectively. Reactions were set up on a 96-well plate and each reaction mix contained (μ l): 5 SYBR Green Master Mix (Thermo Fisher), 1 forward primer, 1 reverse primer, 1 nuclease free water, and 2 cDNA (~1ng). β -actin was the house-keeping gene, postnatal cDNA was the positive control and nuclease free water was the negative control. Each reaction was run as a triplicate.

Once reactions were set up, the 96-well plate was sealed and centrifuged at 1000 x g for 10 seconds. RT-qPCR was performed using a custom-written protocol (Dawn Shepherd, Platt Lab, Oxford) outlined in **Table 2.5** below:

Step	Temperature	Duration	Cycles
UDG activation	50°C	2 minutes	hold
Dual-Lock DNA polymerase	95°C	2 minutes	hold
Denature	95°C	15 seconds	40
Anneal/extend	60°C	30 seconds	

Table 2.5. ‘Dawn Powerup Protocol2’ protocol for RT-qPCR. Credit: Dawn Shepherd, Platt Lab, Oxford.

Results were detected with a CFX96 real-time PCR detection system (BioRad, #184-5384). Data was analysed using the ΔC_t method and normalised against respective β -actin values. Cut-off was 0.05 $1/\Delta C_t$, reflecting the minimum ΔC_t value within the window of β -actin ΔC_t values and the maximum 40x C_t amplification cycles. The threshold for accepting the amplification curve was set to 50 RFU.

2.3.4 Gel electrophoresis of amplification products

Amplification products from the RT-qPCR reaction were checked through running an agarose gel (**Figure 2.6**). To make the gel, 3g agarose powder (Invitrogen, #16500-500) was added to 150 μ l of 1x TAE in a bottle to make a 2% agarose solution. The solution was gently microwaved in 10 second intervals until the agarose powder had fully dissolved, being careful to not let the solution boil over. The bottle was removed from the microwave and 6 μ l ethidium bromide added. Once cool enough to handle, the solution was poured into the gel tray and left to set for 30 minutes. The tray was then filled with 1x TAE buffer, and the well comb gently removed. To prepare the samples for loading, two wells from each triplicate were combined to give ~20 μ l, and 4 μ l of loading dye was added to the sample. Samples were loaded into the gel wells and 2 μ l of DNA ruler loaded into the final well. The gel was sealed and run at 90V for ~30-60

minutes (until the purple ladder band was halfway). Once finished, the gel was imaged under UV light using a Gel Doc.

2.3.5 Western blot

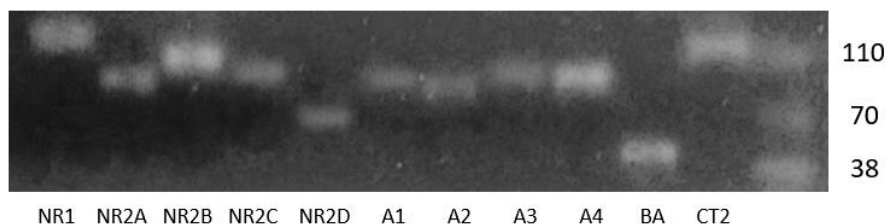


Figure 2.6. PCR gel of custom-designed primers chosen for experiments, tested on postnatal adult mouse whole brain cDNA as a positive control. Tested primers included NMDAR NR1, NR2A-D, AMPAR A1-4, β -actin (BA) and CTIP2 (CT2). The single bands in the expected locations verify these primers work. N.B. NR3A-B and PAX6 were not tested on PCR but verified using methods below (**Supplementary Figure S2.1**).

For protein-level quantification of NR1 in the embryonic LGE (**Experimental Chapter A, Figure 3.3**), E16.5 brains were collected and dissected in the same way as those for cDNA synthesis but in sterile cutting aCSF and processed on collection day.

Dissected tissue was homogenised in RIPA buffer (Sigma Aldrich, #R0278) and protease inhibitor (Thermo Fisher Scientific, #87786). Samples were centrifuged at 5000 x g for 20 minutes, the supernatant collected, and protein concentration quantified using the colorimetric Bicinchoninic acid (BCA) assay. Protein standards were first prepared as per manufacturer's instructions by performing serial dilutions of the Albumin (BSA) Standard (2.0mg/ml). Standards made ($\mu\text{g/ml}$): 200, 40, 20, 10, 5, 2.5, 1, 0.5, 0. Working reagent was then prepared by mixing Micro BCA Reagents MA, MB and MC in a 25:24:1 ratio. To produce the standard curve (**Figure 2.7 A**), 150 μl of each standard was pipetted into a 96-well microplate as a duplicate, and 150 μl WR added to each well. The plate was sealed and incubated at 37°C for 2 hours. The unknown samples were also added to the same plate. Absorbance was then measured at 562nm on a Microplate reader. The average absorbance of the blank standard (0 $\mu\text{g/ml}$) was subtracted from the absorbance of all the other standards and unknowns, and a standard curve produced by plotting the

average blank-corrected reading for each standard vs its concentration. The standard curve was then used to determine the protein concentration of each unknown sample.

Initial suitable concentration of protein to use to get a clear NR1 band was determined by doing a serial dilution Western blot (diluted 1:10 in every lane). The optimal concentration (lane C) was ~200ng/μl. (**Figure 2.7 B**). Samples were stored in Laemmli buffer at -20°C until use.

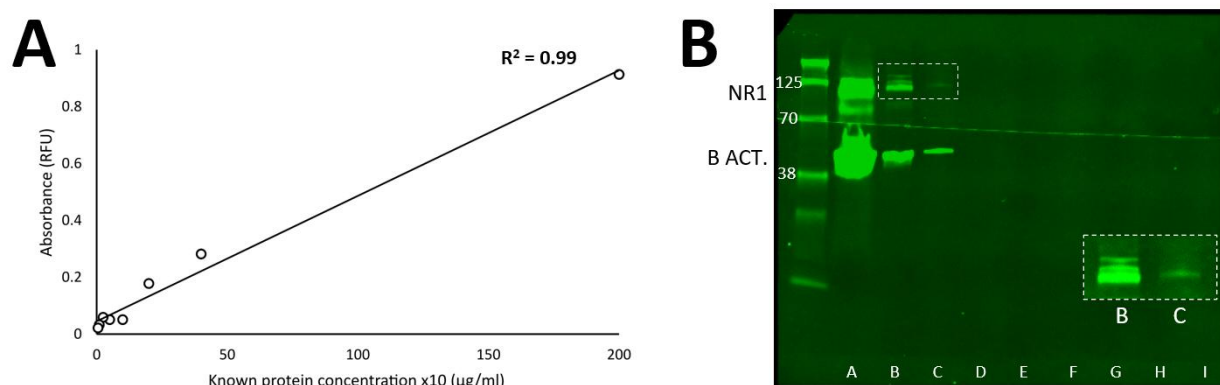


Figure 2.7. Western blot serial protein dilutions and representative standard curve. [A] Representative standard curve of protein concentration against absorbance, using BCA kit standard known protein concentrations. $R^2 = 0.99$. **[B]** Western blot of serial dilutions of protein extracted from a postnatal (p)8 whole brain. A is undiluted protein, and each subsequent lane is diluted 1:10 from the previous lane. The NR1 band (~120kDa) is clearly visible in lanes A-C. Lane C showed a single, clear band for NR1 (inset), and β -actin (~45kDa), suggesting this concentration was close to optimal to use for further experiments. BCA quantification of the protein concentration used in this lane revealed the concentration was 0.17ng/μl, so a concentration of ~0.2ng/μl was used in subsequent experiments.

Blots were performed by loading 3μl of Chameleon Duo protein ladder (LiCor, #928-60000) into the first well of a pre-cast gel, and the samples into the succeeding wells, before running the gel at 120V for 90 minutes. Following this, the blot was transferred to a PVDF membrane. To perform the transfer, filters, sponges and low fluorescence immunoblot PVDF membranes were pre-soaked in Tris Glycine transfer buffer and methanol, before being loaded in a 'sandwich' formation into the transfer cast with the gel and membrane in the middle and running at 150V for 30 minutes. Success of transfer was confirmed by staining with Ponceau S (0.1%) (Biotium, #22001) as per manufacturer's instructions.

The membrane was blocked in TBS LiCor buffer (LiCor, #927-60001) at 4°C overnight and cut horizontally along the ~70kDa band. The top section (bands >70kDa) was incubated in NR1 primary antibody (expected band ~120kDa; 1:1000, Abcam, ab109182, rabbit) and the bottom section (bands <70kDa) in β -actin primary antibody (expected band ~45kDa; 1:1000, LiCor, 926-42210, rabbit). Membranes were washed several times in TBST and incubated with secondary antibody (IRDye® 800CW Donkey anti-rabbit 926-32213, 1:20,000) at room temperature for 1 hour. Membranes were imaged on a LiCor Odyssey 9120 System and analysed in Image Studio Lite (Licor Biosciences). Each band was quantified as a percentage of the β -actin band intensity, whereby the intensity of the β -actin band represented 100%.

2.4 Cell-based assay methods

2.4.1 NMDAR antibody cell-based assay

The cell-based assay experiment to determine affinity and specificity of the polyclonal and monoclonal NR1-IgG and control-IgG antibodies (**Experimental Chapter A, Figure 3.4**) was kindly performed with the help of Dr Khadija Guerti, Karin Van Cotthem and colleagues in the UZA Hospital Lab, Antwerp. Each NR1-IgG antibody was serially diluted to 1:20, 1:500 and 1:12,500 and tested on commercially available EUROIMMUN Anti-Glutamate Receptor (type NMDA) mosaic-6 IIFT kit immunofluorescence chips (comprising HEK293 cells expressing NR1) (fb 112d-1005-6, EUROIMMUN). The control-IgG antibodies were tested at 1:20 only. Dilutions were chosen based on existing literature (**Zappaterra et al. 2013**) and personal correspondence with collaborators.

The assay was performed as per the manufacturer's TITERPLANE protocol (**Figure 2.8**). In brief, 30µl of each sample was applied to the reaction field of each tray and the BIOCHIP slide placed on top, ensuring each sample contacted each BIOCHIP. Samples were incubated at room temperature for 30 minutes, before the BIOCHIP was then flushed and immersed in a dish of PBS-Tween for 5 minutes on a shaker. 25µl of conjugate was pipetted into the reaction fields of a new reagent tray. The BIOCHIP was removed from the dish and blotted gently before being placed on the new reagent tray so the conjugate contacted each BIOCHIP. This was incubated at room temperature for a further 30 minutes and flushed and immersed in a dish of PBS-Tween as above. 10µl mounting medium per reaction field was pipetted onto a cover glass and the BIOCHIP then blotted gently and mounted face downwards. Each sample was imaged at 20x and 40x magnification and the fluorescent binding classified on a 0-4 scale, adapted from existing literature (**Leite et al. 2008, Waters et al. 2008**); 0 – no labelling; 0.5 - weak labelling of few (<20%) transfected cells, with no co-localisation; 1 – weak labelling of some (20-50%) transfected cells, with some co-localisation; 2 – moderate labelling of some (20-50%) transfected cells, with good co-localisation; 3 –

moderate/strong labelling of many (50-80%) transfected cells, with precise co-localisation; 4 – strong labelling of most (>80%) transfected cells, with perfect co-localisation.

[Image redacted in ORA online thesis]

Figure 2.8. Graphical summary of the TITERPLANE protocol. Image credit: EUROIMMUN.

2.5 In utero surgical methods

In our model, surgery was conducted on pregnant dams in order to deliver antibodies, plasmids or other substances to the brains of E14.5 embryos *in utero*, with animals/tissue subsequently being used for a range of immunohistochemical, electrophysiological and/or behavioural studies.

2.5.1 Pre-operative procedures

2.5.1.1 Preparation of substances to be injected intraventricularly *in utero*

Substances injected intraventricularly included patient-derived antibodies, plasmids and FlashTag (all detailed in **Table 2.9** below). These were prepared prior to surgery as follows:

Antibodies: NR1-IgG or control-IgG was taken from the stock solutions we received from collaborators as described in **Section 2.2 Patient-derived antibodies** above. Surgeries either used polyclonal or monoclonal antibodies. Polyclonal antibody concentration was ~30mg/ml, and monoclonal antibody concentration was ~140µg/ml (**Table 2.2**).

Plasmids: Plasmids were prepared using a standard maxiprep and DNA precipitation protocol (Qiagen). Quality and concentration of DNA were assessed using a Nanodrop ND-1000 Spectrophotometer. A 260/280 ratio of between 1.8-2.0 was deemed pure and pure samples taken forward and further diluted with TE buffer to give a final stock concentration of 3.0µg/µl.

The plasmids prepared included:

- 'Tα1-Cre' - the Cre-recombinase gene is under control of part of the Tα1 promoter (**Stancik et al. 2010**)
- 'Cβa-FLEX' - the FLEX cassette is controlled by the chicken β-actin gene. Cre-recombination switches the expression of red TdTomato fluorescent protein to green fluorescent protein (**Franco et al. 2012**)

FlashTag: FlashTag (FT) was used to pulse-label mitotic progenitors at the ventricular wall (Govindan and Jabaudon 2018). FT working solution was prepared by dissolving one vial of CellTrace™ carboxyfluorescein succinimidyl ester (CFSE) (CellTrace CFSE Cell Proliferation Kit, #C34554, Thermo Fisher Scientific) in 8µl DMSO and 1µl Fast Green (0.03%) (Sigma-Aldrich, #F7258), for a final concentration of 10mM. The working solution was stored at -20°C until use.

2.5.1.2 Summary of types of substances injected via *in utero* surgery

Substance(s) injected	Surgery type	Specifics	Ratio	Mixture in surgery pipette
Antibodies only*	Laparotomy	NR1-IgG (poly/mono) Control-IgG (poly/mono)	n/a	9µl IgG (+ 0.5µl FG)
Antibodies + FlashTag (FT)	Laparotomy	NR1-IgG + FT Control-IgG + FT	4:1	4µl IgG + 1µl FT
Plasmids only	IUE	Cβa-FLEx + Tα1-Cre	1:1	4µl plasmid 1 + 4µl plasmid 2 (+ 0.5µl FG)
Plasmids + antibodies	IUE	NR1/control-IgG + Cβa-FLEx + Tα1-Cre	2:1:1	4µl IgG + 2µl plasmid 1 + 2µl plasmid 2 (+ 0.5µl FG)

Table 2.9. Different substances injected in utero. *For embryonic experiments involving antibody injections (Experimental Chapters A and B), embryos on one side of the uterine horn were injected with NR1-IgG and embryos on the other side were injected with the matched control-IgG. For postnatal experiments (Experimental Chapters C and D), the brains of all embryos were injected with either NR1-IgG or control-IgG antibody. FG = Fast-Green (0.03%) dye.

2.5.1.3 Preparing post-operative medicated jelly

At the start of each week, medicated Vetergesic (buprenorphine hydrochloride, 0.1mg/kg, Alstoe Animal Health) and Metacam (meloxicam, 5mg/kg, Boehringer Ingelheim Vetmedica GmbH) jellies were also made to be given to the mice post-operatively. To make the jelly, 1 cube of Hartley's jelly was dissolved in 23.75ml of boiling water, placed on a hot plate with a stirrer. Once dissolved, 47.5ml of cold water was added and the mixture stirred until smooth. 2ml of jelly was then transferred into individual weighing boats using a sterile syringe and labelled with the drug to be added ('Vet' for Vetergesic, 'Met' for

Metacam). Drug dosages were calculated for a 30g pregnant mouse (the approximate weight of a mid-gestational dam). To make Vet jelly, 0.03ml of Vetergesic was added to 2ml jelly weighing boats, to give a final dose of 0.3mg/kg. To make Met jelly, 0.06ml of Metacam was added to 2ml jelly weighing boats, to give a final dose of 1mg/kg. The boats were then transferred to the fridge to cool at 4°C for at least 2 hours.

2.5.1.4 Pre-surgery animal checks

Animals were checked one day prior to surgery to ensure pregnancy, by lightly drawing back the tail and observing for 'bumps' around the abdomen. On the day of surgery, the relevant animal management software (MCMS or Anibio) was checked to ensure that the animal had not been used for previous procedures and the fridge was also checked to ensure the correct medicated jellies had been made.

2.5.1.5 Pre-operative surgical set-up

Surgery was performed in a designated surgery room under a surgical hood, sterilised with 1% Anistel. Autoclave-sterilised surgical tools and PBS were used for every surgery. PBS was pre-warmed prior to surgery in a water bath. A heating mat was also placed under the surgical hood and pre-warmed to 40°C. Glass micropipettes were pulled using a Flaming/Brown Micropipette puller (Sutter Instruments) and filled prior to the surgery with the substances to be injected (**Figure 2.7**). A small volume of Fast Green (0.03%) dye (typically 0.5µl) was added to substances to be injected intraventricularly to allow identification of successful injections.

Two injectable analgesics, Vetergesic (buprenorphine hydrochloride, 0.1mg/kg, Alstoe Animal Health) and Metacam (meloxicam, 5mg/kg, Boehringer Ingelheim Vetmedica GmbH) were also prepared prior to surgery by dilution with sterile PBS.

2.5.2 Intra-operative procedures

2.5.2.1 Laparotomy and *in utero* intraventricular injections

Following surgical set up, a pregnant E14.5 dam was deeply anaesthetised using gaseous isoflurane (2.5-4%, Zoetis). Anaesthetic depth was checked using the tail pinch and the pedal withdrawal reflexes and once adequate, the dam was transferred to the anaesthetic mask in the surgical hood and placed face down on the heating mat. Anaesthesia was adjusted as necessary to maintain depth and respiration rate closely monitored and maintained around 1 breath/second throughout surgery. Once anaesthetised and positioned in the surgical field, the dam was given intra-operative analgesic injections of Vetergesic (0.1mg/kg) and Metacam (1mg/kg) subcutaneously. Viscotears (Bausch and Lomb) was also applied topically to both eyes to prevent drying out. The dam was then placed on its back and the fur covering the abdomen shaved to expose the underlying skin. The surgical site was cleaned using surgical swabs and diluted Hibiscrub and draped. An ~1-2cm long incision was made through the skin and muscle layers of the abdomen, and the uterine horns exposed. Embryos were counted and then working from the left side of the uterus (as viewed from above), each embryo was immobilised using organ holding forceps and one brain ventricle was injected with the relevant substance (**Table 2.9**) using a custom-pulled glass micropipette via mouth pipetting. The total volume injected per embryo was ~0.5-1µl and successful injection was determined by a green (Fast Green (0.03%) dye) colouration observed inside the ventricle. Typically, 100% of the pups were injected. Sham surgical control experiments were also performed where the embryos were exposed but not injected. Post-injection, embryos were carefully replaced, the abdomen filled with warmed PBS and the muscle and skin incisions stitched using vicryl and proline sutures respectively.

2.5.2.2 *In utero* electroporation (IUE)

For IUE of plasmid DNA, surgery was performed in the same way as above, but with an additional electroporation step. After intraventricular injection of the plasmids (**Table 2.9**), a Tweezertrode electrode

(Sonidel) was positioned with the cathode slightly above the primordial ear and the anode close to the opposite cheek (**Baumgart and Baumgart 2016**). Five pulses of 50 millisecond duration, separated by 200-950 milliseconds, at 55 volts were given using a BTX ECM 830 pulse generator (Genetronics). Normally 80% of the pups were injected in each litter. After delivery of the pulses, the embryos were carefully placed back, and the dam sutured and recovered as described above. IUE plasmid labelling was used to label two specific progenitor populations in the LGE ('apical intermediate progenitors' (aiPs) and 'other progenitors' (OPs)) through injecting C β a-FLEX + T α 1-Cre plasmids (**Table 2.9**) alongside the antibodies, since Cre-recombination switches expression of red TdTomato fluorescent protein to green fluorescent protein and therefore labels T α 1+ cells green (termed 'aiPs' as outlined in **Experimental Chapter B**), and leaves a heterogeneous population of T α 1- cells red (a heterogeneous population termed 'OPs').

2.5.3 Post-operative procedures

2.5.3.1 Post-operative care

Post-operatively, dams were placed in a 38°C recovery box and observed closely until fully awake before transfer back to their home cages. Following surgery, dams were checked daily, and the surgical wound inspected closely to ensure stitch integrity and healing. Dams were given a wound score (1-5) and a general health score (1-4) at each check. The first post-operative check was done in the evening of the day of surgery (Day 0). Dams were also given a regime of medicated jelly during the first three days of post-operative checks (Vet jelly on Day 0 and Met jelly on Days 1 and 2).

2.6 Antibody immunohistochemistry methods

2.6.1 Fixation and sectioning of brain tissue

Brain tissue from both wild-type animals, and animals injected with NR1-IgG or control-IgG embryonically, was fixed and sliced for several experiments throughout the thesis as follows:

Embryonic: Pregnant dams were deeply anaesthetised on the relevant embryonic day (E16.5 unless otherwise stated) using isoflurane, and decapitated. Embryonic heads were collected and fixed in 4% PFA solution for ~3 days. Brains were then carefully removed from the skulls and embedded in 5% agarose gel. To make the agar gel, 2.5g agarose powder was combined with 50ml of ddH₂O and heated at 150°C on a hot plate with a slow magnetic stirrer until the agarose powder dissolved. For embedding, brains were placed on a cold metal plate on ice and the gel gently pipetted over them and then left until cooled. This process was repeated on the underside of the brains so that they were fully encased (**Figure 2.10**). The agar gel containing the brains was then cut to form a cube and glued to the plate of the vibratome (Microm HM650V). The vibratome chamber was filled with fresh PBS and brains were coronally sectioned at 60µm and collected using paintbrushes across two to four wells. Two or three brains were sectioned at a time.

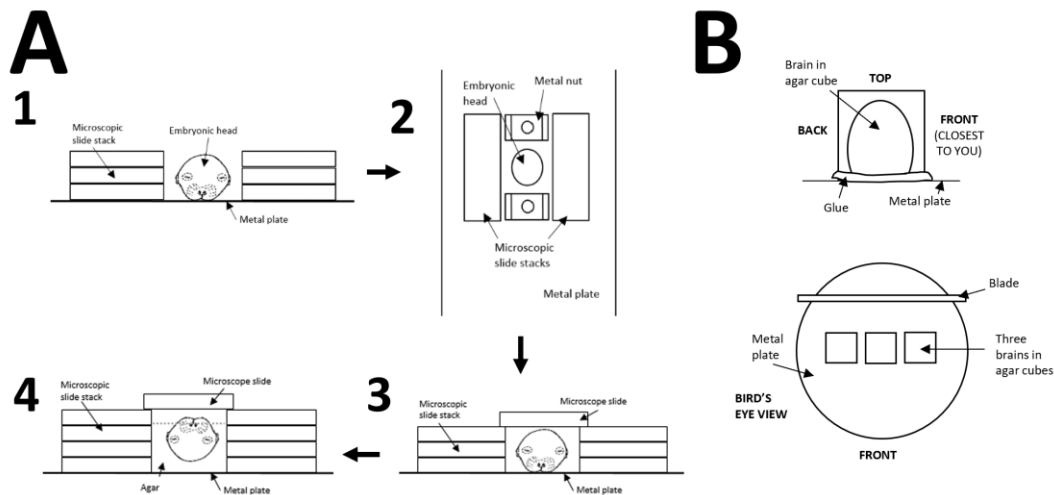


Figure 2.10. Agar embedding embryonic brains. [A] Graphical summary of the agar embedding method for embryonic brains. [B] Diagram showing the orientation of the brain glued on the plate for coronal slicing, with the olfactory bulbs pointing upwards.

Postnatal: Postnatal animals were deeply anaesthetised and decapitated. Brains were extracted and washed carefully in fresh PBS, before being transferred to 4% PFA and fixed for ~5 days. They were then transferred to a Petri dish containing PBS, and the cerebellum removed with a blade to leave a flat surface. Two or three brains were sliced at a time. Brains were then glued to the vibratome plate by the flat surface (i.e. olfactory bulbs pointing towards the ceiling) and sectioned, collected and stored in the same way as the embryonic tissue. Brains were stored at 4°C until use.

2.6.2 Experiments using fixed slices

2.6.2.1 Fluorescent labelling of cells using IUE of plasmid DNA

In some experiments, specific cells or populations were fluorescently labelled using IUE of plasmid DNA, either in wild-type animals, or animals also injected with NR1-IgG or control-IgG. Following labelling, this tissue was fixed and sliced as above, and labelled cells could then be visualised under the microscope. Slices were mounted in Vectashield (Vector Laboratories, #H-1000). Slices were imaged using an Olympus Fluorescence Microscope (model BX40) with a Hamamatsu ORCA-ER camera and HCLImage Live Software

(or an SM 710 confocal microscope with ZEN software (Zeiss) for published IUE data in **Experimental Chapter B, Figure 4.5**) and then analysed in *Image J* (Fiji). In terms of analysis for specific experiments:

To quantify **two specific progenitor populations**, (aIPs and OPs) within the LGE, brains underwent IUE with C β a-FLEX + T α 1-Cre plasmids (**Experimental Chapter B, Figure 4.5**) or IUE of these plasmids injected alongside the NR1-IgG or control-IgG antibodies (**Experimental Chapter B, Figure 4.6**). Specific details of the mechanisms of these plasmids are found under **Section 2.5.2.2 *In utero* electroporation (IUE)** above, but in summary they worked to label a T α 1+ population of cells green ('aIPs') and a T α 1- (heterogeneous) population cells red ('OPs'). Occasionally GFP or RFP primary antibodies were used to boost the signal from the surgical labelling of the aIP and OP populations respectively in the fixed slices. This was done as outlined in **Section 2.6.2.2 Fluorescent antibody staining** below.

To quantify aIPs and OPs in the LGE at E12.5 and 15.5 (**Experimental Chapter B, Figure 4.5 B, 4.6 B**), 40 μ m z-stacks were taken, with an interval of 1 μ m and the **numbers of green (aIP) and red (OP) cells** manually counted offline in a defined region of the LGE. Positive cells were deemed to be those with a fluorescence >2SD above the background.

To determine **cell cycle kinetics of aIPs and OPs** (**Experimental Chapter B, Figure 4.5 G**), brains were electroporated at E15.5 followed by varying time-delays to the point of fixation and then stained with PH3+ primary antibody to label dividing mitotic progenitors amongst the aIP and OP populations. aIP and OP cells co-labelled with PH3 were imaged and counted in the same way as for counting total numbers of aIP and OP cells described in the paragraph above.

The **location of aIP and OP cells** (**Experimental Chapter B, Figure 4.5 F**) in the LGE was determined using the x and y coordinates of labelled cells relative to the ventricular edge, with the same method as used for analysing location of PH3+ cells in **Section 2.6.2.3 Analysis of fluorescent antibody staining** below.

Counting of the basal processes of aIP and OP cells (Experimental Chapter B, Figure 4.5 E) was done in 40µm z-stacks, with 1µm intervals.

To analyse **cell migration (Experimental Chapter B, Figure 4.7)**, FlashTag, which labels mitotic cells at the ventricular wall, was co-injected alongside NR1-IgG or control-IgG antibodies at E14.5. Fixed slices were made at E16.5 and cell migration quantified through measuring the width of the FlashTag band in the LGE at five evenly spaced intervals and taking a mean in images taken at 20x magnification.

2.6.2.2 Fluorescent antibody staining

Several experiments throughout the thesis also involved fluorescent antibody immunohistochemistry staining on fixed embryonic or postnatal mouse brain slices, either wild-type, or surgically injected *in utero* with NR1-IgG or control-IgG. Details of specific antibodies and experiments are given in **Table 2.12** below. The general protocol was as follows: fixed coronal sections containing the LGE/striatum were selected and placed in a fresh 24-well plate in fresh PBS. Typically, 3-6 sections were selected per brain. For embryonic experiments, sections were classified prior to staining as rostral, medial or caudal using the Prenatal Mouse Brain Atlas (**Schambra 2008**) rostral (CORONAL 4-6), medial (CORONAL 8-10) and caudal (CORONAL 12-14) (**Figure 2.11**). For postnatal experiments, only medial sections were used.

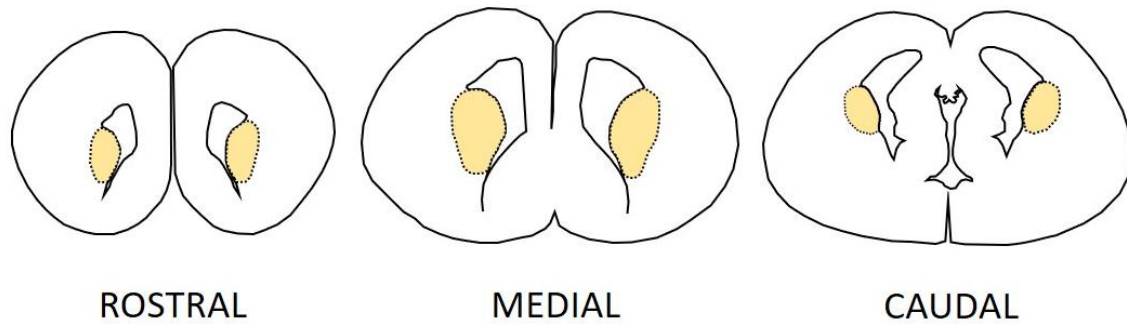


Figure 2.11. E16.5 rostral, medial and caudal slices. Reference diagrams of the morphology of a typical rostral, medial, and caudal slice at E16.5, with the LGE coloured in orange, traced from the Prenatal Mouse Brain Atlas (**Schambra 2008**) - rostral (CORONAL 4-6), medial (CORONAL 8-10) and caudal (CORONAL 12-14).

For certain antibodies (**Table 2.12**), staining was facilitated through antigen retrieval by first incubating sections in 10mM sodium citrate buffer (pH 6.0) in an 80°C water bath for 40 minutes. Slices underwent three 5-minute washes in PBS-Tx (0.1% for embryonic, 0.3% for postnatal) on a 60 RPM shaker and were then incubated in PBS-Tx with 10% normal donkey serum (NDS; Vector Laboratories, #017-000-121) for 2 hours at room temperature. Primary antibody solution, which consisted of the primary antibodies diluted in PBS-Tx, along with 1% NDS, (**Table 2.13**) were then added and plates left to incubate overnight at 4°C on gentle rotation. Slices were then washed a further three times in PBS, before secondary antibodies, diluted 1:500 in PBS only (**Table 2.14**), were added and plates left to incubate at room temperature for 2 hours. Sections were transferred onto labelled glass slides with a paint brush and mounted in Vectashield (Vector Laboratories, #H-1000).

Stage	Type	AR	1° antibodies	2° antibodies (1:500)	Experiment	Ex. Ch.
E	Untx	No	NR1-IgG (human) 1:50 Control-IgG (human) 1:50	Goat-α-human 488	<i>In vitro</i> binding of NR1-IgG antibodies	A
E	Ab inj.	No	None (as they were injected)	Goat-α-human 488	<i>Ex vivo</i> binding of NR1-IgG antibodies	A
E	Ab inj.	No	VGLUT1 (rabbit) 1:500	Goat-α-rabbit 555	Embryonic expression of VGLUT1	A
E	Ab inj.	No	VGLUT2 (mouse) 1:500	Goat-α-mouse 555	Embryonic expression of VGLUT2	A
E	Ab inj.	Yes	CTIP2 (rat) 1:1000 PH3 (rabbit) 1:500	Goat-α-rat 647 Goat-α-rabbit 555	Delineating primordial striatum Expression of LGE proliferating cells	B
E	Ab inj.	No	GFP (chicken) 1:1000 RFP (rat) 1:1000	Goat-α-chicken 488 Goat-α-rat 555	Boosting fluorescence of aIP/OP IUE progenitors	B
E/p	Ab inj.	No	IBA1 (rabbit) 1:500	Goat-α-rabbit 555	Staining embryonic / postnatal microglia	B/C
p	Ab inj.	Yes	CTIP2 (rat) 1:1000 PPE (rabbit) 1:500	Goat-α-rat 647 Goat-α-rabbit 555	Staining all SPNs Staining D2-SPNs	C

Table 2.12. Table of all immunohistochemistry experiments performed. Abbreviations: E = embryonic, p = postnatal; Untx = untreated ie. animals have not undergone any surgery, Ab inj. = animals have been injected embryonically with NR1-IgG or control-IgG antibodies; AR = antigen retrieval ie. whether slices underwent boiling in citrate buffer to facilitate antigen retrieval prior to primary antibody staining; Ex. Ch. = Experimental chapter the specific experiment(s) is found in.

Primary antibody		Species	Supplier	Cat. #	Role
VGLUT1	Vesicular glutamate transporter 1	Guinea pig	Millipore	ab5905	Labels VLUT-1, responsible for uploading glutamate into synaptic vesicles (Vigneault et al. 2015)
VGLUT2	Vesicular glutamate transporter 2	Mouse	Millipore	MAB5504	Labels VLUT-2, responsible for uploading glutamate into synaptic vesicles (Pietrancosta et al. 2020)
NMDAR1	N-methyl-D-aspartate receptor subunit 1	Rabbit	Abcam	ab109182	Labels NR1, the primary subunit of the NMDAR (Tripathi et al. 2024)
CTIP2	COUP TF1-interacting protein 2	Rat	Abcam	ab14865	Labels SPNs, the major cell type of the striatum (~95%) (Arlotta et al. 2008)
PH3	Phospho-histone 3	Rabbit	Millipore	06-570	Mitotic marker expressed by neural progenitors (Taupin 2007)
GFP	Green fluorescent protein	Chicken	Ave Labs	GFP-1020	Boosts GFP labelling of aIPs following IUE of $\text{T}\alpha\text{1-Cre}$ + FLEX (Van Heusden, Macey-Dare et al. 2021)
RFP	Red fluorescent protein	Rat	Chromotek	5f8-100	Boosts RFP labelling of OPs following IUE of FLEX (Van Heusden, Macey-Dare et al. 2021)
IBA1	Ionised calcium-binding adaptor molecule 1	Rabbit	Fujifilm	019-19741	Labels activated microglia (Sasaki et al. 2001, Butovsky et al. 2014)
PPE	Pre-proenkephalin	Rabbit	LifeSpan Biosciences	LS-C23084	Labels D2+ SPNs, one of two major populations of SPNs (Lee et al. 1997, Sharott et al. 2017)

Table 2.13. Table of all primary antibodies used.

Secondary antibody	Supplier	Cat. #
Streptavidin AlexaFluor405	Thermo Fisher Scientific	S32351
Goat-anti-human AlexaFluor488	Thermo Fisher Scientific	A11013
Goat-anti-chicken AlexaFluor488	Life Technologies	A111039
Goat-anti-rabbit AlexaFluor555	Thermo Fisher Scientific	A-215429
Goat-anti-mouse AlexaFluor555	Thermo Fisher Scientific	A-21422
Goat-anti-rat AlexaFluor555	Life Technologies	A-21434
Goat-anti-rat AlexaFluor647	Thermo Fisher Scientific	A-21247

Table 2.14. Table of all secondary antibodies used.

2.6.2.3 Analysis of fluorescent antibody staining

Following staining, slices were imaged using an Olympus Fluorescence Microscope (model BX40) with a Hamamatsu ORCA-ER camera and HCLImage Live Software and then analysed in *Image J* (Fiji). The exception to this was the imaging of the postnatal striatum stained with CTIP2 and PPE for experiments investigating the D1/D2-SPN ratio (**Experimental Chapter C, Figure 5.12**). For these experiments, images were captured on a Zeiss Imager M2 epifluorescence microscope (Carl Zeiss) equipped with a 20x objective (Aperture = 0.8) using Stereoinvestigator software (MBF Biosciences). Unless stated, a single image was taken of the slice at ~5µm depth below the surface. Cases where a z-stack was used have been noted individually below, along with the specific parameters. For embryonic experiments, three sections (rostral, medial, caudal) were analysed per brain (unless otherwise stated). For postnatal experiments, one section (medial) was analysed per brain. In terms of analysis for specific experiments:

Endogenous expression of NR1 (**Experimental Chapter A, Figure 3.3**) was assessed by using NMDAR1 primary antibody at 1:50, with an anti-rabbit secondary antibody on wild-type fixed embryonic slices and imaging at 20x magnification. Images were analysed in *Image J*, where five ~50µm lines were drawn, starting at the edge of the LGE ventricular boarder and extending towards the centre of the LGE. For consistency, the lines were evenly spaced and drawn perpendicular to the ventricular wall. Exposure time was kept constant at 500ms and Gain was set to 0. The values along the five lines were averaged to get a value for the mean signal intensity (i.e. the mean grey value (MGV)) in the slice.

***In vitro* binding of polyclonal and monoclonal NR1-IgG and control-IgG** (**Experimental Chapter A, Figure 3.5**) was assessed by using the antibodies as primaries at 1:50 with an anti-human secondary antibody on wild-type fixed embryonic slices and imaging the sections at 20x magnification. Images were analysed in *Image J*, using the same method as for **Endogenous expression of NR1** above.

Ex vivo binding of polyclonal and monoclonal NR1-IgG and control-IgG (Experimental Chapter A, Figure 3.6 B, C) was assessed by using anti-human secondary antibody on embryonic slices from animals injected at E14.5 with either NR1-IgG or control-IgG. Both total level of antibody and bound antibody only were assessed. To assess total antibody (i.e antibody that was bound to the brain tissue as well as that which was unbound but still present inside the brain), brains were collected and immediately fixed. To assess bound antibody only, when brains were collected, they were cut in half down the midline to expose the ventricles and washed extensively with PBS for 20 minutes to remove any unbound antibody, prior to fixation. Total and bound antibody signal was assessed using the same MGV method as for **Endogenous expression of NR1** described above.

To investigate **regional distribution of the antibody within the LGE (Experimental Chapter A, Figure 3.6 F)**, the MGV values along the perpendicular lines were averaged into 2µm groups. To assess **‘clearance’ of antibody from the brain (Experimental Chapter A, Figure 3.6 E)**, brains were fixed at different timepoints post injection (24 hours and 48 hours). Signal was assessed in the LGE using the same MGV method as for **Endogenous expression of NR1** described above.

Early expression of glutamate transporters (Experimental Chapter A, Figure 3.1) was determined by staining separate wildtype embryonic slices with VGLUT1 or VGLUT2 primary antibodies and the corresponding secondary antibodies (**Tables 2.13, 2.14**). Slices were imaged at 20x and 100x (oil) magnification. Distribution of glutamate transporters was not strictly quantified but evaluated in a qualitative manner.

To assess **primordial striatum size, proliferative zone width and cortical thickness (Experimental Chapter B, Figure 4.2 C-G)**, embryonic slices from either NR1-IgG or control-IgG injected brains were stained with CTIP2 primary antibody to label the SPNs within the primordial striatum. Primordial striatum size was quantified by drawing a boarder around the CTIP-labelled region on images taken at 4x magnification.

Proliferative zone width was measured in by drawing perpendicular lines from the LGE ventricular edge to the edge of the CTIP2-labelled area. Three evenly spaced lines were drawn, and an average was taken. Cortical thickness was measured in a similar manner, by drawing three evenly spaced lines from the cortical ventricular edge up to the top of the cortex and taking the average.

To quantify **numbers of proliferating cells within the LGE and cortex** (Experimental Chapter B, *Figure 4.3*), embryonic slices from either NR1-IgG or control-IgG injected brains were stained with PH3 primary antibody to label the mitotic neural progenitors within the brain. PH3+ cells were counted in a defined LGE or cortical region, standardised across sections, in sections imaged at 20x magnification. Only cells in focus, with intensity >2SD above background were counted. **PH3+ cell location** (Experimental Chapter B, *Figure 4.4*) was determined by placing markers close together along the full ventricular edge, and then comparing the coordinates from the labelled PH3+ cells to each of these counters and determining the one with the shortest distance. There was slight but non-significant variation in the size of the area in which the cells were counted. Therefore, the relationship between number of counted cells and area was assessed, and no significant correlation was found (**Appendix, Supplementary Figures 4.2, S4.3**).

To quantify the **density of embryonic microglial cells within the LGE and cortex** (Experimental Chapter B, *Figure 4.10 B-E*), embryonic slices from either NR1-IgG or control-IgG injected animals were stained with IBA1 primary antibody to label activated microglial cells within the brain. 30µm z-stacks, with 0.5µm intervals were taken of the LGE and cortex, and cells quantified in a defined region, standardised across sections. To count, cells were counted in each layer of the z-stack. Only cells in focus, with intensity >2SD above background were counted. The density of the cells was then calculated by dividing the total number of cells counted by the volume of the z-stack (density = total cells counted/(30*area of region)). **Location of embryonic microglial cells** (Experimental Chapter B, *Figure 4.10 F*) was determined using the x and y coordinates of the counted cells and comparing them to the coordinates of the markers placed close to

one another along the ventricular edge using the same method as for determining PH3+ cell location described in the paragraph above.

To determine the **density of postnatal microglial cells within the striatum and cortex** (**Experimental Chapter C, Figure 5.13**), the same method was used as for embryonic microglia but using a pre-defined region of the postnatal striatum and cortex.

To quantify the **D1/D2 SPN ratio in the striatum** (**Experimental Chapter C, Figure 5.12 C-D**), postnatal slices from either NR1-IgG or control-IgG brains were stained with CTIP2 and PPE primary antibodies to label all SPNs and D2-SPNs within the striatum respectively. One medial slice per brain was then imaged using a Zeiss Imager M2 epifluorescence microscope at 20x magnification. Counting was performed similarly to that described in **Garas et al. (2016)** and **Garas et al. (2018)**. The striatum was delineated within the Stereoinvestigator software (MBF Biosciences) linked to the microscope by tracing around the striatum prior to imaging at 5x magnification, which was clearly defined by the presence of CTIP2. Imaging of the striatum was subsequently performed by capturing a series of tessellated 10µm z-stacks, with 1µm interval steps, between 2-12µm from the section surface, to form a 10µm thick optical dissector. Images were then analysed offline within the Stereoinvestigator software following the inbuilt *Optical Fractionator Workflow*. Counts were performed across the entirety of the dorsal striatum within the coronal section, with the grid size and counting frame both set to 500 x 500µm. A 2µm guard zone at the top of each section was not imaged to prevent surface irregularities influencing results. A neuron was counted if it came into focus within the 10µm stack and had an intensity >2SD above the background. Neurons that were already in focus at the top of the stack were not included. Cells that had both clear CTIP2 nuclear labelling and also showed clear PPE cytoplasmic labelling were counted as PPE+ SPNs (i.e. D2-SPNs). Cells that only exhibited CTIP2 labelling were counted as PPE- SPNs (i.e. D1-SPNs).

The same z-stacks were also used to determine **density of total SPNs** (**Experimental Chapter C, Figure 5.12 E**). Density was calculated using cell counts from three counting frames for each brain and dividing by the counting frame volume (10 x 500 x 500 μ m).

2.6.2.4 TUNEL assay to label apoptotic cells

Identification of apoptotic cells was performed using the Click-iT Plus Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick-End Labelling (TUNEL) Assay (C10618, Thermo Fisher), following manufacturer's protocol, modified to use agarose-embedded tissue (**Experimental Chapter B, Figure 4.8**). Fixed E16.5 slices from brains injected with NR1-IgG or control-IgG were permeabilised with Proteinase K for 15 minutes. They were then incubated in terminal deoxynucleotidyl transferase (TdT) Reaction buffer for 10 minutes at 37°C, then TdT Reaction Mixture consisting of TdT reaction buffer, TdT enzyme, EdUTP nucleotides and Alexa Fluor 594 picolyl azide dye for 1 hour at 37°C. After washing, the Click-iT Plus reaction was performed by adding TUNEL Reaction Cocktail to each section in a light-protected, humidified chamber for 30 minutes at 37°C. Sections were washed with 3% BSA and PBS containing DAPI (Thermo Fisher, 488, 1:10,000). Sections were imaged with an Olympus Fluorescence Microscope (model BX40) with a Hamamatsu ORCA-ER camera and HCLImage Live Software and analysed in *Image J* (Fiji). Cells were counted in a defined LGE region, standardised across sections, in sections imaged at 20x magnification. Only positively labelled cells in focus, with intensity >2SD above background were counted. Positive and negative control sections were included. The positive control was ezDNAse enzyme (Thermo Fisher Scientific) which induces DNA strand breaks and apoptosis (**Appendix, Supplementary Figure S4.5**). The negative control was ddH₂O.

2.7 Electrophysiology methods

2.7.1 Slice preparation

Acute striatal slices were made from postnatal animals at p7-p14, that had been injected with either NR1-IgG or control-IgG at E14.5. Litter sizes and sexes were similarly matched. Animals were deeply anaesthetised with isoflurane, and rapidly decapitated. The brain was transferred to a Petri dish of slicing aCSF containing (in mM): 65 sucrose, 85 NaCl, 25 KCl, 1.25 NaH_2PO_4 , 7 MgCl_2 , 0.5 CaCl_2 , 25 NaHCO_3 and 10 glucose bubbled with carbogen gas (5% CO_2 , 95% O_2 , pH 7.3). The cerebellum was sliced off to create a flat surface and the brain was super-glued with the olfactory bulbs pointing upwards onto the slicing plate. The plate was transferred to the vibratome (Microm HM650) which was also filled with slicing aCSF. 350 μm coronal slices were cut and transferred into a storage beaker containing recording aCSF containing (mM): 130 NaCl, 3.5 KCl, 1.2 NaH_2PO_4 , 2 MgCl_2 , 2 CaCl_2 , 24 NaHCO_3 , 10 glucose (pH7.3), bubbled with carbogen gas at room temperature.

2.7.2 Recording conditions

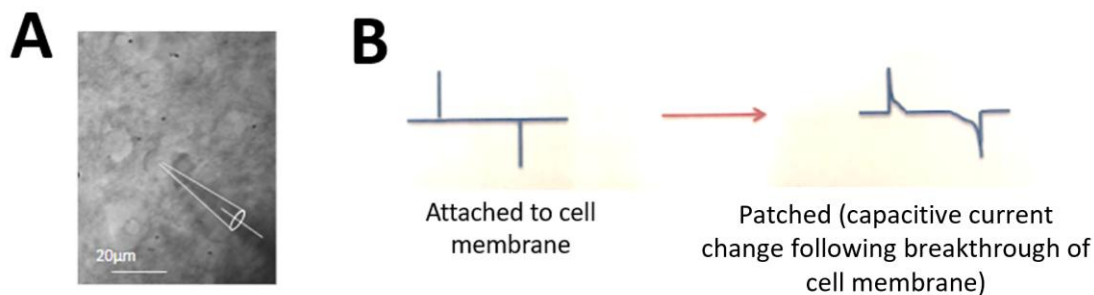


Figure 2.15. Whole-cell patch-clamp recording capacitive current changes [A] A photograph of a cell being patched. [B] Successfully patched cell seal-test shape change.

Recording was performed on a whole-cell patch-clamp rig, perfused with recording aCSF at 2ml/min. Striatal slices were transferred into the recording chamber and held in place with a harp. Whole-cell current-clamp recordings were conducted using pulled borosilicate glass pipettes ($\sim 6\text{M}\Omega$) containing

(mM): 110 KGluc, 40 HEPES, 4 NaCl, 2 ATP-Mg, 0.3 Na-GTP, 0.2 QX-314 and 4mg/ml biocytin (pH 7.3, 295mOsmol/L). To patch a cell, a suitable cell within the dorsal striatum was first located down the rig microscope, and the location marked on the screen. The pipette tip was then located under the microscope, checked for an appropriate seal with a seal test (5mV repeat voltage steps at 20Hz), and a small amount of positive pressure applied via a syringe. The pipette was then slowly lowered to the level of the cell and the positive pressure released once close to the cell to allow attachment to the cell membrane. The cell was left for a couple of minutes to allow the pipette to seal properly with the membrane, and a good seal was shown by a leak current close to 0pA. Once patched, more forceful negative pressure was applied by sucking on a pipette tip to break through the cell lipid bilayer and patch the cell. Successful patch was confirmed by change in the shape of the seal test trace (**Figure 2.15**). Patched neurons could be maintained for at least 30 minutes and occasionally a small amount (<10mA) of current was injected to help stabilise the resting membrane potential. Recordings were made with Multiclamp 700B amplifiers, filtered at 4kHz and acquired at 10kHz using an InstruTECH ITC-18 analog/digital converter (Digitmer) and WinWCP Software (University of Strathclyde).

2.7.3 Stimulation and recording protocols

2.7.3.1 Intrinsic properties

Intrinsic properties of the cells including resting membrane potential (RMP), input resistance (IR), membrane time constant (MTC), and spike threshold, as well as properties of the action potentials generated including amplitude, frequency and duration, and interspike interval (ISI), were assessed by applying a series of hyperpolarising and depolarising current steps (**Experimental Chapter C, Figures 5.1, 5.4, Tables 5.2, 5.3**). The current steps applied were –100 to +100pA in most cases, to enable sufficient SPN depolarisation with consideration of input resistance changes and observations of depolarisation block and action potential failure in SPNs (**Krajeski et al. 2019**). In some of the very young neurons (p7-8), current steps were applied as –50 to +50pA since the cells could not tolerate the greater range. Following

application of current steps, a 'rheobase 10' protocol was applied, starting from 0mV and injecting 10pA current in each step to find the point at which the cells started generating action potentials. The shape and pattern of the action potentials was assessed to ensure that the cell being patched was a SPN and any cells with atypical firing were excluded. The baseline of the cell was also recorded for 2 minutes in both current clamp and voltage clamp mode to check cell stability.

2.7.3.2 Synaptic inputs and connections

The baseline voltage clamp recording, where the cell was clamped at -70mV , was also subsequently analysed for presence of miniature EPSCs (**Experimental Chapter C, Figure 5.8**). Although typically these are recorded in the presence of an action potential blocker such as TTX, in this case we did not use an action potential blocker since it would interfere with subsequent evoked corticostriatal recordings.

In a subset of striatal SPNs, the properties of corticostriatal connections were also investigated (**Experimental Chapter C, Figures 5.9, 5.11**). To do this, a bipolar stimulating electrode (FHC Inc.) was placed in the external capsule close to the striatum. GABA_A receptor antagonist SR95531 (Gabazine, $1\mu\text{M}$, Tocris Biosciences) was perfused through the rig to ensure only glutamatergic transmission was being recorded. The cortical afferents were activated via the electrode every 5 seconds, and evoked excitatory post-synaptic potentials (EPSPs) were recorded from the patched cell (held in current clamp, at resting membrane potential). In the first experiments, the level of stimulation was slowly titrated to find a point where EPSPs were around 50% of their maximum height (typically around $0.6\text{-}1.2 \times 100\mu\text{A}$). This stimulation strength was then used for the rest of the protocol. The EPSPs were then recorded every 5 seconds for 20 repetitions. Next, the paired-pulse ratio (PPR) was assessed by delivering two pulses of stimulation with a short (50ms) interval. Response to trains of stimulation was then evaluated by delivering a train of 10 pulses at 20Hz, and trains repeated every 30 seconds for a maximum of five repetitions. The recordings in just the presence of Gabazine captured EPSPs mediated by combined AMPARs/KARs and NMDARs.

NMDAR antagonist D-(-)-2-amino-5-phosphopentanoic acid (DAP5, 50 μ M, Tocris Biosciences) was then added to the recording solution to block NMDARs and the solution washed through the rig for 10 minutes. The EPSP, PPR and train stimulation protocols were then repeated (**Experimental Chapter C, Figure 5.10**). The recordings captured EPSPs mediated by AMPA/KA only. Following recordings, AMPAR/KAR antagonist CNQX was washed in as a control.

2.7.3.3 Analysis of recordings

Electrophysiological data was analysed both as a whole dataset (p7-14), and in smaller two-day windows (p7-8, p9-10, p11-12 and p13-14) to both look at larger effects and pinpoint effects at more specific timepoints. The mean age of each of these groups was compared between the NR1-IgG and control-IgG cells, to ensure there was no significant difference in the age of any of the groups. Intrinsic properties were also compared between animals injected with polyclonal antibody vs monoclonal antibody for both the NR1-IgG and control-IgG to validate the similar effects of the antibodies (**Experimental Chapter C, Tables 5.2 , 5.3**).

Recordings were converted into .abf files in WinWCP and then analysed offline using custom-written Igor Pro scripts (Wavemetrics). **RMP** was taken as the average baseline in the first 10 seconds of recording. **IR** was calculated from the observed change in membrane potential following hyperpolarisation of the membrane potential with a set current injection. **MTC** was evaluated as the time taken for a change in potential to reach 63% of its final value. **Spike** (i.e. action potential) **threshold** and **rheobase** respectively corresponded to the membrane voltage and current step at which an action potential was first produced. **Spike rate** was assessed as the number of spikes over the time window in which the current step was applied. **ISI** was taken as the time between the peaks of two adjacent spikes. **Spike duration** was evaluated as the duration between the up and down phase of the spike at 25% of the peak amplitude. IV curves were produced by taking the mean voltage produced at each injected current step.

Spontaneous EPSCs (sEPSCs) were defined as downward deflections of $>2SD$ below the baseline (average of whole recording) with a minimum duration of 10ms in baseline traces pre-filtered with a 50Hz low-pass filter.

The traces from evoked EPSPs were used to analyse synaptic properties including amplitude, duration, rise time and decay time. Traces were pre-filtered with a low-pass filter of 500Hz and a high-pass filter of 0.1Hz. **Amplitude** and **duration** were both calculated from the start of the downward phase of the spike until the return to the initial baseline. **Rise time** was evaluated as the time between 20-80% of the peak amplitude, and **decay time** as the time from 100% back down to 50% of the peak amplitude.

Short term plasticity was determined using train stimulation recordings, by taking the amplitude of each EPSP and dividing it by the first EPSP amplitude. A ratio greater than 1 indicated a facilitative effect whereas a ratio less than 1 indicated a depressive effect. In a similar way, **PPR** was calculated by dividing the amplitude of the second peak by the amplitude of the first peak.

The **NMDA/AMPA ratio** was determined using evoked EPSP traces. NMDA-mediated current was first determined by subtracting the amplitude of the mean EPSP from the trace where AMPARs/KARs were pharmacologically isolated with DAP5, from the trace without DAP5 (i.e combined NMDAR and AMPAR/KAR-mediated current). NMDAR-mediated peak amplitude was divided by peak AMPAR/KAR-mediated peak amplitude to give the ratio. To calculate the NMDAR component proportion, NMDAR-mediated peak amplitude was divided by NMDAR and AMPAR/KAR-mediated peak amplitude (i.e no DAP5 trace) and converted to a percentage.

The Gabazine only and Gabazine + DAP5 traces were also compared for synaptic properties of EPSPs. To standardise across stimulation strengths, the results in the Gabazine + DAP5 condition were normalised as a percentage of the Gabazine only condition.

2.8 Morphological reconstruction methods

2.8.1 Embryonic brain weight

To measure embryonic brain weight (**Experimental Chapter B, Figure 4.2 A**), dams that had undergone *in utero* surgery were culled and embryonic heads subsequently collected in a Petri dish containing PBS. Each brain was individually extracted from the skull and blotted dry, before being weighed on an ultra-fine scale (Model 100A, XE Series, Denver Instrument Company).

2.8.2 Analysis of striatal volume using structural MRI

Following behavioural experiments, to analyse striatal volume using structural MRI (**Experimental Chapter C, Figure 5.12 A, B**), brains of animals injected with either NR1-IgG or control-IgG were fixed at postnatal day 23 for 96 hours in 4% PFA. Brains were then transferred to perfluorinate solution to boost contrast and scanned using a 9.4T Biospec with cryocoil scanner for 1 hour. Scans were kindly performed with the help of Johan Van Audekerke and colleagues (BioImaging Lab, Antwerp). Scans of each brain were analysed in ITK Snap software. The striatum was manually contoured in each section of the scan using the drawing function and the contours then stacked to get a total volume. To account for variation in brain size impacting any striatal volume differences observed, the volume of the whole brain was also ascertained using the same method. The volume of the striatum was then normalised to the volume of the total brain and presented as a percentage of the total volume.

2.8.3 SPN reconstructions using DAB labelling

To perform DAB SPN reconstructions (**Experimental Chapter C, Figure 5.5**), following electrophysiological recordings SPNs were filled with biocytin and brain slices were fixed in 4% PFA for ~1 week. Slices were then washed 3 times using PBS and incubated in fresh H₂O₂ on a 60 RPM shaker plate at room temperature for 5 minutes. Slices were washed with PBS four more times and then incubated in ABC Kit solution for 3 hours at room temperature. ABC kit solution was made up by adding 1 drop of solution A and 1 drop of

solution B to every 2500µl of PBS-Tx (0.3%). Following incubation in ABC kit solution, DAB/H₂O₂ solution was prepared in a fume hood, by adding 1 tablet of H₂O₂ and 1 tablet of DAB to every 1000µl of ddH₂O. Slices were washed in TBS 0.05M for 10 minutes and then DAB was carefully pipetted into each well, before waiting 3 to 10 minutes until the neurons were very darkly labelled but the background was still relatively pale. The reaction was stopped by washing the slices 3 times in PBS. Following staining, the slices were taken through 25%, 50%, 75% and 100% glycerol steps, whereby each concentration was added sequentially to the slices for 15 minutes, before replacing with the next concentration up. Slices were mounted in 100% glycerol.

For DAB-labelled neurons, z-stacks of the full neuron, with 0.5µm steps, were taken at 40x magnification. Only cells that had a full dendritic arbour were included. Z-stacks were processed and analysed following similar methods to those found in **Young and Morrison (2018)**. Each z-stack was converted to binary 8-bit images using *Image J*. Where needed, features of the *Image J* toolbar were used to reduce the background and increase the contrast, including *Unsharp Mask* (pixel radius: 3, mask weight: 0.6), *Despeckle*, *Threshold*, and *Remove Outliers*. To obtain **total branch length**, and **numbers of branches**, the image was skeletonised using the *Skeletonise* function, and the skeleton then analysed using the plugin *AnalyzeSkeleton(2D/3D)*. The *Scholl Analysis* function was also applied to the image to evaluate morphological **complexity**.

To evaluate **spine density** (**Experimental Chapter C, Figure 5.6**), z-stacks of primary (1°), secondary (2°), and tertiary (3°) branches were taken at 100x magnification. Branch length was manually evaluated by tracing each branch in *Image J*, and spines individually counted along each branch using the pointer tool. Density was calculated by dividing the number of spines along a branch by the total branch length. Density was evaluated for two 1°, two 2° and two 3° dendrites per cell.

2.8.4 Microglial reconstructions

To reconstruct embryonic and postnatal microglial cells in the LGE/striatum (**Experimental Chapter B, Figure 4.11** and **Experimental Chapter C, Figure 5.14** respectively), fixed slices were stained with IBA1 primary antibody to label activated microglial cells. Z-stacks of full microglia cells, with 0.5µm steps, were then taken at 40x magnification. Only cells that had a full dendritic arbour were included. Cells to be imaged were chosen using a random number generator (www.calculator.net). Z-stacks were opened in *Image J* and converted to binary 8-bit images. They were then skeletonised, and the skeleton analysed as described for the DAB neurons in **Section 2.8.3 SPN reconstructions using DAB labelling** above. Microglia were also analysed with the *FracLac Image J* plugin to obtain further detailed information about subtle morphological differences, summarised in the **Figure 2.16** below.

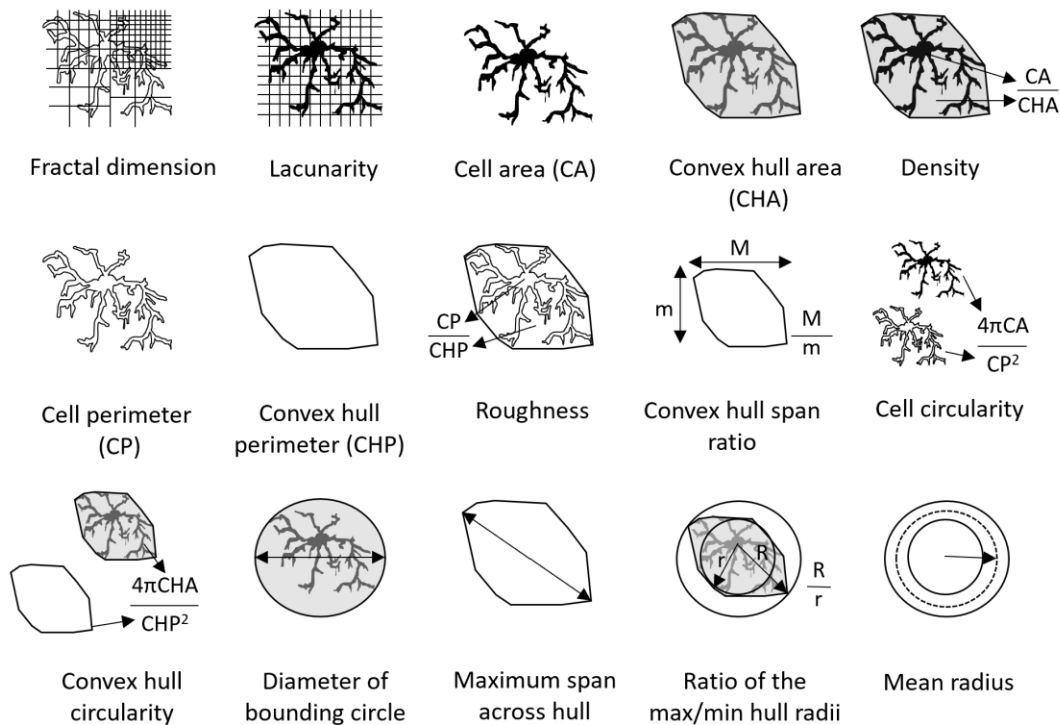


Figure 2.16. Microglial morphological parameters analysed with FracLac analysis. Methods and diagram based on Fernández-Arjona et al. 2017.

2.9 Behavioural methods

2.9.1 Pre-weaning tests

2.9.1.1 Early neurological reflex testing

One way to evaluate early neurological development and neuromotor ability is to assess the development of neurological reflexes. Thus, in this study we tested three motor-related reflexes (surface righting, negative geotaxis and cliff aversion) during the second postnatal week (p7-14) (**Experimental Chapter D, Figure 6.1**). These reflexes were chosen as they require integrity of the basal ganglia and striatum (amongst other regions) for full expression (**Fox 1965, Arakawa and Erzurumlu 2015**). This testing window was chosen since in our set up, earlier disturbance of the nest (i.e. first postnatal week) for behavioural testing was linked to poor survival rates amongst the litters.

At p6, pups from litters either injected with NR1-IgG or control-IgG at E14.5 were individually marked with a distinct tail pattern using a permanent marker to provide identification for the early testing period. Litters were similarly matched for size (either 6 or 7 pups per litter). In the three days prior to testing, the mother and pups were carefully handled daily to familiarise them with the tester.

Reflex testing was done daily from p7-14, at a similar time each evening (~7pm) to correspond to the dark (active) phase of the mouse day. Possible confounding variables including temperature, diet, light/dark cycles, noise, odours and housing were controlled for wherever possible.

At the start of testing each day, pups were transferred to a clean cage containing home cage bedding for familiarity. The tester's hands were deliberately rubbed in the home cage bedding to avoid maternal rejection. Each pup was first weighed, and then underwent the surface righting reflex test, followed by the cliff aversion test, followed by the negative geotaxis test. Between different tests the pup was returned to the clean cage for at least 3 minutes. Pups were returned to their home cage and reunited with their mother post-testing. There were two trials for each test per day, with a 1-minute break in

between. Each test was conducted daily until it could be performed successfully for two consecutive days. The cut off period for each test was 30 seconds.

2.9.1.2 Surface righting reflex

The surface righting reflex involves a sequence of coordinated motor movements for the pup to be able to flip over from its back onto its front (**Figure 2.17**). The reflex typically appears between p1-10 (**Heyser et al. 2004**).

To evaluate surface righting, the pup was placed in a supine position on a flat surface, supported in position by the tester for 5 seconds. The support was then released and the time for the pup to turn over fully onto its abdomen measured using a stopwatch. If the pup had not flipped over before the 30 second cut off, the test was stopped, and it was gently turned over manually by the tester. (**Figure 2.17 A**).

2.9.1.3 Cliff avoidance

The cliff avoidance reflex primarily evaluates the vestibular reflexes, alongside coordination and strength, and develops from p1-14 (**Hill et al. 2008**).

To assess cliff avoidance, the pup was placed on top of a polystyrene box, which was approximately 30cm high, with the front paws and snout over the box edge. The pup was held in place by the tester initially. Once released, the time for the pup to step away from the cliff and remove its paws and snout from the edge was timed using a stopwatch. If the pup fell or had not moved within the 30 second cut off, the test was terminated. (**Figure 2.17 B**).

2.9.1.4 Negative geotaxis

The negative geotaxis reflex assesses motor coordination and vestibular reflexes. It develops between p3-15 (**Heyser et al. 2004**).

To measure negative geotaxis, the pup was placed on a polystyrene lid angled at 45° facing downwards and held in position by the tester. When released, the time to turn 180° so it faced up the slope was measured with a stopwatch. If the pup could not turn within the 30 second cut of the test was stopped (*Figure 2.17 C*).

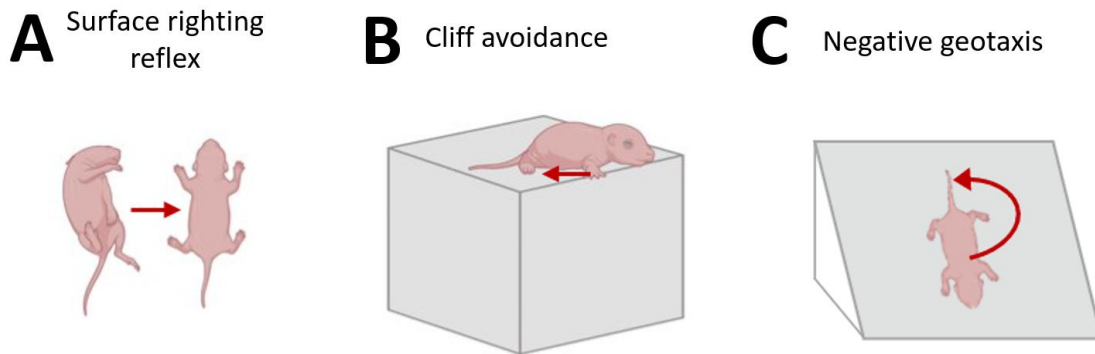


Figure 2.17. Early postnatal reflexes. Graphical summary of the **[A]** surface righting reflex, **[B]** cliff avoidance reflex, and **[C]** negative geotaxis reflex.

2.9.2 Early adolescent behavioural tests

To assess later behaviour changes, two tests, the open-field test and the accelerating rotarod test, were also performed on the NR1-IgG and control-IgG mice at p21-23 (i.e postnatal week 4) (**Experimental Chapter D, Figure 6.2, 6.3**). Open-field was chosen as it captures a wide range of potential alterations, including those linked to key striatal pathways such as motor- and anxiety-related circuits (**Kraeuter et al. 2019**). Accelerating rotarod was chosen as a more specific test for assessing motor coordination and anxiety (**Deacon 2013**). To perform these tests, animals were brought to a dark testing room in their home cages at least 1 hour prior to testing to acclimatise. The open-field test was performed first and the accelerating rotarod second, with a break of at least 5 minutes in between.

The early adolescent behavioural tests were kindly performed with the help of Elke Calus (Department of Biomedical Sciences, Antwerp).

2.9.2.1 Open-field test

The open-field task is primarily a measure of motor- and anxiety-related behaviour (**Kraeuter et al. 2019**).

To perform the test, the mouse was placed in the corner of a brightly illuminated 60 x 60cm clear square Perspex box. Movement around the box was then recorded for 11 minutes (1 minute of acclimatisation and then a 10-minute test period) using AnyMaze Ethovision software. The box was divided into three key zones: 1. centre 2. periphery, and 3. corners (**Figure 2.18 A**). The parameters recorded included total distance and mean velocity during the trial period, as well as frequency, duration, latency to enter, distance moved, and mean velocity within each of the three key zones.

2.9.2.2 Accelerating rotarod

The accelerating rotarod tests motor coordination, balance, cerebellar learning and anxiety (**Moser 2001, Bearzatto et al. 2005, Deacon 2013**).

To perform the test, the mouse was gently lifted from the home cage and placed on the rotating rod, facing away from the direction of rotation (**Figure 2.18 B**). Each mouse first individually underwent two acclimatisation periods, where it was placed on the rod rotating at constant slow starting speed of 4 RPM for 2 minutes. If the mouse fell during this time, it was returned to the home cage. For the test itself, the rotarod can accommodate up to five mice at once and mice in the same group were assessed simultaneously wherever possible with each mouse maintaining a consistent testing slot throughout. To test the mice, they were placed on the rod at a steady initial speed of 4 RPM, which accelerated uniformly up to 40 RPM over a 5-minute period. The time on the rod was measured. Four trials were conducted, with at least 1 minute between trials, during which mice were returned to their home cage. If a mouse fell or jumped off the rod within the first 15 seconds, it was given one further trial. For data analysis, the four trials performed were averaged.

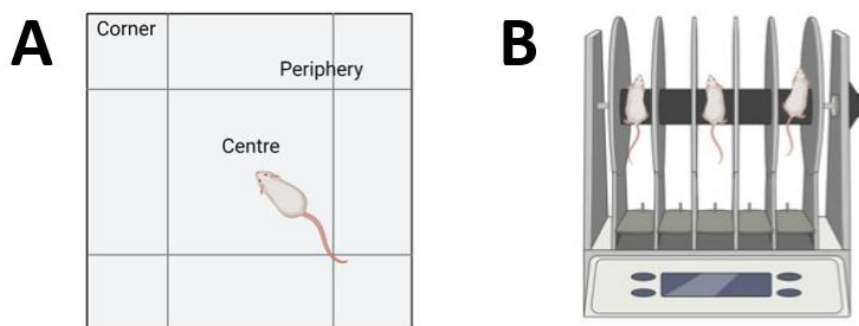


Figure 2.18. Post-weaning behavioural tests. Graphical summary of the **[A]** open-field test and **[B]** accelerating rotarod.

2.10 Rescue model generation

To attempt to ‘rescue’ behavioural alterations seen in our NR1-IgG animals, a model was generated to enhance endogenous glutamatergic transmission using the positive AMPA allosteric modulator AMPAkinone CX516 (**Experimental Chapter D, Figures 6.4, 6.5, 6.6**). Following *in utero* injection of NR1-IgG or control-IgG at E14.5, pups were born and then injected twice a day intraperitoneally with 2.5mg/kg of CX516 between p0-7. They subsequently underwent behavioural testing as described in **Section 2.9 Behavioural methods**. Effects were evaluated by comparing the change between the rescue NR1-IgG and non-rescue NR1-IgG populations, as well as between the rescue NR1-IgG population and the non-rescue and rescue control-IgG populations.

Injections of CX516 and early reflex testing in rescue animals were performed with the kind help of Prof. Dr Tommas Ellender and Yana Van de Poll (both Department of Biomedical Sciences, Antwerp), and open-field and accelerating rotarod experiments in rescue animals were performed with the kind help of Elke Calus (Department of Biomedical Sciences, Antwerp).

2.11 Statistical methods

Details of specific statistical tests used for each experiment are given in the respective results sections within the chapters. All data are presented as mean $\pm$ SEM. For embryonic experiments, to account for inter-litter variation, data is presented as a normalised % change relative to litter-matched controls. All data was initially normality tested using the Shapiro-Wilks test or the Kolmogorov-Smirnov test, and the appropriate two-tailed parametric (one-sample t-test, unpaired t-test, repeated measures (RM) ANOVA, mixed ANOVA) or non-parametric (Wilcoxon signed-rank (WSR), Mann-Whitney U (MWU), Fisher's exact test, Mood's Median (MM)) test applied. In cases of repeated measures, data underwent sphericity and homogeneity assumption testing and the appropriate correction (Greenhouse-Geisser or Huynh-Feldt) applied where necessary. Significance for correlations was determined using the Pearson correlation coefficient and sample size. Data was corrected where appropriate using either Bonferroni or Sidak *post hoc* corrections, to compensate for multiple comparisons. For all statistical tests, $p < 0.05$ was deemed significant. * $p < 0.05$, ** $p < 0.01$.

3. Experimental Chapter A: Defining a novel *in utero* intraventricular model of NR1-IgG antibody delivery to the embryonic mouse brain

3.1 Chapter Introduction

The primary aim of this thesis was to explore how brief embryonic exposure to patient-derived antibodies against the NR1 subunit of the glutamatergic NMDAR impacts the development of the mouse brain and specifically, the striatum.

Over the past two decades, there has been increasing interest in the study of antibody-mediated diseases (reviewed in **Prüss 2021**). One of the most common of these is NMDAR antibody-mediated encephalitis (NMDARE), a disease where antibodies are raised against the NR1 subunit of the glutamatergic NMDAR; a receptor that plays a critical role in neurodevelopment amongst many other functions (**Dupuis et al. 2023**). To study these diseases, a range of models have been developed, which have been useful in providing insights into pathogenicity, and furthering mechanistic and therapeutic understanding (**Rose and Bona 1993, Giannoccaro et al. 2020**). For this thesis, a passive transfer *in utero* exposure mouse model was developed, using direct intraventricular injections of patient-derived polyclonal and monoclonal NMDAR NR1-IgG antibodies (in this **Experimental Chapter A**) to study the embryonic and longer-term postnatal effects of exposure to these antibodies in the developing mouse striatum (**Experimental Chapters B, C and D**). The key benefits of this model are that it allows for study of direct embryonic effects as antibody is delivered to the embryonic brain directly, and practically it requires a much smaller volume of antibody relative to other models (**Giannoccaro et al. 2020**).

3.2 Chapter Brief Background

The mammalian CNS is derived from multipotent neural progenitor cells, which differentiate into the neurons or other cell types (astrocytes or oligodendrocytes) of the mature brain (**Kriegstein and Alvarez-Buylla 2009**). The stepwise process of embryonic neurogenesis is tightly regulated and modulated by multiple extracellular signals (**Jansson and Åkerman 2014, Mira and Morante 2020**). One of the key modulators is glutamate, the principal excitatory neurotransmitter in the CNS. Beyond a well-established role in synaptic transmission, glutamate exhibits many important roles in neurogenesis and development, including regulation of key processes such as proliferation, survival, migration and differentiation of neurons and neuronal precursors. The importance of glutamate in early neurodevelopment is highlighted by relatively high levels of glutamate found in the neuron-generating ('neurogenic') zones of the embryonic brain (**Behar et al. 1999, Haydar et al. 2000**) as well as the presence of functional glutamate receptors on neural stem cells and neural progenitors prior to synapse establishment (**Luk et al. 2003, Canudas et al. 2004, Martins et al. 2006, Gandhi et al. 2008, Nakamichi et al. 2008, Ciceroni et al. 2010**).

3.2.1 The striatum and glutamate

The striatum is the main input nucleus of the basal ganglia, and is involved in motor control, executive function, reward, cognition, and reinforcement (**Nestler et al. 2009, Graybiel et al. 2015, Yager et al. 2015, Kim and Im 2019, Fieblinger 2020**). Most striatal neurons originate from the lateral ganglionic eminence (LGE), one of the four main embryonic regions in the ventral telencephalon. The striatum is composed mostly of GABAergic spiny projection neurons (SPNs), but it is known that glutamate is present in the telencephalic germinal zones during embryogenesis (**Blanton and Kriegstein 1991, Behar et al. 1999**) and plays a fundamental role in many early neurodevelopmental processes within the LGE and striatum (**Komoro and Rakic 1993, Luk et al. 2003, Manent and Represa 2007, Elias et al. 2008, Georgiev et al. 2008**). Pertinently, **Luk et al. (2003)** demonstrated that *in utero* exposure to NMDAR antagonists

during striatal neurogenesis impaired cellular proliferation in the LGE and dramatically reduced adult striatal neuron numbers, highlighting the importance of glutamate and NMDARs in early striatal development. Early distribution of glutamate and its receptors remains to be fully characterised.

3.2.2 Expression of glutamate transporters

Glutamate is transported into synaptic vesicles by three closely related proteins: VGLUT1, 2 and 3 (**Boulland et al. 2004**). Distribution of these transporters shows where glutamate is taken into vesicles and can be used as a pseudo-marker to identify key or possible sites of glutamate action. Although closely related in function, previous work demonstrates complementary expression patterns in different subsets of glutamatergic cell populations (**Herzog et al. 2001, Varoqui et al. 2002, Fremeau et al. 2004**) and dynamic changes over development, with VGLUT2 predominant in very early development (**Boulland et al. 2004**). There have also been regional differences identified over development, and in the early postnatal striatum it has been observed that striatal matrix stains strongly for VGLUT2, with transient VGLUT1 expression in the striatal patches only; a difference that disappears by adulthood.

In terms of cellular expression location, during early postnatal development, it has been shown that immunoreactivity for VGLUT1 and VGLUT2 within telencephalon structures is predominantly found in the neuropil, likely in axon terminals. Additionally, there is co-localisation at single axon terminals in some structures (eg. primary somatosensory cortex), but not all (eg. olfactory bulb) during the first postnatal week (**Nakamura et al. 2005**).

3.2.3 Dynamic expression of glutamate receptors

During development, glutamate and its receptors have many important functions (reviewed in **Henley and Wilkinson 2016**). In this study, the antibodies being explored bind to the NR1 subunit of the NMDAR; one of three types of ionotropic glutamate receptor. Structurally, NMDARs are heterotetrametric channels composed of obligatory NR1 subunits, coupled with NR2 (four variants: NR2A-D) or NR3 (two

variants: NR3A-B) subunits to form various subtypes (**Karakas and Furukawa 2014**). NMDARs are involved in a host of neuronal developmental processes including migration (**Behar et al. 1999**), maturation (**Hirasawa et al. 2003**), synaptogenesis (**Cull-Candy et al. 2001, Tolia et al. 2005, Hardingham and Bading 2010**), neuronal growth (**Wu et al. 1999**) and cell survival (**Hardingham and Bading 2010**). AMPARs are the other key type of ionotropic glutamatergic receptor, and they mediate most fast excitatory neurotransmission. Structurally, AMPARs are also heteromeric assemblies, consisting of four key subunits, A1-A4. Like NMDARs, AMPARs are involved in a range of developmental processes including synapse formation, stabilisation, plasticity and the formation of neural circuits (reviewed in **Henley and Wilkinson 2016**).

Previous studies have indicated the expression patterns and locations of the main ionotropic glutamatergic receptors (NMDARs and AMPARs) and their subunits are dynamic throughout development, which is useful in that the different subunits confer different properties on the receptor subtypes, allowing them to partake in a wide range of functions (**Cull-Candy et al. 2004, Paoletti 2011**).

For NMDARs, subunits exhibit temporal and regional variation in their expression patterns (**General Introduction, Figure 1.4**), and only certain subunits (NR1, NR2B, NR2D and NR3A) are expressed embryonically (**Monyer et al. 1994, Laurie and Seeburg 1994, Paupard et al. 1997, Stocca and Vincini 1998, Nishi et al. 2001, Chatterton et al. 2002, Matsuda 2002, Wong et al. 2002, Aizenman and Cline 2007, Ewald and Cline 2009**). Pertinent to this thesis, NR1 expression begins around E14 and peaks during the third postnatal week. It is the most highly expressed subunit throughout development, with greatest expression in the cortex and hippocampus, and lower expression in the striatum. For AMPARs, all subunits are expressed embryonically, showing temporal variability in expression over development in both striatum and cortex (**Jakowek et al. 1998, Lilliu et al. 2001**).

3.2.4 NMDARE, NR1-IgG antibodies and the striatum

Both NMDARs and AMPARs are developmentally and activity-regulated, and the number, properties, subunit composition and surface dynamics are crucial to correct functioning and normal development (Paoletti et al. 2013, Fan et al. 2014, Lussier et al. 2015, Henley and Wilkinson 2016, Hunter et al. 2021).

NMDAR antibody-mediated encephalitis (NMDARE) is a disease whereby antibodies (NR1-IgG) are raised against the NR1 subunit of the NMDAR. Mechanistically, NR1-IgG antibodies bind to the extracellular domains of the NMDAR NR1 subunit, resulting in altered surface dynamics and impaired interactions with receptor stabilising proteins such as the EphB2 receptor (Hughes et al. 2010, Mikasová et al. 2012, Planagumà 2016, Castillo-Gómez et al. 2017, Ladépêche et al. 2018, Pan et al. 2018). This results in receptor internalisation, leading to decreased surface density and synaptic localisation of NMDARs (Dalmau et al. 2008, Hughes et al. 2010, Mikasová et al. 2012) with serious downstream impacts on NMDAR network and synaptic functioning (Hughes et al. 2010, Mikasová et al. 2012, Moscato et al. 2014, Rosch et al. 2018). Additional likely contributing mechanisms that have recently been identified include the antibody binding leading to a longer NMDAR channel open time prior to internalisation (Lynch et al. 2018) as well as subunit-specific nanoscale redistribution of NMDARs (Ladépêche et al. 2018).

This study focuses on the striatum, the main input nucleus of the basal ganglia, which plays a key role in the regulation of motor and other functions. This study specifically focuses on the impacts of these antibodies during early striatal development and in the context of gestational transfer. If a mother develops NR1-IgG (or other) antibodies during pregnancy, they may be transferred placentally and disrupt the developing brain. Indeed, recent studies suggest that maternal antibody-mediated immune responses against distinct neuronal proteins play roles in neurodevelopmental disorders including ASD, learning disabilities and schizophrenia (Singer et al. 2009, Braunschweig et al. 2012, Brimberg et al. 2016, Coutinho et al. 2017a, Jurek et al. 2019).

There is good rationale to focus on the impacts of NR1-IgG antibodies on the striatum during development:

Pre-clinically, literature suggests regional differences in expression and importance of glutamatergic receptors during development, with NMDARs particularly important for striatal neurogenesis (**Luk et al. 2003, Jansson and Åkerman 2014, Krajewski et al. 2019**). Glutamate is a key modulator of progenitor proliferation, migration, differentiation, and survival (**Jansson and Åkerman 2014**). It was previously shown *in vitro* that proliferation of striatal neuroblasts was sensitive to NMDAR antagonists, and similarly that *in utero* exposure to NMDAR antagonists during the neurogenic period resulted in a significant decrease in progenitor proliferation, DNA synthesis and striatal neurons numbers (**Luk et al. 2003**). *In vivo* studies have demonstrated behavioural deficits consistent with impaired striatal function, including impaired motor coordination (**Jurek et al. 2019, García-Serra et al. 2020**). Recent loss-of-function NR1 mouse studies have also shown that adult genetic rescue of NMDARs fails to reverse many behaviours linked to striatal function (eg. hyperactivity and stereotypy), likely due to the limited increase in striatal NMDAR function (**Mielnik et al. 2020**).

Clinically, the latter symptoms of NMDARE include dyskinesia, dystonia (**Florance et al. 2009**) and movement disorders (**Irani et al. 2010**), which implicate motor circuit dysfunction including those of the basal ganglia and striatum (**Lanciego et al. 2012**). Patients also often have seizures. An important question to explore given this presentation is whether these antibodies directly affect the striatum and striatal progenitors. Additionally, children with NMDARE often experience a more ‘neurological’ than psychiatric presentations (**Armangue et al. 2013, Wright et al. 2015a**).

Overall, these pre-clinical and clinical observations posit the striatum as a potential important site of NMDAR antibody interaction, but so far literature has focussed almost exclusively on the hippocampus and cortex.

Antibodies against the NR1 subunit are particularly interesting in a gestational transfer context. Firstly, NMDARE is the most common autoimmune encephalitis (**Dalmau et al. 2019**), and NR1-IgG is thought to be among the most common anti-neuronal antibody in asymptomatic individuals, with an estimated seroprevalence of 1% (**Dahm et al. 2014, Lang and Prüss 2017**). Secondly, 60-80% of patients are women of child-bearing age (**Irani et al. 2010, Titulaer et al. 2013, Viacoz et al. 2014**) and NMDARE can occur during pregnancy, with several studies reporting a high frequency (>50%) of adverse outcomes including pre-term labour and foetal death (**Kumar et al. 2010, Mathis et al. 2015, Joubert et al. 2020**). Thirdly, a likely pathogenic role for NR1-IgG antibodies in neurodevelopment is widely illustrated in the literature (**Giannoccaro et al. 2020, Hunter et al. 2021**).

3.2.5 Key NR1-IgG and other antibody models

Over the past two decades, models have been developed to study the effects of these antibodies, most recently in the context of gestational transfer and neurodevelopment (reviewed in **Giannoccaro et al. 2020**). Most animal models involve passive transfer of serum, immunoglobulins, antibodies or antibody-producing cells to reproduce disease. Antibodies are derived from patients and may either be polyclonal or monoclonal. Polyclonal antibodies come from several different immune cells and have affinity for the same antigen but variable epitopes, whereas monoclonal antibodies have much greater specificity since they are derived from identical immune cells that are all clones of a specific parent. Prenatal NR1-IgG and other antibody models almost exclusively use intraperitoneal or tail-vein injections to deliver the antibody to the mother during pregnancy, potentially (or shown) to elicit systemic effects (**Jurek et al. 2019, García-Serra et al. 2020, Giannoccaro et al. 2020**).

However, the model involves delivery of both polyclonal and monoclonal NR1-IgG antibodies, derived from patients with NMDARE, directly to the embryonic brain *in utero* using intraventricular injections at E14.5. To our knowledge this is a novel model for studying the impact of NR1-IgG antibodies and therefore

the first chapter of the thesis focuses on characterising some of the key features of this model including how these antibodies bind *in vitro*, and how they distribute and are removed from the brain following embryonic injection.

Additionally, in the subsequent chapters of the thesis both a polyclonal and a monoclonal NR1-IgG antibody are used due to a limited supply of the polyclonal antibody available, so a second aim of this first chapter is comparing these two antibodies within our model.

3.3 Chapter Aims

The aim of **Experimental Chapter A** was to define our novel *in utero* mouse model for exploring the impacts of patient-derived NR1-IgG antibodies on the developing brain, including the following:

- 1) What is the expression and distribution of glutamate transporters in the LGE?
- 2) What are the transcript levels of NR1 and other glutamatergic subunits in the embryonic cortex and the LGE?
- 3) How does protein-level expression of NR1 compare between the LGE and cortex?
- 4) What is the binding efficacy of patient-derived NR1-IgG polyclonal and monoclonal antibodies *in vitro*?
- 5) Do they bind the embryonic mouse brain *in vivo* and how is the binding distributed?
- 6) What is binding like at 24 hours and 48 hours following injection into the embryonic brain?

3.4 Chapter Methods

Methods used in this Chapter are described in detail in **General Methods** and are briefly summarised below (in the approximate order in which they appear):

3.4.1 qPCR methods

(See **General Methods Section 2.3 Transcript and protein quantification methods**)

To quantify NMDAR and AMPAR glutamatergic subunits in the embryonic brain at the transcript-level, E16.5 wild-type brains were first extracted and carefully dissected into the cortex and LGE. Two brains (cortices/LGEs) were combined for each sample. mRNA was extracted using a QIAGEN kit as per the manufacturer's protocol. Quality and concentration of the extracted RNA was assessed using a Nanodrop ND-1000. A 260/280 absorbance ratio of ~2.0 was considered pure, and a concentration of >30ng deemed a cut off for continuation to cDNA generation. cDNA was generated from 1µg RNA using a SuperScript IV-VILO kit as per the manufacturer's protocol, and samples stored at -20°C until use.

RT-qPCR was performed following published methods (**Han et al. 2020**). Briefly, primers targeting the exon-intron region of the NMDAR subunits (1, 2A-D, 3A-B), AMPAR subunits (1-4), CTIP2, PAX6, and β -actin were custom-designed and tested for specificity using postnatal cDNA, with a single pair per receptor used for further experiments. Each reaction mix contained (µl): 5 SYBR Green Master Mix (Thermo Fisher), 1 forward primer, 1 reverse primer, 1 nuclease free water, and 2 cDNA (~1ng). β -actin was the house-keeping gene, postnatal cDNA the positive control and water the negative control. RT-qPCR was performed as per manufacturer's instructions and results detected with a CFX96 real-time PCR detection system (BioRad). Data was analysed using the ΔC_t method and normalised against respective β -actin values. Cut-off was 0.05 $1/\Delta C_t$, and threshold was 50 RFU.

3.4.2 Western blot methods

(See **General Methods Section 2.3 Transcript and protein quantification methods**)

To quantify NR1 subunit expression in the embryonic brain at the protein level, E16.5 brains were first collected and dissected like those for cDNA synthesis but in sterile cutting aCSF, and processed on collection day. Dissected tissue was homogenised in RIPA buffer (Sigma Aldrich) and protease inhibitor (Thermo Fisher). Samples were centrifuged at 3000 x g for 20 minutes, the supernatant collected, and protein concentration quantified using the Bicinchoninic acid (BCA) assay. Samples were diluted to ~200ng/μl.

Blots were performed per manufacturer's instructions. Briefly, samples were loaded into a pre-cast gel and run at 120V for 90 minutes, before transfer to a PVDF membrane. The membrane was blocked in TBS LiCor buffer at 4°C overnight and cut horizontally along the ~70kDa band. The top (bands >70kDa) was incubated in NR1 primary (expected band ~120kDa; 1:1000, Abcam, ab109182, rabbit) and the bottom (bands <70kDa) in β-actin primary (expected band ~45kDa; 1:1000, LiCor, 926-42210, rabbit). Membranes were washed and incubated with secondary antibody (IRDye® 800CW Donkey anti-rabbit 926-32213, 1:20,000) at room temperature for 1 hour. Membranes were imaged on a LiCor Odyssey 9120 System and analysed in Image Studio Lite (Licor Biosciences). Each band was quantified as % β-actin band intensity.

3.4.3 Cell-based assay methods

(See **General Methods Section 2.4 Cell-based assay methods**)

To assess whether the patient-derived polyclonal and monoclonal NR1-IgG antibodies bind the NR1 subunit, an NR1-specific cell-based assay was performed with the help of Dr Khadija Guerti, Karin Van Cotthem and colleagues (UZA Hospital Lab, Antwerp). Each NR1-IgG antibody was serially diluted to 1:20, 1:500 and 1:12,500 and tested on commercially available EUROIMMUN Anti-Glutamate Receptor (type NMDA) mosaic-6 IIFT kit immunofluorescence chips (comprising HEK293 cells expressing NR1) (fb 112d-

1005-6, EUROIMMUN) following the manufacturer's TITREPLANE protocol (**General Methods, Figure 2.8**). The control antibodies were tested at 1:20 only. Each sample was imaged at 20x and 40x magnification and the fluorescent binding then classified on a 0-4 scale, adapted from existing literature (**Leite et al. 2008, Waters et al. 2008**); 0 – no labelling; 0.5 - weak labelling of few (<20%) transfected cells, with no co-localisation; 1 – weak labelling of some (20-50%) transfected cells, with some co-localisation; 2 – moderate labelling of some (20-50%) transfected cells, with good co-localisation; 3 – moderate/strong labelling of many (50-80%) transfected cells, with precise co-localisation; 4 – strong labelling of most (>80%) transfected cells, with perfect co-localisation.

3.4.4 Immunohistochemical methods

(See **General Methods Section 2.6 Antibody immunohistochemical methods**)

For fixed slice experiments, wild-type brains were collected at E16.5, fixed in 4% PFA for ~72 hours, agar embedded and sliced into 60µm coronal sections. All fixed slices for embryonic immunohistochemistry antibody experiments were permeabilised using PBS-Tx (0.1%) and blocked for 2 hours at room temperature in 10% NDS, before addition of primary antibodies at 4°C overnight. Slices were then washed in PBS and secondary antibodies were added for 2 hours at room temperature. Slices were mounted in Vectashield and imaged on an Olympus Fluorescence microscope.

To **label embryonic glutamate transporters**, the primary antibodies VGLUT1 (rabbit, 1:500) or VGLUT2 (mouse, 1:500) were added, and secondary antibodies (1:500) goat-anti-rabbit 555 or goat-anti-mouse 555 used respectively. Antibodies were not used on the same slices due to issues with bleed-through. Slices were imaged at 20x and 100x (oil) magnification. Distribution of VGLUT1 and VGLUT2 was not strictly quantified but evaluated in a qualitative manner.

To assess **endogenous expression of NR1**, slices were incubated in NMDAR1 primary antibody (rabbit, 1:50), followed by goat-anti-rabbit 488 secondary antibody and imaged at 20x magnification. Images were

analysed in *Image J*, where five ~50µm lines were drawn, starting at the edge of the LGE ventricular boarder and extending towards the centre of the LGE. For consistency, the lines were evenly spaced and drawn perpendicular to the ventricular wall. Exposure time was kept constant at 500ms, and Gain was set to 0. The values along the five lines were averaged to get a value for the mean signal intensity (i.e. mean grey value (MGV)) in the slice.

***In vitro* binding of polyclonal and monoclonal NR1-IgG and control-IgG antibodies** was assessed by using the patient-derived NR1-IgG and control-IgG antibodies (details in **Section 2.2 Patient-derived antibodies**) as primaries at 1:50 with an anti-human secondary on wild-type fixed embryonic slices, and imaging the sections at 20x magnification. Images were analysed in *Image J*, using the same method as for endogenous expression of NR1 described above.

3.4.5 In utero surgical methods

(See **General Methods Section 2.5 In utero surgical methods**)

To characterise aspects of how NR1-IgG antibodies behaved within the living mouse model, the patient-derived polyclonal or monoclonal NR1-IgG or control-IgG antibodies were injected at E14.5, and embryonic heads collected and fixed either 24 or 48 hours later. In all experiments, one half of the embryos were injected with NR1-IgG and the other half with control-IgG to provide an intra-litter control. Embryonic heads were collected at E16.5 and fixed in 4% PFA.

After ~72h fixation, there were removed from the skull and embedded in agar, before being sliced using a vibratome into 60µm coronal sections. Slices containing the LGE were mounted in Vectashield and imaged on an Olympus Fluorescence microscope.

***Ex vivo* binding of polyclonal and monoclonal NR1-IgG and control-IgG in fixed slices** was assessed by using anti-human 488 secondary antibody on the slices injected with either NR1-IgG or control-IgG. Both total antibody and bound antibody only were assessed. To assess total antibody (i.e antibody that was

bound to the brain tissue as well as that which was unbound but still present inside the brain), brains were collected and immediately fixed. To assess bound antibody only, when brains were collected, they were cut in half down the midline to expose the ventricles and washed extensively with PBS for 20 minutes to wash out any unbound antibody, prior to fixation. Total and bound antibody signal was assessed using the same method as for endogenous expression of NR1 described above.

To investigate **regional distribution of the antibodies** within the LGE, the MGV values along the perpendicular lines were averaged into 2µm groups. To assess '**clearance**' of antibody from the brain, brains were fixed at different timepoints post injection (24 hours and 48 hours). Signal was assessed in the LGE using the same method as for endogenous expression of NR1 described above.

For embryonic experiments involving *in utero* injection of antibodies at E14.5, and fixation of slices at E16.5, the overall survival rates at collection were: poly NR1-IgG: 66.0%, poly control-IgG: 100.0%, mono NR1-IgG: 75.0%, mono control-IgG: 66.0%.

3.4.6 Statistical methods

(See **General Methods Section 2.11 Statistical methods**)

Details of specific statistical tests used for each experiment are given in the respective results sections in the chapter. All data is presented as mean $\pm$ SEM. All data was initially normality tested using the Shapiro-Wilks or Kolmogorov-Smirnov test, and the appropriate two tailed parametric (paired t-test, unpaired t-test) or non-parametric (Wilcoxon signed-rank (WSR), Mann-Whitney U (MWU), Mood's Median (MM)) test applied. For all statistical tests, $p < 0.05$ was deemed significant. * $p < 0.05$, ** $p < 0.01$.

3.5 Chapter Results

3.5.1 Exploring expression of early glutamate transporters in the embryonic brain

This study aimed to explore how antibodies against the NR1 subunit of the glutamatergic NMDAR affect neurodevelopment, with specific focus on the striatum. To do this, a novel mouse model was used, whereby patient-derived antibodies against the NR1 subunit are injected into the embryonic brain *in utero*. Since the NMDAR is a glutamatergic receptor, a first important step was to gain more understanding about glutamate and its distribution in the healthy developing mouse striatum. Glutamate is transported into synaptic vesicles by three vesicular glutamate transporters (VGLUT1-3), which can act as a pseudo-marker for the distribution of glutamate. Our first experiments used immunohistochemistry to label VGLUT1 and VGLUT2 in coronal fixed E16.5 brain slices (n=3 brains, 1-2 slices per transporter from each brain), to better understand distribution of glutamate transporters in the developing brain and striatum, which is currently poorly defined in the literature. Qualitative analysis of the staining revealed that in the cortex, the transporters appeared to show visual differences in the distribution between the different cortical layers (**Figure 3.1 A, B**).

In the LGE, VGLUT2 staining appeared to be more intense than VGLUT1 at 20x magnification (**Figure 3.1 C, D**), putatively suggesting greater levels of the VGLUT2 transporter. At 100x magnification, individual puncta could be observed, revealing differences in the distribution of these transporters. VGLUT1 appeared to be arranged in puncta around individual cells in the neuropil and potentially also in innervations of those cells such as the corticostriatal axons. VGLUT2 appeared to be arranged along fibre-like structures, which could also be early axons passing through the LGE. They were also arranged in puncta round individual cells though to a lesser degree than VGLUT1 (**Figure 3.1 E, F**). Staining of the p20 postnatal striatum revealed a similar observable staining intensity for both VGLUT1 and VGLUT2, with more pronounced puncta (lower background staining) for VGLUT1 (**Figure 3.1 G, H**).

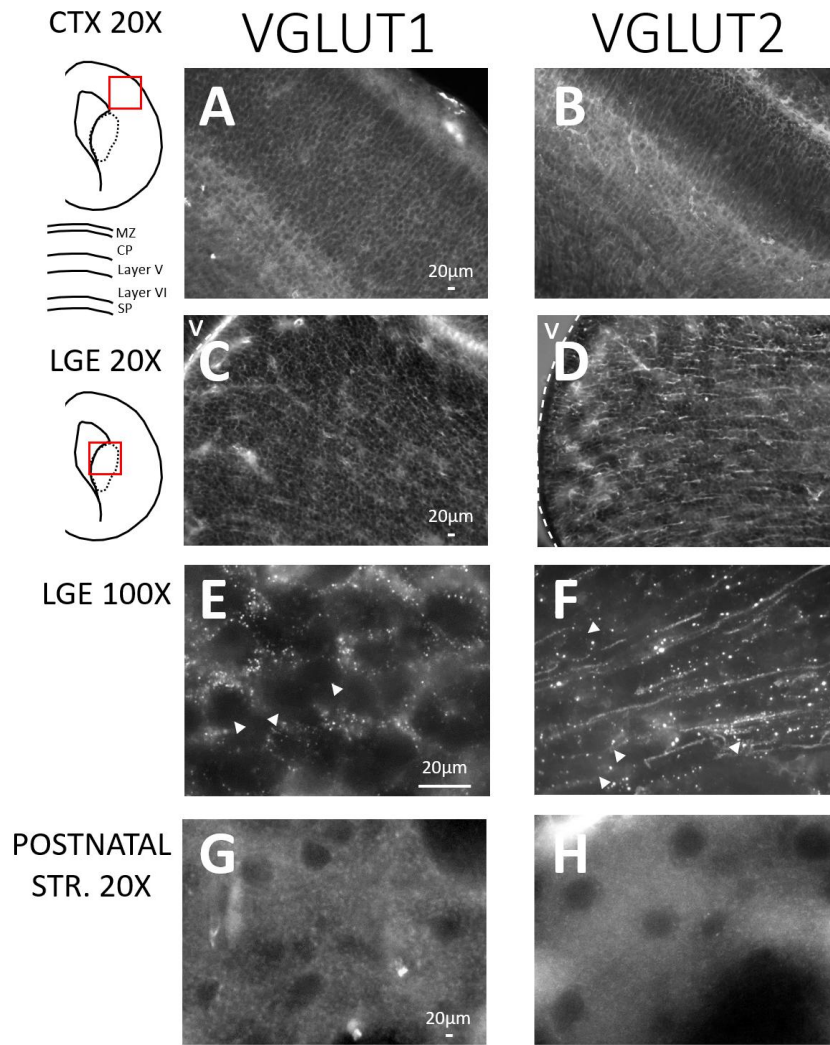


Figure 3.1. Vesicular glutamate transporters in the wild-type embryonic cortex and LGE. Embryonic E16.5 brains were stained with VGLUT1 (rabbit) or VGLUT2 (mouse) primary antibody, followed by the relevant secondary antibodies. **[A-B]** Representative images of VGLUT1 and VGLUT2 staining in the E16.5 cortex of wild-type mice at 20x magnification. Early cortical layers labelled in the left-hand diagram, from outside to inside: MZ = marginal zone, CP = undifferentiated cortical plate, Layer V, Layer VI, SP = subplate. **[C-D]** Representative images of VGLUT1 and VGLUT2 staining in the E16.5 LGE at 20x magnification. v = ventricle. **[E-F]** Representative images of VGLUT1 and VGLUT2 staining in the E16.5 LGE at 100x magnification. Individual puncta are visible, and a difference in the distribution of the staining is revealed. Arrow heads indicate individual cell bodies. **[G-H]** Representative images of VGLUT1 and VGLUT2 staining in the postnatal (p20) striatum at 20x magnification, showing similar staining patterns and intensity.

Overall, these experiments suggest that glutamate can be packaged into structures close to striatal progenitors and neurons, as well as eluding to potential differences in the distribution patterns of VGLUT1 and VGLUT2 in the LGE.

3.5.2 Transcript- and protein-level NR1 target expression in the embryonic brain

3.5.2.1. Transcript-level expression of glutamatergic subunits in the wild-type embryonic brain

Literature suggests regional and temporal differences in expression and importance of glutamatergic receptors during development, and this dynamic expression is a critical feature of many key developmental processes (Gu et al. 1996, Metzger et al. 1998, Hoppmann et al. 2008). Since this thesis explores antibodies that bind to the NR1 subunit of the glutamatergic NMDAR, it was important to first assess and quantify expression of the NR1 subunit and other key glutamatergic subunits in the embryonic brain in healthy wild-type mice. NMDAR and AMPAR subunits were chosen as the focus, since these are the two key ionotropic glutamatergic receptors in the brain and work closely in tandem with one another during neurodevelopment. Experiments by Luk et al. (2003) indicate that striatal neurogenesis may be NMDAR-mediated whereas cortical neurogenesis may be AMPAR-mediated (Luk et al. 2003, Jansson and Åkerman 2014) and so the cortex was chosen as a good comparator region for this set of experiments. Expression was quantified at E16, which falls within the window of NR1-IgG antibody exposure in this model.

The first experiments in this section utilised RT-qPCR to quantify transcript level expression of glutamate receptors and other structural markers in the E16.5 cortex and LGE, relative to a house-keeping gene (β -actin) (**Figure 3.2**). The cortex and LGE were dissected separately out of the embryonic brain and the dissection accuracy was confirmed by detection of significantly higher expression of cortical marker PAX6 in cortex samples (PAX6 cortex: 17.7 ± 0.6 $1/\Delta Ct \cdot 100$, LGE: 5.1 ± 0.01 $1/\Delta Ct \cdot 100$, paired t-test: $p = 4.0 \times 10^{-6}$; $n = 3$ samples (2 brains/sample)) and striatal marker CTIP2 in LGE samples (CTIP2 cortex: 6.0 ± 0.8 $1/\Delta Ct \cdot 100$, LGE: 9.6 ± 0.8 $1/\Delta Ct \cdot 100$, paired t-test: $p = 0.040$; $n = 3$ samples (2 brains/sample); **Figure 3.2 A, B**).

Having confirmed dissection accuracy, transcript-level expression of the NMDAR and AMPAR subunits was next explored. The NMDAR subunits include NR1, NR2A-D and NR3A-B. The AMPAR subunits include A1-4. Analysing NMDAR and AMPAR subunit expression subsequently revealed there was significantly higher expression of the NR1 subunit in the LGE (NR1 cortex: 13.4 ± 0.4 1/ Δ Ct*100, LGE: 16.3 ± 0.6 1/ Δ Ct*100, paired t-test: $p = 0.0002$; $n = 3$ samples (2 brains/sample)), as well as significantly higher expression of AMPAR subunits A2 and A3 (A2 cortex: 14.7 ± 0.4 1/ Δ Ct*100, LGE: 15.3 ± 0.5 1/ Δ Ct*100, paired t-test: $p = 0.00016$; A3 cortex: 8.6 ± 0.2 1/ Δ Ct*100, LGE: 9.5 ± 0.3 1/ Δ Ct*100, paired t-test: $p = 0.030$; $n = 3$ samples (2 brains/sample); **Figure 3.2 C**). NR1, NR2B and A2 were the most highly expressed subunits in both regions.

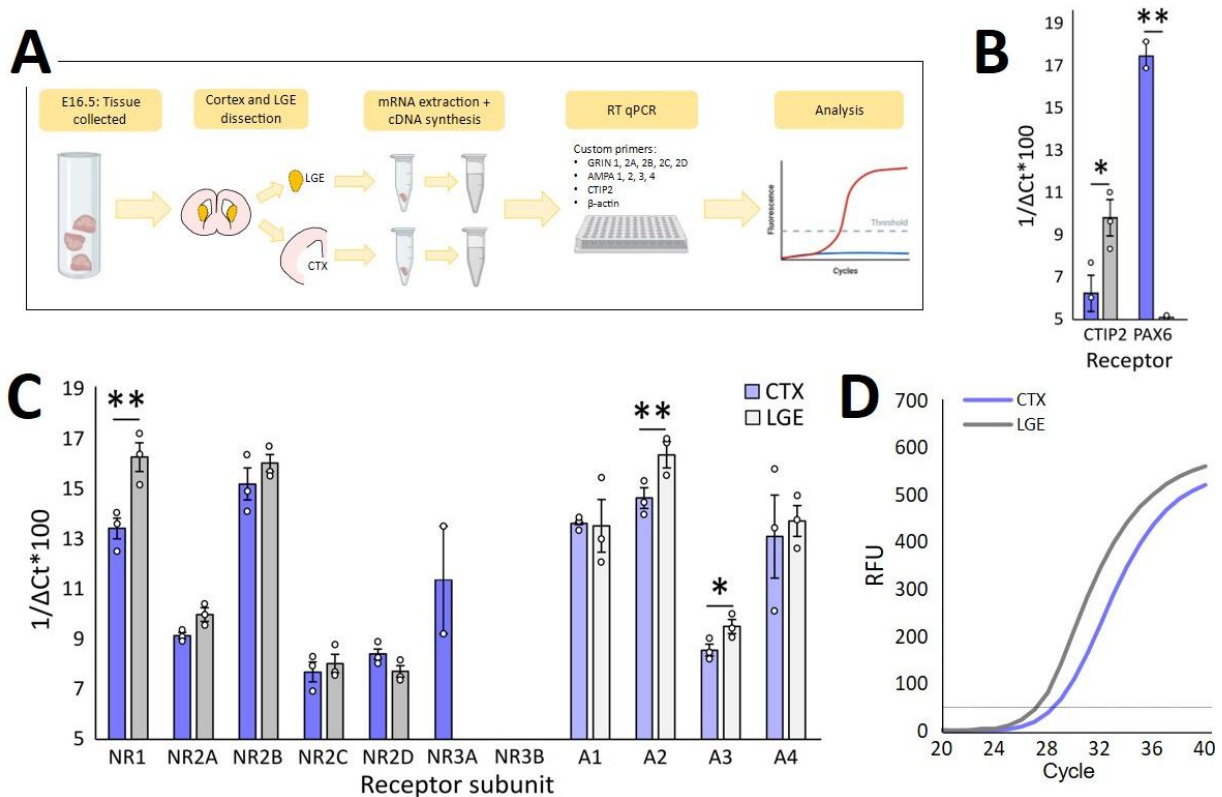


Figure 3.2. Transcript-level glutamate receptor expression in the wild-type embryonic cortex and LGE. [A] Graphical outline of the RT-qPCR methodology to quantify NMDAR and AMPAR subunit expression and delineating cortical (PAX6) and striatal (CTIP2) markers at the transcript level in the E16.5 cortex and LGE. For all panels, $n=3$ samples (2 brains/sample). [B] Bar plot of the expression of CTIP2 (striatal marker) and PAX6 (cortical marker (Manuel et al. 2015)). Expression of CTIP2 was significantly higher in the LGE than cortex and expression of PAX6 was significantly higher in the cortex than LGE, supporting accurate dissection in these samples. [C] Bar plot showing RT-qPCR quantification of the glutamatergic NMDAR subunits (NR1, 2A-D, 3A-B) and AMPAR subunits (A1-4). There was significantly greater expression of NMDAR-NR1, AMPAR-A2, and A3 subunits in the LGE than cortex [D] Mean RT qPCR amplification curve of the NR1 subunit, showing consistent higher expression in the LGE vs CTX.

3.5.2.2. Protein-level expression of NR1 in the wild-type embryonic brain

After identifying differences in transcript-level expression of the NR1 subunit in the LGE vs the cortex, the next experiments sought to explore whether this was also reflected at protein level (Figure 3.3 A). Western blots revealed protein-level expression of NR1 in both the E16.5 cortex and LGE (Figure 3.3 B),

with subtle but significantly greater expression in the LGE (relative NR1 band intensity cortex: 0.6 ± 0.2 % β -actin intensity, LGE: 0.9 ± 0.2 % β -actin intensity, WSR: $p = 0.00012$; $n = 11$ brains; **Figure 3.3 C**).

To further corroborate the above findings, protein-level expression was also assessed with immunohistochemistry using a commercial primary NMDAR NR1-IgG antibody (rabbit, Abcam) applied to E16.5 wild-type fixed sections (**Figure 3.3 D**). Comparison of the relative mean grey value (MGV) signal intensity from the E16.5 cortex and LGE revealed significantly greater expression in LGE compared to cortex (relative endogenous NR1 IHC signal intensity cortex: 1678.3 ± 6.2 MGV, LGE: 2286.9 ± 16.2 MGV, independent t-test: $p = 1.1 \times 10^{-4}$; $n = 3$ brains; **Figure 3.3 E**).

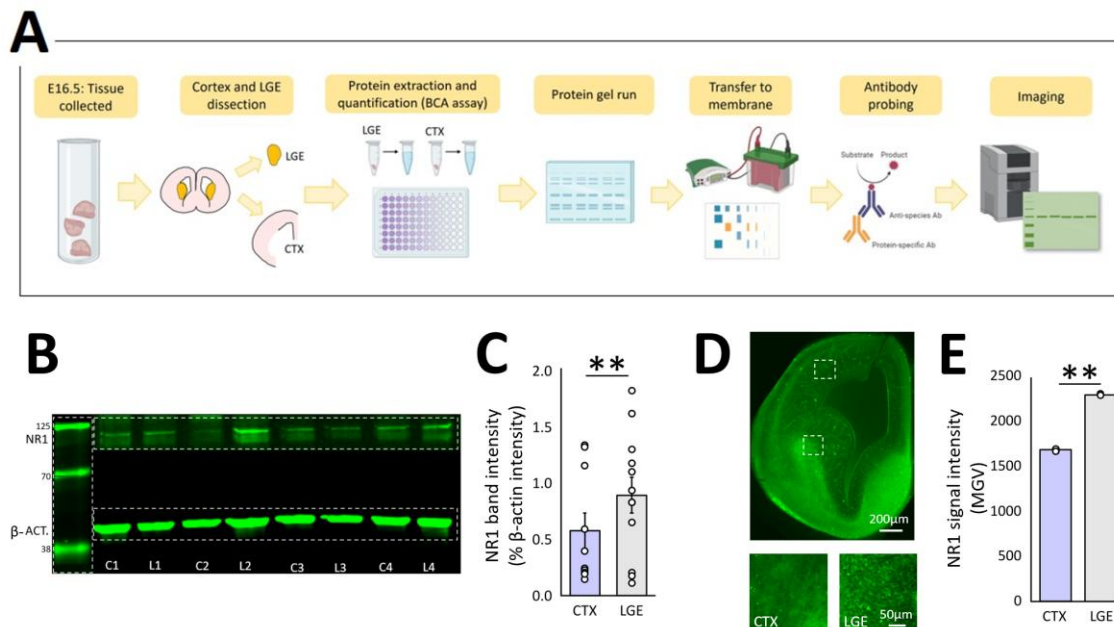


Figure 3.3. Protein-level NR1 expression in the wild-type embryonic cortex and LGE. **[A]** Graphical outline of the Western blot methodology to quantify NR1 in the E16.5 cortex and LGE. $n = 11$ samples (1 brain/sample). **[B]** Representative Western blot of four cortical (C) and LGE (L) samples showing NR1 subunit (expected band ~ 120 kDa) and β -actin (expected band ~ 45 kDa, loading control) bands in all samples. Note the visibly higher levels of NR1 in the LGE, particularly prominent in samples 2 and 4. **[C]** Bar plot of the mean fluorescence intensity of the NR1 band in cortex and LGE samples, relative to the respective β -actin control. **[D]** Representative epifluorescence image of a coronal section of an E16.5 wild-type brain stained for endogenous NR1 using a commercial NMDAR1 antibody (ab109182) with a 488-channel secondary antibody. $n = 3$ brains. **[E]** Fluorescent signal was quantified in the cortex and LGE, showing significantly higher expression of NR1 in LGE than cortex, corroborating what was seen on the Western blot.

Overall, these experiments reveal significant differences in the expression of NR1 and other glutamatergic subunits between the LGE and cortex in the E16.5 brain, and strong expression of NR1 in the E16.5 LGE at the transcript and protein level, that is significantly higher than cortical expression at this developmental stage.

3.5.3 *In vitro* binding of patient-derived NR1-IgG antibodies

3.5.3.1 Binding in an NR1-expressing HEK cell-based assay

Having quantified expression of the NR1 target at both transcript and protein level, the next part of this chapter focused on characterising properties of patient-derived NR1-IgG antibodies used in this project, using both *in vitro* and *ex vivo* experiments.

This project used two NR1-specific IgG antibodies, both derived from patients with NMDARE; one polyclonal antibody and one monoclonal antibody, along with two respectively matched isotype control-IgG antibodies. These antibodies have previously been used in other published research (**Malviya et al. 2017, Wright et al. 2021**) and have been shown to have pathogenic effects (full antibody details in **General Methods, Table 2.1**).

In previous literature, NR1-IgG antibodies have been shown to bind the NR1 subunit, causing internalisation of NMDARs with varying pathological consequences (reviewed in **Giannoccaro et al. 2020**). As the first user of these antibodies in the lab, the first question to answer was whether it could be confirmed that these antibodies actually bind the NR1 subunit. To do this, a cell-based assay was used. The assay itself was performed with the help of Dr Khadija Guerti's lab (UZA Hospital Lab, Antwerp). The polyclonal and monoclonal NR1-IgG antibodies were serially diluted to 1:20, 1:500 and 1:12,500 and applied to EUROIMMUN Anti-Glutamate Receptor (type NMDA) immunofluorescence chips (a cell-based assay with fixed HEK cells expressing NR1) (**Figure 3.4 A, B**). The isotype-matched control-IgG antibodies were only tested at 1:20. The fluorescent binding was subsequently classified based on a visual

classification scale (VCS) of 0-4, adapted from existing literature (Leite et al. 2008, Waters et al. 2008; **Figure 3.4 C**), which is also used for clinical assessment. At a titre of 1:20, i.e. the approximate dilution of the antibody when injected into the E14.5 mouse brain in our model (Zappaterra et al. 2013), both the polyclonal and monoclonal NR1-IgG antibodies bound strongly (poly NR1 VCS: 3, mono NR1 VCS: 4). At the same titre, the polyclonal control-IgG antibody showed only very weak binding, and the monoclonal control-IgG antibody showed no binding (poly ctrl VCS: 0.5, mono ctrl VCS: 0). At 1:20 and 1:500, the polyclonal NR1-IgG antibody showed lower affinity than the monoclonal NR1-IgG antibody (poly NR1 1:20 VCS: 3, 1:500 VCS: 1, mono NR1 1:20 VCS: 4, 1:500 VCS: 2). Each assay was run once (i.e. n = 1).

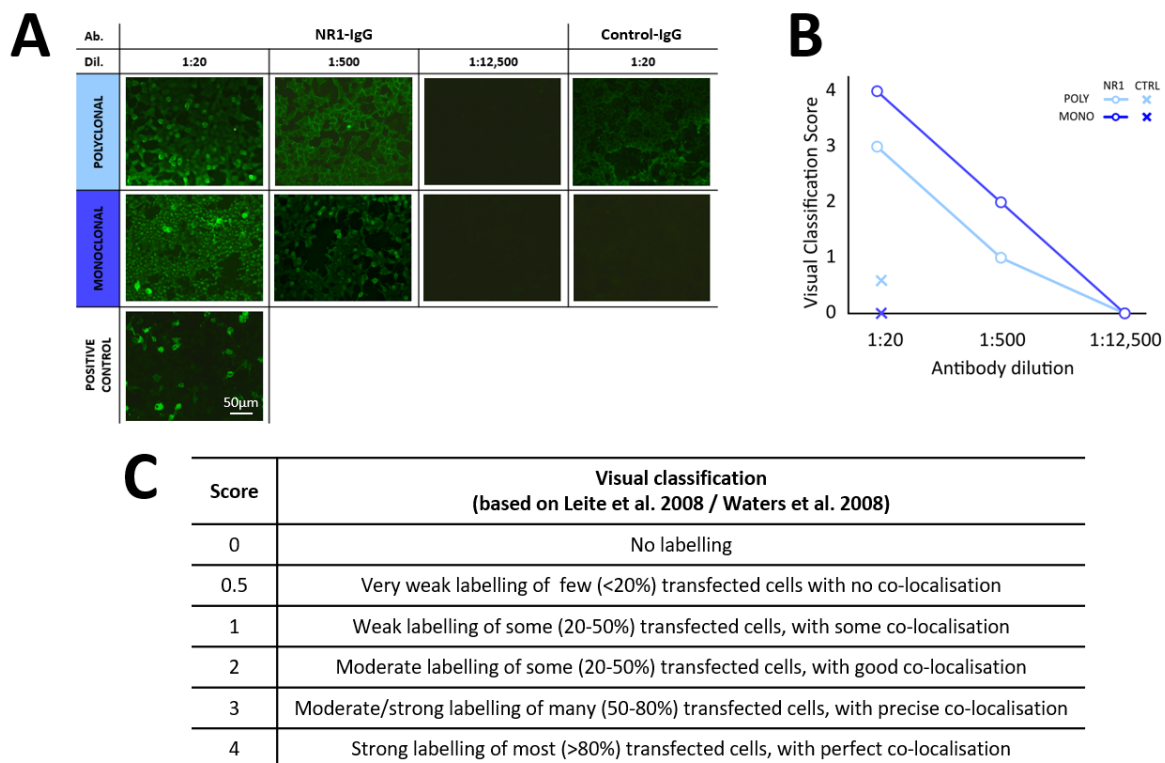


Figure 3.4. NR1-expressing HEK cell-based assay of patient-derived polyclonal and monoclonal NR1-IgG. **[A]** Representative epifluorescence images of NR1-expressing HEK cell immunofluorescence chips, when different titres of polyclonal and monoclonal NR1-IgG antibodies, or isotype-matched control-IgG antibodies were applied. **[B]** Line plot of the HEK cell NR1-IgG antibody binding at 1:20, 1:500 and 1:12,500. Classification of the binding on a 0-4 visual classification scale showed that at 1:20 and 1:500, the monoclonal NR1-IgG antibodies exhibited higher affinity than the polyclonal NR1-IgG antibodies. **[C]** Table of the detailed visual classification scale (VCS). The scale was produced by combining two similar scales in the existing literature (Leite et al. 2008, Waters et al. 2008).

3.5.3.2 Binding in fixed wild-type embryonic brain slices

Having established that the antibodies do indeed bind NR1, the next step was to establish whether they bind neurons and neurites in the embryonic mouse brain, and specifically in our region of interest, the LGE (**Figure 3.5**). Binding of the NR1-IgG antibodies was evaluated by using them as a primary antibody (1:50) on untreated E16.5 fixed coronal sections from wild-type embryos, with an anti-human 488 secondary antibody. The same experiments were also performed with the isotype-matched polyclonal and monoclonal control-IgG antibodies simultaneously for comparison. It is worth noting that the polyclonal and monoclonal antibodies used in this study are thought to be similar in terms of pathogenicity and indeed have been previously combined in other studies (eg. **Wright et al. 2021**). However, in this study it was still important to evaluate and compare both antibodies to ascertain this.

In terms of the polyclonal antibodies, these immunohistochemistry experiments revealed that both NR1-IgG and control-IgG antibodies bound the embryonic LGE (poly NR1-IgG vs 2° only: poly NR1-IgG: 954.0 ± 10.0 MGv, 2°: 274.9 ± 2.4 MGv, independent t-test: $p = 7.2 \times 10^{-5}$; poly control-IgG vs 2° only: poly control-IgG: 412.6 ± 3.1 MGv, independent t-test: $p = 1.3 \times 10^{-5}$; $n = 3$ brains, 1 slice per brain; **Figure 3.5 B**). Mean NR1-IgG binding was significantly higher than polyclonal control-IgG (independent t-test: $p = 6.3 \times 10^{-5}$).

In terms of the monoclonal antibodies, these experiments revealed that both NR1-IgG and control-IgG antibodies also bound the embryonic LGE (mono NR1-IgG vs 2° only: mono NR1-IgG: 1864.7 ± 11.1 MGv, 2°: 274.9 ± 2.4 MGv, independent t-test: $p = 9.5 \times 10^{-7}$; mono control-IgG vs 2° only: mono control-IgG: 558.5 ± 1.4 MGv, independent t-test: $p = 3.0 \times 10^{-5}$; $n = 3$ brains, 1 slice per brain; **Figure 3.5 B**). Mean monoclonal NR1-IgG binding was also significantly higher than monoclonal control-IgG (independent t-test: $p = 7.9 \times 10^{-5}$). Additionally, comparing polyclonal NR1-IgG binding to monoclonal NR1-IgG binding revealed that monoclonal NR1-IgG exhibited significantly stronger binding (poly NR1-IgG vs mono NR1-

IgG: poly NR1-IgG: 954.0 ± 3.3 MGv, mono NR1-IgG: 1864.7 ± 6.2 MGv, independent t-test: $p = 2.4 \times 10^{-6}$).

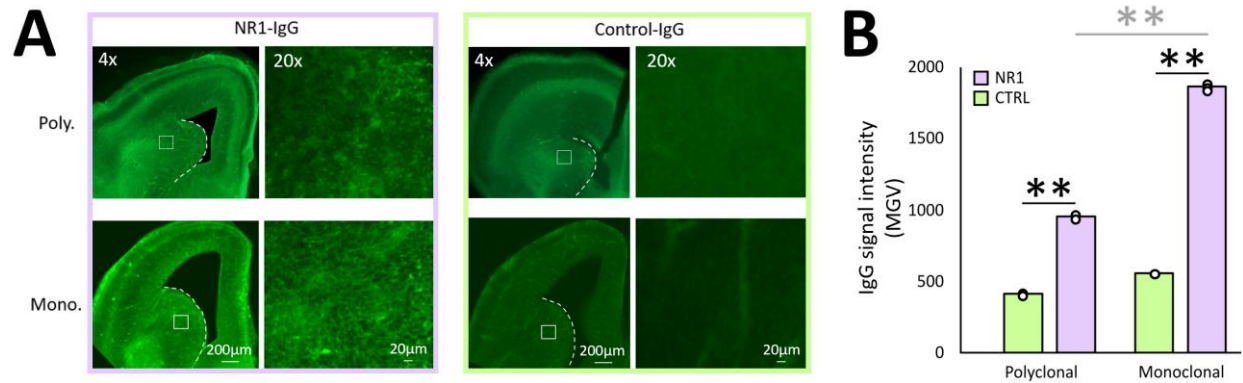


Figure 3.5. Binding of patient-derived NR1-IgG antibodies in fixed wild-type coronal embryonic slices. **[A]** Representative epifluorescence images of E16.5 coronal sections stained with polyclonal or monoclonal NR1-IgG (purple box) or polyclonal or monoclonal matched control-IgG (green box). Images on the left side of the respective boxes are taken at 4x magnification. The dashed line demarks the ventricular boarder of the LGE. Images on the right side are at 20x magnification, zoomed into the LGE. **[B]** Bar plot quantification of the binding of polyclonal and monoclonal NR1-IgG and control-IgG, quantified as the MGv along 3 perpendicular lines in the LGE (drawn from ventricle inwards). Quantification reveals significantly stronger binding of NR1-IgG vs respective control-IgG, for both polyclonal and monoclonal antibodies. Comparison between the polyclonal and monoclonal NR1-IgG antibodies also reveals significantly stronger binding of monoclonal NR1-IgG antibody.

3.5.4 Ex vivo dynamics of patient-derived NR1-IgG antibodies

Within the current literature, many different mouse and other models have been used to study antibody-mediated diseases in development (reviewed in **Giannoccaro et al. 2020**) using a range of antibody delivery methods, most predominantly intraperitoneal or tail-vein injections into the pregnant dam. In this thesis, a novel model was developed, whereby NR1-IgG antibodies were injected directly into the embryonic brain *in utero* and the effects then studied embryonically (**Experimental Chapter B**) and postnatally (**Experimental Chapter C and D**). To our knowledge, this was the first time this delivery method has been used to study the effects of NR1-IgG antibodies in the developing brain, and therefore it was important to characterise the dynamics of these antibodies in the embryonic brain when delivered

in this way. To do this, antibodies were injected at E14.5, with half the litter being injected with NR1-IgG and half being injected with matched control-IgG (**Figure 3.6 A, B**). Different litters were used for the polyclonal and monoclonal antibodies. To assess the binding of the antibodies to the brain tissue, brains were cut in half down the midline to expose the ventricles and washed extensively before fixation and sectioning. Immunohistochemistry experiments applying anti-human 488 secondary antibody to the sections and then quantifying intensity of the fluorescence revealed that NR1-IgG binding was significantly higher than control-IgG binding for both the polyclonal and monoclonal antibodies (poly NR1-IgG vs poly control-IgG: poly NR1-IgG: 516.7 ± 7.4 MGv, poly control-IgG: 487.1 ± 11.4 MGv, independent t-test: $p = 0.032$; mono NR1-IgG vs mono control-IgG: mono NR1-IgG: 760.9 ± 16.8 MGv, mono control-IgG: 462.8 ± 4.0 MGv, independent t-test: $p = 6.4 \times 10^{-5}$; $n(\text{poly NR1-IgG}) = 3$ brains, $n(\text{poly control-IgG}) = 4$ brains, $n(\text{mono NR1-IgG}) = 4$ brains, $n(\text{mono control-IgG}) = 3$ brains, all 1 slice per brain), and that monoclonal NR1-IgG bound more than the polyclonal equivalent (poly NR1-IgG vs mono NR1-IgG: independent t-test: $p = 0.00021$; **Figure 3.6 C**). There was no significant difference between control-IgG antibodies (poly control-IgG vs mono control-IgG: independent t-test: $p = 0.35$).

The percentage of antibody bound 48 hours after injection was assessed through comparing the intensity of the antibody signal following addition of anti-human 488 secondary antibody in slices where brains had been washed thoroughly with PBS following collection before fixation (as above), with those that were immediately fixed in 4% PFA on extraction (so both bound and unbound antibody would be captured). This revealed that binding of monoclonal NR1-IgG antibody was highest at 86.8%, followed by polyclonal NR1-IgG antibody at 53.4% (**Figure 3.6 D**). The binding of the control antibodies was similar in both cases, with polyclonal control antibody at 37.8% and monoclonal control antibody at 31.2%.

The removal ('clearance') of antibody from the brain was next investigated by injecting the brains with the NR1-IgG and control-IgG antibodies at E14.5 and then fixing them at two different timepoints: 24 hours or 48 hours later. Comparison of the binding at the two different timepoints revealed that for

polyclonal antibodies, NR1-IgG showed a trend towards a significantly higher signal at 24 hours than 48 hours (24h vs 48h NR1-IgG: 24h: 626.3 ± 22.9 MGv, 48h: 470.5 ± 3.1 MGv, MWU: $p = 0.083$) and control-IgG showed a significantly higher signal at 24 hours than 48 hours (24h vs 48h control-IgG: 24h: 548.7 ± 28.9 MGv, 48h: 383.1 ± 3.9 MGv, MWU: $p = 0.020$). For the monoclonal antibodies, signal was significantly higher at 24 hours than 48 hours for both NR1-IgG and control-IgG (24h vs 48h NR1-IgG: 24h: 852.8 ± 75.6 MGv, 48h: 549.8 ± 7.7 MGv, MWU: $p = 0.024$; 24h vs 48h control-IgG: 24h: 857.2 ± 86.9 MGv, 48h: 395.7 ± 3.4 MGv, MWU: $p = 0.0020$; **Figure 3.6 E**). N.B. higher signal reflects more antibody present.

Finally, antibody distribution within the LGE was assessed at 48 hours, through measuring and averaging the signal at $2\mu\text{m}$ intervals along perpendicular lines drawn inwards from the ventricle. This revealed that all antibodies had a similar flat distribution profile. For the polyclonal antibodies, there was similar binding for both NR1-IgG and control-IgG close to the ventricle, with differences only appearing at $\sim 20\mu\text{m}$ from the ventricle onwards, with NR1-IgG binding greater than control-IgG binding (MM: 0- $10\mu\text{m}$, 10- $20\mu\text{m}$: $p = 0.15$, 20- $30\mu\text{m}$, 30- $40\mu\text{m}$, 40- $50\mu\text{m}$: $p = 0.040$). For the monoclonal antibodies, NR1-IgG binding was significantly greater than control-IgG binding at all distances measured from the ventricle (MM: 0- $10\mu\text{m}$, 10- $20\mu\text{m}$, 20- $30\mu\text{m}$, 30- $40\mu\text{m}$, 40- $50\mu\text{m}$: $p = 0.040$; **Figure 3.6 F**).

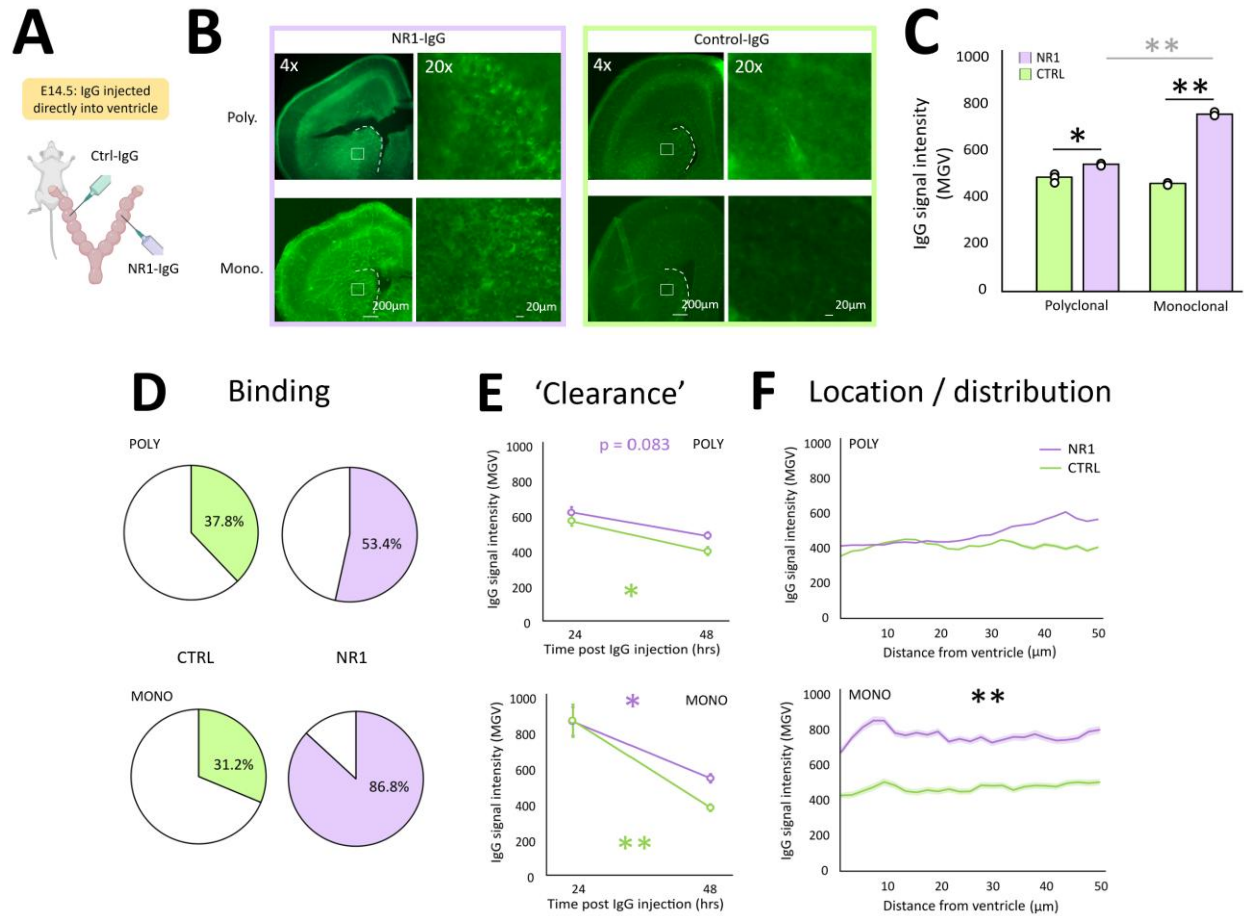


Figure 3.6. Dynamics of patient-derived NR1-IgG antibodies when injected in utero. **[A]** Graphical outline of method, whereby antibodies were intraventricularly injected in utero at E14.5. One half of the uterus was injected with NR1-IgG and the other with control-IgG. **[B]** Representative epifluorescence images of coronal sections fixed at E16.5, 48 hours post-injection of antibodies, following staining with anti-human 488 secondary antibody. Brains were either injected with polyclonal or monoclonal NR1-IgG (purple box), or polyclonal or monoclonal matched control-IgG (green box). **[C]** Quantification of the binding of polyclonal and monoclonal NR1-IgG and control-IgG, quantified as the MGV along 3 perpendicular lines in the LGE (drawn from the ventricle inwards). Quantification revealed significantly stronger binding of NR1-IgG vs respective control-IgG, for both the polyclonal and monoclonal antibodies. Comparison between polyclonal and monoclonal NR1-IgG antibodies also revealed significantly stronger binding of monoclonal NR1-IgG. **[D]** Pie charts showing antibody bound to the LGE at 48 hours, quantified as a percentage of the total antibody present (bound and unbound). Quantification revealed both NR1-IgG antibodies had a large percentage of antibody still bound to the LGE at 48h post-injection. **[E]** Line plots showing the intensity of the fluorescent antibody signal (i.e. binding) at 24 hours and 48 hours post-injection, revealing that for NR1-IgG and control-IgG antibodies, binding was greater at 24 hours than at 48 hours (significant for polyclonal control-IgG, and both monoclonals, and trending towards significance for the polyclonal NR1-IgG). **[F]** Line graphs showing the fluorescent antibody signal intensity relative to the distance from the ventricle, revealing that for the polyclonal antibodies signal was similar in the first 20µm from the ventricle, but for monoclonal antibodies, NR1-IgG signal was significantly higher at all distances measured.

Overall, these results show that the polyclonal and monoclonal NR1-IgG antibodies bind NR1 in a cell-based assay and bind neurons and neurites in the LGE both *in vitro* and *ex vivo*. When further characterised *ex vivo*, this data suggests NR1-IgG and control-IgG antibodies are both removed from the LGE over the first 48 hours and show subtle differences in the way that they are distributed.

3.6 Chapter Discussion

This study aimed to assess how patient-derived NR1-IgG antibodies affect development of the LGE/striatum in a mouse model. This chapter set out to characterise key features of that mouse model, including embryonic distribution of glutamate transporters and receptors in the brain, and *in vitro* and *ex vivo* binding and dynamics of the polyclonal and monoclonal NR1-IgG antibodies in the LGE. The key findings were:

- 1) Vesicular glutamate transporters VGLUT1 and VGLUT2 are both present in the embryonic cortex and LGE, suggesting that glutamate-loaded vesicles are present.
- 2) Transcript- and protein-level expression of NR1 are both higher in the LGE than cortex suggesting that the NR1 subunit is highly expressed and potentially functional.
- 3) Both polyclonal and monoclonal patient-derived NR1-IgGs bind NR1 *in vitro* in a cell-based assay.
- 4) Both NR1-IgG antibodies bind the mouse LGE *in vitro* and *ex vivo*, with the monoclonal antibody exhibiting stronger binding.
- 5) Antibodies exhibit a similar pattern of distribution in the LGE *ex vivo* and show higher binding at 24 hours than 48 hours post-injection.

3.6.1. Vesicular glutamate transporters VGLUT1 and VGLUT2 are both present in the embryonic cortex and LGE, suggesting that glutamate loaded vesicles are present;

Glutamate, the main excitatory neurotransmitter in the mammalian brain, is key for neurogenesis. It is involved in a host of neurodevelopmental processes including tightly regulating proliferation, survival, differentiation and migration of neural progenitor cells, enabling them to eventually form the neurons of the mature brain (Jansson and Åkerman 2014).

To gain understanding about glutamate distribution in the early brain, the first experiments investigated the presence of two primary transporters of glutamate, VGLUT1 and VGLUT2, in the LGE.

Immunohistochemistry labelling revealed the clear presence of both transporters in the LGE at E16.5. These findings specifically show that there is machinery in place that can load glutamate into vesicles during the embryonic periods, which is suggestive that there is loading and releasing of glutamate in the embryonic brain, placing glutamate as an early and possibly important player in striatal development during this stage (**Mount et al. 1993, Forrest et al. 1994, Copani et al. 1998, Monti and Contestabile 2000, Boulland et al. 2004**).

Although not formally quantified, observation of microscope images taken of VGLUT1 and VGLUT2 immunohistochemical antibody staining in the LGE appeared to show a higher staining intensity for VGLUT2, suggesting potential greater prevalence of this transporter in the LGE at this stage. This would align with the quantitative immunoblotting results seen in mature rodent brains which demonstrate that VGLUT1 and VGLUT2 show complimentary distribution patterns between the telencephalic and diencephalic regions respectively, with VGLUT2 expressed most highly in the striatum, thalamus and pons, and VGLUT1 expressed predominantly in the cortex and hippocampus (**Freneau et al. 2001, Herzog et al. 2001, Varoqui et al. 2002, Boulland et al. 2004**). Additionally, it would also fit experimental observations in the literature using immunohistochemical staining of the VGLUT transporters at selected developmental stages from E15 up to p21, that in embryonic and early postnatal development, global expression of VGLUT2 is higher than VGLUT1, with VGLUT1 expression gradually increasing as the brain matures, replacing VGLUT2 in several regions (**Boulland et al. 2004**).

The staining patterns of VGLUT1 and VGLUT2 also appeared different in the embryonic cortex, with VGLUT1 most highly expressed in the marginal zone and SP, with lower-level staining in the undifferentiated cortical plate and layers V and VI, and VGLUT2 most highly expressed in the marginal zone and layers V and VI, but to a lower degree in the undifferentiated cortical plate or subplate. These distribution patterns align closely with the patterns observed by **Nakamura et al. (2005)** using immunohistochemical localisation of VGLUT1 and VGLU2 in p0 mouse brain slices.

In the embryonic LGE, observations here suggest that VGLUT1 and VGLUT2 are arranged in puncta around individual cells. This largely fits literature showing that during the early postnatal development period, immunoreactivity for VGLUT1 and VGLUT2 within telencephalon structures is predominantly found in the neuropil, likely in axon terminals (**Nakamura et al. 2005**). Interestingly, the distribution of VGLUT2 puncta was also observed to be arranged along fibre-like structures, which could be parts of early axons passing through the LGE. It is important to note that interpretation of specific distributions of VGLUT1 and VGLUT2 here may be limited here by use of different secondary antibodies.

The exact reason for why VGLUT2 is observed along fibre-like structures in the LGE additionally to the puncta surrounding cells is somewhat unclear, and whilst there is limited literature on VGLUT distribution in the embryonic brain, there is precedent for differences in VGLUT distribution in the developing striatum and wider brain. This includes observations that VGLUT1 and VGLUT2 are transiently predominantly concentrated in striatal matrix and striatal patches respectively during the first postnatal week, with these differences no longer present by adulthood (**Boulland et al. 2004**). The striatum is also known to be innervated by both VGLUT1- and VGLUT2-expressing neurons (**Härtig et al. 2003**). Thus, what is observed here could be reflect preferential expression of VGLUT2 early on or suggest that certain VGLUT2-expressing axons traversing the striatum develop earlier than VGLUT1-expressing axons, potentially aligning with the wider VGLUT2 to VGLUT1 switch seen in several brain structures during postnatal maturation (**Boulland et al. 2004**). The differences observed early on by us here may have a specific developmental purpose, the exact nature of which requires further experimental work to establish.

In further study, it would also be useful to quantify glutamate itself to identify regional specificity for where it is at its highest levels. Direct visualisation of glutamate was attempted using immunohistochemistry with two commercial antibodies but was unsuccessful in this instance, however other antibodies or methods may enable this. Additionally, this part of the project was somewhat limited by only qualitatively assessing samples, so a helpful extension would be to quantify VGLUT1 and VGLUT2

puncta accurately using stereological methods and high-resolution microscopy. Furthermore, it would be useful to explore potential possible early sources of striatal glutamate such as further characterising early corticostriatal axons entering the striatum (Sohur et al. 2014).

3.6.2. Transcript- and protein-level expression of NR1 are both higher in the LGE than cortex suggesting that the NR1 subunit is highly expressed and potentially functional;

It has been suggested that one mechanism through which glutamate regulates embryonic neurogenesis is directly through glutamate receptors expressed on neural progenitor cells and their derivatives (Zafra et al. 1991, Lee et al. 2002, Schlett 2006, Mattson 2008), and that the morphogenic effects it exerts vary with receptor subtype (Blanton and Kriegstein 1991, Behar et al. 1999, Luk et al. 2003). My lab has previously demonstrated that NMDAR and AMPAR subunit expression is highly dynamic in early postnatal development (Krajeski et al. 2019). Here, differences in transcript-level expression of the subunits of both NMDARs and AMPARs in the embryonic cortex and LGE were identified. From the non-obligate subunits of the NMDAR, our observation that NR2B was the most highly expressed in both the cortex and LGE fits current literature that NR2B is a predominant subunit at this stage (Gambrill and Baria 2011), and plays a key role in neurogenesis, migration and synaptogenesis (Loftis and Janowsky 2003). Interestingly, in the context of this study, it is worth noting that NR1-IgG exposure preferentially clusters NR2B-NMDARs (Ladépêche et al. 2018) and therefore it might be hypothesised that exposure at a point when NR2B is particularly high could have greater effects on processes governed by the NR2B subunit. Moreover, it suggests that NR1/NR2B is a dominant configuration of the NMDAR at this stage.

High levels of transcript-level expression of the NR1 subunit were also seen, which would be expected as the obligate subunit. Comparing the LGE and cortex revealed significantly higher levels of NR1 in the LGE at both the transcript and protein level. Supporting these findings, work by Kovács et al. (2001) showed that cultured embryonic rat striatal neurons exhibited higher or similar transcript-level expression of NR1

compared to cortical neurons. The same study paradoxically found a significantly lower relative protein-level NR1 expression in striatal cultures, contradicting our results. One key reason for this might be due to technical differences in our models. In this study, embryonic mouse brains at E16.5 were used, and utilised freshly dissected striatal and cortical tissue, whereas **Kovács et al. (2001)** used embryonic rat brains at E18-20, from which they grew cortical and striatal cultures. The transformation into cultures could impact how much protein they were able to extract for protein quantification experiments and additionally there may have been differences in the way they dissected the cortical and striatal regions relative to our experiments. Additionally, there could be differences due to the different species or time points used, especially since NMDAR subunits are dynamic over development (**Krajeski et al. 2019**). In further support of our experiments, adult rodent data also demonstrates regional differences in NR1 subunit expression specifically, with highest expression in the striatum, and lowest expression in the cortex (**Goebel and Poosch 1999**). It should also be considered that a limitation in the approach here is that it is very difficult to completely accurately dissect the cortex and the LGE in the embryonic brain, although much care was taken to make this as accurate as possible.

A higher relative number of NR1 subunits would suggest there may be a greater number of NMDARs overall, since NR1 is required to form functional NMDARs. Our observations would align with the notion of specific importance of NMDARs in embryonic striatal development during the neurogenic period, formulated by **Luk et al. (2003)**, following their observations that NMDAR antagonism of rat E15-E18 brains with NMDAR antagonists MK-801 and CGS 19755 reduces proliferation in the LGE and subsequent adult striatal neuron numbers. Interestingly, administration of these drugs in the post-proliferative phase (E18-E21) had no significant effects.

Previous early literature proposes conversely that cortical neurogenesis is primarily AMPA-mediated, with experiments demonstrating that embryonic cortical cultures were sensitive to AMPAR/KAR blockade, and

that proliferation in cortical regions was reduced in brains of rodents injected with AMPAR/KAR but not NMDAR antagonists (Lo Turco et al. 1995, Haydar et al. 2000, Luk et al. 2003). In our experiments however, significantly higher transcript-level expression of A2 and A3 subunits in the LGE relative to the cortex was observed. One hypothesis is that previous experiments largely use AMPAR antagonists to identify predominant mechanisms, whereas here only subunit expression at transcript level was quantified. Since transcript- and protein-level expression do not have a 1:1 relationship there may be high mRNA in the LGE that does not relate to protein-level expression. Furthermore, expression and function are not directly correlated, so even if the LGE does contain more protein subunits, they may be in a non-functional capacity (eg. not expressed in or trafficked to the correct locations). Indeed, it is primarily the long-tailed A1 and A4 subunits which dictate trafficking properties of AMPARs (Henley and Wilkinson 2016) and these show similar expression here. A helpful future extension to this project to address this question would be to also quantify the AMPAR (and remaining NMDAR) subunits at protein level.

3.6.3. Both polyclonal and monoclonal patient-derived NR1-IgGs bind NR1 *in vitro* in a cell-based assay;

NR1-IgG antibodies from patients with NMDARE are thought to bind to the NR1 subunit of the NMDAR, resulting in receptor cross-linking, internalisation, and downstream pathological consequences (Hughes et al. 2010, Castillo-Goméz et al. 2017, Pan et al. 2018). Evaluation of the two main patient-derived NR1-IgG antibodies used in this thesis (one polyclonal and one monoclonal) on an NR1-expressing HEK cell-based assay confirmed the antibodies bound NR1 as expected. For the polyclonal NR1-IgG antibody the affinity was slightly lower than the values reported by Dr Sukhvir Wright (personal correspondence with Dr Sukhvir Wright, data from 2018; **Appendix, Supplementary Figure S3.1**), and the polyclonal control-IgG antibody also showed some very weak non-specific binding, potentially owing to the increased age of these antibodies. It is also important to consider the subjective nature of the VCS which may account for the discrepancy here. There is no published data to our knowledge on the affinity of the monoclonal

antibodies used so these are harder to comment on in the context of the literature. Comparing the polyclonal and monoclonal NR1-IgG antibodies, the observation that the monoclonal NR1-IgG antibody demonstrated a higher affinity fits expectations since monoclonal antibodies are clones of a single parent, and therefore only recognise a single epitope, hence are much more specific than polyclonal antibodies.

3.6.4. Both NR1-IgG antibodies bind the mouse LGE *in vitro* and *ex vivo*, with the monoclonal antibody exhibiting stronger binding;

The next experiments assessed binding in fixed wild-type slices of mouse tissue and revealed binding of NR1-IgG to the neurons and neurites of the LGE for the first time. Binding *in vitro* in wild-type slices appeared to be relatively diffuse in the embryonic brain and LGE, and largely cytoplasmic within the cells of the LGE, similar to the visual patterns of binding seen in cell cultures and brain regions, including the embryonic hippocampus, in several other studies using similar tissue and time points (**Wright et al. 2015b, Jurek et al. 2019, García-Serra et al. 2020**). There was also minimal binding of the control-IgG antibodies as previously reported (**Wright et al. 2021**). Some background binding may be accredited to the increased age of the antibodies. The observation that binding intensity was higher for monoclonal NR1-IgG relative to polyclonal NR1-IgG was also as expected due to the aforementioned differences in specificity.

There are several methods by which these antibodies can be delivered in an animal model to study their impact on neurodevelopment. Here, embryos were injected intraventricularly *in utero* at E14.5. Binding observed 48 hours later for both NR1-IgG antibodies was similar to that seen in embryonic E19 brains, where similar antibodies were delivered by intraperitoneal injection to the dam over E13-E17 (**Jurek et al. 2019**) and also to binding of similar antibodies delivered via osmotic pumps intraventricularly into early postnatal mice; specifically that the antibody was diffusely distributed in the brain including the developing LGE, and largely confined to the cellular cytoplasm (**Wright et al. 2015b**). The diffuse

distribution suggests that the majority of SPNs in the developing striatum express NMDARs which are bound by the antibody.

The increased binding of the monoclonal antibody observed *in vitro* was also seen following *in utero* injections. The finding that the percentage of antibody bound to the LGE was highest for monoclonal NR1-IgG, followed by polyclonal NR1-IgG, with both control-IgG antibodies exhibiting much lower binding, likely testifies to the differing specificity of the antibodies for NR1. The intensity of both the NR1-IgG and control-IgG antibodies decreased significantly between 24 and 48 hours, which may be explained by the flow of CSF clearing the antibodies from the ventricle, or alternatively by internalisation and degradation of the antibody-receptor complex shortly post binding, in line with the literature (**Hughes et al. 2010, Mikasová et al. 2012, Moscato et al. 2014, Planagumà et al. 2016, Castillo-Goméz et al. 2017, Ladépêche et al. 2018, Pan et al. 2018, Huang et al. 2020**).

3.6.5. Antibodies exhibit a similar pattern of distribution in the LGE *ex vivo*, and show higher binding at 24 hours than 48 hours post-injection;

In these experiments, although binding of both NR1-IgG antibodies decreased between 24 hours and 48 hours post-injection, they were both still present at the 48-hour timepoint. This observation aligns with the findings by **Jurek et al. (2019)** who injected a similar NR1-IgG antibody intraperitoneally into pregnant dams from E13 up to E17 and still detected NR1-IgG antibody binding in the embryonic brain tissue at E19 i.e. 48 hours later. Similarly, **García-Serra et al. (2020)** administered NR1-IgG antibodies via maternal tail-vein injections over three consecutive days up until E16 and detected NR1-IgG binding in whole brain slices at E18 i.e. 48 hours later. An interesting extension of the project would be to evaluate binding at further time-points (eg. 12 hours or 72 hours post-injection) to narrow the time window as to when the antibody is still present and bound to the structures in the LGE. This could help direct focus to which

particular developmental processes might be more greatly impacted, since they largely have specific temporal windows in which they occur (**General Introduction, Figure 1.2**).

In these experiments, relatively similar distribution profiles of both the NR1-IgG antibodies across the LGE was seen, which would suggest on a macro-scale that NR1 has an even distribution within the LGE, aligning with the uniform distribution patterns seen in other studies in the embryonic and early postnatal hippocampus (**Wright et al. 2015b, Jurek et al. 2019, García-Serra et al. 2020**), and whole brain sections (**García-Serra et al. 2020**). It is important to consider however that there could be nanoscale differences not observable at this magnification, particularly in light of more recent findings that NR1-IgG binding results in subunit-specific nanoscale redistribution of NMDARs (**Ladépêche et al. 2018**). Further experimental work would be useful to establish this as well as to explore potential differences in binding on specific neuronal subtypes eg. SPNs vs interneurons.

The key advantages of our novel model for investigating NR1-IgG antibodies are that a much smaller volume of antibody is required for each experiment, and that it also removes maternal factors such as variation in the permeability of the blood-brain barrier between different animals or systemic inflammatory effects. Alongside these benefits, the clear binding and similar binding patterns to other delivery methods support this as a likely useful and viable model for studying the impact of these patient-derived NR1-IgG antibodies on the developing brain. It is important to consider that there are limitations to any model. Specific here, results from mice do not always translate across to humans, and the need for general anaesthetic to administer antibodies can also impact developmental parameters such as proliferation (**Sall et al. 2009**). These limitations are acknowledged and comparisons of NR1-IgG data with matched control-IgG data throughout the thesis is also useful to help mitigate these.

3.7 Chapter Summary

This chapter set out to characterise and validate key features of a novel *in utero* intraventricular model of delivering NR1-IgG antibodies directly to the embryonic mouse brain. In this chapter, early distributions of glutamate transporters (as pseudo-markers for glutamate and glutamate action) in the early brain were explored. Glutamatergic subunits were quantified and regional differences identified, and pertinently, high transcript- and protein-level expression of the NR1 subunit in the LGE was found (significantly more so than the cortex). It was also established that the patient-derived polyclonal and monoclonal NR1-IgG antibodies both bind NR1 *in vitro* and also bind in the LGE when injected intraventricularly *in utero* at E14.5, with binding still being detectable 48 hours post-injection. Binding had a similar appearance to other antibody delivery routes in the published literature.

Having established and validated key characteristics of our model, the next chapter explored the embryonic impacts on of the NR1-IgG antibodies the developing mouse striatum.

4. Experimental Chapter B: How does brief *in utero* exposure to NR1-IgG antibodies affect neurogenesis and early striatal formation embryonically?

4.1 Chapter Introduction

In **Experimental Chapter A**, key parameters of our novel *in utero* intraventricular injection model for assessing impact of patient-derived NR1-IgG antibodies on the developing mouse LGE and cortex were defined and validated.

As outlined in **General Introduction Section 1.3.5 Why is the striatum an interesting potential target for NMDAR antibodies?**, evidence suggests that the main input nucleus of the basal ganglia, the striatum, may be of particular importance and interest regarding the effects of NR1-IgG antibodies during early development, but this remains largely unexplored. Therefore, work in **Experimental Chapter B** set out to investigate how these antibodies affect the developing striatum embryonically.

4.2 Chapter Brief Background

4.2.1 Early striatal (and cortical) development

The striatum is the main input nucleus of the basal ganglia and is involved in a wide range of functions including motor control, executive function, reward, cognition, and reinforcement (**Nestler et al. 2009, Graybiel and Grafton 2015, Yager et al. 2015, Kim and Im 2019, Fieblinger 2021**). It originates from the LGE, one of the four main embryonic regions in the ventral telencephalon. Here, during early neurodevelopment, an initial neuroepithelial population differentiates into radial glia and other embryonic progenitors. These subsequently give rise to most neurons (neurogenesis) and glia that compose the striatum (**Marín and Müller 2014**). In rodents, striatal neurogenesis typically occurs between E14.5-18.5 (**Van der Kooy and Fishell 1987, Deacon et al. 1994, Olsson et al. 1995, Chen et al. 2017**). Anatomically, radial glia and other apical progenitors initially line the LGE ventricle ('the ventricular zone' (VZ)). Later in development, a new proliferative area forms basally, known as the 'sub-ventricular zone' (SVZ) (**General Introduction, Figure 1.10**). This zone progressively expands to become the main proliferative region, and this expansion gives rise to a diverse population of intermediate progenitors (IPs) (**Smart 1976, Fentress et al. 1981, Pilz et al. 2013, Kelly et al. 2018**). Specific LGE progenitor pools can be identified and lineage traced into the mature striatum through various methods including using expression of specific markers such as Tα1 (**Van Heusden, Macey-Dare et al. 2021**). The progenitors undergo differentiation towards final identity, concurrent alongside a well characterised sequence of migration steps away from the ventricular wall towards their final destination, eventually resulting in the post-mitotic neuronal subtypes within the striatum, primarily spiny projection neurons (SPNs) (**Villar-Cerviño et al. 2015**).

The cortex is a six-layer structure, responsible for higher cognitive functioning (**Broadmann 1909**). The upper layers (I-IV) connect and process information locally within the cortex (**Gilbert and Wiesel 1979**,

Toyama et al. 1974), whereas the deep layers (V-VI) mainly project over longer distances to the subcortical targets, including the striatum, thalamus and other regions (**Belgard et al. 2011**). Neurogenesis in the cortex follows a similar pattern to the striatal neurogenesis, with an initial pool of radial glial cells amplifying (**Malatesta et al. 2000, Noctor et al. 2001, Noctor et al. 2004**), then asymmetrically dividing, producing a diverse array of progenitors and eventually post-mitotic cortical neurons (**Uzquiano et al. 2018**). The cortex forms in an ‘inside out’ manner, with neurons produced during early neurogenesis (E11.5-14.5) forming the deep cortical layers, and those produced during late neurogenesis (E14.5-17.5) migrating past the early born neurons to form the superficial layers (**Luskin et al. 1988, McConnell and Kaznowski 1991, Walsh and Cepko 1993, Frantz and McConnell 1996, Desai and McConnell 2000, Shen et al. 2006, Gao et al. 2014, Kaplan et al. 2017**).

Converse to the striatum, the cortex primarily develops from the dorsal telencephalon, a region giving rise to glutamatergic excitatory (pyramidal/principal) neurons, which compose ~80% of cortical cells (**Lodato and Arlotta 2015**). The other 20% consists of GABAergic interneurons which are produced in the ventral telencephalic ganglionic eminences and migrate into the developing cortex (**Marín 2013, Bandler et al. 2017, Laclef and Métin 2018**).

4.2.2 Roles of NMDARs in neurogenesis and early neurodevelopment

NMDARs are crucially important in striatal and cortical neurogenesis and wider early neurodevelopment (**Manent et al. 2006, Yozu et al. 2008, Bortone and Polleux 2009, De Marco García et al. 2011, Le Magueresse and Monyer 2013, Kelsch et al. 2014, Akgül and McBain 2016, Chittajallu et al. 2017, Akgül et al. 2019, Hanson et al. 2019, Akgül and McBain 2020, Pagano et al. 2021**).

NMDARs have been shown to be present and functional on radial glial cells and progenitors (**López et al. 1997**) and are involved in regulating both proliferation and differentiation (**Sadikot et al. 1998, Luk et al. 2003, Suzuki et al. 2006, Mochizuki et al. 2007, Li et al. 2011a**). *In vitro* experiments using both gain-of-

function and loss-of-function mutations show that both processes are influenced by activation of genes and genetic pathways that are either 'pro-neural' (i.e. pathways that promote cells to proliferate/differentiate and progress towards becoming neurons), or 'anti-neural' (i.e. pathways that halt differentiation and progression towards becoming neurons). Several studies indicate activation of NMDAR-related mechanisms is typically pro-neural, directing embryonic neural progenitors to exit the cell cycle and undergo post-mitotic neuronal differentiation. For example, **Mochizuki et al. (2007)** demonstrated in cultured neurospheres generated from rat embryonic cortical progenitors that blockade of NMDARs with antagonist MK801 decreased neurosphere diameter, suggesting reduced proliferation, whilst **Hirasawa et al. (2003)** showed that chronic exposure of embryonic rat neocortex to NMDAR antagonist DAP5 has pro-progenitor effects, increasing numbers of proliferating progenitor cells in the neocortical ventricular zone and delaying neuronal migration and maturation, with inhibition of NMDARs linked to increased expression of anti-neural gene *Hes*.

NMDARs have also been shown to play a role in neuronal migration, with *in vitro* experiments demonstrating that activation of NMDARs promotes migration of cortical excitatory neurons and cerebellar granule cells (**Komuro and Rakic 1993, Behar et al. 1999, Hirai et al. 1999, Hirasawa et al. 2003, Mancini and Atchison 2007**). *In vivo* experiments have shown that implants delivering MK801 to the early postnatal cortex result in altered cortical cytoarchitecture (**Marret et al. 1996, Reiprich et al. 2005**), as do *de novo* gain-of-function mutations in the *GRIN1* gene encoding the NR1 subunit in human clinical studies, with both suggesting impaired neuronal migration (**Fry et al. 2018**). Further, study of rodent models of the rare metabolic disorder Zellweger syndrome indicate that NMDAR-mediated mechanisms underly cortical neuronal migration impairments observed (**Gressens et al. 2000**). There is limited literature on the role of NMDARs in early striatal cell migration specifically, but wider literature indicates the importance of NMDARs in migration may be cell-specific, since it has been shown *in vitro* that NMDAR

blockade has no impact on hippocampal interneuron migration, which is instead regulated by AMPAR-mediated mechanisms (**Manent et al. 2006**).

Further, early studies suggest that NMDARs in the early brain regulate cell survival, with **Ikonomidou et al. (1999)** demonstrating *in vivo* in rats that MK801 antagonism of NMDARs during the late embryonic or early postnatal period can trigger widespread neuronal apoptosis across the developing brain. Their specific role in cell survival is not well characterised in the very early brain but in the later adolescent and mature brain, their role appears convoluted, since they have been shown to both promote neuronal survival and cell death, thought to be dependent on a range of factors including receptor activity level, subunit composition and cellular location (**Hardingham and Bading 2010**). The literature suggests that normal NMDAR activity promotes cell survival through multiple genetic pathways such as the PI3K-Akt, ERK and CREB pathways, whilst hyper- or hypoactivation is linked to neuronal death (**Mao et al. 2022**). More recent studies using *in vitro* and *in vivo* stroke models suggest that NMDAR-mediation of neuronal survival may be receptor subtype related, with NMDARs containing NR2A and NR2B subunits promoting neuronal survival and death respectively (**Sattler et al. 1999, Aarts et al. 2002, Lai et al. 2011, Martel et al. 2012, Wu and Tymianski 2018**). However, this view is likely over-simplistic as it does not consider the roles of the other NMDAR non-obligate subunits, which are also suggested to be important (**Nakanishi et al. 2009, Baron et al. 2010, Chung et al. 2016**). Studies have also shown that synaptic vs extrasynaptic NMDAR activity also promotes cell survival and cell death respectively, through promotion or suppression of specific pro-survival or pro-death pathways (**Hardingham and Bading 2010**).

Neurogenic processes have also been shown to be partly mediated by non-neuronal cells such as microglia (**Araki et al. 2021, Pérez-Rodríguez et al. 2021**). These are the immune cells of the CNS, which generally function to monitor and respond to any potential alterations in the physiological microenvironment (**Jurga et al. 2020**). Once changes are recognised, microglia shift to an 'activated' state which includes dynamic

switch in their function and morphology, and release cytokines, alerting nearby cells to the threat and altering their function, triggering an inflammatory state (**Du et al. 2017, Jurga et al. 2020**).

Microglia invade the brain early (~E9.5) and occupy the neuroepithelium where progenitors proliferate, implying a functional role in early neurodevelopment (**Cowan and Petri 2018**). A bi-directional role for microglia in neurogenesis is hypothesised, both supporting cell generation and removing unrequired cells (**Ueno and Yamashita 2014**). *In vitro*, cultured embryonic cortical neurons from mice genetically lacking microglia have shown reductions in proliferation, without changes to neurogenesis or cell survival (**Antony et al. 2011**), whilst *in vivo*, reduced microglia have been shown to lead to decreased cellular differentiation (**Erblich et al. 2011**) or restricted numbers of neural progenitor cells (**Cunningham et al. 2013**), with the latter study finding that microglia regulated progenitor numbers predominantly through phagocytosis of non-dying progenitor cells. Pertinent to this thesis, NMDARs have also been shown to be present and functional on microglia, including embryonically (**Gottlieb and Matute 1997, Eriksson et al. 2000, Tahraoui et al. 2001, Tikka et al. 2001, Kaur et al. 2005, Nair et al. 2006, Newcombe et al. 2008, Liang et al. 2010, Murugan et al. 2011, Kaindl et al. 2012**). Further, it has been shown that activation of microglial NMDARs can trigger neuronal cell death and inflammation in the early brain, with **Kaindl et al. (2012)** demonstrating in embryonic murine cortical microglial cultures that stimulating these microglial NMDARs electrophysiologically triggered microglial activation and neurotoxic factor secretion, inducing neuronal death and removal of these NMDARs through antagonism of loss-of-function mutations significantly reduced neuronal cell death.

4.2.3 Disruption of NMDARs during neurodevelopment by NR1-IgG antibodies

The studies above demonstrate NMDARs are clearly important in neurogenic processes. One way in which they may be impaired during early development is by the binding of anti-neuronal antibodies against the NMDAR NR1 subunit, which can be passed from the mother to the foetus during pregnancy (**Giannoccaro**

et al. 2020). Mechanistically, NR1-IgG antibodies have been shown to primarily cause receptor clustering (**Ladépêche et al. 2018**), cross-linking and internalisation (**Hughes et al. 2010, Castillo-Gómez et al. 2017, Pan et al. 2018**), resulting in decreased surface density and synaptic localisation (**Dalmau et al. 2008, Hughes et al. 2010, Mikasová et al. 2012**).

Pre-clinically, experiments using intraperitoneal or intravenous injections of patient-derived NR1-IgG antibodies in pregnant mice by **Jurek et al. (2019)** and **García-Serra et al. (2020)** respectively have shown they result in reduced cell-surface and synaptic NMDARs, cellular structural changes, microglial activation, and cortical plate thinning in offspring. Pathological effects including increased early postnatal mortality, altered electrophysiological properties, impaired neurodevelopmental reflexes (**Jurek et al. 2019**), poor social-spatial memory and impaired motor coordination (**García-Serra et al. 2020**) have been observed in neonates. Further, effects have been observed into adulthood, including hyperactivity, impaired sensorimotor gating, and reduced brain volumes (**García-Serra et al. 2020**). Using genetic mutation or NMDAR antagonists to induce hypofunction has also been shown to cause major developmental deficits, including cognitive impairment and reduced growth (**Single et al. 2000, Stefani et al. 2005, Jurek et al. 2019**). Clinically, it has also been observed there is a higher NR1-IgG seroprevalence in mothers of children with various neurological diseases (**Jurek et al. 2019**).

4.2.4 The striatum as an interesting, unexplored target of developmental NR1-IgG impacts

The presence of seizures in NMDARE has driven a hippocampal and cortical focus in the literature so far (**Tanaka et al. 2015, Wright et al. 2015b, Würdemann et al. 2016, Malviya et al. 2017, Rosch et al. 2018, Jurek et al. 2019, Kersten et al. 2019, Taraschenko et al. 2019, García-Serra et al. 2020, Giannoccaro et al. 2020, Wright et al. 2021**). This chapter investigates the impact of antibodies against the NR1 subunit delivered *in utero* on neurogenesis and striatal formation embryonically. There are several reasons as to why the striatum presents an interesting, unexplored target derived from pre-clinical and clinical

literature, which are largely outlined in **General Introduction Section 1.3.5 Why is the striatum an interesting potential target for NMDAR antibodies?**. To summarise literature most relevant for this chapter:

Pre-clinically, literature suggests regional differences in expression and importance of glutamatergic receptors during development, with NMDARs particularly important for striatal neurogenesis (**Luk et al. 2003, Jansson and Åkerman 2014, Krajewski et al. 2019**). It has been shown in culture by **Luk et al. (2003)** that proliferation of striatal neuroblasts was sensitive to NMDAR antagonists, and similarly, *in utero* exposure to antagonists during the neurogenic period resulted in a significant decrease in progenitor proliferation, DNA synthesis and striatal neuron numbers. Also pointing towards a heavy involvement of NMDARs in striatal development specifically, **Sadikot et al. (1998)** showed using BrdU time-labelling of dividing striatal progenitors that gestational rats given either competitive antagonist CG-19755 or non-competitive antagonist MK801 during the embryonic proliferative window (E15-18), but not those given them during the post-proliferative period (E18-21) showed a marked reduction of striatal PV interneurons in the adult striatum. The findings in **Experimental Chapter A** also show that the NR1-IgG antibodies remain bound to the LGE for 48h+ post-injection, which would give an extended window for impacts to occur. In our model, the point of NR1-IgG exposure also coincides with the peak of striatal neurogenesis. *In vivo* behavioural studies and clinical literature also support potential involvement of striatal circuits **Florance et al. 2009, Irani et al. 2010, Lanciego et al. 2012, Armangué et al. 2013, Wright et al. 2015a, Jurek et al. 2019, Mielnik et al. 2020, García-Serra et al. 2020** (most relevant to, and hence detailed later in, **Experimental Chapter D**).

4.3 Chapter Aims

The aim of **Experimental Chapter B** was to use our model to explore the embryonic impact of brief *in utero* exposure to NR1-IgG antibodies on the developing striatum, and its precursor region, the lateral ganglionic eminence (LGE), and address the following questions:

- 1) How does NR1-IgG *in utero* exposure impact LGE morphology?
- 2) How does it impact progenitor proliferation in the LGE?
- 3) Are there differences in the impact on specific progenitor populations within the LGE?
- 4) How does it impact cell migration in the LGE?
- 5) How does it impact cell death in the LGE?
- 6) Are similar changes seen when testing other patient-derived NR1-IgG antibodies?
- 7) How does it impact microglia in the LGE?

4.4 Chapter Methods

Methods used in this Chapter are described in detail in **General Methods** and are briefly summarised below (in the approximate order in which they appear):

4.4.1 *In utero* surgical methods

(See **General Methods Section 2.5 *In utero* surgical methods**)

For most experiments in this chapter, embryos were injected intraventricularly with either NR1-IgG or control-IgG at E14.5 and fixed 48 hours later at E16.5. In all experiments, one half of the embryos in the uterus were injected with NR1-IgG and one half with control-IgG, to give an intra-litter control for all experiments. To account for inter-litter variation, each NR1-IgG dataset was compared relative to the mean of its own intra-litter control and data is presented as % change relative to the control-IgG. Either polyclonal or monoclonal antibodies were used in these experiments.

For the *in utero* electroporation (IUE) experiments to label T α 1⁺ expressing ('aIPs') and T α 1⁻ non-expressing ('OPs') progenitor populations in the healthy LGE, the ventricles of embryonic brains were injected with the relevant T α 1-Cre and FLEEx plasmids. For experiments to then assess impact of t NR1-IgG antibodies on these two progenitor pools, IUE of T α 1-Cre and FLEEx plasmids was performed alongside injection of NR1-IgG and control-IgG antibodies. Similarly, for experiments to assess the impact of NR1-IgG antibodies on cell migration, FlashTag, which pulse-labels migrating progenitors at the ventricular wall, was co-injected alongside NR1-IgG and control-IgG antibodies.

Sham control surgeries were also performed in a small number of animals to confirm that impacts seen were not a result of surgery alone, and in these operations the uterus was exposed but no embryos were injected (**Appendix, Supplementary Figures S4.1, S4.4, S4.5**).

For embryonic experiments involving *in utero* injection of antibodies at E14.5, and fixation of slices at E16.5, the overall survival rates at collection were: NR1-IgG: 79.3%, control-IgG: 90.4%.

4.4.2 Immunohistochemical methods

(See **General Methods Section 2.6 Antibody immunohistochemical methods**)

Post-injection with NR1-IgG or control-IgG (+ T α 1-Cre and FLEEx plasmids for the aIP/OP progenitor labelling experiments or + FlashTag for the cell migration experiments), brains were fixed and sliced into 60 μ m coronal sections before immunohistochemistry was performed to assess various parameters. For embryonic experiments, three sections (rostral, medial, caudal) were analysed per brain (unless otherwise stated) (**Figure 4.1 A**). Slices were imaged using an Olympus Fluorescence Microscope (model BX40) with a Hamamatsu ORCA-ER camera and HCLImage Live Software (or an SM 710 confocal microscope with ZEN software (Zeiss) for published IUE data in **Figure 4.5**) and then analysed in *Image J* (Fiji).

4.4.2.1 Gross and regional morphology

Gross morphology was assessed by weighing brains using an ultra-fine scale. Regional morphology was assessed through labelling the primordial striatum with CTIP2 to label SPNs and measuring primordial striatal area and proliferative zone width. **Primordial striatum size** was quantified by drawing a boarder around the CTIP2-labelled region on images taken at 4x magnification. **Proliferative zone width** was measured in by drawing perpendicular lines from the LGE ventricular edge to the edge of the CTIP2-labelled area. Three evenly spaced lines were drawn, and an average was taken. **Cortical thickness** was measured in a similar manner, by drawing three evenly spaced lines from the cortical ventricular edge up to the top of the cortex and taking the average.

4.4.2.2 Progenitor proliferation

LGE and cortical progenitor proliferation was assessed through labelling mitotic progenitors with the mitotic marker PH3. PH3+ cells were counted in a defined LGE or cortex region, standardised across

sections, at 20x magnification. Only cells in focus, with intensity >2SD above background were counted.

PH3+ cell location was determined by placing markers close together along the full ventricular edge and then comparing the coordinates from the labelled PH3+ cells to each of these counters and determining the one with the shortest distance.

4.4.2.3. Quantifying aIP and OP progenitor populations

To quantify aIPs and OPs in the LGE at E12.5 and 15.5, 40µm z-stacks were taken, with an interval of 1µm and the **number of green (aIPs) and red (OPs) cells** manually counted in a defined region of the LGE. Positive cells were deemed to be those with a fluorescence >2SD above the background. To determine **cell cycle kinetics of aIPs and OPs**, brains were electroporated at E15.5 followed by varying time-delays to the point of fixation and then stained with PH3+ primary antibody to label dividing mitotic progenitors amongst the aIP and OP populations. aIPs and OPs co-labelled with PH3 were imaged and counted in the same way as for counting total numbers of aIPs and OPs described earlier in this paragraph. The **location of aIPs and OPs in the LGE** and spread was determined using the x and y coordinates of the labelled cells relative to the ventricular edge, with the same method as used for analysing location of PH3+ cells in the paragraph above. **Counting of basal processes of aIPs and OPs** was done in 40µm z-stacks, with 1µm intervals.

4.4.2.4 Cell migration

Cell migration was assessed by measuring FlashTag band width. FlashTag, which pulse-labels mitotic cells at the ventricular wall, was co-injected alongside NR1-IgG or control-IgG antibodies at E14.5. Fixed slices were made at E16.5 and cell migration quantified through measuring the width of the FlashTag band in the LGE at five evenly spaced intervals and taking a mean, in images taken at 20x magnification.

4.4.2.5. Cell death

Cell death was assessed with a TUNEL assay, following the manufacturer's protocol. Brains injected with NR1-IgG or control-IgG were fixed at 48 hours, sectioned and permeabilised with Proteinase K, prior to incubation with TdT Reaction buffer, then TdT Reaction Mixture and Alexa Fluor 594 picolyl azide dye. After washing, the Click-iT Plus reaction was performed by adding TUNEL Reaction Cocktail to each section in a light-protected, humidified chamber. Sections were washed with 3% BSA and PBS containing DAPI (Thermo Fisher Scientific, 488, 1:10,000), before imaging at 20x magnification. Cells were counted in a defined LGE region, standardised across sections. Only positively labelled cells in focus, with intensity >2SD above background were counted. Positive and negative control sections were included. The positive control was ezDNase enzyme (Thermo Fisher Scientific) which induces DNA strand breaks and apoptosis and negative control was ddH₂O.

4.4.2.6. Microglial density

Microglial density and morphology were assessed through labelling using the marker, IBA1, which is upregulated in activated microglial cells (**Ohsawa et al. 2004**). 30µm z-stacks, with 0.5µm intervals were taken of the striatum and cortex, and cells quantified in a defined region, standardised across sections. To count, cells were counted in each layer of the z-stack. Only cells in focus, with intensity >2SD above background were counted. **Microglial density** was then calculated by dividing the total number of cells counted by the volume of the z-stack (density = total cells counted/(30*area of region)).

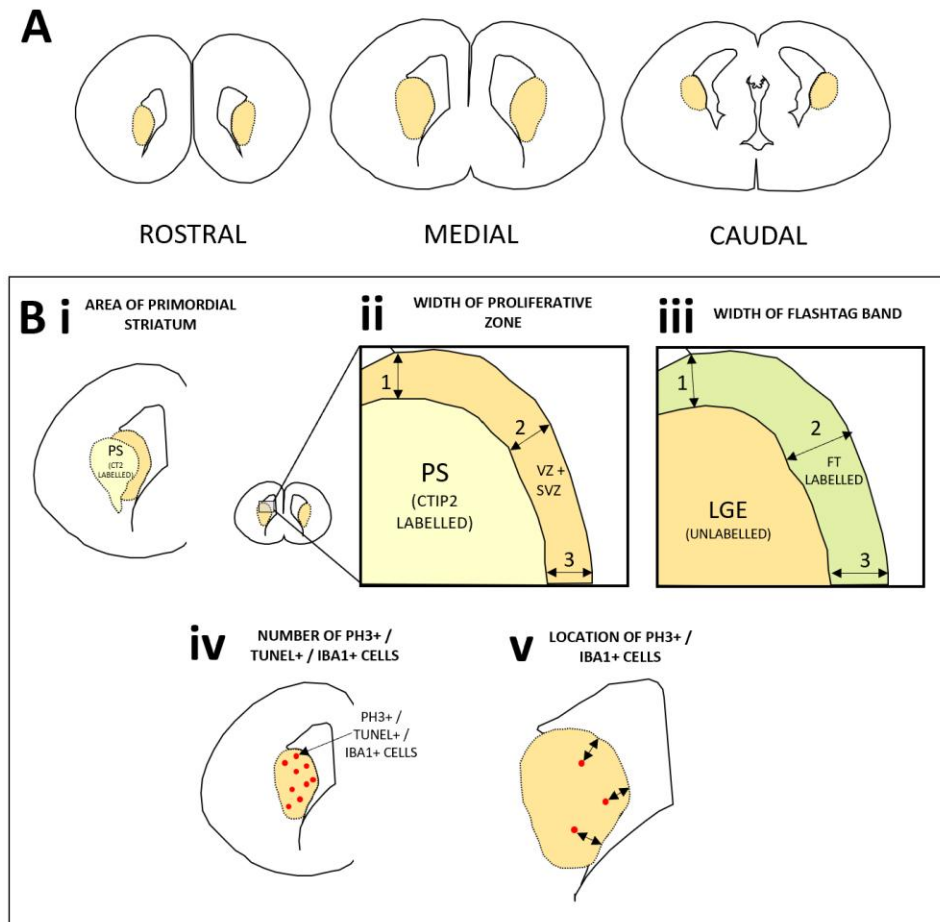


Figure 4.1. Embryonic immunohistochemistry analysis methods [A] Reference diagrams of the morphology of a typical rostral, medial, and caudal slice at E16.5, with the LGE coloured in orange, traced from the Prenatal Mouse Brain Atlas (Schambra 2008) - rostral (CORONAL 4-6), medial (CORONAL 8-10) and caudal (CORONAL 12-14). [B] Graphical summary of immunohistochemical analysis methods to assess *i.* area of primordial striatum (labelled with SPN marker CTIP2), *ii.* width of proliferative zone, *iii.* width of FlashTag band (distance pulse-labelled mitotic progenitors have migrated from the ventricular wall), *iv.* numbers of PH3+ (labelling proliferating progenitors) / TUNEL+ (apoptotic cells) / IBA1+ (microglia), *v.* location of PH3+ / IBA1+ cells. PS = primordial striatum, CT2 = CTIP2, VZ = ventricular zone, SVZ = subventricular zone, FT = FlashTag.

4.4.2.7. Microglial reconstructions

(See General Methods Section 2.8 Morphological reconstruction methods)

Individual microglial cells in the IBA1-labelled striatal sections were imaged at 40x magnification, in z-stacks with a 0.5µm interval, and cells chosen via a random number generator. Z-stacks converted to

binary 8-bit images in *Image J*, and appropriate filters applied to generate a sharp image. To obtain data about **total branch length**, and **numbers of primary branches**, the image was skeletonised using the *Skeletonise* function, and then the skeletonised image analysed using the plugin *AnalyzeSkeleton(2D/3D)*. The *Scholl Analysis* function was also applied to the image to evaluate the complexity of the neuron. The image processing and analysis here followed a similar protocol to **Young and Morrison (2018)**. The microglia skeletons were also analysed with the *FracLac Image J* plugin to obtain further detailed information about subtle morphological differences, following a similar protocol to **Fernández-Arjona et al. (2017) (General Methods, Figure 2.16)**.

4.4.3 Statistical methods

(See **General Methods Section 2.11 Statistical methods**)

Details of specific statistical tests used for each experiment are given in the respective results sections in the chapter. All data is presented as mean $\pm$ SEM. All data was initially normality tested using the Shapiro-Wilks or Kolmogorov-Smirnov test, and the appropriate two tailed parametric (ANOVA, one-sample t-test, unpaired t-test) or non-parametric (Wilcoxon signed-rank (WSR), Mann-Whitney U (MWU)) test applied. Significance for correlations was determined using the Pearson correlation coefficient and sample size. Data was corrected where appropriate using the Bonferroni *post hoc* correction, to compensate for multiple comparisons. For all statistical tests, $p < 0.05$ was deemed significant. * $p < 0.05$, ** $p < 0.01$.

4.5 Chapter Results

4.5.1 Embryonic impacts of NR1-IgG on gross and regional morphology

One impact of NR1-IgG that has been shown following *in utero* exposure is reduced brain and striatal volumes in adulthood (Jurek et al. 2019). Therefore, the first experiments set out to explore whether these impacts also occurred in the embryonic brain. NR1-IgG or control-IgG was intraventricularly injected at E14.5 and brains fixed at E16.5 (**Figure 4.2 A**). On extraction, brains were blotted dry and weighed with an ultra-fine scale. For this dataset $n(\text{NR1-IgG}) = 7$ brains, and $n(\text{control-IgG}) = 6$ brains, 2 litters. There was no significant difference in the weight of the control-IgG and NR1-IgG brains (control-IgG: $40.4 \pm 0.6\text{mg}$, NR1-IgG: $44.1 \pm 2.4\text{mg}$, MWU: $p = 0.15$; **Figure 4.2 B**). The literature also suggests that the striatum may be more sensitive to manipulation of NMDARs than the cortex, with neurogenesis primarily NMDAR-mediated in the striatum but AMPAR-mediated in the cortex (Luk et al. 2003), and therefore immunohistochemistry with SPN marker CTIP2 was utilised to label the primordial striatum, allowing quantification of primordial striatum size as well as proliferative zone width. One rostral, one medial and one caudal section was analysed per brain and averaged to give an overall measurement. For this dataset, $n(\text{NR1-IgG}) = 10$ brains, $n(\text{control-IgG}) = 14$ brains, 4 litters. The primordial striatum size trended towards a decrease in NR1-IgG embryos relative to control-IgG embryos (relative % change NR1-IgG: $-9.4 \pm 5.2\%$, one-sample t-test: $p = 0.070$; **Figure 4.2 C, D**). Cortical thickness showed no significant differences between groups (relative % change NR1-IgG: $-1.4 \pm 2.7\%$, one-sample t-test: $p = 0.76$; **Figure 4.2 E**). The LGE proliferative zone width showed a significant increase in NR1-IgG embryos (relative % change NR1-IgG: $17.5 \pm 9.8\%$, one-sample t-test: $p = 0.040$; **Figure 4.2 F, G**).

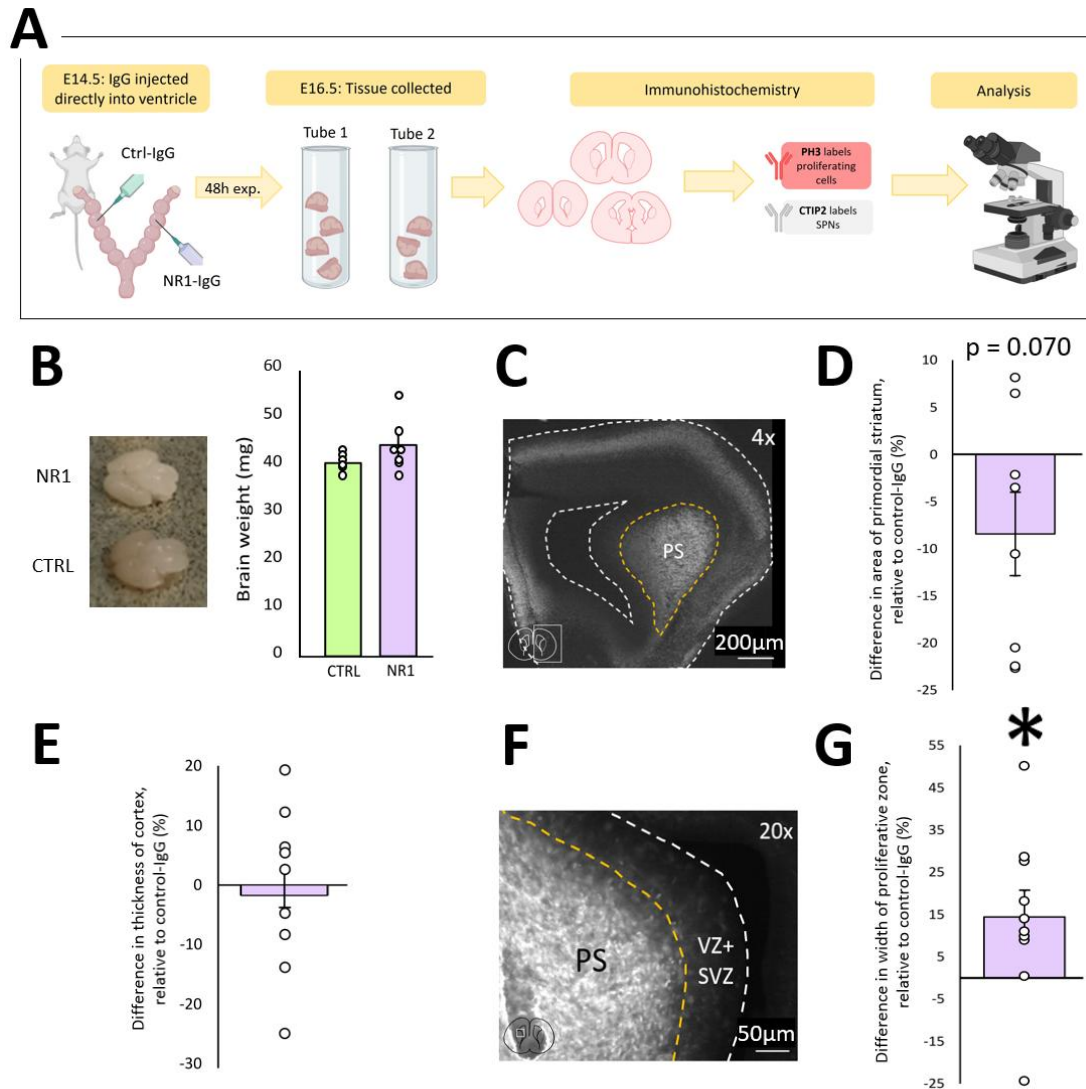


Figure 4.2. Altered morphology in the LGE following brief in utero NR1-IgG exposure. [A] Graphical outline of the methodology to assess morphological features of E16.5 brains, following 48-hour exposure to either patient-derived NR1-IgG or human-derived control-IgG. For [B], $n(\text{NR1-IgG}) = 7$ brains, $n(\text{control-IgG}) = 6$ brains, 2 litters. For other panels, $n(\text{NR1-IgG}) = 9$ brains, $n(\text{control-IgG}) = 14$ brains, 4 litters. Mitotic marker PH3 was used to label proliferating cells, and SPN marker CTIP2 to delineate the primordial striatum (PS). [B] Left: Representative photographs of an NR1-IgG and control-IgG injected E16.5 brains. Right: Bar plot of embryonic brain weight of NR1-IgG and control-IgG embryos. [C] Representative epifluorescence image of the primordial striatum, immunostained with CTIP2 (bright zone) in the E16.5 LGE. [D] Bar plot of the difference the primordial striatum area of NR1-IgG embryos, relative to litter-matched control-IgG embryos. The primordial striatum area in NR1-IgG embryos trends towards a decrease. [E] Bar plot of the difference in cortical thickness of NR1-IgG embryos, relative to litter-matched control-IgG embryos. There was no significant difference in cortical thickness. [F] Representative epifluorescence image of the proliferative zone in the LGE. The proliferative zone spans from the ventricular edge of the LGE, to the edge of the PS, and comprises of the ventricular (VZ) and subventricular (SVZ) zones. The bright zone is the primordial striatum, immunostained with CTIP2. [G] Bar plot of the difference in proliferative zone width of NR1-IgG embryos, relative to litter-matched control-IgG embryos. The proliferative zone width is significantly greater in NR1-IgG embryos. Sham control experiments also demonstrate these findings are not due to surgery alone (Appendix, Supplementary Figure S4.1).

4.5.2 Embryonic impacts of NR1-IgG on progenitor proliferation

4.5.2.1. Effects in the LGE and cortex

NMDARs are present and functional on neuronal progenitors and are involved in regulating both proliferation and differentiation (Sadikot et al. 1998, Luk et al. 2003, Suzuki et al. 2006, Mochizuki et al. 2007, Li et al. 2011a). It was previously shown that *in utero* exposure to NMDAR antagonist MK801 during the neurogenic period resulted in a significant decrease in striatal progenitor proliferation, DNA synthesis and striatal neuron numbers, with little impact on the cortex (Luk et al. 2003). Therefore, progenitor proliferation was next assessed in the same brain slices (one rostral, one medial and one caudal section per sample). For this dataset, n(NR1-IgG) = 9 brains, n(control-IgG) = 14 brains, 4 litters.

Mitotic marker PH3 immunostaining to label proliferating cells also revealed a significant decrease in proliferating cell numbers in the LGE, but not cortex (one-sample t-tests: relative % change NR1-IgG: cortex: $-3.1 \pm 3.5\%$, $p = 0.61$; LGE: $-8.4 \pm 3.4\%$, $p = 0.020$; **Figure 4.3 B, D**).

It is important to note that there was no significant difference in the area of the cortex or LGE that the cells were counted in between the NR1-IgG or control-IgG brains in any level of section (cortex: rostral: MWU: $p = 0.87$; medial: MWU: $p = 0.91$; caudal: MWU: $p = 0.76$ | LGE: rostral: MWU: $p = 0.54$; medial: MWU: $p = 0.89$, caudal: MWU: $p = 0.92$; **Figure 4.3 Ei, Fi**, data in figure), and there were also no significant correlations between area and cell numbers (**Figure 4.3 Eii, Fii**, data and statistics in figure; **Appendix, Supplementary Figures S4.2, S4.3, S4.4**).

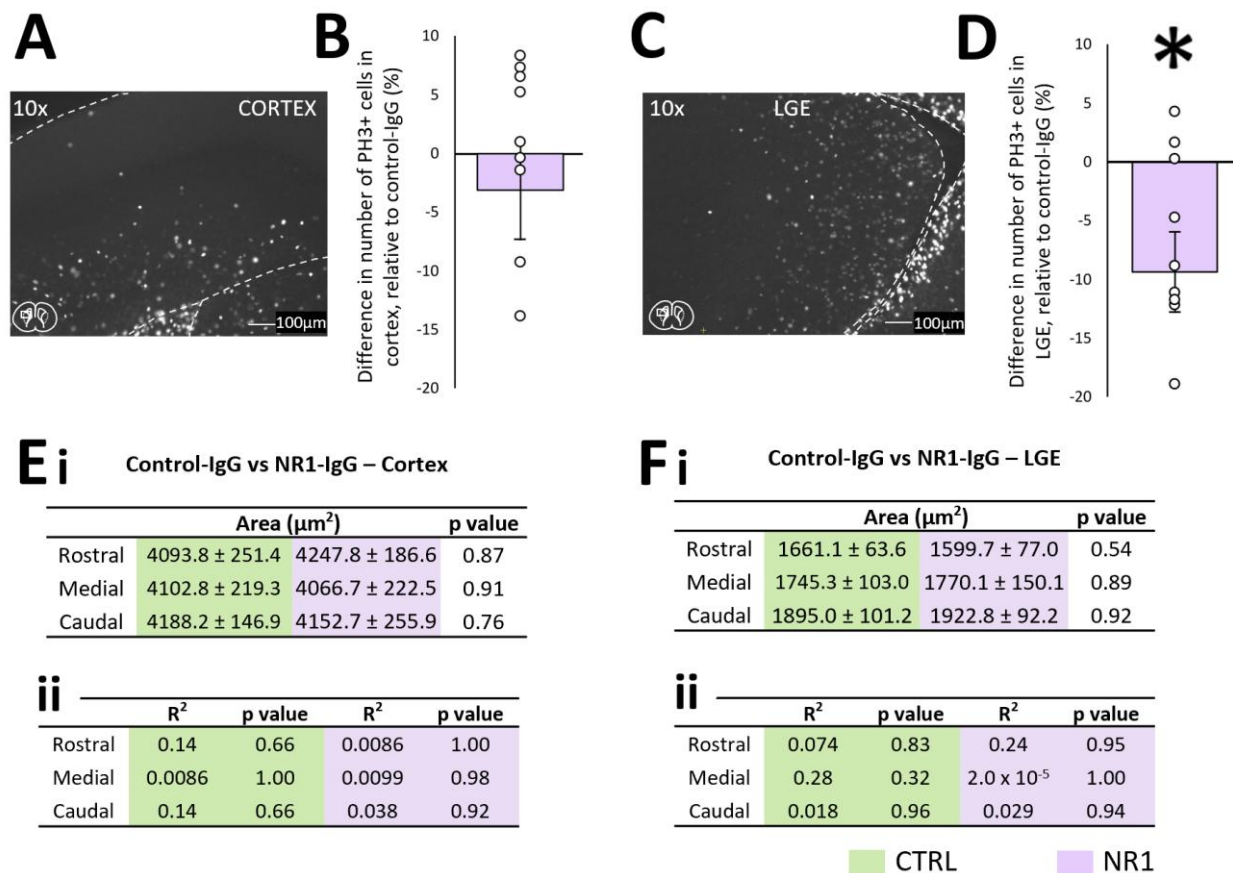


Figure 4.3. Decreased progenitor proliferation in the LGE following brief in utero NR1-IgG exposure. [A] Representative epifluorescence image of the E16.5 cortex, immunostained with PH3 to label proliferating cells. [B] Bar plot of the difference in number of PH3+ cells in the cortex of NR1-IgG embryos, relative to litter-matched control-IgG embryos. There is no significant difference in number of PH3+ cells in the cortex. [C] Similar image to [A] but of the E16.5 LGE instead. [D] Similar bar plot to [B], but of PH3+ cells in the LGE instead. There is a significant decrease in relative number of proliferating cells in the LGE of NR1-IgG embryos. [E] Since we are comparing number of PH3+ cells rather than density, it is important to control for area. The tables state that there were i. no significant differences in area between control-IgG or NR1-IgG cortical regions counted in and ii. also no significant correlation between area of cortex and number of PH3+ cells counted in that area for any section level (rostral, medial, caudal). [F] The same as [E] but for the LGE rather than cortex. The tables similarly state that there were i. no significant differences in area between control-IgG or NR1-IgG LGE regions counted in and ii. also no significant correlation between area of LGE and number of PH3+ cells counted in that area for any of the section level (rostral, medial, caudal). Further supporting figures provided in **Appendix, Supplementary Figures S4.2, S4.3, S4.4.**

4.5.2.2. Regional differences in the LGE

The LGE is a heterogeneous structure, consisting of intermingled cell populations, characterised in part by their location, in addition to cell-cycle kinetics, morphology and transcriptome (Knowles et al. 2021, Van Heusden, Macey-Dare et al. 2021). Cell counts were therefore also analysed individually at the three different levels of the LGE, in one rostral, one medial and one caudal section per brain. For these experiments, $n(\text{NR1-IgG}) = 9$ brains, $n(\text{control-IgG}) = 14$ brains, 4 litters. This split analysis showed the decrease in PH3+ cells in the LGE occurred specifically in medial sections (one-sample t-tests: rostral: $-3.3 \pm 4.4\%$, $p = 0.47$; medial: $-22.4 \pm 4.9\%$, $p = 0.00030$; caudal: $0.5 \pm 5.1\%$, $p = 0.93$; **Figure 4.4 A**). Cell location analysis in medial sections, whereby cells were quantified every 20 μm from the ventricle, revealed the greatest decreases occurred within the first 200 μm of the ventricle (one-sample t-tests: 0-20 μm : $-39.8 \pm 9.1\%$, $p = 0.0010$; 20-40 μm : $-40.5 \pm 9.1\%$, $p = 0.042$; 120-140 μm : $-86.4 \pm 18.0\%$, $p = 0.044$, 140-160 μm : $-47.1 \pm 16.5\%$, $p = 0.021$; 160-180 μm : $-50.1 \pm 14.6\%$, $p = 0.035$, all other intervals $p > 0.05$; **Figure 4.4 B**).

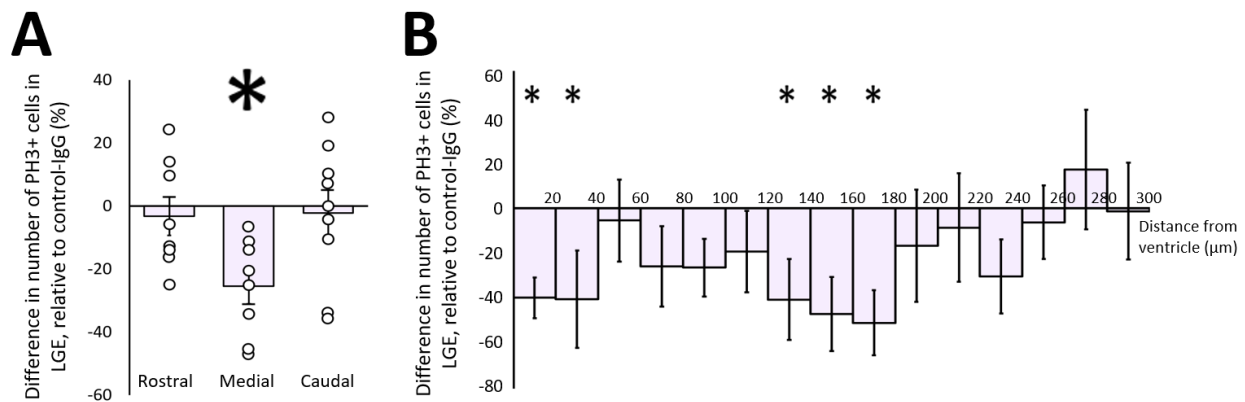


Figure 4.4. Greatest decreases in progenitor proliferation in the medial LGE and close to the ventricle [A] Bar plot of the difference in mean number of proliferative cells in the LGE in NR1-IgG embryos, relative to litter-matched control-IgG embryos, split into rostral, medial, and caudal sections. There was significant decrease in number of proliferating cells specifically in medial sections of NR1-IgG embryos. [B] Bar plot of the difference in mean numbers of proliferating cells in medial sections of the LGE of NR1-IgG embryos, relative to litter-matched control-IgG embryos, split by their distance from the ventricle. There were significant decreases in the first 200 μm from the ventricle.

Overall, these results demonstrate that exposure to NR1-IgG affects proliferating cells in the LGE and in particular those relatively close to the ventricle.

4.5.3 Embryonic impacts of NR1-IgG on specific LGE progenitor populations

As well as location, the heterogeneous progenitors of the LGE can also be classified by the markers they express and the neurons and other cell types they generate (reviewed in **Knowles et al. 2021**). Building on the regional differences observed within the LGE in terms of numbers of proliferating progenitors that are affected by the antibodies shown in the previous section, it was also thought it could be the case that certain specific populations of progenitors might be more greatly impacted by NR1-IgG antibodies than others.

4.5.3.1. Characterising apically dividing Tα1+ and Tα1- progenitor pools in the healthy LGE

To explore this further, it was investigated whether it is possible to label and characterise specific populations of progenitors in the LGE. In the cortex, it is possible to distinguish between different progenitors based on differential gene expression, with *in utero* electroporation previously used to selectively fluorescently pulse-label cortical short neural precursor progenitors in the cortical VZ, based on their differential expression of the tubulin alpha 1 (Tα1) gene (**Gal et al. 2006, Stancik et al. 2010, Tyler and Haydar 2013, Ellender et al. 2019**). Here, a similar method was first used to distinguish a Tα1+ apical progenitor population in the LGE.

This was achieved by electroporating two DNA constructs: Tα1-Cre, where Cre recombinase is controlled by the Tα1 promotor (**Stancik et al. 2010**) and a CβA-FLEX reporter construct containing a FLEX cassette where Cre recombination switches expression from fluorescent protein TdTomato (TdTom; red) to green fluorescent protein (GFP; green) (**Franco et al. 2012**) (**Figure 4.5 A**). Electroporation at E12.5 and E15.5 revealed that Tα1+/GFP+ progenitors formed a large population at the later timepoint (E12.5: $4.8 \pm 4.1\%$ and E15.5: $34.5 \pm 3.0\%$, MWU: $p = 0.0020$; **Figure 4.5 B**). For this experiment, $n(\text{E12.5}) = 250$ cells, 7 brains and $n(\text{E15.5}) = 1,204$ cells, 17 brains.

Additionally, co-labelling with PH3+ revealed mitotic Tα1+/GFP+ progenitors had a rounded morphology with an absent basal process (**Figure 4.5 C**), in contrast to Tα1-/TdTom+ mitotic progenitors which often retained their process (**Figure 4.5 D**) (Tα1+/GFP+ + process: $20.6 \pm 4.7\%$ and Tα1-/TdTom+ + process: $35.9 \pm 7.8\%$, WSR: $p = 0.029$; $n = 961$ cells, 15 brains; **Figure 4.5 E**). Both progenitor pools showed similar migration at 24 hours, with Tα1+/GFP+ cells located slightly further from the ventricle (24h: Tα1+/GFP+: $66.24 \pm 3.57\mu\text{m}$ and Tα1-/TdTom+: $56.45 \pm 2.90\mu\text{m}$, t-test: $p = 0.0055$; $n = 1,434$ cells, 30 brains; **Figure 4.5 F**) (but no difference in locations of mitotic progenitors in each pool specifically). Tα1+/GFP+ mitotic progenitors had faster cell-cycle kinetics, with earlier return to the mitotic phase (16h: Tα1+/GFP+: $19.85 \pm 7.15\%$ and Tα1-/TdTom+: $5.18 \pm 1.72\%$, t-test: $p = 0.025$; $n = 318$ cells, 6 brains; **Figure 4.5 G**), which may have contributed to the locational differences observed.

Note: This work was done with the help of other lab members as published in **Van Heusden, Macey-Dare et al. (2021)**.

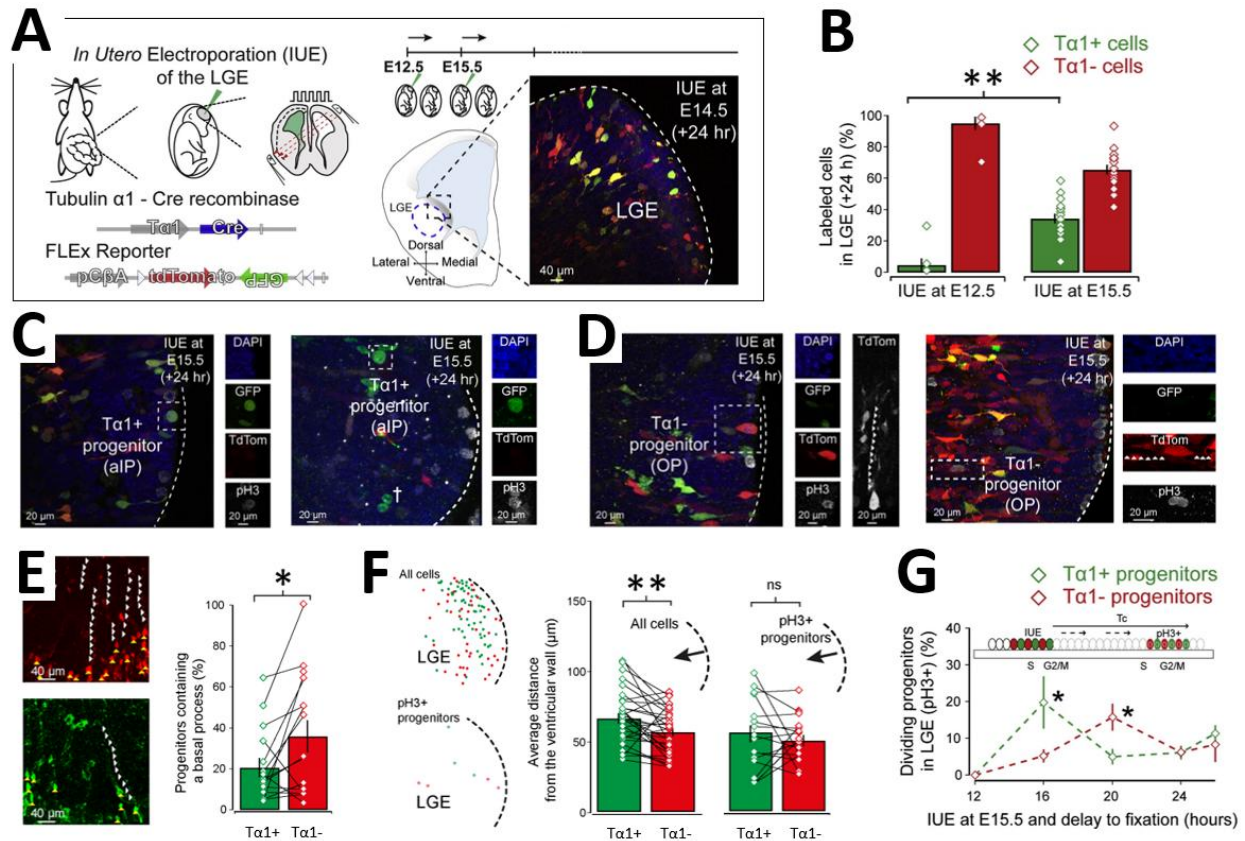


Figure 4.5. Characteristics of two populations of IUE labelled progenitors in the healthy LGE [A] Graphical outline of IUE methodology. Ta1-Cre and FLEX reporter plasmids were able to label Ta1-expressing (Ta1+/GFP+; green fluorescing) and non-Ta1-expressing (Ta1-/TdTom+; red fluorescing) progenitors in the LGE respectively. **[B]** Bar plot showing percentage of Ta1+ and Ta1- progenitors in the LGE when IUE was performed at E12.5 and E15.5, revealing a significantly greater population of Ta1+ progenitors at E15.5. **[C]** Confocal images of the LGE showing mitotic Ta1+ progenitors co-labelled with mitotic marker PH3, located at the ventricle (left) and further away (right), showing rounded morphology and lack of basal processes. **[D]** Similar to [C] but for Ta1- mitotic progenitors, showing elongated morphology and retained basal processes. **[E]** Bar plot quantification of basal processes of LGE progenitors in the proliferative zones, revealing significantly more Ta1- cells retained their basal process. **[F]** Top: Quantification of mean distance from the ventricular wall of Ta1+ and Ta1- cells revealing Ta1+ cells were significantly further from the ventricular wall. Bottom: Quantification of mean distance from the ventricular wall of Ta1+ and Ta1- mitotic progenitors specifically revealing no significant difference in location. **[G]** Line plot of Ta1+ and Ta1- progenitors co-labelled with mitotic marker PH3, demonstrating that Ta1+ progenitors exhibited faster cell cycle kinetics, returning to the mitotic phase earlier than Ta1- progenitors.

4.5.3.1. Differential effects of NR1-IgG on Tα1+ and Tα1- progenitor pools in the LGE

Building on this, a small pilot experiment was also conducted to begin to investigate if either of the Tα1+ or Tα1- populations were more affected by *in utero* exposure to NR1-IgG. To do this, the plasmids above were co-injected alongside the NR1-IgG antibody at E14.5, collected 24 hours later, and numbers of Tα1+ and Tα1- cells assessed (**Figure 4.6**). For this pilot experiment, n(NR1-IgG) = 138 cells, 2 brains, n(control-IgG) = 222 cells, 4 brains, 1 litter.

Electroporation at E15.5 revealed that both Tα1+/GFP+ and Tα1-/TdTom+ populations were able to still be successfully labelled when the plasmids were co-injected alongside the NR1-IgG or control-IgG antibodies, although labelling appeared visually weaker, possibly due to dilution of the plasmid with the antibody (**Figure 4.6 A**).

Quantification of the number of labelled Tα1+/GFP+ and Tα1-/TdTom+ cells revealed no significant differences in the relative proportions of Tα1+/GFP+ cells between NR1-IgG and control-IgG co-injected animals (Tα1+/GFP+: control-IgG: $30.2 \pm 4.0\%$, NR1-IgG: $24.7 \pm 7.3\%$, MWU: $p = 0.64$; **Figure 4.6 B**). The total labelled cell numbers and relative proportion of Tα1+ cells were also similar to those observed in the experiments injecting only plasmids (**Figure 4.5**) above, for both NR1-IgG and control-IgG exposed brains.

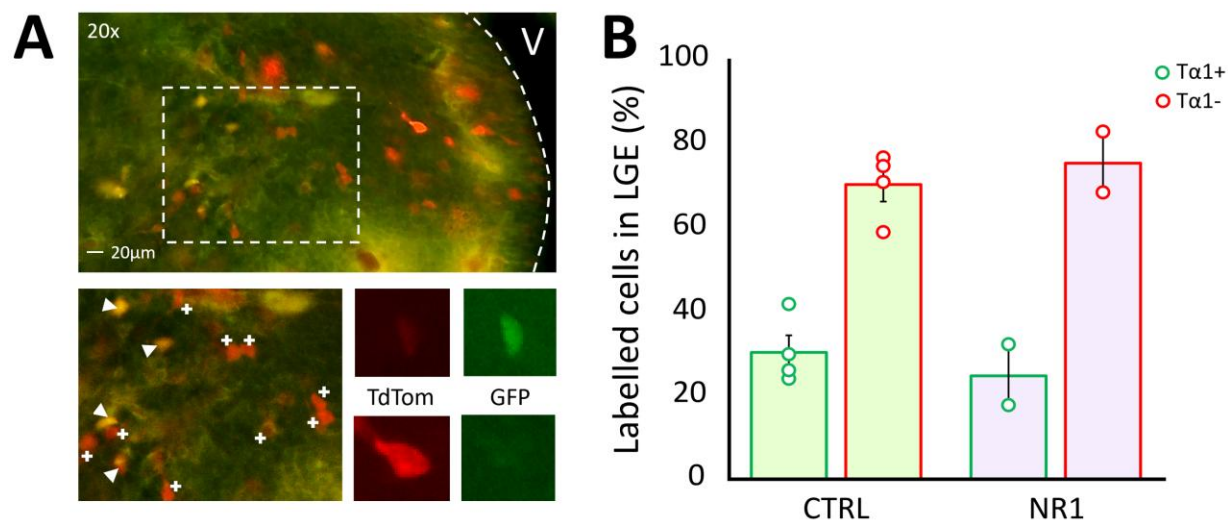


Figure 4.6. Characteristics of two populations of IUE labelled LGE progenitors co-injected with NR1-IgG
[A] Epifluorescence images of the LGE from an E15.5 brain injected with Tα1-Cre and FLEX reporter plasmids alongside NR1-IgG. Tα1-Cre and FLEX reporter plasmids labelled Tα1-expressing (Tα1+/GFP+; green fluorescing) and non-Tα1-expressing (Tα1-/TdTom+; red fluorescing) progenitors in the LGE respectively, when co-injected alongside either NR1-IgG or control-IgG antibodies, though labelling was visually weaker than if plasmids were injected without the antibody. ▲ Tα1+/GFP+ cells, + Tα1-/TdTom+ cells, v = ventricle **[B]** Bar plot showing percentage of Tα1+ and Tα1- progenitors in the LGE when IUE was performed at E15.5 with co-injections of Tα1-Cre and FLEX reporter plasmids alongside either NR1-IgG or control-IgG antibodies, revealing no significant difference between the relative population size (%) of Tα1+ progenitors between NR1-IgG or control-IgG exposed brains.

4.5.4 Embryonic impacts of NR1-IgG on cell migration and cell death

4.5.4.1. Effects on cell migration in the LGE

The paradoxical finding that proliferative zone width increased (**Figure 4.2 F, G**) whilst proliferating cell numbers decreased (**Figure 4.3 C, D**) also warranted further exploration. One hypothesis was impaired migration since glutamate is a trophic factor (Behar et al. 1999, Luhmann et al. 2015) and NMDARs are important for migration (Jansson and Åkerman 2014), with experiments showing that activation of NMDARs promotes neuronal migration in both cortical and cerebellar cells *in vitro* (Komuro and Rakic 1993, Behar et al. 1999, Hirai et al. 1999, Hirasawa et al. 2003, Mancini and Atchison 2007) and that *in*

vivo both antagonists and *de novo* gain-of-function mutations in the *GRIN1* gene encoding the NR1 subunit can alter neuronal cytoarchitecture (Marret et al. 1996, Reiprich et al. 2005, Fry et al. 2018).

Thus, following exposure to NR1-IgG, cells could be getting 'stuck', unable to migrate away from the proliferative zone, leading to increased zone width. To investigate this further, labelling of all cells that reached the ventricle at E14.5 was attempted using FlashTag injected alongside NR1-IgG or control-IgG at E14.5. FlashTag pulse-labels mitotic progenitors at the ventricular wall as an intraventricularly injected cell-permeable agent, and the distance cells have migrated can then be quantified in fixed slices (Govindan et al. 2018). Here, brains were fixed 48 hours post-injection (**Figure 4.7 A, B**). Distance migrated was significantly less in the LGE of NR1-IgG exposed brains, indicating impaired migration (relative % change NR1-IgG: $14.0 \pm 4.1\%$, one-sample t-test: $p=0.00071$; $n(\text{NR1-IgG}) = 7$ brains, $n(\text{control-IgG}) = 5$ brains, 3 litters; **Figure 4.7 C**).

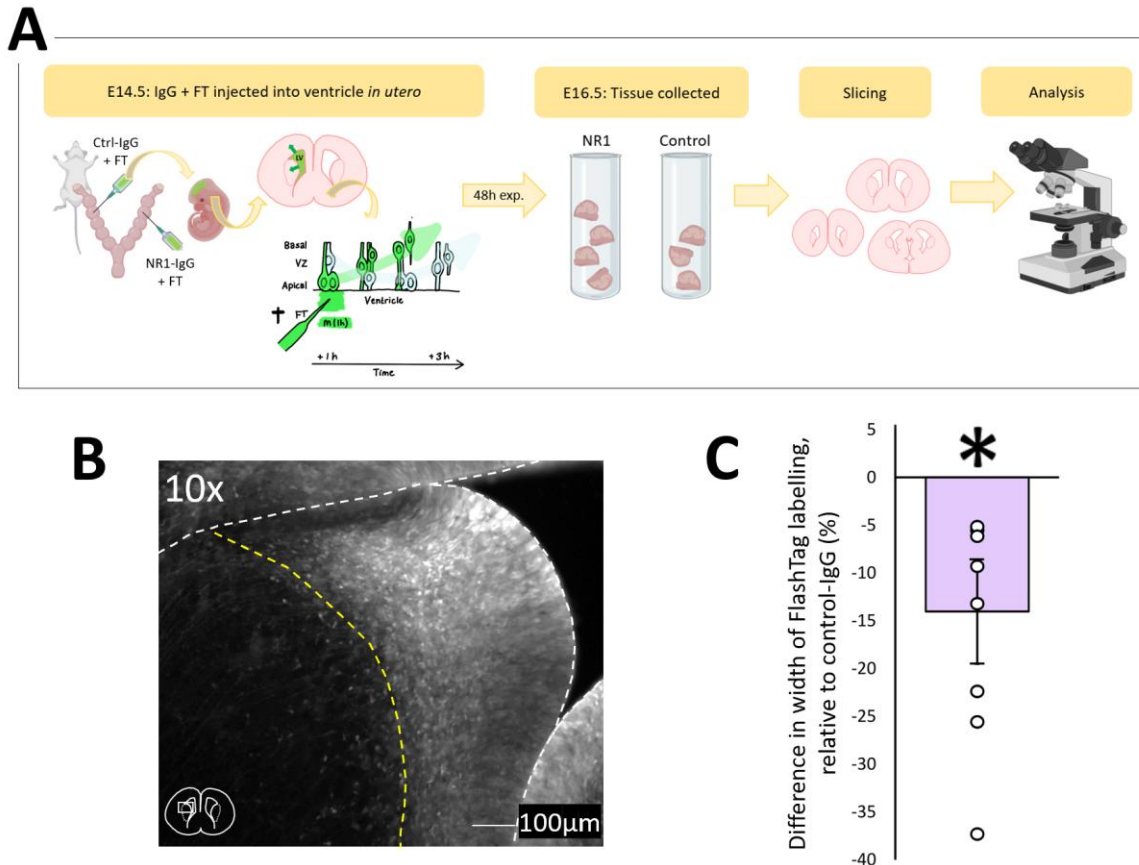


Figure 4.7. Decreased cell migration in the LGE following brief in utero NR1-IgG exposure. [A] Graphical outline of FlashTag methodology to assess cell migration from the ventricle in the E16.5 LGE following 48-hour exposure to either NR1-IgG or control-IgG. † Panel from published protocol (Govindan et al. 2018). [B] Representative epifluorescence image of the E16.5 LGE injected with FlashTag at E14.5. Mitotic ventricular zone progenitors between 1-2 hours post-injection were labelled. [C] Bar plot of the relative difference in FlashTag band width of NR1-IgG embryos. The FlashTag band width in the LGE of NR1-IgG exposed brains was significantly smaller.

4.5.3.2. Effects on cell death in the LGE

Another hypothesis to explore was whether cell death processes were altered since the literature suggests that normal NMDAR activity promotes cell survival via the PI3K-Akt, ERK and CREB genetic pathways, whilst hyper- or hypoactivation has been linked to neuronal death (Mao et al. 2022). This is supported by early studies demonstrating that exposure to NMDAR antagonist MK801 during the late embryonic or early postnatal period can trigger widespread neuronal apoptosis across the developing brain in rats (Ikonomidou et al. 1999), as well as work showing that glutamate acting on NMDARs is critical for

neuroblast survival before they form synapses (Platel et al. 2010). Disruption to the NMDAR could therefore potentially lead to increased dead cells, which could congestate in the proliferative zone region, increasing width. This was investigated using the TUNEL assay, which labels apoptotic cells through incorporation of fluorescently tagged deoxynucleotides into DNA-strand breaks (**Figure 4.8 A, B**).

There was no significant difference in TUNEL+ cell numbers between groups, suggesting cell death likely did not underlie earlier observations (relative % change NR1-IgG: $1.0 \pm 3.8\%$, one-sample t-test: $p = 0.91$; $n(\text{NR1-IgG}) = 4$ brains, $n(\text{control-IgG}) = 4$ brains, 2 litters; **Figure 4.8 C**).

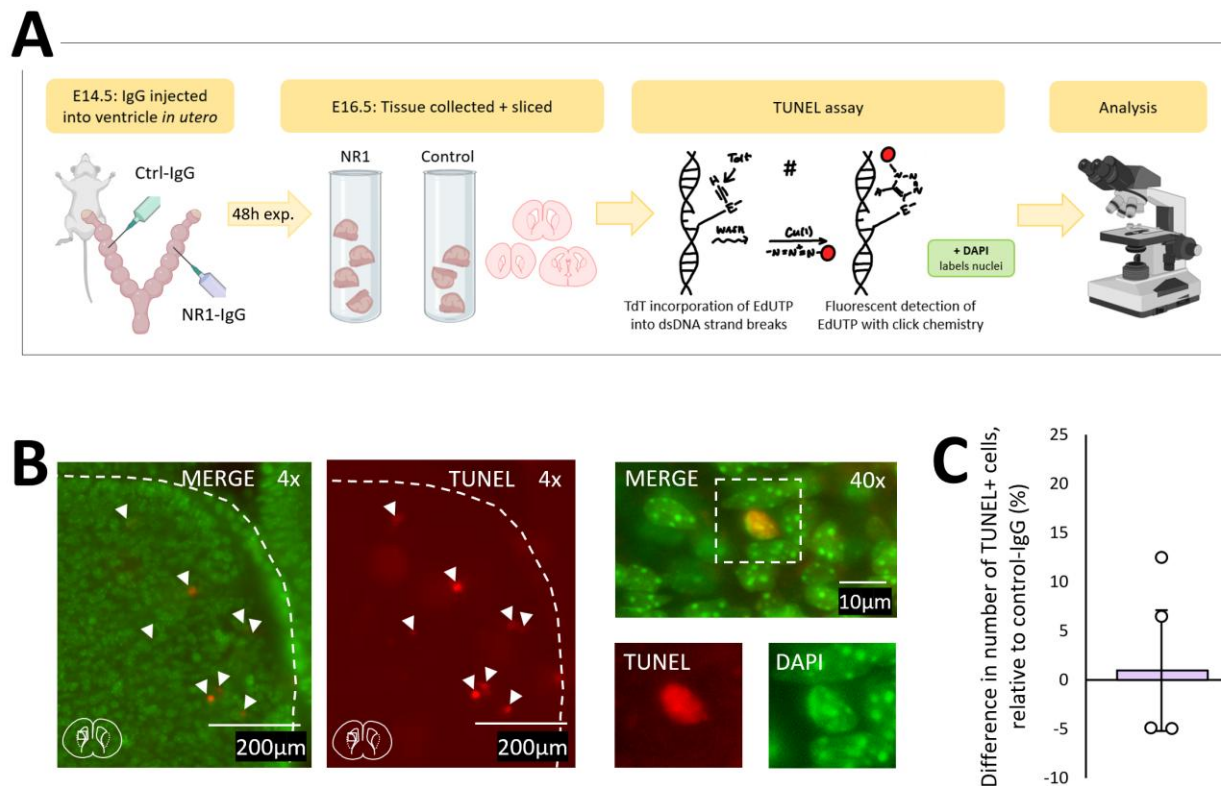


Figure 4.8. No change in cell death in the LGE following brief in utero NR1-IgG exposure. **[A]** Graphical outline of TUNEL methodology to assess cell death in the E16.5 LGE following 48h exposure to either NR1-IgG or control-IgG. **#** Panel from manufacturer's protocol (**Thermo Fisher Click it Plus TUNEL Assay Manual 2021**). **[B]** Example epifluorescence images of the E16.5 LGE processed with the TUNEL+ assay to label apoptotic cells (red). Cellular nuclei are labelled with DAPI (green). **[C]** Bar plot of the relative difference in mean number of TUNEL+ cells in NR1-IgG embryos. There was no significant difference between groups. Further experiments validating both FlashTag and TUNEL assays are given in **Appendix, Supplementary Figure S4.5**.

4.5.5 Validating effects using four further patient-derived polyclonal NR1-IgG antibodies

As a further extension to help validate findings so far, small volumes of patient-derived polyclonal NR1-IgG antibodies from four further NMDARE patients were also tested in our model, using the most robust method and metric in our model which was determined to be the FlashTag cell migration. For these experiments, $n(\text{NR1-IgG}) = 13$ brains (sample A = 2, B = 2, C = 1, D = 4) and $n(\text{control-IgG (combined)}) = 13$ brains, 5 litters. The NR1-IgG samples had variable concentrations, ranging from 3.7 to 16.9 $\mu\text{g}/\mu\text{l}$. The control-IgG samples had concentrations ranging from 14.1 to 42.1 $\mu\text{g}/\mu\text{l}$. Limited patient information was available, but all samples were obtained from confirmed NMDARE patients during the acute phase of illness (**Figure 4.9 A**).

Quantification revealed that injection of these polyclonal NR1-IgG antibodies also impaired cell migration for two of the samples (relative % change NR1-IgG: Sample A: $-16.3 \pm 13.4\%$, one-sample t-test: $p = 0.022$; Sample B: $-18.9 \pm 6.1\%$, one-sample t-test: $p = 0.0011$; Sample C: $-33.2 \pm 13.4\%$, insufficient sample size for statistical evaluation; Sample D: $-1.5 \pm 6.7\%$, one-sample t-test: $p = 0.67$; **Figure 4.9 B**). The relationship between antibody concentration and FlashTag band width was also assessed for the four samples, revealing no significant differences ($R^2 = 0.071$, $p = 0.86$; **Figure 4.9 C**).

Although the data size is small, these results would support our other observations using the main patient-derived polyclonal and monoclonal antibodies.

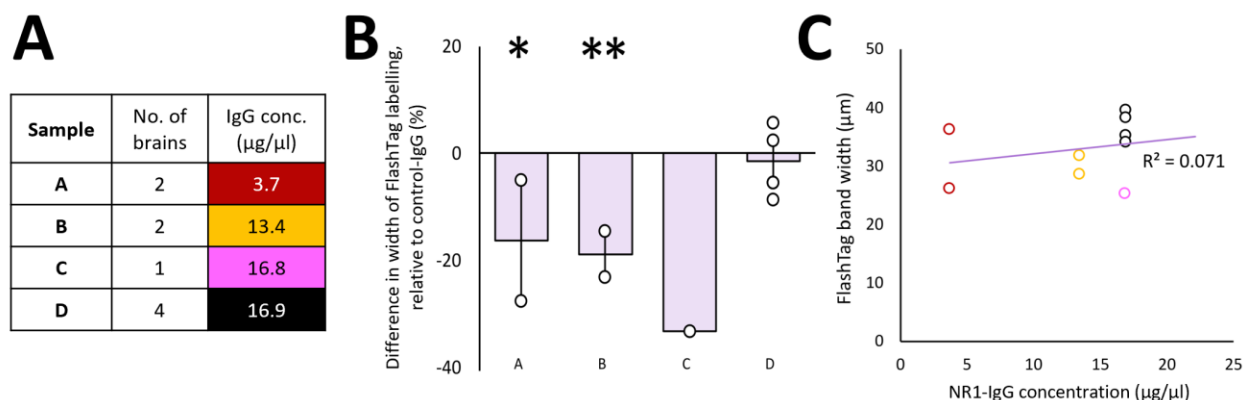


Figure 4.9. Other NR1-IgG antibodies also impair cell migration in the LGE after brief in utero exposure.

[A] Table of the NR1-IgG samples used, showing number of brains injected and concentrations of each antibody. **[B]** Bar plot of relative difference in FlashTag band width in the LGE of NR1-IgG exposed brains relative to control-IgG brains for four NR1-IgG samples (A,B,C,D). The FlashTag band width in NR1-IgG exposed brains was significantly smaller in two of the four samples, similar to what was seen with the main NR1-IgG antibodies used in this study. Sample C could not be statistically evaluated due to insufficient sample size but also suggested a decrease. Each bar represents a different patient-derived NR1-IgG antibody sample (A,B,C,D). **[C]** Graph of the relationship between IgG concentration of the NR1-IgG samples and FlashTag band width, showing no significant correlation between the two.

4.5.6 Embryonic impacts of NR1-IgG on microglia

4.5.6.1 Effects on microglial density in the LGE

With the above findings demonstrating that NR1-IgG antibodies impact the morphology and progenitor proliferation in the developing striatum, the next experiments sought to investigate whether certain changes observed are a direct effect of the antibodies on the developing neuronal population or whether there are intermediary step(s) through other cell types and in particular microglia.

Microglia were chosen as a good initial additional cell type to explore since they are present in the embryonic brain early (~E9.5), prior to the onset of neurogenesis, and occupy the proliferative neuroepithelium, suggesting a role in embryonic neurodevelopment (Cowan and Petri 2018). They are thought to have a bi-directional role in neurogenesis, both supporting and phagocytosing cells (Ueno et al. 2014), with literature showing that impaired microglia can result in decreased differentiation (Erblich

et al. 2011), reduced proliferation (Antony et al. 2011) and fewer neural progenitors (Cunningham et al. 2013). Further, embryonic microglia have been shown to express functional NMDARs (Gottlieb and Matute 1997, Eriksson et al. 2000, Tahraoui et al. 2001, Tikka et al. 2001, Kaur et al. 2005, Nair et al. 2006, Newcombe et al. 2008, Liang et al. 2010, Murugan et al. 2011, Kaindl et al. 2012), and activation of microglial NMDARs can result in neuronal cell death and inflammation in the early brain (Kaindl et al. 2012).

Thus, activated microglial marker IBA1 was used to immunolabel activated microglia in the LGE of NR1-IgG and control-IgG embryos and microglial density and location subsequently assessed (**Figure 4.10 A**). For these experiments, n(NR1-IgG) = 9 brains, n(control-IgG) = 6 brains, 3 litters. Quantification of IBA1+ cells revealed no significant difference in the cortex (relative % change NR1-IgG: cortex: $7.7 \pm 7.1\%$, one-sample t-test: $p = 0.31$; **Figure 4.10 B, C**), but a specific increase in microglia density in the LGE of NR1-IgG exposed brains (relative % change NR1-IgG: LGE: $14.1 \pm 4.2\%$, one-sample t-test: $p = 0.012$; **Figure 4.10 D, E**). Within the LGE of NR1-IgG exposed brains, the greatest density of microglia appeared to be close to the ventricle ((cell count): 0-20 μm : 7.6 ± 2.6 , 20-40 μm : 7.9 ± 3.5 , 40-60 μm : 4.9 ± 3.3 , 60-80 μm : 3.8 ± 2.2 , 80-100 μm : 4.9 ± 2.4 , 100-120 μm : 3.3 ± 2.1 , 120-140 μm : 1.9 ± 1.1 , 140-160 μm : 3.2 ± 1.4 , 160-180 μm : 4.2 ± 2.4 , 180-200 μm : 3.1 ± 1.6 , 200 $\mu\text{m}+$: all <3.5 ; **Figure 4.10 F**).

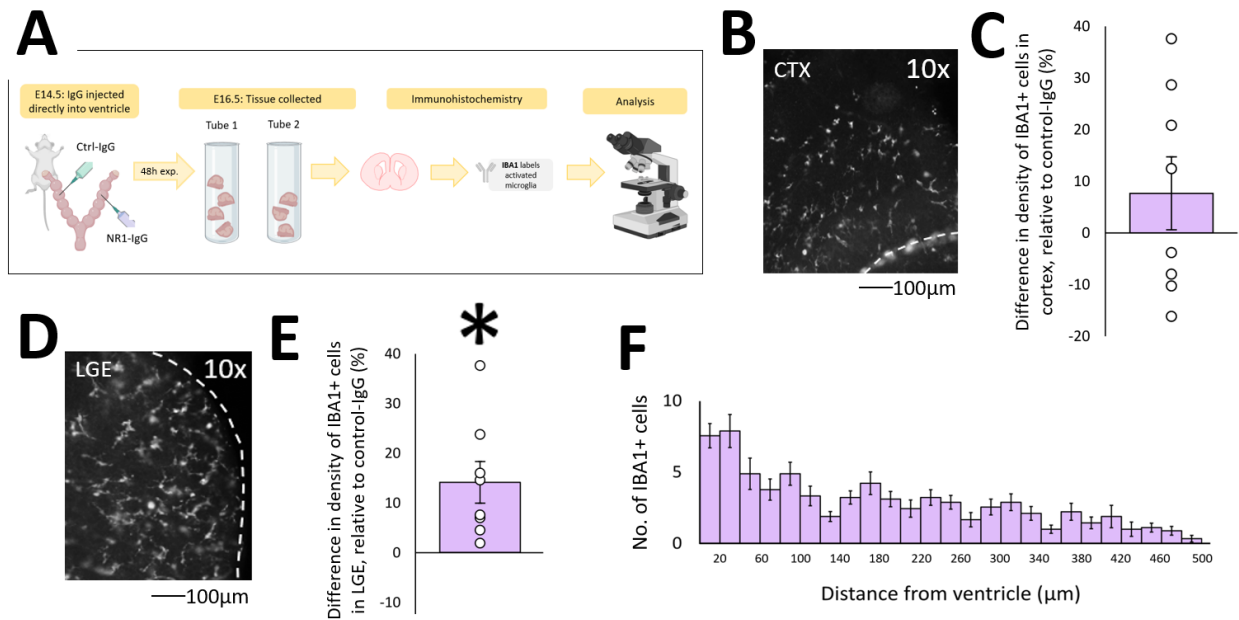


Figure 4.10. Increased microglial density in the LGE after brief in utero NR1-IgG exposure. [A] Graphical outline of the immunohistochemistry methodology to assess microglia in E16.5 brains, following 48-hour exposure to either NR1-IgG or control-IgG. IBA1 was used to label activated microglial cells. [B] Representative epifluorescence image of the E16.5 cortex, immunostained with IBA1 to label activated microglial cells. [C] Bar plot of the difference in number of IBA1+ cells in the cortex of NR1-IgG brains, relative to litter-matched control-IgG brains. There was no significant difference in number of IBA1+ cells in the cortex. [D] Similar image to [A] but of the E16.5 LGE instead. [E] Similar bar plot to [C], but of IBA1+ cells in the LGE instead. There was a significant increase in the relative number of activated microglial cells in the LGE of NR1-IgG brains. [F] Bar plot of mean number of IBA1+ cells in medial sections of the LGE of NR1-IgG brains, split by their distance from the ventricle. The highest numbers of microglial cells were found in the first ~50µm from the ventricle.

4.5.6.2 Effects on microglial morphology in the LGE

Microglia exhibit dynamic morphology, which is closely related to both their maturational state (Zusso et al. 2012) and their function (Brockhaus et al. 1996, Dailey and Waite 1999, Stence et al. 2001, Zusso et al. 2012, Fernández-Arjona et al. 2017). Hence, the morphology of microglia within the LGE was also analysed. Microglia were reconstructed using a similar protocol to that set out in Young and Morrison (2018) (Figure 4.11 A, B). For these experiments, n(NR1-IgG) = 36 cells, 9 brains, n(control-IgG) = 24 cells, 6 brains, 3 litters. Initial analysis of simple morphological parameters such as branch length, soma width

and number of endpoints revealed a significant decrease in the total number of branches (relative % change NR1-IgG: $-13.0 \pm 6.0\%$, one sample t-test: $p = 0.037$; **Figure 4.11 C**), and number of primary branches (relative % change NR1-IgG: $-12.4 \pm 4.9\%$, one sample t-test: $p = 0.017$; **Figure 4.11 D**), and a strong trend towards a decrease in number of end points (relative % change NR1-IgG: $-11.3 \pm 5.6\%$, one sample t-test: $p = 0.051$) but no significant changes in branching complexity when analysed with Scholl analysis (mixed ANOVA: $F_{(1,58)} = 0.22$, $p = 0.64$; **Figure 4.11 E**).

Recent literature (eg. **Fernández-Arjona et al. 2017**) has also begun to utilise more detailed morphological analysis on microglia to uncover subtle but important morphological differences. Hence, an additional set of more fine-scale morphological parameters were analysed based on those detailed in **Fernández-Arjona et al. (2017)** (**General Methods, Figure 2.16**). These revealed a subtle but significant decrease in fractal dimension (a measure of complexity, with high values indicating greater complexity) (relative % change NR1-IgG: $-1.9 \pm 0.8\%$, one sample t-test: $p = 0.022$; **Figure 4.11 F**), and a significant increase in lacunarity (a measure of shape heterogeneity, with higher values indicating greater heterogeneity) (relative % change NR1-IgG: $5.8 \pm 1.8\%$, one sample t-test: $p = 0.0022$; **Figure 4.11 G**). They also revealed subtle but significant decreases in 'density' (cell area/hull area; a measure of compactness of the cell) (relative % change NR1-IgG: $-4.5 \pm 5.5\%$, one sample t-test: $p = 0.033$; **Figure 4.11 H**) and cell circularity (relative % change NR1-IgG: $-3.0 \pm 6.6\%$, one sample t-test: $p = 0.037$; **Figure 4.11 I**). The other parameters tested showed no significant differences (data and statistics in **Appendix, Supplementary Table S4.6**).

Overall, the differences in the morphological parameters observed would suggest a more activated microglial morphological state and/or could also reflect a maturational difference (**Zusso et al. 2012, Torres-Platas et al. 2014, Fernández-Arjona 2017**).

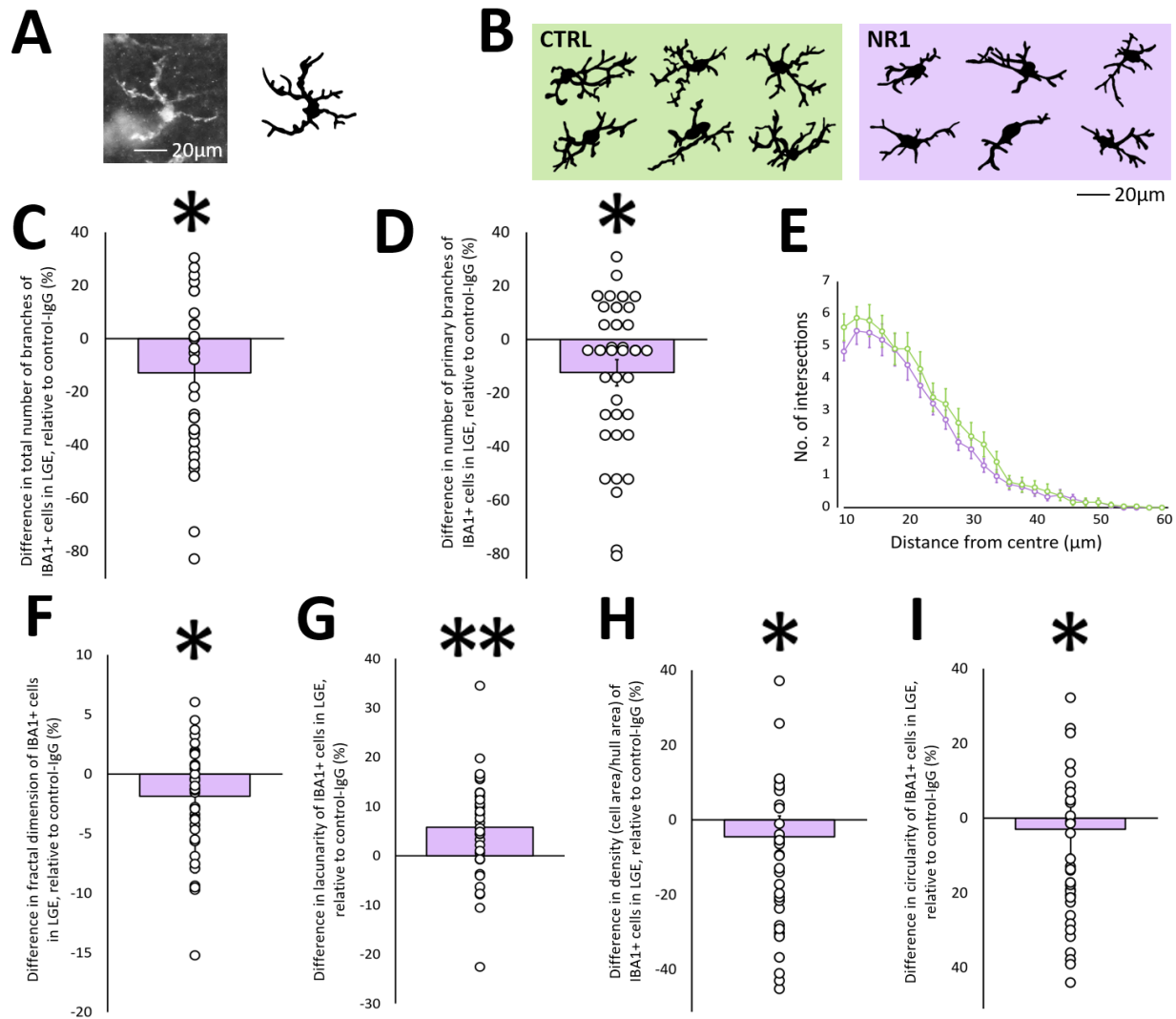


Figure 4.11. Altered microglial morphology in the LGE after brief in utero NR1-IgG exposure. [A] Representative epifluorescence image of an LGE activated microglial cell stained with IBA1, alongside a binary reconstruction of the same microglial cell using Image J. Cells were imaged in a 30µm z-stack, at 1µm intervals, following the protocol outlined in **Young and Morrison (2018)**. [B] Further representative reconstructions of IBA1+ LGE microglia from control-IgG (green) and NR1-IgG (purple) exposed brains. [C] Bar plot of the difference in total number of branches of IBA1+ cells in the LGE of NR1-IgG brains, relative to litter-matched control-IgG brains. LGE IBA1+ microglia exposed to NR1-IgG had significantly fewer branches. [D] Similar to [C], but for relative difference in number of primary branches. LGE IBA1+ microglia exposed to NR1-IgG had significantly fewer primary branches. [E] Scholl analysis of dendritic complexity of IBA+ cells in the LGE of NR1-IgG brains, relative to litter-matched control-IgG brains, showing no significant difference. [F] Bar plot of the difference in fractal dimension of IBA+ cells in the LGE of NR1-IgG brains, relative to litter-matched control-IgG brains. LGE IBA1+ microglia exposed to NR1-IgG had a significantly lower fractal dimension, reflecting reduced complexity. [G] Similar to [F], but for lacunarity. LGE IBA1+ microglia exposed to NR1-IgG had significantly greater lacunarity, revealing a more heterogeneous morphology. [H] Similar to [F], but for 'density' (cell area/hull area). LGE IBA1+ microglia exposed to NR1-IgG had relatively decreased density (cell area/hull area). [I] Similar to [F] but for circularity. LGE IBA1+ microglia exposed to NR1-IgG had significantly decreased circularity.

4.6 Chapter Discussion

In rodents, striatal neurogenesis typically occurs between E14.5-18.5, during which the main striatal neurons (SPNs) are formed, and the literature suggests that striatal neurogenesis may be largely NMDAR-mediated (Luk et al. 2003). Thus, in **Experimental Chapter B**, we explored the embryonic impact of patient-derived NR1-IgG antibodies on development of the striatum embryonically, using the novel *in utero* intraventricular injection model outlined and characterised in **Experimental Chapter A**. Immunohistochemical and morphological studies identified several differences in processes underlying striatal neurogenesis and early striatal formation. Specifically, the key findings in *in utero* NR1-IgG exposed brains were:

1. **Gross LGE morphology:** Differences in gross striatal morphology at E16.5 including a significant decrease in the area of the primordial striatum, and a significant increase in the width of the LGE proliferative zone, with no differences in cortical thickness.
2. **Overall progenitor proliferation:** Significant decrease in PH3+ progenitor proliferation in the LGE, with the greatest decreases in medial sections and in the proliferative zone. No differences in cortex.
3. **Specific progenitor populations:** A large distinct population of T α 1-expressing progenitors could be labelled successfully in the LGE at E15.5 in healthy brains. There was no significant difference in the percentage of labelled T α 1-expressing progenitors in NR1-IgG-exposed brains.
4. **Cell migration and cell death:** Significant decrease in migration of FlashTag pulse-labelled cells away from the ventricular wall in the LGE. No significant differences in cell death in the LGE.
5. **Other patient-derived NR1-IgG antibodies:** Significant decrease in migration of FlashTag pulse-labelled cells when using two (out of four) other patient-derived NR1-IgG antibodies, and suggestive decrease in the third sample (insufficient data for statistical analysis).

6. **Microglia:** Increase in activated microglial density in the LGE and alterations in morphology suggestive of a more activated state and/or maturational differences.

4.6.1 Gross LGE morphology: Significant decrease in the area of the primordial striatum, and significant increase in the width of the LGE proliferative zone;

Since the literature suggests that many regions and processes in the developing brain might be impacted by embryonic NR1-IgG exposure (eg. **Jurek et al. 2019**, **García-Serra et al. 2020**), the first experiments investigated gross brain morphology. Assessment of brain weight between NR1-IgG and control-IgG exposed brains at E16.5 revealed no significant differences. Although there is limited literature on embryonic brain weight to compare to here, **Jurek et al. (2019)** measured brain volumes in mice exposed *in utero* to NR1-IgG at birth and also found no significant differences, aligning with our findings. Interestingly, they did find reduced brain volumes at 8 weeks postnatally, which could suggest potential delayed onset of pathological changes. An alternative explanation for our findings is that interpretation of brain weight is somewhat limited as it is a crude methodology, particularly when measuring very small brains, and hence subtle early differences may not have been detected.

Striatal-specific morphological changes were also assessed more specifically through labelling the primordial striatum with SPN marker CTIP2 in fixed E16.5 coronal brain sections, which showed a trend towards a decrease in primordial striatum size in NR1-IgG exposed brains. Though there is no specific embryonic striatal data to compare to, this could also fit additional observations by **Jurek et al. (2019)** who identified a significant decrease in striatal volume at 8 weeks in *in utero* exposed NR1-IgG brains, and also **Luk et al. (2003)** who found significant decrease in striatal volume at 6-7 weeks following *in utero* exposure to NMDAR antagonists MK801 and CGS-19755. We also observed a significant increase in the width of the proliferative zone in the NR1-IgG exposed LGE. All SPNs are generated by progenitors within the proliferative zone close to the ventricular wall, that proliferate, differentiate, and subsequently

migrate away to form the mature striatum. Hence, these findings together suggest a likely impairment in at least one of these key processes along the developmental trajectory from progenitors to striatal neurons (Knowles et al. 2021).

4.6.2 Overall progenitor proliferation: Significant decrease in PH3+ progenitor proliferation in the LGE, with the greatest decreases in medial sections and in the proliferative zone;

Given the increased proliferative zone width, to assess whether progenitor proliferation may be impaired, proliferating LGE progenitors were labelled with mitotic marker PH3. Here a significant decrease in numbers of proliferating progenitors was found, suggesting impaired proliferation may be one reason behind a reduced primordial striatal size. The decreases occurring most predominantly in medial slices and in the proliferative zone is likely due to increased anatomical exposure to the ventricle, aligning to where the antibody is injected. These observations fit the aforementioned study by Luk et al. (2003) who specifically found decreased LGE progenitor proliferation, DNA synthesis and striatal neuron numbers following *in utero* NMDAR antagonist exposure, as well as broadly with Sadikot et al. (1998) who used a similar protocol and found decreased striatal interneuron numbers. In both studies, effects were seen following antagonist exposure during the embryonic striatal proliferative window (E15-18) but not the post-proliferative period (E18-21), indicating a specific impact on striatal proliferation.

Concordantly, morphology and proliferation in the cortex was also investigated, and no significant differences in either cortical thickness, or in numbers of PH3+ proliferating progenitors in the cortex was identified, suggesting minimal impact on cortical neurogenesis. In support of these findings, Luk et al. (2003) identified using *in utero* exposure to NMDAR antagonists (MK801, CGS-19755) and AMPAR antagonists (NBQX), that embryonic cortical neurogenesis is more AMPAR/KAR-mediated, which could explain why in the cortex here there was minimal impact from antibodies against the NMDAR. This would

also largely fit with the transcriptional glutamatergic receptor findings observed in **Experimental Chapter A**.

Other literature is more mixed as to the impact of impairments to the NMDAR *in utero* on cortical neurogenesis. For example, **Lee et al. (2008)** suggests that NMDAR-specific antibodies *in utero* can result in cortical plate thinning embryonically. One explanation for this discrepancy is that they administered antibodies to pregnant dams at E12, whereas in this study the antibodies were administered at E14.5. Since the deep layers form around E11.5-14.5, and the superficial layers form from E14.5-17.5 (**Luskin et al. 1988, McConell and Kaznowski 1991, Walsh and Cepko 1993, Frantz and McConnell 1996, Desai and McConnell 2000, Shen et al. 2006, Gao et al. 2014, Kaplan et al. 2017**), the later injection timepoint here likely missed the deep layer formation window which may have minimised impact on the cortex. An alternative explanation is delayed onset, and in this case these findings might also align with **García-Serra et al. (2020)** who found that *in utero* exposure to NR1-IgG from E14-16 results in reduced cortical thickness at p21 (no assessment of cortical thickness was done in their study before this point). It is also important to consider that counting individual antibody-stained progenitors may be limited by challenges accurately visualising the cells, for example through discrepancy in the strength of the antibody staining between slices, or subjective differences in what counts as a 'labelled' cell. Much care was taken to minimise these impacts through using a standardised threshold above background staining as criteria for including or excluding a cell and having one individual only count all slices.

Studies assessing impacts on proliferation specifically also somewhat disagree with one another; **Luk et al. (2003)** found no changes in proliferation following *in utero* NMDAR antagonist exposure similarly to us, whereas **Mochizuki et al. (2007)** observed decreased cortical progenitor proliferation *in vitro* following NMDAR antagonist MK801 exposure, and **Hiwasawa et al. (2003)** found increased numbers of proliferating cortical progenitors *in vitro* following chronic embryonic exposure to NMDAR antagonist DAP5. One explanation is that both **Mochizuki et al. (2007)** and **Hiwasawa et al. (2003)** conducted their

studies *in vitro* using cultures derived at E17-18, whereas **Luk et al. (2003)** conducted their studies earlier, between E15-18. Other studies would suggest that the very late embryonic/early postnatal period may be a particularly vulnerable time for cortical development, with several experiments showing that delivering NMDAR antagonists (MK801) to the early postnatal brain also results in altered cortical cytoarchitecture (**Marret et al. 1996, Reiprich et al. 2005**). Other experimental differences may also underlie the discrepancies observed including *in vitro* vs *in vivo* administration of antagonists, and different antagonist types, concentrations and exposure times used.

4.6.3 Specific progenitor populations: No significant difference in the percentage of labelled

Tα1-expressing progenitors;

Returning to focus on the LGE, more in-depth analysis of the location of the PH3+ labelled cells in the LGE demonstrated that the decreases were greatest in medial sections and notably, close to the ventricle, within the proliferative zone. One explanation for this is that since antibodies are injected directly into the ventricles, the proliferative zone is most exposed and therefore this would be the region where you would expect the greatest impacts.

Since the LGE has been shown to consist of heterogeneous progenitor populations classified by their location (amongst other characteristics) (**Knowles et al. 2021**), the regional differences observed in density of proliferating cells within the LGE raised the additional question as to whether specific progenitor type(s) were more greatly impacted by the NR1-IgG antibody. This was an important question, since previous work has demonstrated important and distinct roles for individual embryonic progenitor types in other brain regions in shaping postnatal circuitry (**Yu et al. 2009, Yu et al. 2012, Cadwell et al. 2020, Tyler et al. 2015, Ellender et al. 2019**). Experiments using IUE of two reporter plasmids were successfully able to label two populations of apically dividing populations in the healthy LGE (Tα1-expressing and non-Tα1-expressing progenitors), revealing a large distinct LGE population of Tα1-

expressing progenitors at E15.5. Injection of IgG antibodies alongside the plasmids in a small pilot experiment revealed no significant differences in the size of the T α 1-expressing and non-T α 1-expressing progenitor populations, putatively suggesting the total population was not more greatly impacted, despite the earlier experiments revealing faster cell-cycle kinetics, with earlier return to the mitotic phase. One explanation for this could be that they are located further away from the ventricle and therefore receive less exposure to the antibody injected into the ventricle. Alternatively, it could be that only proliferating T α 1-expressing progenitors are affected rather than the whole population, which was not assessed here but is an important question for future studies. Results may also have been limited by antibodies being marginally diluted by co-injected plasmids or by variability between confocal vs epifluorescence imaging modalities.

4.6.4 Cell migration and cell death: Significant decrease in cell migration but no significant differences in cell death in the LGE;

Considering the observation of an increase in proliferative zone width in conjunction with a decrease in the number of progenitors close to the ventricle raised the question as to whether cell migration might also be affected. One hypothesis could be that cells were getting 'stuck' within the proliferative zone, unable to migrate away into the primordial striatum. To address this, antibodies were co-injected with FlashTag, a marker that pulse-labels mitotic progenitors at the ventricular wall. Measurement of FlashTag band width revealed a significant decrease in cell migration in the LGE. Although there is limited literature on migration specifically in the embryonic striatum *in vivo*, this observation would be consistent with *in vitro* studies by **Pregno et al. (2011)** who demonstrated that the migration of embryonic primordial striatal-derived progenitors can be positively modulated by NMDAR activation, via NRG1/ErbB4 pathways. Unfortunately, the methodology here did not allow us to explore cortical migration specifically because the FlashTag band spanned most of the cortical width and therefore was unable to be accurately

measured. However, there is also a relatively large body of literature suggesting that NMDARs play a role in cortical migration (**Komuro and Rakic 1993, Behar 1999, Hirai et al. 1999, Hirasawa et al. 2003, Mancini and Atchison 2007, Jansson et al. 2012**). Of note here, **Behar et al. (1999)** found that in embryonic cortical slice cultures *in vitro*, glutamate acting on NMDARs stimulated cell migration, with the largest effect on the VZ/SVZ. Thus, this could also tentatively offer an explanation as to why we also observe predominant impacts in the striatal VZ/SVZ, as it may be the case that since NMDARs have the greatest effect on migration in these zones, hence disruption here by NR1-IgG antibodies would confer the greatest impacts. Exploring this is an important extension to future work.

A further potential mechanism for the decreased number of proliferating progenitors and smaller primordial striatum along with an increased proliferative zone width is cell death, since NMDAR activity plays a role in regulating neuronal survival and NMDAR hyper- or hypoactivation is linked to neuronal death (**Mao et al. 2022**). Increased numbers of dead cells due to NMDAR disruption could potentially congregate in the proliferative zone region, increasing zone width. A TUNEL assay to label apoptotic cells revealed that there were no differences in cell death in the LGE of NR1-IgG exposed brains, suggesting cell death was not an underlying mechanism here. This finding contrasts with experiments by **Ikonomidou et al. (1999)** demonstrating *in vivo* that MK801 antagonism of NMDARs during the late embryonic or early postnatal period triggers widespread neuronal apoptosis across the developing brain. One explanation for this discrepancy could be that this is due to mechanistic (eg. temporal or clearing) differences in antagonistic receptor blockade vs antibody exposure, since other experiments delivering patient-derived NR1-IgG antibodies intraventricularly into postnatal rat brains for prolonged periods showed no significant difference in cell survival in the hippocampus when assessed with a TUNEL assay, broadly fitting our observations (**Hughes et al. 2010**). Alternatively, pro-survival and pro-death mechanisms could also be disrupted to an approximately equal degree, since the NMDAR plays a key role in both (**Hardingham and Bading 2010**), resulting in no overall differences observed. It is also important to consider potential

limitations with the TUNEL staining protocol that may play a part in results discrepancy here, such as the potential for cells to be obscured by background staining, or for cells to be damaged through incubation at high temperatures.

It is important to consider that more than one mechanism may underly these findings. In future work, it would be interesting to investigate whether there is also a delay in differentiation, since NMDARs have been shown to play a significant role in differentiation of neural progenitors via both the Notch and bHLH gene pathways (**Ohtsuka et al. 2001**, **Cau et al. 2002**, **Kageyama et al. 2005**, **Yoon et al. 2005**). Since CTIP2 controls the differentiation of SPNs (**Arlotta et al. 2008**), a delay could result in reduced CTIP2 expression, giving the appearance of a larger 'proliferative zone' as seen here.

The role of oedema is another interesting possibility to explore, although the finding of no significant difference in brain weight between NR1-IgG and control-IgG injected brains earlier in this chapter suggests that this is a less likely case.

4.6.5 Other patient-derived NR1-IgG antibodies;

To help corroborate observations in this chapter, polyclonal NR1-IgG antibodies derived from four further NMDARE patients during acute illness were also assessed. Due to small volume size, these were tested on one robust impact metric: FlashTag cell migration. Experiments here revealed a similar significant decrease in cell migration in two of the four samples, a suggested decrease in the third sample (which could not be formally statistically assessed due to insufficient size), and no change in migration in the fourth sample. Although a small dataset, these results overall support the observations using the main patient-derived polyclonal and monoclonal NR1-IgG antibodies. The similar effects observed here would align with previous work demonstrating similar pathogenic effects of several different polyclonal and monoclonal NR1-IgG antibodies (**Kreye et al. 2016**, **Malviya et al. 2017**, **Wright et al. 2021**). Since the four samples had different concentrations, potential correlation between concentration and cell migration was

assessed and it was found that no significant correlation was present. This is somewhat in contrast with **Hughes et al. (2010)** who found *in vitro* that a higher NR1-IgG antibody titre decreased total and surface levels of NMDAR protein more than a lower titre antibody from the same patient, however it is important to remember that titre and concentration are directly linked, and that two antibodies of the same concentration may have different titres, hence our results are not directly comparable to **Hughes et al. (2010)**. Beyond this, one explanation for a potential discrepancy here could be that here comparisons are being made between different concentrations from different patients and therefore there may be other differences in antibody characteristics specific to the patient also impacting results. Alternatively, characteristics beyond just titre may have impacted the results observed by **Hughes et al. (2010)** since on collection of the lower antibody titre the patient was in a much less acute clinical phase. Results here could also be limited somewhat by age of these antibodies.

4.6.6. Microglia: Increase in activated microglial density in the LGE and alterations in morphology suggestive of a more activated state and/or maturational differences;

Based on the observations here that brief embryonic exposure to NR1-IgG likely impacts several neurogenic processes in the LGE, the final question addressed in this chapter was whether observed changes were due to the NR1-IgG antibodies acting on neurons and neural precursors directly, or whether there may be additional involvement of other intermediary cell types. It is well known that microglia, the immune cells of the brain, are present in the brain prior to onset of neurogenesis, and likely play a functional role in early neurodevelopment (**Cowan and Petri 2018**). Further, NMDARs are present and functional on embryonic microglia, and therefore their functioning could be disrupted by NR1-IgG antibodies (**Gottlieb and Matute 1997, Eriksson et al. 2000, Tahraoui et al. 2001, Tikka et al. 2001, Kaur et al. 2005, Nair et al. 2006, Newcombe et al. 2008, Liang et al. 2010, Murugan et al. 2011, Kaindl et al. 2012**). IBA1 was used to immunohistochemically label and quantify activated microglia, which revealed an increase in activated microglial density specifically in the LGE of NR1-IgG exposed brains. The highest

numbers of activated microglial cells were found close to the ventricle, in the proliferative zone, a similar region to where the greatest decreases in proliferating progenitors were identified. Previous literature suggests that one of the key functions of microglia in neurogenesis is phagocytosis of excessive new-born cells (**Sierra et al. 2010**). A simple explanation for why these two observations were seen in tandem is that greater numbers of activated microglia in the proliferative zone could result in excessive phagocytosis of new-born cells, decreasing cell numbers and impairing striatal neurogenesis. This would align with experiments by **Cunningham et al. (2013)** who found that *in utero* activation of microglia through maternal immune activation significantly decreased number of neuronal progenitors.

Microglial morphology is also reflective of both activation and maturational state (**Brockhaus et al. 1996, Dailey and Waite 1999, Stence et al. 2001, Zusso et al. 2012, Fernández-Arjona et al. 2017**). Activated microglia are typically amoeboid, with few, thick processes and a large, irregular soma, whereas resting state microglia exhibit a more ramified, larger morphology with many extended processes (**Torres-Platas 2014**). In the study here, reconstruction and analysis of simple morphological characteristics of IBA1+ microglia revealed a significant decrease in total number of branches and number of primary branches, likely reflective of an activated morphology. Further detailed analysis uncovered subtle morphological differences including significant decreases in fractal dimension and cell circularity, also supporting a more activated state (**Fernández-Arjona 2017**). These findings would be consistent with **García-Serra et al. (2020)** who observed microglial activation in the early postnatal brain following *in utero* NR1-IgG antibody exposure, as well as wider literature reporting that *in utero* exposure to maternal immune activation alters embryonic and early postnatal microglial phenotype and disrupts neurogenesis (**Loayza et al. 2023**).

It is important to note that differences in embryonic microglial morphology could also reflect a delay in microglial maturation, since it is the most common morphology found in embryonic brains (**Zusso et al. 2012**), before a *Runx1*-mediated shift to a more ramified resting morphology during the late embryonic

and early postnatal stages (**Zusso et al. 2012, Prinz and Priller 2014**). However, they are more likely to reflect microglial activation since IBA1 is upregulated in activated microglia (**Ohsawa et al. 2004**).

Considering our other observations, *in vitro* studies on neuronal cultures from mice genetically lacking microglia have shown reduced proliferation, without impaired cell survival (**Antony et al. 2011**), whilst *in vivo*, reduced microglia have been shown to result in decreased differentiation (**Erblich et al. 2011**) and progenitor numbers (**Cunningham et al. 2013**). It has also been shown that antagonism and loss-of-function mutations of microglial NMDARs reduces neuronal death which could explain why we do not observe changes in cell death in these results (**Kaindl et al. 2012**), as the microglial NMDARs are also likely bound by the NR1-IgG antibody and internalised (**Moscato et al. 2014**). Overall, the literature here largely supports these experimental findings, suggesting that some of the intermediary steps via microglia that play a role in neurogenesis are also impacted by NR1-IgG antibodies.

Whilst our discussion here largely focuses on microglia as the predominant immune cell type (~90%) in the embryonic brain, it is important to acknowledge that IBA1 may also label other macrophage-linked cell types including barrier-associated macrophages, that are also present in the foetal brain and play differing roles in early brain immunity and pathology (reviewed in **Silvin et al. 2023**). More detailed investigation into the role and impact on these other cell types is an interesting question for future work.

4.7 Chapter Summary

This chapter set out to explore the embryonic impact of patient-derived NR1-IgG antibodies on the developing mouse striatum. Following short exposure to NR1-IgG during embryonic neurogenesis, the LGE showed altered gross and cellular morphology including an increase in proliferative zone width and a decrease in proliferating progenitors in the LGE, especially those close to the ventricle. Analysis of a specific Tα1+ progenitor population in the LGE demonstrated that it could be successfully labelled at E15.5 with IUE, but that it showed no significant change in population size following NR1-IgG antibody exposure, despite the relative proximity to the ventricle. Additionally, a decrease in LGE cell migration was found, but no differences in cell death identified. Assessment of other patient NR1-IgG antibody samples mostly demonstrated similar decreases in cell migration but also some variability between different samples. Investigation into microglia also found increased density and altered morphology of activated microglia in the LGE.

Building on the observed disruption in the embryonic brain seen here, the next chapter assessed impact of *in utero* NR1-IgG exposure on development of early postnatal striatal cells and circuits.

5. Experimental Chapter C: How does brief *in utero* exposure to patient-derived NR1-IgG affect striatal cellular and circuit development postnatally?

5.1 Chapter Introduction

Experimental Chapter B demonstrated that short *in utero* exposure to patient-derived NR1-IgG antibodies results in changes embryonically in the developing striatum, including decreased progenitor proliferation and migration, and increased density of activated microglia. Building on from this, **Experimental Chapter C** set out to explore whether these changes impacted the early postnatal striatum using a two-pronged approach: the first part focused on electrophysiological changes including intrinsic properties of striatal SPNs, formation of synapses, and integration of SPNs into corticostriatal circuits, and the second part focused on morphological changes including morphology of the gross striatum, SPNs and striatal microglia.

5.2 Chapter Brief Background

The late embryonic and early postnatal phases are highly dynamic periods for striatal development (**Krajeski et al. 2019**). The mature mouse striatum has been shown to consist almost exclusively (~95%) of spiny projection neurons (SPNs) (**Tepper et al. 2010**), which can be sub-divided into two populations: D1-expressing and D2-expressing SPNs. They are associated with the direct and indirect pathways in the striatum respectively (**Day et al. 2008, Gertler et al. 2008**). Correct development of both types of SPN, and careful balance of activity within these pathways is key for correct motor and cognitive functioning (**Graybiel et al. 1994, Grillner et al. 2005, Yin and Knowlton 2006, Kravitz et al. 2010, Tecuapetla et al. 2016**), with disruption to early striatal development implicated in a host of neurodevelopmental disorders including OCD (**Graybiel and Rauch 2000**), Tourette's syndrome (**McNaught and Mink 2011, Albin et al. 2018**), ADHD (**Del Campo et al. 2011**), ASD (**Shepherd 2013**) and schizophrenia (**Shepherd 2013**).

5.2.1 Cellular development of SPNs in the early postnatal period

Over the first postnatal weeks, SPNs mature both electrophysiologically and morphologically (**Krajeski et al. 2019, Lebouc et al. 2020**). Both *in vivo* and *ex vivo* recording studies of early postnatal SPNs have shown that they are 'electrically immature' shortly after birth, characterised by a high intrinsic excitability and high input resistance, and thus only able to generate small action potentials (**Tepper et al. 1998, Belleau and Warren 2000, Dehorter et al. 2011, Peixoto et al. 2016**). In the following weeks, they undergo a progressive decrease in intrinsic excitability and resting membrane potential (**Lieberman et al. 2018, Krajeski et al. 2019**), coupled with the ability to produce larger action potentials at greater firing frequencies (**Peixoto et al. 2016, Krajeski et al. 2019**). There is concurrent morphological maturation from a radial to reticular cellular layout, parallel to substantial increase in size and complexity of their dendritic arbour (**Krajeski et al. 2019**). SPN morphological development facilitates the maturation of intrinsic

properties, enabling greater expression of inward rectifying K⁺ channels, which contributes to their decrease in intrinsic excitability (**Lieberman et al. 2018**).

Full electrical maturity is only reached during the fourth postnatal week when membrane potential bistability emerges in the shape of up and down states, crucial for circuit integration, input processing and mature action potential generation (**Wilson and Kawaguchi 1996, Murer et al. 2002, Mahon et al. 2003, Carter and Sabatini 2004**). The functional reason for this distinct temporal evolution of intrinsic properties is not fully understood but it is suggested that initial high excitability may contribute to synaptogenesis and early synaptic plasticity through facilitated activation of dendritic and synaptic voltage-dependent currents (**Carter et al. 2007, Carter and Sabatini 2004, Peixoto et al. 2016**) or enhancement of early network activity patterns (**Khazipov et al. 2004, Colonnese et al. 2010, Kirkby et al. 2013**).

It is worth being aware that some differences have been reported in the electrical and morphological development of D1- and D2-SPNs. D1-SPNs are suggested to be born earlier (**Marchand and Lajoie 1986, Van der Kooy and Fishell 1987, Kelly et al. 2018**) and my lab and others have shown them to be electrically more mature during the initial postnatal phase, with a lower intrinsic excitability, and ability to generate larger action potentials than their D2-expressing counterparts (**Peixoto et al. 2016, Krajeski et al. 2019**). Morphologically, my lab has previously found that D1- and D2-SPNs develop in parallel (**Krajeski et al. 2019**), but others have identified morphological differences in developing and mature D1- and D2-SPNs, including a larger total dendritic length and number of primary dendrites in D1-SPNs (**Gertler et al. 2008, Benthall et al. 2018**).

5.2.2 SPNs and the development of striatal circuits

As SPNs mature they develop synapses, enabling local striatal communication and integration into wider neural circuits. SPNs predominantly synapse via their dendritic spines, and synapse-like structures are observed on SPN neurites from ~E14. Spine density progressively increases over early development,

peaking around the second postnatal week, corresponding to the emergence of complex, coordinated motor behaviours (**Tepper et al. 1998, Gertler et al. 2008, Dehorter et al. 2011, Peixoto et al. 2016, Krajeski et al. 2019**). Synaptic development and maturation are closely regulated by several mechanisms, including neuromodulatory systems that influence cAMP-dependent protein kinase through G-protein coupled receptors (**Kozorovitskiy et al. 2015, Speranza et al. 2017**) and a shift in the NMDA/AMPA ratio towards a bias in AMPAR recruitment, which takes place over the first postnatal weeks (**Petralia et al. 1999, Peixoto et al. 2016, Krajeski et al. 2019**).

Locally, SPNs mainly form gap junctions with one another over the first postnatal week, which are progressively replaced by precise, non-random inhibitory synapses as the striatum develops (**Krajeski et al. 2019**). More widely, striatal SPNs also receive long-range excitatory connections from two main structures: the cortex (a focus of this thesis) and the thalamus (**Kemp and Powell 1971, Buchwald et al. 1973, Smith et al. 2004**). Corticostriatal pathways have roles in an array of cognitive and motor functions (**Pennartz et al. 2009**) and disruption to these pathways is implicated in multiple neurodevelopmental disorders (**Shepherd 2013**). Such projections are present and functional in the striatum by the first postnatal week, and strengthen over the first three postnatal weeks, via increasing AMPAR-mediated transmission (**Sohur et al. 2014, Krajeski et al. 2019**). Afferent corticostriatal activity is thought to be an important regulator of early postnatal SPN synaptogenesis, with studies during the second postnatal week demonstrating that increased cortical activity accelerates maturation and potentiation (**Kozorovitskiy et al. 2015, Peixoto et al. 2016**). Additionally, there is a rapid increase in both cortical and striatal activity during this time window.

My lab has also previously identified that maturation of excitatory corticostriatal synapses occurs mostly in parallel for both D1- and D2-SPNs, but with some differences, including stronger NMDAR-mediated inputs from cortex onto D1-SPNs during the second postnatal week, posited to be due to transient changes in receptor expression or differential input maturation (**Krajeski et al. 2019**). Changes in postnatal

network activity have also been shown to impact SPN excitatory synapse maturation, with silencing of D2-SPNs during development demonstrated to increase SPN excitatory synaptic inputs, and the converse true for silencing of D1-SPNs (**Kozorovitskiy et al. 2012**). Additionally, local striatal inhibitory synapses have been shown to exhibit non-random and relative biases from the second postnatal week onwards including highly interconnected D2-SPNs (**Krajeski et al. 2019**).

5.2.3 Influence of microglia on shaping early postnatal circuits

Microglia have been shown to influence many neurogenic processes in the embryonic brain, and evidence suggests their role extends into the early postnatal development of neural circuitry, with particular involvement in synaptogenesis, refinement and axonal growth (**General Introduction, Section 1.4.2 Roles of microglia in neurodevelopment**). Of most relevance here, within the striatum, microglial activation has been recently demonstrated to induce local cytokine expression and a negative affective state, as well as a prostaglandin-dependent reduction in SPN excitability (**Klawonn et al. 2021**). More widely, experiments have also shown their role in influencing synaptic strength, either through TNF α release (**Zhong et al. 2010**) or promotion of surface AMPAR expression (**Beattie et al. 2002, Cingolani et al. 2008**).

5.2.4 The importance of NMDARs in early postnatal cellular and circuit development

The early postnatal period is clearly an important time for striatal cellular and circuit maturation. NMDARs have been shown to play important roles here, including in development of neuronal intrinsic properties and morphology, and development of local and long-range synaptic connections. Their functions are given in detail in **General Introduction, Section 1.1.5 Roles of NMDARs in neuronal and synaptic development**, but to briefly summarise the most relevant literature here:

In terms of **neuronal cellular development**, several studies indicate NMDARs are important for early electrophysiological and morphological maturation in numerous cell types. NR1 knock-out studies in both primary sensory and thalamic neurons have been linked to increased early intrinsic excitability, along with

reduced HCN channel function in the latter (**Pagadala et al. 2013, Hou and Zhang 2017**), whilst similar knock-out studies in thalamic and cortical neurons have demonstrated morphological alterations including reduced numbers of dendrites, decreased spine density and altered spine morphology (**Ultanir et al. 2007, Hou and Zhang 2017**). *In utero* exposure to NR1-IgG antibodies has also been demonstrated to reduce spine density in the hippocampus (**García-Serra et al. 2020**). Regarding the striatum specifically, SPNs from NR1 knock-out mice have demonstrated severe morphological alterations including decreased dendritic length and a smaller cell body (**Beutler et al. 2011**). NMDAR subunit composition has also been shown to influence SPN dendritic spine morphology, with antagonism of NR2A (with NVP-AAM007) in corticostriatal slices increasing spine head width, but antagonism of NR2B (with ifenprodil) preventing D1-agonist induced morphological changes (**Vastagh et al. 2012**). Limited work has been done on the role of NMDARs in the development of SPN intrinsic properties, but based on findings in other cell types, as well the high striatal expression of NR1 in early development (**Experimental Chapter A**), one would expect that they likely play an important role here.

Regarding **neuronal circuit development**, NMDARs have a well-established role in development and plasticity of glutamatergic synapses (**Malinow and Malenka 2002, Song and Hyganir 2002**). They are particularly important for glutamatergic transmission during the early postnatal period, since glutamatergic synapses contain very few AMPARs (**Isaac et al. 1995, Liao et al. 1995, Isaac et al. 1997**). They are also responsible for the developmental increase in synaptic AMPARs (**Liao et al. 1999, Adesnik et al. 2008, Zhang et al. 2013**), and pruning of redundant synaptic inputs (**Ruthazer et al. 2003, Zhang et al. 2013**); both critical in synaptic and circuit maturation. Considering corticostriatal circuits specifically, NMDARs exhibit dynamic expression during the early postnatal period (**Monyer et al. 1994, Dehorter et al. 2011, Krajcski et al. 2019**). *In vitro*, it has been demonstrated in corticostriatal cultures that SPNs with fewer cortical inputs display significantly decreased dendritic length and reduced complexity, likely mediated by NMDARs (**Buren et al. 2016**). Further, in healthy physiological development it has been

shown that specific NMDAR-mediated corticostriatal inputs occurs alongside reduction in an immature striatal activity pattern in the second postnatal week, correlated to the onset of locomotion, highlighting their importance in corticostriatal synaptic maturation (**Dehorter et al. 2011**).

5.2.5 Focusing on the impact of NR1-IgG antibodies on the early postnatal striatum

Previous studies have shown that embryonic disruption to NMDARs (and other key targets) can have impacts spanning into the early postnatal period. **Jurek et al. (2019)** has previously shown that *in utero* exposure to patient-derived NR1-IgG antibodies results in altered early postnatal electrophysiological properties of cortical neurons including significantly decreased amplitudes of spontaneous EPSCs (sEPSCs), whilst **García-Serra et al. (2020)** has demonstrated that similar *in utero* NR1-IgG antibody exposure results in cortical plate thinning as well as microglial activation in offspring. Considering the striatum, **Luk et al. (2003)** has demonstrated that *in utero* exposure to NMDAR antagonist MK801 in mice leads to a significant decrease in the number of early postnatal SPNs, but the detailed impacts of NR1-IgG antibodies on early striatal cellular and circuit development have not yet been explored. Both our data from **Experimental Chapter B** and **Luk et al. (2003)** suggest heavy impacts on embryonic striatal progenitors following *in utero* disruption to the NMDAR and would therefore posit striatal circuits as likely targets of consequent postnatal disruption. Based on the important roles that NMDARs play in early postnatal cellular and circuit development described above, such consequences might include more hyperexcitable SPNs with a simpler morphology, as well as impairments in the development of local and long-range SPN synapses (**Hou and Zhang 2017**).

5.3 Chapter Aims

The aim of **Experimental Chapter C** was to build on the embryonic findings of the impact of brief *in utero* exposure to NR1-IgG antibodies on the developing striatum observed in the previous chapter and explore if these translated into more prolonged impacts in the cells and circuits of the early postnatal striatum.

This chapter aimed to address the following:

- 1) How does *in utero* exposure to NR1-IgG antibodies affect the intrinsic properties of early postnatal SPNs?
- 2) How does it impact corticostriatal connections in the early postnatal brain?
- 3) How does it impact the NMDA/AMPA ratio in early postnatal SPNs?
- 4) How does it affect the gross morphology of the early postnatal striatum?
- 5) How does it affect the ratio and density of D1 and D2-SPNs?
- 6) How does it affect the morphology of early postnatal SPNs?
- 7) How does it impact early postnatal striatal microglia?

5.4 Chapter Methods

Methods used in this Chapter are described in detail in **General Methods** and are briefly summarised below (in the approximate order in which they appear):

5.4.1 *In utero* surgical methods

(See **General Methods Section 2.5 *In utero* surgical methods**)

The experiments in this chapter used a similar *in utero* antibody model to **Experimental Chapter B**, whereby patient-derived NR1-IgG or matched control-IgG antibodies were injected intraventricularly at E14.5. However, due to the postnatal use of these animals, the entire litter was injected with either NR1-IgG or control-IgG (rather than having an intra-litter control as for the embryonic experiments).

For postnatal experiments involving *in utero* injection of antibodies at E14.5, and use of slices/brains postnatally, the overall survival rates at birth were: NR1-IgG: 77.3%, control-IgG: 75.0%.

5.4.2 Electrophysiological methods

(See **General Methods Section 2.7 Electrophysiological methods**)

Following surgery to inject either NR1-IgG or control-IgG antibodies, acute striatal slices were made from postnatal animals ranging from p7-14. In brief, animals were deeply anaesthetised and decapitated before the brains were rapidly removed and kept on ice-cold cutting aCSF. 350µm acute coronal sections were then made using a vibrating microtome. Slices containing the striatum were collected and transferred to a storage beaker containing recording aCSF bubbled with carbogen gas and left to rest for at least 1 hour at room temperature.

Whole-cell patch-clamp recordings of SPNs were then made on a rig perfused with recording aCSF at 2ml/min. Briefly, slices were transferred to the recording chamber and a suitable cell within the dorsal striatum located. A borosilicate glass pipette filled with potassium gluconate recording solution was

lowered to the cell surface, and steps of negative pressure applied to first attach to the cell membrane and then 'patch' the cell (i.e. break through the membrane). Successful patch was confirmed by change in the shape of the seal test trace (**General Methods, Figure 2.15**). Patched neurons could be maintained for at least 30 minutes and in a small number of neurons a small amount (<10mA) of current was injected to help stabilise the resting membrane potential.

5.4.2.1 Intrinsic properties

Intrinsic properties including **resting membrane potential (RMP)**, **input resistance (IR)**, **membrane time constant (MTC)** and **spike threshold**, as well as action potential properties including **amplitude**, **frequency** and **duration** were assessed through applying a series of hyperpolarising and depolarising current steps in current clamp mode (-100 to +100pA for most neurons, or -50 to +50pA for some immature p7-8 neurons which could not tolerate the wider range of current steps).

5.4.2.2 Spontaneous events

Neurons were also recorded in voltage clamp mode, with the cell clamped at -70mV and analysed for the presence of **spontaneous EPSCs (sEPSCs)**.

5.4.2.3 Corticostriatal connections (evoked events)

In a subset of cells, the corticostriatal connections were also assessed by placing a bipolar electrode in the external capsule, close to the striatum. GABA_A receptor antagonist SR95531 (Gabazine, 1μM) was perfused through the rig to ensure only excitatory transmission was recorded. Cortical afferents were activated via the electrode every 5 seconds, and stimulation strength titrated until evoked **EPSPs** were ~50% of their maximum height. Using this stimulation strength, EPSPs were recorded every 5 seconds for 20 repetitions. **Paired-pulse ratio (PPR)** was also recorded by delivering two pulses of stimulation at 20Hz, and response to **train stimulation** evaluated by delivering a train of 10 pulses at 20Hz.

Recordings in just the presence of Gabazine captured EPSPs mediated by combined AMPARs/KARs and NMDARs. All recordings were repeated in the presence of NMDAR antagonist DAP5 (50 μ M) to capture evoked EPSPs mediated by AMPA/KA only. Competitive AMPA/KA antagonist CNQX was washed in at the end of experiments as a control measure to confirm that only glutamatergic AMPA/KA EPSPs were being captured in our results.

5.4.2.4 Analysis of recordings

Electrophysiological data was analysed both as a whole dataset (p7-14) and in smaller two-day time windows (p7-8, p9-10, p11-12 and p13-14) to both look at larger effects and also pinpoint effects at in more specific windows. The mean age of each of these groups was compared between NR1-IgG and control-IgG cells, to ensure there was no significant difference in the age of any of the groups. Intrinsic properties were also compared between animals injected with polyclonal antibody vs animals injected with polyclonal antibody for both the NR1-IgG and control-IgG to validate the similar effects of the antibodies.

Recordings were analysed using custom-written Igor Pro scripts. For intrinsic properties, traces were not pre-filtered. For spontaneous events, traces were low-pass filtered at 50Hz. For evoked events, traces were pre-filtered with a low-pass filter of 500Hz and a high-pass filter of 0.1Hz. Recordings were analysed as follows:

Parameter	Analysis
<i>Intrinsic properties</i>	
Resting membrane potential (RMP)	Average of Vm baseline in first 10 seconds of recording
Input resistance (IR)	Observed change in membrane potential following membrane hyperpolarisation with set current step
Membrane time constant (MTC)	Time taken for a change in potential to reach 63% final value
Spike threshold	Membrane voltage at which action potential was first produced

Rheobase	Current step at which action potential was first produced
Spike rate	Number of spikes in the time window in which the current step was applied
Interspike interval (ISI)	Time between two adjacent spikes
Spike amplitude	Height of spike above baseline
Spike duration	Duration between the up and down phase of the spike at 25% peak amplitude
<i>Spontaneous events</i>	
sEPSCs	Downward deflections >2SD below baseline (average of whole recording) with a minimum duration of 10ms in baseline traces
<i>Evoked events (single-stimulation recordings)</i>	
EPSP amplitude	Height change from downwards phase of spike until return to initial baseline
EPSP duration	Duration from downwards phase of spike until return to initial baseline
Rise time	Time between 20-80% of the peak amplitude
Decay time	Time from 100% back down to 50% peak amplitude
NMDA/AMPA ratio	$\frac{EPSP\ amplitude_{(gabazine\ only)} - EPSP\ amplitude_{(gabazine+DAP5)}}{EPSP\ amplitude_{(gabazine\ only)}}$ Where an EPSP in the presence of Gabazine only, corresponds to an EPSP mediated by both NMDA and AMPA/KA receptors, and an EPSP in the presence of Gabazine and NMDAR antagonist DAP5 corresponds to an EPSP mediated only by AMPA/KA receptors
<i>Evoked events (multi-stimulation recordings to evaluate short term plasticity)</i>	
Paired-pulse ratio (PPR)	Peak amplitude of the second peak divided by peak amplitude of the first peak
Train stimulation	Peak amplitude of each EPSP divided by the amplitude of the first EPSP

5.4.3 Morphological methods

(See **General Methods Section 2.8 Morphological reconstruction methods**)

5.4.3.1 SPN reconstructions

SPNs used for electrophysiological recordings were filled with biocytin and subsequently labelled with DAB, a dark precipitate, allowing their morphology to be visualised. DAB-labelled neurons were visualised using the Olympus Fluorescence Microscope and HCLImage Live Software. Z-stacks of the full neuron, with 0.5µm steps, were taken at 40x magnification. Z-stacks were processed and analysed following similar

methods to those found in **Young and Morrison (2018)**. Z-stacks of each cell were converted to binary 8-bit images in *Image J* before filtering to remove any background objects. To obtain data about **total branch length**, and **numbers of branches**, the image was skeletonised using the *Skeletonise* function and analysed using the *AnalyzeSkeleton(2D/3D)* function. The *Scholl Analysis* function was also applied to the image to evaluate the **complexity** of dendritic arborisation.

To evaluate **spine density**, z-stacks of primary (1°), secondary (2°) and tertiary (3°) branches were taken at 100x magnification. Branch length was manually evaluated by tracing each branch in *Image J*, and spines individually counted along each branch using the pointer tool. Density was calculated by dividing the number of spines along a branch by the total branch length. Density was evaluated for two 1°, two 2° and two 3° dendrites per cell.

5.4.3.2 MRI analysis of striatal volume

Brains of animals injected with either NR1-IgG or control-IgG were fixed at p23, transferred to perfluorinate solution, and scanned using a 9.4T Biospec with cryocoil scanner for 1 hour. Scans were analysed using ITK Snap software. The left and right dorsal striatum was manually contoured in each transverse section from the top of the brain down to the level of the third ventricle, and contours stacked to determine total striatal volume. The volume of the whole brain was ascertained using the same contouring method. Brain volume excluded the cerebellum and olfactory bulbs as these were incomplete in some brains. The volume of the dorsal striatum was normalised to the volume of the total brain as a percentage of total volume, to mitigate any differences due to different total brain volumes.

5.4.3.3 Striatal cell composition and density

(See **General Methods Section 2.6 Antibody immunohistochemical methods**)

To determine the **D1/D2-SPN ratio** in the dorsal striatum, brains used for the MRI experiments were subsequently sliced into 60µm coronal sections and stained with CTIP2 and PPE primary antibodies to

delineate all SPNs and D2-SPNs within the striatum respectively. One medial slice per brain was imaged using a Zeiss Imager M2 epifluorescence microscope at 20x magnification. The striatum was imaged using a series of tessellated 10µm z-stacks to form a 10µm thick optical dissector. Images were analysed using Stereoinvestigator software following the inbuilt optical fractionator workflow. Counts were performed across the entirety of the dorsal striatum, with the grid size and counting frame both set to 500 x 500µm. A neuron was counted if it came into focus in the z-stack and had an intensity >2SD above the background. Cells that had both clear CTIP2 nuclear labelling and also showed clear PPE cytoplasmic labelling were counted as PPE+ SPNs (i.e. D2-SPNs). Cells that only exhibited CTIP2 labelling were counted as PPE- SPNs (i.e. D1-SPNs).

The same z-stacks were also used to determine **density of total SPNs**. Density was calculated using cell counts from three counting frames for each brain and dividing by the counting frame volume (10 x 500 x 500µm).

5.4.3.4 Microglial density

Fixed slices from the MRI scanned brains were also stained with IBA1 primary antibody to label activated microglial cells. Slices were imaged using an Olympus Fluorescence Microscope with HCLImage Live software. 30µm z-stacks, with 0.5µm intervals were taken of the striatum and cortex, and cells quantified in a defined region, standardised across sections. To count, cells were counted in each layer of the z-stack. Only cells in focus, with intensity >2SD above background were counted. The density of the cells was then calculated by dividing the total number of cells counted by the volume of the z-stack (density = total cells counted/(30*area of region).

5.4.3.5 Microglial reconstructions

(See **General Methods Section 2.8 Morphological reconstruction methods**)

Individual cells in the IBA1-labelled striatal sections were imaged at 40x magnification, in z-stacks with a 0.5 μm interval, and cells chosen via a random number generator. Z-stacks were converted to binary 8-bit images in *Image J*, before skeletonisation and analysis as described for DAB-labelled SPNs above.

5.4.4 Statistical methods

(See **General Methods Section 2.11 Statistical methods**)

Details of specific statistical tests used for each experiment are given in the respective results sections in the chapter. All data is presented as mean $\pm$ SEM. All data was initially normality tested using the Shapiro-Wilks or Kolmogorov-Smirnov test, and the appropriate two tailed parametric (ANOVA, one-sample t-test, unpaired t-test) or non-parametric (Wilcoxon signed-rank (WSR), Mann-Whitney U (MWU)) test applied. Significance for correlations was determined using the Pearson correlation coefficient and n number. Data was corrected where appropriate using the Bonferroni *post hoc* correction, to compensate for multiple comparisons. For all statistical tests, $p < 0.05$ was deemed significant. * $p < 0.05$, ** $p < 0.01$.

5.5 Chapter Results

5.5.1 Postnatal impact of NR1-IgG antibodies on the intrinsic properties of SPNs

5.5.1.1 Impact on intrinsic properties of early postnatal (p7-14) SPNs

The striatum consists predominantly of SPNs, which arise from progenitors in the LGE and migrate post-mitotically into the striatum (**Knowles et al. 2021**). **Experimental Chapter B** demonstrated that *in utero* exposure to the NR1-IgG antibodies during striatal neurogenesis significantly impacts striatal progenitors, including decreased overall proliferation (i.e. lower numbers of mitotic PH3+ cells) and distance migrated away from the LGE ventricle. Current literature supports the idea that there may also be long-term consequences postnatally including significant decreases in numbers of early postnatal SPNs, disruption of development of electrophysiological properties of cortical neurons and cortical plate thinning, as well as lasting cognitive deficits (**Single et al. 2000, Stefani and Moghaddam 2005, Jurek et al. 2019, García-Serra et al. 2020**). Building on this, the next logical question to explore was whether the SPNs generated from these altered progenitors were also functionally impacted.

Previous literature suggests that NMDARs are important in the maturation of intrinsic properties, with NR1 knock-out studies in primary sensory and thalamic neurons demonstrating increased excitability throughout the early postnatal period (**Ewald et al. 2009, Pagadala et al. 2013, Hou and Zhang 2017**). Hence, the first experiments here aimed to explore whether the *in utero* exposure to NR1-IgG impacted intrinsic properties of early postnatal SPNs. This was investigated by injecting embryos either NR1-IgG or control-IgG at E14.5, making acute striatal slices in the second postnatal week (p7-14) and performing whole-cell patch-clamp electrophysiology of SPNs. This timeframe was chosen because the second postnatal week is a highly dynamic period when the excitatory synaptic inputs to the striatum rapidly mature, concordantly with rapid improvements in motor abilities of the pups (**Dehorter et al. 2011, Krajewski et al. 2019**). In these initial experiments, SPNs were analysed as a large-scale data set with SPNs

from the full p7-14 age range, with the average age of the groups similarly matched to ensure age discrepancy was not influencing findings (mean age: NR1-IgG SPNs = 10.9 ± 0.3 days, control-IgG SPNs = 10.7 ± 0.3 days). For this dataset, n(NR1-IgG SPNs) = 61 cells, 18 brains, 6 litters, and n(control-IgG SPNs) = 47 cells, 15 brains, 6 litters. These experiments revealed that *in utero* NR1-IgG exposure led to a significant depolarisation of resting membrane potential (RMP) (control-IgG: -67.0 ± 0.5 mV, NR1-IgG: -64.9 ± 0.50 mV, MWU: $p = 0.0013$; **Figure 5.1 C**), higher threshold (control-IgG: -40.7 ± 0.8 mV, NR1-IgG: -35.9 ± 0.9 mV, independent t-test: $p = 6.4 \times 10^{-5}$; **Figure 5.1 D**), higher input resistance (IR) (control-IgG: 409.6 ± 35.2 m Ω , NR1-IgG: 603.4 ± 61.2 M Ω , MWU: $p = 0.012$; **Figure 5.1 E**) and longer membrane time constant (MTC) (control-IgG: 15.9 ± 1.4 ms, NR1-IgG: 22.3 ± 2.0 ms, MWU: $p = 0.026$; **Figure 5.1 F**), and a significant decrease in first spike amplitude (control-IgG: 73.2 ± 2.5 mV, NR1-IgG: 65.8 ± 2.3 mV, independent t-test: $p = 0.030$; **Figure 5.1 G**) and second spike amplitude (control-IgG: 65.6 ± 2.5 mV, NR1-IgG: 56.9 ± 2.3 mV, independent t-test: $p = 0.013$; **Figure 5.1 H**). There was no significant difference in first spike duration, second spike duration, spike rate, or inward rectifying current (**Figure 5.1 I-L** respectively), or any other intrinsic properties tested (all data and statistics in **Appendix, Supplementary Table S5.1**).

These results suggest that key intrinsic properties of postnatal SPNs are affected by *in utero* NR1-IgG antibody exposure.

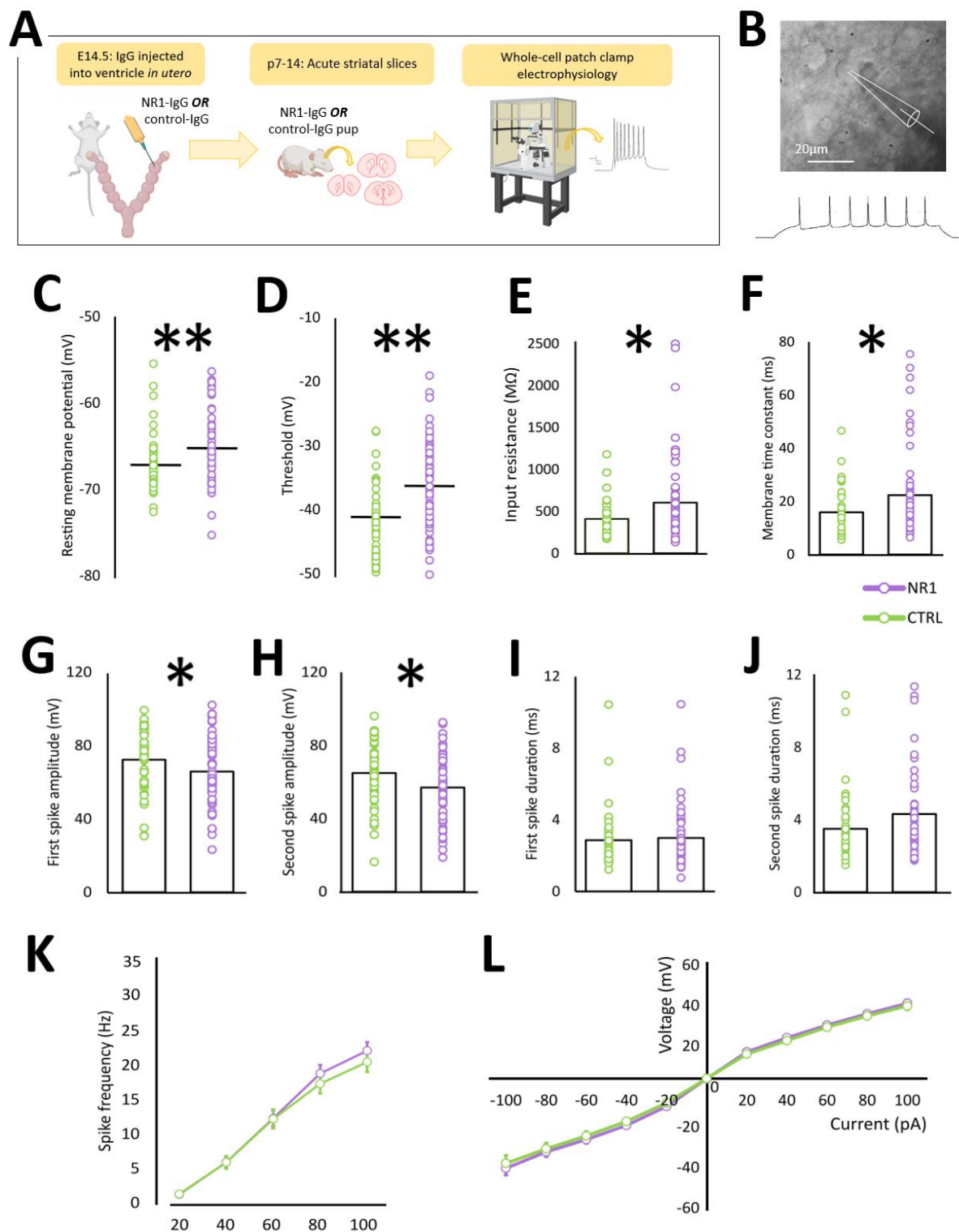


Figure 5.1. Altered intrinsic properties of early postnatal SPNs following brief *in utero* NR1-IgG exposure. (Description on next page).

Figure 5.1. Altered intrinsic properties of early postnatal SPNs following brief in utero NR1-IgG exposure (cont.). **[A]** Graphical outline of the whole-cell patch-clamp electrophysiology method to assess SPN intrinsic properties of p7-14 mice, following short exposure to NR1-IgG or control-IgG at E14.5. $n(\text{NR1-IgG}) = 61$ cells, 18 brains, 4 litters, mean age = 10.9 ± 0.3 days old; $n(\text{control-IgG}) = 47$ cells, 15 brains, 4 litters, mean age = 10.7 ± 0.3 days old. **[B]** Representative 4x image of an SPN being patched in the p10 striatum with example characteristic trace. **[C-L]** Hyperpolarising and depolarising steps were used to characterise the intrinsic properties of SPNs from the second postnatal week. These experiments revealed that there was a significant increase in **[C]** resting membrane potential (RMP), **[D]** threshold, **[E]** input resistance (IR) and **[F]** membrane time constant (MTC), as well as a significant decrease in **[G]** first and **[H]** second spike amplitude in the NR1-IgG exposed SPNs relative to the controls. Conversely, there were no differences in **[I]** first or **[J]** second spike duration, **[K]** spike rate or **[L]** inward rectifying current.

5.5.1.2 Comparison of the effects of polyclonal vs monoclonal antibodies on SPN intrinsic properties

As a control measure, it was important to ensure that similar results were being obtained with both the polyclonal and monoclonal NR1-IgG and control-IgG antibodies since, due to limited antibody supplies, both were used for the electrophysiological studies and other experiments in this thesis. Data was compared from p9 upwards, as no slices younger than p9 were made with polyclonal antibody injected animals. $n(\text{NR1-IgG SPNs}) = 56$ cells, 14 brains, 5 litters; age = 11.8 ± 0.2 days, $n(\text{control-IgG SPNs}) = 41$ cells, 13 brains, 5 litters; age = 11.2 ± 0.3 days. Comparing the impacts on postnatal intrinsic properties from the control-IgG antibodies revealed no significant differences (**Table 5.2**) and comparing the NR1-IgG antibodies showed very minimal differences (**Table 5.3**), with only spike threshold being slightly lower in the polyclonal antibody, providing reassuring evidence that both sets of NR1-IgG and control-IgG antibodies were having similar effects in our model.

	p9-14						
	Polyclonal ctrl-IgG			Monoclonal ctrl-IgG		p value	
Resting membrane potential (mV)	-66.6	±	1.0	-68.1	±	0.3	0.18
Input resistance (MΩ)	385.6	±	41.4	364.8	±	45.9	0.52
Membrane time constant (ms)	16.3	±	2.0	12.6	±	1.2	0.22
Spike threshold (mV)	-42.2	±	0.9	-41.6	±	1.1	0.63
Rheobase (pA)	62.1	±	9.1	63.7	±	4.8	0.31
Time to first spike (ms)	892.7	±	26.0	939.7	±	22.3	0.18
First ISI (ms)	51.6	±	6.6	59.2	±	6.3	0.43
Second ISI (ms)	48.1	±	5.5	53.1	±	5.5	0.68
Third ISI (ms)	52.7	±	7.0	52.6	±	5.4	0.97
Fourth ISI (ms)	45.5	±	3.9	54.6	±	4.7	0.32
First spike amplitude (mV)	75.6	±	3.4	72.7	±	3.8	0.73
Second spike amplitude (mV)	68.9	±	3.6	64.6	±	3.8	0.41
First spike duration (ms)	2.3	±	0.2	3.0	±	0.2	0.062
Second spike duration (ms)	3.2	±	0.3	4.2	±	0.6	0.25
ANOVA							
Spike rate (100 pA) (Hz)	19.0	±	2.4	18.9	±	1.8	F(1,36) = 0.30, p = 0.59
Spike rate (80 pA) (Hz)	15.7	±	2.2	15.9	±	1.8	
Spike rate (60 pA) (Hz)	11.7	±	2.0	10.3	±	2.0	
Spike rate (40 pA) (Hz)	6.6	±	1.6	4.0	±	1.3	
Spike rate (20 pA) (Hz)	1.7	±	0.7	0.3	±	0.2	
Voltage (-100pA) (mV)	-44.7	±	7.0	-39.7	±	5.4	F(1,36) = 0.80, p = 0.059
Voltage (-80pA) (mV)	-37.8	±	5.4	-31.5	±	3.4	
Voltage (-60pA) (mV)	-30.9	±	4.1	-25.4	±	2.6	
Voltage (-40pA) (mV)	-23.1	±	2.8	-18.8	±	1.9	
Voltage (-20pA) (mV)	-12.9	±	1.8	-10.7	±	1.1	
Voltage (0pA) (mV)	0.2	±	0.2	-0.1	±	0.1	
Voltage (20pA) (mV)	10.7	±	1.5	12.9	±	1.3	
Voltage (40pA) (mV)	16.2	±	1.8	20.7	±	1.3	
Voltage (60pA) (mV)	21.6	±	2.3	28.3	±	1.4	
Voltage (80pA) (mV)	27.9	±	3.0	33.4	±	1.7	
Voltage (100pA) (mV)	32.0	±	3.5	39.3	±	2.3	

Table 5.2. Similar effects of in utero exposure to polyclonal and monoclonal control-IgG antibodies on early postnatal SPN intrinsic properties. Table comparing intrinsic properties of SPNs from animals injected with polyclonal control-IgG vs monoclonal control-IgG antibodies, revealing no significant differences between the two. All data was first assessed for normality and then significance assessed using either ANOVA, independent t-test (parametric data) or Mann-Whitney-U (MWU) test (non-parametric data), with Bonferroni post hoc corrections to correct for multiple comparisons where necessary.

	p9-14						
	Polyclonal NR1-IgG			Monoclonal NR1-IgG		p value	
Resting membrane potential (mV)	-65.3	±	1.0	-65.9	±	0.5	0.74
Input resistance (MΩ)	462.2	±	51.3	421.2	±	31.3	0.76
Membrane time constant (ms)	18.9	±	1.8	14.8	±	1.2	0.064
Spike threshold (mV)	-38.8	±	1.6	-36.2	±	1.0	0.031
Rheobase (pA)	54.4	±	4.8	56.9	±	3.0	0.59
Time to first spike (ms)	914.7	±	22.8	920.3	±	18.9	0.86
First ISI (ms)	48.9	±	7.5	49.7	±	4.6	0.77
Second ISI (ms)	45.1	±	4.8	41.8	±	2.8	0.89
Third ISI (ms)	44.5	±	3.7	42.4	±	3.1	0.64
Fourth ISI (ms)	51.1	±	4.1	45.7	±	3.1	0.39
First spike amplitude (mV)	65.0	±	4.3	70.7	±	3.1	0.30
Second spike amplitude (mV)	55.1	±	3.9	64.0	±	2.9	0.085
First spike duration (ms)	2.7	±	0.4	2.5	±	0.1	0.55
Second spike duration (ms)	3.7	±	0.4	3.3	±	0.2	0.89
ANOVA							
Spike rate (100 pA) (Hz)	20.1	±	1.5	23.5	±	1.9	F(1,47) = 0.30, p = 0.59
Spike rate (80 pA) (Hz)	16.5	±	1.7	20.4	±	1.8	
Spike rate (60 pA) (Hz)	11.4	±	1.9	12.2	±	1.5	
Spike rate (40 pA) (Hz)	6.2	±	1.5	3.8	±	0.9	
Spike rate (20 pA) (Hz)	1.2	±	0.5	0.0	±	0.0	
Voltage (-100pA) (mV)	-46.0	±	5.1	-35.6	±	2.3	F(1,47) = 2.77, p = 0.10
Voltage (-80pA) (mV)	-37.4	±	3.7	-30.8	±	1.8	
Voltage (-60pA) (mV)	-30.6	±	3.1	-25.7	±	1.5	
Voltage (-40pA) (mV)	-23.5	±	2.5	-19.9	±	1.2	
Voltage (-20pA) (mV)	-14.3	±	1.8	-11.6	±	0.8	
Voltage (0pA) (mV)	-0.2	±	0.3	0.0	±	0.1	
Voltage (20pA) (mV)	13.4	±	1.8	12.8	±	0.6	
Voltage (40pA) (mV)	19.2	±	2.2	20.7	±	0.7	
Voltage (60pA) (mV)	25.0	±	2.7	26.7	±	0.8	
Voltage (80pA) (mV)	29.7	±	2.9	32.1	±	1.0	
Voltage (100pA) (mV)	34.7	±	3.5	36.8	±	1.3	

Table 5.3. Similar effects of in utero exposure to polyclonal and monoclonal NR1-IgG antibodies on early postnatal SPN intrinsic properties. Table comparing intrinsic properties of SPNs from animals injected with polyclonal NR1-IgG vs monoclonal NR1-IgG antibodies, revealing the only significant difference between the two was a slightly decreased spike threshold in the polyclonal NR1-IgG SPNs. All data was first assessed for normality and then significance assessed using either ANOVA, independent t-test (parametric data) or Mann-Whitney-U (MWU) test (non-parametric data), with Bonferroni post hoc corrections to correct for multiple comparisons where necessary.

5.5.1.3 Impacts on early postnatal SPN intrinsic properties within specific two-day time windows

It is known that the second postnatal week is a highly dynamic period in the progressive development of SPN electrical properties (Tepper et al. 1998, Dehorter et al. 2011, Korovitskiy et al. 2012, Peixoto et al. 2016, Krajewski et al. 2019), and in the corresponding development of the motor skills of pups, where even day-by-day differences are visible (Fox 1965). Having identified that there were significant differences in several intrinsic properties in p7-14 SPNs, analysis next sought to investigate whether there were specific smaller time windows in which properties were particularly affected. Data was split into smaller two-day time windows (p7-8, p9-10, p11-12 and p13-14) and intrinsic properties compared in these subsets. For this dataset, n(NR1-IgG SPNs) = p7-8: 14 cells, p9-10: 14 cells, p11-12: 17 cells, p13-14: 16 cells; n(control-IgG SPNs) = p7-8: 6 cells, p9-10: 19 cells, p11-12: 10 cells, p13-14: 12 cells. This analysis revealed significant differences in several intrinsic properties at specific timepoints.

Firstly, differences in membrane properties were analysed. Mean resting membrane potential trended towards being higher at p7-8 and p9-10 and was significantly higher from p11 onwards (p7-8: independent t-test: $p = 0.38$; p9-10: MWU: $p = 0.16$; p11-12: independent t-test: $p = 0.018$; p13-14: independent t-test: $p = 0.015$; **Figure 5.4 A**; full data in **Appendix, Supplementary Table S5.2**). Input resistance was also significantly higher at the earlier timepoints up to p12, with no significant difference at p13-14 (p7-8: independent t-test: $p = 0.026$; p9-10: MWU: $p = 0.025$; p11-12: MWU: $p = 0.0070$; p13-14: MWU: $p = 0.61$; **Figure 5.4 B**; full data in **Appendix, Supplementary Table S5.2**). Membrane time constant was significantly higher across all timepoints except p9-10 (p7-8: independent t-test: $p = 0.032$; p9-10: MWU: $p = 0.26$; p11-12: independent t-test: $p = 0.039$; p13-14: MWU: $p = 0.0070$; **Figure 5.4 C**; full data in **Appendix, Supplementary Table S5.2**).

Next, differences in several properties of action potentials were explored. Here it was found that threshold was significantly higher at p9-10 and p11-12 but appeared to have normalised by p13-14 (p7-8:

independent t-test: $p = 0.27$; p9-10: independent t-test: $p = 0.0010$; p11-12: independent t-test: $p = 0.0040$; p13-14: independent t-test: $p = 0.98$; **Figure 5.4 D**; full data in **Appendix, Supplementary Table S5.2**). Both first and second spike amplitude showed either a trend towards being reduced or showed significant reduction in the NR1-IgG exposed SPNs up to p12, but differences normalised after this (first spike amplitude: p7-8: independent t-test: $p = 0.25$; p9-10: independent t-test: $p = 0.022$; p11-12: independent t-test: $p = 0.020$; p13-14: independent t-test: $p = 0.24$ | second spike amplitude: p7-8: independent t-test: $p = 0.20$; p9-10: independent t-test: $p = 0.051$; p11-12: independent t-test: $p = 0.028$; p13-14: independent t-test: $p = 0.65$; **Figure 5.4 E, F**; full data in **Appendix, Supplementary Table S5.2**). Conversely, first and second spike duration only showed a significantly reduced duration in NR1-IgG SPNs at p13-14 (first spike duration: p7-8: MWU: $p = 0.34$; p9-10: MWU: $p = 0.13$; p11-12: independent t-test: $p = 0.57$; p13-14: independent t-test: $p = 0.0080$ | second spike duration: p7-8: MWU: $p = 0.21$; p9-10: MWU: $p = 0.060$; p11-12: MWU: $p = 0.92$; p13-14: independent t-test: $p = 0.012$; **Figure 5.4 G, H**; full data in **Appendix, Supplementary Table S5.2**).

Lastly, spike rate and inward rectifying currents were investigated. Spike rate showed no significant differences at any of the timepoints, though there was a trend towards a decrease at p7-8 (overall: p7-8: ANOVA: $F_{(1,7)} = 3.90$, $p = 0.089$; p9-10: ANOVA: $F_{(1,29)} = 0.00021$, $p = 0.99$; p11-12: ANOVA: $F_{(1,24)} = 0.81$, $p = 0.38$; p13-14: ANOVA: $F_{(1,29)} = 2.92$, $p = 0.098$; **Figure 5.4 I**; full data in **Appendix, Supplementary Table S5.2**). Inward rectifying current showed a trend towards a reduced inwards rectifying current at p7-8, and a significantly increased inward rectifying current at p13-14 (overall: p7-8: ANOVA: $F_{(1,7)} = 4.73$, $p = 0.066$; p9-10: ANOVA: $F_{(1,29)} = 2.56$, $p = 0.12$; p11-12: ANOVA: $F_{(1,24)} = 0.38$, $p = 0.55$; p13-14: ANOVA: $F_{(1,28)} = 15.02$, $p = 0.00059$; **Figure 5.4 J, K**; full data in **Appendix, Supplementary Table S5.2**).

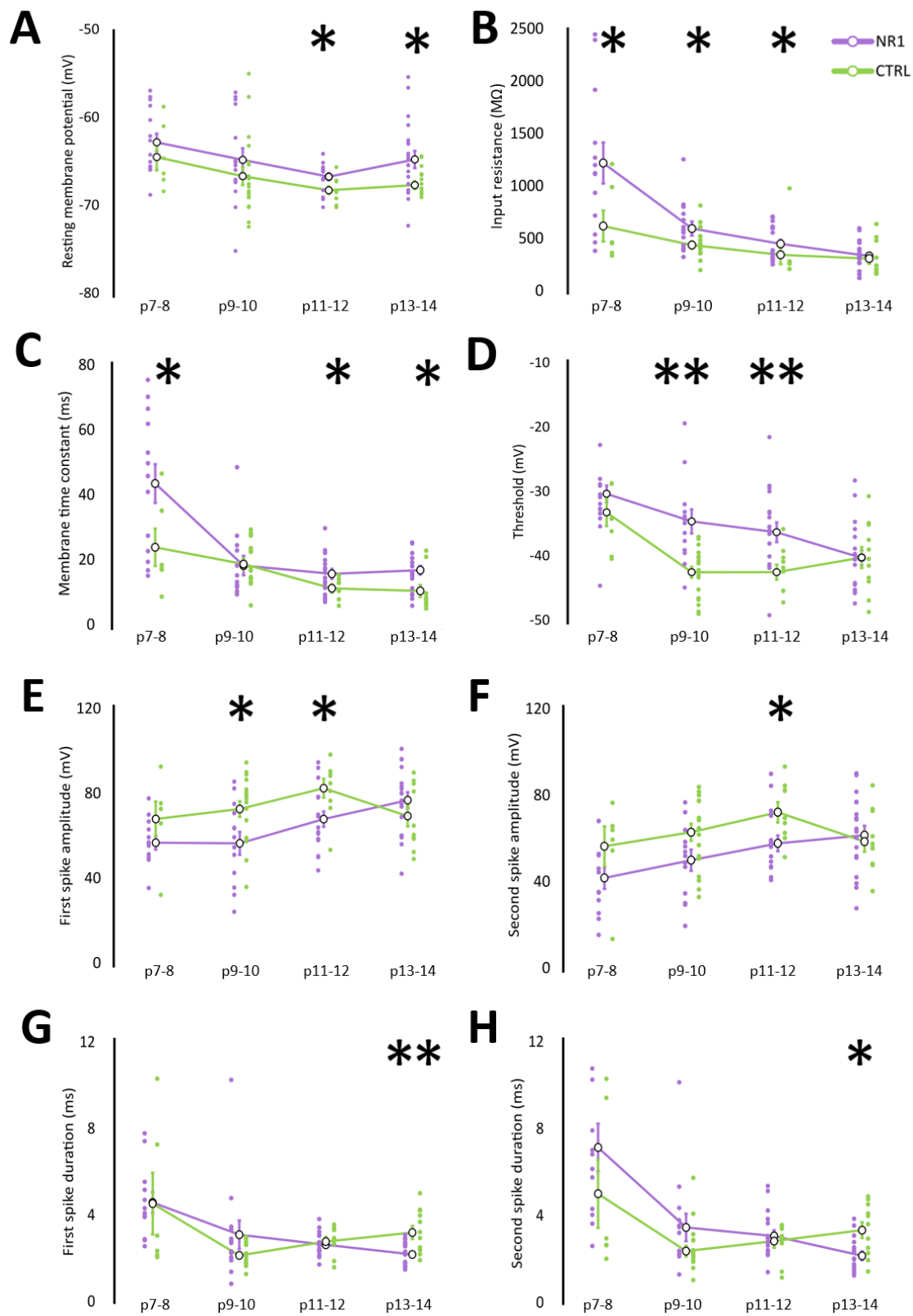


Figure 5.4. Effects of in utero NR1-IgG exposure on SPN intrinsic properties in specific two-day time windows. (Description on next page).

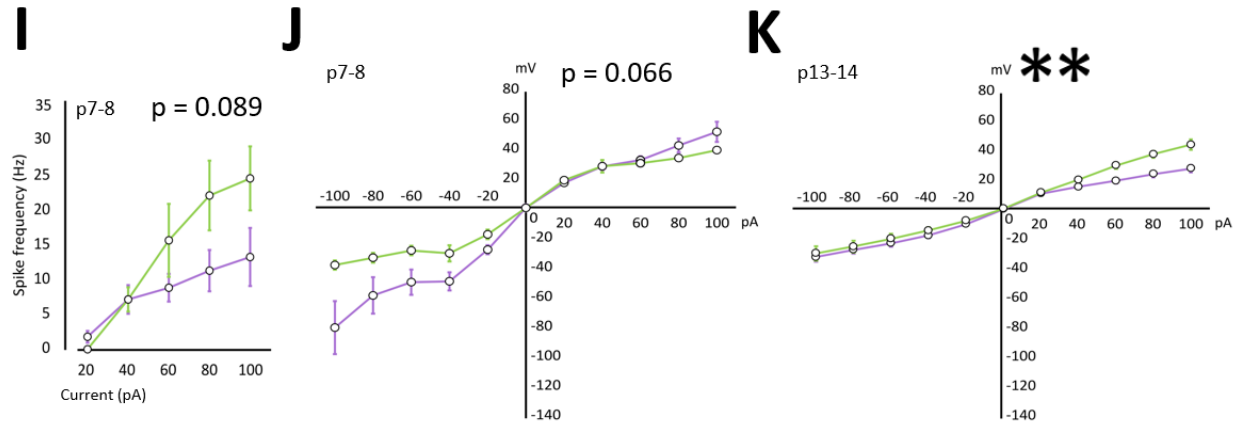


Figure 5.4. Effects of in utero NR1-IgG exposure on SPN intrinsic properties in specific two-day time windows (cont.). [A] Line graph of resting membrane potential of NR1-IgG vs control-IgG exposed SPNs compared in specific two-day time windows during the second postnatal week revealed that there was a trend towards a higher resting membrane potential, which was significant at the later timepoints. [B] Comparison of threshold in the same time windows revealed significant differences at p9-10 and p11-12. [C] Input resistance showed significant differences at earlier timepoints, which normalised by p13-14. [D] Comparisons of membrane time constant showed it was significantly higher in NR1-IgG exposed SPNs at all time points except p9-10. [E] First spike amplitude showed a trend towards a reduction at p7-8, a significant reduction at p9-10 and p11-12, and normalisation at p13-14. [F] Second spike amplitude showed a very similar trend. [G] Conversely, first spike duration showed no significant differences except a significantly reduced duration at p13-14. [H] Second spike duration followed the same trend as first spike duration. [I] Spike rate showed no significant differences, but there was a trend towards a reduced spike rate at p7-8. [J] Inward rectifying current showed a trend towards a reduced inward rectifying current at p7-8. [K] Conversely, there were no significant differences at p9-10 or p11-12, but a significantly greater inward rectifying current at p13-14. Please also see full data in **Appendix, Supplementary Table S5.2**.

Overall, these results indicate there are several significant differences in intrinsic properties of second postnatal week SPNs exposed to NR1-IgG *in utero*, and that when analysed in smaller two-day time windows, these differences are non-uniform across time, with several differing occurring at earlier timepoints eg. p7-8 or p9-10 and exhibiting some form of subsequent normalisation later on in development eg. p13-14.

5.5.2 Postnatal impact of NR1-IgG antibodies on SPN morphology

SPN morphology has previously shown to develop concurrently to electrical maturation, with complexity and size of dendritic arbour closely linked to intrinsic excitability (**Gertler et al. 2008, Cazorla et al. 2012 Liebermann et al. 2018, Krajewski et al. 2019**). Having identified differences in the SPN intrinsic properties, the next experiments therefore sought to explore if these differences were also reflected in cellular morphology. In all whole-cell patch-clamp recordings, biocytin was added to the internal recording solution, which allowed for subsequent DAB immunohistochemical labelling and reconstruction of neurons (**Figure 5.5 A, B**). Due to occasional biocytin leakage or poor DAB labelling, not all cells recorded could be reconstructed, so for this section the n numbers are as follows: n(NR1-IgG SPNs) = 16 cells, 8 brains, 4 litters, mean age 10.6 ± 0.4 days, and n(control-IgG SPNs) = 14 cells, 6 brains, 3 litters, mean age = 10.7 ± 0.6 days.

Several parameters were investigated relating to dendritic arborisation and morphology including numbers of primary (1°), secondary (2°) and tertiary (3°) branches, and complexity of dendritic arbour. Analysis of these reconstructions revealed that NR1-IgG *in utero* exposed SPNs exhibited similar dendritic arborisation to control-IgG *in utero* exposed SPNs, including no significant differences in branch length (control-IgG: $853.1 \pm 66.0\mu\text{m}$, NR1-IgG: $819.9 \pm 65.9\mu\text{m}$, independent t-test: $p = 0.73$; **Figure 5.5 C**), number of primary branches (control-IgG: 6.3 ± 0.5 , NR1-IgG: 5.8 ± 0.4 , MWU: $p = 0.61$; **Figure 5.5 D**) or dendritic complexity (Scholl analysis: RM ANOVA: $F_{(1,27)} = 0.12$, $p = 0.91$; **Figure 5.5 F**). However, there was a trend towards a decrease in total number of branches in NR1-IgG exposed SPNs (control-IgG: 92.1 ± 6.2 , NR1-IgG: 79.1 ± 5.8 , independent t-test: $p = 0.14$; **Figure 5.5 E**).

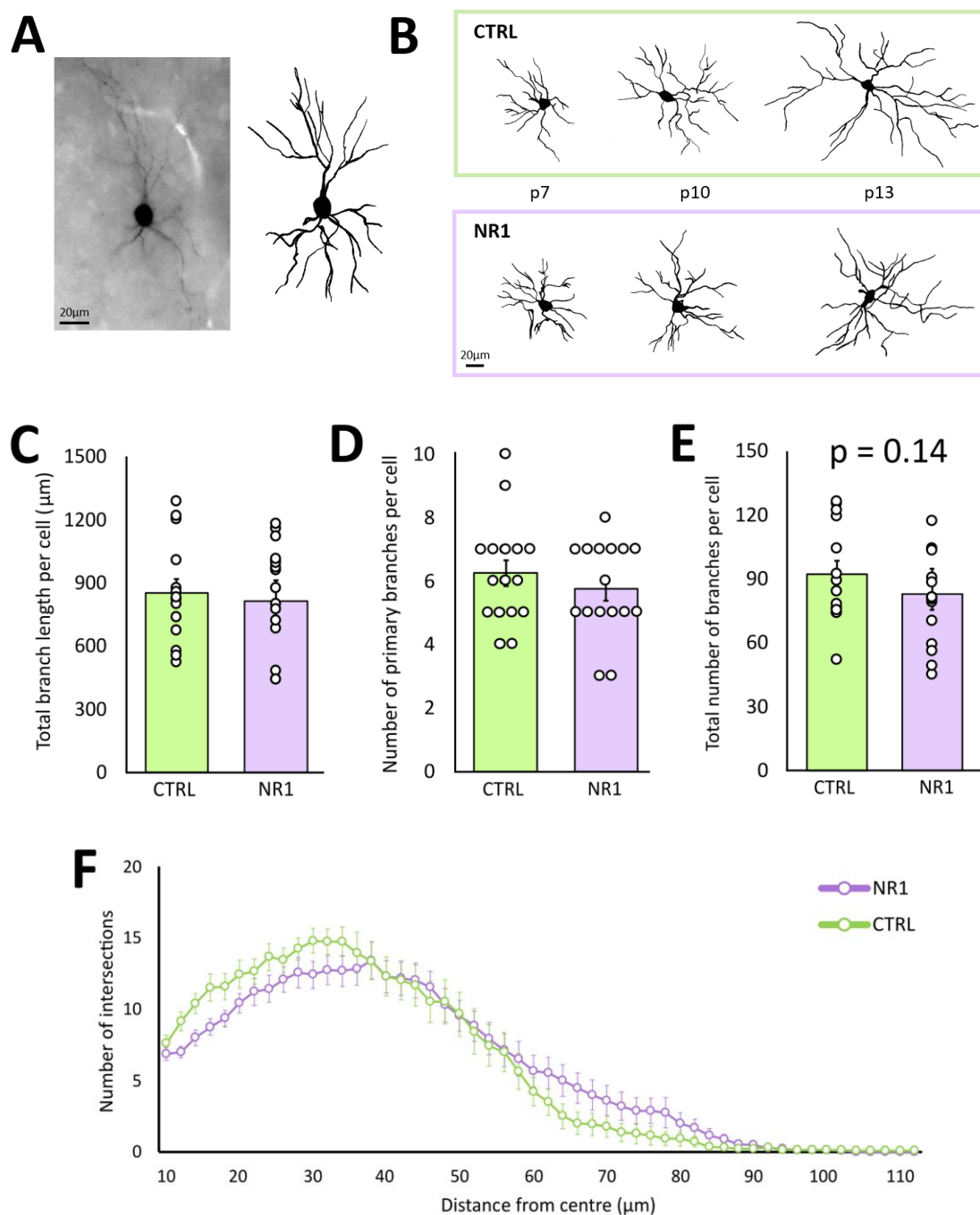


Figure 5.5. No change in dendritic arborisation of early postnatal SPNs following in utero NR1-IgG exposure. **[A]** Epifluorescence image of a DAB-labelled SPN, along with the neuronal reconstruction in Image J. **[B]** Representative reconstructions of control-IgG (green) and NR1-IgG (purple) in utero exposed SPNs labelled with DAB immunohistochemistry. **[C-F]** SPNs exposed in utero to NR1-IgG exhibited similar morphology to those exposed to control-IgG, including no significant differences in **[C]** total branch length, **[D]** number of primary branches, **[E]** total number of branches, or **[F]** dendritic complexity assessed using Scholl analysis, although there was a trend towards a decrease in total number of branches in NR1-IgG exposed SPNs.

A key morphological feature of SPNs is their dendritic spines (Somogyi et al. 1981, Bolam and Izzo 1988, Yung et al. 1996, Doig et al. 2010). Analysis of spine density on primary, secondary and tertiary dendrites in high-magnification images (100x objective) revealed that although there was no significant difference in spine density averaged across all branch types (control-IgG: 0.59 ± 0.04 , NR1-IgG: 0.54 ± 0.03 , independent t-test: $p = 0.40$; **Figure 5.6 B**), there was a significant reduction in spine density specifically on secondary dendrites (2° : control-IgG: 0.76 ± 0.04 , NR1-IgG: 0.60 ± 0.05 , independent t-test: $p = 0.048$; **Figure 5.6 D**). No significant differences were detected on primary or tertiary dendrites, although 6/8 NR1-IgG exposed SPNs exhibited a reduced tertiary spine density relative to the mean of the control-IgG SPNs (i.e large range) (1° : control-IgG: 0.23 ± 0.08 , NR1-IgG: 0.16 ± 0.05 , independent t-test: $p = 0.46$; **Figure 5.6 C** | 3° : control-IgG: 0.79 ± 0.01 , NR1-IgG: 0.71 ± 0.04 , independent t-test: $p = 0.20$; **Figure 5.6 E**). Due to the high quality of staining required to accurately count spines, only a subset of cells could be analysed for spine density. For this analysis, $n(\text{NR1-IgG SPNs}) = 8$ cells, $n(\text{control-IgG SPNs}) = 5$ cells.

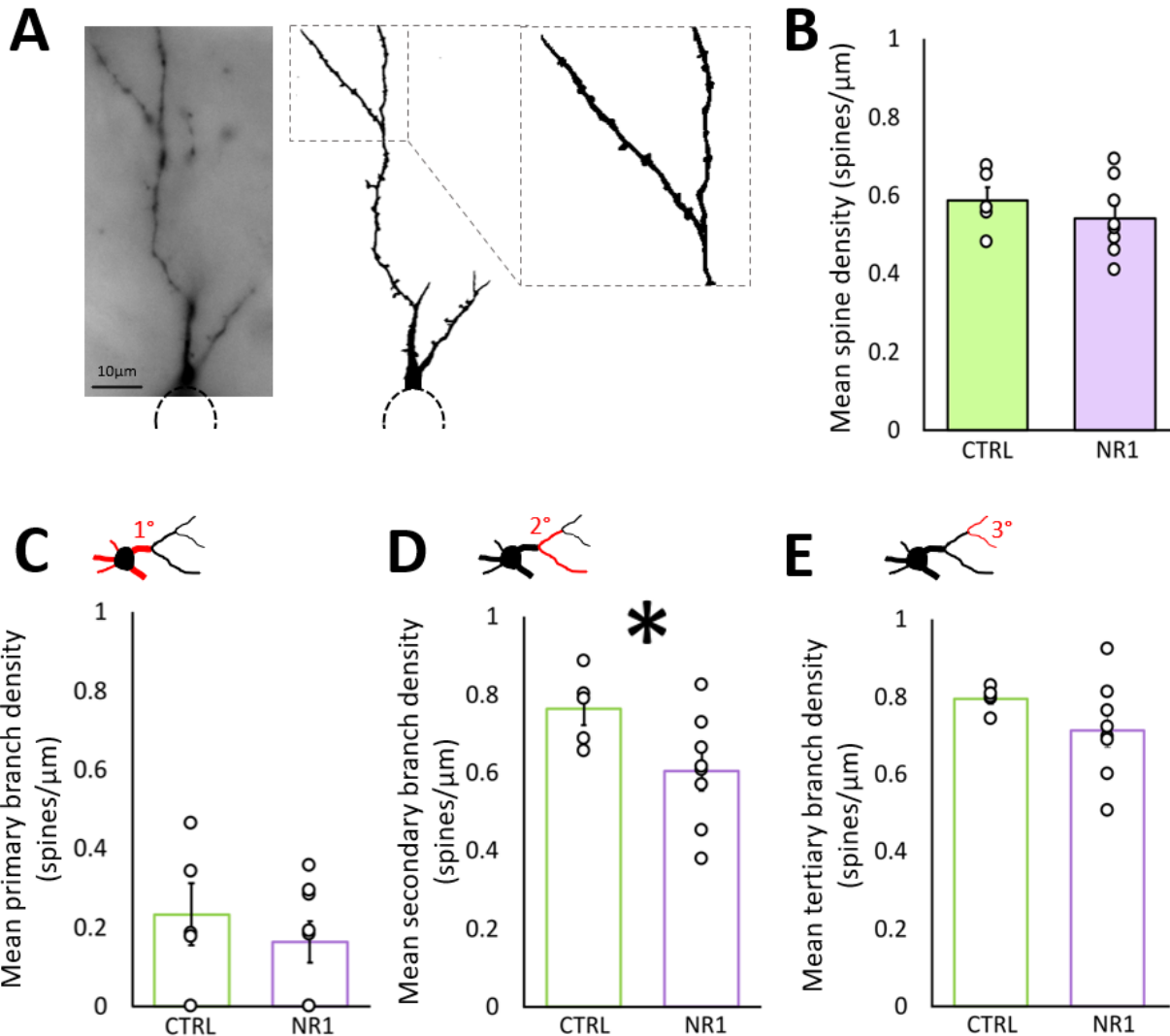


Figure 5.6. Reduced spine density on secondary dendrites of early postnatal SPNs following in utero NR1-IgG exposure. [A] 100x microscope representative image of a branch of an SPN labelled with DAB, along with its reconstruction, with dendritic spines clearly visible. [B] Bar plot of mean spine density showing no significant difference in mean spine density when averaged across the primary, secondary, and tertiary branch types for cells exposed to NR1-IgG vs control-IgG in utero. [C-E] However, bar plots of primary, secondary, and tertiary branch spine density reveal that there was significantly reduced spine density specifically on 2° dendrites, and many analysed 3° branches also exhibit a decrease.

Data was also split into two-day time windows to see if morphological features were more affected at specific timepoints. n(NR1-IgG SPNs): p7-8 = 2 cells, p9-10 = 3 cells, p11-12 = 9 cells, p13-14 = 2 cells; n(control-IgG SPNs): p7-8: 4 cells, p9-10: 1 cell, p11-12: 6 cells, p13-14: 3 cells. Although this analysis was very limited due to the reduced numbers of neurons able to be reconstructed and hence robust statistical

comparisons were not possible, the trends putatively suggest that the greatest morphological differences may be at p13-14, with the most divergence in total branch length, total number of branches, number of primary branches, and dendritic complexity at this time point (**Figure 5.7**, full data in legend).

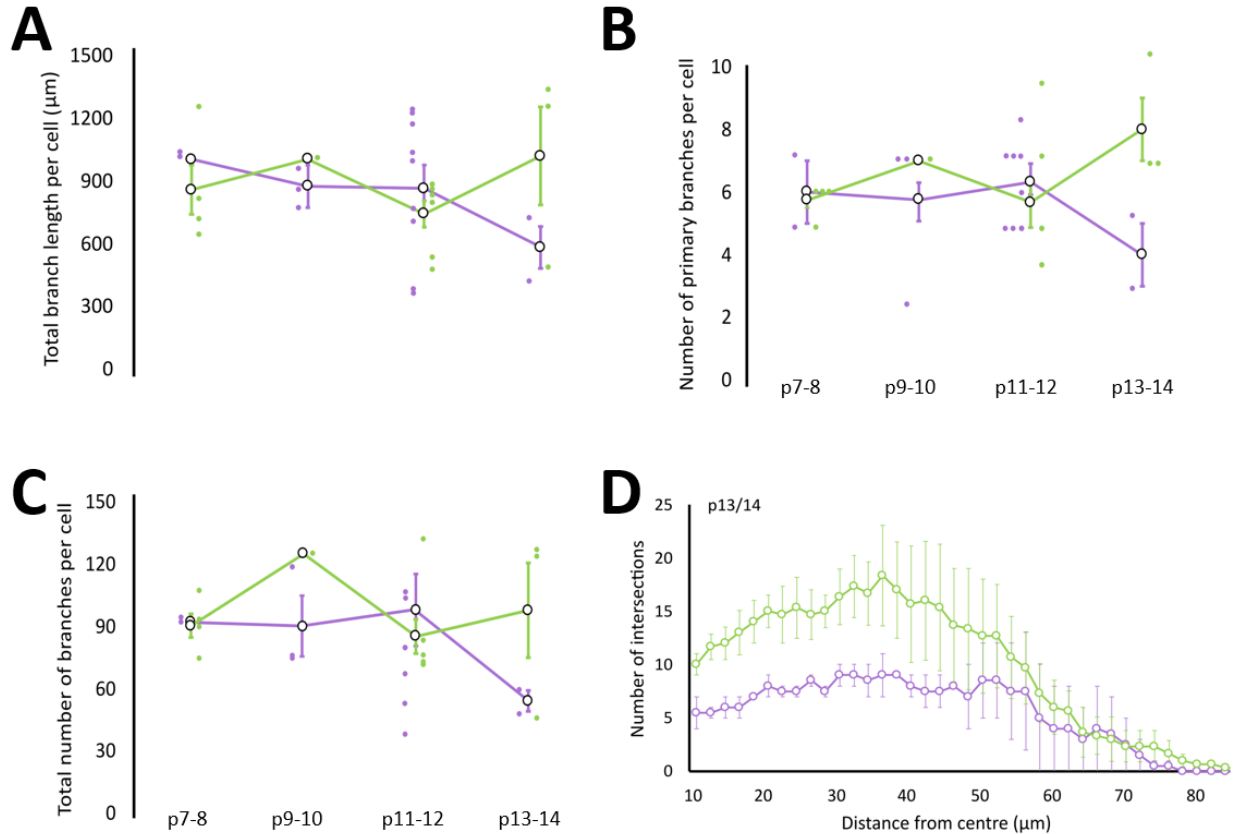


Figure 5.7. Greatest differences in SPN morphology putatively occur at the end of the second postnatal week. [A-C] Line plots of [A] total branch length, [B] number of primary branches and [C] total number of branches respectively for NR1-IgG and control-IgG in utero exposed SPNs suggest NR1-IgG SPNs show the largest relative reduction in these parameters at p13-14, with very little difference at earlier timepoints. Total branch length: p7-8: control-IgG: $857.2 \pm 118.3\mu\text{m}$, NR1-IgG: $1003.2 \pm 10.1\mu\text{m}$; p9-10: control-IgG: $1004.5 \pm 0.0\mu\text{m}$, NR1-IgG: $877.1 \pm 46.8\mu\text{m}$; p11-12: control-IgG: $742.2 \pm 62.6\mu\text{m}$, NR1-IgG: $866.5 \pm 96.5\mu\text{m}$; p13-14: control-IgG: $1018.8 \pm 233.7\mu\text{m}$, NR1-IgG: $582.5 \pm 100.6\mu\text{m}$. Number of primary branches: p7-8: control-IgG: 5.8 ± 0.2 , NR1-IgG: 6.0 ± 1.0 ; p9-10: control-IgG: 7.0 ± 0.0 , NR1-IgG: 5.7 ± 1.3 ; p11-12: control-IgG: 5.7 ± 0.8 , NR1-IgG: 6.1 ± 0.4 ; p13-14: control-IgG: 8.0 ± 1.0 , NR1-IgG: 4.0 ± 1.0 . Total number of branches: p7-8: control-IgG: 90.3 ± 5.8 , NR1-IgG: 92.0 ± 1.0 ; p9-10: control-IgG: 125.0 ± 0.0 , NR1-IgG: 92.0 ± 12.5 ; p11-12: control-IgG: 85.2 ± 8.3 , NR1-IgG: 99.4 ± 16.1 ; p13-14: control-IgG: 97.7 ± 22.9 , NR1-IgG: 54.0 ± 5.0 . [D] Scholl analysis also putatively suggests reduced dendritic complexity at p13-14, particularly close to the soma, with no obvious differences at earlier timepoints.

Overall, these morphological studies indicate that the dendritic arbour and complexity of SPNs exposed to NR1-IgG *in utero* is largely similar to SPNs exposed to control-IgG, but with a trend towards a lower number of dendritic branches as well as a significant reduction in spine density of secondary dendrites. Analysis at specific two-day time windows putatively suggests the greatest differences in morphology may occur towards the end of the second postnatal week.

Correlations between intrinsic properties and morphology were also briefly explored and summarised in **Appendix, Supplementary Table S5.3** and **Supplementary Figure S5.4**.

5.5.3 Postnatal impact of NR1-IgG antibodies on excitatory synaptic inputs and corticostriatal connections

Correct intrinsic electrophysiological functioning is important for SPN integration in the striatum, synapse formation, and appropriate communication with other neurons (**Carter and Sabatini 2004**). In healthy development, the glutamatergic synapses onto SPNs undergo a protracted development over the early postnatal period, with a maturational peak at ~p14 in mice, concordant with maturation of cellular intrinsic properties and development of high-level, coordinated motor behaviours (**Tepper et al. 1998, Dehorter et al. 2011, Peixoto et al. 2016, Krajeski et al. 2019**).

Given the fact that both disruption to SPN intrinsic properties and changes in spine density (i.e. the site of most SPN glutamatergic synapses (**Peixoto et al. 2016**)) were seen here, the next part of this project set out to explore whether these changes co-occurred with wider changes in excitatory synaptic input properties.

5.5.3.1 Impacts on early postnatal SPN excitatory synaptic inputs

Firstly, SPN excitatory synaptic inputs were assessed by undertaking whole-cell patch-clamp recordings of spontaneous EPSCs (sEPSCs) whilst holding the membrane at -70mV (**Figure 5.8 A**). It was observed that

sEPSCs could be detected from the earliest recording day (p7) for both NR1-IgG and control-IgG SPNs. Both amplitude of sEPSCs, where changes are thought to largely reflect post-synaptic alterations, and frequency of sEPSCs, where changes are through to reflect pre-synaptic release events, were assessed. For this dataset, n(NR1-IgG SPNs) = 52 cells (p7-8: 14 cells, p9-10: 7 cells, p11-12: 12 cells, p13-14: 19 cells; mean age: 11.0 ± 0.3 days), 15 brains, 4 litters; n(control-IgG SPNs) = 39 cells (p7-8: 5 cells, p9-10: 18 cells, p11-12: 6 cells, p13-14: 10 cells; mean age: 10.7 ± 0.3 days), 13 brains, 3 litters.

When analysing the dataset overall, no significant difference in either amplitude (control-IgG: 4.9 ± 0.4 pA, NR1-IgG: 4.4 ± 0.3 pA, MWU: $p = 0.24$; **Figure 5.8 B**) or frequency (control-IgG: 0.8 ± 0.1 pA, NR1-IgG: 0.8 ± 0.1 pA, MWU: $p = 0.86$; **Figure 5.8 C**) of sEPSCs was found. However, when the data was split into two-day time windows, a trend towards a decrease in amplitude was observed in the p11-12 NR1-IgG exposed SPNs, which reached significance at p13-14 (p7-8: control-IgG: 1.4 ± 0.6 pA, NR1-IgG: 3.8 ± 0.6 pA, independent t-test: $p = 0.92$; p9-10: control-IgG: 4.5 ± 0.8 pA, NR1-IgG: 5.4 ± 0.7 pA, independent t-test: $p = 0.44$; p11-12: control-IgG: 6.6 ± 0.5 pA, NR1-IgG: 4.8 ± 0.8 pA, independent t-test: $p = 0.14$; p13-14: control-IgG: 5.2 ± 0.4 pA, NR1-IgG: 3.9 ± 0.4 pA, independent t-test: 0.048 ; **Figure 5.8 D**). Conversely, there were no significant differences seen in sEPSC frequency at any of the timepoints (p7-8: control-IgG: 0.6 ± 0.2 Hz, NR1-IgG: 0.7 ± 0.2 Hz, independent t-test: $p = 0.72$; p9-10: control-IgG: 0.7 ± 0.1 Hz, NR1-IgG: 0.8 ± 0.2 Hz, independent t-test: $p = 0.99$; p11-12: control-IgG: 0.7 ± 0.1 Hz, NR1-IgG: 0.6 ± 0.1 Hz, MWU: $p = 0.13$; p13-14: control-IgG: 1.0 ± 0.2 Hz, NR1-IgG: 1.1 ± 0.1 Hz, independent t-test: $p = 0.61$; **Figure 5.8 E**).

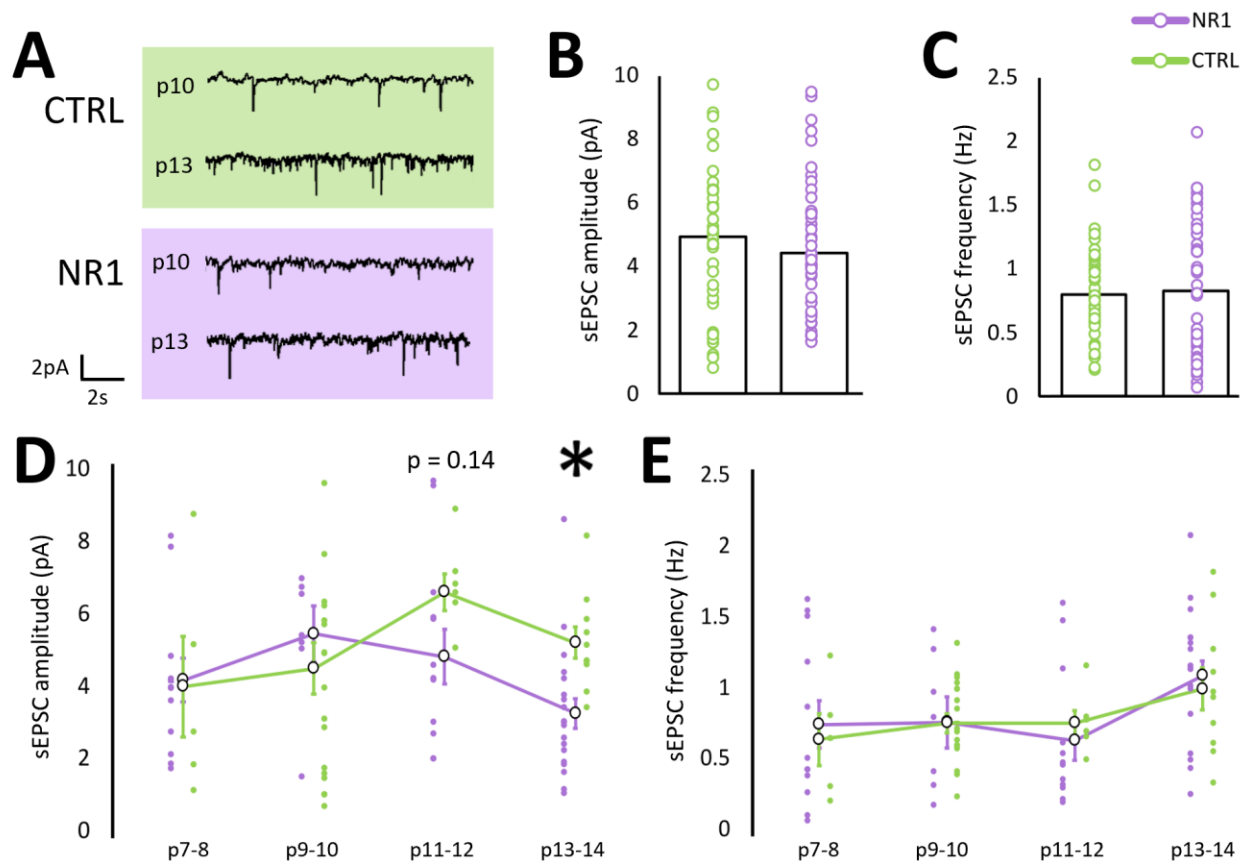


Figure 5.8. Reduced sEPSC amplitude in early postnatal SPN excitatory synapses following in utero NR1-IgG exposure. **[A]** Representative traces showing sEPSCs for the NR1-IgG and control-IgG exposed SPNs. sEPSCs were recorded as downwards deflections in the baseline recordings of SPNs voltage-clamped at -70mV. **[B-C]** Bar plots of sEPSC amplitude and frequency for p7-14 SPNs exposed to either control-IgG or NR1-IgG in utero. When the dataset was analysed as a whole, there was no significant difference in either amplitude or frequency. **[D]** However, when the sEPSC amplitude data was analysed in smaller two-day time windows, there was a trend towards a decrease in amplitude at p11-12, and a significant decrease at p13-14 in NR1-IgG exposed SPNs. **[E]** Conversely there was no significant difference in sEPSC frequency in any of the two-day time windows.

5.5.3.2 Impacts on early postnatal SPN corticostriatal connections

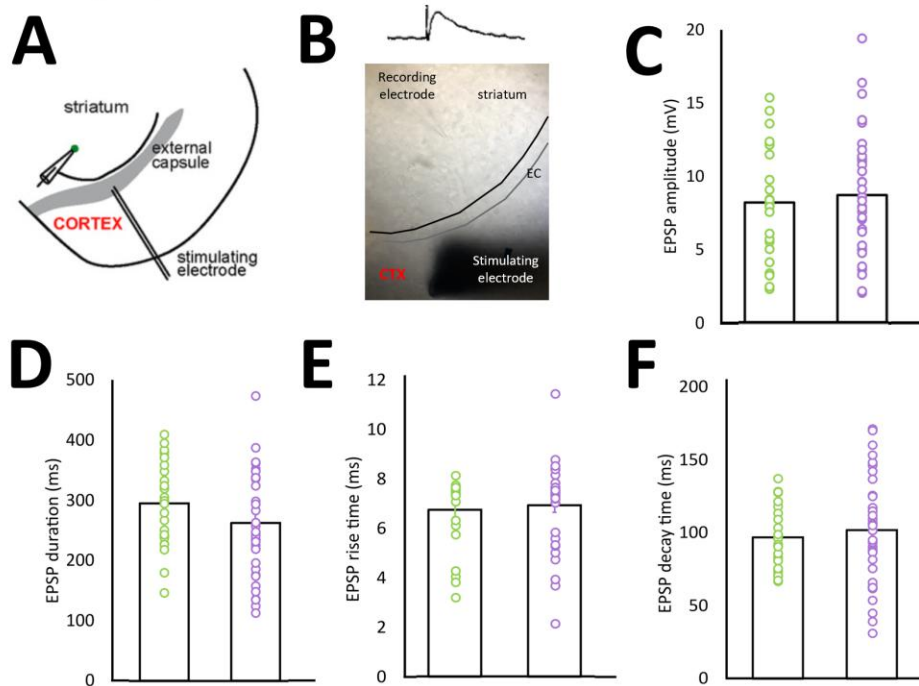
One of the main excitatory afferents to SPNs in the striatum arises from neurons in the cortex (**Kemp and Powell 1971, Buchwald et al. 1973, Smith et al. 2004**). To investigate cortical inputs, whole-cell patch-clamp recordings of dorsal SPNs from p7-14 were undertaken and excitatory cortical afferents were stimulated using single and train stimulation via a tungsten bipolar electrode next to the corticostriatal

border (**Figure 5.9 A, B**). For this dataset, n(NR1-IgG SPNs) = 35 cells, 15 brains, 4 litters, mean age = 10.5 $\pm$ 0.4 days; n(control-IgG SPNs) = 25 cells, 12 brains, 3 litters, mean age = 10.8 $\pm$ 0.4 days.

The first experiments captured evoked excitatory responses mediated by both NMDA and AMPA/KA glutamate receptors, by performing baseline recordings of EPSPs in the presence of GABA_A receptor antagonist Gabazine to block any influence of inhibitory inputs. Stimulation strength was controlled to be as constant as possible between all animals and slices used. Experiments using single stimulation pulses in these conditions revealed no significant differences in several post-synaptic properties including EPSP amplitude (control-IgG: 8.2 $\pm$ 0.7mV, NR1-IgG: 8.9 $\pm$ 0.7mV, independent t-test: p = 0.52; **Figure 5.9 C**), duration (control-IgG: 295.3 $\pm$ 14.3ms, NR1-IgG: 262.1 $\pm$ 16.8ms, independent t-test: p = 0.14; **Figure 5.9 D**), rise time (control-IgG: 6.7 $\pm$ 0.3ms, NR1-IgG: 6.9 $\pm$ 0.3ms, MWU: p = 0.77; **Figure 5.9 E**) and decay time (control-IgG: 96.5 $\pm$ 4.4ms, NR1-IgG: 101.6 $\pm$ 6.4ms, independent t-test: p = 0.52; **Figure 5.9 F**).

Possible pre-synaptic changes which might influence short-term corticostriatal synaptic plasticity were also investigated using 20Hz trains of stimulation. Here it was identified that there were pre-synaptic differences including a significantly higher normalised EPSP amplitude during train stimulation (overall: ANOVA: $F_{(1,53)} = 38.24$, p = 9.0×10^{-8}) which showed a summing pattern, relative to the depressing pattern seen in control-IgG SPNs (**Figure 5.9 G**), and also a trend towards a higher paired-pulse ratio (PPR) (control-IgG: 1.8 $\pm$ 0.1, NR1-IgG: 2.1 $\pm$ 0.1, independent t-test: p = 0.071; **Figure 5.9 H**). There were no significant differences in other pre- or post-synaptic properties tested (**Appendix, Supplementary Table S5.5**).

Post-synaptic



Pre-synaptic

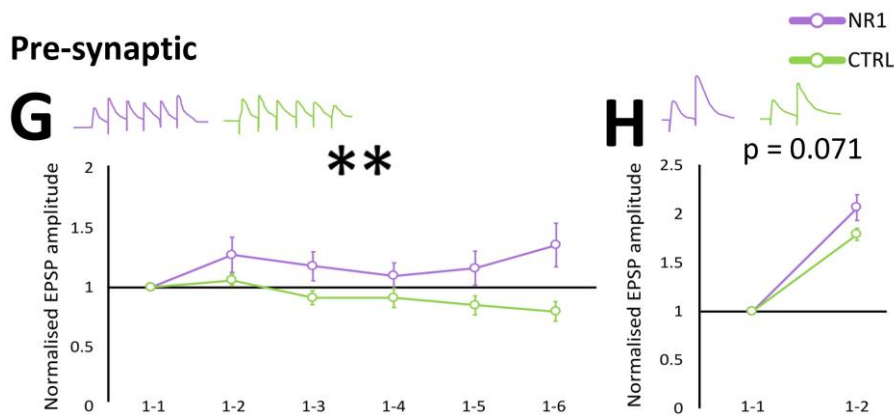


Figure 5.9. Altered cortical excitatory synaptic properties in early postnatal SPNs following in utero NR1-IgG exposure. **[A]** Diagram showing the set-up of the cortical stimulation whole-cell patch-clamp recordings, including placement of stimulation electrode in the external capsule close to the corticostriatal border, and placement of the recording electrode in the striatum. Diagram from *Krajeski et al. 2019*. **[B]** Dot contrast image showing placement of the stimulation and recording electrodes in the cortex and striatum respectively. EC = external capsule, CTX = cortex. Above: Example EPSP trace from a representative SPN recording showing the stimulation artefact followed by the EPSP wave. **[C-F]** Bar plots of the post-synaptic properties **[C]** EPSP amplitude, **[D]** duration, **[E]** rise time and **[F]** decay time respectively for the p7-14 control-IgG and NR1-IgG exposed SPNs, showing no significant difference in any of these properties in NR1-IgG exposed SPNs. **[G]** Graph of EPSP amplitude across a 20Hz train stimulation showing a significant difference between NR1-IgG and control-IgG exposed SPNs, with NR1-IgG SPNs exhibiting short-term summation and control-IgG SPNs exhibiting short-term depression. **[H]** Graph of PPR showing a trend towards a higher ratio in NR1-IgG exposed SPNs. Above **[G]** and **[H]** are representative traces of train stimulation and PPR respectively.

One of the key mechanisms by which NR1-IgG is thought to cause pathological disruption in the brain is through binding to the NR1 subunit of the NMDAR, which has been demonstrated to induce receptor internalisation, resulting in reduced NMDAR dependent glutamatergic signalling (**Dalmau et al. 2008, Hughes et al. 2010, Mikasová et al. 2012, Moscato et al. 2014, Rosch et al. 2018**). Here, experiments were performed to investigate whether evidence of internalisation could be observed. To capture this, the NMDA/AMPA ratio was studied by recording from the same neurons as above and, after assessing baseline parameters, then adding NMDAR antagonist DAP5 to the aCSF to capture residual responses solely governed by AMPA/KA receptors. Analysis of the ratio of peak amplitude from the NMDA and AMPA/KA EPSPs vs that from the AMPA/KA only EPSPs enabled calculation of the NMDA/AMPA ratio, which revealed a significantly higher normalised EPSP amplitude for NR1-IgG SPNs relative to control-IgG SPNs (control-IgG: $62.0 \pm 3.8\%$, NR1-IgG: $73.8 \pm 2.8\%$, independent t-test: $p = 0.017$; **Figure 5.10 A**), and a significantly reduced NMDA/AMPA ratio (control-IgG: $0.8 \pm 0.1\text{mV}$, NR1-IgG: $0.4 \pm 0.1\text{mV}$, MWU: $p = 0.022$; **Figure 5.10 B**). Analysis of other post-synaptic EPSP properties in the presence of DAP5 did not show any significant differences (**Appendix, Supplementary Table S5.5**), but train stimulation in the presence of DAP5 revealed a depression in the NR1-IgG exposed SPNs, converse to the summation seen when NMDARs were not antagonised (**Figure 5.10 C**).

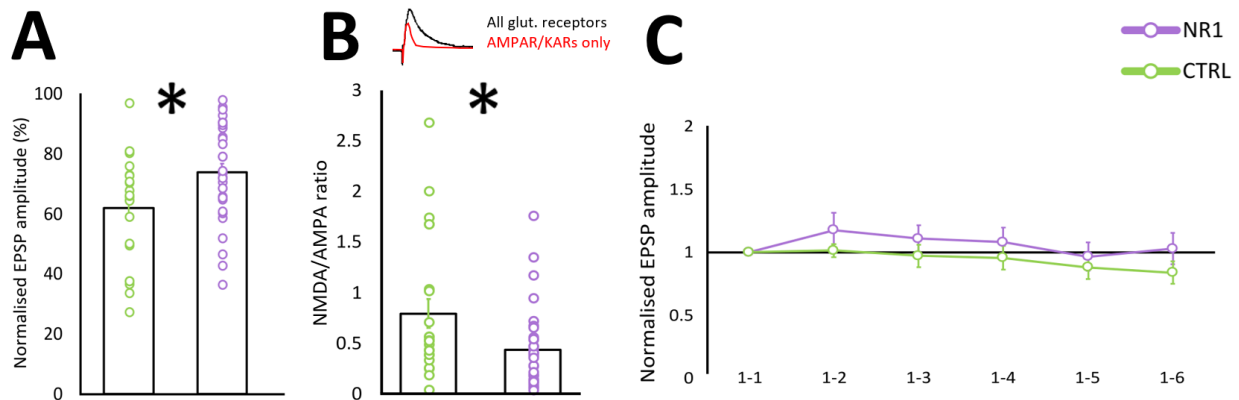


Figure 5.10. Reduced NMDA/AMPA ratio in early postnatal SPNs following in utero NR1-IgG exposure.

[A] Bar plot of EPSP amplitude for control-IgG and NR1-IgG exposed SPNs recorded in the presence of NMDAR antagonist DAP5, normalised against the same recording without DAP5 present, which revealed a significantly higher normalised amplitude for NR1-IgG exposed SPNs. **[B]** Bar plot of NMDA/AMPA ratio, revealing a significant decrease in NMDA/AMPA ratio in NR1-IgG exposed SPNs. The trace above provides an example cortical stimulation trace from the same neuron showing an EPSP when all ionotropic glutamate receptors are active (black) and when only AMPAR/KARs are active (i.e. DAP5 washed in to antagonise NMDARs (red)). **[C]** Graph of EPSP amplitude across a 20Hz train stimulation showing that in the presence of DAP5, both NR1-IgG and control-IgG exposed SPNs exhibited short-term depression.

5.5.3.3 Impacts on early postnatal SPN corticostriatal connections within specific two-day time windows

Using a similar approach as for intrinsic properties and synaptic inputs, corticostriatal data was also further analysed in smaller two-day time windows to establish if there were any specific timepoints within the second postnatal week which might differ. For this analysis, the n numbers were as follows: n(NR1-IgG SPNs): p7-8: 9 cells, p9-10: 8 cells, p11-12: 11 cells, p13-14: 7 cells; n(control-IgG SPNs): p7-8: 4 cells, p9-10: 7 cells, p11-12: 7 cells, p13-14: 7 cells.

Analysis of several post-synaptic properties during this period revealed no significant differences in EPSP amplitude, duration, rise time or decay time (data in **Appendix, Supplementary Table S5.6, Supplementary Figure S5.7**).

However, analysis of pre-synaptic properties revealed significant differences following 20Hz train stimulation at both p7-8 (ANOVA: $F_{(1,11)} = 7.40$, $p = 0.020$; **Figure 5.11 A**) and p13-14 (ANOVA: $F_{(1,10)} = 10.81$, $p = 0.0070$, **Figure 5.11 B**), with NR1-IgG exposed SPNs exhibiting short-term summation in both cases, converse to the short-term depression at p7-8 and no change either way at p13-14 seen in control-IgG exposed SPNs. PPR analysis also revealed a significant difference at p7-8 (p7-8: control-IgG: 1.5 ± 0.03 , NR1-IgG: 2.4 ± 0.2 , MWU: $p = 0.0030$; **Figure 5.11 C**). There were no significant differences in either train stimulation or PPR at p9-10 and p11-12, or in PPR at p13-14 (data and statistics in **Appendix, Supplementary Table S5.6**).

Further, analysis of NMDA/AMPA ratio revealed a diverging trend between NR1-IgG and control-IgG SPNs, with the largest differences observed at p13-14 (p7-8: control-IgG: 0.3 ± 0.04 , NR1-IgG: 0.3 ± 0.1 , MWU: $p = 0.37$; p9-10: control-IgG: 0.7 ± 0.3 , NR1-IgG: 0.6 ± 0.2 , MWU: $p = 0.96$; p11-12: control-IgG: 0.7 ± 0.1 , NR1-IgG: 0.5 ± 0.1 , independent t-test: $p = 0.25$, p13-14: control-IgG: 1.2 ± 0.3 , NR1-IgG: 0.5 ± 0.2 , MWU: $p = 0.13$; **Figure 5.11 D**).

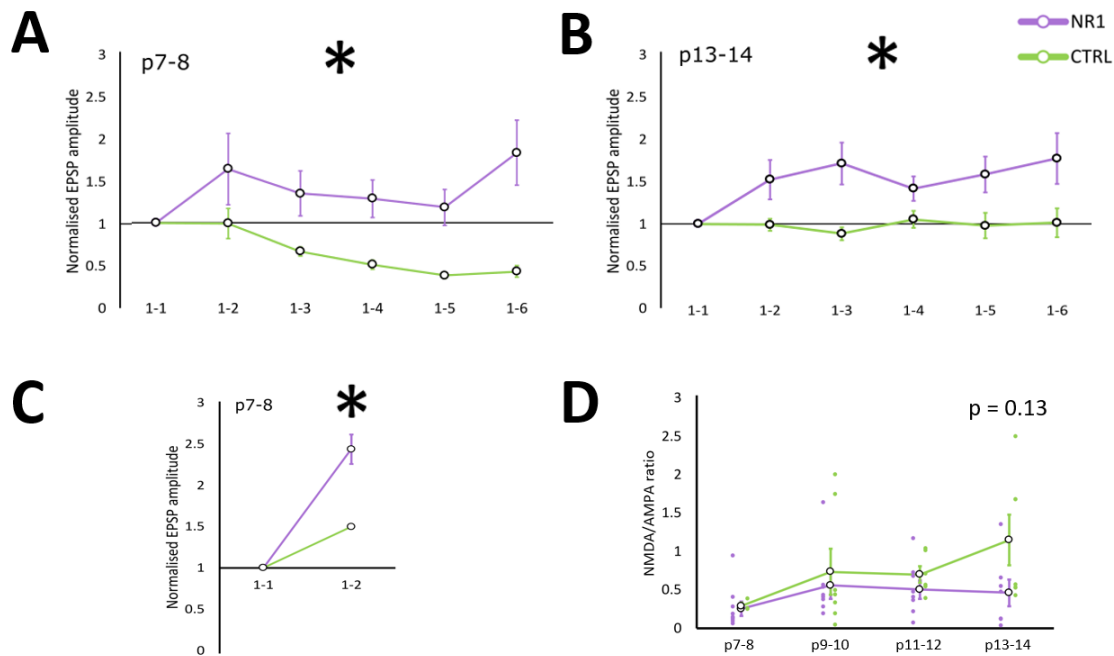


Figure 5.11. In utero NR1-IgG exposure predominantly affects pre-synaptic properties of early postnatal SPNs in specific short time windows. (Description on next page).

Figure 5.11. *In utero* NR1-IgG exposure predominantly affects pre-synaptic properties of early postnatal SPNs in specific short time windows (cont.). [A-B] Graphs of 20Hz train stimulation of [A] p7-8 and [B] p13-14 SPNs respectively, exposed to control-IgG or NR1-IgG *in utero*, showing a significant difference between groups at both timepoints, with NR1-IgG exposed SPNs exhibiting short-term summation, contrary to control-IgG exposed SPNs, which exhibited short term depression at p7-8 and neither depression nor summation at p13-14. There were no significant differences at p9-10 or p11-12 during train stimulation. [C] Graphs of PPR at p7-8 of control-IgG and NR1-IgG *in utero* exposed SPNs, which revealed a significantly higher paired-pulse ratio in NR1-IgG exposed SPNs at this time point. There were no significant differences at p9-10, p11-12 or p13-14. [D] Graph of NMDA/AMPA ratio split into two-day time windows. Although there were no significant differences at any of the time points, there was a diverging trend, with the largest difference seen at p13-14. (Also see **Appendix, Supplementary Table S5.6, S5.7**).

Overall, these results reveal an impact of NR1-IgG exposure *in utero* on the development of early postnatal glutamatergic synapses. Analysis of spontaneous sEPSCs showed that sEPSC amplitude decreased in the latter half of the second postnatal week, without concurrent change in sEPSC frequency, indicating potential post-synaptic alterations. Consistent with post-synaptic changes, evaluation of corticostriatal connections using evoked responses in both the presence and absence of NMDAR antagonist DAP5 showed a significantly reduced NMDA/AMPA ratio, with most difference at p13-14.

Interestingly however, there were no significant differences observed in the individual corticostriatal post-synaptic properties examined including EPSP amplitude, duration, rise time and decay time overall or at any two-day timepoints. Instead, there were significant changes in pre-synaptic properties and short-term plasticity, notably a facilitative effect upon train stimulation, which became depressive on NMDAR blockade with DAP5. Pre-synaptic effects showed most differences at p7-8 and p13-14 when analysed in two-day time windows.

Correlations between NMDA/AMPA ratio and intrinsic properties and synaptic properties were also briefly explored and are summarised in **Appendix, Supplementary Table S5.8**.

5.5.4 Postnatal impact of NR1-IgG antibodies on striatal morphology and cell composition

Having identified impacts of *in utero* NR1-IgG exposure on individual SPNs, experiments next sought to assess the scale of the impact of *in utero* NR1-IgG exposure on the striatum as a whole, through analysis of regional morphology, striatal cell density and composition.

The first experiments involved MRI-scanning third-week postnatal brains that had been injected with NR1-IgG or control-IgG *in utero* and performing volumetric analysis of the whole brain and striatum (**Figure 5.12 A**). For this dataset, $n(\text{NR1-IgG}) = 5$ brains, 2 litters, $n(\text{control-IgG}) = 6$ brains, 2 litters. The making of these MRI scans was kindly supported by Johan Van Audekerke and colleagues (BioImaging Lab, Antwerp). The volume of the dorsal striatum was quantified as a percentage of the whole brain to control for any differences in total brain volume between animals. This analysis revealed that there was a significant reduction in striatal volume in NR1-IgG exposed brains relative to control-IgG exposed brains (control-IgG: $5.1 \pm 0.2\%$ total brain volume, NR1-IgG: $4.4 \pm 0.2\%$ total brain volume, independent t-test: $p = 0.040$; **Figure 5.12 B**).

Cellular composition of the striatum was also assessed at this stage, specifically the ratio of D1- to D2-SPNs. Both populations have important and distinct functional roles in the striatum and differentially regulate motor behaviour and cognition (Graybiel et al. 1994, Grillner et al. 2005, Yin and Knowlton 2006, Kravitz et al. 2010, Tecuapetla et al. 2016), with imbalance linked to impaired neurodevelopment (and wider neurological disorders) (Graybiel and Rauch 2000, McNaught and Mink 2011, Langen et al. 2011, Del Campo et al. 2011, Albin et al. 2018, Shepherd et al. 2013). 50 μm coronal sections were made of the MRI-scanned NR1-IgG and control-IgG injected brains and these were stained for CTIP2 (SPN marker) and PPE (D2+ SPN marker). The ratio of D1- to D2-SPNs was then obtained through subsequent careful stereological counting (**Figure 5.12 C**). For this dataset, $n(\text{NR1-IgG}) = 7$ brains, 3 litters, $n(\text{control-IgG}) = 10$ brains, 3 litters. These experiments revealed a significant decrease in the proportion of D2-SPNs in NR1-

IgG *in utero* exposed brains (control-IgG: $48.0 \pm 1.0\%$, NR1-IgG: $34.0 \pm 3.0\%$, independent t-test: $p = 0.00069$; **Figure 5.12 D**).

To evaluate if this reduction in volume and decrease in D2-SPNs might be due to reduced cell numbers, the density of SPNs was also analysed in the same slices. This revealed a trend towards a decrease in SPN density in NR1-IgG exposed brains (control-IgG: $2.5 \pm 0.2 \text{ cells} \cdot 10^5/\text{mm}^3$, NR1-IgG: $2.2 \pm 0.1 \text{ cells} \cdot 10^5/\text{mm}^3$, independent t-test: $p = 0.073$; **Figure 5.12 E**).

Overall, these results demonstrate significant impact on both regional morphology and cellular composition in the postnatal striatum of brains exposed to NR1-IgG *in utero* that are still present in the fourth postnatal week, including a decrease in striatal volume and SPN cell density, as well as a shifted D1/D2 ratio, with a decrease in D2-SPNs.

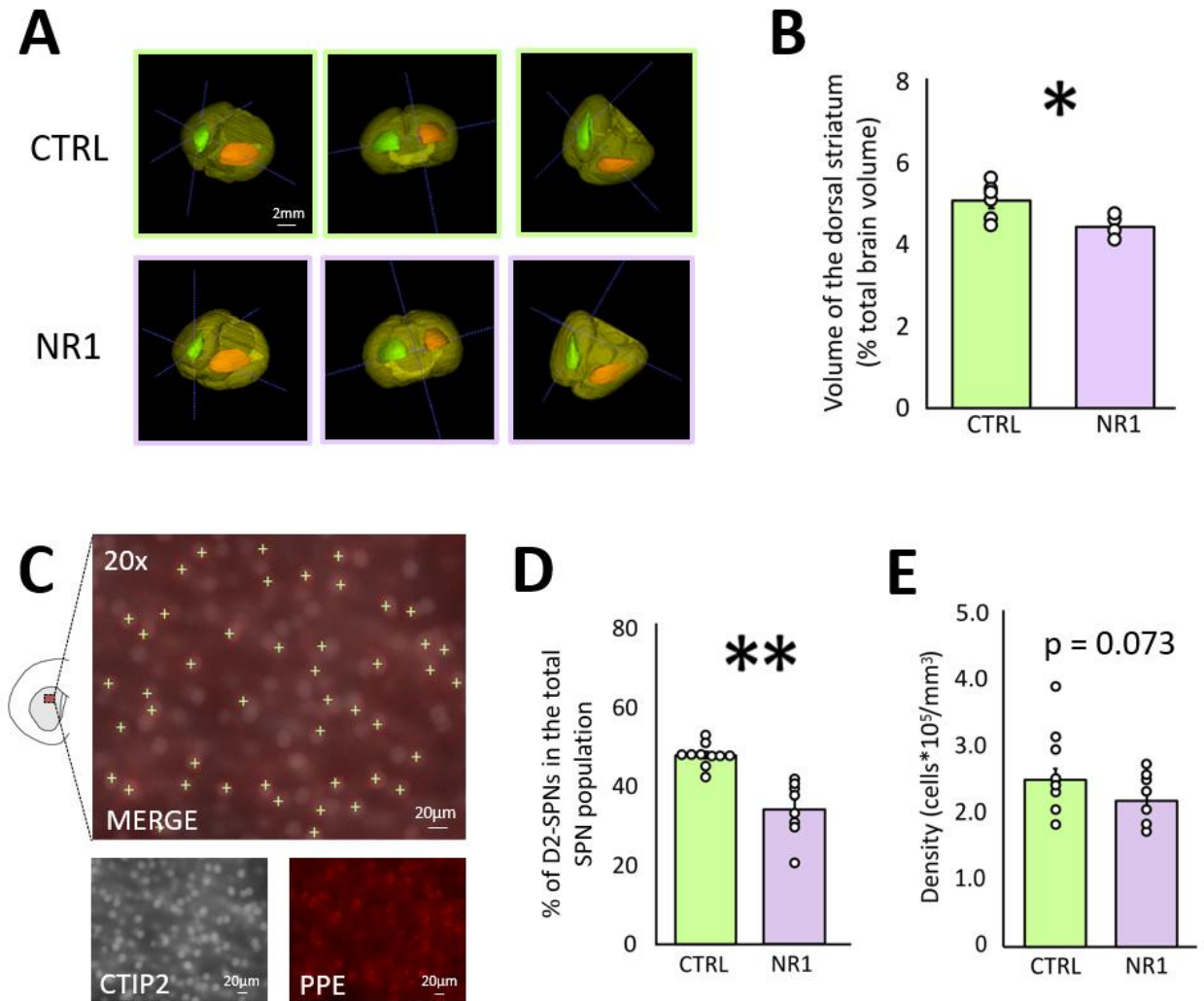


Figure 5.12. Alterations in striatal size, cell composition and density following in utero NR1-IgG exposure. **[A]** 3D reconstructions of a representative control-IgG and NR1-IgG in utero exposed brain fixed at p23, showing the whole brain in yellow, left dorsal striatum in red and right dorsal striatum in green. **[B]** Bar plot of the normalised dorsal striatum volume for control-IgG and NR1-IgG in utero exposed brains, fixed at p24, showing a significant decrease in dorsal striatal volume of NR1-IgG exposed brains. **[C]** Representative 20x image from a p23 striatum, showing immunostaining for CTIP2 labelling all SPNs (1:1000, white) and PPE, labelling D2-SPNs (1:500, red). Yellow + symbols represent cells counted that are labelled for both CTIP2 and PPE (i.e. D2-SPNs). Cells that are only CTIP2 labelled are counted as D1-SPNs. **[D]** Bar plot of the proportion of D2-SPNs in the total SPN population (i.e. D1/D2 ratio), showing close to the expected 50:50 D1/D2 ratio for control-IgG exposed brains, but a significantly reduced proportion of D2-SPNs in NR1-IgG exposed brains. **[E]** Bar plot of density of cells in the striatum evaluated in control-IgG and NR1-IgG exposed p23 brains, revealing a trend towards a reduced SPN density in the striatum of NR1-IgG exposed brains.

5.5.5 Postnatal impact of NR1-IgG antibodies on microglia in the striatum

In **Experimental Chapter B**, it was observed that as well as having impacts on embryonic progenitors and neurons, NR1-IgG *in utero* exposure also resulted in both increased density and altered morphology of embryonic microglia. Further, *in utero* exposure to other antibodies such as CASPR2 has previously demonstrated that microglial activation can persist postnatally (**Coutinho et al. 2017b**). Therefore, the final part of this chapter explored whether *in utero* exposure to NR1-IgG had a prolonged impact on microglia that was observable in the postnatal period. Microglia were visualised using IBA1 immunolabelling and analysed using similar methods as for embryonic microglia to assess their density and morphological features.

Microglial density in the cortex and striatum was first examined, since in the embryonic brain a significantly higher density in the striatum of NR1-IgG exposed brains relative to control-IgG exposed brains had been observed. This analysis revealed a trend towards a significant increase in microglial density in the cortex of NR1-IgG exposed brains (control-IgG: 6.0 ± 0.3 cells* 10^3 /mm³, NR1-IgG: 6.9 ± 0.5 cells* 10^3 /mm³, independent t-test: $p = 0.15$; **Figure 5.13 A**), and a significantly higher density of microglia in the striatum of NR1-IgG exposed brains (control-IgG: 8.0 ± 0.7 cells* 10^3 /mm³, NR1-IgG: 10.7 ± 0.5 cells* 10^3 /mm³, independent t-test: $p = 0.020$; **Figure 5.13 B**). For this analysis, n(NR1-IgG) = 5 brains, 2 litters; n(control-IgG) = 7 brains, 3 litters.

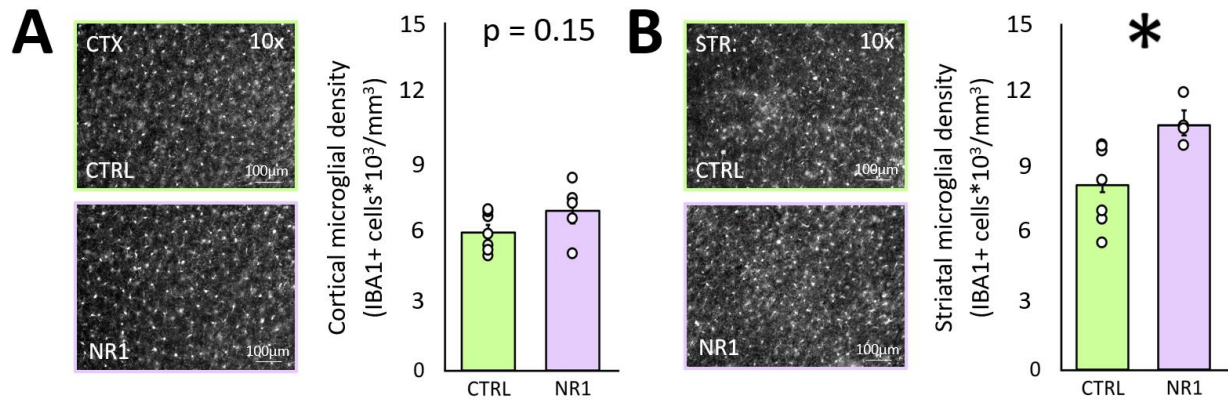


Figure 5.13. Increased striatal microglial density following in utero NR1-IgG exposure. **[A]** Representative epifluorescence images of the cortex from control-IgG and NR1-IgG in utero exposed brains taken at p23 in fixed tissue coronal slices and immunostained with IBA1 to label activated microglial cells, next to a bar plot of the microglial density in the cortex, showing a trend towards an increase in microglial density in the cortex of NR1-IgG exposed brains. **[B]** Similar to **[A]** but for the striatum rather than the cortex. The bar plot shows there was a significantly increased density of microglia in the striatum of NR1-IgG in utero exposed brains.

Potential alterations in microglial morphology were also investigated to establish if the microglial morphological changes observed embryonically persisted into the postnatal brain. For this analysis, individual microglial cells were imaged at 40x magnification and reconstructed digitally in *Image J* (**Figure 5.14 A, B**). For these experiments, n(NR1-IgG microglia) = 40 cells, 5 brains, 2 litters; n(control-IgG microglia) = 48 cells, 6 brains, 3 litters. This analysis revealed significant differences in the microglial morphology including a significant decrease in total branch length (control-IgG: $657.9 \pm 19.1\mu\text{m}$, NR1-IgG: $485.0 \pm 17.2\mu\text{m}$, independent t-test: $p = 3.4 \times 10^{-9}$; **Figure 5.14 C**), number of 1° branches (control-IgG: 6.2 ± 0.2 , NR1-IgG: 5.4 ± 0.2 , independent t-test: $p = 7.9 \times 10^{-5}$; **Figure 5.14 D**) and total number of branches (control-IgG: 87.6 ± 3.2 , NR1-IgG: 69.3 ± 3.0 , independent t-test: $p = 0.013$; **Figure 5.14 E**), as well as reduced dendritic complexity (Scholl analysis: ANOVA: $F_{(1, 86)} = 39.47$, $p = 1.32 \times 10^{-8}$; **Figure 5.14 F**), all indicators of a more activated state.

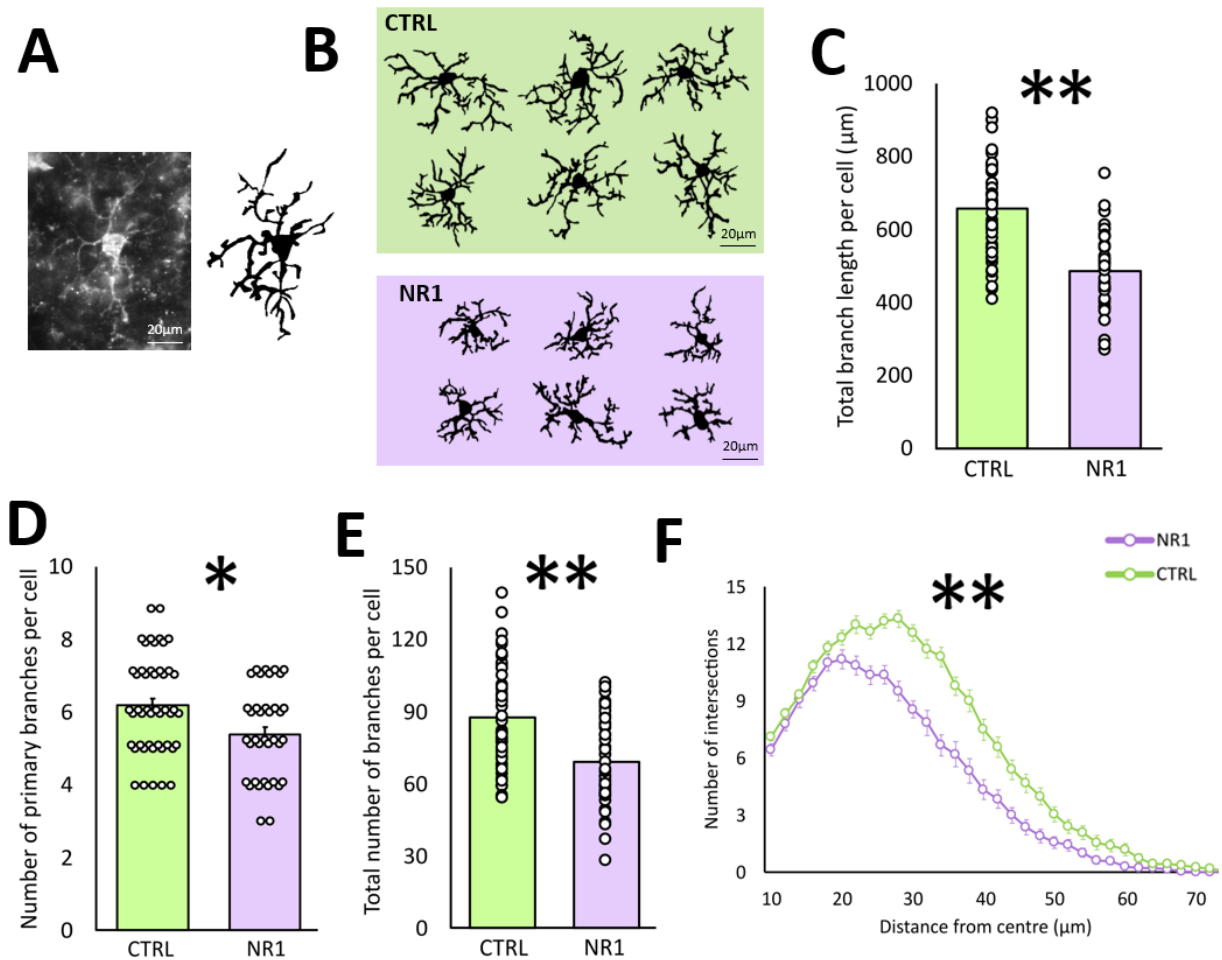


Figure 5.14. Morphological alterations in striatal microglia following in utero NR1-IgG exposure. [A] Left: Representative epifluorescence image of a striatal activated microglial cell, stained with IBA1, prior to any image processing. Cells were imaged in a 30 μ m z-stack, at 1 μ m intervals. Right: Binary reconstruction of the same microglial cell, following the protocol outlined in **Young and Morrison 2018**. **[B]** Further representative reconstructions of IBA1+ striatal microglial cells from control-IgG (green) and NR1-IgG (purple) in utero exposed brains. **[C]** Bar plot of total branch length per cell for control-IgG and NR1-IgG exposed microglia, revealing a significant decrease in total branch length in microglia exposed to NR1-IgG in utero. **[D-E]** Similarly, bar plots of number of primary branches per cell and total number of branches per cell respectively, revealing a significant decrease in both parameters in striatal microglia from NR1-IgG in utero exposed brains. **[F]** Scholl analysis also revealed a significant decrease in complexity of microglia from the striatum of NR1-IgG in utero exposed brains.

Overall, these results indicate that the microglia exposed to NR1-IgG *in utero* show a persistently increased density and a more activated morphological state that is still detectable in the fourth postnatal week.

5.6 Chapter Discussion

Experimental Chapter C built on the embryonic findings in **Experimental Chapter B** and explored the impact of *in utero* exposure to NR1-IgG on the early postnatal striatum. Electrophysiological and morphological studies identified several alterations in early postnatal cells and circuits. Specifically, the key findings in NR1-IgG exposed SPNs/brains were:

1. **SPN intrinsic properties:** Differences in early postnatal (p7-14) SPN intrinsic properties, including an increase in resting membrane potential, threshold, input resistance and membrane time constant, and a decrease in first and second spike amplitude, suggestive of relative electrical immaturity. Effects were non-uniform across time when analysed in two-day time windows, with several showing more significant effects earlier on (eg. p7-8), and subsequent normalisation.
2. **SPN morphology:** Subtle differences in early postnatal SPN morphology, including a trend towards a reduction in the total number of branches per cell, and a significant decrease in spines on secondary dendrites.
3. **SPN excitatory synaptic inputs:** Reduced sEPSC amplitude in the latter half of the second postnatal week (p11-14), but no changes in sEPSC frequency, suggestive of a post-synaptic effect.
4. **NMDA/AMPA ratio:** Significantly reduced NMDA/AMPA ratio, with greatest difference at p13-14.
5. **Corticostriatal connections:** Changes in pre-synaptic properties and short-term plasticity, including a facilitative effect upon train stimulation, which became depressive upon antagonising NMDARs with DAP5. No differences in post-synaptic properties analysed including EPSP amplitude, duration, rise time or decay time.
6. **Regional morphology and cell composition:** Decreased striatal volume, a trend towards decreased SPN cell density and a significantly shifted D1/D2 ratio (fewer D2-SPNs) in the fourth postnatal week.

7. **Microglia:** Increase in activated microglia density and alterations in morphology suggestive of a more activated state in the fourth postnatal week striatum.

5.6.1 SPN intrinsic properties: Differences in early postnatal (p7-14) SPN intrinsic properties, suggestive of relative electrical immaturity; and

5.6.2 SPN morphology: Subtle differences in early postnatal SPN morphology, including a significant decrease in spines on secondary dendrites;

Firstly, significant differences in the development of intrinsic properties of SPNs exposed to NR1-IgG *in utero* and recorded in the second postnatal week (p7-14) were identified. When looking at the dataset as a whole, it was found that SPNs exhibited significantly higher resting membrane potential, threshold, input resistance, membrane time constant, and a significantly lower first and second spike amplitude. For several properties, effects were most prominent early on (eg. p7-8), with subsequent normalisation (eg. p13-14).

Previous work by my lab and others has identified that as SPNs mature, they undergo changes in their intrinsic properties including a progressive decrease in their input resistance and a hyperpolarisation of their resting membrane potential (Lieberman et al. 2018), enabling generation of more mature action potentials at higher frequencies (Peixoto et al. 2016). Hence our observations here suggest that NR1-IgG *in utero* exposed SPNs were relatively electrically immature, and therefore unable to generate the large action potentials seen in control-IgG SPNs at the same stage.

Morphological reconstruction of a subset of these cells revealed similar dendritic arbour between NR1-IgG and control-IgG *in utero* exposed SPNs, though there was a trend towards a decrease in total number of branches in the NR1-IgG exposed group. A significantly reduced spine density on secondary dendrites was also identified, and a slight trend towards a reduced density on tertiary dendrites. Earlier studies have also shown that SPNs undergo a concurrent maturation in their morphology as their intrinsic properties

mature, including elaboration of their dendritic arbour (**Krajeski et al. 2019**) and protracted increase in spine density, particularly along non-primary dendrites (**Tepper et al. 1998, Gertler et al. 2008, Dehorter et al. 2011, Peixoto et al. 2016, Krajeski et al. 2019**). Hence morphological differences observed here also fit with a picture of relative morphological immaturity in NR1-IgG *in utero* exposed SPNs.

Although there is limited existing research on the influence of NR1-IgG antibodies on SPN intrinsic properties, the relative electrophysiological and morphological immaturity observed is consistent with findings from other studies using early genetic deletion of NR1 to remove NMDARs in SPNs and other neuronal cell types. For example, **Pagadala et al. (2013)** found in mice that knock-out of NR1 in primary sensory neurons showed increased intrinsic excitability, which could also be replicated by NMDAR antagonist DAP5, and **Hou and Zhang (2017)** also found that mosaic deletion of NR1 in early thalamic neurons resulted in increased intrinsic excitability throughout the early postnatal period, as well as morphological alterations including reduction in number of dendrites, similar to what was found here. **Beutler et al. (2011)** also showed NR1 knock-out mice had severely altered SPN morphology including smaller cell bodies and decreased dendritic length. Additionally, deletion of NR1 has been shown to lead to a decrease in spine density as well as altered spine morphology in developing cortical neurons (**Ultanir et al. 2007**), and NR1-IgG antibodies have been shown to decrease spine density in hippocampal neurons (**García-Serra et al. 2020**). Further, NMDAR antagonism using MK801 in the early postnatal period has been shown to decrease dendritic arborisation of hippocampal neurons (**Eldhart et al. 2010**). Hence our results support the idea that *in utero* NR1-IgG exposure disrupts early function of NMDARs (**Hughes et al. 2010, Moscato et al. 2014**), resulting in improper development of SPN electrical and morphological cellular properties.

Conversely, there are some studies that do not completely fit our findings. **Hughes et al. (2010)** found, similarly to us, that there was no change in dendritic branching or complexity of hippocampal neurons *in vitro* treated with CSF from NMDARE patients but also found no impact on the number of dendritic spines.

Their negative findings about numbers of spines could be due to their use of CSF as opposed to IgG, which likely contains a lower concentration of the antibody. It could also be due to *in vitro* vs *ex vivo* techniques, or due to the use of hippocampal neurons rather than SPNs which have a far lower spine density and therefore may be less impacted. **García-Serra et al. (2020)** found a decrease in dendritic spines on hippocampal and cortical neurons in their studies using similar *in vivo* IgG methods to our study, suggesting that methodology rather than neuronal cell type may account for differences here. Interestingly, **García-Serra et al. (2020)** also identified an increase in dendritic arbour in both hippocampal and cortical neurons at p21 following NR1-IgG *in utero* exposure, converse to our observations of no change in dendritic arbour in SPNs, which may suggest that the impact of NR1-IgG on neurons is more cell-type specific, a theory supported by studies of SPNs and other neurons in NR1 knock-out models (**Beutler et al. 2011, Hou and Zhang 2017**). Additionally, it could be due to the different timepoints studied (p21 vs p7-14 in our study). It is also important to consider here that recording and reconstructing cells and spines in slices used in both experiments in this thesis and other studies above has limitations and conditions are highly different from the *in vivo* brain. Although care is taken to maintain conditions as close to *in vivo* as possible, precise ionic conditions and temperatures are unlikely to be completely reflective, and making slices will sever a subset of dendrites and axons, reducing connectivity. Biocytin and DAB staining may also not completely fill all dendrites, axons and spines, and thus reconstructions may not capture the full arbour or spine density.

Mechanistically, maturation of SPN intrinsic excitability is largely governed by a family of potassium channels (**Karschin et al. 1996, Shen et al. 2007, Cazorla et al. 2012**). The exact role of NMDARs in maturation of SPN neuronal excitability specifically is not well understood, however **Liebermann et al. (2018)** have identified that maturation of D1-SPN intrinsic excitability arises from PIP₂-Kir₂ interactions driven by dopamine signalling in the critical period. It has previously been suggested that one mechanism by which dopamine signalling regulates PIP₂ synthesis is through calcium entry via NMDARs (**Nakano-**

Kobayashi et al. 2007, Unoki et al. 2012) and thus it may be the case that loss of surface NMDARs reduces PIP₂ synthesis and hence PIP₂-Kir2 interactions, reducing membrane insertion of Kir2 channels, and therefore impairing maturation of intrinsic excitability specifically in D1-SPNs. Given that we also show later in this chapter that the D1/D2 ratio is shifted towards fewer D2-SPNs in NR1-IgG *in utero* exposed brains, the impact on D1-SPNs could be particularly pertinent. The documented quicker intrinsic property maturational timescale of D1-SPNs relative to D2-SPNs (**Krajeski et al. 2019**) might also be mitigated in this case.

Development of neuronal morphology is dependent on interplay between intrinsic mechanisms, extracellular signalling and synaptic activity (**Scott and Luo 2001, Cline 2001, Hua and Smith 2004**). It has previously also been shown by **Carzola et al. (2012)** that downregulation of Kir2 channels also results in decreased SPN dendritic arborisation, including dendritic length, and numbers of branch nodes and tips, and hence the mechanism proposed above could also fit the trends towards decrease in total number of branches in our NR1-IgG exposed SPNs observed here. Beyond this, *in vivo* imaging and other experiments suggest that NMDAR-mediated synaptic activity promotes branch formation and stabilisation (**Dailey and Smith 1996, Maletic-Savatic et al. 1999, Niell et al. 2004**) and also stabilises spines and synapses, including stabilising the actin cytoskeleton (**Star et al. 2002**) and increasing F-actin in spines (**Fukazawa et al. 2003**). Pertinently here, NMDAR-mediated cortical inputs onto SPNs (which work in this chapter discussed further on shows exhibit alterations following NR1-IgG exposure) also influence SPN morphology, with **Buren et al. (2016)** demonstrating a significant decrease in dendritic length and complexity of SPNs with reduced cortical inputs *in vitro*. Interestingly, it has recently been shown that extrasynaptic NMDARs bidirectionally modulate intrinsic excitability in GABAergic neurons, with NMDAR activation increasing excitability and inhibition reducing it (**Yao et al. 2022**), which may putatively suggest that there are greater impacts on synaptic NMDARs in this study, or that synaptic NMDARs have more influence, in the context of the converse observations seen here. Logically, it might be expected that NR1-IgG probably binds all

NMDARs (both synaptic and extrasynaptic) on young neurons, however recent work has demonstrated that NR1-IgG binding onto synaptic and extrasynaptic NMDARs differs between inhibitory and excitatory cortical neurons (**Andrzejak et al. 2022**). Thus, there may be differential binding and/or impacts on SPN NMDARs, which is an important future question to explore.

5.6.3 SPN excitatory synaptic inputs: Reduced sEPSC amplitude but no changes in sEPSC frequency, suggestive of a post-synaptic effect;

It was also identified that there were subtle differences in the development of early excitatory synaptic inputs onto NR1-IgG exposed SPNs. Recordings of sEPSCs revealed no significant differences in either frequency or amplitude when the p7-14 dataset was analysed as a whole, but when data was further evaluated in smaller two-day time windows, a diverging trend was observed, with a trend decrease in amplitude seen at p11-12, which further decreased and reached significance at p13-14.

Observations of decreased sEPSC amplitude but not frequency point towards post-synaptic changes. These findings somewhat align with those of **Jurek et al. (2019)**, who also demonstrate that *in utero* exposure to NR1-IgG antibodies resulted in decreased amplitude of sEPSCs in hippocampal pyramidal neurons in the early postnatal period, with no significant changes in frequency. In their study, they observed the differences shortly after birth (p0) which normalised by p7, whereas in the study here the differences occurred in the latter half of the second postnatal week (p11-12 and p13-14). This could be explained by differences in the model used (IP injections into the mother vs our direct intraventricular injection into the embryonic brain) or different cell types (hippocampal neurons vs SPNs). Although injections here were conducted at similar developmental timepoints, **Jurek et al. (2019)** injected into the dam rather than embryo meaning antibodies had to cross the placenta and then into the embryonic brain, and were therefore likely reach the brain later than our injections. Additionally, hippocampal development starts slightly earlier than striatal development (E8.5 vs E10) (**Nazzari and Francis 2013, Kelly**

et al. 2018). Combined, the hippocampal neurons may have been more mature by the time the antibody reached them, resulting in impacts at an earlier timepoint. It could also be the case that certain neuronal types are impacted earlier by the antibody than others, possibly either due to location, or NMDAR subunit composition, or some other as yet unestablished neuronal-type specific factors. Indeed, in NMDARE, symptomatic temporal dichotomy is well documented (**Irani et al. 2010, Barry et al. 2015**), with initial symptoms typically being psychiatric before progressing to motor symptoms, and therefore it might be speculated that there could be a potential relative delay in the effect on motor-associated circuits such as those of the striatum. Although in this study individual contributions of NMDAR and AMPARs to sEPSCs were not isolated, **Hughes et al. (2010)** used whole-cell patch clamp recordings of cultured hippocampal neurons with wash in of NMDAR antagonist DAP5 or AMPAR antagonist CNQX and found that NR1-IgG antibodies decreased NMDAR-mediated (but not AMPAR-mediated) mEPSCs (another measure of the influence of excitatory neurotransmitters on a neuron, albeit it without capturing spontaneous events), suggesting that pharmacological isolation of the glutamatergic components may also be a useful extension for more detailed mechanistic understanding in SPNs.

5.6.4 NMDA/AMPA ratio: Significantly reduced NMDA/AMPA ratio, with greatest difference at p13-14;

Previous work by my lab and others has demonstrated that in healthy development there is a large increase in mEPSC amplitude during the second postnatal week (**Dehorter et al. 2009, Peixoto et al. 2016, Krajewski et al. 2019**), likely reflective of changes post-synaptically. The literature suggests that increase in response amplitudes correlates with larger AMPAR-mediated currents, paralleling the decrease in the NMDA/AMPA ratio as the developmental insertion of AMPARs into the post-synaptic membrane occurs (**Colwell et al. 1998, Hurst et al. 2001, Peixoto et al. 2016**). However, evoked corticostriatal response data here revealed a decreased NMDA/AMPA ratio in spite of a decrease in sEPSC amplitude, which could be explained by the decreased ratio reflecting a decrease in NMDARs secondary to NR1-IgG antibody binding

and receptor internalisation (Hughes et al. 2010, Moscato et al. 2014), rather than the addition of AMPARs. Alternatively, it may reflect findings by Colwell et al. (1998) that NMDAR function is delayed specifically in striatal SPNs, with contributions increasing approximately two-fold between the first and third postnatal weeks. A trending decrease in total branch length, number of primary branches and total number of branches over the second postnatal week was observed, which could explain the parallel decreases in sEPSC amplitudes seen, since dendritic geometry and function are highly intertwined, modulating integration and propagation of synaptic signals, and ability to generate action potential output (Häusser et al. 2000, Mcgee 2000, Poiraz and Mel 2001, Spruston 2008). It is also important to consider that experiments here using DAP5 may not have captured the full extent of NMDAR currents since SPNs were recorded at -70mV in potassium gluconate internal (to allow for capture of other intrinsic and synaptic properties) rather than +40mV in caesium gluconate internal (usually used for recording NMDAR-mediated currents since these conditions release the Mg²⁺ block from the NMDAR), therefore limiting interpretation of NMDA/AMPA ratio results.

5.6.5 Corticostriatal connections: Changes in pre-synaptic properties and short-term plasticity, but no differences in post-synaptic properties analysed;

In further experiments, recordings of electrically evoked corticostriatal responses showed changes in pre-synaptic properties and short-term plasticity, including a facilitative effect upon train stimulation, which became depressive upon antagonising NMDARs. Counterintuitively, no differences were found in the post-synaptic properties analysed, including EPSP amplitude, duration, rise time or decay time.

The facilitative effect seen upon train stimulation and increased PPR in NR1-IgG exposed SPNs suggests a pre-synaptic effect and lower release probability. Although NMDARs are classically thought of as post-synaptic receptors and are found at most excitatory synapses post-synaptically, pre-synaptic NMDARs also exist in specific brain regions and at specific sets of synapses (Braisser et al. 2008, Huang et al. 2011,

Buchanan et al. 2012, Banerjee et al. 2014, Larsen et al. 2014). Pertinent to our study, **Park et al. (2014)** have identified an essential role of pre-synaptic NMDARs in activity dependent long-term potentiation at mouse corticostriatal synapses through triggering activity-induced pre-synaptic secretion of BDNF. Although yet to be studied, given pre-synaptic NMDARs are present at these synapses, they also likely have a role in short-term plasticity. It would be expected that pre-synaptic NMDARs would be affected by *in utero* NR1-IgG exposure in a similar manner to post-synaptic NMDARs since they have congruent structures and therefore the pre-synaptic short-term plasticity effects observed here may result from pre-synaptic NMDAR internalisation. Alternatively, impact on post-synaptic NMDARs identified in our spontaneous recordings may also be able to explain the pre-synaptic effects observed; indeed, recent experiments by **Kesner et al. (2020)** looking at pre- and post-synaptic NMDARs in *Xenopus* tadpole visual circuit neurons identified that pre-synaptic release probabilities can be modulated by post-synaptic NMDARs, posited via retrograde messengers such as BDNF or nitric oxide.

An unexpected finding here was the lack of alterations seen in the post-synaptic properties of the corticostriatal evoked responses. A likely explanation for this is that the changes were subtle, (corroborated by the sEPSC results), and therefore possibly occluded by variable stimulation strengths in the evoked experiments. Indeed, it is highly challenging to normalise between slices as there are many variables that are difficult to totally control for including stimulation strength, electrode placement and slice cutting angle, thus these factors may have limited detection of more subtle differences in these results. Alternatively, it could be that the mechanism of action of NR1-IgG in corticostriatal pathways more specifically involves pre-synaptic NMDARs. Indeed, regional specificity in the pathological mechanisms of NR1-IgG has been recently shown, with **Andrzejak et al. (2022)** using whole-cell patch-clamp recordings in cultured cortical neurons to identify that NR1-IgG antibodies selectively impair synaptic transmission and NMDAR-mediated currents of cortical inhibitory (not excitatory) neurons only, contrary to what is seen in the hippocampus.

5.6.5.1 Analysis in two-day time windows

When analysing our results in smaller two-day time windows we observed dynamic variation within both electrophysiological and morphological properties. The variation in all these properties is likely due to the dynamic expression of NMDAR subunits during the early postnatal period (**Monyer et al. 1994, Krajcsi et al. 2019**), which result in NMDAR subtypes with marked variability in pharmacological, biophysical and signalling properties (**Paoletti et al. 2013**). In particular, there is a well-characterised developmental switch of NR2B to NR2A subunits in NMDARs in the early postnatal period, coinciding with synaptogenesis (**Sheng et al. 1994, Pagano et al. 2021**). NR2B- and NR2A-containing receptors are thought to exert opposite signalling effects, slowing or promoting synaptic maturation and stabilisation respectively (**Ohno et al. 2010, Gray et al. 2011, Wang et al. 2011a, Henle et al. 2012, Kelsch et al. 2014, Gray et al. 2018, Wang et al. 2021**). It is unclear what the specific effects of NR1-IgG are on subunit composition within our study, but one possibility is that it delays the switch, leading to altered receptor temporal integration properties and slower maturation of synapses (**Flint et al. 1997, Stocca and Vincini 1998, Barth and Malenka 2001**). The fact that the switch occurs at a similar timepoint to the point in which alterations are seen certainly warrants further investigation in future studies.

Different properties also showed different patterns of variation, with most intrinsic properties and pre-synaptic properties impacted more at earlier (eg. p7-8) timepoints, and post-synaptic (sEPSC amplitudes), morphology and NMDA/AMPA ratio exhibiting greater differences later on (eg. p13-14). The exact mechanisms underpinning this are yet to be identified, but it could be hypothesised that there may be interactive temporal effects between what is observed here. For example, early impacts on pre-synaptic properties could result in a decrease in spontaneous neurotransmitter release (**Abrahamsson et al. 2017**), consequently resulting in adaptive upregulation of AMPARs in the post-synaptic neuron (**Sutton et al. 2006, Jakawich et al. 2010**), leading to the changes in post-synaptic sEPSCs and decreased NMDA/AMPA ratio observed later on. Likewise, impacts on early intrinsic properties and pre-synaptic properties could

also impact subsequent morphological development, since proper morphological development has previously been shown to be dependent on intrinsic mechanisms and synaptic activity, amongst other parameters (Scott and Luo 2001, Cline 2001, Hua and Smith 2004).

5.6.6 Regional morphology and cell composition: Decreased striatal volume, a trend towards decreased SPN cell density and a significantly shifted D1/D2 ratio (fewer D2-SPNs) in the fourth postnatal week;

These experiments also showed that *in utero* NR1-IgG exposure resulted in a significant decrease in the volume of the postnatal striatum (assessed in the fourth postnatal week), as well as a significantly shifted D1/D2 ratio, with a decrease in D2-SPNs, and a trend towards a decrease in SPN density.

The decrease in striatal volume observed aligns with the study by Jurek et al. (2019) which also found that *in utero* exposure to NR1-IgG resulted in a decreased striatal volume. Clinical literature also broadly fits a decrease in striatal volume with progressive striatal necrosis reported in NMDARE patients (Tzoulis et al. 2013), although there is difference between SPN survival and SPNs not being generated to start with. The trend towards decreased density of SPNs observed would also align with early seminal studies by Luk et al. (2003), showing that *in utero* exposure to NMDAR antagonist MK801 resulted in significant reduction in total number of adult SPNs, and also with the earlier observations in **Experimental Chapter B** of reduced numbers of LGE proliferating progenitors.

A trend towards a decrease in the density of SPNs was observed, which likely explains the reduction in overall striatal volume seen, since SPNs compose the predominant (95%) cell type of the striatum. Alterations in morphology and reductions in dendritic arbour of SPNs may also have contributed. Indeed, over the second postnatal week, a general negative relationship between dendritic arbour and developmental age (i.e. the oldest (p13-14) cells exhibiting the smallest branch length and total numbers of branches) was observed and it may be the case that this decrease continues further as time goes on,

so that cells are significantly smaller by the fourth postnatal week when the volumetric analysis was conducted. Indeed, **Beutler et al. (2011)** has previously shown that cell size and dendritic arbour are still significantly decreased at 8-10 weeks in an NR1 knock-out mouse model.

The shifted D1/D2 ratio with fewer D2-SPNs may result from the later birthdate of D2-SPNs (**Marchand and Lajoie 1986, Van der Kooy and Fishell 1987, Kelly et al. 2018**), meaning that their generation is more greatly impacted by the NR1-IgG antibody. Alternatively, it has been posited that D2-SPNs have an increased expression of NMDARs (**Kreitzer et al. 2007**), which could leave them more susceptible to the impact of NR1-IgG antibodies. The same paper demonstrated that D2-pathway synapses have a significantly larger NMDA/AMPA ratio than D1-pathway synapses, hence the D1/D2 ratio shift could also underlie the decrease in NMDA/AMPA ratio we observed in the experiments here. The shift in D1/D2 ratio is an important finding since the imbalance in the two pathways is well established in a host of neurodevelopmental disorders including Tourette's (**McNaught and Mink 2011, Albin et al. 2018**), OCD (**Grabiel and Rauch 2000, Langen et al. 2011**), ADHD (**Del Campo et al. 2011**) and ASD (**Shepherd et al. 2013**), and provides a starting point for mechanistic insights into how NR1-IgGs may disrupt striatal development specifically. A useful focus for future experiments would be to establish whether nuclei downstream of the basal ganglia exhibit different activity patterns because of the imbalance in the D1/D2 pathway.

5.6.7 Microglia: Increase in activated microglia density and alterations in morphology suggestive of a more activated state in the fourth postnatal week striatum;

In the final experiments of this chapter, an increased density of and altered morphology in microglia was also observed in the striatum in the fourth postnatal week.

This finding parallels observations by **García-Serra et al. (2020)**, who found microglial activation in the early postnatal brain following *in utero* exposure to NR1-IgG antibodies through maternal tail-vein

injection. It also fits findings about prenatal exposure to antibodies and microglial activation more widely such as **Coutinho et al. (2017b)**, who also observed persistent activation of microglial cells as well as synaptic loss in the postnatal brain following *in utero* exposure to CASPR2 antibodies. Additionally, it fits several clinical autopsy studies which identified significant microgliosis post-mortem in brains from patients with NMDARE (**Dalmau et al. 2008, Tüzün et al. 2009, Bien et al. 2012**).

Functionally, NMDARs and microglia are closely linked and take part in many important neuronal-microglial interactions, including circuit refinement, microglia-mediated synaptic pruning and neuroplasticity (**Anderson and Vetter 2019**). There is a well-established link between perinatal immune challenges (eg. exposure to pathological antibodies *in utero*) and postnatal microglial activation. Any such challenge can lead to microglial activation and result in excessive microglia-mediated synaptic pruning (**Paolicelli and Ferretti 2017**). More specifically, recent work by **Rahman et al. (2023)** identified *in vitro* that microglia play an active role in removal of NR1-IgG bound NMDARs and associated post-synaptic proteins and synapses. They suggest that mechanistically bound NR1-IgG antibodies engage microglia via their Fc region through FcγR or complement binding, leading to specific removal by microglia. Excessive microglial pruning of synapses would align with other observations here such as reduced numbers of dendritic spines and also fit studies by **Klawonn et al. (2021)** that chemogenetic activation of microglia in the dorsal striatum induces local cytokine expression, and a prostaglandin-dependent reduction of excitability of striatal neurons.

Alongside synaptic pruning, microglia have also been shown to partake in a number of other functions, including inducing cell death in malfunctioning neurons (**Wake et al. 2009, Hornik et al. 2016**) and clearing the extracellular space by phagocytosing cellular debris (**Neumann et al. 2009, Herzog et al. 2019**). Such processes in excess due to increased activation could thus also underlie the decreases in striatal volume observed, as well as disruption to circuits and pathways.

Beyond responding to NMDARs bound by NR1-IgG antibodies on surrounding neurons, microglia themselves also express NMDARs on their surface (**Gottlieb and Matute 1997, Eriksson et al. 2000, Tahraoui et al. 2001, Tikka et al. 2001, Kaur et al. 2005, Nair et al. 2006, Newcombe et al. 2008, Liang et al. 2010, Murugan et al. 2011, Kaindl et al. 2012**), which are also likely to be bound and internalised upon NR1-IgG antibody exposure (**Moscato et al. 2014**), thus providing a second mechanism by which microglial function may be altered and having an impact on neuronal development. Removal of other microglial surface proteins such as BDNF has previously been shown to alter neuronal-microglial interactions and reduce neuronal expression of glutamate receptors including the NR2B subunit, and result in synaptic deficits including impaired NMDAR-mediated mEPSCs (**Parkhurst et al. 2013**), similar to what was found with sEPSCs in these results. Interestingly it has also been shown that the NR2A subunit specifically regulates certain neuronal-microglial interactions (**Eyo et al. 2018**) and therefore a possible delay in the NR2B-NR2A switch secondary to *in utero* NR1-IgG antibody exposure could constitute a third mechanism by which neuronal-microglial interactions are impaired. The specific mechanisms by which microglia and their interactions with developing striatal neurons are impacted by *in utero* NR1-IgG antibody exposure is an important topic for future work.

5.7 Chapter Summary

This chapter set out to investigate striatal cellular and circuit alterations following *in utero* exposure to NR1-IgG, building off embryonic impacts in the previous chapter. In this chapter, alterations in several intrinsic properties and morphological characteristics of early postnatal SPNs were identified, which showed non-uniform impairment in specific two-day time windows, the specific timepoint of which was variable (eg. most intrinsic property effects were most prominent at p7-8 whilst post-synaptic effects on sEPSC amplitudes, and alterations in NMDA/AMPA ratio were most prominent at p13-14). Impairments in early corticostriatal and other glutamatergic synapses were seen, with both pre- and post-synaptic NMDARs likely functionally impaired. A reduced NMDA/AMPA ratio was also observed, which correlated to several altered intrinsic and synaptic properties. Further, reduced striatal volume, trend towards decreased SPN cell density, and significantly shifted D1/D2 ratio towards fewer D2-SPNs were all identified. Lastly, the increased activated microglial density and altered microglial morphology observed embryonically was shown to persist into the fourth week postnatal brain. These findings overall suggest that the postnatal brain of NR1-IgG antibody *in utero* exposed animals is prolongedly altered well into the critical periods of striatal development.

Having established multiple cellular and circuit changes within the early postnatal striatum following *in utero* exposure to NR1-IgG, the final experimental chapter investigated whether there are impacts on behaviour of young and adolescent mice, and secondly whether some of the cellular or behavioural phenotype can be rescued through boosting AMPAR transmission via early postnatal administration of an AMPAkinase.

6. Experimental Chapter D: How does brief *in utero* exposure to patient-derived NR1-IgG impact postnatal behaviour and can the behavioural phenotype be rescued using an AMPAkinine?

6.1 Chapter Introduction

In **Experimental Chapters B and C**, it was demonstrated that direct *in utero* exposure to patient-derived NR1-IgG antibodies results in aberrant effects on the neurons and circuits of the developing striatum at both embryonic and postnatal stages. Building on this, **Experimental Chapter D** set out to firstly explore whether these cellular and circuit changes observed translated into possible behavioural changes. Secondly, it aimed to explore whether it was possible to rescue some of the behavioural phenotype through boosting activity at a different glutamate receptor, specifically the AMPAR, using early postnatal administration of an AMPAkinine.

6.2 Chapter Brief Background

6.2.1 NMDAR antibodies and behaviour

Patients with NMDARE are typically young women of child-bearing age who present with a multi-stage clinical syndrome, typically starting with initial psychiatric symptoms before later progressing onto motor (amongst other) symptoms (**Dalmau 2008, Irani 2010**), along with reversal to a developmentally ‘childlike’ state (**Quoted by S. Irani in Room 5. Series 1. 1. Bex, BBC Sounds Podcast**).

It has also been shown in multiple studies that autoimmune diseases are more prevalent in mothers of children with a range of neurodevelopmental and neuropsychiatric conditions, including ASD, learning disabilities and schizophrenia (**Singer et al. 2009, Braunschweig et al. 2012, Brimberg et al. 2016, Coutinho et al. 2017a, Jurek et al. 2019**). Clinical literature supports the specific link between NMDAR antibodies and potential neurodevelopmental behavioural phenotypes. **Coutinho et al. (2017a)** describes a relatively high incidence of NMDAR antibodies (5.8%) during pregnancy and found that NMDAR antibodies showed a non-significant trend towards being more frequent in mothers of children with neurodevelopmental disorders. **Jurek et al. (2019)** also found a marginal increase in NMDAR antibody titres in mothers of children with a range of neuropsychiatric disorders relative to mothers with healthy children. Mutations in genes encoding subunits of the NMDAR have also been found in children with a range of neurodevelopmental and epileptic disorders (**Burnashev and Szepietowski 2015**). Clinical case reports suggest that in pregnant NMDARE patients, foetal exposure to NMDAR antibodies may be associated with neurological deficits in some neonates (**Jagota et al. 2014, Hilderink et al. 2015, Shi et al. 2017, Chourasia et al. 2018, Kalam et al. 2019, Joubert et al. 2020, Scorrano et al. 2021**).

These neurodevelopmental disorders encompass a wide range of behaviours including impairments in motor learning, cognitive function, social interactions, and anxiety behaviour (**ICD-11, WHO**). Further, studies in gestational transfer models of NR1-IgG and other antibody exposure also report congruent

behavioural deficits in offspring in the early postnatal period that can also persist into adulthood. **Jurek et al. (2019)** found *in utero* exposure to NR1-IgG resulted in impaired neurodevelopmental reflexes, hyperactivity, reduced anxiety and altered sensorimotor gating in adolescence and adulthood, whilst **García-Serra et al. (2020)** found delayed innate reflexes, poor motor coordination, and deficits in memory and nest-building. More widely, **Singer et al. (2009)** found that *in utero* injection of IgG from mothers of children with autism resulted in increased activity in the open-field test in adolescence, as well as increased anxiety and alterations in sociability during adulthood, and **Coutinho et al. (2017b)** identified that *in utero* exposure to CASPR2 antibodies resulted in altered social interactions in adulthood.

Although the above studies have largely investigated these behaviours in the context of the cortex, hippocampus, or brain more widely, many of the reported behavioural deficits also have a likely link to striatal and basal ganglia circuits, which are integral for many of these complex behaviours reportedly affected (reviewed in **Graybiel and Grafton 2015, Lago et al. 2017**).

6.2.2 Behavioural testing in antibody models

As seen in the studies mentioned above, various behavioural tests can be used to capture potential disease pathology in antibody models. Early neonatal reflex testing during the first two postnatal weeks is useful, since more complex testing is not generally possible during this period, and it provides a reflection of the maturational state of the developing nervous system. Various reflexes can be assessed but three of the most common and relevant to our study here are surface righting, negative geotaxis, and cliff avoidance, which primarily capture coordinated motor movements, vestibular reflexes, and strength, and require integrity of the basal ganglia and striatum for full expression (**Fox 1965, Arakawa and Erzurumlu 2015**).

To assess later behavioural changes, the open-field is a good assessment as it captures a wide range of potential alterations, including anxiolytic behaviours and motor deficits (**Kraeuter et al. 2019**). The

accelerating rotarod is also a good test for assessing more specific motor coordination and balance as well as anxiety (**Deacon 2013**). These tests are particularly useful in the context of our study as the striatum and basal ganglia both have pivotal functions in motor- and anxiety-related behaviour (**Yin et al. 2009, Thorn et al. 2010, Graybiel and Grafton 2015, Lago et al. 2017**).

6.2.3 Rescuing deficits following early NMDAR disruption

Within the literature, there are various studies exploring potential ways of rescuing deficits brought about by impairments to NMDARs. **Bostrom et al. (2015)** showed that impairments in NMDAR-dependent synaptic plasticity in a mouse model of the neurodevelopmental disorder Fragile X syndrome, could be rescued by treatment with NMDAR co-agonists glycine or D-serine. **Planagumà et al. (2016)** also used administration of EphB2 (a ligand shown to be disrupted by NR1-IgG antibodies, de-stabilising the NMDAR receptor (**Mikasová et al. 2012**)) in mice infused with CSF from NMDARE patients and found that it prevented the pathogenic effects of the antibodies in the behavioural paradigms they assessed, including memory and depressive-behaviour. More recently, **Mielnik et al. (2021)** used postnatal tamoxifen-inducible Cre recombinase injections to rescue the consequences of early NMDAR deficiency (brought about by a loss-of-function allele of *GRIN1*) in adulthood, through restoring function to the NMDAR, and **García-Serra et al. (2021)** also demonstrated that blockade of the neonatal FcRn receptor 6 hours before the injection of NR1-IgG prevented the placental transfer of NR1-IgG and subsequent pathogenic effects in the offspring.

There is also exciting novel work from Huntington's disease models. **Schellino et al. (2023)** recently used embryonic stem cell-derived human progenitor grafts to replace degenerated striatal neurons in circuits in a rat model of Huntington's disease, showing that grafts can wire into both local and long-range striatal circuits, and improve elements of motor function. Additionally, recent work by **Braz et al. (2022)** demonstrated rescue of the Huntington's disease phenotype and disrupted glutamatergic signalling by

boosting transmission via the main other ionotropic glutamate receptor AMPAR with AMPAkinine CX516. Huntington's involves a large component of striatal specific pathology, and there is evidence to suggest it encompasses alterations in NMDARs and NMDAR-mediated transmission, including early loss of SPNs with high levels of NMDARs, and altered function of NMDARs at corticostriatal synapses (reviewed in **Fan and Raymond 2007**). Therefore, such rescue might also be relevant in the case of dysfunction caused by NR1-IgG antibodies.

6.3 Chapter Aims

The aim of **Experimental Chapter D** was to explore if the earlier embryonic and postnatal morphological, cellular and circuit changes following *in utero* NR1-IgG exposure seen in **Experimental Chapters B and C** were reflected in changes in early postnatal behaviour, and whether aspects of an altered behavioural phenotype could be rescued by boosting glutamatergic transmission through AMPARs. To our knowledge, this was the first time this approach had been used as a potential rescue in an antibody model.

This chapter aimed to specifically address the following:

1. How does *in utero* exposure to NR1-IgG antibodies affect development of early postnatal reflexes?
2. How does this antibody exposure affect motor and other behaviours in the late adolescent period?
3. Can boosting AMPAR transmission during the first postnatal week using AMPAkin CX516 rescue aspects of the behavioural phenotype observed?

6.4 Chapter Methods

Methods used in this Chapter are described in detail in **General Methods** and are briefly summarised below (in the approximate order in which they appear):

6.4.1 *In utero* surgical methods

(See **General Methods Section 2.5 *In utero* surgical methods**)

The experiments in this chapter used the same *in utero* antibody model as **Experimental Chapter C**, whereby patient-derived NR1-IgG or matched control-IgG antibodies were injected intraventricularly at E14.5 and animals then used for postnatal experiments.

For postnatal experiments involving *in utero* injection of antibodies at E14.5, and use of animals postnatally, the overall survival rates at birth were: NR1-IgG: 63.9%, control-IgG: 75.8%.

6.4.2 Behavioural methods

(See **General Methods Section 2.9 Behavioural methods**)

6.4.2.1 Early reflex behaviour

Behavioural impact of NR1-IgG *in utero* injections was evaluated through three developmental reflex tests performed daily during the second postnatal week: surface righting, negative geotaxis and cliff aversion. This testing window was chosen since in our set up, earlier disturbance of the nest (i.e. first postnatal week) for behavioural testing was linked to poor survival rates amongst the litters. At p6, pups were marked with a distinct tail pattern using a permanent marker and reflex testing was then done daily from p7-14 at a similar time each day. Pups were weighed primarily as a control measure of maternal care and transferred to a clean cage. There was no significant difference in mean weight between groups (evaluated here between p7-p10, to allow comparison to rescue groups) (control-IgG: $5.0 \pm 1.5\text{g}$, NR1-IgG: $4.6 \pm 0.8\text{g}$; MWU: $p = 0.13$).

They first underwent the **surface righting reflex test** whereby each pup was placed on its back and the time taken for it to flip back over onto its front recorded. They next underwent the **cliff avoidance reflex** test whereby each pup was placed at the edge of a 'cliff' (30cm high polystyrene box) with paws and snout over the edge, and the time taken for it to withdraw its paws from the edge evaluated. Lastly, they underwent the **negative geotaxis reflex**, whereby each pup was placed on a 45° polystyrene slope facing downwards, and the time to turn 180° to face up the slope recorded.

Between tests, the pup was returned to the clean cage for at least 3 minutes. Pups were returned to their home cage and reunited with their mother post-testing. There were two trials for each test per day, with a 1-minute break in between. Each test was conducted daily until it could be performed successfully for two consecutive days. The cut off period for each test was 30 seconds.

6.4.2.2 Open-field and accelerating rotarod

To assess whether the behavioural impact extended into early adolescence, two tests, the open-field test and the accelerating rotarod test, were also performed on the NR1-IgG and control-IgG mice at p21-23.

To perform the **open-field test**, each mouse was placed in the corner of a brightly illuminated 60 x 60cm clear square Perspex box. Movement around the box was then recorded for 11 minutes (1 minute of acclimatisation and then a 10-minute test period) using AnyMaze Ethovision software. The box was had three key zones, 1. centre, 2. periphery, and 3. corners (**General Methods, Figure 2.18 A**). The parameters recorded included total distance and mean velocity during the trial period, as well as frequency, duration, latency to enter, distance moved, and mean velocity within each of the three key zones.

To perform the **accelerating rotarod test**, the mouse was placed on the rotating rod, facing away from the direction of rotation. Each mouse first individually underwent two acclimatisation periods, where it was placed on the rod rotating at constant slow starting speed of 4 RPM for 2 minutes. Mice then underwent four trials, whereby they were placed on the rod at a steady initial speed of 4 RPM, which

accelerated uniformly up to 40 RPM over a 5-minute period, and the time on rod measured. If a mouse fell or jumped off the rod within the first 15 seconds, it was given one further trial. There was a least 1-minute rest period between trials, where mice were returned to their home cage. The rotarod can hold up to 5 mice at once and mice in the same group were assessed simultaneously wherever possible, with each mouse keeping the same testing slot.

Open-field and accelerating rotarod tests were performed with the kind help of Elke Calus (Department of Biomedical Sciences, Antwerp).

6.4.3 Rescue methods

(See **General Methods Section 2.10 Rescue model generation**)

To attempt to rescue alterations seen in our NR1-IgG animals, a model was generated whereby endogenous glutamatergic transmission was enhanced in our NR1-IgG and control-IgG animals using the positive AMPA allosteric modulator AMPAkinone CX516. Following *in utero* injection of NR1-IgG or control-IgG at E14.5, pups were born and then injected twice a day intraperitoneally with 2.5mg/kg of CX516 between p0-7. They subsequently underwent behavioural testing as described in the section above. Effects were evaluated by comparing the change between the rescue NR1-IgG and non-rescue NR1-IgG populations, as well as between the rescue NR1-IgG population and the non-rescue and rescue control-IgG populations.

As with non-rescue pups, rescue pups from both NR1-IgG and control-IgG groups were weighed during reflex experiments (evaluated from p7-p10), and there was no significant difference between either rescue groups (rescue control-IgG: $5.0 \pm 0.6g$, rescue NR1-IgG: 4.1 ± 0.8 ; MWU: $p = 0.10$) or between the rescue groups and their non-rescue counterparts (non-rescue control-IgG vs rescue control-IgG: MWU: $p = 0.50$; non-rescue NR1-IgG vs rescue NR1-IgG: MWU: $p = 0.054$).

Injections of CX516 and early reflex testing in rescue animals were performed with the kind help of Prof. Dr Tommas Ellender and Yana Van de Poll (both Department of Biomedical Sciences, Antwerp), and open-field and rotarod experiments in rescue animals were performed with the kind help of Elke Calus (Department of Biomedical Sciences, Antwerp).

6.4.4 Statistical methods

(See **General Methods Section 2.11 Statistical methods**)

Details of specific statistical tests used for each experiment are given in the respective results sections within the chapter. All data was initially tested for normality using the Shapiro-Wilks test, and the appropriate two-tailed parametric (repeated measures (RM) ANOVA, one-sample t-test, unpaired t-test) or non-parametric (Wilcoxon signed-rank (WSR), Mann-Whitney U (MWU), Fisher's exact test) test applied. For RM ANOVA statistics, non-significant Day or Day x Group F and p values are typically omitted from the main text and only the Group F and p values are reported, to assist with ease of reading (but all Group, Day and Group x Day data is available in **Appendix, Supplementary Tables S6.1, S6.4**). Data was corrected where appropriate using Bonferroni or Sidak *post hoc* corrections, to compensate for multiple comparisons. For all statistical tests, $p < 0.05$ was deemed significant. * $p < 0.05$, ** $p < 0.01$.

6.5 Chapter Results

6.5.1 Postnatal impact of NR1-IgG antibodies on early reflex behaviour

The literature suggests that *in utero* NR1-IgG exposure may also be reflected in the early behavioural phenotype (Jurek et al. 2019). The first experiments in this chapter thus set out to explore whether the early cellular and circuit changes observed in the previous **Experimental Chapters B and C** were reflected in early postnatal reflexes (**Figure 6.1 A, B**). Since the dorsal striatum has been shown to be critical in motor learning and habitual behaviour (Graybiel and Grafton 2015), we chose to evaluate the development of three early motor-related reflexes in the p7-14-time window that require intact striatal and basal ganglia circuitry for full expression (Fox 1965, Arakawa and Erzurumlu 2015).

Daily evaluation of surface righting, negative geotaxis and cliff avoidance in our NR1-IgG and control-IgG *in utero* exposed animals over p7-14 revealed that all three reflexes emerged much later in the NR1-IgG animals. For this dataset, n(NR1-IgG) = 19 pups, 3 litters and n(control-IgG) = 19 pups, 3 litters. Surface righting and negative geotaxis showed a similar delay of ~1 day (surface righting: control-IgG: 7.5 ± 0.2 days, NR1-IgG: 8.3 ± 0.2 days; MWU: $p = 0.018$; negative geotaxis: control-IgG: 7.7 ± 0.3 days, NR1-IgG: 8.6 ± 0.3 days, MWU: $p = 0.025$, **Figure 6.1 Di, Ei**) and similar daily progression in terms of the percentage of the population with the reflex present (data in **Appendix, Supplementary Table S6.2**: surface righting: Fisher's exact tests: p7: $p = 0.022$, p8: $p = 0.27$, p9: $p = 0.23$, p10+: $p = 1.00$; negative geotaxis: Fisher's exact tests: p7: $p = 0.049$, p8: $p = 0.017$, p9: $p = 0.45$, p10+: $p = 1.00$; **Figure 6.1 Dii, Eii**). Cliff avoidance showed a much greater delay (control-IgG: 7.8 ± 0.3 days, NR1-IgG: 10.2 ± 0.6 days; **Figure 6.1 Fi**), with differences in % population exhibiting the reflex still significant on p12 (data in **Appendix, Supplementary Table S6.2**: Fisher's exact tests: p7: $p = 0.19$, p8: $p = 0.049$, p9: $p = 0.0050$, p10: $p = 0.013$, p11: $p = 5.9 \times 10^{-12}$, p12: $p = 7.1 \times 10^{-12}$, p13+: $p = 1.00$; **Figure 6.1 Fii**).

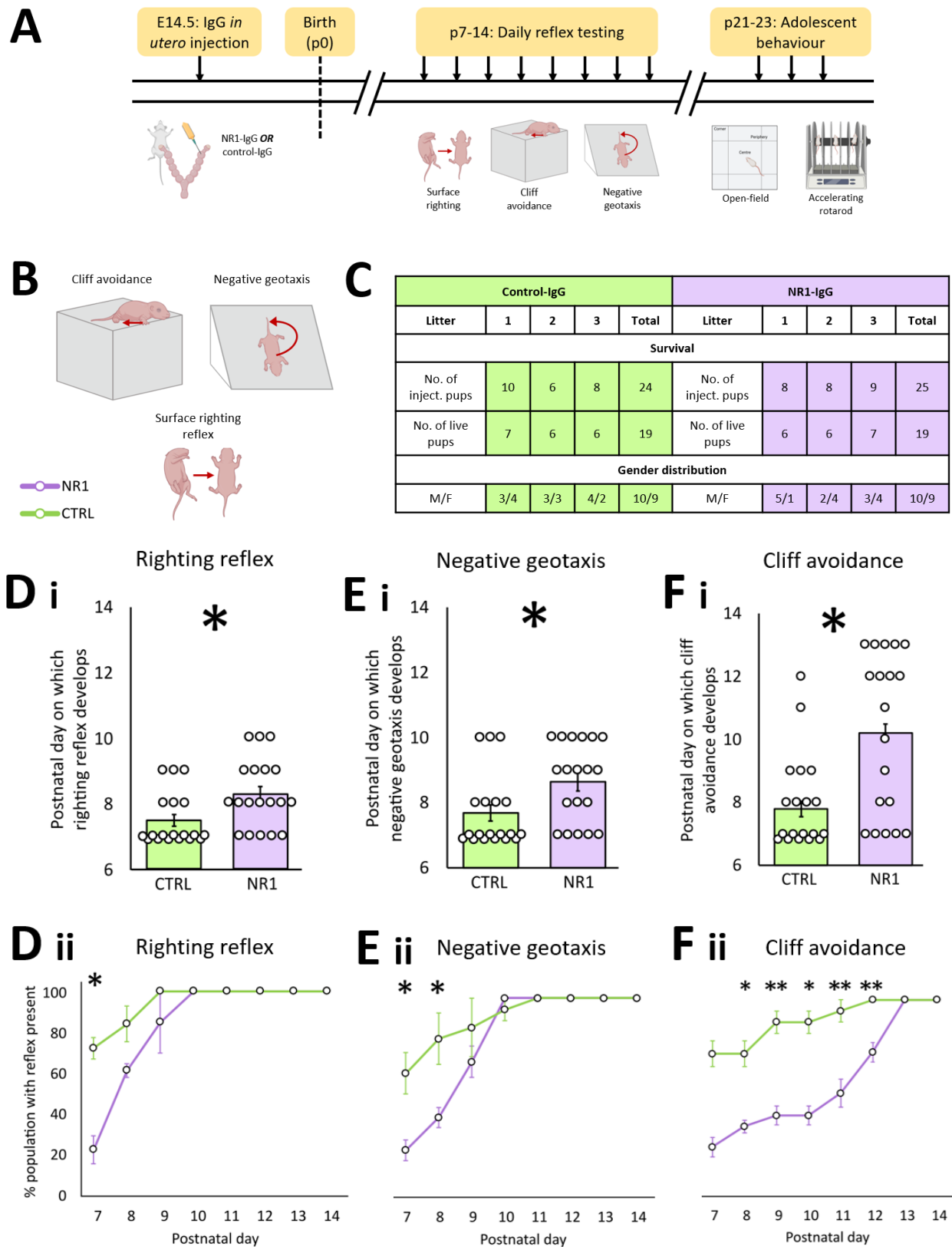


Figure 6.1. Altered early postnatal reflexes following in utero NR1-IgG exposure. (Description on next page).

Figure 6.1. Altered early postnatal reflexes following in utero NR1-IgG exposure (cont.). **[A]** Overview of the behavioural testing protocol for both early postnatal reflexes (p7-14) and early adolescent behaviours (p21-23). Animals were injected at E14.5 with NR1-IgG or control-IgG and then underwent early reflex testing between p7-14, and open-field and accelerating rotarod testing between p21-23. **[B]** Diagrams of the three postnatal reflexes tested: surface righting, cliff avoidance and negative geotaxis. **[C]** Table of key parameters of litters used for early reflex behaviour testing. **[Di-Fi]** Bar plots of the postnatal day on which **[Di]** surface righting reflex, **[Ei]** negative geotaxis, and **[Fi]** cliff avoidance respectively developed in control-IgG and NR1-IgG in utero exposed mice, showing all three emerged significant later in the NR1-IgG population. **[Dii-Fii]** Line graphs showing the % population that had the **[Dii]** surface righting, **[Eii]** negative geotaxis and **[Fii]** cliff avoidance reflexes respectively on each testing day, showing a significantly smaller percentage of the NR1-IgG exposed population has righting reflex on p7, negative geotaxis on p7 and p8, and cliff avoidance on p8-p12.

Overall, this data suggests mice exposed *in utero* to NR1-IgG have delays in the development of several early motor-related reflexes including surface righting, negative geotaxis and cliff avoidance.

6.5.2 Postnatal impact of NR1-IgG antibodies on early adolescent open-field behaviour

The literature also suggests that NR1-IgG may impact on a range of later postnatal behaviours (Jurek et al. 2019, García-Serra et al. 2020). Having observed a significant delay in early developmental motor-related reflexes, the next experiments aimed to explore whether behavioural impairments extended into the early adolescent period. To do this, NR1-IgG and control-IgG mice underwent both open-field and accelerating rotarod testing daily at the start of the fourth postnatal week (p21-23). Given the literature suggests a wide range of different behavioural alterations following *in utero* exposure to NR1-IgG (and other antibodies) (Singer et al. 2009, Coutinho et al. 2017b, Jurek et al. 2019, García-Serra et al. 2020), the open-field test (**Figure 6.2 A**) was selected as a first pass test to capture an array of behavioural elements including general motor- and anxiety-related behaviours (Deacon 2013). For both the open-field and later accelerating rotarod experiments, n(control-IgG) = 10 animals, 4 litters and n(NR1-IgG) = 9 animals, 3 litters.

Overall open-field behaviour: Looking at behaviour overall in the open-field, there was no significant difference in total distance covered over the 10-minute trial window between NR1-IgG and control-IgG animals, when averaged across the three daily trials (control-IgG: $2650.4 \pm 287.3\text{cm}$, NR1-IgG: $2676.4 \pm 142.7\text{cm}$, RM ANOVA: Group: $F_{(1,13)} = 0.0080$, $p = 0.93$). Whilst there was a significant difference in the distance covered by day, there was no interaction between group and day (RM ANOVA: Day: $F_{(1.40, 18.22)} = 16.91$, $p = 0.00023$; Group x Day: $F_{(2,26)} = 0.12$, $p = 0.89$; **Figure 6.2 B; Appendix, Supplementary Table S6.1**). Similarly, evaluating mean velocity over the three trials, there was no significant difference between groups (control-IgG: $4.4 \pm 0.5\text{cm/s}$, NR1-IgG: $4.5 \pm 0.2\text{ cm/s}$, RM ANOVA: Group: $F_{(1,13)} = 0.0050$, $p = 0.94$) but a significant difference in distance covered each day (RM ANOVA: Day: $F_{(1.40, 18.24)} = 17.18$, $p = 0.00021$) with no interaction between group and day (RM ANOVA: Group x Day: $F_{(1.40, 18.24)} = 0.13$, $p = 0.80$; **Figure 6.2 C; Appendix, Supplementary Table S6.1**). Overall, the gross parameters total distance and velocity showed no significant difference between NR1-IgG and control-IgG animals.

Specific open-field regions: However, looking at behaviour related to specific regions of the open-field, there was a significant difference in many of the centre-related parameters, including a significant increase in normalised distance moved in the centre (control-IgG: $4.5 \pm 0.7\%$, NR1-IgG: $6.5 \pm 0.4\%$, RM ANOVA: Group: $F_{(1,13)} = 6.98$, $p = 0.020$) and number of visits to the centre (control-IgG: 6.9 ± 0.9 visits, NR1-IgG: 12.6 ± 1.6 visits: RM ANOVA: Group: $F_{(1,12)} = 6.22$, $p = 0.028$) in NR1-IgG mice (**Figure 6.2 D, E; Appendix, Supplementary Table S6.1**). There was also a trend towards a lower velocity in the centre in NR1-IgG mice (control-IgG: $11.3 \pm 1.9\text{cm/s}$, NR1-IgG: $8.1 \pm 0.8\text{cm/s}$, RM ANOVA: Group: $F_{(1,13)} = 3.16$, $p = 0.10$; **Figure 6.2 F, Appendix, Supplementary Table S6.1**). These three parameters showed no significant effects of Day and no Group x Day interactions (statistics in **Appendix, Supplementary Table S6.1**).

Looking at corner-related parameters, there was a trend towards a significant decrease in normalised duration the NR1-IgG animals spent in the corners (control-IgG: $38.4 \pm 5.9\%$, NR1-IgG: $28.7 \pm 3.1\%$, RM

ANOVA: Group: $F_{(1,13)} = 2.52$, $p = 0.14$), with a trend towards a significant interaction between group and day (Day: $F_{(2,26)} = 0.81$, $p = 0.46$, Group x Day: $F_{(2,26)} = 2.12$, $p = 0.14$; **Figure 6.2 G; Appendix, Supplementary Table S6.1**). Exploring this further through looking at data from each individual trial day revealed NR1-IgG exposed mice spent a significantly less relative amount of time in the corners on the first day relative to control-IgG exposed mice (control-IgG: $40.0 \pm 4.3\%$, NR1-IgG: $21.3 \pm 1.6\%$, independent t-test: $p = 0.00041$), which normalised to the control over the next two days (Day 2: control-IgG: $38.6 \pm 8.3\%$, NR1-IgG: $30.1 \pm 4.8\%$, independent t-test: $p = 0.36$; Day 3: control-IgG: $36.7 \pm 7.5\%$, NR1-IgG: $34.7 \pm 5.5\%$, independent t-test: $p = 0.83$; **Figure 6.2 H; Appendix, Supplementary Table S6.1**).

Overall, these results suggest that NR1-IgG mice spent more time in the centre of the open-field, possibly as a reflection of reduced anxiety.

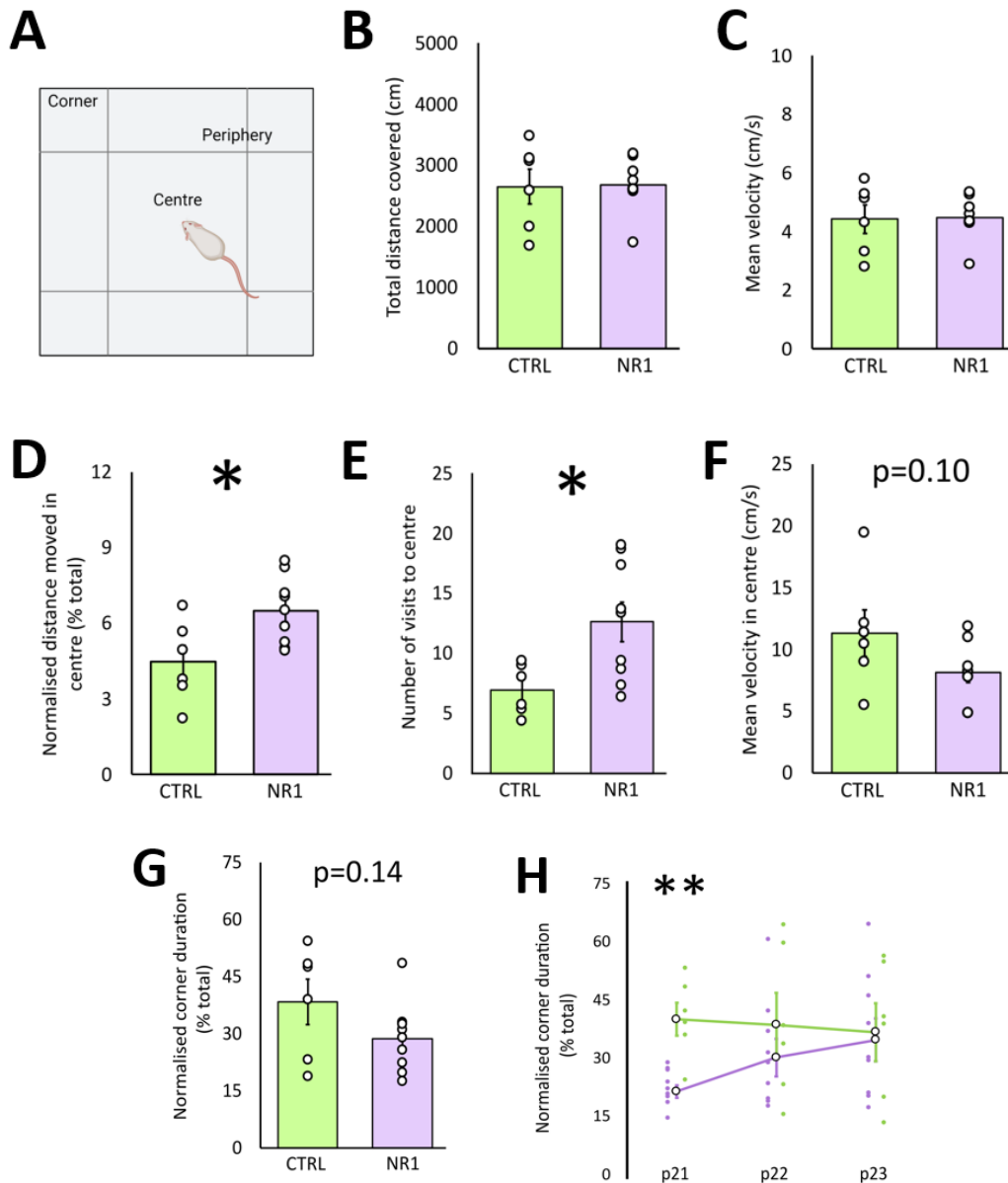


Figure 6.2. Altered anxiety-related open-field behaviours following in utero NR1-IgG exposure. [A] Diagram of the open-field test, showing the three key regions (centre, periphery, corners). [B-C] Bar plots of [B] total distance covered and [C] mean velocity of the animals respectively during the 10-minute testing window, averaged over the three test days. There was no significant difference in either distance covered or mean velocity. [D-E] Conversely, analysis of parameters relating to exploration of the centre region of the open-field showed that [D] normalised distance covered in the centre and [E] number of visits to the centre were both significantly higher in NR1-IgG exposed mice. [F] There was also a trend to a decrease in the velocity of NR1-IgG exposed mice in the centre of the field. [G] Additionally, there was a trend towards NR1-IgG exposed mice spending less time in the corners of the open-field compared to controls when averaged over the three-day period. [H] Analysing this data separately on each of the three days showed that NR1-IgG mice spent significantly less time in the corners on the first testing day, but this normalised to results seen in control-IgG mice over the next two testing days.

6.5.3 Postnatal impact of NR1-IgG antibodies on early adolescent accelerating rotarod behaviour

The accelerating rotarod was also performed as a specific test for balance and motor coordination (**Figure 6.3 A**). Evaluating the mean time spent on the rod across the three trial days revealed an unexpected significant increase in the mean time NR1-IgG exposed animals spent on the rotarod relative to control-IgG animals (control-IgG: $93.3 \pm 10.3s$, NR1-IgG: $145.1 \pm 14.2s$, RM ANOVA: Group: $F_{(1,13)} = 7.17$, $p = 0.019$, **Figure 6.3 B; Appendix, Supplementary Table S6.1**). Comparing days individually revealed a trend towards a significant difference across all three trial days (Day 1: RM ANOVA: Group: $F_{(1,10)} = 3.15$, $p = 0.10$; Day 2: RM ANOVA: Group: $F_{(1,10)} = 2.63$, $p = 0.14$; Day 3: RM ANOVA: Group: $F_{(1,10)} = 3.51$, $p = 0.090$; **Figure 6.3 C**, data in legend).

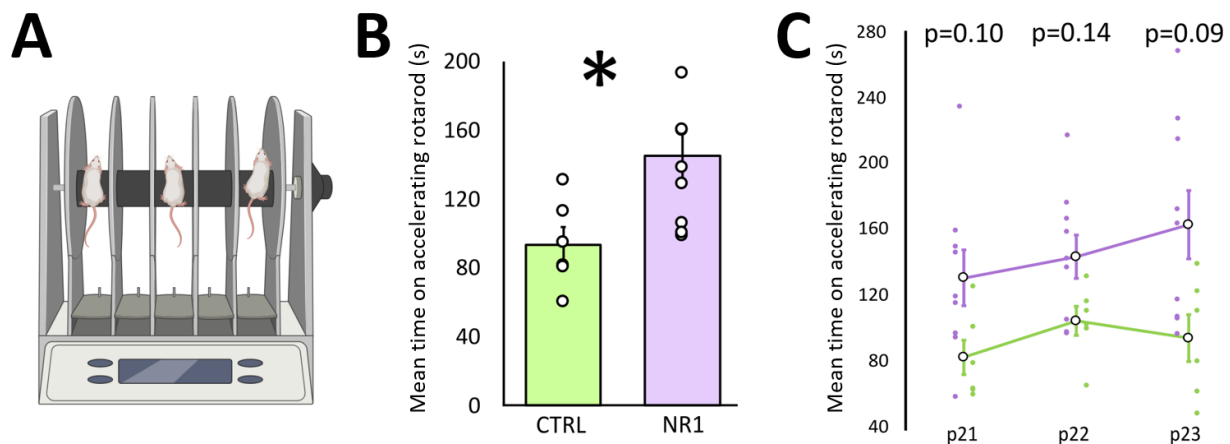


Figure 6.3. Increased time on accelerating rotarod following in utero NR1-IgG exposure. **[A]** Diagram of the accelerating rotarod set up. **[B]** Bar plot of the mean time animals spent on the rotarod, averaged over the three days, showing an unexpected significant increase in the time spent on the rod in NR1-IgG in utero exposed animals. **[C]** Analysing data separately for each day revealed a consistent trend difference and somewhat diverging trend between the NR1-IgG and control-IgG exposed animals. Data: Day 1: control-IgG: $82.2 \pm 10.4s$, NR1-IgG: $130.1 \pm 16.8s$; Day 2: control-IgG: $104.0 \pm 8.8s$, NR1-IgG: $142.9 \pm 13.2s$; Day 3: control-IgG: $93.7 \pm 14.2s$, NR1-IgG: $162.3 \pm 20.7s$.

Overall, these results suggest that there are impacts predominantly in anxiety-related behaviours in the open-field test in NR1-IgG *in utero* exposed animals when tested in the fourth postnatal week, such as more visits to the centre of the field concomitant with a trend towards a reduced proportion of time spent

in the corners, relative to control-IgG animals. There is little evidence to suggest motor impairment from the open-field data, with distance covered and mean velocity very similar for both groups. On the accelerating rotarod test, NR1-IgG exposed mice consistently remained on the rod for significantly longer than control-IgG exposed mice, an unexpected and interesting finding.

6.5.4 Effects of boosting glutamatergic transmission with AMPAkinine CX516 on behaviour in

NR1-IgG *in utero* exposed mice

In this chapter so far, a number of behavioural alterations have been observed in our NR1-IgG exposed animals, including a delay in the development of several motor-related reflexes, and differences in anxiety-related open-field behaviours and time spent on the accelerating rotarod in the early adolescent period. Experiments in the previous chapter showed that glutamatergic transmission and intrinsic excitability were impaired in the NR1-IgG exposed mice which likely underlies some of the behavioural changes seen. Since literature suggests that NR1-IgG binds the NR1 subunit, resulting in cross-linking and removal of NMDARs from the cell surface (Hughes et al. 2010, Mikasová et al. 2012, Moscato et al. 2014), the next line of thought was whether the impaired glutamatergic transmission could be alleviated instead through boosting AMPAR-mediated transmission instead.

Here the positive AMPA allosteric modulator AMPAkinine CX516 was chosen for this purpose, as recent work by Braz et al. (2022) successfully used this approach and demonstrated rescue of specific Huntington's disease phenotypes which are suggested to result from a deficit in glutamatergic signalling. Huntington's disease is a complex disorder and consists in part of a significant striatal pathology with loss of D2-SPNs. There is also evidence to suggest alterations in NMDARs and NMDAR-mediated transmission including early loss of SPNs with high levels of NMDARs and altered function of NMDARs at corticostriatal synapses in Huntington's disease models (reviewed in Fan and Raymond 2007).

To perform these rescue experiments, the same protocol as **Braz et al. (2022)** was utilised with twice-daily IP injections of CX516 (2.5mg/kg) in the NR1-IgG and control-IgG *in utero* exposed animals from p0-7, before evaluation of early reflex behaviours and open-field/rotarod behaviours using the same procedures as for the non-rescue animals (**Figures 6.4 A, B**) For these experiments, n(NR1-IgG) = 4 pups, 2 litters and n(control-IgG) = 6 pups, 1 litter.

6.5.4.1 Effects on early reflex behaviour

As a baseline control, the impact of the rescue on control-IgG exposed animals was first compared to the non-rescue control-IgG animals (i.e. those also injected with control-IgG but not given the rescue injections). This revealed no significant differences for any of the reflexes, showing the rescue had no significant impact on control-IgG animals in terms of postnatal day the reflexes developed on (surface righting: non-rescue control-IgG: 7.5 ± 0.2 days, rescue control-IgG: 7.0 ± 0.0 days, MWU: $p = 0.27$; negative geotaxis: non-rescue control-IgG: 7.7 ± 0.3 days, rescue control-IgG: 7.3 ± 0.3 days, MWU: $p = 0.51$; cliff avoidance: non-rescue control-IgG: 7.8 ± 0.3 days, rescue control-IgG: 7.2 ± 0.2 days, MWU: $p = 0.22$) or in terms of % population with the reflex on any one day (data in **Appendix, Supplementary Tables S6.2, S6.3**, Fisher's exact tests: surface righting: p7: $p = 0.16$, p8: $p = 0.26$, p9+: $p = 1.00$; negative geotaxis: p7: $p = 0.58$, p8: $p = 0.40$, p9: $p = 0.26$, p10+: $p = 1.00$; cliff avoidance: p7: $p = 0.34$, p8: $p = 0.34$, p9+: $p = 1.00$).

Moving on to then look at NR1-IgG animals with and without the CX516 rescue:

Surface righting reflex: Comparison of surface righting reflex revealed a significant increase in the % population with the reflex present on p7, p8 and p9 (data in **Appendix, Supplementary Tables S6.2 A, S6.3 A**, Fisher's exact tests: p7: $p = 0.0020$, p8: $p = 0.0080$, p9: $p = 0.022$, p10+: $p = 1.00$; **Figure 6.4 Cii**), and a trend towards a significantly earlier postnatal day on which the reflex developed in rescue NR1-IgG

exposed animals relative to non-rescue NR1-IgG exposed animals (non-rescue NR1-IgG: 8.3 ± 0.2 days, rescue NR1-IgG: 7.3 ± 0.3 days, MWU: $p = 0.081$; **Figure 6.4 Ci**). NR1-IgG rescue animals also showed no significant differences compared to the non-rescue control-IgG or rescue control-IgG animals for either day on which the reflex developed (non-rescue control-IgG vs rescue NR1-IgG: MWU: $p = 0.79$, rescue control-IgG vs rescue NR1-IgG: MWU: $p = 0.61$; **Figure 6.4 Ci**) or % population with the reflex present on any day except for p7 (data in **Appendix, Supplementary Tables S6.2 A, S6.3 A**, Fisher's exact tests: non-rescue control-IgG vs rescue NR1-IgG: p7+: $p = 1.00$, rescue control-IgG vs rescue NR1-IgG: p7: $p = 0.0016$, p8+: $p = 1.00$; **Figure 6.4 Cii**).

Negative geotaxis reflex: The negative geotaxis reflex showed a similar picture, with a significant increase in % population with the reflex present on p7, p8 and p9 (data in **Appendix, Supplementary Tables S6.2 B, S6.3 B**, Fisher's exact tests: p7: $p = 0.01$, p8: $p = 0.00062$, p9: $p = 0.016$, p10+: $p = 1.00$; **Figure 6.4 Dii**), and a trend towards a significantly earlier day on which the reflex developed in rescue vs non-rescue NR1-IgG animals (non-rescue NR1-IgG: 8.6 ± 0.3 days, rescue NR1-IgG: 7.3 ± 0.3 days, MWU: $p = 0.054$; **Figure 6.4 Di**), but no significant differences between rescue NR1-IgG animals and either rescue or non-rescue control-IgG animals for either postnatal day on which the reflex developed (non-rescue control-IgG vs rescue NR1-IgG: MWU: $p = 0.67$, rescue control-IgG vs rescue NR1-IgG: MWU: $p = 0.91$; **Figure 6.4 Di**) or % population with the reflex present after p8 (data in **Appendix, Supplementary Tables S6.2 B, S6.3 B**, Fisher's exact tests: non-rescue control-IgG vs rescue NR1-IgG: p7+: $p = 1.00$, rescue control-IgG vs rescue NR1-IgG: p7: $p = 0.004$, p8: $p = 0.004$, p9+: $p = 1.00$; **Figure 6.4 Dii**).

Cliff avoidance reflex: The cliff avoidance reflex showed the greatest difference between the rescue NR1-IgG animals and non-rescue NR1-IgG animals, with a decrease in the mean postnatal day on which the reflex developed by over 3 days (non-rescue NR1-IgG: 10.2 ± 0.6 days, rescue NR1-IgG: 7.5 ± 0.3 days, MWU: $p = 0.097$; **Figure 6.4 Ei**). There was a significantly greater % population with the reflex on p7, p8,

p9 and p10, with 100% of the rescue population showing the reflex by p8 (data in **Appendix, Supplementary Tables S6.2 C, S6.3 C**, Fisher's exact tests: p7: $p = 0.014$, p8: $p = 0.00025$, p9: $p = 0.00062$, p10: $p = 7.0 \times 10^{-5}$; **Figure 6.4 Eii**). Further, there was no significant differences between rescue NR1-IgG animals and either rescue control-IgG or non-rescue control-IgG animals in terms of the mean postnatal day on which the reflex developed (non-rescue control-IgG vs rescue NR1-IgG: MWU: $p = 0.79$, rescue control-IgG vs rescue NR1-IgG: MWU: $p = 0.48$; **Figure 6.4 Ei**) or differences in % population with the reflex present on any day after p7 (data in **Appendix, Supplementary Tables S6.2 C, S6.3 C**, Fisher's exact tests: non-rescue control-IgG vs rescue NR1-IgG: p7: $p = 1.00$, p8: $p = 0.69$, p9+: $p = 1.00$, rescue control-IgG vs rescue NR1-IgG: p7: $p = 0.0020$, p8+: $p = 1.00$; **Figure 6.4 Eii**).

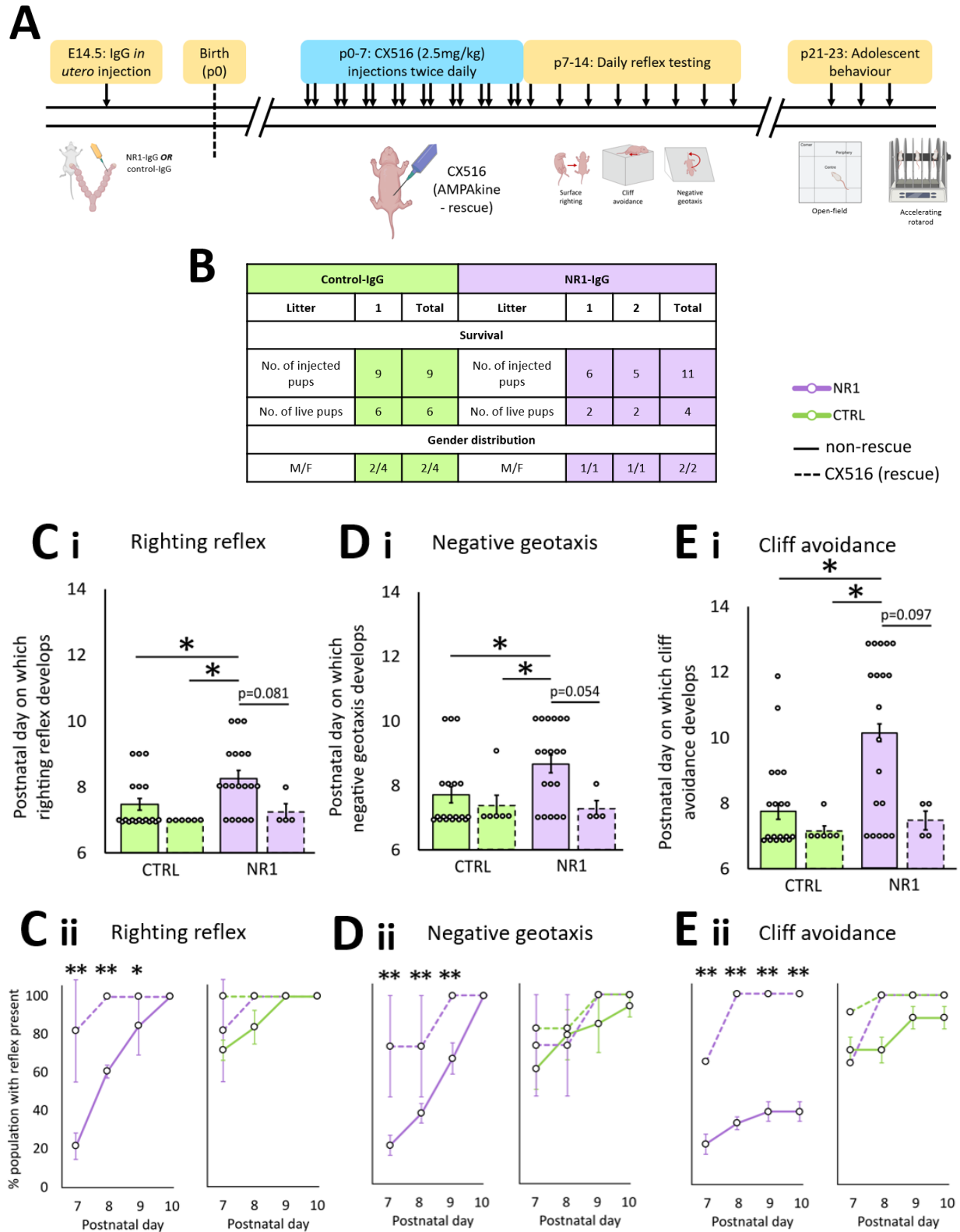


Figure 6.4. Rescue of early postnatal reflex delays in NR1-IgG in utero exposed animals using AMPAkinase CX516. (Description on next page).

Figure 6.4. Rescue of early postnatal reflex delays in NR1-IgG in utero exposed animals using AMPAkinine CX516 (cont.). **[A]** Diagram of the rescue protocol for in utero exposed NR1-IgG and control-IgG animals. Animals were injected with NR1-IgG or control-IgG at E14.5 and then given twice daily IP injections of AMPAkinine CX516 between p0-7. Early postnatal reflexes and open-field and accelerating rotarod behaviour were evaluated at p7-14 and p21-23 respectively, in the same manner as for the non-rescue animals. **[B]** Table of key parameters for the litters used in the rescue model. **[Ci-Ei]** Bar plots of the mean postnatal day on which the [Ci] surface righting, [Di] negative geotaxis and [Ei] cliff avoidance reflexes respectively developed for non-rescue control-IgG and NR1-IgG mice, and also for rescue control-IgG and NR1-IgG mice, showing that CX516 injections normalised the delays seen in non-rescue NR1-IgG animals back towards the results seen in control-IgG animals. **[Cii-Eii]** Graphs of % population with [Cii] surface righting, [Dii] negative geotaxis and [Eii] cliff avoidance reflexes respectively over p7-10, for non-rescue NR1-IgG and rescue NR1-IgG animals, as well as for non-rescue control-IgG animals, rescue control-IgG animals and rescue NR1-IgG animals, showing that NR1-IgG significantly increased % population of NR1-IgG animals with these reflexes on p7, p8 and p9 for all three reflexes, normalising them to the levels seen in both control-IgG populations.

Overall, these experiments suggest that boosting glutamatergic transmission with AMPAkinine CX516 can to some extent rescue delays in early reflex development initially seen in our NR1-IgG *in utero* exposed animals with no significant impact on development of reflexes in control-IgG animals. However, these results should be interpreted with some caution due to the small number of animals and litters tested in these first rescue experiments.

6.5.4.2 Effects on early adolescent open-field behaviour

Overall open-field behaviour: It was also evaluated whether the alterations seen in the p21-23 open-field and rotarod tests in NR1-IgG *in utero* exposed mice could be rescued using the same approach. In the open-field test, looking at overall parameters, rescue NR1-IgG and control-IgG animals both showed very similar results to non-rescue NR1-IgG and control-IgG animals, with no significant differences between rescue NR1-IgG animals and any of the other three groups (RM ANOVAs: non-rescue NR1-IgG vs rescue NR1-IgG: Group: $F_{(1,11)} = 0.36$, $p = 0.56$; non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,8)} = 0.43$, $p = 0.53$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,7)} = 0.23$, $p = 0.65$; **Figure 6.5 A**, data in figure legend; **Appendix, Supplementary Table S6.4**).

Mean velocity also showed a similar pattern, with no significant differences between rescue NR1-IgG animals and any of the three other groups (RM ANOVAs: non-rescue NR1-IgG vs rescue NR1-IgG: Group: $F_{(1,11)} = 0.76$, $p = 0.40$; non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,10)} = 0.62$, $p = 0.50$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,8)} = 0.00023$, $p = 0.99$; **Figure 6.5 B**, data in figure legend; **Appendix, Supplementary Table S6.4**).

Specific open-field regions: Looking at parameters relating to the centre and corners of the open-field, there was a trend towards a significant decrease in normalised distance moved in the centre for rescue NR1-IgG animals, relative to non-rescue NR1-IgG animals (non-rescue NR1-IgG: $6.5 \pm 0.4\%$, rescue NR1-IgG: $4.8 \pm 0.5\%$, RM ANOVA: Group: $F_{(1,10)} = 2.35$, $p = 0.16$), and normalisation to both rescue and non-rescue control-IgG animals (non-rescue control-IgG: $4.5 \pm 0.7\%$, rescue control-IgG: $4.9 \pm 0.5\%$; RM ANOVAs: non-rescue control-IgG vs rescue NR1-IgG: $F_{(1,10)} = 0.23$, $p = 0.65$, Group x Day: $F_{(2,20)} = 4.85$, $p = 0.019$, Day: $F_{(2,20)} = 1.16$, $p = 0.33$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,7)} = 0.23$, $p = 0.65$; **Figure 6.5 C**; **Appendix, Supplementary Table S6.4**).

Conversely, there were no significant differences in number of visits to the centre between rescue NR1-IgG animals and non-rescue NR1-IgG animals (non-rescue NR1-IgG: 12.6 ± 1.7 visits, rescue NR1-IgG: 10.8 ± 1.4 visits, RM ANOVA: Group: $F_{(1,10)} = 0.030$, $p = 0.87$), but interestingly there was a significant difference between the two control-IgG groups, with rescue control-IgG animals showing a significantly higher number of visits to the centre than non-rescue control-IgG animals, with a similar number of visits to both non-rescue NR1-IgG and rescue NR1-IgG groups (non-rescue control-IgG: 6.9 ± 0.9 visits, rescue control-IgG: 10.9 ± 1.4 visits; RM ANOVAs: non-rescue control-IgG vs rescue control-IgG: Group: $F_{(1,9)} = 5.46$, $p = 0.044$, Group x Day: $F_{(1,12, 10.06)} = 3.76$, $p = 0.078$, Day: $F_{(1,12, 10.06)} = 7.05$, $p = 0.0050$; non-rescue NR1-IgG vs rescue control-IgG: Group: $F_{(1,13)} = 0.56$, $p = 0.47$; rescue NR1-IgG vs rescue control-IgG: Group: $F_{(1,7)} = 0.35$, $p = 0.58$, Group x Day: $F_{(1,11, 7.77)} = 8.81$, $p = 0.58$; **Figure 6.5 D**; **Appendix, Supplementary Table S6.4**).

There was also a significant effect of day between rescue and non-rescue control-IgG groups, with analysis of data from individual days revealing the largest differences in rescue control-IgG animals occurred on the first postnatal day, normalising to non-rescue control-IgG over the subsequent days (Day 1: non-rescue control-IgG: 8.2 ± 2.0 visits, rescue control-IgG: 17.7 ± 3.5 visits, independent t-test: $p = 0.051$; Day 2: non-rescue control-IgG: 5.6 ± 0.8 visits, rescue control-IgG: 10.0 ± 2.0 visits, independent t-test: $p = 0.079$; Day 3: non-rescue control-IgG: 6.7 ± 0.8 visits, rescue control-IgG: 5.0 ± 1.2 visits, independent t-test: $p = 0.25$; **Appendix, Supplementary Table S6.4**).

Mean velocity in the centre circle showed no significant difference between rescue NR1-IgG and non-rescue NR1-IgG animals (RM ANOVA: Group: $F_{(1,10)} = 0.42$, $p = 0.53$), but there was a slight trend towards a difference between rescue control-IgG and rescue NR1-IgG animals (RM ANOVA: Group: $F_{(1,7)} = 2.34$, $p = 0.17$; **Figure 6.5 E**, data in figure legend; **Appendix, Supplementary Table S6.4**).

Analysis of normalised corner duration, which showed a trend towards a significant decrease in non-rescue NR1-IgG animals, revealed no significant differences between rescue NR1-IgG and non-rescue NR1-IgG animals (RM ANOVA: Group: $F_{(1,11)} = 0.098$, $p = 0.76$) or between rescue NR1-IgG animals and either of the controls (RM ANOVAs: non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,8)} = 1.08$, $p = 0.33$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,8)} = 0.25$, $p = 0.63$; **Figure 6.5 F**, data in figure legend; **Appendix, Supplementary Table S6.4**).

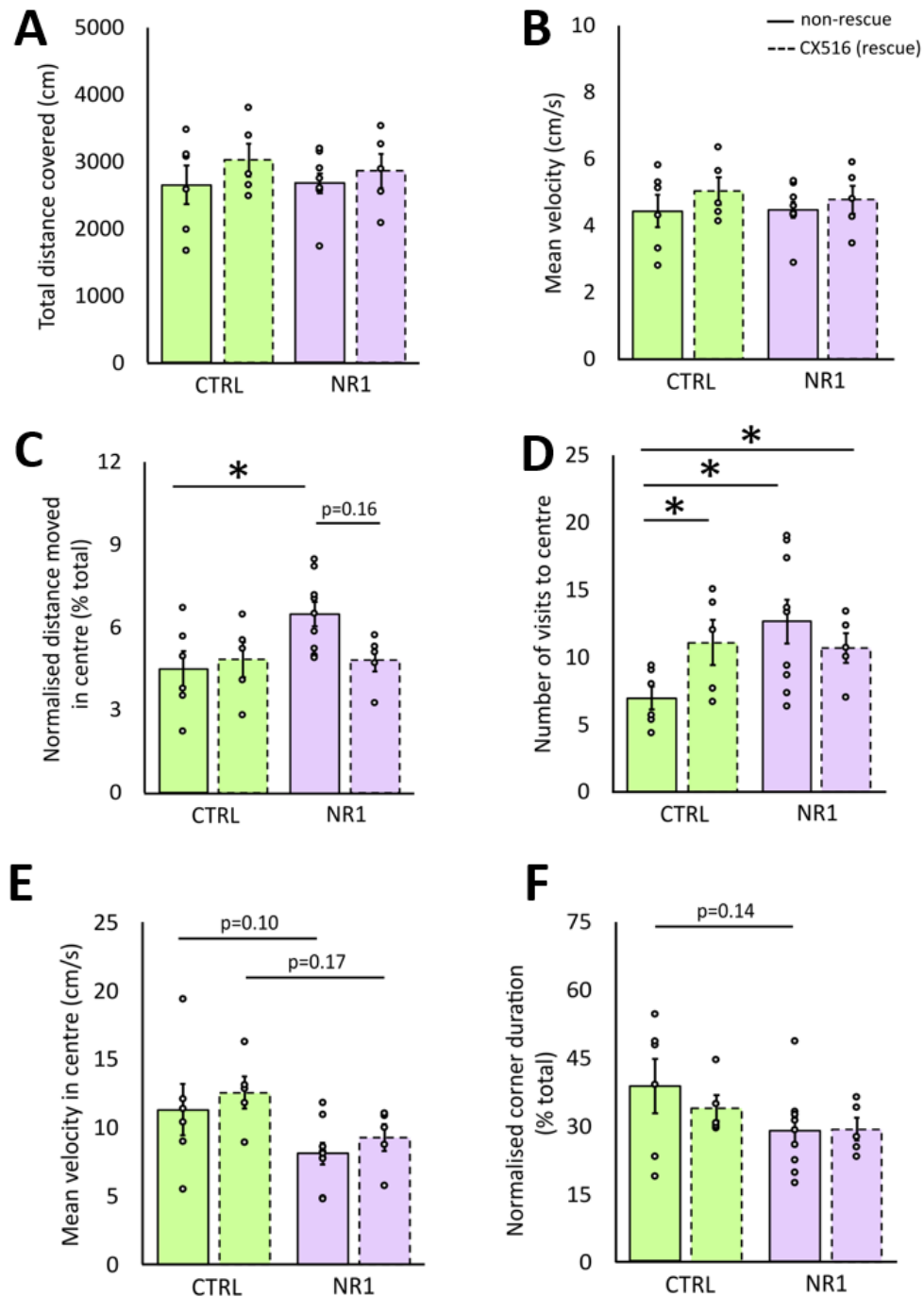


Figure 6.5. Partial rescue of alterations in open-field behaviour in NR1-IgG in utero exposed animals using AMPA/kine CX516. **[A]** Graph showing total distance covered in the open-field for non-rescue NR1-IgG and control-IgG animals as well as for rescue NR1-IgG and control-IgG animals (i.e. those given the CX516 treatment). Analysis revealed no significant differences between the groups. Data: control-IgG: 2650.4 ± 287.3 cm, NR1-IgG: 2676.4 ± 142.7 cm, rescue control-IgG: 3022.5 ± 247.6 cm, rescue NR1-IgG: 2858.3 ± 255.0 cm. **[B]** Graph showing mean velocity of animals in the open-field for non-rescue NR1-IgG and control-IgG animals as well as for rescue NR1-IgG and control-IgG animals (i.e. those given the CX516 treatment).

Figure 6.5. Effects of early postnatal injection of AMPAkinine CX516 on alterations in p21-23 open-field behaviour that occur following in utero NR1-IgG exposure (cont.). Analysis revealed no significant differences between the groups. Data: control-IgG: $4.4 \pm 0.5\text{cm/s}$, NR1-IgG: $4.5 \pm 0.2\text{cm/s}$, rescue control-IgG: $5.0 \pm 0.4\text{cm/s}$, rescue NR1-IgG: $4.8 \pm 0.4\text{cm/s}$. **[C]** Graph of normalised distance moved in the centre for non-rescue and rescue NR1-IgG and control-IgG populations, showing the significant increase in non-rescue NR1-IgG animals is normalised in rescue NR1-IgG animals. **[D]** Graph of number of visits to the centre, showing no differences between rescue NR1-IgG and non-rescue NR1-IgG populations, but an interesting significant increase in number of visits to the centre in rescue control-IgG animals relative to non-rescue control-IgG animals. **[E]** Graph of mean velocity in the centre showing no significant differences between rescue NR1-IgG animals and non-rescue NR1-IgG animals, although there was a slight trend towards a difference between rescue NR1-IgG animals and rescue control-IgG animals. Data: non-rescue NR1-IgG: $8.1 \pm 0.8\text{cm/s}$, rescue NR1-IgG: $9.1 \pm 1.2\text{cm/s}$, non-rescue control-IgG: $11.3 \pm 1.9\text{cm/s}$, rescue control-IgG: $12.1 \pm 1.1\text{cm/s}$; **[F]** Graph of normalised corner duration showing no significant differences between rescue NR1-IgG animals and non-rescue NR1-IgG animals or either control-IgG group. Data: non-rescue NR1-IgG: $28.7 \pm 3.1\%$, rescue NR1-IgG: $30.3 \pm 3.0\%$, non-rescue control-IgG: $38.4 \pm 5.9\%$, rescue-control-IgG: $32.4 \pm 2.7\%$.

6.5.4.3 Effects on early adolescent rotarod behaviour

Lastly, the impact of the CX516 rescue on the alterations previously observed in mean time spent on the accelerating rotarod in our non-rescue NR1-IgG exposed animals was evaluated. This analysis revealed that CX516 rescue normalised the mean time spent on the accelerating rotarod in rescue NR1-IgG animals, with a trend towards a significant decrease in rescue NR1-IgG animals relative to non-rescue NR1-IgG animals (non-rescue NR1-IgG: $127.6 \pm 32.1\text{s}$, rescue NR1-IgG: $145.1 \pm 14.2\text{s}$, RM ANOVA: Group: $F_{(1,11)} = 24.22$, $p = 0.11$) and no significant differences between rescue NR1-IgG animals and either of the control-IgG groups (non-rescue control-IgG: $93.3 \pm 10.3\text{s}$, rescue control-IgG: $109.0 \pm 15.8\text{s}$; RM ANOVAs: non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,8)} = 0.25$, $p = 0.63$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,8)} = 0.072$, $p = 0.80$; **Figure 6.6 A; Appendix, Supplementary Table S6.4**).

Splitting the data by day revealed a reasonably consistent difference between non-rescue and rescue NR1-IgG animals, with trends towards significance on the first and second days (Day 1: RM ANOVA: Group: $F_{(1,10)} = 1.82$, $p = 0.21$; Day 2: RM ANOVA: Group: $F_{(1,10)} = 3.14$, $p = 0.11$; Day 3: RM ANOVA: Group: $F_{(1,10)} =$

0.54, $p = 0.48$, Group $\times$ Day: $F_{(3,30)} = 4.00$, $p = 0.016$, Day: $F_{(3,30)} = 1.32$, $p = 0.29$), and a normalisation of these differences to non-rescue and rescue control-IgG groups (Day 1: RM ANOVAs: non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,7)} = 0.077$, $p = 0.79$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,6)} = 0.010$, $p = 0.92$; Day 2: RM ANOVAs: non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,6)} = 0.026$, $p = 0.88$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,7)} = 1.48$, $p = 0.18$; RM ANOVAs: non-rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,6)} = 1.27$, $p = 0.30$; rescue control-IgG vs rescue NR1-IgG: Group: $F_{(1,7)} = 0.0050$, $p = 0.94$; **Figure 6.6 B**, data in figure legend; **Appendix, Supplementary Table S6.4**).

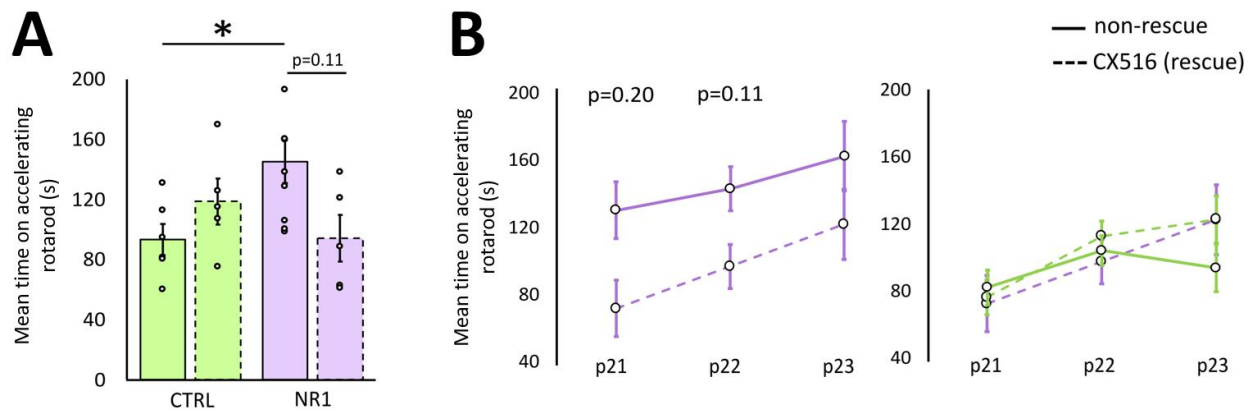


Figure 6.6. Rescue of alterations in accelerating rotarod behaviour in NR1-IgG in utero exposed animals using AMPA/kine CX516. **[A]** Graphs showing mean time on rotarod for non-rescue NR1-IgG and control-IgG animals, as well as rescue (i.e. CX516 treated) NR1-IgG and control-IgG animals, showing normalisation of the significantly higher time non-rescue NR1-IgG animals spent on the rotarod in rescue NR1-IgG animals, aligning with both control-IgG groups. **[B]** Graphs of mean time on rotarod split by individual trial day for non-rescue NR1-IgG and rescue NR1-IgG animals (left) and non-rescue control-IgG, rescue control-IgG and rescue NR1-IgG animals (right), showing a consistent difference between rescue and non-rescue NR1-IgG animals, and normalisation of these differences in rescue NR1-IgG animals to non-rescue and rescue control-IgG groups. Data: Day 1: non-rescue NR1-IgG: $130.1 \pm 16.8s$, rescue NR1-IgG: $77.7 \pm 17.4s$, non-rescue control-IgG: $82.2 \pm 10.4s$, rescue control-IgG: $81.4s$; Day 2: non-rescue NR1-IgG: $142.9 \pm 13.2s$, rescue NR1-IgG: $102.6 \pm 12.6s$, non-rescue control-IgG: $104.0 \pm 8.8s$, rescue control-IgG: $117.8 \pm 17.2s$; Day 3: non-rescue NR1-IgG: $162.3 \pm 20.7s$, rescue NR1-IgG: $127.6 \pm 32.1s$, non-rescue control-IgG: $93.7 \pm 10.3s$, rescue control-IgG: $127.8 \pm 26.8s$.

Overall, this data reveals a minor effect of CX516 rescue on open-field behaviour, with normalisation of the distance moved in the centre to the values seen in control-IgG animals, but no significant impact on other open-field parameters including number of visits to the centre, mean velocity in the centre or normalised corner duration. Interestingly, there was a significant difference between the number of visits to the centre amongst controls, with rescue control-IgG animals exhibiting a significantly greater number of visits to the centre than their non-rescue counterparts, more in line with what was observed in NR1-IgG exposed animals. The CX516 did however rescue the abnormal increased time spent on the rotarod seen in NR1-IgG animals, normalising it to the results seen in control-IgG animals.

6.6 Chapter Discussion

Experimental Chapter D first explored whether the embryonic and postnatal cellular and circuit alterations observed in the previous chapters were reflected in the early behavioural phenotype. Subsequently it investigated whether early behavioural alterations observed could be ‘rescued’ through boosting glutamatergic transmission by positive allosteric modulation of AMPARs using the AMPAkinase CX516.

The key findings in this chapter were:

1. **Significant delays in the development of certain early postnatal reflexes** (surface righting, negative geotaxis and cliff avoidance) in NR1-IgG *in utero* exposed animals.
2. **Alterations in anxiety-related aspects of open-field behaviour suggestive of reduced anxiety** including a significant increase in normalised distance moved in the centre, number of visits to the centre, and a decrease in normalised duration in corners in NR1-IgG *in utero* exposed animals.
3. **Minimal differences in motor-related aspects of open-field behaviour** including total distance covered and mean velocity.
4. **A surprising significant increase in time spent on the accelerating rotarod** in NR1-IgG *in utero* exposed animals.
5. **Rescue of early postnatal reflex delays** in NR1-IgG *in utero* exposed animals through boosting glutamatergic transmission with CX516 AMPAkinase injections.
6. **Minimal rescue of open-field behaviour following CX516 rescue treatment** with normalisation of distance moved in the centre by NR1-IgG *in utero* exposed animals to values seen in the control-IgG animals, but no significant impacts on other parameters.
7. **Normalisation of the time spent on the rotarod following CX516 rescue treatment** seen in NR1-IgG *in utero* exposed animals to levels seen in control-IgG animals.

6.6.1 Significant delays in the development of certain early postnatal reflexes (surface righting, negative geotaxis and cliff avoidance) in NR1-IgG *in utero* exposed animals;

The emergence of reflexes occurs at specific points during the early postnatal period and provides a good behavioural readout of the maturational state of the nervous system over this time (**Fox 1965**). In our first experiments, we tested three motor-related reflexes that require an intact basal ganglia and striatum for full expression (**Fox 1965, Arakawa and Erzurumlu 2015**). We found significant delays in the development of all three reflexes (surface righting, negative geotaxis, and cliff avoidance) in NR1-IgG *in utero* exposed animals relative to control-IgG animals. Similar delays in the emergence of these reflexes have also been observed in a closely related NR1-IgG *in utero* exposure model by **Jurek et al. (2019)**, although they observe the greatest delays in negative geotaxis, whilst we observe the most significant delays in cliff avoidance, in terms of percentage of the population with the reflex on a given tested day. Reflexes here also appeared somewhat later in both NR1-IgG and control-IgG animals relative to their study, possibly due to their early reflex testing start point (p0 rather than p7). Indeed, one limiting factor of the approach in this thesis is that a proportion of animals already had the reflexes present on the first testing day so differences may have been greater than currently evaluated. **García-Serra et al. (2020)** found in a similar placental transfer model of NR1-IgG that the time to perform the surface righting and negative geotaxis reflexes was extended in the early postnatal period, confirming that development of these reflexes is affected by early NR1-IgG exposure.

This also translates broadly to the clinical picture, with one study showing up to half of the babies from pregnant NMDARE patients show obvious neonatal developmental impairments (**Shi et al. 2017**). More widely, similar delays in early postnatal reflexes have also been reported in NR1 deficient mice (**Single et al. 2000**) and on administration of competitive NMDAR antagonist CGP to newborn rats (**Mikulecká and Mareš 2002**). Mechanistically, the gross impairments observed in these experiments and by others following disruption to the NMDAR either by antibodies or other means is likely explained by their critical

role as the primary conductors of glutamatergic transmission at this developmental stage (reviewed in **Hansen et al. 2021**). Corroborating this, blockade of glutamate release in mice using riluzole (which inhibits NMDARs and KARs and potentiates GABA_ARs (**Bellingham 2011**)) has been shown to induce loss of righting reflex (among other early reflexes) (**Irifune et al. 2007**).

6.6.2 Alterations in anxiety-related aspects of open-field behaviour suggestive of reduced anxiety;

More complex anxiety and motor-related behaviours were also tested during early adolescence (p21-23) using both the open-field and accelerating rotarod tests.

In the open-field test, it was found that there were several alterations in anxiety-related parameters, with NR1-IgG *in utero* exposed animals showing measures of significantly reduced anxiety behaviour including more visits to the centre of the field, a greater proportion of their total distance moved being in the centre of the field, and trends towards reduced velocity in the centre and proportion of time spent in the corners.

Contextualising these findings in the current literature, the only study that specifically explores the impact of NR1-IgG *in utero* exposure on anxiety-related behaviours is **Jurek et al. (2019)**, who similarly to results here also observed decreased levels of anxiety in mice exposed to NR1-IgG *in utero* (assessed using the elevated plus maze test), including a significant increase in number of visits and proportional duration of time spent in the open arms. **Mielnik et al. (2021)** similarly found decreased anxiety in the elevated plus maze task in animals with a congenital global loss-of-function allele of *GRIN1* when behaviourally assessed in adulthood (p98+). More widely, intravenous injection of NMDAR partial antagonist ketamine in dams at E14 was shown to result in increased anxiety in the offspring using the open-field test (**Zhao et al. 2014**), whilst *in utero* prenatal induced stress was found to result in increased anxiety in conjunction with decreased NR1 and NR2B in the striatum (**Sun et al. 2013**). The discrepancy in these studies relative to our results may be due to alternative mechanisms underlying the specific alterations to NMDARs in each

case due to the different inducers (NR1-IgG vs. pre-natal stress or antagonism). Indeed, it has been recently shown using *in vitro* hippocampal slices that NMDAR antagonist MK801 inhibits internalisation of the NMDAR following binding of a monoclonal NR1-IgG antibody, whilst the NMDAR antagonist DAP5 inhibits both binding and internalisation (**Sharma et al. 2018**).

Interestingly, three separate studies exposing mice to NR1-IgG antibodies, via stereotaxic NR1-IgG injections or prolonged NMDARE patient CSF infusions, at a later postnatal time point (p56-70), found no difference in the open-field or elevated plus maze tasks (**Planagumà et al. 2015, Taraschenko et al. 2019, Kersten et al. 2019**). One possible explanation for the differences between these results and ours is age at which the mice were exposed, highlighting a possible increased vulnerability of anxiety-related circuits to an NMDAR antibody-mediated insult during the late embryonic and early postnatal periods, relative to in the more mature brain (**Lago et al. 2017**).

Additionally, experiments exposing mice to specific NR1 peptides found differing effects. **Wagnon et al. (2020)** found increased anxiety in the O-maze task (a task which evaluates preference for protected or novel areas) in p70 mice when tested 2 weeks after active immunisation with the GluN1₃₅₉₋₃₇₈ peptide, whilst **Wilke et al. (2021)** found exposure to GluN1₃₅₉₋₃₇₈ or GluN1₁₆₈₋₁₈₇ at p56 had no impact on open-field or elevated plus maze behaviours in mice tested 2-3 weeks later. These differences could also be due to age of exposure or other methodological differences but may also be explained by differences in downstream mechanisms at the NMDAR such as varying levels of cross-linking and internalisation due to the use of active immunisation vs passive transfer (**Giannoccaro et al. 2020**). It is important to consider the limitations of behavioural tests in this thesis and the wider literature, including possible subjectivity in the way tests may have been conducted between different experimental groups despite best efforts to standardise procedures.

6.6.3 Minimal differences in motor-related aspects of open-field behaviour;

The open-field also captures some components of motor behaviour including hyperactivity. In the analysis here, no significant differences in any motor-related component were found, including no difference in total distance travelled or mean velocity in NR1-IgG *in utero* exposed mice relative to controls. Additionally, no gross abnormalities in the movements of the mice were observed at any point during open-field testing. However, in the accelerating rotarod studies, which more specifically test motor coordination and balance, it was unexpectedly found that the NR1-IgG *in utero* exposed animals remained on the rod for significantly longer than the control-IgG exposed animals.

In the context of the literature, **Jurek et al. (2019)** also found no difference in velocity in the elevated plus maze but observed hyperactive movements during the dark phase of the day/night cycle of home cage 24-hour recordings. Unfortunately, they did not perform the rotarod task in their study so no direct comparisons can be made there. Converse to our results, **García-Serra et al. (2020)** reported ‘poor motor coordination’ in their animals following placental *in utero* transfer of NR1-IgG when tested on the rotarod at 1 month of age (~p28). However, exploring this further, the discrepancy is likely due to differences in the rotarod protocols used. **García-Serra et al. (2020)** also performed 3 days of trials, but with fewer trials per day (2 trials vs our 4), with a much longer inter-trial period (1.5 hours between vs. our 1 minute). Experiments in this thesis found a significant difference in the three-day trial average, and a trend towards longer rotarod occupation for the NR1-IgG group when analysing individual days, with a consistent difference between NR1-IgG and control-IgG animal times maintained across the three trial days. On the other hand, **García-Serra et al. (2020)** only compared individual trials and found significant diverging improvement in the times of control-IgG animals from trial 4 onwards, with NR1-IgG animal times remaining stable across trials, suggesting their protocol captured an element of motor-learning in their control-IgG animals that the one used here did not, rather than any decrease in motor performance in their NR1-IgG animals. Other reasons underlying the differences may be due to differential exposure to

NR1-IgG (*in utero* direct injection vs placental transfer), differential age of testing (p21 vs p28) or differences in specific antibodies used.

Considering the literature more widely, **Mielnik et al. (2021)** found significant increase in distance travelled in the open-field in their *GRIN1* loss-of-function genetic model tested at p98. **Beutler et al. (2011)** found normal open-field behaviour (observed over 24 hours) but a decrease in rotarod performance in a similar model tested between p56 and p70. *In utero* NMDAR antagonist models had mixed results, with *in utero* exposure to NMDAR antagonist CGP 39551 (E6-p16) leading to decreased locomotion in adulthood (p98-p112) including reduced mean velocity and total distance travelled in the open-field test (**Collison et al. 2016**), whilst *in utero* exposure to PCP (E6-18) resulted in increased locomotion in an open-field beam break task at 7 weeks (**Lu et al. 2010**). These differences may be due to different mechanisms in how the NMDAR is disrupted (NR1-IgG vs. genetic disruption or antagonism) but they may also point towards an interesting possibility of later postnatal disruption to locomotor activity following *in utero* disruption to the NMDAR, which may not have been captured by the relatively early behavioural test point (p21-23) used here, thus presenting an interesting extension to this work.

Most studies delivering NR1-IgG antibodies at a later postnatal time point (typically p56-70) using either intraventricular infusion of NMDAR patients CSF or stereotaxic injections showed no significant differences in locomotor activity on tasks including the open-field test (**Kersten et al. 2019, Taraschenko et al. 2019**), horizontal and vertical tests (**Planagumà et al. 2015**), or rotarod test (**Taraschenko et al. 2019**), assessed 5 days to 2 weeks post-exposure. Similarly, exposure to specific NR1 peptides such as GluN1₃₅₉₋₃₇₈ and GluN1₁₆₈₋₁₈₇ at a similar point resulted in no significant motor alterations in several studies, including in distance moved in the open-field test (**Wilke et al. 2021**), or any differences in the horizontal and vertical tests (**Wagnon et al. 2020**), assessed 1-2 weeks later. These studies suggest that the underlying motor circuits may be more robust to disruption by NMDAR antibodies when exposed at this later postnatal timepoint than they are when the insult is received embryonically. This could be

because a larger number of neurons or circuits may be impacted if progenitors are affected, or due to transient critical expression or function of NMDARs on progenitors or neurons in early development (reviewed in **Ewald and Cline, 2009, Hansen et al. 2021**). The one study where a difference was noted was **Sharma et al. (2018)**, who found an increase in wheel running in p42-56 mice injected with an NR1-IgG monoclonal antibody. One possible explanation is that this study captures more acute effects of a more severe insult, since testing was performed almost directly post injection of the antibody (day 4 onwards), and monoclonal antibodies were used which have a much higher titre than CSF from patients. In the other studies, the lower insult may have led to no effect, or an effect that resolves in the time window before testing. Indeed, there are reports that alterations in behaviour due to NR1-IgG antibody exposure (albeit *in utero*) may undergo reversal in the subsequent postnatal period (**García-Serra et al. 2020**).

6.6.4 A surprising significant increase in time spent on the accelerating rotarod;

Considering these studies, our accelerating rotarod results showing a consistent longer time spent on the rod for NR1-IgG animals relative to control-IgG animals are somewhat surprising. It is possible that the increased time on the rod as it accelerates reflects a degree of motor hyperactivity in these animals. Mechanistically, increased movements could be explained by the decreased proportion of D2-SPNs relative to D1-SPNs identified in **Experimental Chapter C**, since D2-SPNs are classically associated with inhibition of movement (**Gerfen and Surmeier 2011, Gallo 2019**). Although no differences in hyperactivity in the open-field test were observed, this latter test may have included aspects of both anxiety and novelty that might have affected overall activity. Another hypothesis is that the increased time on the rod could reflect decreased anxiety in these animals. As the rod accelerates, the mice are thought to become increasingly stressed (**Rozas and Guerra 1997, Mizoguchi et al. 2002**). Theoretically speaking, animals with a lower level of anxiety, as seen with NR1-IgG animals in the open-field test, may feel less stressed and therefore be able to remain on the rod for longer. **Beutler et al. (2011)** offers some evidence to

support this, with reduced fear learning observed in their NR1 knock-out animals relative to controls when assessed with an active avoidance task. A third possibility is that in the absence of D2-SPNs, the greater proportion of D1-SPNs may play a more prominent role, and therefore NR1-IgG animals may paradoxically have an improved level of motor learning, since activity in D1- but not D2-SPNs has been shown to underpin accelerated rotarod motor learning (**Liang et al. 2022**). This hypothesis would fit the general trend of daily improvement seen in NR1-IgG animals, that is not seen in control-IgG animals. It is also important to consider the possibility that there may have been a difference in the size of the animals, since it is easier for smaller animals to stay on the rod for longer. Although animals did not appear grossly different in size, they were not formally measured or weighed prior to open-field/accelerating rotarod experiments, and so this could have been an unseen limiting factor. Indeed, literature suggests that disruption to the NR1 subunit eg. in genetic knock-out mice, can result in smaller body size relative to controls (**Beutler et al. 2011**). A useful extension of this project would be to conduct experiments to determine underlying mechanisms more conclusively.

Mechanistically, the alterations we observe in both motor- and anxiety-related behaviours suggest potential involvement of the striatum and associated circuits including those mediating stress and anxiety (eg. BNST/amygdala and cortex (**Smith et al. 2016, Jurek et al. 2019**)). Considering the striatum specifically as the main focus of study, the dorsal striatum is classically associated with regulation of motor and cognitive processes through its connections to the premotor, motor and parietal cortices (**Porter et al. 2015**), whilst there is an established role for the ventral striatum in emotional regulation through its association with the ventral prefrontal cortex, and an emerging role for its involvement in anxiety pathways (**Groenwegen et al. 1987, Fudge et al. 2002, Liljeholm and O'Doherty 2012, Avery et al. 2014, Calhoun and Tye 2015, Torrisi et al. 2015, Lago et al. 2017**). The fact that alterations to both these behavioural types were observed suggests potential wider-scale disruption to the striatal circuitry than has already been established through the studies of dorsal corticostriatal connections in the previous

chapter, and thus it would be useful to extend to explore ventral striatal connections in future work. Beyond the striatum, the BNST has similar developmental origin and has a key role in stress and anxiety circuits, with implications in several disorders including PTSD and generalised anxiety disorder (**Ahearn et al. 2007, Buff et al. 2017, Clauss et al. 2019, Feola et al. 2023**). The disruption to progenitors in the LGE observed in **Experimental Chapter B** therefore likely also includes BNST progenitors, and thus knock on disruption to BNST neurons and related circuits may underlie some of the anxiety-related behavioural alterations observed here; an important area to explore in future studies.

6.6.5 Rescue of early postnatal reflex delays through boosting glutamatergic transmission with CX516 AMPAkinic injections;

6.6.6 Minimal rescue of open-field behaviour following CX516 rescue treatment; and

6.6.7 Normalisation of the time spent on the rotarod following CX516 rescue treatment;

The alterations observed in behaviour are likely underpinned by abnormalities in glutamatergic transmission, possibly brought about by the cross-linking and internalisation of the NMDARs following exposure to the NR1-IgG antibodies *in utero*. Thus, the next experiments sought to explore whether boosting glutamatergic transmission through the other major type of glutamate receptor, AMPARs, using AMPAkinic CX516 could rescue the behavioural phenotype observed. It was found that this manipulation rescued the delays observed in the surface righting, negative geotaxis and cliff avoidance reflexes, and restored alterations seen in accelerating rotarod behaviour back to the level of control-IgG animals, but only had a minimal effect on anxiety-related open-field behaviour, with restoration of relative distance moved in the centre back to control-IgG animal levels, but no impacts on other open-field parameters.

To our knowledge, this is the first time this type of rescue has been used in an antibody model. However, the same rescue protocol used previously in a Huntington's disease model, which also entails a level of disruption to glutamatergic signalling and intrinsic excitability in the cortex and striatum (**Cummings et al.**

2009, Raymond et al. 2011, Heikkinen et al. 2012, Indersmitten et al. 2015, Kovalenko et al. 2018). Use of the CX516 rescue protocol during the first postnatal week appeared to rescue many of the motor, sensory and cognitive deficits observed in their Huntington's disease animals when assessed at p21, including normalisation of the percentage of distance covered in the centre of the open-field to the level of control animals, in line with what was observed here, as well as normalisation of latency in the horizontal ladder test (a measure of motor coordination) (**Braz et al. 2022**). Further, aligning with findings here, they also observed a decreased latency in righting reflex at three timepoints in the first postnatal week, which was significantly improved following CX516 treatment.

Since their model tested similar electrophysiological and morphological parameters to the ones assessed in this thesis (albeit in cortical II/III neurons rather than SPNs) it provides potential mechanistic insights into how the rescue may work in this model. In their pathological phenotype they observe reduced mEPSC amplitude and transiently increased neuronal excitability in the early postnatal period, along with delays in dendritic arborisation, decreased spine density and numbers of (DARPP32+) SPNs, and reductions in striatal volumes; all characteristics that were rescued in their CX516 treated animals. Their phenotype is suggested to result from low glutamatergic activity, supported by defective development of dendritic arbours and altered neuronal physiological function in other studies (**Mandal et al. 2011**). AMPA/kine CX516 may not only mitigate cellular morphological and electrophysiological disruption through helping to increase glutamatergic transmission but might also mitigate deficits in synaptic plasticity through up-regulation of BDNF, a neurotrophin involved in promoting activity-driven actin polymerisation of dendritic spines and stable synapse formation (**Rex et al. 2007, Lynch et al. 2007, Simmons et al. 2009, Mandal et al. 2011**).

Interestingly, in their model, they observed that wild-type (control) mice treated with CX516 showed several alterations including changes in spine and synapse density, reduced numbers of SPNs, and increased latency in the righting reflex. This suggests that glutamatergic transmission in developing

postnatal circuits is finely balanced and may be impaired by too much as well as too little glutamatergic transmission (**Braz et al. 2022, Rosen and Schulkin 2022**). This may explain the significant difference found here between rescue and non-rescue control-IgG animals in visits to the centre in the open-field test, with rescue control-IgG animals showing decreased anxiety in this parameter, similar to what was seen in the NR1-IgG non-rescue group. In the open-field test very limited rescue of most parameters studied was seen, whereas in the accelerating rotarod test near complete restoration of the alterations was observed, suggesting that the AMPAkinase may have differential ability to rescue specific circuits or that anxiety-related circuits may have additional disruption beyond impaired glutamatergic transmission.

An important final consideration is that in the study by **Braz et al. (2022)**, the rescue only delayed the onset rather than fully preventing the Huntington's disease phenotype from emerging. An important future study for our lab therefore is to assess the efficacy of the same rescue at a much later postnatal timepoint to see if the rescue remains. It is also important to expand the current small-scale pilot testing of reflexes and p21-23 behaviour much further before any robust conclusions are drawn. Lastly, it would also be interesting to trial other rescue approaches in this *in utero* model such as EphB2 (**Planagumà et al. 2016**), which have shown promise in rescuing defects induced with NR1-IgG infusions postnatally, or embryonic stem-cell derived striatal progenitor grafts, which have shown recent promise in rescuing early striatal progenitor impairments and some of the subsequent postnatal impacts (**Schellino et al. 2023**).

6.7 Chapter Summary

This chapter set out to investigate whether the postnatal cellular and circuit changes observed in mice exposed to NR1-IgG *in utero* in the previous chapter (**Experimental Chapter C**), translated into a postnatal behavioural phenotype. It also set out to assess whether any behavioural changes observed could be rescued through boosting glutamatergic transmission with the AMPAkinic CX516. In this chapter, several behavioural alterations were identified in the early postnatal period, including a significant delay in three neurodevelopmental reflexes (surface righting, negative geotaxis and cliff avoidance) in the second postnatal week, and in anxiety-related parameters in the open-field test, along with an unexpected increase in time spent on the accelerating rotarod, both assessed in the fourth postnatal week (p21-23). Further, bi-daily intraperitoneal injections of AMPAkinic CX516 during the first postnatal week in NR1-IgG *in utero* exposed animals were able to rescue the delays in developmental reflexes and restore alterations in the accelerating rotarod test, whilst having minimal impact on changes observed in the open-field test.

Overall, these results demonstrate that mice exposed *in utero* to NR1-IgG display early postnatal behavioural alterations in reflex development, and some motor- and anxiety-related behaviours, which can be partially rescued through administration of AMPAkinic CX516 during the first postnatal week.

7. General Discussion

7.1. Summary of Findings

Neurodevelopmental consequences of *in utero* exposure to maternal antibodies such as those against the NMDAR have become an increasing area of interest in recent years (reviewed in **Giannoccaro et al. 2020, Prüss 2021**), with studies linking them to a range of pathological outcomes and neurodevelopmental disorders including ASD, learning disabilities and schizophrenia (**Singer et al. 2009, Braunschweig et al. 2012, Brimberg et al. 2016, Coutinho et al. 2017a, Jurek et al. 2019**). Whilst much literature so far has focused on the hippocampus and cortex, both pre-clinical and clinical literature point to the striatum as an important, as of yet unexplored target (**Luk et al. 2003, Florance et al. 2009, Irani et al. 2010, Lanciego et al. 2012, Armangue et al. 2013, Jansson et al. 2014, Wright et al. 2015a, Jurek et al. 2019, Krajewski et al. 2019, Mielnik et al. 2020, García-Serra et al. 2020**). Thus, the overarching aim of this DPhil project was to explore the impact of patient-derived antibodies against the NMDAR on the development of the striatum in a gestational mouse model.

Experimental Chapter A established and validated key characteristics of a novel *in utero* intraventricular mouse model for studying the impacts of NR1-IgG on the developing mouse brain and striatum. Key model features characterised included exploring early distribution of glutamate transporters and receptors in the brain, and *in vitro* and *ex vivo* binding and dynamics of NR1-IgG antibodies in the striatal precursor, the LGE. Regional differences in embryonic expression of glutamatergic subunits were identified, including high transcript- and protein-level expression of the NR1 subunit in the LGE (**Figures 3.2, 3.3**), and it was established that both patient-derived antibodies being utilised for this study (polyclonal and monoclonal NR1-IgG) bound the NR1 subunit *in vitro* (**Figures 3.4, 3.5**), and also importantly bound the LGE following *in utero* intraventricular injection (**Figure 3.6**), with similar binding patterns to other antibody delivery

routes in the literature (Wright et al. 2015b). Taken together, these findings establish and validate key characteristics of the *in utero* intraventricular NR1-IgG antibody injection model.

Experimental Chapter B aimed to use the *in utero* model to explore how NR1-IgG antibodies impacted the developing striatum embryonically as compared to control-IgG antibodies. In this chapter, immunohistochemical and morphological studies found several differences in processes underlying striatal neurogenesis of NR1-IgG injected embryonic brains including reductions in the size of the primordial striatum (**Figure 4.2**), decreased progenitor proliferation particularly within proliferative zones close to the ventricle (**Figures 4.3, 4.4**), and impaired cell migration away from the ventricular wall (**Figure 4.7**). Immunohistochemical labelling and reconstruction of microglia also revealed increases in activated microglial density and altered morphology, suggestive of an increased activated state and/or maturational differences (**Figures 4.10, 4.11**). Combined, these findings show that *in utero* exposure to NR1-IgG impacts multiple important embryonic processes underlying striatal neurogenesis and early striatal formation.

Experimental Chapter C set out to investigate whether embryonic changes observed following *in utero* NR1-IgG exposure also impacted the early postnatal striatum. In this chapter, electrophysiological recordings from early postnatal SPNs revealed differences in development of several intrinsic properties, together suggestive of relative electrical immaturity (**Figure 5.1**), decreased amplitude of excitatory synaptic inputs onto SPNs suggestive of post-synaptic effects (**Figure 5.8**), and alterations in corticostriatal connections predominantly in pre-synaptic properties and short-term plasticity (**Figure 5.9**), along with a reduced NMDA/AMPA ratio (**Figure 5.10**). Morphological and immunohistochemical studies in the early postnatal period also identified differences in both gross and individual SPN morphology, including a significantly reduced striatal volume (**Figure 5.12**), decreased SPN spine density (**Figure 5.6**) and shifted D1/D2 ratio towards fewer D2-SPNs (**Figure 5.12**). Analysis of early postnatal microglia also revealed increased activated microglial density and altered morphology, similar to findings embryonically (**Figures**

5.13, Figure 5.14). Taken together, these findings overall suggest that the postnatal brain and striatum of NR1-IgG *in utero* exposed animals is prolongedly altered well into critical periods of striatal development.

Experimental Chapter D first explored whether the previously observed cellular and circuit changes were reflected in the early behavioural phenotype. Analysis of key early reflexes (surface righting, negative geotaxis and cliff avoidance) revealed significant delays in their development (**Figure 6.1**). Open-field experiments in the third postnatal week showed alterations suggestive of a reduced anxiety phenotype including greater numbers of visits to the centre of the field (**Figure 6.2**), whilst parallel accelerating rotarod experiments revealed a surprising increase in time spent on the rod (**Figure 6.3**). The second part of this chapter explored whether it was possible to rescue the aberrant behavioural phenotype through facilitating activity of glutamatergic AMPARs, using the AMPAkinase CX516, following *in utero* exposure to NR1-IgG. These experiments revealed a rescue of the early postnatal reflex delays observed previously (**Figure 6.4**) and normalisation of time spent on the rotarod (**Figure 6.6**), as well as a trend towards normalisation of some open-field behaviour (**Figure 6.5**). **Overall, these experiments show that *in utero* exposure to NR1-IgG is reflected in a postnatal behavioural phenotype including delayed reflexes and alterations in anxiety-related behaviours. This phenotype can be partially rescued through boosting activity of an alternative ionotropic glutamate receptor, AMPARs.**

The key findings in this thesis are summarised below in **Figure 7.1**.

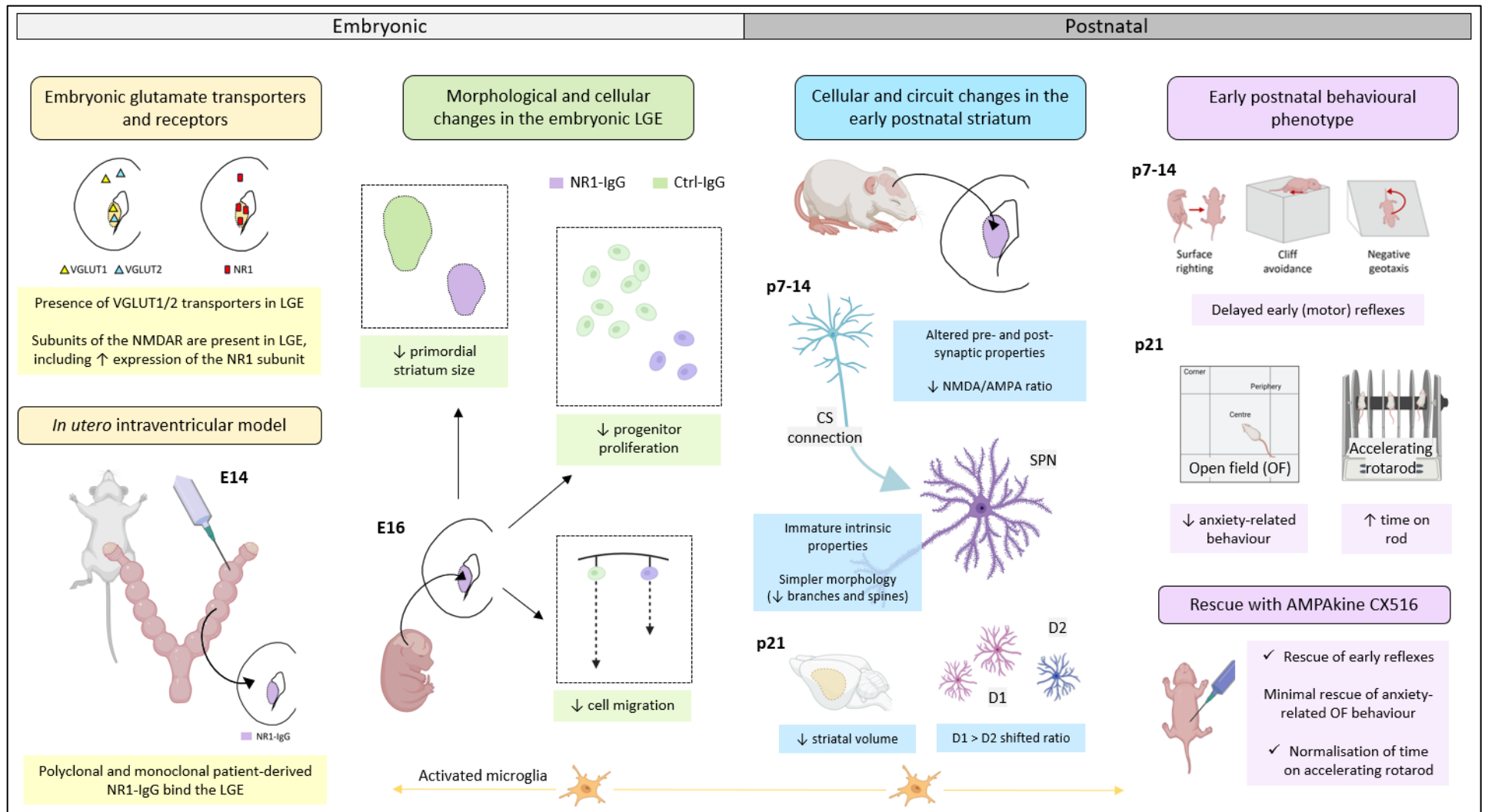


Figure 7.1. Graphical summary of key thesis findings.

7.2. Implication of Findings

7.2.1. The *in utero* intraventricular model for studying maternal antibody impacts

Over the past three decades, many antibody-mediated encephalitic disorders have been identified (Crisp et al. 2016). In line with these discoveries, rodent models have been developed to study the effects of antibodies in an adult context (Giannocaro et al. 2020), with a more recent shift of focus to utilising developmental models to study antibodies in the context of gestational transfer, with literature increasingly linking them to a range of neurodevelopmental disorders including ADHD, ASD and schizophrenia (Shi et al. 2017, Giannocaro et al. 2020, Prüss 2021).

Models for studying impacts of antibody transfer in a gestational context typically involve the passive transfer of either antibodies or CSF derived from patients to pregnant dams, via either intraperitoneal or tail-vein injections (Jurek et al. 2019, García-Serra et al. 2020, Giannocaro et al. 2020), with antibodies crossing into the foetus and through their blood-brain barrier to exert effects. Whilst these models are highly useful, the reliance on maternal-to-foetal transfer is caveated for studying direct impacts and mechanisms of these antibodies on the foetal brain. Peripheral injections often require a much greater volume of antibodies and multiple days of administration (Braunschewig et al. 2012, Chang et al. 2020). Not only may this be impractical with limited antibody resources, but it also poses the risk of inducing a maternal local and/or systemic immune response. Systemic inflammation releases pro-inflammatory cytokines (eg. TNF α) which can upregulate expression of receptors on peripheral neurons, increasing responsiveness to stimuli such as antibodies (Perry 2004, Aktas et al. 2007, Hoogland et al. 2015), and can also lead to post-translational autoantigen modification, altering antibody-antigen interactions, confounding interpretation of direct antibody impacts in the CNS (Dutheil et al. 2016, Spielman et al. 2018, Sun et al. 2022). Further, it can increase blood-brain barrier permeability, enabling entry of antibodies and other inflammatory molecules into the CNS and leading

to central sensitisation (**Abbott et al. 2010, Elwood et al. 2017, Galea 2021**). As such, the potential for systemic inflammation can confound findings by inducing uncertainty as to whether results observed are a consequence of direct antibody-target interactions in the foetal brain or arise from maternal immune activation and/or inflammation.

Additionally, administration of the antibody to the mother reduces standardisation of volume of antibody reaching each foetal brain, which can be impacted by a range of maternal factors including maternal inflammatory response (**Braunschweig et al. 2012, Elwood et al. 2017, Galea 2021**) and placental structure (**Niewiesk 2014**). Furthermore, maternal administration of antibody poses potential issues secondary to differences with FcRn-IgG interaction in mice vs humans, since such interactions are highly species and IgG isotype dependent (**Roopenian and Akilesh 2007**). Moreover, in mice, a large proportion of IgG transfer occurs postnatally via breast milk, whereas in humans it occurs exclusively *in utero* (**Jacobson et al. 1999, Van de Perre 2003, Palmeira et al. 2012**). Therefore, peripheral administration in a gestational mouse model is unlikely to capture the full impacts of IgG transfer on the developing mouse brain, with only limited detection of IgG in the foetal brain before E17.5 in such models (**Braniste et al. 2014, Coutinho 2016**).

This study has helped address these caveats through the development of an *in utero* intraventricular injection model to study NR1-IgG antibodies against the NMDAR, derived from NMDARE patients. Development of this model advances the literature twofold: firstly, to my knowledge this is the first study of NR1-IgG antibodies using direct *in utero* intraventricular injections, and secondly, this is the first study characterising key parameters of these NR1-IgG antibody injections intraventricularly *in utero* including antibody binding, distribution and ‘clearance’ from the LGE following injection.

The usefulness of direct intraventricular administration of NMDAR antibodies is established in several postnatal studies (reviewed in **Giannocaro et al. 2020**). Direct *in utero* injections of maternal antibodies

into a mouse model have not previously been used to study NMDAR antibodies but have been used in the study of impacts of autism-specific maternal antibodies (**Camacho et al. 2014, Martínez-Cerdeño et al. 2016, Ariza et al. 2017**). These studies primarily focus on impacts on the cortex and cortical neurogenesis. They support the utility of the *in utero* intraventricular approach in uncovering mechanisms of maternal antibody impacts directly on the foetal brain from small volume samples, identifying gross and cellular morphological and proliferative changes in the embryonic neocortex and adult cortical neurons, as well as impacts on adult behaviour following *in utero* intraventricular injections of 0.5-1 μ l antibody. However, direct *in utero* injection is not without its own caveats. One key consideration is the need for surgical procedure since anaesthetic agents can impact cell proliferation amongst other parameters (**Sall et al. 2009**). This study addresses this through having surgical control-IgG injected comparator animals, but this should be considered when comparing to wider literature. Further, direct injection discounts any impact peripheral antibodies might have, which are an important part of disease manifestation for other antibodies (eg. CASPR2 and LGI1 (**Kowal et al. 2004, Huerta et al. 2006**)). This could include reduced transfer of microglia from the yolk sac into the brain, which literature suggests are important in management of anti-neuronal antibodies in the brain (**Coutinho et al. 2017b, García-Serra et al. 2020**). This may be less of an issue for NMDAR antibodies, given human autopsy data suggesting the impacts here are unrelated to complement-mediated mechanisms (**Martínez-Hernández et al. 2011, Bien et al. 2012**). Additionally, our data still demonstrates significantly increased microglial density in both the embryonic and postnatal striatum following direct intraventricular NR1-IgG injections at E14.5.

Characterisation of key model parameters such as antibody binding profile and rate of removal from the brain is useful in validating the model against other approaches and assessing usefulness for studying impacts of these specific antibodies *in utero*. This study utilised patient-derived polyclonal and monoclonal NR1-IgG antibodies, both of which have been used in the literature previously,

demonstrating pathogenic effects (Kreye et al. 2016, Malviya et al. 2017). The experiments in **Experimental Chapter A** confirmed both antibodies bound NR1 similarly to previous studies but also found that monoclonal antibodies demonstrated increased binding as expected, likely due to their greater specificity. NR1-IgG binding observed in embryonic brains following the direct *in utero* intraventricular injections was present in embryonic brains 48 hours post-delivery, similar to what has previously been seen in maternal antibody delivery routes such as via maternal intraperitoneal or tail-vein injections, with similar binding patterns to those seen in embryonic hippocampus and whole brain sections (Wright et al. 2015b, Jurek et al. 2019, García-Serra et al. 2020), providing evidence to help validate our approach. This study also importantly validated that there was very little difference between the impacts of these two antibodies via whole-cell patch-clamp electrophysiological studies in **Experimental Chapter C**. However, results in **Experimental Chapter B** using FlashTag labelling to compare impacts on cellular migration of four other patient-derived polyclonal NR1-IgG antibodies showed some differences between samples, with two samples significantly impacting cell migration, and one sample having no effect. Across the literature there is variability in outcomes from NMDAR passive transfer models (Giannocaro et al. 2020) which may be due to a host of factors, amongst which the specific antibodies used must be considered. These findings here highlight the importance of validating parameters of individual antibodies within the chosen model prior to study. Indeed, it is well documented in the clinical context that there is a sub-population of asymptomatic NMDAR-antibody carriers, pertaining to the fact that not all NMDAR antibodies necessarily result in pathological outcomes (Dahm et al. 2014, Lang and Prüss 2017). Why only some of these antibodies exert pathogenic effects is not fully understood and an interesting direction for future work.

A final reflection regarding the usefulness of the *in utero* intraventricular model developed here is the transient nature of antibody delivery, with a single *in utero* intraventricular injection of the antibody being used. This approach is advantageous in removing confounding factors that may result from

multiple injections including permanent mechanical damage to the developing brain and consequences of repeat surgical intervention. Additionally, it enables specific focus on impacts at a particular point of neurodevelopment, such as the peak of striatal neurogenesis here. However, it may be less reflective of the *in utero* setting where persistent antibody production is one of the main issues faced by NMDARE patients (Titulaer et al. 2013, Dalmau et al. 2019, Prüss 2021). Counterbalancing this, NMDARE detection has improved greatly in recent years, with individuals receiving treatment often based off a high degree of clinical suspicion prior to confirmatory tests returning. Therefore, in rapid treatment of a mother presenting with NMDARE, the typical expectation would be high titres driving clinical severity of disease would be reduced rapidly with immunotherapy and adjunct treatments, thus also reducing time of foetal brain exposure to the same antibodies, since the foetus is not generating antibodies itself (Palmeira et al. 2012, Irani et al. 2013, Titulaer et al. 2013, Sechi and Flanagan 2021). Considering the second population of mothers where there are circulating subclinical levels of NMDAR antibodies, whilst these are likely there for the duration of pregnancy, there is likely to be limited transfer to the foetus, which studies comparing volumes of antibodies in foetal brains following variable administration routes demonstrating that much higher volumes an antibody are needed in maternal intraperitoneal injections to reach the same level in the foetal brain as a singular *in utero* intraventricular injection (Chang et al. 2020). Even with singular *in utero* injection, experiments in this thesis demonstrate persistent impacts both embryonically and postnatally, translating into a pathological behavioural phenotype, but it is acknowledged that some impacts may still be reduced relative to what they may be following a longer exposure.

7.2.2. *In utero* NMDAR antibody exposure and the developing brain

Potential pathogenic transfer of antibodies from mother to foetus during pregnancy has become an area of increasing interest in recent decades, owing to evidence of broad and serious impacts on

neurodevelopment and function (**Giannoccaro et al. 2020**). Within this, antibodies against the NMDAR are an important focus but their impacts are not yet well understood.

NMDARE manifests as a complex neuropsychiatric syndrome (reviewed in **Dalmau et al. 2019**). Clinical literature supports the link between NMDAR antibody exposure *in utero* and a wide range of potential neurodevelopmental impacts, including developmental delay, seizures, neuromuscular symptoms and abnormal fine movement (reviewed in **Scorrano et al. 2024**). Existing pre-clinical studies utilising *in utero* exposure models have identified alterations across a spectrum of behavioural paradigms, including impaired neurodevelopmental reflexes, hyperactivity, reduced anxiety, altered sensorimotor gating, poor coordination and deficits in memory and nest building, pertaining to widescale disruption in the brain across multiple regions (**Jurek et al. 2019, García-Serra et al. 2020**). How does *in utero* exposure to NMDAR antibodies result in these functional postnatal deficits? What key regions, cells and circuits are involved?

Current literature demonstrates that NR1-IgG *in utero* exposure results in widespread binding in the brain at, or just prior to, birth, along with concurrent global reduction of NMDAR density (**Jurek et al. 2019, García-Serra et al. 2020**), likely due to receptor internalisation (**Huang et al. 2020**). It has been shown that in the embryonic brain, *in utero* exposure to these antibodies can result in cortical plate thinning embryonically (**Lee et al. 2009**), but little work has been done so far to explore embryonic impacts beyond this. This thesis extends the literature through offering one of the first studies exploring impacts of these antibodies on embryonic (cortical and striatal) progenitors. Within the LGE it was identified that proliferation of progenitors and subsequent migration of cells away into the primordial striatum was impaired. This offers an early mechanistic insight into how NMDAR antibodies *in utero* may disrupt neurogenesis, aligning with work using NMDAR antagonists *in vitro* and *in utero* (**Luk et al. 2003**). In the cortex no significant change in progenitor proliferation was observed, but previous studies identify compaction of the cortex both embryonically (**Lee et al. 2008**) and postnatally

(García-Serra et al. 2020) with NR1-IgG exposure at earlier time points (E12; during cortical neurogenesis (Götz and Huttner 2005)), suggesting that migration in the cortex may also be impaired, a mechanism supported by literature demonstrating that antagonism of NMDARs *in vitro* blocks migration from the ventricular zone into the cortical plate (Behar et al. 1999, Reiprich et al. 2005). This also suggests that time of impact within the gestational period can result in regional differences in impacts, which may partially explain the wide range of symptoms and syndromes seen and differing levels of impact (Jurek et al. 2019, Scorrano et al. 2024). Understanding impacts on progenitors and how neurodevelopment is disrupted at the earliest timepoints is pivotal for furthering mechanistic understanding and informing novel potential therapeutic management. Indeed, this study identifies reduced density of postnatal SPNs and reduced striatal volumes, similar to the reduced volumes across multiple brain regions including the cortex and striatum seen in previous similar work (Jurek et al. 2019), highlighting the widespread potential postnatal consequences of early disruption to progenitors and neurogenic processes.

Most current literature explores the early postnatal brain to investigate impacts of *in utero* exposure to NMDAR antibodies. Previous work has pointed to both the hippocampus and cortex as key players. At a cellular level, it has been shown that cortical and hippocampal cells in the early postnatal brain exhibit altered morphology, along with impairment in how they electrically mature and synapse together to form connections and circuits, with noted impairments in glutamatergic synapse density, development of excitatory synaptic inputs and altered functional connectivity (Jurek et al. 2019, García-Serra 2020, Kuchling et al. 2023). This study extends the literature through identifying specific striatal impacts for the first time. Impacts on SPNs have some parallels to those on cortical and hippocampal cells, including findings of slower maturation of cellular (SPN) intrinsic properties, alterations in excitatory synaptic development, and differences in morphology, which posits that the

mechanism(s) by which these antibodies cause impact is at least to a degree translatable between neuronal subtypes.

Can these observations help explain behavioural phenotypes and clinical symptoms observed? Behavioural impacts seen in both this study and wider literature, particularly on motor- and anxiety-related behaviours, suggest *in utero* disruption to the development of striatal and related circuits underpins aspects of the behavioural phenotype. In the clinical context, babies and children with NMDARE typically exhibit a more 'neurological' than psychiatric presentation (**Armangue et al. 2013, Wright et al. 2015a**), along with reported 20% higher incidence of motor symptoms compared to adults (95% vs 75%) (**Titulaer et al. 2013**), positing that early striatal disruption might be particularly important in the neurodevelopmental setting. Likewise, presentation of babies born to mothers with NMDARE is variable but with suggestion of striatal disruption in several cases including abnormal movement and developmental delay (**Magley et al. 2012, Jagota et al. 2014, Kokubun et al. 2016**). It is important to consider here that impacts may also result from treatments received by the mother during pregnancy (**Scorrano et al. 2024**). The clinical literature currently lacks long-term follow up of these patients and likewise lacks data on children exposed to subclinical levels of NMDAR antibodies, thus translation into these contexts can only be speculated on currently. However, studies indicating improper development of the striatum underpinning a range of neurodevelopmental disorders (**Singer et al. 2009, Braunschweig et al. 2012, Brimberg et al. 2016, Coutinho et al. 2017a, Jurek et al. 2019**) along with reports of increased NMDAR antibodies in mothers of children with similar disorders (**Jurek et al. 2019**) is suggestive of a possible link and an important direction for future study.

7.2.3. Management, rescue and reversibility of NMDAR antibody impacts

7.2.3.1 Management of NMDARE patients

Management of antibody-mediated diseases such as NMDARE presents an ongoing clinical challenge. The acute phase of the disease can be very severe, with mortality estimated around 5% (**Irani et al. 2010, Titulaer et al. 2013**). Although outcomes are generally positive, persistent psychiatric and neurological sequelae are not uncommon, and relapse occurs in around 15% (**Titulaer et al. 2013, Finke et al. 2017, De Bruijn et al. 2018**). Further, approaches to treatment are heterogeneous at present owing to a lack of randomised control trials and incomplete understanding of complex disease mechanisms (**Bartolini and Muscal 2017, Kahn et al. 2017**).

Early initiation of treatment is associated with improved long-term outcomes (**Irani et al. 2013, Titulaer et al. 2013**). Management of NMDARE focuses on reduction and removal of circulating antibodies. First line therapy typically involves expeditious immunosuppressive and immunomodulatory treatments including steroids, IVIG and plasmapheresis (**Titulaer et al. 2013, Nosadini et al. 2015, Malviya et al. 2017, Zhang et al. 2017a**). Alongside immunotherapy, tumour removal is performed if relevant, as well as management of seizures with anti-epileptic drugs (**Neyens et al. 2018, De Bruijn et al. 2019, Wang et al. 2019**), and other supportive measures (**Titulaer et al. 2013, Nosadini et al. 2015**). For patients unresponsive to first-line management after 4 weeks (~50% cases), treatment is escalated to second line options, with the mainstay of treatment typically being rituximab or other anti-CD20 monoclonal antibodies, along with other options including cyclophosphamide, azathioprine and mycophenolate mofetil (**Titulaer et al. 2013, Nosadini et al. 2015, Damato et al. 2016, Lee et al. 2016**). Although rituximab is highly effective in depleting B-cells, the relapses that often occur following its discontinuation suggest it cannot prevent autoreactive B-cells reoccurrence (**Chamberlain et al. 2016**).

Novel methods of antibody reduction continue to be developed. For NMDARE, two different approaches to deplete plasma cells offer promising approaches: protease inhibitor bortezomib and anti-CD38 monoclonal antibody daratumumab, both of which have shown to improve clinical outcomes and produce profound decrease in antibody titres in a small number of case reports of severe refractory NMDARE (**Scheibe et al. 2017, Ratuszny et al. 2020**). More widely, other approaches have shown potential clinical benefit in a range of antibody-mediated diseases that may also offer potential benefit to NMDARE patients. In neuromyelitis optica spectrum disorder (NMOSD), clinical benefits of depleting B-cells with anti-CD19 monoclonal antibody inebilizumab (**Cree et al. 2019**), inactivating complement with eculizumab (**Pittock et al. 2019**) and inhibiting the IL-6 pathway using satralizumab (**Yamamura et al. 2019**) have been shown in high-powered randomised control trials. Both bortezomib and daratumumab have also demonstrated utility beyond NMDARE, with positive clinical impacts also seen in NMOSD (**Zhang et al. 2017b**), and CASPR2 encephalitis (**Scheibe et al. 2020**) respectively, highlighting translatability of approaches between different antibody-mediated disorders. Several therapeutic options currently being experimentally investigated for antibody-mediated and other immunological disorders are also likely translatable here, the most exciting of which is the engineering of highly selective CAAR-T cells to identify and deplete B-cells that are specific to a singular antibody type such as NR1-IgG, with the key advent of reducing immunosuppressive side effects seen in the current less targeted therapeutic regimes (**Ellebrecht et al. 2016, Parvathaneni and Scott 2018**). Steric blockade of IgG binding using high-affinity molecules is another promising future option that may offer fewer side effects (**Duan et al. 2020**).

7.2.3.2 How should *in utero* NMDAR antibody exposure be managed?

The management of children potentially exposed to NMDAR antibodies during pregnancy presents a different clinical and ethical challenge. Antibodies are transplacentally transferred rather than being produced by the foetus itself (**Palmeira et al. 2012, Giannocco et al. 2020**), and literature suggests

that neurological deficits arise from transient exposure to antibodies during critical periods of foetal neurodevelopment rather than persistent antibody production (as is seen in NMDARE patients (**Titulaer et al. 2013, Chamberlain et al. 2016**)). Work in this thesis and other studies would suggest that even a short exposure has the potential to result in long-term neurological sequelae (**Brimberg et al. 2012, Jurek et al. 2019**), thus management here must largely focus on therapies to rescue or counteract underlying deficits resulting from early NMDAR disruption following transient antibody exposure, as well as prevention of transplacental transfer of antibodies if they are detected early enough.

To prevent transplacental transfer of antibodies, experimental evidence suggests that several methods of blocking the neonatal FcRn receptor offer promise. In rodents, daily administration of an antibody against the FcRn 6 hours prior to NR1-IgG injection at E14-16 prohibited transfer of antibodies to the foetus and prevented pathological alterations seen in the offspring of untreated dams (**García-Serra et al. 2021**). More broadly, in myasthenia gravis, inhibiting FcRn with efgartigimod or rozanolixizumab has demonstrated reduction in IgG titres in clinical trials (**Howard et al. 2019, Bril et al. 2021**). However, these approaches are largely reliant on antibodies being detected prior to transfer to the foetus, and therefore likely only applicable to a very small subset of individuals.

What the best method might be to counteract early *in utero* NMDAR disruption following NR1-IgG exposure is an interesting and open question. There are two broad approaches here; either help support functioning of undisrupted NMDARs or counteract NMDAR disruption through upregulating other potentially compensatory molecules/pathways. Experimental approaches to support existing function that have been trialled with success in animal models of early NMDAR disruption include stabilising NMDARs at the cell surface with stabilising protein EphB2 (**Mikasová et al. 2012, Planagumà et al. 2016**), and upregulating function with NMDAR co-agonists such as glycine or D-serine (**Bostrom et al. 2015**). Translating this clinically, small-molecule agonists for EphB2 may provide a useful

therapeutic option, with drugs such as doxazosin which agonise other Ephrin receptors already in clinical use for other diseases (**Petty et al. 2012**), and glycine being a licenced therapeutic option in the management of other neurodevelopmental (and wider) disorders including schizophrenia (**Javitt et al. 2001**).

Counteracting early NMDAR disruption through upregulating other potentially compensatory molecules and pathways also offers good therapeutic potential, particularly upregulating other methods of glutamatergic transmission. In this vein, the final part of the thesis explored whether boosting glutamatergic transmission through the other major type of glutamate receptor (AMPA) could 'rescue' the early behavioural phenotype in animals exposed to NMDAR antibodies *in utero* and it was identified that the phenotype was able to be partially rescued through this approach. To my knowledge, this is the first time this type of rescue has been used in an antibody model and offers a potential therapeutic postnatal management option for babies exposed to NMDAR antibodies *in utero*. This approach was adopted from a model of neurodegenerative Huntington's disease which also demonstrated mitigation of cellular, morphological and electrophysiological disruption in the early behavioural phenotype (**Braz et al. 2022**). In their study, the rescue only delayed onset rather than fully preventing pathological phenotype emergence, but this approach may be more clinically useful in the NMDAR antibody context, owing to its neurodevelopmental rather than neurodegenerative nature, without the build-up of pathological proteins. AMPAkinase may also be particularly useful in a striatal context, with studies of pain demonstrating their full potentiation of the corticostriatal pathway (**Zeng et al. 2021**). It is also worth noting however that AMPAkinase have been investigated for other neurodevelopmental disorders including schizophrenia and ADHD and shown *in vitro* promise but disappointing translatability to the clinical setting so far (**Johnson and Simon 2002, Lynch and Gall 2006, Goff et al. 2008, Kanju et al. 2008, Greer and Ren 2009**).

Beyond AMPARs, metabotropic glutamate receptors (mGluRs) also have roles in early neurogenesis. Relevant to the findings in this thesis, within the striatum mGluR3 and mGluR5 have a suggested role in progenitor proliferation in negative and positive directions respectively (**Gandhi et al. 2008, Vernon et al. 2011**), with antagonism of mGluR5 also shown to result in significantly fewer adult striatal neurons *in vitro* (**Gandhi et al. 2008**). There is ongoing research into the use of potential mGlu drugs for treatment of a range of neurological disorders, notably neurodevelopmental disorder Fragile X syndrome (**Nicoletti et al. 2011**).

Regenerative medicine approaches may also offer therapeutic potential, including the replacement of defective neurons or repair of damaged circuits through cell transplantation. Established *in vitro* methods using human embryonic stem cells present the option to produce large-scale neuronal populations through carefully defined differentiation protocols (**Grade and Götz 2017**). Relevant to the work in this thesis, recent experiments have generated human striatal progenitors neurochemically and morphologically similar to human SPNs which survived up to 6-months post transplantation into a rat model of Huntington's disease, forming local and long-range connections and improving impaired motor behaviour (**Schellino et al. 2023**). This method could be useful in increasing the reduced striatal neuronal numbers in our model to physiological levels, and might offer the possibility of addressing finer-scale circuit alterations such as specific deficits in D2-SPN numbers. Clinical studies of neurodegenerative diseases indicate at least a degree of translatability, with human embryonic dopamine neuron transplants in patients with severe Parkinson's demonstrating clinical benefit in younger patients, and continued survival at one year (**Freed et al. 2001**), although other trials have shown poorer success rates, and there are ongoing issues with post-transplant cell survival, risk of tumour formation and ethical factors that also need to be considered (**Liu et al. 2021**).

The findings from the rescue model in this thesis raise the possibility that in the clinical setting, treating *in utero* exposed babies with a similar rescue during the early postnatal period could alter disease

course. A final interesting consideration surrounds the difficulties in identifying which babies might warrant treatment to mitigate downstream pathological neurological outcomes. Whilst babies born to mothers with NMDARE during pregnancy are a clear potential candidate, antibodies against NMDAR are also harboured by a relatively large (1%) asymptomatic population (**Dahm et al. 2014, Lang and Prüss 2017**), and it has been shown that even subclinical titres of maternal antibodies have the potential to accumulate in the foetal brain at titres high enough to permanently alter synaptic function (**Jurek et al. 2019**). These findings are reflected clinically in the observation of a marginal increase in NMDAR antibody titres in a population of mothers of children with a mixed range of neuropsychiatric disorders relative to healthy controls (**Jurek et al. 2019**). However, it is likely not all babies exposed to NMDAR antibodies will experience neurodevelopmental consequences. Indeed, even in children born to mothers with NMDARE, antibody pathogenicity appears likely restricted to cases of higher serum antibody titres, with many neonates born apparently healthy (**Jagota et al. 2014, Hilderink et al. 2015, Chourasia et al. 2017, Shi et al. 2017, Kalam et al. 2019, Joubert et al. 2020**). Furthermore, there is also literature pointing to potential natural reversibility of antibody impacts, with several pre-clinical studies demonstrating reversal of elements of the early behavioural phenotypes in NR1-IgG *in utero* exposed animals as they reached adulthood (**Jurek et al. 2019, García-Serra et al. 2020**). It has also been demonstrated in a mouse congenital loss-of-function model that gene editing to restore NR1 function can produce robust cognitive improvements, hinting at the possibility that neurodevelopmental impairment secondary to early NMDAR disruption may be able to be treated much further down the developmental timeline (**Mielnik et al. 2020**). Should we be testing all mothers and babies for these antibodies? Would it be appropriate to administer a rescue to any baby that has these antibodies present? Is it possible to rescue them later down the developmental timeline? What is clear is that the clinical literature is currently lacking and future study of pregnant NMDARE patients

and/or asymptomatic people with NMDAR antibodies with close long-term follow up of their babies is an important step in terms of accurately assessing outcomes and guiding clinical management.

7.3 Future Directions

Work in this thesis has identified significant impacts on the developing striatum both embryonically and postnatally following *in utero* exposure to NMDAR NR1-IgG antibodies, with translation into the observed behavioural phenotype. It provides the basis for several interesting avenues of future exploration.

Firstly, it would be interesting to further explore impacts of NMDAR antibodies on progenitors in the striatum and other regions. It is increasingly apparent that embryonic progenitor diversity directly reflects onto neuronal diversity, functionality and synaptic and circuit connectivity across the brain, including in the striatum (**Yu et al. 2009, Tyler et al. 2015, Kelly et al. 2018, Ellender et al. 2019, Guillamon-Vivancos et al. 2019, Matsushima and Graybiel 2020, Van Heusden, Macey-Dare et al. 2021**). Here it was found that progenitor proliferation and migration were impaired in the LGE embryonically, but that proliferation of a sub-population of Tα1-expressing progenitors appeared unimpacted. Are specific progenitor sub-populations impacted more than others? What governs this? What knock on effects may this have in striatal formation and function postnatally?

Secondly, it would be helpful to explore impacts on specific NMDAR subtypes. In this thesis and more widely, developmental and regional variation in NMDAR subtype has been identified, with different subtypes also established to have differing developmental functions (**Cull-Candy and Leszkiewicz 2004, Lau and Zukin 2007, Mony et al. 2009, Traynelis et al. 2010, Paoletti 2011**). How does NR1-IgG *in utero* exposure influence receptor subunit composition? Are specific receptor subtypes more heavily impacted? Is there any impact on the NR2B to NR2A developmental switch?

Thirdly, the behavioural phenotype observed in this study as well as others (**Jurek et al. 2019, García-Serra et al. 2020, Kuchling et al. 2023**), along with the clinical spectrum of symptoms associated with NMDARE and exposure to NMDAR antibodies, points to complex and multi-regional impacts across the

brain, unlikely to be limited to just the striatum, cortex and/or hippocampus. Thus, it is important to consider, what regions might be useful to investigate next? From this study, the BNST might present a good option since it holds a similar developmental origin to the striatum and has an emerging important role in multiple anxiety-related disorders (**Ahearn et al. 2007, Buff et al. 2017, Clauss et al. 2019, Feola et al. 2023**). The amygdala may also be useful to explore owing to the extensive psychiatric symptoms often seen in NMDARE patients (**Dalmau et al. 2019**) and its position within basal ganglia circuitry. Likewise, the thalamus as the other key connection of the striatum, and thalamostriatal connections (**Smith et al. 2014**) are certainly important to study.

Fourthly, in this thesis a shift in the D1/D2 ratio of postnatal SPNs was identified, with fewer D2-SPNs present following *in utero* exposure to NMDAR antibodies. Experimental evidence suggests the imbalance of the striatal direct and indirect pathways underpins a range of neurodevelopmental disorders including Tourette's (**McNaught and Mink 2011, Albin et al. 2018**), OCD (**Grabiell and Rauch 2000, Langen et al. 2011**), ADHD (**Del Campo et al. 2011**) and ASD (**Shepherd et al. 2013**). Thus, this finding provides a useful starting point for mechanistic insights into how NMDAR antibodies may disrupt striatal development specifically. In future it will be useful to understand why is the ratio shifted? Are D2-SPNs impacted in other ways eg. their intrinsic properties, synapses etc? Do downstream nuclei exhibit different activity patterns because of this purported imbalance?

Furthermore, in this thesis several alterations in microglia both embryonically and postnatally were observed, supporting the idea that altered neurodevelopment can emerge from disruption to both neural circuitry and regulatory immune pathways (**Zhan et al. 2014**), and that microglia may remain activated after an initial immune challenge during the late embryonic or early postnatal period (**Bilbo and Schwarz 2012**). Precise mechanisms for how NMDAR antibodies influence microglia would be useful to explore in future work. Do they impact microglia directly? Do microglia play a role in any of the specific neuronal impacts observed?

Additionally, in this thesis several alterations in early postnatal behaviour were found. There is mixed evidence about the long-term consequences of NMDAR antibodies and other antibodies following *in utero* exposure, with some studies suggesting these effects may at least partially reverse during adulthood (**Jurek et al. 2019, García-Serra et al. 2020**). It is important to therefore investigate if the altered behavioural phenotype seen normalises at later timepoints. What mechanisms potentially underly phenotype reversibility? Does this mitigate need for development of potential rescue options?

Finally, there is recent development of the first mouse model with a fully functional human immune system (**Chupp et al. 2024**). This offers an exciting potential extension to the model developed here, with likely reduction in inter-species confounding factors and thus much higher degree of clinical translatability. Such a model may be a particularly useful future tool in modelling *in utero* NMDAR antibody-mediated impacts and other antibody-mediated diseases given the high degrees of immunological complexity and multiple interacting mechanisms that are continuing to be uncovered (**Dalmau et al. 2019, Giannocco 2020, Prüss 2021**).

7.4 Conclusion

In conclusion, this thesis develops a novel *in utero* model for studying the impact of NMDAR antibodies, identifies impacts of these antibodies on embryonic progenitors in the striatum and on striatal cells and circuits postnatally, explores how this translates to an early postnatal behavioural phenotype, and demonstrates the phenotype can be partially rescued through boosting AMPARs using an AMPAkin. The work in this thesis offers potential translatability into the clinical setting as a strong starting point for how exposure to NMDAR antibodies *in utero* can alter striatal development specifically, as well as broader neurodevelopment from the earliest stages.

Together with previous and ongoing studies of NMDAR antibody and other antibody impacts, I hope it will help to provide a springboard in developing novel therapies and improving clinical outcomes for NMDARE patients and those exposed to NMDAR antibodies *in utero*.

8. Bibliography

- Aarts, M.,** Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J. W., Wang, Y. T., Salter, M. W., and Tymianski, M. (2002). Treatment of ischemic brain damage by perturbing NMDA receptor- PSD-95 protein interactions. *Science*, 298(5594), 846–850.
- Abbott, N. J.,** Patabendige, A. A., Dolman, D. E., Yusof, S. R., and Begley, D. J. (2010). Structure and function of the blood-brain barrier. *Neurobiology of Disease*, 37(1), 13–25.
- Abrahamsson, T.,** Chou, C. Y. C., Li, S. Y., Mancino, A., Costa, R. P., Brock, J. A., Nuro, E., Buchanan, K. A., Elgar, D., Blackman, A. V., Tudor-Jones, A., Oyrer, J., Farmer, W. T., Murai, K. K., and Sjöström, P. J. (2017). Differential Regulation of Evoked and Spontaneous Release by Presynaptic NMDA Receptors. *Neuron*, 96(4), 839–855.e5.
- Adesnik, H.,** Li, G., During, M. J., Pleasure, S. J., and Nicoll, R. A. (2008). NMDA receptors inhibit synapse unsilencing during brain development. *Proceedings of the National Academy of Sciences of the United States of America*, 105(14), 5597–5602.
- Ahearn, E. P.,** Juergens, T., Smith, T., Krahn, D., and Kalin, N. (2007). Fear, anxiety, and the neuroimaging of PTSD. *Psychopharmacology Bulletin*, 40(3), 88–103.
- Aizenman, C. D., and Cline, H. T.** (2007). Enhanced visual activity *in vivo* forms nascent synapses in the developing retinotectal projection. *Journal of Neurophysiology*, 97(4), 2949–2957.
- Ajami, B.,** Bennett, J. L., Krieger, C., Tetzlaff, W., and Rossi, F. M. (2007). Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nature Neuroscience*, 10(12), 1538–1543.
- Ajami, B.,** Bennett, J. L., Krieger, C., McNagny, K. M., and Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nature Neuroscience*, 14(9), 1142–1149.
- Akazawa, C.,** Shigemoto, R., Bessho, Y., Nakanishi, S., and Mizuno, N. (1994). Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. *The Journal of Comparative Neurology*, 347(1), 150–160.
- Åkerman, C. J., and Cline, H. T.** (2006). Depolarizing GABAergic conductances regulate the balance of excitation to inhibition in the developing retinotectal circuit *in vivo*. *The Journal of Neuroscience*, 26(19), 5117–5130.
- Akgül, G., and McBain, C. J.** (2016). Diverse roles for ionotropic glutamate receptors on inhibitory interneurons in developing and adult brain. *The Journal of Physiology*, 594(19), 5471–5490.
- Akgül, G.,** Abebe, D., Yuan, X. Q., Auville, K., and McBain, C. J. (2019). The Role of AMPARs in the Maturation and Integration of Caudal Ganglionic Eminence-Derived Interneurons into Developing Hippocampal Microcircuits. *Scientific Reports*, 9(1), 5435.
- Akgül, G., and McBain, C. J.** (2020). AMPA receptor deletion in developing MGE-derived hippocampal interneurons causes a redistribution of excitatory synapses and attenuates postnatal network oscillatory activity. *Scientific Reports*, 10(1), 1333.
- Aktas, O.,** Ullrich, O., Infante-Duarte, C., Nitsch, R., and Zipp, F. (2007). Neuronal damage in brain inflammation. *Archives of neurology*, 64(2), 185–189.
- Albin, R. L.,** Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences*, 12(10), 366–375.
- Albin, R. L.** (2018). Tourette syndrome: a disorder of the social decision-making network. *Brain*, 141(2), 332–347.
- Al-Diwani, A.,** Theorell, J., Damato, V., Bull, J., McGlashan, N., Green, E., Kienzler, A. K., Harrison, R., Hassanali, T., Campo, L., Browne, M., Easton, A., Soleymani Majd, H., Tenaka, K., Iorio, R., Dale, R. C., Harrison, P., Geddes, J., Quested, D., Sharp, D., ... Irani, S. R. (2022). Cervical lymph nodes and ovarian teratomas as germinal centres in NMDA receptor-antibody encephalitis. *Brain*, 145(8), 2742–2754.

- Alexander, G. E.,** DeLong, M. R., and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience*, 9, 357–381.
- Alexander, G. E., and Crutcher, M. D.** (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends in Neurosciences*, 13(7), 266–271.
- Al-Hallaq, R. A.,** Conrads, T. P., Veenstra, T. D., and Wenthold, R. J. (2007). NMDA di-heteromeric receptor populations and associated proteins in rat hippocampus. *The Journal of Neuroscience*, 27(31), 8334–8343.
- Allen, N. J.,** Káradóttir, R., and Attwell, D. (2004). Reversal or reduction of glutamate and GABA transport in CNS pathology and therapy. *European Journal of Physiology*, 449(2), 132–142.
- Allyson, J.,** Dontigny, E., Auberson, Y., Cyr, M., and Massicotte, G. (2010). Blockade of NR2A-containing NMDA receptors induces Tau phosphorylation in rat hippocampal slices. *Neural Plasticity*, 2010, 340168.
- Al-Mubarak, B.,** Soriano, F. X., and Hardingham, G. E. (2009). Synaptic NMDAR activity suppresses FOXO1 expression via a cis-acting FOXO binding site: FOXO1 is a FOXO target gene. *Channels*, 3(4), 233–238.
- Almutairi, M. M.,** Gong, C., Xu, Y. G., Chang, Y., and Shi, H. (2016). Factors controlling permeability of the blood-brain barrier. *Cellular and Molecular Life Sciences*, 73(1), 57–77.
- Alvarez, V. A.,** Ridenour, D. A., and Sabatini, B. L. (2007). Distinct structural and ionotropic roles of NMDA receptors in controlling spine and synapse stability. *The Journal of Neuroscience*, 27(28), 7365–7376.
- Alvarez-Buylla, A.,** García-Verdugo, J. M., and Tramontin, A. D. (2001). A unified hypothesis on the lineage of neural stem cells. *Nature Reviews. Neuroscience*, 2(4), 287–293.
- Anderson, S. R., and Vetter, M. L.** (2019). Developmental roles of microglia: A window into mechanisms of disease. *Developmental Dynamics*, 248(1), 98–117.
- Andreae, L. C., and Burrone, J.** (2015). Spontaneous Neurotransmitter Release Shapes Dendritic Arbors via Long-Range Activation of NMDA Receptors. *Cell Reports*, 10(6), 873–882.
- Andrzejak, E.,** Rabinovitch, E., Kreye, J., Prüss, H., Rosenmund, C., Ziv, N. E., Garner, C. C., and Ackermann, F. (2022). Patient-Derived Anti-NMDAR Antibody Disinhibits Cortical Neuronal Networks through Dysfunction of Inhibitory Neuron Output. *The Journal of Neuroscience*, 42(15), 3253–3270.
- Anthony, T. E., and Heintz, N.** (2008). Genetic lineage tracing defines distinct neurogenic and gliogenic stages of ventral telencephalic radial glial development. *Neural Development*, 3, 30.
- Antony, J. M.,** Paquin, A., Nutt, S. L., Kaplan, D. R., and Miller, F. D. (2011). Endogenous microglia regulate development of embryonic cortical precursor cells. *Journal of Neuroscience Research*, 89(3), 286–298.
- Arakawa, H., and Erzurumlu, R. S.** (2015). Role of whiskers in sensorimotor development of C57BL/6 mice. *Behavioural Brain Research*, 287, 146–155.
- Araki, T.,** Ikegaya, Y., and Koyama, R. (2021). The effects of microglia- and astrocyte-derived factors on neurogenesis in health and disease. *The European Journal of Neuroscience*, 54(5), 5880–5901.
- Arcuri, C.,** Mecca, C., Bianchi, R., Giambanco, I., and Donato, R. (2017). The Pathophysiological Role of Microglia in Dynamic Surveillance, Phagocytosis and Structural Remodeling of the Developing CNS. *Frontiers in Molecular Neuroscience*, 10, 191.
- Ariza, J.,** Hurtado, J., Rogers, H., Ikeda, R., Dill, M., Steward, C., Creary, D., Van de Water, J., and Martínez-Cerdeño, V. (2017). Maternal autoimmune antibodies alter the dendritic arbor and spine numbers in the infragranular layers of the cortex. *PloS one*, 12(8), e0183443.
- Arlotta, P.,** Molyneaux, B. J., Jabaudon, D., Yoshida, Y., and Macklis, J. D. (2008). Ctip2 controls the differentiation of medium spiny neurons and the establishment of the cellular architecture of the striatum. *The Journal of Neuroscience*, 28(3), 622–632.

- Armangué, T.,** Spatola, M., Vlagea, A., Mattozzi, S., Cárceles-Cordon, M., Martínez-Heras, E., Llufríu, S., Muchart, J., Erro, M. E., Abaira, L., Moris, G., Monros-Giménez, L., Corral-Corral, Í., Montejo, C., Toledo, M., Bataller, L., Secondi, G., Ariño, H., Martínez-Hernández, E., Juan, M., ... Spanish Herpes Simplex Encephalitis Study Group (2018). Frequency, symptoms, risk factors, and outcomes of autoimmune encephalitis after herpes simplex encephalitis: a prospective observational study and retrospective analysis. *The Lancet. Neurology*, 17(9), 760–772.
- Armangué, T.,** Titulaer, M. J., Málaga, I., Bataller, L., Gabilondo, I., Graus, F., Dalmau, J., and Spanish Anti-N-methyl-D-Aspartate Receptor (NMDAR) Encephalitis Work Group (2013). Pediatric anti-N-methyl-D-aspartate receptor encephalitis-clinical analysis and novel findings in a series of 20 patients. *The Journal of Pediatrics*, 162(4), 850–856.e2.
- Arnoux, I.,** Hoshiko, M., Mandavy, L., Avignone, E., Yamamoto, N., and Audinat, E. (2013). Adaptive phenotype of microglial cells during the normal postnatal development of the somatosensory "Barrel" cortex. *Glia*, 61(10), 1582–1594.
- Arriza, J. L.,** Eliasof, S., Kavanaugh, M. P., and Amara, S. G. (1997). Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proceedings of the National Academy of Sciences of the United States of America*, 94(8), 4155–4160.
- Asahi, M.,** Hoshimaru, M., Hojo, M., Matsuura, N., Kikuchi, H., and Hashimoto, N. (1998). Induction of the N-methyl-D-aspartate receptor subunit 1 in the immortalized neuronal progenitor cell line HC2S2 during differentiation into neurons. *Journal of Neuroscience Research*, 52(6), 699–708.
- Atallah, H. E.,** McCool, A. D., Howe, M. W., and Graybiel, A. M. (2014). Neurons in the ventral striatum exhibit cell-type-specific representations of outcome during learning. *Neuron*, 82(5), 1145–1156.
- Atladóttir, H. O.,** Pedersen, M. G., Thorsen, P., Mortensen, P. B., Deleuran, B., Eaton, W. W., and Parner, E. T. (2009). Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics*, 124(2), 687–694.
- Avery, S. N.,** Clauss, J. A., Winder, D. G., Woodward, N., Heckers, S., and Blackford, J. U. (2014). BNST neurocircuitry in humans. *NeuroImage*, 91, 311–323.
- Báez-Mendoza, R., and Schultz, W.** (2013). The role of the striatum in social behavior. *Frontiers in Neuroscience*, 7, 233.
- Bahn, S.,** Volk, B., and Wisden, W. (1994). Kainate receptor gene expression in the developing rat brain. *The Journal of Neuroscience*, 14(9), 5525–5547.
- Bai, F.,** Bergeron, M., and Nelson, D. L. (2003). Chronic AMPA receptor potentiator (LY451646) treatment increases cell proliferation in adult rat hippocampus. *Neuropharmacology*, 44(8), 1013–1021.
- Bain, G.,** Kitchens, D., Yao, M., Huettner, J. E., and Gottlieb, D. I. (1995). Embryonic stem cells express neuronal properties *in vitro*. *Developmental Biology*, 168(2), 342–357.
- Balleine, B. W., and Dickinson, A.** (1998). Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology*, 37(4-5), 407–419.
- Balleine, B. W.,** Delgado, M. R., and Hikosaka, O. (2007). The role of the dorsal striatum in reward and decision-making. *The Journal of Neuroscience*, 27(31), 8161–8165.
- Balleine, B. W., and O'Doherty, J. P.** (2010). Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology*, 35(1), 48–69.
- Balsters, J. H.,** Zerbi, V., Sallet, J., Wenderoth, N., and Mars, R. B. (2020). Primate homologs of mouse cortico-striatal circuits. *eLife*, 9, e53680.
- Bandler, R. C.,** Mayer, C., and Fishell, G. (2017). Cortical interneuron specification: the juncture of genes, time and geometry. *Current Opinion in Neurobiology*, 42, 17–24.
- Banerjee, A.,** González-Rueda, A., Sampaio-Baptista, C., Paulsen, O., and Rodríguez-Moreno, A. (2014). Distinct mechanisms of spike timing-dependent LTD

at vertical and horizontal inputs onto L2/3 pyramidal neurons in mouse barrel cortex. *Physiological reports*, 2(3), e00271.

Banerjee, A., Larsen, R. S., Philpot, B. D., and Paulsen, O. (2016). Roles of Presynaptic NMDA Receptors in Neurotransmission and Plasticity. *Trends in Neurosciences*, 39(1), 26–39.

Bannai, H., Lévi, S., Schweizer, C., Inoue, T., Launey, T., Racine, V., Sibarita, J. B., Mikoshiba, K., and Triller, A. (2009). Activity-dependent tuning of inhibitory neurotransmission based on GABAAR diffusion dynamics. *Neuron*, 62(5), 670–682.

Barbera, G., Liang, B., Zhang, L., Gerfen, C. R., Culurciello, E., Chen, R., Li, Y., and Lin, D. T. (2016). Spatially Compact Neural Clusters in the Dorsal Striatum Encode Locomotion Relevant Information. *Neuron*, 92(1), 202–213.

Bariselli, S., Fobbs, W. C., Creed, M. C., and Kravitz, A. V. (2019). A competitive model for striatal action selection. *Brain Research*, 1713, 70–79.

Baron, A., Montagne, A., Cassé, F., Launay, S., Maubert, E., Ali, C., and Vivien, D. (2010). NR2D-containing NMDA receptors mediate tissue plasminogen activator-promoted neuronal excitotoxicity. *Cell Death and Differentiation*, 17(5), 860–871.

Barria, A., and Malinow, R. (2005). NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron*, 48(2), 289–301.

Barry, H., Byrne, S., Barrett, E., Murphy, K. C., and Cotter, D. R. (2015). Anti-N-methyl-d-aspartate receptor encephalitis: review of clinical presentation, diagnosis and treatment. *British Journal of Psychiatry Bulletin*, 39(1), 19–23.

Barth, A. L., and Malenka, R. C. (2001). NMDAR EPSC kinetics do not regulate the critical period for LTP at thalamocortical synapses. *Nature Neuroscience*, 4(3), 235–236.

Bartolini, L., and Muscal, E. (2017). Differences in treatment of anti-NMDA receptor encephalitis: results of a worldwide survey. *Journal of Neurology*, 264(4), 647–653.

Baumgart, J., and Baumgart, N. (2016). Cortex-, Hippocampus-, Thalamus-, Hypothalamus-, Lateral Septal Nucleus- and Striatum-specific *In utero* Electroporation in the C57BL/6 Mouse. *Journal of Visualized Experiments*, (107), e53303.

Bearzatto, B., Servais, L., Cheron, G., and Schiffmann, S. N. (2005). Age dependence of strain determinant on mice motor coordination. *Brain Research*, 1039(1-2), 37–42.

Beattie, E. C., Stellwagen, D., Morishita, W., Bresnahan, J. C., Ha, B. K., Von Zastrow, M., Beattie, M. S., and Malenka, R. C. (2002). Control of synaptic strength by glial TNF α . *Science*, 295(5563), 2282–2285.

Beauchamp, A., Yee, Y., Darwin, B. C., Raznahan, A., Mars, R. B., and Lerch, J. P. (2022). Whole-brain comparison of rodent and human brains using spatial transcriptomics. *eLife*, 11, e79418.

Behar, T. N., Scott, C. A., Greene, C. L., Wen, X., Smith, S. V., Maric, D., Liu, Q. Y., Colton, C. A., and Barker, J. L. (1999). Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *The Journal of Neuroscience*, 19(11), 4449–4461.

Belgard, T. G., Marques, A. C., Oliver, P. L., Abaan, H. O., Sirey, T. M., Hoerder-Suabedissen, A., García-Moreno, F., Molnár, Z., Margulies, E. H., and Ponting, C. P. (2011). A transcriptomic atlas of mouse neocortical layers. *Neuron*, 71(4), 605–616.

Belin, D., Jonkman, S., Dickinson, A., Robbins, T. W., and Everitt, B. J. (2009). Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction. *Behavioural Brain Research*, 199(1), 89–102.

Belleau, M. L., and Warren, R. A. (2000). Postnatal development of electrophysiological properties of nucleus accumbens neurons. *Journal of Neurophysiology*, 84(5), 2204–2216.

Bellingham M. C. (2011). A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis:

what have we learned in the last decade?. *CNS Neuroscience and Therapeutics*, 17(1), 4–31.

Ben-Ari, Y., Gaiarsa, J. L., Tyzio, R., and Khazipov, R. (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiological Reviews*, 87(4), 1215–1284.

Benthall, K. N., Ong, S. L., and Bateup, H. S. (2018). Corticostriatal Transmission Is Selectively Enhanced in Striatonigral Neurons with Postnatal Loss of Tsc1. *Cell Reports*, 23(11), 3197–3208.

Bergeron, R., Meyer, T. M., Coyle, J. T., and Greene, R. W. (1998). Modulation of N-methyl-D-aspartate receptor function by glycine transport. *Proceedings of the National Academy of Sciences of the United States of America*, 95(26), 15730–15734.

Bernier, L. P., Bohlen, C. J., York, E. M., Choi, H. B., Kamyabi, A., Dissing-Olesen, L., Hefendehl, J. K., Collins, H. Y., Stevens, B., Barres, B. A., and MacVicar, B. A. (2019). Nanoscale Surveillance of the Brain by Microglia via cAMP-Regulated Filopodia. *Cell Reports*, 27(10), 2895–2908.e4.

Beutler, L. R., Eldred, K. C., Quintana, A., Keene, C. D., Rose, S. E., Postupna, N., Montine, T. J., and Palmiter, R. D. (2011). Severely impaired learning and altered neuronal morphology in mice lacking NMDA receptors in medium spiny neurons. *PLoS one*, 6(11), e28168.

Bien, C. G., Vincent, A., Barnett, M. H., Becker, A. J., Blümcke, I., Graus, F., Jellinger, K. A., Reuss, D. E., Ribalta, T., Schlegel, J., Sutton, I., Lassmann, H., and Bauer, J. (2012). Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain*, 135(Pt 5), 1622–1638.

Bilbo, S. D., and Schwarz, J. M. (2012). The immune system and developmental programming of brain and behavior. *Frontiers in Neuroendocrinology*, 33(3), 267–286.

Bilimoria, P. M., and Bonni, A. (2008). Cultures of cerebellar granule neurons. *CSH Protocols*, 2008, pdb.prot5107.

Billups, D., and Attwell, D. (2003). Active release of glycine or D-serine saturates the glycine site of NMDA receptors at the cerebellar mossy fibre to granule cell synapse. *The European Journal of Neuroscience*, 18(11), 2975–2980.

Blanton, M. G., and Kriegstein, A. R. (1991). Appearance of putative amino acid neurotransmitters during differentiation of neurons in embryonic turtle cerebral cortex. *The Journal of Comparative Neurology*, 310(4), 571–592.

Bolam, J. P., and Izzo, P. N. (1988). The postsynaptic targets of substance P-immunoreactive terminals in the rat neostriatum with particular reference to identified spiny striatonigral neurons. *Experimental Brain Research*, 70(2), 361–377.

Bolam, J. P., Hanley, J. J., Booth, P. A., and Bevan, M. D. (2000). Synaptic organisation of the basal ganglia. *Journal of Anatomy*, 196 (Pt 4)(Pt 4), 527–542.

Bortone, D., and Polleux, F. (2009). KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. *Neuron*, 62(1), 53–71.

Bostrom, C. A., Majaess, N. M., Morch, K., White, E., Eadie, B. D., and Christie, B. R. (2015). Rescue of NMDAR-dependent synaptic plasticity in Fmr1 knock-out mice. *Cerebral Cortex*, 25(1), 271–279.

Boulland, J. L., Qureshi, T., Seal, R. P., Rafiki, A., Gundersen, V., Bergersen, L. H., Fremieu, R. T., Jr, Edwards, R. H., Storm-Mathisen, J., and Chaudhry, F. A. (2004). Expression of the vesicular glutamate transporters during development indicates the widespread corelease of multiple neurotransmitters. *The Journal of Comparative Neurology*, 480(3), 264–280.

Brambell, F. W., and Hemmings, W. A. (1949). The passage into the embryonic yolk-sac cavity of maternal plasma proteins in rabbits. *The Journal of Physiology*, 108(2), 177–185.

Brambell, F. W., and Halliday, R. (1956). The route by which passive immunity is transmitted from mother to foetus in the rat. *Proceedings of the Royal*

Society of London. Series B, Biological Sciences, 145(919), 170–178.

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Ng, L. G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B. T., Diamond, B., and Pettersson, S. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science Translational Medicine*, 6(263), 263ra158.

Brasier, D. J., and Feldman, D. E. (2008). Synapse-specific expression of functional presynaptic NMDA receptors in rat somatosensory cortex. *The Journal of Neuroscience*, 28(9), 2199–2211.

Braunschweig, D., Golub, M. S., Koenig, C. M., Qi, L., Pessah, I. N., Van de Water, J., and Berman, R. F. (2012). Maternal autism-associated IgG antibodies delay development and produce anxiety in a mouse gestational transfer model. *Journal of Neuroimmunology*, 252(1-2), 56–65.

Braz, B. Y., Wennagel, D., Ratié, L., de Souza, D. A. R., Deloulme, J. C., Barbier, E. L., Buisson, A., Lanté, F., and Humbert, S. (2022). Treating early postnatal circuit defect delays Huntington's disease onset and pathology in mice. *Science*, 377(6613), eabq5011.

Bril, V., Benatar, M., Andersen, H., Vissing, J., Brock, M., Greve, B., Kiessling, P., Woltering, F., Griffin, L., Van den Bergh, P., and MG0002 Investigators (2021). Efficacy and Safety of Rozanolixumab in Moderate to Severe Generalized Myasthenia Gravis: A Phase 2 Randomized Control Trial. *Neurology*, 96(6), e853–e865.

Brimberg, L., Mader, S., Jeganathan, V., Berlin, R., Coleman, T. R., Gregersen, P. K., Huerta, P. T., Volpe, B. T., and Diamond, B. (2016). Caspr2-reactive antibody cloned from a mother of an ASD child mediates an ASD-like phenotype in mice. *Molecular Psychiatry*, 21(12), 1663–1671.

Brockhaus, J., Möller, T., and Kettenmann, H. (1996). Phagocytosing ameboid microglial cells studied in a mouse corpus callosum slice preparation. *Glia*, 16(1), 81–90.

Brown, S. P., and Hestrin, S. (2009). Intracortical circuits of pyramidal neurons reflect their long-range axonal targets. *Nature*, 457(7233), 1133–1136.

Buchanan, K. A., Blackman, A. V., Moreau, A. W., Elgar, D., Costa, R. P., Lalanne, T., Tudor Jones, A. A., Oyler, J., and Sjöström, P. J. (2012). Target-specific expression of presynaptic NMDA receptors in neocortical microcircuits. *Neuron*, 75(3), 451–466.

Buchwald, N. A., Price, D. D., Vernon, L., and Hull, C. D. (1973). Caudate intracellular response to thalamic and cortical inputs. *Experimental Neurology*, 38(2), 311–323.

Buff, C., Brinkmann, L., Bruchmann, M., Becker, M. P. I., Tupak, S., Herrmann, M. J., and Straube, T. (2017). Activity alterations in the bed nucleus of the stria terminalis and amygdala during threat anticipation in generalized anxiety disorder. *Social Cognitive and Affective Neuroscience*, 12(11), 1766–1774.

Buren, C., Tu, G., Parsons, M. P., Sepers, M. D., and Raymond, L. A. (2016). Influence of cortical synaptic input on striatal neuronal dendritic arborization and sensitivity to excitotoxicity in corticostriatal coculture. *Journal of Neurophysiology*, 116(2), 380–390.

Burnashev, N., and Szepietowski, P. (2015). NMDA receptor subunit mutations in neurodevelopmental disorders. *Current opinion in pharmacology*, 20, 73–82.

Burzomato, V., Frugier, G., Pérez-Otaño, I., Kittler, J. T., and Attwell, D. (2010). The receptor subunits generating NMDA receptor mediated currents in oligodendrocytes. *The Journal of Physiology*, 588(Pt 18), 3403–3414.

Bustos, F. J., Jury, N., Martínez, P., Ampuero, E., Campos, M., Abarzúa, S., Jaramillo, K., Ibing, S., Mardones, M. D., Haensgen, H., Kzhyskowska, J., Tevy, M. F., Neve, R., Sanhueza, M., Varela-Nallar, L., Montecino, M., and Van Zundert, B. (2017). NMDA receptor subunit composition controls dendritogenesis of hippocampal neurons through CAMKII, CREB-P, and H3K27ac. *Journal of Cellular Physiology*, 232(12), 3677–3692.

- Butovsky, O.,** Jedrychowski, M. P., Moore, C. S., Cialic, R., Lanser, A. J., Gabriely, G., Koeglsperger, T., Dake, B., Wu, P. M., Doykan, C. E., Fanek, Z., Liu, L., Chen, Z., Rothstein, J. D., Ransohoff, R. M., Gygi, S. P., Antel, J. P., and Weiner, H. L. (2014). Identification of a unique TGF- β -dependent molecular and functional signature in microglia. *Nature Neuroscience*, 17(1), 131–143.
- Cadwell, C. R.,** Scala, F., Fahey, P. G., Kobak, D., Mulherkar, S., Sinz, F. H., Papadopoulos, S., Tan, Z. H., Johnsson, P., Hartmanis, L., Li, S., Cotton, R. J., Tolias, K. F., Sandberg, R., Berens, P., Jiang, X., and Tolias, A. S. (2020). Cell type composition and circuit organization of clonally related excitatory neurons in the juvenile mouse neocortex. *eLife*, 9, e52951.
- Calabresi, P.,** Maj, R., Pisani, A., Mercuri, N. B., and Bernardi, G. (1992). Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *The Journal of Neuroscience*, 12(11), 4224–4233.
- Calhoon, G. G., and Tye, K. M.** (2015). Resolving the neural circuits of anxiety. *Nature Neuroscience*, 18(10), 1394–1404.
- Camacho, J.,** Jones, K., Miller, E., Ariza, J., Noctor, S., de Water, J. V., and Martínez-Cerdeño, V. (2014). Embryonic intraventricular exposure to autism-specific maternal autoantibodies produces alterations in autistic-like stereotypical behaviors in offspring mice. *Behavioural Brain Research*, 266, 46–51.
- Camdessanché, J. P.,** Streichenberger, N., Cavillon, G., Rogemond, V., Jousserand, G., Honnorat, J., Convers, P., and Antoine, J. C. (2011). Brain immunohistopathological study in a patient with anti-NMDAR encephalitis. *European Journal of Neurology*, 18(6), 929–931.
- Canudas, A. M.,** Di Giorgi-Gerevini, V., Iacovelli, L., Nano, G., D'Onofrio, M., Arcella, A., Giangaspero, F., Busceti, C., Ricci-Vitiani, L., Battaglia, G., Nicoletti, F., and Melchiorri, D. (2004). PHCCC, a specific enhancer of type 4 metabotropic glutamate receptors, reduces proliferation and promotes differentiation of cerebellar granule cell neuroprecursors. *The Journal of Neuroscience*, 24(46), 10343–10352.
- Carter, A. G., and Sabatini, B. L.** (2004). State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons. *Neuron*, 44(3), 483–493.
- Carter, A. G.,** Soler-Llavina, G. J., and Sabatini, B. L. (2007). Timing and location of synaptic inputs determine modes of subthreshold integration in striatal medium spiny neurons. *The Journal of Neuroscience*, 27(33), 8967–8977.
- Casado, M.,** López-Guajardo, A., Mellström, B., Naranjo, J. R., and Lerma, J. (1996). Functional N-methyl-D-aspartate receptors in clonal rat pheochromocytoma cells. *The Journal of Physiology*, 490 (Pt 2)(Pt 2), 391–404.
- Castillo-Gómez, E.,** Oliveira, B., Tapken, D., Bertrand, S., Klein-Schmidt, C., Pan, H., Zafeiriou, P., Steiner, J., Jurek, B., Trippe, R., Prüss, H., Zimmermann, W. H., Bertrand, D., Ehrenreich, H., and Hollmann, M. (2017). All naturally occurring autoantibodies against the NMDA receptor subunit NR1 have pathogenic potential irrespective of epitope and immunoglobulin class. *Molecular Psychiatry*, 22(12), 1776–1784.
- Cataldi, S.,** Stanley, A. T., Miniaci, M. C., and Sulzer, D. (2022). Interpreting the role of the striatum during multiple phases of motor learning. *The FEBS journal*, 289(8), 2263–2281.
- Cau, E.,** Casarosa, S., and Guillemot, F. (2002). Mash1 and Ngn1 control distinct steps of determination and differentiation in the olfactory sensory neuron lineage. *Development*, 129(8), 1871–1880.
- Cazorla, M.,** Shegda, M., Ramesh, B., Harrison, N. L., and Kellendonk, C. (2012). Striatal D2 receptors regulate dendritic morphology of medium spiny neurons via Kir2 channels. *The Journal of Neuroscience*, 32(7), 2398–2409.
- Cepeda, C.,** Galvan, L., Holley, S. M., Rao, S. P., André, V. M., Botelho, E. P., Chen, J. Y., Watson, J. B., Deisseroth, K., and Levine, M. S. (2013). Multiple sources of striatal inhibition are differentially affected in Huntington's disease mouse models. *The Journal of Neuroscience*, 33(17), 7393–7406.

- Chamberlain, S. E.,** Yang, J., and Jones, R. S. (2008). The role of NMDA receptor subtypes in short-term plasticity in the rat entorhinal cortex. *Neural Plasticity*, 2008, 872456.
- Chamberlain, N.,** Massad, C., Oe, T., Cantaert, T., Herold, K. C., and Meffre, E. (2016). Rituximab does not reset defective early B-cell tolerance checkpoints. *The Journal of Clinical Investigation*, 126(1), 282–287.
- Chang, G. Q.,** Karatayev, O., Boorgu, D. S. S. K., and Leibowitz, S. F. (2020). Third Ventricular Injection of CCL2 in Rat Embryo Stimulates CCL2/CCR2 Neuroimmune System in Neuroepithelial Radial Glia Progenitor Cells: Relation to Sexually Dimorphic, Stimulatory Effects on Peptide Neurons in Lateral Hypothalamus. *Neuroscience*, 443, 188–205.
- Chang, H.,** Ma, J., Feng, K., Feng, N., Wang, X., Sun, J., Guo, T., Wei, Y., Xu, Y., Wang, H., Yin, L., and Zhang, X. (2023). Elevated blood and cerebrospinal fluid biomarkers of microglial activation and blood–brain barrier disruption in anti-NMDA receptor encephalitis. *Journal of Neuroinflammation*, 20(1), 172.
- Chatterton, J. E.,** Awobuluyi, M., Premkumar, L. S., Takahashi, H., Talantova, M., Shin, Y., Cui, J., Tu, S., Sevarino, K. A., Nakanishi, N., Tong, G., Lipton, S. A., and Zhang, D. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature*, 415(6873), 793–798.
- Chen, V. S.,** Morrison, J. P., Southwell, M. F., Foley, J. F., Bolon, B., and Elmore, S. A. (2017). Histology Atlas of the Developing Prenatal and Postnatal Mouse Central Nervous System, with Emphasis on Prenatal Days E7.5 to E18.5. *Toxicologic Pathology*, 45(6), 705–744.
- Chen, J.,** Ding, Y., Zheng, D., Wang, Z., Pan, S., Ji, T., Shen, H. Y., and Wang, H. (2018). Elevation of YKL-40 in the CSF of Anti-NMDAR Encephalitis Patients Is Associated With Poor Prognosis. *Frontiers in Neurology*, 9, 727.
- Cherubini, E.,** Gaiarsa, J. L., and Ben-Ari, Y. (1991). GABA: an excitatory transmitter in early postnatal life. *Trends in Neurosciences*, 14(12), 515–519.
- Chini, M., and Hanganu-Opatz, I. L.** (2021). Prefrontal Cortex Development in Health and Disease: Lessons from Rodents and Humans. *Trends in Neurosciences*, 44(3), 227–240.
- Chittajallu, R.,** Wester, J. C., Craig, M. T., Barksdale, E., Yuan, X. Q., Akgül, G., Fang, C., Collins, D., Hunt, S., Pelkey, K. A., and McBain, C. J. (2017). Afferent specific role of NMDA receptors for the circuit integration of hippocampal neurogliaform cells. *Nature Communications*, 8(1), 152.
- Chourasia, N.,** Watkins, M. W., Lankford, J. E., Kass, J. S., and Kamdar, A. (2018). An Infant Born to a Mother With Anti-N-Methyl-d-Aspartate Receptor Encephalitis. *Pediatric Neurology*, 79, 65–68.
- Christensen, J.,** Sørensen, J. C., Ostergaard, K., and Zimmer, J. (1999). Early postnatal development of the rat corticostriatal pathway: an anterograde axonal tracing study using biocytin pellets. *Anatomy and embryology*, 200(1), 73–80.
- Chrivia, J. C.,** Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R., and Goodman, R. H. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature*, 365(6449), 855–859.
- Chronister, R. B.,** Farnell, K. E., Marco, L. A., and White, L. E., Jr (1976). The rodent neostriatum: a Golgi analysis. *Brain Research*, 108(1), 37–46.
- Chung, C.,** Marson, J. D., Zhang, Q. G., Kim, J., Wu, W. H., Brann, D. W., and Chen, B. S. (2016). Neuroprotection Mediated through GluN2C-Containing N-methyl-D-aspartate (NMDA) Receptors Following Ischemia. *Scientific Reports*, 6, 37033.
- Chupp, D. P.,** Rivera, C. E., Zhou, Y., Xu, Y., Ramsey, P. S., Xu, Z., Zan, H., and Casali, P. (2024). A humanized mouse that mounts mature class-switched, hypermutated and neutralizing antibody responses. *Nature Immunology*, 25(8), 1489–1506.
- Ciabarra, A. M.,** Sullivan, J. M., Gahn, L. G., Pecht, G., Heinemann, S., and Sevarino, K. A. (1995). Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *The Journal of Neuroscience*, 15(10), 6498–6508.

Ciceroni, C., Mosillo, P., Mastrantoni, E., Sale, P., Ricci-Vitiani, L., Biagioni, F., Stocchi, F., Nicoletti, F., and Melchiorri, D. (2010). mGlu3 metabotropic glutamate receptors modulate the differentiation of SVZ-derived neural stem cells towards the astrocytic lineage. *Glia*, 58(7), 813–822.

Cingolani, L. A., Thalhammer, A., Yu, L. M., Catalano, M., Ramos, T., Colicos, M. A., and Goda, Y. (2008). Activity-dependent regulation of synaptic AMPA receptor composition and abundance by beta3 integrins. *Neuron*, 58(5), 749–762.

Citri, A., and Malenka, R. C. (2008). Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology*, 33(1), 18–41.

Clark, A. K., Gruber-Schoffnegger, D., Drdla-Schutting, R., Gerhold, K. J., Malcangio, M., and Sandkühler, J. (2015). Selective activation of microglia facilitates synaptic strength. *The Journal of Neuroscience*, 35(11), 4552–4570.

Clauss, J. A., Avery, S. N., Benningfield, M. M., and Blackford, J. U. (2019). Social anxiety is associated with BNST response to unpredictability. *Depression and anxiety*, 36(8), 666–675.

Cline, H. T., Debski, E. A., and Constantine-Paton, M. (1987). N-methyl-D-aspartate receptor antagonist desegregates eye-specific stripes. *Proceedings of the National Academy of Sciences of the United States of America*, 84(12), 4342–4345.

Cline, H. T., and Constantine-Paton, M. (1989). NMDA receptor antagonists disrupt the retinotectal topographic map. *Neuron*, 3(4), 413–426.

Cline H. T. (2001). Dendritic arbor development and synaptogenesis. *Current Opinion in Neurobiology*, 11(1), 118–126.

Cline H. (2005). Synaptogenesis: a balancing act between excitation and inhibition. *Current Biology*, 15(6), R203–R205.

Cline, H., and Haas, K. (2008). The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the

synaptotrophic hypothesis. *The Journal of Physiology*, 586(6), 1509–1517.

Collison, K. S., Inglis, A., Shibin, S., Andres, B., Ubungen, R., Thiam, J., Mata, P., and Al-Mohanna, F. A. (2016). Differential effects of early-life NMDA receptor antagonism on aspartame-impaired insulin tolerance and behavior. *Physiology and Behavior*, 167, 209–221.

Colonnese, M. T., and Constantine-Paton, M. (2006). Developmental period for N-methyl-D-aspartate (NMDA) receptor-dependent synapse elimination correlated with visuotopic map refinement. *The Journal of Comparative Neurology*, 494(5), 738–751.

Colonnese, M. T., Kaminska, A., Minlebaev, M., Milh, M., Bloem, B., Lescure, S., Moriette, G., Chiron, C., Ben-Ari, Y., and Khazipov, R. (2010). A conserved switch in sensory processing prepares developing neocortex for vision. *Neuron*, 67(3), 480–498.

Colwell, C. S., Cepeda, C., Crawford, C., and Levine, M. S. (1998). Postnatal development of glutamate receptor-mediated responses in the neostriatum. *Developmental Neuroscience*, 20(2-3), 154–163.

Comi, A. M., Zimmerman, A. W., Frye, V. H., Law, P. A., and Peeden, J. N. (1999). Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *Journal of Child Neurology*, 14(6), 388–394.

Condé, H., Bénita, M., Dormont, J. F., Schmied, A., and Cadoret, A. (1981). Control of reaction time performance involves the striatum. *Journal de Physiologie*, 77(1), 97–105.

Copani, A., Casabona, G., Bruno, V., Caruso, A., Condorelli, D. F., Messina, A., Di Giorgi Gerevini, V., Pin, J. P., Kuhn, R., Knöpfel, T., and Nicoletti, F. (1998). The metabotropic glutamate receptor mGlu5 controls the onset of developmental apoptosis in cultured cerebellar neurons. *The European Journal of Neuroscience*, 10(6), 2173–2184.

Corbin, J. G., Gaiano, N., Machold, R. P., Langston, A., and Fishell, G. (2000). The Gsh2 homeodomain gene controls multiple aspects of telencephalic development. *Development*, 127(23), 5007–5020.

Corlew, R., Wang, Y., Ghermazien, H., Erisir, A., and Philpot, B. D. (2007). Developmental switch in the contribution of presynaptic and postsynaptic NMDA receptors to long-term depression. *The Journal of Neuroscience*, 27(37), 9835–9845.

Corlew, R., Brasier, D. J., Feldman, D. E., and Philpot, B. D. (2008). Presynaptic NMDA receptors: newly appreciated roles in cortical synaptic function and plasticity. *The Neuroscientist*, 14(6), 609–625.

Cottrell, J. R., Dubé, G. R., Egles, C., and Liu, G. (2000). Distribution, density, and clustering of functional glutamate receptors before and after synaptogenesis in hippocampal neurons. *Journal of Neurophysiology*, 84(3), 1573–1587.

Coutinho E. Central Nervous System Autoimmunity in Neuropsychiatric Disorders. (2016). *DPhil Thesis*.

Coutinho, E., Jacobson, L., Pedersen, M. G., Benros, M. E., Nørgaard-Pedersen, B., Mortensen, P. B., Harrison, P. J., and Vincent, A. (2017a). CASPR2 autoantibodies are raised during pregnancy in mothers of children with mental retardation and disorders of psychological development but not autism. *Journal of Neurology, Neurosurgery, and Psychiatry*, 88(9), 718–721.

Coutinho, E., Menassa, D. A., Jacobson, L., West, S. J., Domingos, J., Moloney, T. C., Lang, B., Harrison, P. J., Bennett, D. L. H., Bannerman, D., and Vincent, A. (2017b). Persistent microglial activation and synaptic loss with behavioral abnormalities in mouse offspring exposed to CASPR2-antibodies *in utero*. *Acta Neuropathologica*, 134(4), 567–583.

Cowan, M., and Petri, W. A., Jr (2018). Microglia: Immune Regulators of Neurodevelopment. *Frontiers in Immunology*, 9, 2576.

Cox, J., and Witten, I. B. (2019). Striatal circuits for reward learning and decision-making. *Nature Reviews. Neuroscience*, 20(8), 482–494.

Cree, B. A. C., Bennett, J. L., Kim, H. J., Weinshenker, B. G., Pittock, S. J., Wingerchuk, D. M., Fujihara, K., Paul, F., Cutter, G. R., Marignier, R., Green, A. J., Aktas, O., Hartung, H. P., Lublin, F. D., Drappa, J., Barron, G., Madani, S., Ratchford, J. N., She, D., Cimbora, D., ... N-MOMentum study investigators (2019). Inebilizumab for the treatment of neuromyelitis optica spectrum disorder (N-MOMentum): a double-blind, randomised placebo-controlled phase 2/3 trial. *Lancet*, 394(10206), 1352–1363.

Crisp, S. J., Kullmann, D. M., and Vincent, A. (2016). Autoimmune synaptopathies. *Nature reviews. Neuroscience*, 17(2), 103–117.

Croen, L. A., Grether, J. K., Yoshida, C. K., Odouli, R., and Van de Water, J. (2005). Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Archives of Pediatrics and Adolescent Medicine*, 159(2), 151–157.

Cronk, J. C., Derecki, N. C., Ji, E., Xu, Y., Lampano, A. E., Smirnov, I., Baker, W., Norris, G. T., Marin, I., Coddington, N., Wolf, Y., Turner, S. D., Aderem, A., Klibanov, A. L., Harris, T. H., Jung, S., Litvak, V., and Kipnis, J. (2015). Methyl-CpG Binding Protein 2 Regulates Microglia and Macrophage Gene Expression in Response to Inflammatory Stimuli. *Immunity*, 42(4), 679–691.

Cserép, C., Szabadits, E., Szőnyi, A., Watanabe, M., Freund, T. F., and Nyiri, G. (2012). NMDA receptors in GABAergic synapses during postnatal development. *PloS one*, 7(5), e37753.

Cserép, C., Pósfai, B., Lénárt, N., Fekete, R., László, Z. I., Lele, Z., Orsolits, B., Molnár, G., Heindl, S., Schwarcz, A. D., Ujvári, K., Környei, Z., Tóth, K., Szabadits, E., Sperlág, B., Baranyi, M., Csiba, L., Hortobágyi, T., Maglóczy, Z., Martinecz, B., ... Dénes, Á. (2020). Microglia monitor and protect neuronal function through specialized somatic purinergic junctions. *Science*, 367(6477), 528–537.

Cui, G., Okamoto, T., and Morikawa, H. (2004). Spontaneous opening of T-type Ca²⁺ channels contributes to the irregular firing of dopamine neurons in neonatal rats. *The Journal of Neuroscience*, 24(49), 11079–11087.

Cui, G., Jun, S. B., Jin, X., Pham, M. D., Vogel, S. S., Lovinger, D. M., and Costa, R. M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature*, 494(7436), 238–242.

Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Current Opinion in Neurobiology*, 11(3), 327–335.

Cull-Candy, S. G., and Leszkiewicz, D. N. (2004). Role of distinct NMDA receptor subtypes at central synapses. *Science's STKE: Signal Transduction Knowledge Environment*, 2004(255), re16.

Cummings, D. M., André, V. M., Uzgil, B. O., Gee, S. M., Fisher, Y. E., Cepeda, C., and Levine, M. S. (2009). Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. *The Journal of Neuroscience*, 29(33), 10371–10386.

Cunningham, C. L., Martínez-Cerdeño, V., and Noctor, S. C. (2013). Microglia regulate the number of neural precursor cells in the developing cerebral cortex. *The Journal of Neuroscience*, 33(10), 4216–4233.

Dahm, L., Ott, C., Steiner, J., Stepniak, B., Teegen, B., Saschenbrecker, S., Hammer, C., Borowski, K., Begemann, M., Lemke, S., Rentzsch, K., Probst, C., Martens, H., Wienands, J., Spalletta, G., Weissenborn, K., Stöcker, W., and Ehrenreich, H. (2014). Seroprevalence of autoantibodies against brain antigens in health and disease. *Annals of Neurology*, 76(1), 82–94.

Daikhin, Y., and Yudkoff, M. (2000). Compartmentation of brain glutamate metabolism in neurons and glia. *The Journal of Nutrition*, 130(4S Suppl), 1026S–31S.

Dailey, M. E., and Smith, S. J. (1996). The dynamics of dendritic structure in developing hippocampal slices. *The Journal of Neuroscience*, 16(9), 2983–2994.

Dailey, M. E., and Waite, M. (1999). Confocal imaging of microglial cell dynamics in hippocampal slice cultures. *Methods*, 18(2), 222–177

Dalmau, J., Tüzün, E., Wu, H. Y., Masjuan, J., Rossi, J. E., Voloschin, A., Baehring, J. M., Shimazaki, H., Koide, R., King, D., Mason, W., Sansing, L. H., Dichter, M. A., Rosenfeld, M. R., and Lynch, D. R. (2007). Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Annals of Neurology*, 61(1), 25–36.

Dalmau, J., Gleichman, A. J., Hughes, E. G., Rossi, J. E., Peng, X., Lai, M., Dessain, S. K., Rosenfeld, M. R., Balice-Gordon, R., and Lynch, D. R. (2008). Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *The Lancet. Neurology*, 7(12), 1091–1098.

Dalmau J. (2016). NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: The 2016 Cotzias Lecture. *Neurology*, 87(23), 2471–2482.

Dalmau, J., and Graus, F. (2018). Antibody-Mediated Encephalitis. *The New England Journal of Medicine*, 378(9), 840–851.

Dalmau, J., Armangué, T., Planagumà, J., Radosevic, M., Mannara, F., Leypoldt, F., Geis, C., Lancaster, E., Titulaer, M. J., Rosenfeld, M. R., and Graus, F. (2019). An update on anti-NMDA receptor encephalitis for neurologists and psychiatrists: mechanisms and models. *The Lancet. Neurology*, 18(11), 1045–1057.

Dalsgaard, S., Waltoft, B. L., Leckman, J. F., and Mortensen, P. B. (2015). Maternal history of autoimmune disease and later development of tourette syndrome in offspring. *Journal of the American Academy of Child and Adolescent Psychiatry*, 54(6), 495–501.e1.

Damato, V., Evoli, A., and Iorio, R. (2016). Efficacy and Safety of Rituximab Therapy in Neuromyelitis Optica Spectrum Disorders: A Systematic Review and Meta-analysis. *JAMA Neurology*, 73(11), 1342–1348.

Dawes, J. M., Weir, G. A., Middleton, S. J., Patel, R., Chisholm, K. I., Pettingill, P., Peck, L. J., Sheridan, J., Shakir, A., Jacobson, L., Gutierrez-Mecinas, M., Galino, J., Walcher, J., Kühnemund, J., Kuehn, H., Sanna, M. D., Lang, B., Clark, A. J., Themistocleous, A. C., Iwagaki, N., ... Bennett, D. L. (2018). Immune or Genetic-Mediated Disruption of CASPR2 Causes

Pain Hypersensitivity Due to Enhanced Primary Afferent Excitability. *Neuron*, 97(4), 806–822.e10.

Day, M., Wokosin, D., Plotkin, J. L., Tian, X., and Surmeier, D. J. (2008). Differential excitability and modulation of striatal medium spiny neuron dendrites. *The Journal of Neuroscience*, 28(45), 11603–11614.

De Bruijn, M. A. A. M., Aarsen, F. K., Van Oosterhout, M. P., Van der Knoop, M. M., Catsman-Berrevoets, C. E., Schreurs, M. W. J., Bastiaansen, D. E. M., Sillevs Smitt, P. A. E., Neuteboom, R. F., Titulaer, M. J., and CHANCE Study Group (2018). Long-term neuropsychological outcome following pediatric anti-NMDAR encephalitis. *Neurology*, 90(22), e1997–e2005.

De Bruijn, M. A. A. M., Van Sonderen, A., Van Coevorden-Hameete, M. H., Bastiaansen, A. E. M., Schreurs, M. W. J., Rouhl, R. P. W., Van Donselaar, C. A., Majoie, M. H. J. M., Neuteboom, R. F., Sillevs Smitt, P. A. E., Thijs, R. D., and Titulaer, M. J. (2019). Evaluation of seizure treatment in anti-LGI1, anti-NMDAR, and anti-GABA_BR encephalitis. *Neurology*, 92(19), e2185–e2196.

De Marco García, N. V., Karayannis, T., and Fishell, G. (2011). Neuronal activity is required for the development of specific cortical interneuron subtypes. *Nature*, 472(7343), 351–355.

De Oliveira, R. W., Oliveira, C. L., Guimarães, F. S., and Campos, A. C. (2019). Cannabinoid signalling in embryonic and adult neurogenesis: possible implications for psychiatric and neurological disorders. *Acta Neuropsychiatrica*, 31(1), 1–16.

Deacon, T. W., Pakzaban, P., and Isacson, O. (1994). The lateral ganglionic eminence is the origin of cells committed to striatal phenotypes: neural transplantation and developmental evidence. *Brain Research*, 668(1-2), 211–219.

Deacon R. M. (2013). Measuring motor coordination in mice. *Journal of Visualized Experiments*, (75), e2609.

Dehnes, Y., Chaudhry, F. A., Ullensvang, K., Lehre, K. P., Storm-Mathisen, J., and Danbolt, N. C. (1998). The glutamate transporter EAAT4 in rat cerebellar

Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. *The Journal of Neuroscience*, 18(10), 3606–3619.

Dehorter, N., Guigoni, C., López, C., Hirsch, J., Eusebio, A., Ben-Ari, Y., and Hammond, C. (2009). Dopamine-deprived striatal GABAergic interneurons burst and generate repetitive gigantic IPSCs in medium spiny neurons. *The Journal of Neuroscience*, 29(24), 7776–7787.

Dehorter, N., Michel, F. J., Marissal, T., Rotrou, Y., Matrot, B., López, C., Humphries, M. D., and Hammond, C. (2011). Onset of Pup Locomotion Coincides with Loss of NR2C/D-Mediated Cortico-Striatal EPSCs and Dampening of Striatal Network Immature Activity. *Frontiers in Cellular Neuroscience*, 5, 24.

Deisseroth, K., Singla, S., Toda, H., Monje, M., Palmer, T. D., and Malenka, R. C. (2004). Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron*, 42(4), 535–552.

Del Campo, N., Chamberlain, S. R., Sahakian, B. J., and Robbins, T. W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry*, 69(12), e145–e157.

Del Pino, I., Rico, B., and Marín, O. (2018). Neural circuit dysfunction in mouse models of neurodevelopmental disorders. *Current Opinion in Neurobiology*, 48, 174–182.

Denny-Brown, D., and Yanagisawa, N. (1976). The role of the basal ganglia in the initiation of movement. *Research Publications - Association for Research in Nervous and Mental Disease*, 55, 115–149.

Desai, A. R., and McConnell, S. K. (2000). Progressive restriction in fate potential by neural progenitors during cerebral cortical development. *Development*, 127(13), 2863–2872.

Diering, G. H., and Huganir, R. L. (2018). The AMPA Receptor Code of Synaptic Plasticity. *Neuron*, 100(2), 314–329.

Dietrich, M. R., Ankeny, R. A., and Chen, P. M. (2014). Publication trends in model organism research. *Genetics*, 198(3), 787–794.

Ding, J., Peterson, J. D., and Surmeier, D. J. (2008). Corticostriatal and thalamostriatal synapses have distinctive properties. *The Journal of Neuroscience*, 28(25), 6483–6492.

Ding, Y. W., Pan, S. Y., Xie, W., Shen, H. Y., and Wang, H. H. (2018). Elevated Soluble Fas and FasL in Cerebrospinal Fluid and Serum of Patients With Anti-N-methyl-D-aspartate Receptor Encephalitis. *Frontiers in Neurology*, 9, 904.

Diniz, L. P., Tortelli, V., Garcia, M. N., Araújo, A. P., Melo, H. M., Silva, G. S., Felice, F. G., Alves-Leon, S. V., Souza, J. M., Romão, L. F., Castro, N. G., and Gomes, F. C. (2014). Astrocyte transforming growth factor beta 1 promotes inhibitory synapse formation via CaM kinase II signaling. *Glia*, 62(12), 1917–1931.

Dissing-Olesen, L., LeDue, J. M., Rungta, R. L., Hefendehl, J. K., Choi, H. B., and MacVicar, B. A. (2014). Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. *The Journal of Neuroscience*, 34(32), 10511–10527.

Doig, N. M., Moss, J., and Bolam, J. P. (2010). Cortical and thalamic innervation of direct and indirect pathway medium-sized spiny neurons in mouse striatum. *The Journal of Neuroscience*, 30(44), 14610–14618.

Dolan, R. J., and Dayan, P. (2013). Goals and habits in the brain. *Neuron*, 80(2), 312–325.

Donahue, C. H., Liu, M., and Kreitzer, A. C. (2018). Distinct value encoding in striatal direct and indirect pathways during adaptive learning. *BioRxiv*.

Dornn, A. L., Yuan, K., Barker, A. J., Schreiner, C. E., and Froemke, R. C. (2010). Developmental sensory experience balances cortical excitation and inhibition. *Nature*, 465(7300), 932–936.

Du, L., Zhang, Y., Chen, Y., Zhu, J., Yang, Y., and Zhang, H. L. (2017). Role of Microglia in Neurological Disorders and Their Potentials as a Therapeutic

Target. *Molecular Neurobiology*, 54(10), 7567–7584.

Duan, T., Tradtrantip, L., Phuan, P. W., Bennett, J. L., and Verkman, A. S. (2020). Affinity-matured 'aquaporumab' anti-aquaporin-4 antibody for therapy of seropositive neuromyelitis optica spectrum disorders. *Neuropharmacology*, 162, 107827.

Dufour, M. A., Woodhouse, A., Amendola, J., and Goillard, J. M. (2014). Non-linear developmental trajectory of electrical phenotype in rat substantia nigra pars compacta dopaminergic neurons. *eLife*, 3, e04059.

Dupont, E., Hanganu, I. L., Kilb, W., Hirsch, S., and Luhmann, H. J. (2006). Rapid developmental switch in the mechanisms driving early cortical columnar networks. *Nature*, 439(7072), 79–83.

Dupuis, J. P., Nicole, O., and Groc, L. (2023). NMDA receptor functions in health and disease: Old actor, new dimensions. *Neuron*, 111(15), 2312–2328.

Durand, G. M., and Zukin, R. S. (1993). Developmental regulation of mRNAs encoding rat brain kainate/AMPA receptors: a northern analysis study. *Journal of Neurochemistry*, 61(6), 2239–2246.

Dutheil, S., Ota, K. T., Wohleb, E. S., Rasmussen, K., and Duman, R. S. (2016). High-Fat Diet Induced Anxiety and Anhedonia: Impact on Brain Homeostasis and Inflammation. *Neuropsychopharmacology*, 41(7), 1874–1887.

Eblen, F., and Graybiel, A. M. (1995). Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. *The Journal of Neuroscience*, 15(9), 5999–6013.

Elhardt, M., Martínez, L., and Tejada-Simon, M. V. (2010). Neurochemical, behavioral and architectural changes after chronic inactivation of NMDA receptors in mice. *Neuroscience Letters*, 468(2), 166–171.

Elias, G. M., Elias, L. A., Apostolides, P. F., Kriegstein, A. R., and Nicoll, R. A. (2008). Differential trafficking

of AMPA and NMDA receptors by SAP102 and PSD-95 underlies synapse development. *Proceedings of the National Academy of Sciences of the United States of America*, 105(52), 20953–20958.

Ellebrecht, C. T., Bhoj, V. G., Nace, A., Choi, E. J., Mao, X., Cho, M. J., Di Zenzo, G., Lanzavecchia, A., Seykora, J. T., Cotsarelis, G., Milone, M. C., and Payne, A. S. (2016). Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science*, 353(6295), 179–184.

Ellenbroek, B., and Youn, J. (2016). Rodent models in neuroscience research: is it a rat race?. *Disease Models and Mechanisms*, 9(10), 1079–1087.

Ellender, T. J., Avery, S. V., Mahfooz, K., Scaber, J., von Klemperer, A., Nixon, S. L., Buchan, M. J., Van Rheede, J. J., Gatti, A., Waites, C., Pavlou, H. J., Sims, D., Newey, S. E., and Åkerman, C. J. (2019). Embryonic progenitor pools generate diversity in fine-scale excitatory cortical subnetworks. *Nature Communications*, 10(1), 5224.

Elwood, E., Lim, Z., Naveed, H., and Galea, I. (2017). The effect of systemic inflammation on human brain barrier function. *Brain, Behavior, and Immunity*, 62, 35–40.

Engelhardt B. (2003). Development of the blood-brain barrier. *Cell and Tissue Research*, 314(1), 119–129.

Engert, F., and Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature*, 399(6731), 66–70.

Erblich, B., Zhu, L., Etgen, A. M., Dobrenis, K., and Pollard, J. W. (2011). Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. *PloS one*, 6(10), e26317.

Eriksson, C., Zou, L. P., Ahlenius, S., Winblad, B., and Schultzberg, M. (2000). Inhibition of kainic acid induced expression of interleukin-1 beta and interleukin-1 receptor antagonist mRNA in the rat brain by NMDA receptor antagonists. *Brain Research. Molecular Brain Research*, 85(1-2), 103–113.

Erreger, K., Dravid, S. M., Banke, T. G., Wyllie, D. J., and Traynelis, S. F. (2005). Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. *The Journal of Physiology*, 563(Pt 2), 345–358.

Everitt, B. J., and Robbins, T. W. (2013). From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neuroscience and Biobehavioral Reviews*, 37(9 Pt A), 1946–1954.

Ewald, R. C., and Cline, H. T. (2009). NMDA Receptors and Brain Development. In A. M. Van Dongen (Ed.), *Biology of the NMDA Receptor*. CRC Press/Taylor and Francis.

Eyo, U. B., Peng, J., Swiatkowski, P., Mukherjee, A., Bispo, A., and Wu, L. J. (2014). Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. *The Journal of Neuroscience*, 34(32), 10528–10540.

Eyo, U. B., Gu, N., De, S., Dong, H., Richardson, J. R., and Wu, L. J. (2015). Modulation of microglial process convergence toward neuronal dendrites by extracellular calcium. *The Journal of Neuroscience*, 35(6), 2417–2422.

Eyo, U. B., Bispo, A., Liu, J., Sabu, S., Wu, R., DiBona, V. L., Zheng, J., Murugan, M., Zhang, H., Tang, Y., and Wu, L. J. (2018). The GluN2A Subunit Regulates Neuronal NMDA receptor-Induced Microglia-Neuron Physical Interactions. *Scientific Reports*, 8(1), 828.

Fairman, W. A., Vandenberg, R. J., Arriza, J. L., Kavanaugh, M. P., and Amara, S. G. (1995). An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature*, 375(6532), 599–603.

Fan, M. M., and Raymond, L. A. (2007). N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Progress in Neurobiology*, 81(5-6), 272–293.

Fan, X., Jin, W. Y., and Wang, Y. T. (2014). The NMDA receptor complex: a multifunctional machine at the glutamatergic synapse. *Frontiers in Cellular Neuroscience*, 8, 160.

Fentress, J. C., Stanfield, B. B., and Cowan, W. M. (1981). Observation on the development of the striatum in mice and rats. *Anatomy and Embryology*, 163(3), 275–298.

Feola, B., Flook, E. A., Gardner, H., Phan, K. L., Gwirtsman, H., Olatunji, B., and Blackford, J. U. (2023). Altered bed nucleus of the stria terminalis and amygdala responses to threat in combat veterans with posttraumatic stress disorder. *Journal of Traumatic Stress*, 36(2), 359–372.

Fernández-Arjona, M. D. M., Grondona, J. M., Granados-Durán, P., Fernández-Llebrez, P., and López-Ávalos, M. D. (2017). Microglia Morphological Categorization in a Rat Model of Neuroinflammation by Hierarchical Cluster and Principal Components Analysis. *Frontiers in Cellular Neuroscience*, 11, 235.

Ferrari, D. C., Mdzomba, B. J., Dehorter, N., López, C., Michel, F. J., Libersat, F., and Hammond, C. (2012). Midbrain dopaminergic neurons generate calcium and sodium currents and release dopamine in the striatum of pups. *Frontiers in Cellular Neuroscience*, 6, 7.

Fieblinger T. (2021). Striatal Control of Movement: A Role for New Neuronal (Sub-) Populations?. *Frontiers in Human Neuroscience*, 15, 697284.

Finke, C., Prüss, H., Heine, J., Reuter, S., Kopp, U. A., Wegner, F., Then Bergh, F., Koch, S., Jansen, O., Münte, T., Deuschl, G., Ruprecht, K., Stöcker, W., Wandinger, K. P., Paul, F., and Bartsch, T. (2017). Evaluation of Cognitive Deficits and Structural Hippocampal Damage in Encephalitis With Leucine-Rich, Glioma-Inactivated 1 Antibodies. *JAMA Neurology*, 74(1), 50–59.

Fishell, G., and Van der Kooy, D. (1989). Pattern formation in the striatum: developmental changes in the distribution of striatonigral projections. *Brain Research. Developmental Brain Research*, 45(2), 239–255.

Fishell, G., and Van der Kooy, D. (1991). Pattern formation in the striatum: neurons with early projections to the substantia nigra survive the cell death period. *The Journal of Comparative Neurology*, 312(1), 33–42.

Fizman, M. L., Erdélyi, F., Szabó, G., and Vicini, S. (2007). Presynaptic AMPA and kainate receptors increase the size of GABAergic terminals and enhance GABA release. *Neuropharmacology*, 52(8), 1631–1640.

Flint, A. C., Maisch, U. S., Weishaupt, J. H., Kriegstein, A. R., and Monyer, H. (1997). NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *The Journal of Neuroscience*, 17(7), 2469–2476.

Florance, N. R., Davis, R. L., Lam, C., Szperka, C., Zhou, L., Ahmad, S., Campen, C. J., Moss, H., Peter, N., Gleichman, A. J., Glaser, C. A., Lynch, D. R., Rosenfeld, M. R., and Dalmau, J. (2009). Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. *Annals of Neurology*, 66(1), 11–18.

Flores, C. E., Nikonenko, I., Mendez, P., Fritschy, J. M., Tyagarajan, S. K., and Müller, D. (2015). Activity-dependent inhibitory synapse remodeling through gephyrin phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*, 112(1), E65–E72.

Florio, M., and Huttner, W. B. (2014). Neural progenitors, neurogenesis and the evolution of the neocortex. *Development*, 141(11), 2182–2194.

Forrest, D., Yuzaki, M., Soares, H. D., Ng, L., Luk, D. C., Sheng, M., Stewart, C. L., Morgan, J. I., Connor, J. A., and Curran, T. (1994). Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. *Neuron*, 13(2), 325–338.

Fox M. W. (1966). Neuro-behavioral ontogeny. A synthesis of ethological and neurophysiological concepts. *Brain Research*, 2(1), 3–20.

Frade, J. M., and Barde, Y. A. (1998). Microglia-derived nerve growth factor causes cell death in the developing retina. *Neuron*, 20(1), 35–41.

Franco, S. J., Gil-Sanz, C., Martínez-Garay, I., Espinosa, A., Harkins-Perry, S. R., Ramos, C., and Müller, U. (2012). Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science*, 337(6095), 746–749.

Frantz, G. D., and McConnell, S. K. (1996). Restriction of late cerebral cortical progenitors to an upper-layer fate. *Neuron*, 17(1), 55–61.

Freed, C. R., Greene, P. E., Breeze, R. E., Tsai, W. Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J. Q., Eidelberg, D., and Fahn, S. (2001). Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *The New England Journal of Medicine*, 344(10), 710–719.

Freneau, R. T., Jr, Voglmaier, S., Seal, R. P., and Edwards, R. H. (2004). VGLUTs define subsets of excitatory neurons and suggest novel roles for glutamate. *Trends in Neurosciences*, 27(2), 98–103.

Fry, A. E., Fawcett, K. A., Zelnik, N., Yuan, H., Thompson, B. A. N., Shemer-Meiri, L., Cushion, T. D., Mugalaasi, H., Sims, D., Stoodley, N., Chung, S. K., Rees, M. I., Patel, C. V., Brueton, L. A., Layet, V., Giuliano, F., Kerr, M. P., Banne, E., Meiner, V., Lerman-Sagie, T., ... Pilz, D. T. (2018). De novo mutations in GRIN1 cause extensive bilateral polymicrogyria. *Brain*, 141(3), 698–712.

Fudge, J. L., Kunishio, K., Walsh, P., Richard, C., and Haber, S. N. (2002). Amygdaloid projections to ventromedial striatal subterritories in the primate. *Neuroscience*, 110(2), 257–275.

Fujiyama, F., Kuramoto, E., Okamoto, K., Hioki, H., Furuta, T., Zhou, L., Nomura, S., and Kaneko, T. (2004). Presynaptic localization of an AMPA-type glutamate receptor in corticostriatal and thalamostriatal axon terminals. *The European Journal of Neuroscience*, 20(12), 3322–3330.

Fukata, Y., Chen, X., Chiken, S., Hirano, Y., Yamagata, A., Inahashi, H., Sanbo, M., Sano, H., Goto, T., Hirabayashi, M., Kornau, H. C., Prüss, H., Nambu, A., Fukai, S., Nicoll, R. A., and Fukata, M. (2021). LGI1-ADAM22-MAGUK configures transsynaptic nanoalignment for synaptic transmission and epilepsy prevention. *Proceedings of the National Academy of Sciences of the United States of America*, 118(3), e2022580118.

Fukazawa, Y., Saitoh, Y., Ozawa, F., Ohta, Y., Mizuno, K., and Inokuchi, K. (2003). Hippocampal LTP is accompanied by enhanced F-actin content

within the dendritic spine that is essential for late LTP maintenance *in vivo*. *Neuron*, 38(3), 447–460.

Furukawa, H., Singh, S. K., Mancusso, R., and Gouaux, E. (2005). Subunit arrangement and function in NMDA receptors. *Nature*, 438(7065), 185–192.

Furuta, A., Rothstein, J. D., and Martin, L. J. (1997a). Glutamate transporter protein subtypes are expressed differentially during rat CNS development. *The Journal of Neuroscience*, 17(21), 8363–8375.

Furuta, A., Martin, L. J., Lin, C. L., Dykes-Hoberg, M., and Rothstein, J. D. (1997b). Cellular and synaptic localization of the neuronal glutamate transporters excitatory amino acid transporter 3 and 4. *Neuroscience*, 81(4), 1031–1042.

Gal, J. S., Morozov, Y. M., Ayoub, A. E., Chatterjee, M., Rakic, P., and Haydar, T. F. (2006). Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. *The Journal of Neuroscience*, 26(3), 1045–1056.

Galea I. (2021). The blood-brain barrier in systemic infection and inflammation. *Cellular and Molecular Immunology*, 18(11), 2489–2501.

Gallo E. F. (2019). Disentangling the diverse roles of dopamine D2 receptors in striatal function and behavior. *Neurochemistry International*, 125, 35–46.

Gallo, S., Vitacolonna, A., and Crepaldi, T. (2023). NMDA Receptor and Its Emerging Role in Cancer. *International Journal of Molecular Sciences*, 24(3), 2540.

Gambrill, A. C., and Barria, A. (2011). NMDA receptor subunit composition controls synaptogenesis and synapse stabilization. *Proceedings of the National Academy of Sciences of the United States of America*, 108(14), 5855–5860.

Gan, Q., Salussolia, C. L., and Wollmuth, L. P. (2015). Assembly of AMPA receptors: mechanisms and

regulation. *The Journal of Physiology*, 593(1), 39–48.

Gandhi, R., Luk, K. C., Rymar, V. V., and Sadikot, A. F. (2008). Group I mGluR5 metabotropic glutamate receptors regulate proliferation of neuronal progenitors in specific forebrain developmental domains. *Journal of Neurochemistry*, 104(1), 155–172.

Gangarossa, G., Espallergues, J., Mailly, P., De Bundel, D., de Kerchove d'Exaerde, A., Hervé, D., Girault, J. A., Valjent, E., and Krieger, P. (2013). Spatial distribution of D1R- and D2R-expressing medium-sized spiny neurons differs along the rostro-caudal axis of the mouse dorsal striatum. *Frontiers in Neural Circuits*, 7, 124.

Gao, X. B., and Van den Pol, A. N. (2000). GABA release from mouse axonal growth cones. *The Journal of Physiology*, 523 Pt 3(Pt 3), 629–637.

Gao, P., Postiglione, M. P., Krieger, T. G., Hernández, L., Wang, C., Han, Z., Streicher, C., Papusheva, E., Insolera, R., Chugh, K., Kodish, O., Huang, K., Simons, B. D., Luo, L., Hippenmeyer, S., and Shi, S. H. (2014). Deterministic progenitor behavior and unitary production of neurons in the neocortex. *Cell*, 159(4), 775–788.

Gao, J., Stevenson, T. J., Douglass, A. D., Barrios, J. P., and Bonkowsky, J. L. (2018). The Midline Axon Crossing Decision Is Regulated through an Activity-Dependent Mechanism by the NMDA Receptor. *eNeuro*, 5(2), ENEURO.0389-17.2018.

Garas, F. N., Shah, R. S., Kormann, E., Doig, N. M., Vinciati, F., Nakamura, K. C., Dorst, M. C., Smith, Y., Magill, P. J., and Sharott, A. (2016). Secretagogin expression delineates functionally-specialized populations of striatal parvalbumin-containing interneurons. *eLife*, 5, e16088.

Garas, F. N., Kormann, E., Shah, R. S., Vinciati, F., Smith, Y., Magill, P. J., and Sharott, A. (2018). Structural and molecular heterogeneity of calretinin-expressing interneurons in the rodent and primate striatum. *The Journal of Comparative Neurology*, 526(5), 877–898.

García-Serra, A., Radosevic, M., Pupak, A., Brito, V., Ríos, J., Aguilar, E., Maudes, E., Ariño, H., Spatola, M., Mannara, F., Pedreño, M., Joubert, B., Ginés, S., Planagumà, J., and Dalmau, J. (2020). Placental transfer of NMDAR antibodies causes reversible alterations in mice. *Neurology(R) Neuroimmunology and Neuroinflammation*, 8(1), e915.

García-Serra, A., Radosevic, M., Ríos, J., Aguilar, E., Maudes, E., Landa, J., Sabater, L., Martínez-Hernández, E., Planagumà, J., and Dalmau, J. (2021). Blocking Placental Class G Immunoglobulin Transfer Prevents NMDA Receptor Antibody Effects in Newborn Mice. *Neurology(R) Neuroimmunology and Neuroinflammation*, 8(6), e1061.

Georgiev, D., Taniura, H., Kambe, Y., Takarada, T., and Yoneda, Y. (2008). A critical importance of polyamine site in NMDA receptors for neurite outgrowth and fasciculation at early stages of P19 neuronal differentiation. *Experimental Cell Research*, 314(14), 2603–2617.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr, and Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, 250(4986), 1429–1432.

Gerfen, C. R., and Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annual Review of Neuroscience*, 34, 441–466.

Gertler, T. S., Chan, C. S., and Surmeier, D. J. (2008). Dichotomous anatomical properties of adult striatal medium spiny neurons. *The Journal of Neuroscience*, 28(43), 10814–10824.

Ghassabian, A., Bongers-Schokking, J. J., de Rijke, Y. B., Van Mil, N., Jaddoe, V. W., de Muinck Keizer-Schrama, S. M., Hooijkaas, H., Hofman, A., Visser, W., Roman, G. C., Visser, T. J., Verhulst, F. C., and Tiemeier, H. (2012). Maternal thyroid autoimmunity during pregnancy and the risk of attention deficit/hyperactivity problems in children: the Generation R Study. *Thyroid*, 22(2), 178–186.

Giannoccaro, M. P., Wright, S. K., and Vincent, A. (2020). *In vivo* Mechanisms of Antibody-Mediated Neurological Disorders: Animal Models and

Potential Implications. *Frontiers in Neurology*, 10, 1394.

Gilbert, C. D., and Wiesel, T. N. (1979). Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. *Nature*, 280(5718), 120–125.

Gill, I., Droubi, S., Giovedi, S., Fedder, K. N., Bury, L. A., Bosco, F., Sceniak, M. P., Benfenati, F., and Sabo, S. L. (2015). Presynaptic NMDA receptors - dynamics and distribution in developing axons *in vitro* and *in vivo*. *Journal of Cell Science*, 128(4), 768–780.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., Stanley, E. R., Samokhvalov, I. M., and Merad, M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*, 330(6005), 841–845.

Gladding, C. M., and Raymond, L. A. (2011). Mechanisms underlying NMDA receptor synaptic/extrasynaptic distribution and function. *Molecular and Cellular Neurosciences*, 48(4), 308–320.

Goasdoué, K., Miller, S.M., Colditz, P.B., and Björkman, T. (2017). Review: The blood-brain barrier; protecting the developing fetal brain. *Placenta*, 54, 111–116.

Goebel, D. J., and Poosch, M. S. (1999). NMDA receptor subunit gene expression in the rat brain: a quantitative analysis of endogenous mRNA levels of NR1Com, NR2A, NR2B, NR2C, NR2D and NR3A. *Brain Research. Molecular Brain Research*, 69(2), 164–170.

Goff, D. C., Lamberti, J. S., Leon, A. C., Green, M. F., Miller, A. L., Patel, J., Manschreck, T., Freudenreich, O., and Johnson, S. A. (2008). A placebo-controlled add-on trial of the Ampakine, CX516, for cognitive deficits in schizophrenia. *Neuropsychopharmacology*, 33(3), 465–472.

Gomes-Leal W. (2012). Microglial physiopathology: how to explain the dual role of microglia after acute neural disorders?. *Brain and Behavior*, 2(3), 345–356.

Gottlieb, M., and Matute, C. (1997). Expression of ionotropic glutamate receptor subunits in glial cells of the hippocampal CA1 area following transient forebrain ischemia. *Journal of Cerebral Blood Flow and Metabolism*, 17(3), 290–300.

Götz, M., and Huttner, W. B. (2005). The cell biology of neurogenesis. *Nature Reviews. Molecular Cell Biology*, 6(10), 777–788.

Govindan, S., Oberst, P., and Jabaudon, D. (2018). *In vivo* pulse labeling of isochronic cohorts of cells in the central nervous system using FlashTag. *Nature Protocols*, 13(10), 2297–2311.

Grace, A. A., and Onn, S. P. (1989). Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded *in vitro*. *The Journal of Neuroscience*, 9(10), 3463–3481.

Grade, S., and Götz, M. (2017). Neuronal replacement therapy: previous achievements and challenges ahead. *NPJ Regenerative Medicine*, 2, 29.

Graus, F., Titulaer, M. J., Balu, R., Benseler, S., Bien, C. G., Cellucci, T., Cortese, I., Dale, R. C., Gelfand, J. M., Geschwind, M., Glaser, C. A., Honnorat, J., Höftberger, R., Iizuka, T., Irani, S. R., Lancaster, E., Leypoldt, F., Prüss, H., Rae-Grant, A., Reindl, M., ... Dalmau, J. (2016). A clinical approach to diagnosis of autoimmune encephalitis. *The Lancet. Neurology*, 15(4), 391–404.

Gray, J. A., Shi, Y., Usui, H., During, M. J., Sakimura, K., and Nicoll, R. A. (2011). Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion *in vivo*. *Neuron*, 71(6), 1085–1101.

Graybiel, A. M., Aosaki, T., Flaherty, A. W., and Kimura, M. (1994). The basal ganglia and adaptive motor control. *Science*, 265(5180), 1826–1831.

Graybiel, A. M., and Rauch, S. L. (2000). Toward a neurobiology of obsessive-compulsive disorder. *Neuron*, 28(2), 343–347.

Graybiel A. M. (2008). Habits, rituals, and the evaluative brain. *Annual Review of Neuroscience*, 31, 359–387.

Graybiel, A. M., and Grafton, S. T. (2015). The striatum: where skills and habits meet. *Cold Spring Harbor Perspectives in Biology*, 7(8), a021691.

Greer, J. J., and Ren, J. (2009). Ampakine therapy to counter fentanyl-induced respiratory depression. *Respiratory Physiology and Neurobiology*, 168(1-2), 153–157.

Gressens, P., Baes, M., Leroux, P., Lombet, A., Van Veldhoven, P., Janssen, A., Vamecq, J., Marret, S., and Evrard, P. (2000). Neuronal migration disorder in Zellweger mice is secondary to glutamate receptor dysfunction. *Annals of Neurology*, 48(3), 336–343.

Grillner, S., Hellgren, J., Ménard, A., Saitoh, K., and Wikström, M. A. (2005). Mechanisms for selection of basic motor programs--roles for the striatum and pallidum. *Trends in Neurosciences*, 28(7), 364–370.

Groc, L., Heine, M., Cousins, S. L., Stephenson, F. A., Lounis, B., Cognet, L., and Choquet, D. (2006). NMDA receptor surface mobility depends on NR2A-2B subunits. *Proceedings of the National Academy of Sciences of the United States of America*, 103(49), 18769–18774.

Groenewegen, H. J., Vermeulen-Van der Zee, E., te Kortschot, A., and Witter, M. P. (1987). Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience*, 23(1), 103–120.

Groenewegen, H. J., Wright, C. I., Beijer, A. V., and Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences*, 877, 49–63.

Gu, J. G., Albuquerque, C., Lee, C. J., and MacDermott, A. B. (1996). Synaptic strengthening through activation of Ca²⁺-permeable AMPA receptors. *Nature*, 381(6585), 793–796.

Gu, X., Zhou, L., and Lu, W. (2016). An NMDA Receptor-Dependent Mechanism Underlies

Inhibitory Synapse Development. *Cell Reports*, 14(3), 471–478.

Guan, K., Chang, H., Rolletschek, A., and Wobus, A. M. (2001). Embryonic stem cell-derived neurogenesis. Retinoic acid induction and lineage selection of neuronal cells. *Cell and Tissue Research*, 305(2), 171–176.

Guillamon-Vivancos, T., Tyler, W. A., Medalla, M., Chang, W. W., Okamoto, M., Haydar, T. F., and Luebke, J. I. (2019). Distinct Neocortical Progenitor Lineages Fine-tune Neuronal Diversity in a Layer-specific Manner. *Cerebral Cortex*, 29(3), 1121–1138.

Gundersen, V., Holtén, A. T., and Storm-Mathisen, J. (2004). GABAergic synapses in hippocampus exocytose aspartate on to NMDA receptors: quantitative immunogold evidence for co-transmission. *Molecular and Cellular Neurosciences*, 26(1), 156–165.

Gutmann, D. H., and Kettenmann, H. (2019). Microglia/Brain Macrophages as Central Drivers of Brain Tumor Pathobiology. *Neuron*, 104(3), 442–449.

Guzman, J. N., Sánchez-Padilla, J., Chan, C. S., and Surmeier, D. J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. *The Journal of Neuroscience*, 29(35), 11011–11019.

Haber, S. N., and Knutson, B. (2010). The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*, 35(1), 4–26.

Hadzic, M., Jack, A., and Wahle, P. (2017). Ionotropic glutamate receptors: Which ones, when, and where in the mammalian neocortex. *The Journal of Comparative Neurology*, 525(4), 976–1033.

Han, S., Márquez-Gómez, R., Woodman, M., and Ellender, T. (2020). Histaminergic Control of Corticostriatal Synaptic Plasticity during Early Postnatal Development. *The Journal of Neuroscience*, 40(34), 6557–6571.

Hanamsagar, R., Alter, M. D., Block, C. S., Sullivan, H., Bolton, J. L., and Bilbo, S. D. (2017). Generation of a microglial developmental index in mice and in

humans reveals a sex difference in maturation and immune reactivity. *Glia*, 65(9), 1504–1520.

Hansen, K. B., Yi, F., Perszyk, R. E., Menniti, F. S., and Traynelis, S. F. (2017). NMDA Receptors in the Central Nervous System. *Methods in Molecular Biology*, 1677, 1–80.

Hansen, K. B., Wollmuth, L. P., Bowie, D., Furukawa, H., Menniti, F. S., Sobolevsky, A. I., Swanson, G. T., Swanger, S. A., Greger, I. H., Nakagawa, T., McBain, C. J., Jayaraman, V., Low, C. M., Dell'Acqua, M. L., Diamond, J. S., Camp, C. R., Perszyk, R. E., Yuan, H., and Traynelis, S. F. (2021). Structure, Function, and Pharmacology of Glutamate Receptor Ion Channels. *Pharmacological Reviews*, 73(4), 298–487.

Hanson, E., Armbruster, M., Lau, L. A., Sommer, M. E., Klaft, Z. J., Swanger, S. A., Traynelis, S. F., Moss, S. J., Noubary, F., Chadchankar, J., and Dulla, C. G. (2019). Tonic Activation of GluN2C/GluN2D-Containing NMDA Receptors by Ambient Glutamate Facilitates Cortical Interneuron Maturation. *The Journal of Neuroscience*, 39(19), 3611–3626.

Hardingham, G. E., and Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nature Reviews. Neuroscience*, 11(10), 682–696.

Harlow, D. E., Saul, K. E., Komuro, H., and Macklin, W. B. (2015). Myelin Proteolipid Protein Complexes with α v Integrin and AMPA Receptors *In vivo* and Regulates AMPA-Dependent Oligodendrocyte Progenitor Cell Migration through the Modulation of Cell-Surface GluR2 Expression. *The Journal of Neuroscience*, 35(34), 12018–12032.

Harris, A. Z., and Pettit, D. L. (2007). Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. *The Journal of Physiology*, 584(Pt 2), 509–519.

Hartfuss, E., Galli, R., Heins, N., and Götz, M. (2001). Characterization of CNS precursor subtypes and radial glia. *Developmental Biology*, 229(1), 15–30.

Härtig, W., Riedel, A., Grosche, J., Edwards, R. H., Fremeau, R. T., Jr, Harkany, T., Brauer, K., and

Arendt, T. (2003). Complementary distribution of vesicular glutamate transporters 1 and 2 in the nucleus accumbens of rat: Relationship to calretinin-containing extrinsic innervation and calbindin-immunoreactive neurons. *The Journal of Comparative Neurology*, 465(1), 1–10.

Haselmann, H., Mannara, F., Werner, C., Planagumà, J., Miguez-Cabello, F., Schmidl, L., Grünewald, B., Petit-Pedrol, M., Kirmse, K., Classen, J., Demir, F., Klöcker, N., Soto, D., Dose, S., Dalmau, J., Hallermann, S., and Geis, C. (2018). Human Autoantibodies against the AMPA Receptor Subunit GluA2 Induce Receptor Reorganization and Memory Dysfunction. *Neuron*, 100(1), 91–105.e9.

Häusser, M., Spruston, N., and Stuart, G. J. (2000). Diversity and dynamics of dendritic signaling. *Science*, 290(5492), 739–744.

Hawkins R. A. (2009). The blood-brain barrier and glutamate. *The American Journal of Clinical Nutrition*, 90(3), 867S–874S.

Haydar, T. F., Wang, F., Schwartz, M. L., and Rakic, P. (2000). Differential modulation of proliferation in the neocortical ventricular and subventricular zones. *The Journal of Neuroscience*, 20(15), 5764–5774.

Hediger M. A. (1999). Glutamate transporters in kidney and brain. *The American Journal of Physiology*, 277(4), F487–F492.

Heikkinen, T., Lehtimäki, K., Vartiainen, N., Puoliväli, J., Hendricks, S. J., Glaser, J. R., Bradaia, A., Wadel, K., Touller, C., Kontkanen, O., Yrjänheikki, J. M., Buisson, B., Howland, D., Beaumont, V., Munoz-Sanjuan, I., and Park, L. C. (2012). Characterization of neurophysiological and behavioral changes, MRI brain volumetry and 1H MRS in zQ175 knock-in mouse model of Huntington's disease. *PLoS one*, 7(12), e50717.

Heindl, S., Gesierich, B., Benakis, C., Llovera, G., Duering, M., and Liesz, A. (2018). Automated Morphological Analysis of Microglia After Stroke. *Frontiers in Cellular Neuroscience*, 12, 106.

Héja, L., Karacs, K., and Kardos, J. (2006). Role for GABA and Glu plasma membrane transporters in

the interplay of inhibitory and excitatory neurotransmission. *Current Topics in Medicinal Chemistry*, 6(10), 989–995.

Henle, F., Dehmel, M., Leemhuis, J., Fischer, C., and Meyer, D. K. (2012). Role of GluN2A and GluN2B subunits in the formation of filopodia and secondary dendrites in cultured hippocampal neurons. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 385(2), 171–180.

Henley, J. M., and Wilkinson, K. A. (2016). Synaptic AMPA receptor composition in development, plasticity and disease. *Nature reviews. Neuroscience*, 17(6), 337–350.

Henson, M. A., Roberts, A. C., Pérez-Otaño, I., and Philpot, B. D. (2010). Influence of the NR3A subunit on NMDA receptor functions. *Progress in Neurobiology*, 91(1), 23–37.

Heppner, F. L., Skutella, T., Hailer, N. P., Haas, D., and Nitsch, R. (1998). Activated microglial cells migrate towards sites of excitotoxic neuronal injury inside organotypic hippocampal slice cultures. *The European Journal of Neuroscience*, 10(10), 3284–3290.

Herzog, E., Bellenchi, G. C., Gras, C., Bernard, V., Ravassard, P., Bedet, C., Gasnier, B., Giros, B., and El Mestikawy, S. (2001). The existence of a second vesicular glutamate transporter specifies subpopulations of glutamatergic neurons. *The Journal of Neuroscience*, 21(22), RC181.

Herzog, C., Pons Garcia, L., Keatinge, M., Greenald, D., Moritz, C., Peri, F., and Herrgen, L. (2019). Rapid clearance of cellular debris by microglia limits secondary neuronal cell death after brain injury *in vivo*. *Development*, 146(9), dev174698.

Hestrin, S., and Galarreta, M. (2005). Electrical synapses define networks of neocortical GABAergic neurons. *Trends in Neurosciences*, 28(6), 304–309.

Heyser C. J. (2004). Assessment of developmental milestones in rodents. *Current Protocols in Neuroscience*, Chapter 8.

Hikida, T., Kimura, K., Wada, N., Funabiki, K., and Nakanishi, S. (2010). Distinct roles of synaptic

transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron*, 66(6), 896–907.

Hilderink, M., Titulaer, M. J., Schreurs, M. W., Keizer, K., and Bunt, J. E. (2015). Transient anti-NMDAR encephalitis in a newborn infant due to transplacental transmission. *Neurology(R) Neuroimmunology and Neuroinflammation*, 2(4), e126.

Hill, J. M., Lim, M. A., and Stone, M. M. (2008). *Neuromethods 39: Neuropeptide Techniques*. New Jersey: Humana Press. Developmental milestones in the newborn mouse.

Hirai, K., Yoshioka, H., Kihara, M., Hasegawa, K., Sakamoto, T., Sawada, T., and Fushiki, S. (1999). Inhibiting neuronal migration by blocking NMDA receptors in the embryonic rat cerebral cortex: a tissue culture study. *Brain Research. Developmental Brain Research*, 114(1), 63–67.

Hirasawa, T., Wada, H., Kohsaka, S., and Uchino, S. (2003). Inhibition of NMDA receptors induces delayed neuronal maturation and sustained proliferation of progenitor cells during neocortical development. *Journal of Neuroscience Research*, 74(5), 676–687.

Hoogland, I. C., Houbolt, C., Van Westerloo, D. J., Van Gool, W. A., and Van de Beek, D. (2015). Systemic inflammation and microglial activation: systematic review of animal experiments. *Journal of Neuroinflammation*, 12, 114.

Hoppmann, V., Wu, J. J., Sjøviknes, A. M., Helvik, J. V., and Becker, T. S. (2008). Expression of the eight AMPA receptor subunit genes in the developing central nervous system and sensory organs of zebrafish. *Developmental Dynamics*, 237(3), 788–799.

Horak, M., and Wenthold, R. J. (2009). Different roles of C-terminal cassettes in the trafficking of full-length NR1 subunits to the cell surface. *The Journal of Biological Chemistry*, 284(15), 9683–9691.

Horiuchi, M., Smith, L., Maezawa, I., and Jin, L. W. (2017). CX₃CR1 ablation ameliorates motor and respiratory dysfunctions and improves survival of a

Rett syndrome mouse model. *Brain, Behavior, and Immunity*, 60, 106–116.

Hornik, T. C., Vilalta, A., and Brown, G. C. (2016). Activated microglia cause reversible apoptosis of pheochromocytoma cells, inducing their cell death by phagocytosis. *Journal of Cell Science*, 129(1), 65–79.

Hou, G., and Zhang, Z. W. (2017). NMDA Receptors Regulate the Development of Neuronal Intrinsic Excitability through Cell-Autonomous Mechanisms. *Frontiers in Cellular Neuroscience*, 11, 353.

Houdebine, L. M. (2004). Chapter 6–The Mouse as an Animal Model for Human Diseases. In: *The Laboratory Mouse*.

Howard, J. F., Jr, Bril, V., Burns, T. M., Mantegazza, R., Bilinska, M., Szczudlik, A., Beydoun, S., Garrido, F. J. R. R., Piehl, F., Rottoli, M., Van Damme, P., Vu, T., Evoli, A., Freimer, M., Mozaffar, T., Ward, E. S., Dreier, T., Ulrichs, P., Verschuere, K., Guglietta, A., ... Efgartigimod MG Study Group (2019). Randomized phase 2 study of FcRn antagonist efgartigimod in generalized myasthenia gravis. *Neurology*, 92(23), e2661–e2673.

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., and Malinow, R. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron*, 52(5), 831–843.

Hua, J. Y., and Smith, S. J. (2004). Neural activity and the dynamics of central nervous system development. *Nature Neuroscience*, 7(4), 327–332.

Huang, Y. H., Ishikawa, M., Lee, B. R., Nakanishi, N., Schlüter, O. M., and Dong, Y. (2011). Searching for presynaptic NMDA receptors in the nucleus accumbens. *The Journal of Neuroscience*, 31(50), 18453–18463.

Huang, Q., Xie, Y., Hu, Z., and Tang, X. (2020). Anti-N-methyl-D-aspartate receptor encephalitis: A review of pathogenic mechanisms, treatment, prognosis. *Brain Research*, 1727, 146549.

Huerta, P. T., Kowal, C., DeGiorgio, L. A., Volpe, B. T., and Diamond, B. (2006). Immunity and behavior: antibodies alter emotion. *Proceedings of the National Academy of Sciences of the United States of America*, 103(3), 678–683.

Huettnner J. E. (2003). Kainate receptors and synaptic transmission. *Progress in Neurobiology*, 70(5), 387–407.

Huganir, R. L., and Nicoll, R. A. (2013). AMPARs and synaptic plasticity: the last 25 years. *Neuron*, 80(3), 704–717.

Hughes, E. G., Peng, X., Gleichman, A. J., Lai, M., Zhou, L., Tsou, R., Parsons, T. D., Lynch, D. R., Dalmau, J., and Balice-Gordon, R. J. (2010). Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *The Journal of Neuroscience*, 30(17), 5866–5875.

Hunnicut, B. J., Jongbloets, B. C., Birdsong, W. T., Gertz, K. J., Zhong, H., and Mao, T. (2016). A comprehensive excitatory input map of the striatum reveals novel functional organization. *eLife*, 5, e19103.

Hunt, D. L., and Castillo, P. E. (2012). Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Current Opinion in Neurobiology*, 22(3), 496–508.

Hunter, D., Jamet, Z., and Groc, L. (2021). Autoimmunity and NMDA receptor in brain disorders: Where do we stand?. *Neurobiology of Disease*, 147, 105161.

Hurst, R. S., Cepeda, C., Shumate, L. W., and Levine, M. S. (2001). Delayed postnatal development of NMDA receptor function in medium-sized neurons of the rat striatum. *Developmental Neuroscience*, 23(2), 122–134.

Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., Tenkova, T. I., Stefovsk, V., Turski, L., and Olney, J. W. (1999). Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science*, 283(5398), 70–74.

Indersmitten, T., Tran, C. H., Cepeda, C., and Levine, M. S. (2015). Altered excitatory and inhibitory

inputs to striatal medium-sized spiny neurons and cortical pyramidal neurons in the Q175 mouse model of Huntington's disease. *Journal of Neurophysiology*, 113(7), 2953–2966.

Innocenti, G. M., and Price, D. J. (2005). Exuberance in the development of cortical networks. *Nature reviews. Neuroscience*, 6(12), 955–965.

Irani, S. R., Bera, K., Waters, P., Zuliani, L., Maxwell, S., Zandi, M. S., Friesse, M. A., Galea, I., Kullmann, D. M., Beeson, D., Lang, B., Bien, C. G., and Vincent, A. (2010). N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain*, 133(Pt 6), 1655–1667.

Irani, S. R., Stagg, C. J., Schott, J. M., Rosenthal, C. R., Schneider, S. A., Pettingill, P., Pettingill, R., Waters, P., Thomas, A., Voets, N. L., Cardoso, M. J., Cash, D. M., Manning, E. N., Lang, B., Smith, S. J., Vincent, A., and Johnson, M. R. (2013). Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain*, 136(Pt 10), 3151–3162.

Irani, S. (2022, January 11). *Room 5 Medical Mysteries*. BBC Sounds Podcast. Episode 2/9, Series 1.

Isaac, J. T., Crair, M. C., Nicoll, R. A., and Malenka, R. C. (1997). Silent synapses during development of thalamocortical inputs. *Neuron*, 18(2), 269–280.

Isaac, J. T., Nicoll, R. A., and Malenka, R. C. (1995). Evidence for silent synapses: implications for the expression of LTP. *Neuron*, 15(2), 427–434.

Iwasato, T., Erzurumlu, R. S., Huerta, P. T., Chen, D. F., Sasaoka, T., Ulupinar, E., and Tonegawa, S. (1997). NMDA receptor-dependent refinement of somatotopic maps. *Neuron*, 19(6), 1201–1210.

Iwasato, T., Datwani, A., Wolf, A. M., Nishiyama, H., Taguchi, Y., Tonegawa, S., Knöpfel, T., Erzurumlu, R. S., and Itohara, S. (2000). Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature*, 406(6797), 726–731.

Jacobson, L., Polizzi, A., Morriss-Kay, G., and Vincent, A. (1999). Plasma from human mothers of fetuses with severe arthrogryposis multiplex congenita causes deformities in mice. *The Journal of Clinical Investigation*, 103(7), 1031–1038.

Jagota, P., Vincent, A., and Bhidayasiri, R. (2014). Transplacental transfer of NMDA receptor antibodies in an infant with cortical dysplasia. *Neurology*, 82(18), 1662–1663.

Jakowec, M. W., Jackson-Lewis, V., Chen, X., Langston, J. W., and Przedborski, S. (1998). The postnatal development of AMPA receptor subunits in the basal ganglia of the rat. *Developmental Neuroscience*, 20(1), 19–33.

Jakowich, S. K., Nasser, H. B., Strong, M. J., McCartney, A. J., Perez, A. S., Rakesh, N., Carruthers, C. J., and Sutton, M. A. (2010). Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. *Neuron*, 68(6), 1143–1158.

Jansson, L. C., Louhivuori, L., Wigren, H. K., Nordström, T., Louhivuori, V., Castrén, M. L., and Åkerman, K. E. (2012). Brain-derived neurotrophic factor increases the motility of a particular N-methyl-D-aspartate /GABA-responsive subset of neural progenitor cells. *Neuroscience*, 224, 223–234.

Jansson, L. C., and Åkerman, K. E. (2014). The role of glutamate and its receptors in the proliferation, migration, differentiation and survival of neural progenitor cells. *Journal of Neural Transmission*, 121(8), 819–836.

Javitt, D. C., Silipo, G., Cienfuegos, A., Shelley, A. M., Bark, N., Park, M., Lindenmayer, J. P., Suckow, R., and Zukin, S. R. (2001). Adjunctive high-dose glycine in the treatment of schizophrenia. *The International Journal of Neuropsychopharmacology*, 4(4), 385–391.

Jelitai, M., Schlett, K., Varju, P., Eisel, U., and Madarász, E. (2002). Regulated appearance of NMDA receptor subunits and channel functions during *in vitro* neuronal differentiation. *Journal of Neurobiology*, 51(1), 54–65.

- Jiang, W.**, Wolfe, K., Xiao, L., Zhang, Z. J., Huang, Y. G., and Zhang, X. (2004). Ionotropic glutamate receptor antagonists inhibit the proliferation of granule cell precursors in the adult brain after seizures induced by pentylentetrazol. *Brain Research*, 1020(1-2), 154–160.
- Johnson, S. A., and Simmon, V. F.** (2002). Randomized, double-blind, placebo-controlled international clinical trial of the Ampakine CX516 in elderly participants with mild cognitive impairment: a progress report. *Journal of Molecular Neuroscience*, 19(1-2), 197–200.
- Joubert, B.**, García-Serra, A., Planagumà, J., Martínez-Hernández, E., Kraft, A., Palm, F., Iizuka, T., Honnorat, J., Leypoldt, F., Graus, F., and Dalmau, J. (2020). Pregnancy outcomes in anti-NMDA receptor encephalitis: Case series. *Neurology(R) Neuroimmunology and Neuroinflammation*, 7(3), e668.
- Jourdain, P.**, Bergersen, L. H., Bhaukaurally, K., Bezzi, P., Santello, M., Domercq, M., Matute, C., Tonello, F., Gundersen, V., and Volterra, A. (2007). Glutamate exocytosis from astrocytes controls synaptic strength. *Nature Neuroscience*, 10(3), 331–339.
- Jurek, B.**, Chayka, M., Kreye, J., Lang, K., Kraus, L., Fidzinski, P., Kornau, H. C., Dao, L. M., Wenke, N. K., Long, M., Rivalan, M., Winter, Y., Leubner, J., Herken, J., Mayer, S., Mueller, S., Boehm-Sturm, P., Dirnagl, U., Schmitz, D., Kölch, M., ... Prüss, H. (2019). Human gestational N-methyl-d-aspartate receptor autoantibodies impair neonatal murine brain function. *Annals of Neurology*, 86(5), 656–670.
- Jurga, A. M.**, Paleczna, M., and Kuter, K. Z. (2020). Overview of General and Discriminating Markers of Differential Microglia Phenotypes. *Frontiers in Cellular Neuroscience*, 14, 198.
- Kabakci, K.**, Hedrich, K., Leung, J. C., Mitterer, M., Vieregge, P., Lencer, R., Hagenah, J., Garrels, J., Witt, K., Klostermann, F., Svetel, M., Friedman, J., Kostic, V., Bressman, S. B., Breakefield, X. O., Ozelius, L. J., Pramstaller, P. P., and Klein, C. (2004). Mutations in DYT1: extension of the phenotypic and mutational spectrum. *Neurology*, 62(3), 395–400.
- Kageyama, R.**, Ohtsuka, T., Hatakeyama, J., and Ohsawa, R. (2005). Roles of bHLH genes in neural stem cell differentiation. *Experimental Cell Research*, 306(2), 343–348.
- Kahn, I.**, Helman, G., Vanderver, A., and Wells, E. (2017). Anti- N-Methyl-d-Aspartate (NMDA) Receptor Encephalitis. *Journal of Child Neurology*, 32(2), 243–245.
- Kaindl, A. M.**, Degos, V., Peineau, S., Gouadon, E., Chhor, V., Loron, G., Le Charpentier, T., Josserand, J., Ali, C., Vivien, D., Collingridge, G. L., Lombet, A., Issa, L., Rene, F., Loeffler, J. P., Kavelaars, A., Verney, C., Mantz, J., and Gressens, P. (2012). Activation of microglial N-methyl-D-aspartate receptors triggers inflammation and neuronal cell death in the developing and mature brain. *Annals of Neurology*, 72(4), 536–549.
- Kalam, S.**, Baheerathan, A., McNamara, C., and Singh-Curry, V. (2019). Anti-NMDAR encephalitis complicating pregnancy. *Practical neurology*, 19(2), 131–135.
- Kalb R. G.** (1994). Regulation of motor neuron dendrite growth by NMDA receptor activation. *Development*, 120(11), 3063–3071.
- Kalueff, A. V.**, Stewart, A. M., Song, C., Berridge, K. C., Graybiel, A. M., and Fentress, J. C. (2016). Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nature Reviews. Neuroscience*, 17(1), 45–59.
- Kanju, P. M.**, Parameshwaran, K., Sims, C., Bahr, B. A., Shonesy, B. C., and Suppiramaniam, V. (2008). Ampakine CX516 ameliorates functional deficits in AMPA receptors in a hippocampal slice model of protein accumulation. *Experimental Neurology*, 214(1), 55–61.
- Kaplan, E. S.**, Ramos-Laguna, K. A., Mihalas, A. B., Daza, R. A. M., and Hevner, R. F. (2017). Neocortical Sox9+ radial glia generate glutamatergic neurons for all layers, but lack discernible evidence of early laminar fate restriction. *Neural Development*, 12(1), 14.
- Karakas, E.**, Simorowski, N., and Furukawa, H. (2011). Subunit arrangement and

phenylethanolamine binding in GluN1/GluN2B NMDA receptors. *Nature*, 475(7355), 249–253.

Karakas, E., and Furukawa, H. (2014). Crystal structure of a heterotetrameric NMDA receptor ion channel. *Science*, 344(6187), 992–997.

Karperien, A., Ahammer, H., and Jelinek, H. F. (2013). Quantitating the subtleties of microglial morphology with fractal analysis. *Frontiers in Cellular Neuroscience*, 7, 3.

Karschin, C., Dissmann, E., Stühmer, W., and Karschin, A. (1996). IRK(1-3) and GIRK(1-4) inwardly rectifying K⁺ channel mRNAs are differentially expressed in the adult rat brain. *The Journal of Neuroscience*, 16(11), 3559–3570.

Kato, G., Inada, H., Wake, H., Akiyoshi, R., Miyamoto, A., Eto, K., Ishikawa, T., Moorhouse, A. J., Strassman, A. M., and Nabekura, J. (2016). Microglial Contact Prevents Excess Depolarization and Rescues Neurons from Excitotoxicity. *eNeuro*, 3(3), ENEURO.0004-16.2016.

Kaur, C., Sivakumar, V., and Ling, E. A. (2005). Expression of N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) GluR2/3 receptors in the developing rat pineal gland. *Journal of Pineal Research*, 39(3), 294–301.

Kaur, C., Dheen, S. T., and Ling, E. A. (2007). From blood to brain: amoeboid microglial cell, a nascent macrophage and its functions in developing brain. *Acta Pharmacologica Sinica*, 28(8), 1087–1096.

Kawaguchi Y. (1993). Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *The Journal of Neuroscience*, 13(11), 4908–4923.

Kehoe, L. A., Bernardinelli, Y., and Müller, D. (2013). GluN3A: an NMDA receptor subunit with exquisite properties and functions. *Neural Plasticity*, 2013, 145387.

Kelly, S. M., Raudales, R., He, M., Lee, J. H., Kim, Y., Gibb, L. G., Wu, P., Matho, K., Osten, P., Graybiel, A.

M., and Huang, Z. J. (2018). Radial Glial Lineage Progression and Differential Intermediate Progenitor Amplification Underlie Striatal Compartments and Circuit Organization. *Neuron*, 99(2), 345–361.e4.

Kelsch, W., Li, Z., Wieland, S., Senkov, O., Herb, A., Göngrich, C., and Monyer, H. (2014). GluN2B-containing NMDA receptors promote glutamate synapse development in hippocampal interneurons. *The Journal of Neuroscience*, 34(48), 16022–16030.

Kemp, J. M., and Powell, T. P. (1971). The site of termination of afferent fibres in the caudate nucleus. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 262(845), 413–427.

Kersten, M., Rabbe, T., Blome, R., Porath, K., Sellmann, T., Bien, C. G., Köhling, R., and Kirschstein, T. (2019). Novel Object Recognition in Rats With NMDAR Dysfunction in CA1 After Stereotactic Injection of Anti-NMDAR Encephalitis Cerebrospinal Fluid. *Frontiers in Neurology*, 10, 586.

Kesner, P., Schohl, A., Warren, E. C., Ma, F., and Ruthazer, E. S. (2020). Postsynaptic and Presynaptic NMDARs Have Distinct Roles in Visual Circuit Development. *Cell Reports*, 32(4), 107955.

Khalaf-Nazzal, R., and Francis, F. (2013). Hippocampal development - old and new findings. *Neuroscience*, 248, 225–242.

Khazipov, R., Sirota, A., Leinekugel, X., Holmes, G. L., Ben-Ari, Y., and Buzsáki, G. (2004). Early motor activity drives spindle bursts in the developing somatosensory cortex. *Nature*, 432(7018), 758–761.

Kidd, F. L., and Isaac, J. T. (1999). Developmental and activity-dependent regulation of kainate receptors at thalamocortical synapses. *Nature*, 400(6744), 569–573.

Kim, G. J., Shatz, C. J., and McConnell, S. K. (1991). Morphology of pioneer and follower growth cones in the developing cerebral cortex. *Journal of Neurobiology*, 22(6), 629–642.

Kim, B., and Im, H. I. (2019). The role of the dorsal striatum in choice impulsivity. *Annals of the New York Academy of Sciences*, 1451(1), 92–111.

Kim, M. H., Radaelli, C., Thomsen, E. R., Monet, D., Chartrand, T., Jorstad, N. L., Mahoney, J. T., Taormina, M. J., Long, B., Baker, K., Bakken, T. E., Campagnola, L., Casper, T., Clark, M., Dee, N., D'Orazi, F., Gamlin, C., Kalmbach, B. E., Kebede, S., Lee, B. R., ... Lein, E. (2023). Target cell-specific synaptic dynamics of excitatory to inhibitory neuron connections in supragranular layers of human neocortex. *eLife*, 12, e81863.

Kirkby, L. A., Sack, G. S., Firl, A., and Feller, M. B. (2013). A role for correlated spontaneous activity in the assembly of neural circuits. *Neuron*, 80(5), 1129–1144.

Klaus, A., Martins, G. J., Paixao, V. B., Zhou, P., Paninski, L., and Costa, R. M. (2017). The Spatiotemporal Organization of the Striatum Encodes Action Space. *Neuron*, 95(5), 1171–1180.e7.

Klavinskis-Whiting, S., Bitzenhofer, S., Hanganu-Opatz, I., and Ellender, T. (2023). Generation and propagation of bursts of activity in the developing basal ganglia. *Cerebral Cortex*, 33(20), 10595–10613.

Klawonn, A. M., Fritz, M., Castany, S., Pignatelli, M., Canal, C., Similä, F., Tejeda, H. A., Levinsson, J., Jaarola, M., Jakobsson, J., Hidalgo, J., Heilig, M., Bonci, A., and Engblom, D. (2021). Microglial activation elicits a negative affective state through prostaglandin-mediated modulation of striatal neurons. *Immunity*, 54(2), 225–234.e6.

Knowles, R., Dehorter, N., and Ellender, T. (2021). From Progenitors to Progeny: Shaping Striatal Circuit Development and Function. *The Journal of Neuroscience*, 41(46), 9483–9502.

Kokubun, N., Komagamine, T., and Hirata, K. (2016). Pregnancy and delivery in anti-NMDA receptor encephalitis survivors. *Neurology. Clinical Practice*, 6(5), e40–e43.

Komuro, H., and Rakic, P. (1993). Modulation of neuronal migration by NMDA receptors. *Science*, 260(5104), 95–97.

Kornau, H. C., Kreye, J., Stumpf, A., Fukata, Y., Parthier, D., Sammons, R. P., Imbrosci, B., Kurpjuweit, S., Kowski, A. B., Fukata, M., Prüss, H., and Schmitz, D. (2020). Human Cerebrospinal Fluid Monoclonal LGI1 Autoantibodies Increase Neuronal Excitability. *Annals of Neurology*, 87(3), 405–418.

Kovács, A. D., Cebers, G., Cebere, A., Moreira, T., and Liljequist, S. (2001). Cortical and striatal neuronal cultures of the same embryonic origin show intrinsic differences in glutamate receptor expression and vulnerability to excitotoxicity. *Experimental Neurology*, 168(1), 47–62.

Kovalenko, M., Milnerwood, A., Giordano, J., St Claire, J., Guide, J. R., Stromberg, M., Gillis, T., Sapp, E., DiFiglia, M., MacDonald, M. E., Carroll, J. B., Lee, J. M., Tappan, S., Raymond, L., and Wheeler, V. C. (2018). HttQ111/+ Huntington's Disease Knock-in Mice Exhibit Brain Region-Specific Morphological Changes and Synaptic Dysfunction. *Journal of Huntington's Disease*, 7(1), 17–33.

Kowal, C., DeGiorgio, L. A., Nakaoka, T., Hetherington, H., Huerta, P. T., Diamond, B., and Volpe, B. T. (2004). Cognition and immunity; antibody impairs memory. *Immunity*, 21(2), 179–188.

Kozorovitskiy, Y., Peixoto, R., Wang, W., Saunders, A., and Sabatini, B. L. (2015). Neuromodulation of excitatory synaptogenesis in striatal development. *eLife*, 4, e10111.

Kraeuter, A. K., Guest, P. C., and Sarnyai, Z. (2019). The Open-field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. *Methods in Molecular Biology*, 1916, 99–103.

Krajeski, R. N., Macey-Dare, A., Van Heusden, F., Ebrahimjee, F., and Ellender, T. J. (2019). Dynamic postnatal development of the cellular and circuit properties of striatal D1 and D2 spiny projection neurons. *The Journal of Physiology*, 597(21), 5265–5293.

- Kravitz, A. V.,** Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., Deisseroth, K., and Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature*, 466(7306), 622–626.
- Kravitz, A. V., and Kreitzer, A. C.** (2012). Striatal mechanisms underlying movement, reinforcement, and punishment. *Physiology*, 27(3), 167–177.
- Kreitzer, A. C., and Malenka, R. C.** (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature*, 445(7128), 643–647.
- Kreye, J.,** Wenke, N. K., Chayka, M., Leubner, J., Murugan, R., Maier, N., Jurek, B., Ly, L. T., Brandl, D., Rost, B. R., Stumpf, A., Schulz, P., Radbruch, H., Hauser, A. E., Pache, F., Meisel, A., Harms, L., Paul, F., Dirnagl, U., Garner, C., ... Prüss, H. (2016). Human cerebrospinal fluid monoclonal N-methyl-D-aspartate receptor autoantibodies are sufficient for encephalitis pathogenesis. *Brain*, 139(Pt 10), 2641–2652.
- Kriegstein, A., and Alvarez-Buylla, A.** (2009). The glial nature of embryonic and adult neural stem cells. *Annual Review of Neuroscience*, 32, 149–184.
- Kuchling, J.,** Jurek, B., Kents, M., Kreye, J., Geis, C., Wickel, J., Mueller, S., Koch, S. P., Boehm-Sturm, P., Prüss, H., and Finke, C. (2024). Impaired functional connectivity of the hippocampus in translational murine models of NMDA-receptor antibody associated neuropsychiatric pathology. *Molecular Psychiatry*, 29(1), 85–96.
- Kumar, S. S., and Huguenard, J. R.** (2003). Pathway-specific differences in subunit composition of synaptic NMDA receptors on pyramidal neurons in neocortex. *The Journal of Neuroscience*, 23(31), 10074–10083.
- Kumar, M. A.,** Jain, A., Dechant, V. E., Saito, T., Rafael, T., Aizawa, H., Dysart, K. C., Katayama, T., Ito, Y., Araki, N., Abe, T., Balice-Gordon, R., and Dalmau, J. (2010). Anti-N-methyl-D-aspartate receptor encephalitis during pregnancy. *Archives of Neurology*, 67(7), 884–887.
- Kunchok, A.,** Zekeridou, A., and McKeon, A. (2019). Autoimmune glial fibrillary acidic protein astrocytopathy. *Current Opinion in Neurology*, 32(3), 452–458.
- Kupferschmidt, D. A.,** Juczewski, K., Cui, G., Johnson, K. A., and Lovinger, D. M. (2017). Parallel, but Dissociable, Processing in Discrete Corticostriatal Inputs Encodes Skill Learning. *Neuron*, 96(2), 476–489.e5.
- Kwok, R. P.,** Lundblad, J. R., Chrivia, J. C., Richards, J. P., Bächinger, H. P., Brennan, R. G., Roberts, S. G., Green, M. R., and Goodman, R. H. (1994). Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature*, 370(6486), 223–226.
- Kwon, H. B., and Sabatini, B. L.** (2011). Glutamate induces de novo growth of functional spines in developing cortex. *Nature*, 474(7349), 100–104.
- Laclef, C., and Métin, C.** (2018). Conserved rules in embryonic development of cortical interneurons. *Seminars in Cell and Developmental Biology*, 76, 86–100.
- Ladépêche, L.,** Planagumà, J., Thakur, S., Suárez, I., Hara, M., Borbely, J. S., Sandoval, A., Laparra-Cuervo, L., Dalmau, J., and Lakadamyali, M. (2018). NMDA Receptor Autoantibodies in Autoimmune Encephalitis Cause a Subunit-Specific Nanoscale Redistribution of NMDA Receptors. *Cell Reports*, 23(13), 3759–3768.
- Lago, T.,** Davis, A., Grillon, C., and Ernst, M. (2017). Striatum on the anxiety map: Small detours into adolescence. *Brain Research*, 1654(Pt B), 177–184.
- Lai, T. W.,** Shyu, W. C., and Wang, Y. T. (2011). Stroke intervention pathways: NMDA receptors and beyond. *Trends in Molecular Medicine*, 17(5), 266–275.
- Lancaster, E.,** Lai, M., Peng, X., Hughes, E., Constantinescu, R., Raizer, J., Friedman, D., Skeen, M. B., Grisold, W., Kimura, A., Ohta, K., Iizuka, T., Guzman, M., Graus, F., Moss, S. J., Balice-Gordon, R., and Dalmau, J. (2010). Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *The Lancet. Neurology*, 9(1), 67–76.

Lanciego, J. L., Luquin, N., and Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor perspectives in medicine*, 2(12), a009621.

Landwehrmeyer, G. B., Standaert, D. G., Testa, C. M., Penney, J. B., Jr, and Young, A. B. (1995). NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. *The Journal of Neuroscience*, 15(7 Pt 2), 5297–5307.

Lang, K., and Prüss, H. (2017). Frequencies of neuronal autoantibodies in healthy controls: Estimation of disease specificity. *Neurology(R) Neuroimmunology and Neuroinflammation*, 4(5), e386.

Langen, M., Kas, M. J. H., Staal, W. G., Van Engeland, H., and Durston, S. (2011). The neurobiology of repetitive behavior: Of mice. *Neuroscience and Biobehavioral Reviews*, 35(3), 345–355.

Larsen, R. S., Corlew, R. J., Henson, M. A., Roberts, A. C., Mishina, M., Watanabe, M., Lipton, S. A., Nakanishi, N., Pérez-Otaño, I., Weinberg, R. J., and Philpot, B. D. (2011). NR3A-containing NMDARs promote neurotransmitter release and spike timing-dependent plasticity. *Nature Neuroscience*, 14(3), 338–344.

Larsen, R. S., Smith, I. T., Miriyala, J., Han, J. E., Corlew, R. J., Smith, S. L., and Philpot, B. D. (2014). Synapse-specific control of experience-dependent plasticity by presynaptic NMDA receptors. *Neuron*, 83(4), 879–893.

Lau, C. G., and Zukin, R. S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nature reviews. Neuroscience*, 8(6), 413–426.

Lau, D., and Bading, H. (2009). Synaptic activity-mediated suppression of p53 and induction of nuclear calcium-regulated neuroprotective genes promote survival through inhibition of mitochondrial permeability transition. *The Journal of Neuroscience*, 29(14), 4420–4429.

Laurie, D. J., and Seeburg, P. H. (1994). Regional and developmental heterogeneity in splicing of the rat

brain NMDAR1 mRNA. *The Journal of Neuroscience*, 14(5 Pt 2), 3180–3194.

Lauri, S. E., Segerstråle, M., Vesikansa, A., Maingret, F., Mülle, C., Collingridge, G. L., Isaac, J. T., and Taira, T. (2005). Endogenous activation of kainate receptors regulates glutamate release and network activity in the developing hippocampus. *The Journal of Neuroscience*, 25(18), 4473–4484.

Lauri, S. E., Vesikansa, A., Segerstråle, M., Collingridge, G. L., Isaac, J. T., and Taira, T. (2006). Functional maturation of CA1 synapses involves activity-dependent loss of tonic kainate receptor-mediated inhibition of glutamate release. *Neuron*, 50(3), 415–429.

Lauri, S. E., Ryazantseva, M., Orav, E., Vesikansa, A., and Taira, T. (2021). Kainate receptors in the developing neuronal networks. *Neuropharmacology*, 195, 108585.

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience*, 39(1), 151–170.

Le Magueresse, C., and Monyer, H. (2013). GABAergic interneurons shape the functional maturation of the cortex. *Neuron*, 77(3), 388–405.

Lebouc, M., Richard, Q., Garret, M., and Baufreton, J. (2020). Striatal circuit development and its alterations in Huntington's disease. *Neurobiology of Disease*, 145, 105076.

Lee, T., Kaneko, T., Taki, K., and Mizuno, N. (1997). Preprodynorphin-, preproenkephalin-, and preprotachykinin-expressing neurons in the rat neostriatum: an analysis by immunocytochemistry and retrograde tracing. *The Journal of Comparative Neurology*, 386(2), 229–244.

Lee, J., Duan, W., and Mattson, M. P. (2002). Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *Journal of neurochemistry*, 82(6), 1367–1375.

- Lee, H. K.,** Min, S. S., Gallagher, M., and Kirkwood, A. (2005). NMDA receptor-independent long-term depression correlates with successful aging in rats. *Nature Neuroscience*, 8(12), 1657–1659.
- Lee, J. Y.,** Huerta, P. T., Zhang, J., Kowal, C., Bertini, E., Volpe, B. T., and Diamond, B. (2009). Neurotoxic autoantibodies mediate congenital cortical impairment of offspring in maternal lupus. *Nature Medicine*, 15(1), 91–96.
- Lee, D.,** Seo, H., and Jung, M. W. (2012). Neural basis of reinforcement learning and decision making. *Annual Review of Neuroscience*, 35, 287–308.
- Lee, W. J.,** Lee, S. T., Byun, J. I., Sunwoo, J. S., Kim, T. J., Lim, J. A., Moon, J., Lee, H. S., Shin, Y. W., Lee, K. J., Kim, S., Jung, K. H., Jung, K. Y., Chu, K., and Lee, S. K. (2016). Rituximab treatment for autoimmune limbic encephalitis in an institutional cohort. *Neurology*, 86(18), 1683–1691.
- Leite, M. I.,** Jacob, S., Viegas, S., Cossins, J., Clover, L., Morgan, B. P., Beeson, D., Willcox, N., and Vincent, A. (2008). IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain*, 131(Pt 7), 1940–1952.
- Leyh, J.,** Paeschke, S., Mages, B., Michalski, D., Nowicki, M., Bechmann, I., and Winter, K. (2021). Classification of Microglial Morphological Phenotypes Using Machine Learning. *Frontiers in Cellular Neuroscience*, 15, 701673.
- Leypoldt, F.,** Höftberger, R., Titulaer, M. J., Armangue, T., Gresa-Arribas, N., Jahn, H., Rostásy, K., Schlumberger, W., Meyer, T., Wandinger, K. P., Rosenfeld, M. R., Graus, F., and Dalmau, J. (2015). Investigations on CXCL13 in anti-N-methyl-D-aspartate receptor encephalitis: a potential biomarker of treatment response. *JAMA Neurology*, 72(2), 180–186.
- Li, M.,** Zhang, D. Q., Wang, X. Z., and Xu, T. J. (2011). NR2B-containing NMDA receptors promote neural progenitor cell proliferation through CaMKIV/CREB pathway. *Biochemical and Biophysical Research Communications*, 411(4), 667–672.
- Li, Y.,** Tanaka, K., Wang, L., Ishigaki, Y., and Kato, N. (2015). Induction of Memory Deficit in Mice with Chronic Exposure to Cerebrospinal Fluid from Patients with Anti-N-Methyl-D-Aspartate Receptor Encephalitis. *The Tohoku Journal of Experimental Medicine*, 237(4), 329–338.
- Li, L.,** Sultan, S., Heigele, S., Schmidt-Salzmann, C., Toni, N., and Bischofberger, J. (2017). Silent synapses generate sparse and orthogonal action potential firing in adult-born hippocampal granule cells. *eLife*, 6, e23612.
- Liang, J.,** Takeuchi, H., Jin, S., Noda, M., Li, H., Doi, Y., Kawanokuchi, J., Sonobe, Y., Mizuno, T., and Suzumura, A. (2010). Glutamate induces neurotrophic factor production from microglia via protein kinase C pathway. *Brain Research*, 1322, 8–23.
- Liang, B.,** Zhang, L., Zhang, Y., Werner, C. T., Beachler, N. J., Denman, A. J., Li, Y., Chen, R., Gerfen, C. R., Barbera, G., and Lin, D. T. (2022). Striatal direct pathway neurons play leading roles in accelerating rotarod motor skill learning. *iScience*, 25(5), 104245.
- Liao, D.,** Hessler, N. A., and Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature*, 375(6530), 400–404.
- Liao, D.,** Zhang, X., O'Brien, R., Ehlers, M. D., and Huganir, R. L. (1999). Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nature Neuroscience*, 2(1), 37–43.
- Lieberman, O. J.,** McGuirt, A. F., Mosharov, E. V., Pigulevskiy, I., Hobson, B. D., Choi, S., Frier, M. D., Santini, E., Borgkvist, A., and Sulzer, D. (2018). Dopamine Triggers the Maturation of Striatal Spiny Projection Neuron Excitability during a Critical Period. *Neuron*, 99(3), 540–554.e4.
- Liljeholm, M., and O'Doherty, J. P.** (2012). Contributions of the striatum to learning, motivation, and performance: an associative account. *Trends in Cognitive Sciences*, 16(9), 467–475.

- Lilliu, V.,** Pernas-Alonso, R., Trelles, R. D., di Porzio, U., Zuddas, A., and Perrone-Capano, C. (2001). Ontogeny of AMPA receptor gene expression in the developing rat midbrain and striatum. *Brain Research. Molecular Brain Research*, 96(1-2), 133–141.
- Lilliu, V.,** Perrone-Capano, C., Pernas-Alonso, R., Diaz Trelles, R., Luca Colucci d'Amato, G., Zuddas, A., and di Porzio, U. (2002). Ontogeny of kainate receptor gene expression in the developing rat midbrain and striatum. *Brain Research. Molecular Brain Research*, 104(1), 1–10.
- Lipman, N. S.,** Jackson, L. R., Trudel, L. J., and Weis-Garcia, F. (2005). Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. *ILAR journal*, 46(3), 258–268.
- Lisman, J.,** Yasuda, R., and Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. *Nature reviews. Neuroscience*, 13(3), 169–182.
- Liu, D.,** Bobrovskaya, L., and Zhou, X. F. (2021). Cell Therapy for Neurological Disorders: The Perspective of Promising Cells. *Biology*, 10(11), 1142.
- Loayza, M.,** Lin, S., Carter, K., Ojeda, N., Fan, L. W., Ramarao, S., Bhatt, A., and Pang, Y. (2023). Maternal immune activation alters fetal and neonatal microglia phenotype and disrupts neurogenesis in mice. *Pediatric Research*, 93(5), 1216–1225.
- Lodato, S., and Arlotta, P.** (2015). Generating neuronal diversity in the mammalian cerebral cortex. *Annual Review of Cell and Developmental Biology*, 31, 699–720.
- Loftis, J. M., and Janowsky, A.** (2003). The N-methyl-D-aspartate receptor subunit NR2B: localization, functional properties, regulation, and clinical implications. *Pharmacology and Therapeutics*, 97(1), 55–85.
- Loomba, S.,** Straehle, J., Gangadharan, V., Heike, N., Khalifa, A., Motta, A., Ju, N., Sievers, M., Gempt, J., Meyer, H. S., and Helmstaedter, M. (2022). Connectomic comparison of mouse and human cortex. *Science*, 377(6602), eabo0924.
- López de Armentia, M., and Sah, P.** (2003). Development and subunit composition of synaptic NMDA receptors in the amygdala: NR2B synapses in the adult central amygdala. *The Journal of Neuroscience*, 23(17), 6876–6883.
- López, T.,** López-Colomé, A. M., and Ortega, A. (1997). NMDA receptors in cultured radial glia. *FEBS letters*, 405(2), 245–248.
- Lorenzetti, V.,** Cousijn, J., and Preedy, V. R. (2016). Cannabis use disorders and brain morphology. In *Neuropathology of Drug Addictions and Substance Misuse* (pp. 773–785). Academic Press.
- LoTurco, J. J.,** Owens, D. F., Heath, M. J., Davis, M. B., and Kriegstein, A. R. (1995). GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron*, 15(6), 1287–1298.
- LoTurco, J. J.,** Blanton, M. G., and Kriegstein, A. R. (1991). Initial expression and endogenous activation of NMDA channels in early neocortical development. *The Journal of Neuroscience*, 11(3), 792–799.
- Lu, L.,** Mamiya, T., Lu, P., Toriumi, K., Mouri, A., Hiramatsu, M., Kim, H. C., Zou, L. B., Nagai, T., and Nabeshima, T. (2010). Prenatal exposure to phencyclidine produces abnormal behaviour and NMDA receptor expression in postpubertal mice. *The International Journal of Neuropsychopharmacology*, 13(7), 877–889.
- Lu, W.,** Gray, J. A., Granger, A. J., During, M. J., and Nicoll, R. A. (2011). Potentiation of synaptic AMPA receptors induced by the deletion of NMDA receptors requires the GluA2 subunit. *Journal of Neurophysiology*, 105(2), 923–928.
- Luhmann, H. J.,** Fukuda, A., and Kilb, W. (2015). Control of cortical neuronal migration by glutamate and GABA. *Frontiers in Cellular Neuroscience*, 9, 4.
- Luján, R.,** Shigemoto, R., and López-Bendito, G. (2005). Glutamate and GABA receptor signalling in the developing brain. *Neuroscience*, 130(3), 567–580.
- Luk, K. C.,** Kennedy, T. E., and Sadikot, A. F. (2003). Glutamate promotes proliferation of striatal

neuronal progenitors by an NMDA receptor-mediated mechanism. *The Journal of Neuroscience*, 23(6), 2239–2250.

Lukens, J. R., and Eyo, U. B. (2022). Microglia and Neurodevelopmental Disorders. *Annual Review of Neuroscience*, 45, 425–445.

Lüscher, C., and Malenka, R. C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harbor Perspectives in Biology*, 4(6), a005710.

Luskin, M. B., Pearlman, A. L., and Sanes, J. R. (1988). Cell lineage in the cerebral cortex of the mouse studied *in vivo* and *in vitro* with a recombinant retrovirus. *Neuron*, 1(8), 635–647.

Lussier, M. P., Sanz-Clemente, A., and Roche, K. W. (2015). Dynamic Regulation of N-Methyl-D-aspartate (NMDA) and α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptors by Posttranslational Modifications. *The Journal of Biological Chemistry*, 290(48), 28596–28603.

Lynch, D. R., Rattelle, A., Dong, Y. N., Roslin, K., Gleichman, A. J., and Panzer, J. A. (2018). Anti-NMDA Receptor Encephalitis: Clinical Features and Basic Mechanisms. *Advances in pharmacology*, 82, 235–260.

Lynch, G., and Gall, C. M. (2006). Ampakines and the threefold path to cognitive enhancement. *Trends in Neurosciences*, 29(10), 554–562.

Lynch, G., Rex, C. S., and Gall, C. M. (2007). LTP consolidation: substrates, explanatory power, and functional significance. *Neuropharmacology*, 52(1), 12–23.

Maffei, A., Nelson, S. B., and Turrigiano, G. G. (2004). Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. *Nature Neuroscience*, 7(12), 1353–1359.

Magee J. C. (2000). Dendritic integration of excitatory synaptic input. *Nature Reviews. Neuroscience*, 1(3), 181–190.

Magley, J., Towner, D., Taché, V., and Apperson, M. L. (2012). Pregnancy outcome in anti-N-methyl-D-aspartate receptor encephalitis. *Obstetrics and Gynecology*, 120(2 Pt 2), 480–483.

Mahon, S., Deniau, J. M., and Charpier, S. (2003). Various synaptic activities and firing patterns in cortico-striatal and striatal neurons *in vivo*. *Journal of Physiology*, 97(4-6), 557–566.

Malatesta, P., Hartfuss, E., and Götz, M. (2000). Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development*, 127(24), 5253–5263.

Malenka, R. C., and Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron*, 44(1), 5–21.

Maletic-Savatic, M., Malinow, R., and Svoboda, K. (1999). Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science*, 283(5409), 1923–1927.

Malinow, R., and Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience*, 25, 103–126.

Malviya, M., Barman, S., Golombeck, K. S., Planagumà, J., Mannara, F., Strutz-Seebohm, N., Wrzos, C., Demir, F., Baksmeier, C., Steckel, J., Falk, K. K., Gross, C. C., Kovac, S., Bönnte, K., Johnen, A., Wandinger, K. P., Martín-García, E., Becker, A. J., Elger, C. E., Klöcker, N., ... Goebels, N. (2017). NMDAR encephalitis: passive transfer from man to mouse by a recombinant antibody. *Annals of Clinical and Translational Neurology*, 4(11), 768–783.

Mancini, J. D., and Atchison, W. D. (2007). The NR2B subunit in NMDA receptors is functionally important during cerebellar granule cell migration. *Neuroscience Letters*, 429(2-3), 87–90.

Mandal, M., Wei, J., Zhong, P., Cheng, J., Duffney, L. J., Liu, W., Yuen, E. Y., Twelvetrees, A. E., Li, S., Li, X. J., Kittler, J. T., and Yan, Z. (2011). Impaired α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking and function by mutant huntingtin. *The Journal of Biological Chemistry*, 286(39), 33719–33728.

Manent, J. B., Jorquera, I., Ben-Ari, Y., Aniksztejn, L., and Represa, A. (2006). Glutamate acting on AMPA but not NMDA receptors modulates the migration of hippocampal interneurons. *The Journal of Neuroscience*, 26(22), 5901–5909.

Manent, J. B., and Represa, A. (2007). Neurotransmitters and brain maturation: early paracrine actions of GABA and glutamate modulate neuronal migration. *The Neuroscientist*, 13(3), 268–279.

Manto, M., Dalmau, J., Didelot, A., Rogemond, V., and Honnorat, J. (2010). *In vivo* effects of antibodies from patients with anti-NMDA receptor encephalitis: further evidence of synaptic glutamatergic dysfunction. *Orphanet Journal of Rare Diseases*, 5, 31.

Manuel, M. N., Mi, D., Mason, J. O., and Price, D. J. (2015). Regulation of cerebral cortical neurogenesis by the Pax6 transcription factor. *Frontiers in Cellular Neuroscience*, 9, 70.

Mao, R., Zong, N., Hu, Y., Chen, Y., and Xu, Y. (2022). Neuronal Death Mechanisms and Therapeutic Strategy in Ischemic Stroke. *Neuroscience Bulletin*, 38(10), 1229–1247.

Maragakis, N. J., and Rothstein, J. D. (2004). Glutamate transporters: animal models to neurologic disease. *Neurobiology of Disease*, 15(3), 461–473.

Marchand, R., and Lajoie, L. (1986). Histogenesis of the striopallidal system in the rat. Neurogenesis of its neurons. *Neuroscience*, 17(3), 573–590.

Maric, D., Liu, Q. Y., Grant, G. M., Andreadis, J. D., Hu, Q., Chang, Y. H., Barker, J. L., Joseph, J., Stenger, D. A., and Ma, W. (2000). Functional ionotropic glutamate receptors emerge during terminal cell division and early neuronal differentiation of rat neuroepithelial cells. *Journal of Neuroscience Research*, 61(6), 652–662.

Marín O. (2013). Cellular and molecular mechanisms controlling the migration of neocortical interneurons. *The European Journal of Neuroscience*, 38(1), 2019–2029.

Marín, O., and Müller, U. (2014). Lineage origins of GABAergic versus glutamatergic neurons in the neocortex. *Current Opinion in Neurobiology*, 26, 132–141.

Marín-Teva, J. L., Dusart, I., Colin, C., Gervais, A., Van Rooijen, N., and Mallat, M. (2004). Microglia promote the death of developing Purkinje cells. *Neuron*, 41(4), 535–547.

Markowitz, J. E., Gillis, W. F., Beron, C. C., Neufeld, S. Q., Robertson, K., Bhagat, N. D., Peterson, R. E., Peterson, E., Hyun, M., Linderman, S. W., Sabatini, B. L., and Datta, S. R. (2018). The Striatum Organizes 3D Behavior via Moment-to-Moment Action Selection. *Cell*, 174(1), 44–58.e17.

Marret, S., Gressens, P., and Evrard, P. (1996). Arrest of neuronal migration by excitatory amino acids in hamster developing brain. *Proceedings of the National Academy of Sciences of the United States of America*, 93(26), 15463–15468.

Marsden, K. C., Shemesh, A., Bayer, K. U., and Carroll, R. C. (2010). Selective translocation of Ca²⁺/calmodulin protein kinase IIα (CaMKIIα) to inhibitory synapses. *Proceedings of the National Academy of Sciences of the United States of America*, 107(47), 20559–20564.

Marshall, J. J., and Mason, J. O. (2019). Mouse vs man: Organoid models of brain development and disease. *Brain Research*, 1724, 146427.

Martel, M. A., Ryan, T. J., Bell, K. F., Fowler, J. H., McMahon, A., Al-Mubarak, B., Komiyama, N. H., Horsburgh, K., Kind, P. C., Grant, S. G., Wyllie, D. J., and Hardingham, G. E. (2012). The subtype of GluN2 C-terminal domain determines the response to excitotoxic insults. *Neuron*, 74(3), 543–556.

Martin, L. J., Furuta, A., and Blackstone, C. D. (1998). AMPA receptor protein in developing rat brain: glutamate receptor-1 expression and localization change at regional, cellular, and subcellular levels with maturation. *Neuroscience*, 83(3), 917–928.

Martin, S. J., Grimwood, P. D., and Morris, R. G. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Annual Review of Neuroscience*, 23, 649–711.

Märtn, A., Calvigioni, D., Tzortzi, O., Fuzik, J., Wärnberg, E., and Meletis, K. (2019). A Spatiomolecular Map of the Striatum. *Cell Reports*, 29(13), 4320–4333.e5.

Martínez-Cerdeño, V., Camacho, J., Fox, E., Miller, E., Ariza, J., Kienzle, D., Plank, K., Noctor, S. C., and Van de Water, J. (2016). Prenatal Exposure to Autism-Specific Maternal Autoantibodies Alters Proliferation of Cortical Neural Precursor Cells, Enlarges Brain, and Increases Neuronal Size in Adult Animals. *Cerebral Cortex*, 26(1), 374–383.

Martínez-Hernández, E., Horvath, J., Shiloh-Malawsky, Y., Sangha, N., Martínez-Lage, M., and Dalmau, J. (2011). Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. *Neurology*, 77(6), 589–593.

Martins, R. A., Linden, R., and Dyer, M. A. (2006). Glutamate regulates retinal progenitors cells proliferation during development. *The European Journal of Neuroscience*, 24(4), 969–980.

Ueno, M., and Yamashita, T. (2014). Bidirectional tuning of microglia in the developing brain: from neurogenesis to neural circuit formation. *Current Opinion in Neurobiology*, 27, 8–15.

Mason, J. O., and Price, D. J. (2016). Building brains in a dish: Prospects for growing cerebral organoids from stem cells. *Neuroscience*, 334, 105–118.

Massey, P. V., Johnson, B. E., Moulton, P. R., Auberson, Y. P., Brown, M. W., Molnár, E., Collingridge, G. L., and Bashir, Z. I. (2004). Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *The Journal of Neuroscience*, 24(36), 7821–7828.

Massie, A., Vandesande, F., and Arckens, L. (2001). Expression of the high-affinity glutamate transporter EAAT4 in mammalian cerebral cortex. *Neuroreport*, 12(2), 393–397.

Mathis, S., Pin, J. C., Pierre, F., Ciron, J., Iljicsov, A., Lamy, M., and Neau, J. P. (2015). Anti-NMDA Receptor Encephalitis During Pregnancy: A Case Report. *Medicine*, 94(26), e1034.

Matsuda, K., Kamiya, Y., Matsuda, S., and Yuzaki, M. (2002). Cloning and characterization of a novel NMDA receptor subunit NR3B: a dominant subunit that reduces calcium permeability. *Brain Research. Molecular Brain Research*, 100(1-2), 43–52.

Matsushima, A., and Graybiel, A. M. (2020). Combinatorial Developmental Controls on Striatonigral Circuits. *Cell Reports*, 31(11), 107778.

Matsuzaki, F., and Shitamukai, A. (2015). Cell Division Modes and Cleavage Planes of Neural Progenitors during Mammalian Cortical Development. *Cold Spring Harbor Perspectives in Biology*, 7(9), a015719.

Mattson M. P. (2008). Glutamate and neurotrophic factors in neuronal plasticity and disease. *Annals of the New York Academy of Sciences*, 1144, 97–112.

Mayer, M. L., Westbrook, G. L., and Guthrie, P. B. (1984). Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones. *Nature*, 309(5965), 261–263.

Mayer M. L. (2011). Emerging models of glutamate receptor ion channel structure and function. *Structure*, 19(10), 1370–1380.

McAllister, A. K., Katz, L. C., and Lo, D. C. (1996). Neurotrophin regulation of cortical dendritic growth requires activity. *Neuron*, 17(6), 1057–1064.

McAllister, D. L., Kaplan, B. J., Edworthy, S. M., Martin, L., Crawford, S. G., Ramsey-Goldman, R., Manzi, S., Fries, J. F., and Sibley, J. (1997). The influence of systemic lupus erythematosus on fetal development: cognitive, behavioral, and health trends. *Journal of the International Neuropsychological Society*, 3(4), 370–376.

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science*, 245(4921), 978–982.

McConnell, S. K., and Kaznowski, C. E. (1991). Cell cycle dependence of laminar determination in developing neocortex. *Science*, 254(5029), 282–285.

- McKay, S.,** Griffiths, N. H., Butters, P. A., Thubron, E. B., Hardingham, G. E., and Wyllie, D. J. (2012). Direct pharmacological monitoring of the developmental switch in NMDA receptor subunit composition using TCN 213, a GluN2A-selective, glycine-dependent antagonist. *British Journal of Pharmacology*, 166(3), 924–937.
- McKinney R. A.** (2010). Excitatory amino acid involvement in dendritic spine formation, maintenance and remodelling. *The Journal of Physiology*, 588(Pt 1), 107–116.
- McNaught, K. S., and Mink, J. W.** (2011). Advances in understanding and treatment of Tourette syndrome. *Nature Reviews. Neurology*, 7(12), 667–676.
- Meldrum B. S.** (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *The Journal of Nutrition*, 130(4S Suppl), 1007S–15S.
- Merriman, H.,** Warmsley, J., Bond, S., Gregory, R., Staples, J., Rippon, E., and Knight, R. (2022). Series 1. 1. Bex. [Audio podcast episode]. In *Room 5*. BBC Sounds.
- Messersmith, E. K.,** Feller, M. B., Zhang, H., and Shatz, C. J. (1997). Migration of neocortical neurons in the absence of functional NMDA receptors. *Molecular and Cellular Neurosciences*, 9(5-6), 347–357.
- Métin, C.,** Denizot, J. P., and Ropert, N. (2000). Intermediate zone cells express calcium-permeable AMPA receptors and establish close contact with growing axons. *The Journal of Neuroscience*, 20(2), 696–708.
- Metzger, F.,** Wiese, S., and Sendtner, M. (1998). Effect of glutamate on dendritic growth in embryonic rat motoneurons. *The Journal of Neuroscience*, 18(5), 1735–1742.
- Mi, D.,** Li, Z., Lim, L., Li, M., Moissidis, M., Yang, Y., Gao, T., Hu, T. X., Pratt, T., Price, D. J., Sestan, N., and Marín, O. (2018). Early emergence of cortical interneuron diversity in the mouse embryo. *Science*, 360(6384), 81–85.
- Mielnik, C. A.,** Binko, M. A., Chen, Y., Funk, A. J., Johansson, E. M., Intson, K., Sivananthan, N., Islam, R., Milenkovic, M., Horsfall, W., Ross, R. A., Groc, L., Salahpour, A., McCullumsmith, R. E., Tripathy, S., Lambe, E. K., and Ramsey, A. J. (2021). Consequences of NMDA receptor deficiency can be rescued in the adult brain. *Molecular psychiatry*, 26(7), 2929–2942.
- Mikasová, L.,** De Rossi, P., Bouchet, D., Georges, F., Rogemond, V., Didelot, A., Meissirel, C., Honnorat, J., and Groc, L. (2012). Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. *Brain*, 135(Pt 5), 1606–1621.
- Mikulecká, A., and Mares, P.** (2002). NMDA receptor antagonists impair motor performance in immature rats. *Psychopharmacology*, 162(4), 364–372.
- Milnerwood, A. J.,** Gladding, C. M., Pouladi, M. A., Kaufman, A. M., Hines, R. M., Boyd, J. D., Ko, R. W., Vasuta, O. C., Graham, R. K., Hayden, M. R., Murphy, T. H., and Raymond, L. A. (2010). Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron*, 65(2), 178–190.
- Mink J. W.** (1996). The basal ganglia: focused selection and inhibition of competing motor programs. *Progress in Neurobiology*, 50(4), 381–425.
- Minocha, S.,** Valloton, D., Arsenijevic, Y., Cardinaux, J. R., Guidi, R., Hornung, J. P., and Lebrand, C. (2017). Nkx2.1 regulates the generation of telencephalic astrocytes during embryonic development. *Scientific Reports*, 7, 43093.
- Mira, H., and Morante, J.** (2020). Neurogenesis From Embryo to Adult - Lessons From Flies and Mice. *Frontiers in Cell and Developmental Biology*, 8, 533.
- Miyachi, S.,** Hikosaka, O., Miyashita, K., Kárádi, Z., and Rand, M. K. (1997). Differential roles of monkey striatum in learning of sequential hand movement. *Experimental Brain Research*, 115(1), 1–5.

Miyamoto, A., Wake, H., Ishikawa, A. W., Eto, K., Shibata, K., Murakoshi, H., Koizumi, S., Moorhouse, A. J., Yoshimura, Y., and Nabekura, J. (2016). Microglia contact induces synapse formation in developing somatosensory cortex. *Nature Communications*, 7, 12540.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., and Tabira, T. (2002). Chronic stress impairs rotarod performance in rats: implications for depressive state. *Pharmacology, Biochemistry, and Behavior*, 71(1-2), 79–84.

Mochizuki, N., Takagi, N., Kurokawa, K., Kawai, T., Besshoh, S., Tanonaka, K., and Takeo, S. (2007). Effect of NMDA receptor antagonist on proliferation of neurospheres from embryonic brain. *Neuroscience Letters*, 417(2), 143–148.

Mohanty, S., Kim, J., Ganesan, L. P., Phillips, G. S., Hua, K., Jarjoura, D., Hayton, W. L., Robinson, J. M., and Anderson, C. L. (2010). IgG is transported across the mouse yolk sac independently of FcγRIIb. *Journal of Reproductive Immunology*, 84(2), 133–144.

Molnár, Z., Clowry, G. J., Šestan, N., Alzu'bi, A., Bakken, T., Hevner, R. F., Hüppi, P. S., Kostović, I., Rakic, P., Anton, E. S., Edwards, D., Garcez, P., Hoerder-Suabedissen, A., and Kriegstein, A. (2019). New insights into the development of the human cerebral cortex. *Journal of Anatomy*, 235(3), 432–451.

Monti, B., and Contestabile, A. (2000). Blockade of the NMDA receptor increases developmental apoptotic elimination of granule neurons and activates caspases in the rat cerebellum. *The European Journal of Neuroscience*, 12(9), 3117–3123.

Mony, L., Kew, J. N., Gunthorpe, M. J., and Paoletti, P. (2009). Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. *British Journal of Pharmacology*, 157(8), 1301–1317.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., and Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional

properties of four NMDA receptors. *Neuron*, 12(3), 529–540.

Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., and Everall, I. P. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry*, 68(4), 368–376.

Morley, P., MacPherson, P., Whitfield, J. F., Harris, E. W., and McBurney, M. W. (1995). Glutamate receptor-mediated calcium surges in neurons derived from P19 cells. *Journal of Neurochemistry*, 65(3), 1093–1099.

Morphis, L. G., and Gitlin, D. (1970). Maturation of the maternofetal transport system for human gamma-globulin in the mouse. *Nature*, 228(5271), 573.

Morris R. G. (2013). NMDA receptors and memory encoding. *Neuropharmacology*, 74, 32–40.

Moscato, E. H., Peng, X., Jain, A., Parsons, T. D., Dalmau, J., and Balice-Gordon, R. J. (2014). Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Annals of Neurology*, 76(1), 108–119.

Moser V. C. (2001). Neurobehavioral screening in rodents. *Current Protocols in Toxicology, Chapter 11*, Unit11.2.

Mostafa, G. A., and Shehab, A. A. (2010). The link of C4B null allele to autism and to a family history of autoimmunity in Egyptian autistic children. *Journal of Neuroimmunology*, 223(1-2), 115–119.

Mostafa, G. A., El-Sherif, D. F., and Al-Ayadhi, L. Y. (2014). Systemic auto-antibodies in children with autism. *Journal of Neuroimmunology*, 272(1-2), 94–98.

Mount, H. T., Dreyfus, C. F., and Black, I. B. (1993). Purkinje cell survival is differentially regulated by metabotropic and ionotropic excitatory amino acid receptors. *The Journal of Neuroscience*, 13(7), 3173–3179.

- Muir, J.,** Arancibia-Carcamo, I. L., MacAskill, A. F., Smith, K. R., Griffin, L. D., and Kittler, J. T. (2010). NMDA receptors regulate GABAA receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the $\gamma 2$ subunit. *Proceedings of the National Academy of Sciences of the United States of America*, 107(38), 16679–16684.
- Muñoz-Manchado, A. B.,** Bengtsson Gonzales, C., Zeisel, A., Munguba, H., Bekkouche, B., Skene, N. G., Lönnerberg, P., Ryge, J., Harris, K. D., Linnarsson, S., and Hjerling-Leffler, J. (2018). Diversity of Interneurons in the Dorsal Striatum Revealed by Single-Cell RNA Sequencing and PatchSeq. *Cell Reports*, 24(8), 2179–2190.e7.
- Murer, M. G.,** Tseng, K. Y., Kasanetz, F., Belluscio, M., and Riquelme, L. A. (2002). Brain oscillations, medium spiny neurons, and dopamine. *Cellular and Molecular Neurobiology*, 22(5-6), 611–632.
- Murugan, M.,** Sivakumar, V., Lu, J., Ling, E. A., and Kaur, C. (2011). Expression of N-methyl D-aspartate receptor subunits in amoeboid microglia mediates production of nitric oxide via NF- κ B signaling pathway and oligodendrocyte cell death in hypoxic postnatal rats. *Glia*, 59(4), 521–539.
- Nägerl, U. V.,** Eberhorn, N., Cambridge, S. B., and Bonhoeffer, T. (2004). Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron*, 44(5), 759–767.
- Nair, A., and Bonneau, R. H.** (2006). Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *Journal of Neuroimmunology*, 171(1-2), 72–85.
- Nakamichi, N.,** Yoshida, K., Ishioka, Y., Makanga, J. O., Fukui, M., Yoneyama, M., Kitayama, T., Nakamura, N., Taniura, H., and Yoneda, Y. (2008). Group III metabotropic glutamate receptor activation suppresses self-replication of undifferentiated neocortical progenitor cells. *Journal of Neurochemistry*, 105(5), 1996–2012.
- Nakamura, K.,** Hioki, H., Fujiyama, F., and Kaneko, T. (2005). Postnatal changes of vesicular glutamate transporter (VGLUT)1 and VGLUT2 immunoreactivities and their colocalization in the mouse forebrain. *The Journal of Comparative Neurology*, 492(3), 263–288.
- Nakanishi, N.,** Tu, S., Shin, Y., Cui, J., Kurokawa, T., Zhang, D., Chen, H. S., Tong, G., and Lipton, S. A. (2009). Neuroprotection by the NR3A subunit of the NMDA receptor. *The Journal of Neuroscience*, 29(16), 5260–5265.
- Nakano-Kobayashi, A.,** Yamazaki, M., Unoki, T., Hongu, T., Murata, C., Taguchi, R., Katada, T., Frohman, M. A., Yokozeki, T., and Kanaho, Y. (2007). Role of activation of PIP5Kgamma661 by AP-2 complex in synaptic vesicle endocytosis. *The EMBO Journal*, 26(4), 1105–1116.
- Namba, T.,** Mochizuki, H., Onodera, M., Mizuno, Y., Namiki, H., and Seki, T. (2005). The fate of neural progenitor cells expressing astrocytic and radial glial markers in the postnatal rat dentate gyrus. *The European Journal of Neuroscience*, 22(8), 1928–1941.
- Nestler, E. J.,** Hyman, S. E., and Malenka, R. C. (2009). *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* (2nd ed.). New York: McGraw-Hill Medical.
- Neumann, H.,** Kotter, M. R., and Franklin, R. J. (2009). Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain*, 132(Pt 2), 288–295.
- Newcombe, J.,** Uddin, A., Dove, R., Patel, B., Turski, L., Nishizawa, Y., and Smith, T. (2008). Glutamate receptor expression in multiple sclerosis lesions. *Brain Pathology*, 18(1), 52–61.
- Neyens, R. R.,** Gaskill, G. E., and Chalela, J. A. (2018). Critical Care Management of Anti-N-Methyl-D-Aspartate Receptor Encephalitis. *Critical Care Medicine*, 46(9), 1514–1521.
- Nicoletti, F.,** Bockaert, J., Collingridge, G. L., Conn, P. J., Ferraguti, F., Schoepp, D. D., Wroblewski, J. T., and Pin, J. P. (2011). Metabotropic glutamate receptors: from the workbench to the bedside. *Neuropharmacology*, 60(7-8), 1017–1041.
- Niell, C. M.,** Meyer, M. P., and Smith, S. J. (2004). *In vivo* imaging of synapse formation on a growing

dendritic arbor. *Nature Neuroscience*, 7(3), 254–260.

Niewiesk S. (2014). Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Frontiers in Immunology*, 5, 446.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science*, 308(5726), 1314–1318.

Nishi, M., Hinds, H., Lu, H. P., Kawata, M., and Hayashi, Y. (2001). Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. *The Journal of Neuroscience*, 21(23), RC185.

Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S., and Kriegstein, A. R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature*, 409(6821), 714–720.

Noctor, S. C., Martínez-Cerdeño, V., Ivic, L., and Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature Neuroscience*, 7(2), 136–144.

Nosadini, M., Mohammad, S. S., Ramanathan, S., Brilot, F., and Dale, R. C. (2015). Immune therapy in autoimmune encephalitis: a systematic review. *Expert Review of Neurotherapeutics*, 15(12), 1391–1419.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., and Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*, 307(5950), 462–465.

Nugent, F. S., Penick, E. C., and Kauer, J. A. (2007). Opioids block long-term potentiation of inhibitory synapses. *Nature*, 446(7139), 1086–1090.

Ohno, T., Maeda, H., Murabe, N., Kamiyama, T., Yoshioka, N., Mishina, M., and Sakurai, M. (2010). Specific involvement of postsynaptic GluN2B-containing NMDA receptors in the developmental

elimination of corticospinal synapses. *Proceedings of the National Academy of Sciences of the United States of America*, 107(34), 15252–15257.

Oh sawa, K., Imai, Y., Sasaki, Y., and Kohsaka, S. (2004). Microglia/macrophage-specific protein IBA1 binds to fimbrin and enhances its actin-bundling activity. *Journal of Neurochemistry*, 88(4), 844–856.

Ohtsuka, T., Sakamoto, M., Guillemot, F., and Kageyama, R. (2001). Roles of the basic helix-loop-helix genes Hes1 and Hes5 in expansion of neural stem cells of the developing brain. *The Journal of Biological Chemistry*, 276(32), 30467–30474.

Olah, M., Biber, K., Vinet, J., and Boddeke, H. W. (2011). Microglia phenotype diversity. *CNS and Neurological Disorders Drug Targets*, 10(1), 108–118.

Olson, L., and Seiger, A. (1972). Early prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. *Zeitschrift für Anatomie und Entwicklungsgeschichte*, 137(3), 301–316.

Olsson, M., Campbell, K., Victorin, K., and Björklund, A. (1995). Projection neurons in fetal striatal transplants are predominantly derived from the lateral ganglionic eminence. *Neuroscience*, 69(4), 1169–1182.

Pachernegg, S., Strutz-Seebohm, N., and Hollmann, M. (2012). GluN3 subunit-containing NMDA receptors: not just one-trick ponies. *Trends in Neurosciences*, 35(4), 240–249.

Pagadala, P., Park, C. K., Bang, S., Xu, Z. Z., Xie, R. G., Liu, T., Han, B. X., Tracey, W. D., Jr, Wang, F., and Ji, R. R. (2013). Loss of NR1 subunit of NMDARs in primary sensory neurons leads to hyperexcitability and pain hypersensitivity: involvement of Ca²⁺-activated small conductance potassium channels. *The Journal of Neuroscience*, 33(33), 13425–13430.

Pagano, J., Giona, F., Beretta, S., Verpelli, C., and Sala, C. (2021). N-methyl-d-aspartate receptor function in neuronal and synaptic development and signaling. *Current Opinion in Pharmacology*, 56, 93–101.

- Palmeira, P.,** Quinello, C., Silveira-Lessa, A. L., Zago, C. A., and Carneiro-Sampaio, M. (2012). IgG placental transfer in healthy and pathological pregnancies. *Clinical and Developmental Immunology*, 2012, 985646.
- Pan, H.,** Oliveira, B., Saher, G., Dere, E., Tapken, D., Mitjans, M., Seidel, J., Wesolowski, J., Wakhloo, D., Klein-Schmidt, C., Ronnenberg, A., Schwabe, K., Trippe, R., Mätz-Rensing, K., Berghoff, S., Al-Krinawe, Y., Martens, H., Begemann, M., Stöcker, W., Kaup, F. J., ... Ehrenreich, H. (2019). Uncoupling the widespread occurrence of anti-NMDAR1 autoantibodies from neuropsychiatric disease in a novel autoimmune model. *Molecular Psychiatry*, 24(10), 1489–1501.
- Paoletti P.** (2011). Molecular basis of NMDA receptor functional diversity. *The European Journal of Neuroscience*, 33(8), 1351–1365.
- Paoletti, P.,** Bellone, C., and Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nature reviews. Neuroscience*, 14(6), 383–400.
- Paolicelli, R. C.,** Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T. A., Guiducci, E., Dumas, L., Ragozzino, D., and Gross, C. T. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science*, 333(6048), 1456–1458.
- Paolicelli, R. C., and Ferretti, M. T.** (2017). Function and Dysfunction of Microglia during Brain Development: Consequences for Synapses and Neural Circuits. *Frontiers in Synaptic Neuroscience*, 9, 9.
- Papadia, S.,** Soriano, F. X., Léveillé, F., Martel, M. A., Dakin, K. A., Hansen, H. H., Kaindl, A., Siffringer, M., Fowler, J., Stefovskaja, V., McKenzie, G., Craigmiles, M., Corriveau, R., Ghazal, P., Horsburgh, K., Yankner, B. A., Wyllie, D. J., Ikonomidou, C., and Hardingham, G. E. (2008). Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nature Neuroscience*, 11(4), 476–487.
- Paridaen, J. T., and Huttner, W. B.** (2014). Neurogenesis during development of the vertebrate central nervous system. *EMBO reports*, 15(4), 351–364.
- Park, H.,** Popescu, A., and Poo, M. M. (2014). Essential role of presynaptic NMDA receptors in activity-dependent BDNF secretion and corticostriatal LTP. *Neuron*, 84(5), 1009–1022.
- Parker, J. G.,** Marshall, J. D., Ahanonu, B., Wu, Y. W., Kim, T. H., Grewe, B. F., Zhang, Y., Li, J. Z., Ding, J. B., Ehlers, M. D., and Schnitzer, M. J. (2018). Diametric neural ensemble dynamics in parkinsonian and dyskinetic states. *Nature*, 557(7704), 177–182.
- Parkhurst, C. N.,** Yang, G., Ninan, I., Savas, J. N., Yates, J. R., 3rd, Lafaille, J. J., Hempstead, B. L., Littman, D. R., and Gan, W. B. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*, 155(7), 1596–1609.
- Parvathaneni, K., and Scott, D. W.** (2018). Engineered FVIII-expressing cytotoxic T cells target and kill FVIII-specific B-cells *in vitro* and *in vivo*. *Blood Advances*, 2(18), 2332–2340.
- Paupard, M. C.,** Friedman, L. K., and Zukin, R. S. (1997). Developmental regulation and cell-specific expression of N-methyl-D-aspartate receptor splice variants in rat hippocampus. *Neuroscience*, 79(2), 399–409.
- Peixoto, R. T.,** Wang, W., Croney, D. M., Kozorovitskiy, Y., and Sabatini, B. L. (2016). Early hyperactivity and precocious maturation of corticostriatal circuits in Shank3B(-/-) mice. *Nature Neuroscience*, 19(5), 716–724.
- Pennartz, C. M.,** Berke, J. D., Graybiel, A. M., Ito, R., Lansink, C. S., Van der Meer, M., Redish, A. D., Smith, K. S., and Voorn, P. (2009). Corticostriatal Interactions during Learning, Memory Processing, and Decision Making. *The Journal of Neuroscience*, 29(41), 12831–12838.
- Pérez-Rodríguez, D. R.,** Blanco-Luquin, I., and Mendioroz, M. (2021). The Participation of Microglia in Neurogenesis: A Review. *Brain Sciences*, 11(5), 658.
- Perry V. H.** (2004). The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative

disease. *Brain, Behavior, and Immunity*, 18(5), 407–413.

Personius, K. E., Slusher, B. S., and Udin, S. B. (2016). Neuromuscular NMDA Receptors Modulate Developmental Synapse Elimination. *The Journal of Neuroscience*, 36(34), 8783–8789.

Petit-Pedrol, M., Armangue, T., Peng, X., Bataller, L., Cellucci, T., Davis, R., McCracken, L., Martínez-Hernández, E., Mason, W. P., Kruer, M. C., Ritacco, D. G., Grisold, W., Meaney, B. F., Alcalá, C., Sillevius-Smitt, P., Titulaer, M. J., Balice-Gordon, R., Graus, F., and Dalmau, J. (2014). Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABAA receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. *The Lancet. Neurology*, 13(3), 276–286.

Petit-Pedrol, M., Sell, J., Planagumà, J., Mannara, F., Radošević, M., Haselmann, H., Ceanga, M., Sabater, L., Spatola, M., Soto, D., Gasull, X., Dalmau, J., and Geis, C. (2018). LGI1 antibodies alter Kv1.1 and AMPA receptors changing synaptic excitability, plasticity and memory. *Brain*, 141(11), 3144–3159.

Petralia, R. S., and Wenthold, R. J. (1992). Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *The Journal of Comparative Neurology*, 318(3), 329–354.

Petralia, R. S., Yokotani, N., and Wenthold, R. J. (1994). Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *The Journal of Neuroscience*, 14(2), 667–696.

Petralia, R. S., Esteban, J. A., Wang, Y. X., Partridge, J. G., Zhao, H. M., Wenthold, R. J., and Malinow, R. (1999). Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature Neuroscience*, 2(1), 31–36.

Petralia, R. S., Wang, Y. X., Hua, F., Yi, Z., Zhou, A., Ge, L., Stephenson, F. A., and Wenthold, R. J. (2010). Organization of NMDA receptors at extrasynaptic locations. *Neuroscience*, 167(1), 68–87.

Petrini, E. M., Ravasenga, T., Hausrat, T. J., Iurilli, G., Olcese, U., Racine, V., Sibarita, J. B., Jacob, T. C., Moss, S. J., Benfenati, F., Medini, P., Kneussel, M., and Barberis, A. (2014). Synaptic recruitment of gephyrin regulates surface GABAA receptor dynamics for the expression of inhibitory LTP. *Nature Communications*, 5, 3921.

Petty, A., Myshkin, E., Qin, H., Guo, H., Miao, H., Tochtrop, G. P., Hsieh, J. T., Page, P., Liu, L., Lindner, D. J., Acharya, C., MacKerell, A. D., Jr, Ficker, E., Song, J., and Wang, B. (2012). A small molecule agonist of EphA2 receptor tyrosine kinase inhibits tumor cell migration *in vitro* and prostate cancer metastasis *in vivo*. *PloS one*, 7(8), e42120.

Pfeiffer, T., Avignone, E., and Nägerl, U. V. (2016). Induction of hippocampal long-term potentiation increases the morphological dynamics of microglial processes and prolongs their contacts with dendritic spines. *Scientific Reports*, 6, 32422.

Pietrancosta, N., Djibo, M., Daumas, S., El Mestikawy, S., and Erickson, J. D. (2020). Molecular, Structural, Functional, and Pharmacological Sites for Vesicular Glutamate Transporter Regulation. *Molecular Neurobiology*, 57(7), 3118–3142.

Pilz, G. A., Shitamukai, A., Reillo, I., Pacary, E., Schwausch, J., Stahl, R., Ninkovic, J., Snippert, H. J., Clevers, H., Godinho, L., Guillemot, F., Borrell, V., Matsuzaki, F., and Götz, M. (2013). Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. *Nature Communications*, 4, 2125.

Piña-Crespo, J. C., Talantova, M., Micu, I., States, B., Chen, H. S., Tu, S., Nakanishi, N., Tong, G., Zhang, D., Heinemann, S. F., Zamponi, G. W., Stys, P. K., and Lipton, S. A. (2010). Excitatory glycine responses of CNS myelin mediated by NR1/NR3 "NMDA" receptor subunits. *The Journal of Neuroscience*, 30(34), 11501–11505.

Pittock, S. J., Berthele, A., Fujihara, K., Kim, H. J., Levy, M., Palace, J., Nakashima, I., Terzi, M., Totolyan, N., Viswanathan, S., Wang, K. C., Pace, A., Fujita, K. P., Armstrong, R., and Wingerchuk, D. M. (2019). Eculizumab in Aquaporin-4-Positive Neuromyelitis Optica Spectrum Disorder. *The New England Journal of Medicine*, 381(7), 614–625.

- Pla, R.,** Stanco, A., Howard, M. A., Rubin, A. N., Vogt, D., Mortimer, N., Cobos, I., Potter, G. B., Lindtner, S., Price, J. D., Nord, A. S., Visel, A., Schreiner, C. E., Baraban, S. C., Rowitch, D. H., and Rubenstein, J. L. R. (2018). Dlx1 and Dlx2 Promote Interneuron GABA Synthesis, Synaptogenesis, and Dendritogenesis. *Cerebral Cortex*, 28(11), 3797–3815.
- Planagumà, J.,** Leypoldt, F., Mannara, F., Gutiérrez-Cuesta, J., Martín-García, E., Aguilar, E., Titulaer, M. J., Petit-Pedrol, M., Jain, A., Balice-Gordon, R., Lakadamyali, M., Graus, F., Maldonado, R., and Dalmau, J. (2015). Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain*, 138(Pt 1), 94–109.
- Planagumà, J.,** Haselmann, H., Mannara, F., Petit-Pedrol, M., Grünewald, B., Aguilar, E., Röpke, L., Martín-García, E., Titulaer, M. J., Jercog, P., Graus, F., Maldonado, R., Geis, C., and Dalmau, J. (2016). Ephrin-B2 prevents N-methyl-D-aspartate receptor antibody effects on memory and neuroplasticity. *Annals of Neurology*, 80(3), 388–400.
- Platel, J. C.,** Dave, K. A., Gordon, V., Lacar, B., Rubio, M. E., and Bordey, A. (2010). NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. *Neuron*, 65(6), 859–872.
- Poirazi, P., and Mel, B. W.** (2001). Impact of active dendrites and structural plasticity on the memory capacity of neural tissue. *Neuron*, 29(3), 779–796.
- Polgár, E.,** Watanabe, M., Hartmann, B., Grant, S. G., and Todd, A. J. (2008). Expression of AMPA receptor subunits at synapses in laminae I–III of the rodent spinal dorsal horn. *Molecular Pain*, 4, 5.
- Pollak, T. A.,** Lennox, B. R., Müller, S., Benros, M. E., Prüss, H., Tebartz Van Elst, L., Klein, H., Steiner, J., Frodl, T., Bogerts, B., Tian, L., Groc, L., Hasan, A., Baune, B. T., Endres, D., Haroon, E., Yolken, R., Benedetti, F., Halaris, A., Meyer, J. H., ... Bechter, K. (2020). Autoimmune psychosis: an international consensus on an approach to the diagnosis and management of psychosis of suspected autoimmune origin. *The Lancet. Psychiatry*, 7(1), 93–108.
- Poluch, S.,** Drian, M. J., Durand, M., Astier, C., Benyamin, Y., and König, N. (2001). AMPA receptor activation leads to neurite retraction in tangentially migrating neurons in the intermediate zone of the embryonic rat neocortex. *Journal of Neuroscience Research*, 63(1), 35–44.
- Poluch, S., and König, N.** (2002). AMPA receptor activation induces GABA release from neurons migrating tangentially in the intermediate zone of embryonic rat neocortex. *The European Journal of Neuroscience*, 16(2), 350–354.
- Porter, J. N.,** Roy, A. K., Benson, B., Carlisi, C., Collins, P. F., Leibenluft, E., Pine, D. S., Luciana, M., and Ernst, M. (2015). Age-related changes in the intrinsic functional connectivity of the human ventral vs. dorsal striatum from childhood to middle age. *Developmental Cognitive Neuroscience*, 11, 83–95.
- Pregno, G.,** Zamburlin, P., Gambarotta, G., Farcito, S., Licheri, V., Fregnan, F., Perroteau, I., Lovisolo, D., and Bovolin, P. (2011). Neuregulin1/ErbB4-induced migration in ST14A striatal progenitors: calcium-dependent mechanisms and modulation by NMDA receptor activation. *BMC Neuroscience*, 12, 103.
- Preuss T. M.** (1995). Do rats have prefrontal cortex? The rose-woolsey-akert program reconsidered. *Journal of Cognitive Neuroscience*, 7(1), 1–24.
- Prinz, M., and Priller, J.** (2014). Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nature Reviews. Neuroscience*, 15(5), 300–312.
- Prüss H.** (2021). Autoantibodies in neurological disease. *Nature Reviews. Immunology*, 21(12), 798–813.
- Rabacchi, S.,** Bailly, Y., Delhay-Bouchaud, N., and Mariani, J. (1992). Involvement of the N-methyl D-aspartate (NMDA) receptor in synapse elimination during cerebellar development. *Science*, 256(5065), 1823–1825.
- Rahman, K. A.,** Orlando, M., Boulos, A., Andrzejak, E., Schmitz, D., Ziv, N. E., Prüss, H., Garner, C. C., and Ichkova, A. (2023). Microglia actively remove NR1

autoantibody-bound NMDA receptors and associated post-synaptic proteins in neuron microglia co-cultures. *Glia*, 71(8), 1804–1829.

Rajan, I., and Cline, H. T. (1998). Glutamate receptor activity is required for normal development of tectal cell dendrites *in vivo*. *The Journal of Neuroscience*, 18(19), 7836–7846.

Rajan, I., Witte, S., and Cline, H. T. (1999). NMDA receptor activity stabilizes presynaptic retinotectal axons and postsynaptic optic tectal cell dendrites *in vivo*. *Journal of Neurobiology*, 38(3), 357–368.

Ramoá, A. S., Mower, A. F., Liao, D., and Jafri, S. I. (2001). Suppression of cortical NMDA receptor function prevents development of orientation selectivity in the primary visual cortex. *The Journal of Neuroscience*, 21(12), 4299–4309.

Ratuszny, D., Skripuletz, T., Wegner, F., Groß, M., Falk, C., Jacobs, R., Ruschulte, H., Stangel, M., and Sühs, K. W. (2020). Case Report: Daratumumab in a Patient With Severe Refractory Anti-NMDA Receptor Encephalitis. *Frontiers in Neurology*, 11, 602102.

Rauen T. (2000). Diversity of glutamate transporter expression and function in the mammalian retina. *Amino acids*, 19(1), 53–62.

Rauner, C., and Köhr, G. (2011). Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. *The Journal of Biological Chemistry*, 286(9), 7558–7566.

Ray, W. J., and Gottlieb, D. I. (1993). Expression of ionotropic glutamate receptor genes by P19 embryonal carcinoma cells. *Biochemical and Biophysical Research Communications*, 197(3), 1475–1482.

Raymond, L. A., André, V. M., Cepeda, C., Gladding, C. M., Milnerwood, A. J., and Levine, M. S. (2011). Pathophysiology of Huntington's disease: time-dependent alterations in synaptic and receptor function. *Neuroscience*, 198, 252–273.

Redgrave, P., Rodriguez, M., Smith, Y., Rodriguez-Oroz, M. C., Lehericy, S., Bergman, H., Agid, Y.,

DeLong, M. R., and Obeso, J. A. (2010). Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. *Nature reviews. Neuroscience*, 11(11), 760–772.

Reiprich, P., Kilb, W., and Luhmann, H. J. (2005). Neonatal NMDA receptor blockade disturbs neuronal migration in rat somatosensory cortex *in vivo*. *Cerebral Cortex*, 15(3), 349–358.

Reshef, R., Kudryavitskaya, E., Shani-Narkiss, H., Isaacson, B., Rimmerman, N., Mizrahi, A., and Yirmiya, R. (2017). The role of microglia and their CX3CR1 signaling in adult neurogenesis in the olfactory bulb. *eLife*, 6, e30809.

Rex, C. S., Lin, C. Y., Kramár, E. A., Chen, L. Y., Gall, C. M., and Lynch, G. (2007). Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. *The Journal of Neuroscience*, 27(11), 3017–3029.

Riou, M., Stroebel, D., Edwardson, J. M., and Paoletti, P. (2012). An alternating GluN1-2-1-2 subunit arrangement in mature NMDA receptors. *PloS one*, 7(4), e35134.

Ritter, L. M., Vazquez, D. M., and Meador-Woodruff, J. H. (2002). Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. *Brain Research. Developmental Brain Research*, 139(2), 227–236.

Rocha, M., and Sur, M. (1995). Rapid acquisition of dendritic spines by visual thalamic neurons after blockade of N-methyl-D-aspartate receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 92(17), 8026–8030.

Roitman, M. F., Wheeler, R. A., and Carelli, R. M. (2005). Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron*, 45(4), 587–597.

Roopenian, D. C., and Akilesh, S. (2007). FcRn: the neonatal Fc receptor comes of age. *Nature reviews. Immunology*, 7(9), 715–725.

- Rosch, R. E.,** Wright, S., Cooray, G., Papadopoulou, M., Goyal, S., Lim, M., Vincent, A., Upton, A. L., Baldeweg, T., and Friston, K. J. (2018). NMDA-receptor antibodies alter cortical microcircuit dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, 115(42), E9916–E9925.
- Rose, N. R., and Bona, C.** (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunology Today*, 14(9), 426–430.
- Rosen, J. B., and Schulkin, J.** (2022). Hyperexcitability: From Normal Fear to Pathological Anxiety and Trauma. *Frontiers in Systems Neuroscience*, 16, 727054.
- Ross, G.,** Sammaritano, L., Nass, R., and Lockshin, M. (2003). Effects of mothers' autoimmune disease during pregnancy on learning disabilities and hand preference in their children. *Archives of pediatrics and adolescent medicine*, 157(4), 397–402.
- Rossi, D. J., and Slater, N. T.** (1993). The developmental onset of NMDA receptor-channel activity during neuronal migration. *Neuropharmacology*, 32(11), 1239–1248.
- Rozas, G.,** Guerra, M. J., and Labandeira-García, J. L. (1997). An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. *Brain Research. Brain Research Protocols*, 2(1), 75–84.
- Rumbaugh, G.,** Prybylowski, K., Wang, J. F., and Vicini, S. (2000). Exon 5 and spermine regulate deactivation of NMDA receptor subtypes. *Journal of Neurophysiology*, 83(3), 1300–1306.
- Ruthazer, E. S.,** Åkerman, C. J., and Cline, H. T. (2003). Control of axon branch dynamics by correlated activity *in vivo*. *Science*, 301(5629), 66–70.
- Ryazantseva, M.,** Englund, J., Shintyapina, A., Huupponen, J., Shteinikov, V., Pitkänen, A., Partanen, J. M., and Lauri, S. E. (2020). Kainate receptors regulate development of glutamatergic synaptic circuitry in the rodent amygdala. *eLife*, 9, e52798.
- Sadikot, A. F.,** Burhan, A. M., Bélanger, M. C., and Sasseville, R. (1998). NMDA receptor antagonists influence early development of GABAergic interneurons in the mammalian striatum. *Brain Research. Developmental Brain Research*, 105(1), 35–42.
- Sall, J. W.,** Stratmann, G., Leong, J., McKleroy, W., Mason, D., Shenoy, S., Pleasure, S. J., and Bickler, P. E. (2009). Isoflurane inhibits growth but does not cause cell death in hippocampal neural precursor cells grown in culture. *Anesthesiology*, 110(4), 826–833.
- Salter, M. W., and Kalia, L. V.** (2004). Src kinases: a hub for NMDA receptor regulation. *Nature Reviews. Neuroscience*, 5(4), 317–328.
- Salussolia, C. L.,** Prodromou, M. L., Borker, P., and Wollmuth, L. P. (2011). Arrangement of subunits in functional NMDA receptors. *The Journal of Neuroscience*, 31(31), 11295–11304.
- Sanz-Clemente, A.,** Nicoll, R. A., and Roche, K. W. (2013). Diversity in NMDA receptor composition: many regulators, many consequences. *The Neuroscientist*, 19(1), 62–75.
- Sasaki, Y.,** Ohsawa, K., Kanazawa, H., Kohsaka, S., and Imai, Y. (2001). IBA1 is an actin-cross-linking protein in macrophages/microglia. *Biochemical and Biophysical Research Communications*, 286(2), 292–297.
- Sattler, R.,** Xiong, Z., Lu, W. Y., Hafner, M., MacDonald, J. F., and Tymianski, M. (1999). Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science*, 284(5421), 1845–1848.
- Saunders, N. R.,** Liddelow, S. A., and Dziegielewska, K. M. (2012). Barrier mechanisms in the developing brain. *Frontiers in Pharmacology*, 3, 46.
- Sceniak, M. P.,** Berry, C. T., and Sabo, S. L. (2012). Facilitation of neocortical presynaptic terminal development by NMDA receptor activation. *Neural Development*, 7, 8.
- Schambra, U.** (2008). *Prenatal Mouse Brain Atlas*. 1st ed. Boston: Springer.

Scheibe, F., Prüss, H., Mengel, A. M., Kohler, S., Nümann, A., Köhnlein, M., Ruprecht, K., Alexander, T., Hiepe, F., and Meisel, A. (2017). Bortezomib for treatment of therapy-refractory anti-NMDA receptor encephalitis. *Neurology*, 88(4), 366–370.

Scheibe, F., Ostendorf, L., Reincke, S. M., Prüss, H., von Brünneck, A. C., Köhnlein, M., Alexander, T., Meisel, C., and Meisel, A. (2020). Daratumumab treatment for therapy-refractory anti-CASPR2 encephalitis. *Journal of Neurology*, 267(2), 317–323.

Schellino, R., Besusso, D., Parolisi, R., Gómez-González, G. B., Dallere, S., Scaramuzza, L., Ribodino, M., Campus, I., Conforti, P., Parmar, M., Boido, M., Cattaneo, E., and Buffo, A. (2023). hESC-derived striatal progenitors grafted into a Huntington's disease rat model support long-term functional motor recovery by differentiating, self-organizing and connecting into the lesioned striatum. *Stem Cell Research and Therapy*, 14(1), 189.

Schlett K. (2006). Glutamate as a modulator of embryonic and adult neurogenesis. *Current Topics in Medicinal Chemistry*, 6(10), 949–960.

Schmitt, S. E., Pargeon, K., Frechette, E. S., Hirsch, L. J., Dalmau, J., and Friedman, D. (2012). Extreme delta brush: a unique EEG pattern in adults with anti-NMDA receptor encephalitis. *Neurology*, 79(11), 1094–1100.

Schüler, T., Mesic, I., Madry, C., Bartholomäus, I., and Laube, B. (2008). Formation of NR1/NR2 and NR1/NR3 heterodimers constitutes the initial step in N-methyl-D-aspartate receptor assembly. *The Journal of Biological Chemistry*, 283(1), 37–46.

Scorrano, G., Dono, F., Corniello, C., Consoli, S., Evangelista, G., Di Ludovico, A., Chiarelli, F., Anzellotti, F., Di Iorio, A., and Sensi, S. L. (2024). Perinatal outcome in anti-NMDAR encephalitis during pregnancy-a systematic review with individual patients' data analysis. *Neurological Sciences*, 45(9), 4211–4227.

Scott, E. K., and Luo, L. (2001). How do dendrites take their shape?. *Nature Neuroscience*, 4(4), 359–365.

Sechi, E., and Flanagan, E. P. (2021). Antibody-Mediated Autoimmune Diseases of the CNS: Challenges and Approaches to Diagnosis and Management. *Frontiers in Neurology*, 12, 673339.

Sedel, F., Béchade, C., Vyas, S., and Triller, A. (2004). Macrophage-derived tumor necrosis factor alpha, an early developmental signal for motoneuron death. *The Journal of Neuroscience*, 24(9), 2236–2246.

Seiger, A., and Olson, L. (1973). Late prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations. *Zeitschrift für Anatomie und Entwicklungsgeschichte*, 140(3), 281–318.

Setlow, B., Schoenbaum, G., and Gallagher, M. (2003). Neural encoding in ventral striatum during olfactory discrimination learning. *Neuron*, 38(4), 625–636.

Seutin, V., Massotte, L., Scuvée-Moreau, J., and Dresse, A. (1998). Spontaneous apamin-sensitive hyperpolarizations in dopaminergic neurons of neonatal rats. *Journal of Neurophysiology*, 80(6), 3361–3364.

Sharma, R., Al-Saleem, F. H., Panzer, J., Lee, J., Puligedda, R. D., Felicori, L. F., Kattala, C. D., Rattelle, A. J., Ippolito, G., Cox, R. H., Lynch, D. R., and Dessain, S. K. (2018). Monoclonal antibodies from a patient with anti-NMDA receptor encephalitis. *Annals of Clinical and Translational Neurology*, 5(8), 935–951.

Sharott, A., Vinciati, F., Nakamura, K. C., and Magill, P. J. (2017). A Population of Indirect Pathway Striatal Projection Neurons Is Selectively Entrained to Parkinsonian Beta Oscillations. *The Journal of Neuroscience*, 37(41), 9977–9998.

Sharpe, N. A., and Tepper, J. M. (1998). Postnatal development of excitatory synaptic input to the rat neostriatum: an electron microscopic study. *Neuroscience*, 84(4), 1163–1175.

Shen, Q., Wang, Y., Dimos, J. T., Fasano, C. A., Phoenix, T. N., Lemischka, I. R., Ivanova, N. B., Stifani, S., Morrissey, E. E., and Temple, S. (2006). The timing of cortical neurogenesis is encoded within

lineages of individual progenitor cells. *Nature Neuroscience*, 9(6), 743–751.

Shen, W., Tian, X., Day, M., Ulrich, S., Tkatch, T., Nathanson, N. M., and Surmeier, D. J. (2007). Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. *Nature Neuroscience*, 10(11), 1458–1466.

Sheng, M., Cummings, J., Roldan, L. A., Jan, Y. N., and Jan, L. Y. (1994). Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature*, 368(6467), 144–147.

Shepherd G. M. (2013). Corticostriatal connectivity and its role in disease. *Nature reviews. Neuroscience*, 14(4), 278–291.

Shi, Y. C., Chen, X. J., Zhang, H. M., Wang, Z., and Du, D. Y. (2017). Anti-N-Methyl-d-Aspartate receptor (NMDAR) encephalitis during pregnancy: Clinical analysis of reported cases. *Taiwanese Journal of Obstetrics and Gynecology*, 56(3), 315–319.

Shi, Y., Wang, M., Mi, D., Lu, T., Wang, B., Dong, H., Zhong, S., Chen, Y., Sun, L., Zhou, X., Ma, Q., Liu, Z., Wang, W., Zhang, J., Wu, Q., Marín, O., and Wang, X. (2021). Mouse and human share conserved transcriptional programs for interneuron development. *Science*, 374(6573), eabj6641.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., Tsirka, S. E., and Maletic-Savatic, M. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*, 7(4), 483–495.

Silberberg, G., and Bolam, J. P. (2015). Local and afferent synaptic pathways in the striatal microcircuitry. *Current Opinion in Neurobiology*, 33, 182–187.

Silvin, A., Qian, J., and Ginhoux, F. (2023). Brain macrophage development, diversity and dysregulation in health and disease. *Cellular & Molecular Immunology*, 20(11), 1277–1289.

Simister N. E. (2003). Placental transport of immunoglobulin G. *Vaccine*, 21(24), 3365–3369.

Simmons, D. A., Rex, C. S., Palmer, L., Pandeyarajan, V., Fedulov, V., Gall, C. M., and Lynch, G. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proceedings of the National Academy of Sciences of the United States of America*, 106(12), 4906–4911.

Simon, D. K., Prusky, G. T., O'Leary, D. D., and Constantine-Paton, M. (1992). N-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. *Proceedings of the National Academy of Sciences of the United States of America*, 89(22), 10593–10597.

Sin, W. C., Haas, K., Ruthazer, E. S., and Cline, H. T. (2002). Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. *Nature*, 419(6906), 475–480.

Singer, H. S., Morris, C., Gause, C., Pollard, M., Zimmerman, A. W., and Pletnikov, M. (2009). Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: A pregnant dam mouse model. *Journal of Neuroimmunology*, 211(1-2), 39–48.

Single, F. N., Rozov, A., Burnashev, N., Zimmermann, F., Hanley, D. F., Forrest, D., Curran, T., Jensen, V., Hvalby, O., Sprengel, R., and Seeburg, P. H. (2000). Dysfunctions in mice by NMDA receptor point mutations NR1(N598Q) and NR1(N598R). *The Journal of Neuroscience*, 20(7), 2558–2566.

Sipe, G. O., Lowery, R. L., Tremblay, M. È., Kelly, E. A., Lamantia, C. E., and Majewska, A. K. (2016). Microglial P2Y₁₂ is necessary for synaptic plasticity in mouse visual cortex. *Nature Communications*, 7, 10905.

Smart I. H. (1976). A pilot study of cell production by the ganglionic eminences of the developing mouse brain. *Journal of Anatomy*, 121(Pt 1), 71–84.

Smith, Y., Raju, D. V., Pare, J. F., and Sidibe, M. (2004). The thalamostriatal system: a highly specific network of the basal ganglia circuitry. *Trends in Neurosciences*, 27(9), 520–527.

- Smith, Y.,** Galvan, A., Ellender, T. J., Doig, N., Villalba, R. M., Huerta-Ocampo, I., Wichmann, T., and Bolam, J. P. (2014). The thalamostriatal system in normal and diseased states. *Frontiers in Systems Neuroscience*, 8, 5.
- Smith, J. B.,** Klug, J. R., Ross, D. L., Howard, C. D., Hollon, N. G., Ko, V. I., Hoffman, H., Callaway, E. M., Gerfen, C. R., and Jin, X. (2016). Genetic-Based Dissection Unveils the Inputs and Outputs of Striatal Patch and Matrix Compartments. *Neuron*, 91(5), 1069–1084.
- Sohur, U. S.,** Padmanabhan, H. K., Kotchetkov, I. S., Menezes, J. R., and Macklis, J. D. (2014). Anatomic and molecular development of corticostriatal projection neurons in mice. *Cerebral Cortex*, 24(2), 293–303.
- Soltys, Z.,** Orzylowska-Sliwinska, O., Zaremba, M., Orłowski, D., Piechota, M., Fiedorowicz, A., Janeczko, K., and Oderfeld-Nowak, B. (2005). Quantitative morphological study of microglial cells in the ischemic rat brain using principal component analysis. *Journal of Neuroscience Methods*, 146(1), 50–60.
- Somogyi, P.,** Bolam, J. P., and Smith, A. D. (1981). Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscopic study using the Golgi-peroxidase transport-degeneration procedure. *The Journal of Comparative Neurology*, 195(4), 567–584.
- Song, I., and Huganir, R. L.** (2002). Regulation of AMPA receptors during synaptic plasticity. *Trends in Neurosciences*, 25(11), 578–588.
- Soria, J. M., and Valdeolmillos, M.** (2002). Receptor-activated calcium signals in tangentially migrating cortical cells. *Cerebral Cortex*, 12(8), 831–839.
- Specht, L. A.,** Pickel, V. M., Joh, T. H., and Reis, D. J. (1981). Light-microscopic immunocytochemical localization of tyrosine hydroxylase in prenatal rat brain. I. Early ontogeny. *The Journal of Comparative Neurology*, 199(2), 233–253.
- Speranza, L.,** Labus, J., Volpicelli, F., Guseva, D., Lacivita, E., Leopoldo, M., Bellenchi, G. C., di Porzio, U., Bijata, M., Perrone-Capano, C., and Ponimaskin, E. (2017). Serotonin 5-HT₇ receptor increases the density of dendritic spines and facilitates synaptogenesis in forebrain neurons. *Journal of Neurochemistry*, 141(5), 647–661.
- Spielman, L. J.,** Gibson, D. L., and Klegeris, A. (2018). Unhealthy gut, unhealthy brain: The role of the intestinal microbiota in neurodegenerative diseases. *Neurochemistry International*, 120, 149–163.
- Špírková, A.,** Kovaříková, V., Šefčíková, Z., Pisko, J., Kšiňanová, M., Koppel, J., Fabian, D., and Čikoš, Š. (2022). Glutamate can act as a signaling molecule in mouse preimplantation embryos†. *Biology of Reproduction*, 107(4), 916–927.
- Spitzer N. C.** (2006). Electrical activity in early neuronal development. *Nature*, 444(7120), 707–712.
- Sprengel, R.,** Suchanek, B., Amico, C., Brusa, R., Burnashev, N., Rozov, A., Hvalby, O., Jensen, V., Paulsen, O., Andersen, P., Kim, J. J., Thompson, R. F., Sun, W., Webster, L. C., Grant, S. G., Eilers, J., Konnerth, A., Li, J., McNamara, J. O., and Seeburg, P. H. (1998). Importance of the intracellular domain of NR2 subunits for NMDA receptor function *in vivo*. *Cell*, 92(2), 279–289.
- Spruston N.** (2008). Pyramidal neurons: dendritic structure and synaptic integration. *Nature Reviews. Neuroscience*, 9(3), 206–221.
- Stancik, E. K.,** Navarro-Quiroga, I., Sellke, R., and Haydar, T. F. (2010). Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. *The Journal of Neuroscience*, 30(20), 7028–7036.
- Star, E. N.,** Kwiatkowski, D. J., and Murthy, V. N. (2002). Rapid turnover of actin in dendritic spines and its regulation by activity. *Nature Neuroscience*, 5(3), 239–246.
- Stefani, M. R., and Moghaddam, B.** (2005). Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive

deficits relevant to schizophrenia. *Biological Psychiatry*, 57(4), 433–436.

Stence, N., Waite, M., and Dailey, M. E. (2001). Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. *Glia*, 33(3), 256–266.

Stenman, J., Toresson, H., and Campbell, K. (2003). Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. *The Journal of Neuroscience*, 23(1), 167–174.

Stocca, G., and Vicini, S. (1998). Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. *The Journal of Physiology*, 507 (Pt 1)(Pt 1), 13–24.

Storey, G. P., Opitz-Araya, X., and Barria, A. (2011). Molecular determinants controlling NMDA receptor synaptic incorporation. *The Journal of Neuroscience*, 31(17), 6311–6316.

Strahan, J. A., Walker, W. H., 2nd, Montgomery, T. R., and Forger, N. G. (2017). Minocycline causes widespread cell death and increases microglial labeling in the neonatal mouse brain. *Developmental Neurobiology*, 77(6), 753–766.

Stratoulis, V., Venero, J. L., Tremblay, M. È., and Joseph, B. (2019). Microglial subtypes: diversity within the microglial community. *The EMBO Journal*, 38(17), e101997.

Streit W. J. (2001). Microglia and macrophages in the developing CNS. *Neurotoxicology*, 22(5), 619–624.

Strübing, C., Ahnert-Hilger, G., Shan, J., Wiedenmann, B., Hescheler, J., and Wobus, A. M. (1995). Differentiation of pluripotent embryonic stem cells into the neuronal lineage *in vitro* gives rise to mature inhibitory and excitatory neurons. *Mechanisms of Development*, 53(2), 275–287.

Sucher, N. J., Brose, N., Deitcher, D. L., Awobuluyi, M., Gasic, G. P., Bading, H., Cepko, C. L., Greenberg, M. E., Jahn, R., Heinemann, S. F., and Lipton, S. A.

(1993). Expression of endogenous NMDAR1 transcripts without receptor protein suggests post-transcriptional control in PC12 cells. *The Journal of Biological Chemistry*, 268(30), 22299–22304.

Sun, H., Jia, N., Guan, L., Su, Q., Wang, D., Li, H., and Zhu, Z. (2013). Involvement of NR1, NR2A different expression in brain regions in anxiety-like behavior of prenatally stressed offspring. *Behavioural Brain Research*, 257, 1–7.

Sun, Y., Koyama, Y., and Shimada, S. (2022). Inflammation From Peripheral Organs to the Brain: How Does Systemic Inflammation Cause Neuroinflammation?. *Frontiers in Aging Neuroscience*, 14, 903455.

Sur, M., and Leamey, C. A. (2001). Development and plasticity of cortical areas and networks. *Nature Reviews. Neuroscience*, 2(4), 251–262.

Sutherland, M. L., Delaney, T. A., and Noebels, J. L. (1996). Glutamate transporter mRNA expression in proliferative zones of the developing and adult murine CNS. *The Journal of Neuroscience*, 16(7), 2191–2207.

Sutton, M. A., Ito, H. T., Cressy, P., Kempf, C., Woo, J. C., and Schuman, E. M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell*, 125(4), 785–799.

Suzuki, M., Nelson, A. D., Eickstaedt, J. B., Wallace, K., Wright, L. S., and Svendsen, C. N. (2006). Glutamate enhances proliferation and neurogenesis in human neural progenitor cell cultures derived from the fetal cortex. *The European Journal of Neuroscience*, 24(3), 645–653.

Sweeten, T. L., Bowyer, S. L., Posey, D. J., Halberstadt, G. M., and McDougale, C. J. (2003). Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Pediatrics*, 112(5), e420.

Swinnen, N., Smolders, S., Avila, A., Notelaers, K., Paesen, R., Ameloot, M., Brône, B., Legendre, P., and Rigo, J. M. (2013). Complex invasion pattern of the cerebral cortex by microglial cells during

development of the mouse embryo. *Glia*, 61(2), 150–163.

Symmonds, M., Moran, C. H., Leite, M. I., Buckley, C., Irani, S. R., Stephan, K. E., Friston, K. J., and Moran, R. J. (2018). Ion channels in EEG: isolating channel dysfunction in NMDA receptor antibody encephalitis. *Brain*, 141(6), 1691–1702.

Szabadits, E., Cserép, C., Szonyi, A., Fukazawa, Y., Shigemoto, R., Watanabe, M., Itohara, S., Freund, T. F., and Nyiri, G. (2011). NMDA receptors in hippocampal GABAergic synapses and their role in nitric oxide signaling. *The Journal of Neuroscience*, 31(16), 5893–5904.

Tahraoui, S. L., Marret, S., Bodénant, C., Leroux, P., Dommergues, M. A., Evrard, P., and Gressens, P. (2001). Central role of microglia in neonatal excitotoxic lesions of the murine periventricular white matter. *Brain Pathology*, 11(1), 56–71.

Tan, Y. L., Yuan, Y., and Tian, L. (2020). Microglial regional heterogeneity and its role in the brain. *Molecular Psychiatry*, 25(2), 351–367

Tao, H. W., and **Poo, M. M.** (2005). Activity-dependent matching of excitatory and inhibitory inputs during refinement of visual receptive fields. *Neuron*, 45(6), 829–836.

Taraschenko, O., Fox, H. S., Pittock, S. J., Zekeridou, A., Gafurova, M., Eldridge, E., Liu, J., Dravid, S. M., and Dingledine, R. (2019). A mouse model of seizures in anti-N-methyl-D-aspartate receptor encephalitis. *Epilepsia*, 60(3), 452–463.

Tashiro, A., Sandler, V. M., Toni, N., Zhao, C., and Gage, F. H. (2006). NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature*, 442(7105), 929–933.

Taupin P. (2007). BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. *Brain Research Reviews*, 53(1), 198–214.

Taverna, S., Ilijic, E., and Surmeier, D. J. (2008). Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of

Parkinson's disease. *The Journal of Neuroscience*, 28(21), 5504–5512.

Tecuapetla, F., Jin, X., Lima, S. Q., and Costa, R. M. (2016). Complementary Contributions of Striatal Projection Pathways to Action Initiation and Execution. *Cell*, 166(3), 703–715.

Tecuapetla, F., Matias, S., Dugue, G. P., Mainen, Z. F., and Costa, R. M. (2014). Balanced activity in basal ganglia projection pathways is critical for contraversive movements. *Nature Communications*, 5, 4315.

Temple S. (2001). The development of neural stem cells. *Nature*, 414(6859), 112–117.

Teng, L., Qin, Q., Zhou, Z., Zhou, F., Cao, C., Yang, J., and Ding, J. (2023). Glutamate secretion by embryonic stem cells as an autocrine signal to promote proliferation. *Scientific Reports*, 13(1), 19069.

Tepper, J. M., and **Trent, F.** (1993). *In vivo* studies of the postnatal development of rat neostriatal neurons. *Progress in Brain Research*, 99, 35–50.

Tepper, J. M., Sharpe, N. A., Koós, T. Z., and Trent, F. (1998). Postnatal development of the rat neostriatum: electrophysiological, light- and electron-microscopic studies. *Developmental Neuroscience*, 20(2-3), 125–145.

Tepper, J. M., Tecuapetla, F., Koós, T., and Ibáñez-Sandoval, O. (2010). Heterogeneity and diversity of striatal GABAergic interneurons. *Frontiers in Neuroanatomy*, 4, 150.

Tepper, J. M., Koós, T., Ibanez-Sandoval, O., Tecuapetla, F., Faust, T. W., and Assous, M. (2018). Heterogeneity and Diversity of Striatal GABAergic Interneurons: Update 2018. *Frontiers in Neuroanatomy*, 12, 91.

Tetreault, N. A., Hakeem, A. Y., Jiang, S., Williams, B. A., Allman, E., Wold, B. J., and Allman, J. M. (2012). Microglia in the cerebral cortex in autism. *Journal of Autism and Developmental Disorders*, 42(12), 2569–2584.

Thermo Fisher Scientific. Click iT Plus TUNEL Assay Manual. (2021).

Thorn, C. A., Atallah, H., Howe, M., and Graybiel, A. M. (2010). Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. *Neuron*, 66(5), 781–795.

Tikka, T. M., and Koistinaho, J. E. (2001). Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *Journal of Immunology*, 166(12), 7527–7533.

Titulaer, M. J., McCracken, L., Gabilondo, I., Armangué, T., Glaser, C., Iizuka, T., Honig, L. S., Benseler, S. M., Kawachi, I., Martínez-Hernández, E., Aguilar, E., Gresa-Arribas, N., Ryan-Florange, N., Torrents, A., Saiz, A., Rosenfeld, M. R., Balice-Gordon, R., Graus, F., and Dalmau, J. (2013). Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *The Lancet. Neurology*, 12(2), 157–165.

Tolias, K. F., Bikoff, J. B., Burette, A., Paradis, S., Harrar, D., Tavazoie, S., Weinberg, R. J., and Greenberg, M. E. (2005). The Rac1-GEF Tiam1 couples the NMDA receptor to the activity-dependent development of dendritic arbors and spines. *Neuron*, 45(4), 525–538.

Torres-Platas, S. G., Comeau, S., Rachalski, A., Bo, G. D., Cruceanu, C., Turecki, G., Giros, B., and Mechawar, N. (2014). Morphometric characterization of microglial phenotypes in human cerebral cortex. *Journal of Neuroinflammation*, 11, 12.

Torrisi, S., O'Connell, K., Davis, A., Reynolds, R., Balderston, N., Fudge, J. L., Grillon, C., and Ernst, M. (2015). Resting state connectivity of the bed nucleus of the stria terminalis at ultra-high field. *Human Brain Mapping*, 36(10), 4076–4088.

Tovar, K. R., and Westbrook, G. L. (2002). Mobile NMDA receptors at hippocampal synapses. *Neuron*, 34(2), 255–264.

Toyama, M., Maeda, T., and Shimizu, N. (1974). Detailed noradrenaline pathways of locus coeruleus

neuron to the cerebral cortex with use of 6-hydroxydopa. *Brain Research*, 79(1), 139–144.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., and Dingledine, R. (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews*, 62(3), 405–496.

Tremblay, M. È., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biology*, 8(11), e1000527.

Tripathi, M. K., Ojha, S. K., Kartawy, M., Khaliulin, I., Hamoudi, W., and Amal, H. (2024). Mutations associated with autism lead to similar synaptic and behavioral alterations in both sexes of male and female mouse brain. *Scientific Reports*, 14(1), 10.

Tu, W., Xu, X., Peng, L., Zhong, X., Zhang, W., Soundarapandian, M. M., Balel, C., Wang, M., Jia, N., Zhang, W., Lew, F., Chan, S. L., Chen, Y., and Lu, Y. (2010). DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell*, 140(2), 222–234.

Turetsky, D. M., Huettner, J. E., Gottlieb, D. I., Goldberg, M. P., and Choi, D. W. (1993). Glutamate receptor-mediated currents and toxicity in embryonal carcinoma cells. *Journal of Neurobiology*, 24(9), 1157–1169.

Turrero García, M., and Harwell, C. C. (2017). Radial glia in the ventral telencephalon. *FEBS letters*, 591(24), 3942–3959.

Tüzün, E., Zhou, L., Baehring, J. M., Bannykh, S., Rosenfeld, M. R., and Dalmau, J. (2009). Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. *Acta Neuropathologica*, 118(6), 737–743.

Tyler, W. A., and Haydar, T. F. (2013). Multiplex genetic fate mapping reveals a novel route of neocortical neurogenesis, which is altered in the Ts65Dn mouse model of Down syndrome. *The Journal of Neuroscience*, 33(12), 5106–5119.

Tyler, W. A., Medalla, M., Guillamon-Vivancos, T., Luebke, J. I., and Haydar, T. F. (2015). Neural precursor lineages specify distinct neocortical pyramidal neuron types. *The Journal of Neuroscience*, 35(15), 6142–6152.

Tzoulis, C., Vedeler, C., Haugen, M., Storstein, A., Tran, G. T., Gjerde, I. O., Biermann, M., Schwarzmüller, T., and Bindoff, L. A. (2013). Progressive striatal necrosis associated with anti-NMDA receptor antibodies. *BMC Neurology*, 13, 55.

Ueno, M., Fujita, Y., Tanaka, T., Nakamura, Y., Kikuta, J., Ishii, M., and Yamashita, T. (2013). Layer V cortical neurons require microglial support for survival during postnatal development. *Nature Neuroscience*, 16(5), 543–551.

Ueno, M., and Yamashita, T. (2014). Bidirectional tuning of microglia in the developing brain: from neurogenesis to neural circuit formation. *Current Opinion in Neurobiology*, 27, 8–15.

Ultanir, S. K., Kim, J. E., Hall, B. J., Deerinck, T., Ellisman, M., and Ghosh, A. (2007). Regulation of spine morphology and spine density by NMDA receptor signaling *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America*, 104(49), 19553–19558.

Unoki, T., Matsuda, S., Kakegawa, W., Van, N. T., Kohda, K., Suzuki, A., Funakoshi, Y., Hasegawa, H., Yuzaki, M., and Kanaho, Y. (2012). NMDA receptor-mediated PIP5K activation to produce PI(4,5)P₂ is essential for AMPA receptor endocytosis during LTD. *Neuron*, 73(1), 135–148.

Urowitz, M. B., Gladman, D. D., MacKinnon, A., Ibañez, D., Bruto, V., Rovet, J., and Silverman, E. (2008). Neurocognitive abnormalities in offspring of mothers with systemic lupus erythematosus. *Lupus*, 17(6), 555–560.

Uzquiano, A., Gladwyn-Ng, I., Nguyen, L., Reiner, O., Götz, M., Matsuzaki, F., and Francis, F. (2018). Cortical progenitor biology: key features mediating proliferation versus differentiation. *Journal of Neurochemistry*, 146(5), 500–525.

Valjent, E., and Gangarossa, G. (2021). The Tail of the Striatum: From Anatomy to Connectivity and Function. *Trends in Neurosciences*, 44(3), 203–214.

Van de Perre P. (2003). Transfer of antibody via mother's milk. *Vaccine*, 21(24), 3374–3376.

Van der Kooy, D., and Fishell, G. (1987). Neuronal birthdate underlies the development of striatal compartments. *Brain Research*, 401(1), 155–161.

Van Heusden, F.*, Macey-Dare, A.*, Gordon, J., Krajewski, R., Sharott, A., and Ellender, T. (2021). Diversity in striatal synaptic circuits arises from distinct embryonic progenitor pools in the ventral telencephalon. *Cell Reports*, 35(4), 109041.

* joint first author

Van Horn, M. R., Strasser, A., Miraucourt, L. S., Pollegioni, L., and Ruthazer, E. S. (2017). The Gliotransmitter d-Serine Promotes Synapse Maturation and Axonal Stabilization *In vivo*. *The Journal of Neuroscience*, 37(26), 6277–6288.

Van Zundert, B., Zhao, J. P., and Constantine-Paton, M. (2010). Synaptic drive at developing synapses: transient upregulation of kainate receptors. *Developmental Neurobiology*, 70(11), 737–750.

Vance, K. M., Hansen, K. B., and Traynelis, S. F. (2012). GluN1 splice variant control of GluN1/GluN2D NMDA receptors. *The Journal of Physiology*, 590(16), 3857–3875.

Varju, P., Schlett, K., Eisel, U., and Madarász, E. (2001). Schedule of NMDA receptor subunit expression and functional channel formation in the course of *in vitro*-induced neurogenesis. *Journal of Neurochemistry*, 77(6), 1444–1456.

Varoqui, H., Schäfer, M. K., Zhu, H., Weihe, E., and Erickson, J. D. (2002). Identification of the differentiation-associated Na⁺/PI transporter as a novel vesicular glutamate transporter expressed in a distinct set of glutamatergic synapses. *The Journal of Neuroscience*, 22(1), 142–155.

Vashishta, A., Habas, A., Pruunsild, P., Zheng, J. J., Timmusk, T., and Hetman, M. (2009). Nuclear factor of activated T-cells isoform c4 (NFATc4/NFAT3) as a

mediator of antiapoptotic transcription in NMDA receptor-stimulated cortical neurons. *The Journal of Neuroscience*, 29(48), 15331–15340.

Vastagh, C., Gardoni, F., Bagetta, V., Stanic, J., Zianni, E., Giampà, C., Picconi, B., Calabresi, P., and Di Luca, M. (2012). N-methyl-D-aspartate (NMDA) receptor composition modulates dendritic spine morphology in striatal medium spiny neurons. *The Journal of Biological Chemistry*, 287(22), 18103–18114.

Velmeshev, D., Schirmer, L., Jung, D., Haeussler, M., Perez, Y., Mayer, S., Bhaduri, A., Goyal, N., Rowitch, D. H., and Kriegstein, A. R. (2019). Single-cell genomics identifies cell type-specific molecular changes in autism. *Science*, 364(6441), 685–689.

Venance, L., Glowinski, J., and Giaume, C. (2004). Electrical and chemical transmission between striatal GABAergic output neurones in rat brain slices. *The Journal of Physiology*, 559(Pt 1), 215–230.

Vercelli, A., Marini, G., and Tredici, G. (2003). Anatomical organization of the telencephalic connections of the parafascicular nucleus in adult and developing rats. *The European Journal of Neuroscience*, 18(2), 275–289.

Vernon, A. C., Smith, E. J., Stevanato, L., and Modo, M. (2011). Selective activation of metabotropic glutamate receptor 7 induces inhibition of cellular proliferation and promotes astrocyte differentiation of ventral mesencephalon human neural stem/progenitor cells. *Neurochemistry International*, 59(3), 421–431.

Viaccoz, A., Desestret, V., Ducray, F., Picard, G., Cavillon, G., Rogemond, V., Antoine, J. C., Delattre, J. Y., and Honnorat, J. (2014). Clinical specificities of adult male patients with NMDA receptor antibodies encephalitis. *Neurology*, 82(7), 556–563.

Vicente, A. M., Galvão-Ferreira, P., Tecuapetla, F., and Costa, R. M. (2016). Direct and indirect dorsolateral striatum pathways reinforce different action strategies. *Current Biology*, 26(7), R267–R269.

Vicini, S., Wang, J. F., Li, J. H., Zhu, W. J., Wang, Y. H., Luo, J. H., Wolfe, B. B., and Grayson, D. R. (1998). Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. *Journal of Neurophysiology*, 79(2), 555–566.

Vigneault, É., Poirel, O., Riad, M., Prud'homme, J., Dumas, S., Turecki, G., Fasano, C., Mechawar, N., and El Mestikawy, S. (2015). Distribution of vesicular glutamate transporters in the human brain. *Frontiers in Neuroanatomy*, 9, 23.

Villar-Cerviño, V., Kappeler, C., Nóbrega-Pereira, S., Henkemeyer, M., Rago, L., Nieto, M. A., and Marín, O. (2015). Molecular mechanisms controlling the migration of striatal interneurons. *The Journal of Neuroscience*, 35(23), 8718–8729.

Vinet, É., Pineau, C. A., Clarke, A. E., Scott, S., Fombonne, É., Joseph, L., Platt, R. W., and Bernatsky, S. (2015). Increased Risk of Autism Spectrum Disorders in Children Born to Women With Systemic Lupus Erythematosus: Results From a Large Population-Based Cohort. *Arthritis and Rheumatology*, 67(12), 3201–3208.

Vitaliani, R., Mason, W., Ances, B., Zwerdling, T., Jiang, Z., and Dalmau, J. (2005). Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. *Annals of Neurology*, 58(4), 594–604.

Vogel, S., Klumpers, F., Schröder, T. N., Oplaat, K. T., Krugers, H. J., Oitzl, M. S., Joëls, M., Doeller, C. F., and Fernández, G. (2017). Stress Induces a Shift Towards Striatum-Dependent Stimulus-Response Learning via the Mineralocorticoid Receptor. *Neuropsychopharmacology*, 42(6), 1262–1271.

Volianskis, A., France, G., Jensen, M. S., Bortolotto, Z. A., Jane, D. E., and Collingridge, G. L. (2015). Long-term potentiation and the role of N-methyl-D-aspartate receptors. *Brain Research*, 1621, 5–16.

Voorn, P., Kalsbeek, A., Jorritsma-Byham, B., and Groenewegen, H. J. (1988). The pre- and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat. *Neuroscience*, 25(3), 857–887.

- Wagnon, I.,** Hélie, P., Bardou, I., Regnault, C., Lesec, L., Leprince, J., Naveau, M., Delaunay, B., Toutirais, O., Lemauff, B., Etard, O., Vivien, D., Agin, V., Macrez, R., Maubert, E., and Docagne, F. (2020). Autoimmune encephalitis mediated by B-cell response against N-methyl-d-aspartate receptor. *Brain*, 143(10), 2957–2972.
- Wake, H.,** Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *The Journal of Neuroscience*, 29(13), 3974–3980.
- Wakselman, S.,** Béchade, C., Roumier, A., Bernard, D., Triller, A., and Bessis, A. (2008). Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. *The Journal of Neuroscience*, 28(32), 8138–8143.
- Walsh, C., and Cepko, C. L.** (1993). Clonal dispersion in proliferative layers of developing cerebral cortex. *Nature*, 362(6421), 632–635.
- Wang, C. C.,** Held, R. G., Chang, S. C., Yang, L., Delpire, E., Ghosh, A., and Hall, B. J. (2011). A critical role for GluN2B-containing NMDA receptors in cortical development and function. *Neuron*, 72(5), 789–805.
- Wang, H. Y.,** Li, T., Li, X. L., Zhang, X. X., Yan, Z. R., and Xu, Y. (2019). Anti-N-methyl-D-aspartate receptor encephalitis mimics neuroleptic malignant syndrome: case report and literature review. *Neuropsychiatric Disease and Treatment*, 15, 773–778.
- Wang, H.,** Lv, S., Stroebel, D., Zhang, J., Pan, Y., Huang, X., Zhang, X., Paoletti, P., and Zhu, S. (2021). Gating mechanism and a modulatory niche of human GluN1-GluN2A NMDA receptors. *Neuron*, 109(15), 2443–2456.e5.
- Warren, N.,** Grote, V., O’Gorman, C., and Siskind, D. (2019). Electroconvulsive therapy for anti-N-methyl-d-aspartate (NMDA) receptor encephalitis: A systematic review of cases. *Brain Stimulation*, 12(2), 329–334.
- Washbourne, P.,** Bennett, J. E., and McAllister, A. K. (2002). Rapid recruitment of NMDA receptor transport packets to nascent synapses. *Nature Neuroscience*, 5(8), 751–759.
- Watabe-Uchida, M.,** Zhu, L., Ogawa, S. K., Vamanrao, A., and Uchida, N. (2012). Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron*, 74(5), 858–873.
- Watanabe, M.,** Inoue, Y., Sakimura, K., and Mishina, M. (1992). Developmental changes in distribution of NMDA receptor channel subunit mRNAs. *Neuroreport*, 3(12), 1138–1140.
- Waters, P.,** Jarius, S., Littleton, E., Leite, M. I., Jacob, S., Gray, B., Geraldles, R., Vale, T., Jacob, A., Palace, J., Maxwell, S., Beeson, D., and Vincent, A. (2008). Aquaporin-4 antibodies in neuromyelitis optica and longitudinally extensive transverse myelitis. *Archives of Neurology*, 65(7), 913–919.
- Whish, S.,** Dziegielewska, K. M., Mollgard, K., Noor, N. M., Liddel, S. A., Habgood, M. D., Richardson, S. J., and Saunders, N. R. (2015). The inner CSF-brain barrier: developmentally controlled access to the brain via intercellular junctions. *Frontiers in Neuroscience*, 9, 16.
- Whitten, W. K.** (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *The Journal of Endocrinology*, 13(4), 399–404.
- Wichterle, H.,** Garcia-Verdugo, J. M., Herrera, D. G., and Alvarez-Buylla, A. (1999). Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. *Nature Neuroscience*, 2(5), 461–466.
- Wichterle, H.,** Turnbull, D. H., Nery, S., Fishell, G., and Alvarez-Buylla, A. (2001). *In utero* fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development*, 128(19), 3759–3771.
- Wierzb-Bobrowicz, T.,** Gwiazda, E., Kosno-Kruszewska, E., Lewandowska, E., Lechowicz, W., Bertrand, E., Szpak, G. M., and Schmidt-Sidor, B. (2002). Morphological analysis of active microglia--

rod and ramified microglia in human brains affected by some neurological diseases (SSPE, Alzheimer's disease and Wilson's disease). *Folia Neuropathologica*, 40(3), 125–131.

Wilke, J. B. H., Hindermann, M., Berghoff, S. A., Zihlsler, S., Arinrad, S., Ronnenberg, A., Barnkothe, N., Steixner-Kumar, A. A., Röglin, S., Stöcker, W., Hollmann, M., Nave, K. A., Lühder, F., and Ehrenreich, H. (2021). Autoantibodies against NMDA receptor 1 modify rather than cause encephalitis. *Molecular Psychiatry*, 26(12), 7746–7759.

Williams K. (1993). Ifenprodil discriminates subtypes of the N-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Molecular Pharmacology*, 44(4), 851–859.

Wilson, C. J., and Groves, P. M. (1980). Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular inject of horseradish peroxidase. *The Journal of Comparative Neurology*, 194(3), 599–615.

Wilson, C. J., Groves, P. M., Kitai, S. T., and Linder, J. C. (1983). Three-dimensional structure of dendritic spines in the rat neostriatum. *The Journal of Neuroscience*, 3(2), 383–388.

Wilson, C. J., and Kawaguchi, Y. (1996). The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *The Journal of Neuroscience*, 16(7), 2397–2410.

Wisden, W., and Seeburg, P. H. (1993). A complex mosaic of high-affinity kainate receptors in rat brain. *The Journal of Neuroscience*, 13(8), 3582–3598.

Wong, W. T., and Wong, R. O. (2001). Changing specificity of neurotransmitter regulation of rapid dendritic remodeling during synaptogenesis. *Nature Neuroscience*, 4(4), 351–352.

Wong, H. K., Liu, X. B., Matos, M. F., Chan, S. F., Pérez-Otaño, I., Boysen, M., Cui, J., Nakanishi, N., Trimmer, J. S., Jones, E. G., Lipton, S. A., and Sucher,

N. J. (2002). Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. *The Journal of Comparative Neurology*, 450(4), 303–317.

Wong, H. H., Chou, C. Y. C., Watt, A. J., and Sjöström, P. J. (2023). Comparing mouse and human brains. *eLife*, 12, e90017.

Woodhall, G., Evans, D. I., Cunningham, M. O., and Jones, R. S. (2001). NR2B-containing NMDA autoreceptors at synapses on entorhinal cortical neurons. *Journal of Neurophysiology*, 86(4), 1644–1651.

World Health Organization (WHO). International Classification of Diseases, Eleventh Revision (ICD-11). (2019/2021).

Wright, S., Hacohen, Y., Jacobson, L., Agrawal, S., Gupta, R., Philip, S., Smith, M., Lim, M., Wassmer, E., and Vincent, A. (2015a). N-methyl-D-aspartate receptor antibody-mediated neurological disease: results of a UK-based surveillance study in children. *Archives of Disease in Childhood*, 100(6), 521–526.

Wright, S., Hashemi, K., Stasiak, L., Bartram, J., Lang, B., Vincent, A., and Upton, A. L. (2015b). Epileptogenic effects of NMDAR antibodies in a passive transfer mouse model. *Brain*, 138(Pt 11), 3159–3167.

Wright, S. K., Rosch, R. E., Wilson, M. A., Upadhy, M. A., Dhangar, D. R., Clarke-Bland, C., Wahid, T. T., Barman, S., Goebels, N., Kreye, J., Prüss, H., Jacobson, L., Bassett, D. S., Vincent, A., Greenhill, S. D., and Woodhall, G. L. (2021). Multimodal electrophysiological analyses reveal that reduced synaptic excitatory neurotransmission underlies seizures in a model of NMDAR antibody-mediated encephalitis. *Communications Biology*, 4(1), 1106.

Wu, G. Y., Zou, D. J., Rajan, I., and Cline, H. (1999). Dendritic dynamics *in vivo* change during neuronal maturation. *The Journal of Neuroscience*, 19(11), 4472–4483.

Wu, Q. J., and Tymianski, M. (2018). Targeting NMDA receptors in stroke: new hope in neuroprotection. *Molecular Brain*, 11(1), 15.

Würdemann, T., Kersten, M., Tokay, T., Guli, X., Kober, M., Rohde, M., Porath, K., Sellmann, T., Bien, C. G., Köhling, R., and Kirschstein, T. (2016). Stereotactic injection of cerebrospinal fluid from anti-NMDA receptor encephalitis into rat dentate gyrus impairs NMDA receptor function. *Brain Research*, 1633, 10–18.

Yager, L. M., Garcia, A. F., Wunsch, A. M., and Ferguson, S. M. (2015). The ins and outs of the striatum: role in drug addiction. *Neuroscience*, 301, 529–541.

Yamamura, T., Kleiter, I., Fujihara, K., Palace, J., Greenberg, B., Zakrzewska-Pniewska, B., Patti, F., Tsai, C. P., Saiz, A., Yamazaki, H., Kawata, Y., Wright, P., and De Seze, J. (2019). Trial of Satralizumab in Neuromyelitis Optica Spectrum Disorder. *The New England Journal of Medicine*, 381(22), 2114–2124.

Yamasaki, M., Okada, R., Takasaki, C., Toki, S., Fukaya, M., Natsume, R., Sakimura, K., Mishina, M., Shirakawa, T., and Watanabe, M. (2014). Opposing role of NMDA receptor GluN2B and GluN2D in somatosensory development and maturation. *The Journal of Neuroscience*, 34(35), 11534–11548.

Yang, J. W., Hanganu-Opatz, I. L., Sun, J. J., and Luhmann, H. J. (2009). Three patterns of oscillatory activity differentially synchronize developing neocortical networks *in vivo*. *The Journal of Neuroscience*, 29(28), 9011–9025.

Yang, X., Gong, R., Qin, L., Bao, Y., Fu, Y., Gao, S., Yang, H., Ni, J., Yuan, T. F., and Lu, W. (2022). Trafficking of NMDA receptors is essential for hippocampal synaptic plasticity and memory consolidation. *Cell Reports*, 40(7), 111217.

Yao, L., Rong, Y., Ma, X., Li, H., Deng, D., Chen, Y., Yang, S., Peng, T., Ye, T., Liang, F., Xu, N., and Zhou, Q. (2022). Extrasynaptic NMDA Receptors Bidirectionally Modulate Intrinsic Excitability of Inhibitory Neurons. *The Journal of Neuroscience*, 42(15), 3066–3079.

Yasuda, M., Johnson-Venkatesh, E. M., Zhang, H., Parent, J. M., Sutton, M. A., and Umemori, H. (2011). Multiple forms of activity-dependent competition refine hippocampal circuits *in vivo*. *Neuron*, 70(6), 1128–1142.

Ye, G. L., Song Liu, X., Pasternak, J. F., and Trommer, B. L. (2000). Maturation of glutamatergic neurotransmission in dentate gyrus granule cells. *Brain Research. Developmental Brain Research*, 124(1-2), 33–42.

Yin, H. H., Knowlton, B. J., and Balleine, B. W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *The European Journal of Neuroscience*, 19(1), 181–189.

Yin, H. H., Ostlund, S. B., Knowlton, B. J., and Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *The European Journal of Neuroscience*, 22(2), 513–523.

Yin, H. H., and Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews. Neuroscience*, 7(6), 464–476.

Yin, H. H., Mulcare, S. P., Hilário, M. R., Clouse, E., Holloway, T., Davis, M. I., Hansson, A. C., Lovinger, D. M., and Costa, R. M. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nature Neuroscience*, 12(3), 333–341.

Yoon, K., and Gaiano, N. (2005). Notch signaling in the mammalian central nervous system: insights from mouse mutants. *Nature Neuroscience*, 8(6), 709–715.

Young, K., and Morrison, H. (2018). Quantifying Microglia Morphology from Photomicrographs of Immunohistochemistry Prepared Tissue Using Image J. *Journal of Visualized Experiments*, (136), 57648.

Yozu, M., Tabata, H., Konig, N., and Nakajima, K. (2008). Migratory behavior of presumptive interneurons is affected by AMPA receptor activation in slice cultures of embryonic mouse neocortex. *Developmental Neuroscience*, 30(1-3), 105–116.

Yu, Y. C., Bultje, R. S., Wang, X., and Shi, S. H. (2009). Specific synapses develop preferentially among sister excitatory neurons in the neocortex. *Nature*, 458(7237), 501–504.

- Yu, Y. C.,** He, S., Chen, S., Fu, Y., Brown, K. N., Yao, X. H., Ma, J., Gao, K. P., Sosinsky, G. E., Huang, K., and Shi, S. H. (2012). Preferential electrical coupling regulates neocortical lineage-dependent microcircuit assembly. *Nature*, 486(7401), 113–117.
- Yung, K. K.,** Smith, A. D., Levey, A. I., and Bolam, J. P. (1996). Synaptic connections between spiny neurons of the direct and indirect pathways in the neostriatum of the rat: evidence from dopamine receptor and neuropeptide immunostaining. *The European Journal of Neuroscience*, 8(5), 861–869.
- Zafra, F.,** Castrén, E., Thoenen, H., and Lindholm, D. (1991). Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America*, 88(22), 10037–10041.
- Zalocusky, K. A.,** Ramakrishnan, C., Lerner, T. N., Davidson, T. J., Knutson, B., and Deisseroth, K. (2016). Nucleus accumbens D2R cells signal prior outcomes and control risky decision-making. *Nature*, 531(7596), 642–646.
- Zappaterra, M. W.,** LaMantia, A. S., Walsh, C. A., and Lehtinen, M. K. (2013). Isolation of cerebrospinal fluid from rodent embryos for use with dissected cerebral cortical explants. *Journal of Visualized Experiments*, (73), e50333.
- Zeng, F.,** Zhang, Q., Liu, Y., Sun, G., Li, A., Talay, R. S., and Wang, J. (2021). AMPAkinases potentiate the corticostriatal pathway to reduce acute and chronic pain. *Molecular Brain*, 14(1), 45.
- Zhan, Y.,** Paolicelli, R. C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., Vyssotski, A. L., Bifone, A., Gozzi, A., Ragozzino, D., and Gross, C. T. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nature Neuroscience*, 17(3), 400–406.
- Zhang, L. I.,** Tao, H. W., and Poo, M. (2000). Visual input induces long-term potentiation of developing retinotectal synapses. *Nature Neuroscience*, 3(7), 708–715.
- Zhang, Z. W.,** Peterson, M., and Liu, H. (2013). Essential role of postsynaptic NMDA receptors in developmental refinement of excitatory synapses. *Proceedings of the National Academy of Sciences of the United States of America*, 110(3), 1095–1100.
- Zhang, L.,** Wu, M. Q., Hao, Z. L., Chiang, S. M., Shuang, K., Lin, M. T., Chi, X. S., Fang, J. J., Zhou, D., and Li, J. M. (2017a). Clinical characteristics, treatments, and outcomes of patients with anti-N-methyl-D-aspartate receptor encephalitis: A systematic review of reported cases. *Epilepsy and Behavior*, 68, 57–65.
- Zhang, C.,** Tian, D. C., Yang, C. S., Han, B., Wang, J., Yang, L., and Shi, F. D. (2017b). Safety and Efficacy of Bortezomib in Patients With Highly Relapsing Neuromyelitis Optica Spectrum Disorder. *JAMA Neurology*, 74(8), 1010–1012.
- Zhao, T.,** Li, Y., Wei, W., Savage, S., Zhou, L., and Ma, D. (2014). Ketamine administered to pregnant rats in the second trimester causes long-lasting behavioral disorders in offspring. *Neurobiology of Disease*, 68, 145–155.
- Zhao, M.,** Jiang, X. F., Zhang, H. Q., Sun, J. H., Pei, H., Ma, L. N., Cao, Y., and Li, H. (2021). Interactions between glial cells and the blood-brain barrier and their role in Alzheimer's disease. *Ageing Research Reviews*, 72, 101483.
- Zhong, Y.,** Zhou, L. J., Ren, W. J., Xin, W. J., Li, Y. Y., Zhang, T., and Liu, X. G. (2010). The direction of synaptic plasticity mediated by C-fibers in spinal dorsal horn is decided by Src-family kinases in microglia: the role of tumor necrosis factor- α . *Brain, Behavior, and Immunity*, 24(6), 874–880.
- Zhou, Q.,** Homma, K. J., and Poo, M. M. (2004). Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron*, 44(5), 749–757.
- Zhu, S., and Paoletti, P.** (2015). Allosteric modulators of NMDA receptors: multiple sites and mechanisms. *Current Opinion in Pharmacology*, 20, 14–23.

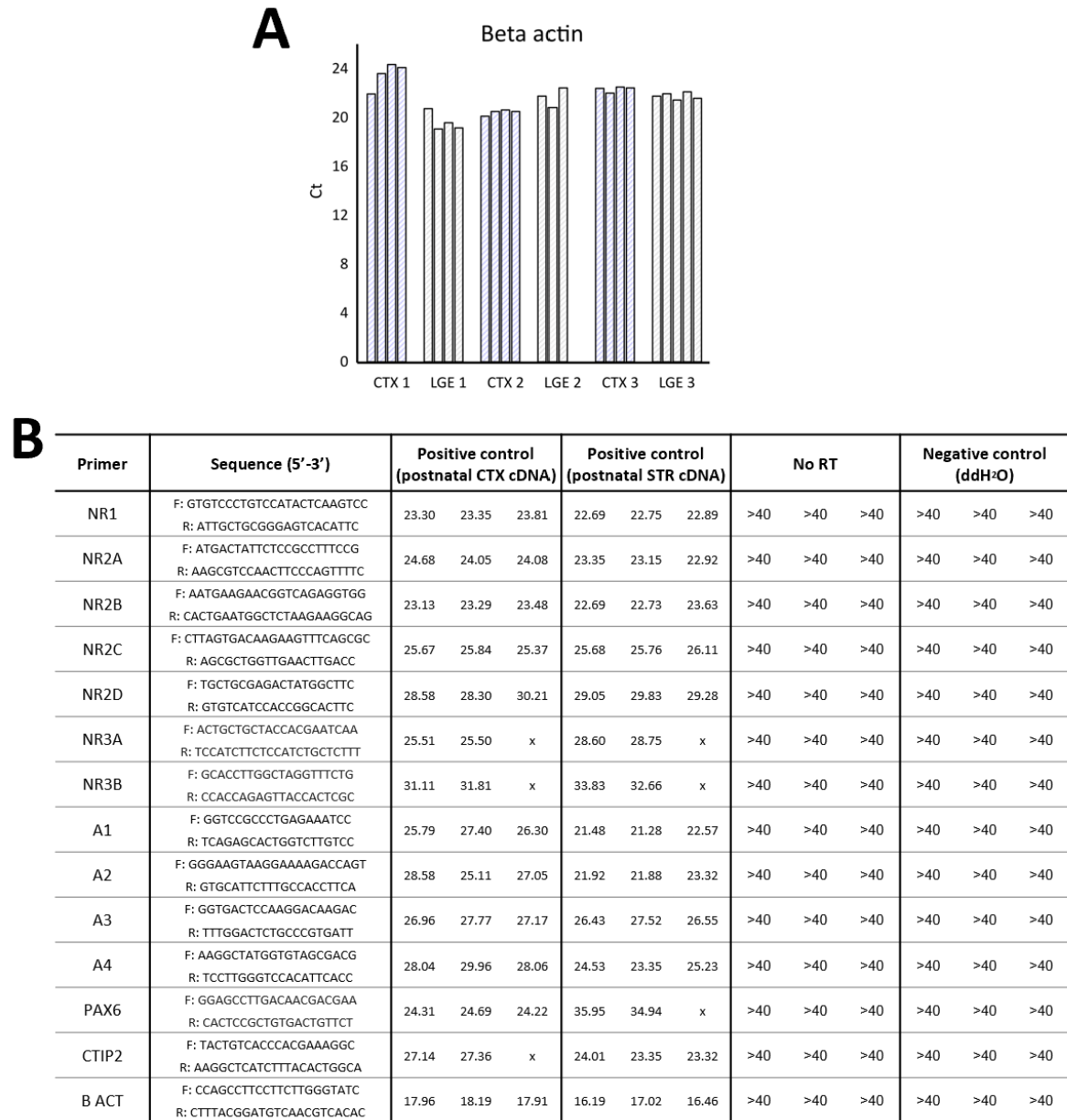
Zlokovic, B. V., Skundric, D. S., Segal, M. B., Lipovac, M. N., Mackic J. B., and Davson, H. (1990). A saturable mechanism for transport of immunoglobulin G across the blood brain barrier of the guinea pig. *Experimental Neurology*, 107(3): 263-270.

Zorumski, C. F., and Izumi, Y. (2012). NMDA receptors and metaplasticity: mechanisms and

possible roles in neuropsychiatric disorders. *Neuroscience and Biobehavioral Reviews*, 36(3), 989–1000.

Zusso, M., Methot, L., Lo, R., Greenhalgh, A. D., David, S., and Stifani, S. (2012). Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor Runx1. *The Journal of Neuroscience*, 32(33), 11285–11298.

9. Appendix



Supplementary Figure S2.1. Supporting validation data and controls for custom-designed primers. [A] Bar plot of Ct values of beta (β) actin (house-keeping gene) from all experiments from all cortex and LGE samples used in qPCR, showing similar values between experiments. The consistency of these results confirms the quality of the cDNA used. The slight variation is accounted for when quantifying and comparing results by normalising to the respective β -actin value in each experiment. [B] Table of all custom-designed primers used and their respective sequences. All primers were verified with RT-qPCR, using postnatal adult cortical and striatal cDNA as a positive control, no RT, and ddH₂O as a negative control.



Sukhvir Wright
To: Anezka Macey-Dare



Mon 22/08/2022 10:06

Hi Anezka
Here is the end point titration for the IgG you have from me

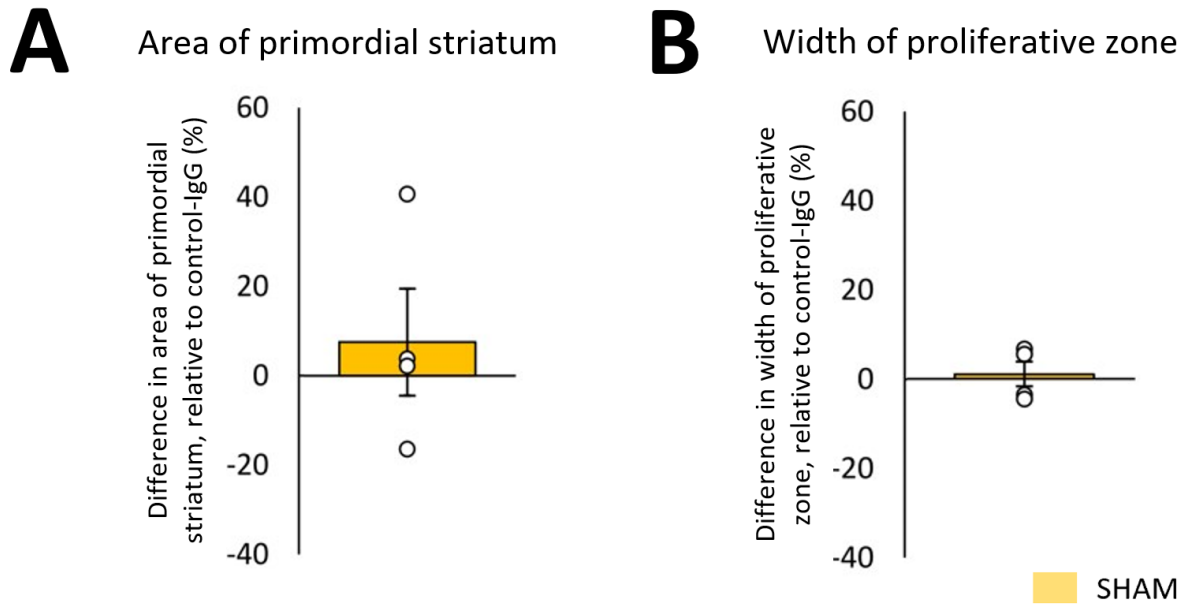
			α H&L	α H&L	α H&L	α H&L	α H&L
			NMDA	NMDA	NMDA	NMDA	NMDA
			20	100	500	2500	12500
22/3/2018	1	NMDA IgG	4	3	1.5	0.5	0
22/3/2018	2	CONTROL	0	0	0	0	0

Concentration is the amount of IgG per ml or microlitre
The titre gives an idea of the avidity of the antibody, so if it's still binding in the CBA at 1 in 100000 for example its very "strong"

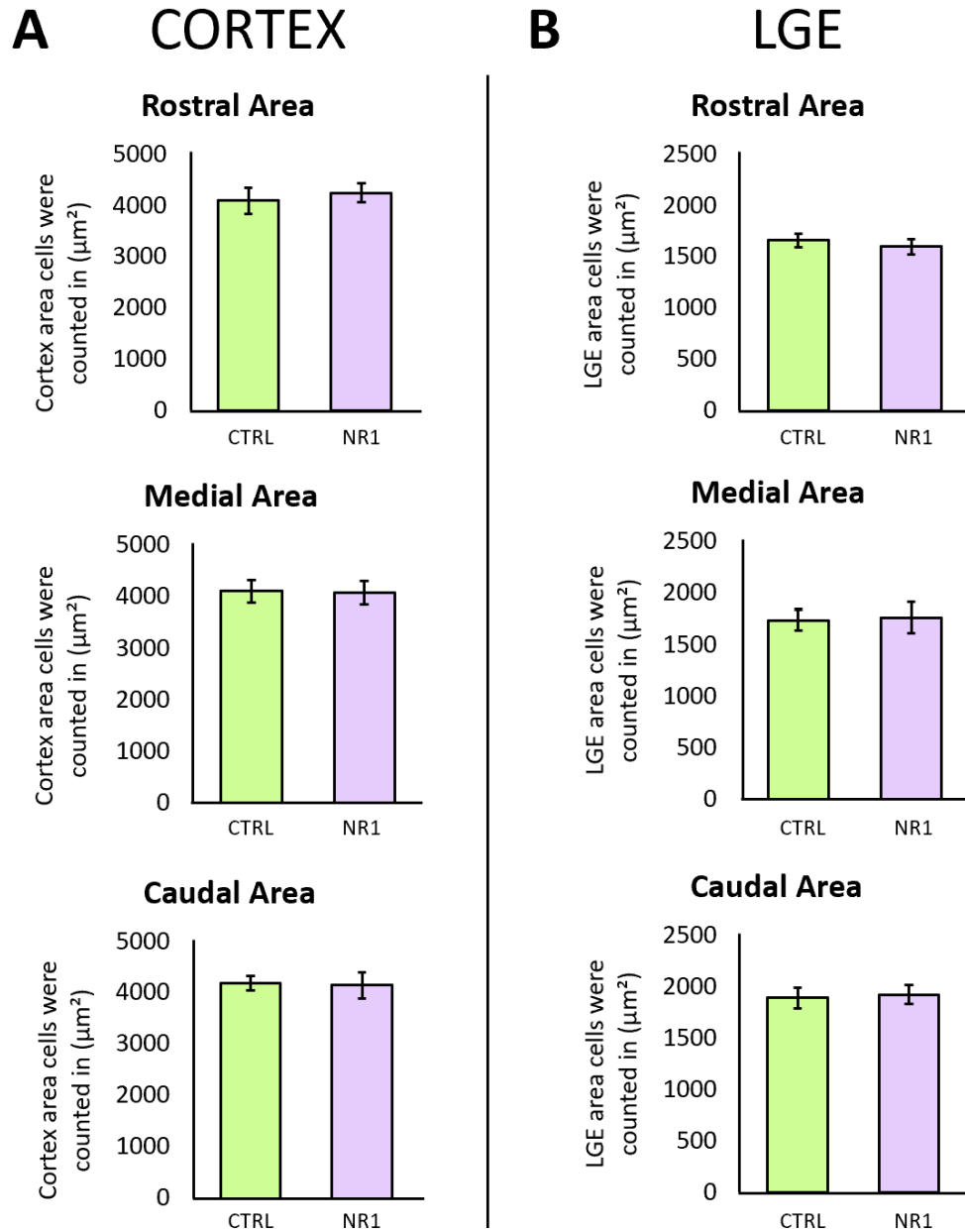
BW
Sukhe

Dr Sukhvir Wright

Supplementary Figure S3.1. Personal correspondence with Dr Sukhvir Wright detailing experimental end point titrations for the NR1-IgG and control-IgG polyclonal antibodies tested on NR1-expressing HEK cells in 2018.

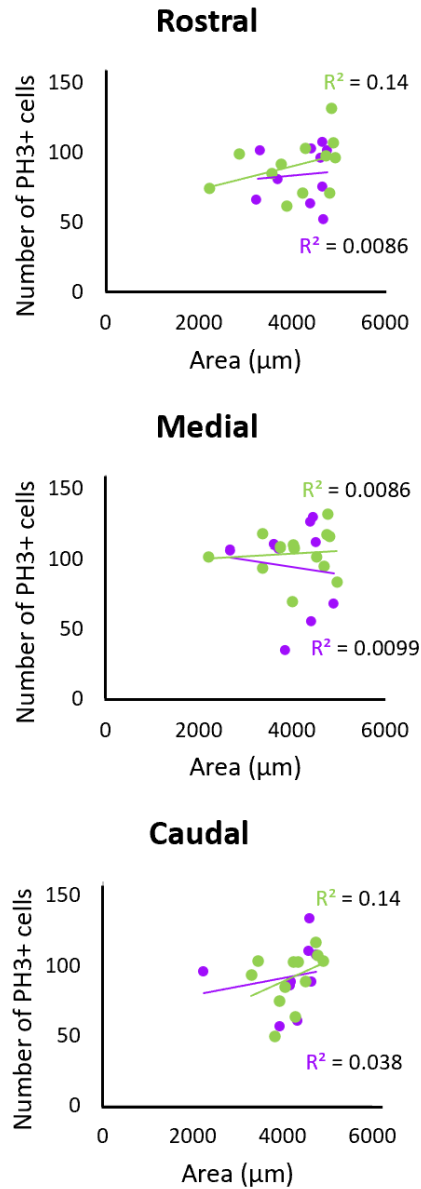


Supplementary Figure S4.1. Supporting control data for morphology-related IHC in Figure 4.2. [A] Bar plot of the difference in primordial striatum size of sham control embryos, relative to control-IgG treated embryos (orange). $n(\text{sham}) = 4$ brains, 1 litter. The size of the primordial striatum in sham control embryos showed no significant difference compared to control-IgG embryos (one-sample t -test: $p = 0.57$). **[B]** Bar plot of the difference in width of the proliferative zone of sham control embryos, relative to control-IgG embryos (orange). Proliferative zone width in sham control embryos showed no significant difference compared to control-IgG embryos (one sample t -test: $p = 0.75$).

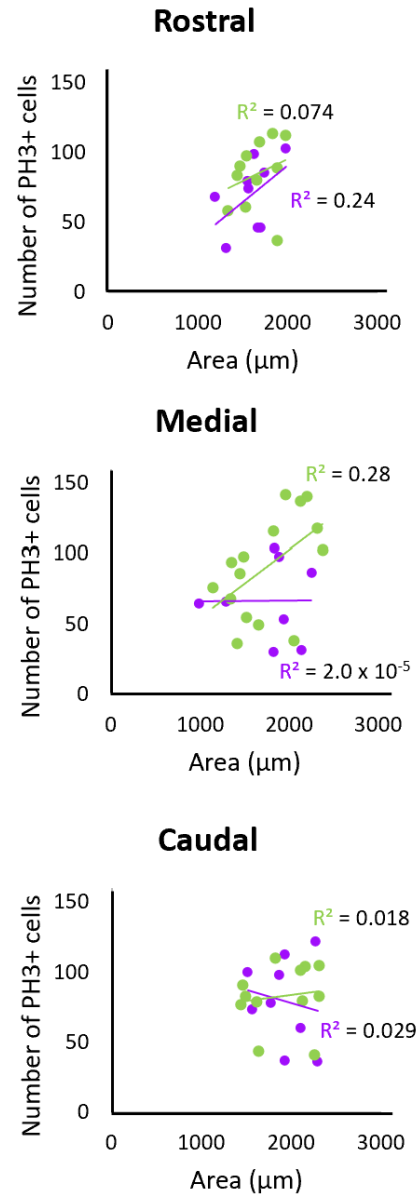


Supplementary Figure S4.2. No significant difference in the areas that PH3+ cells (Figure 4.3) were counted in. Since the numbers of PH3+ cells (rather than density) were being compared, it was important to control for the area cells were counted in. Bar plots showing there were no significant differences in the area that PH3+ cells were counted in for either **[A]** the cortex or **[B]** the LGE, for any section level (rostral, medial, caudal). *p*-values given in main **Figure 4.3**.

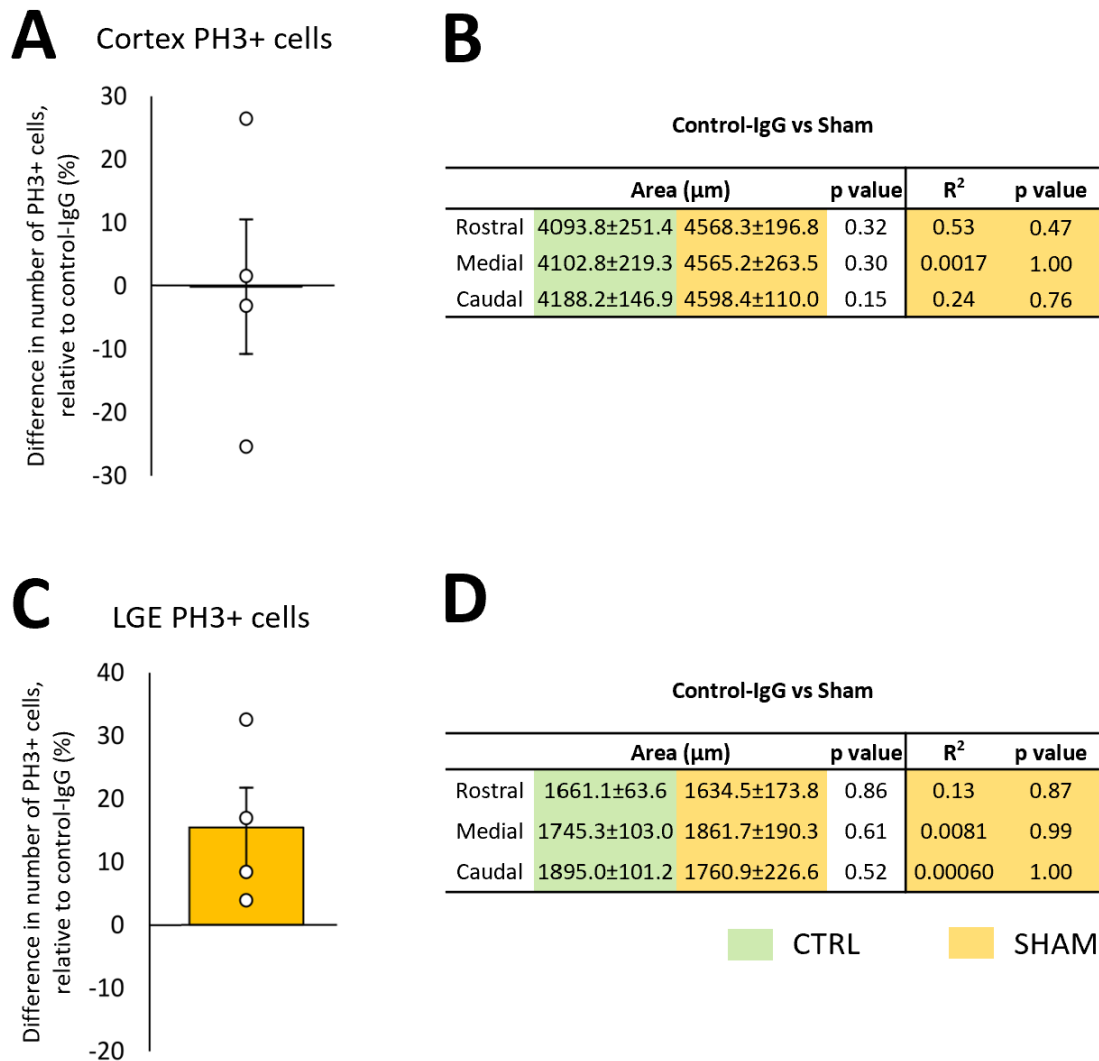
A CORTEX



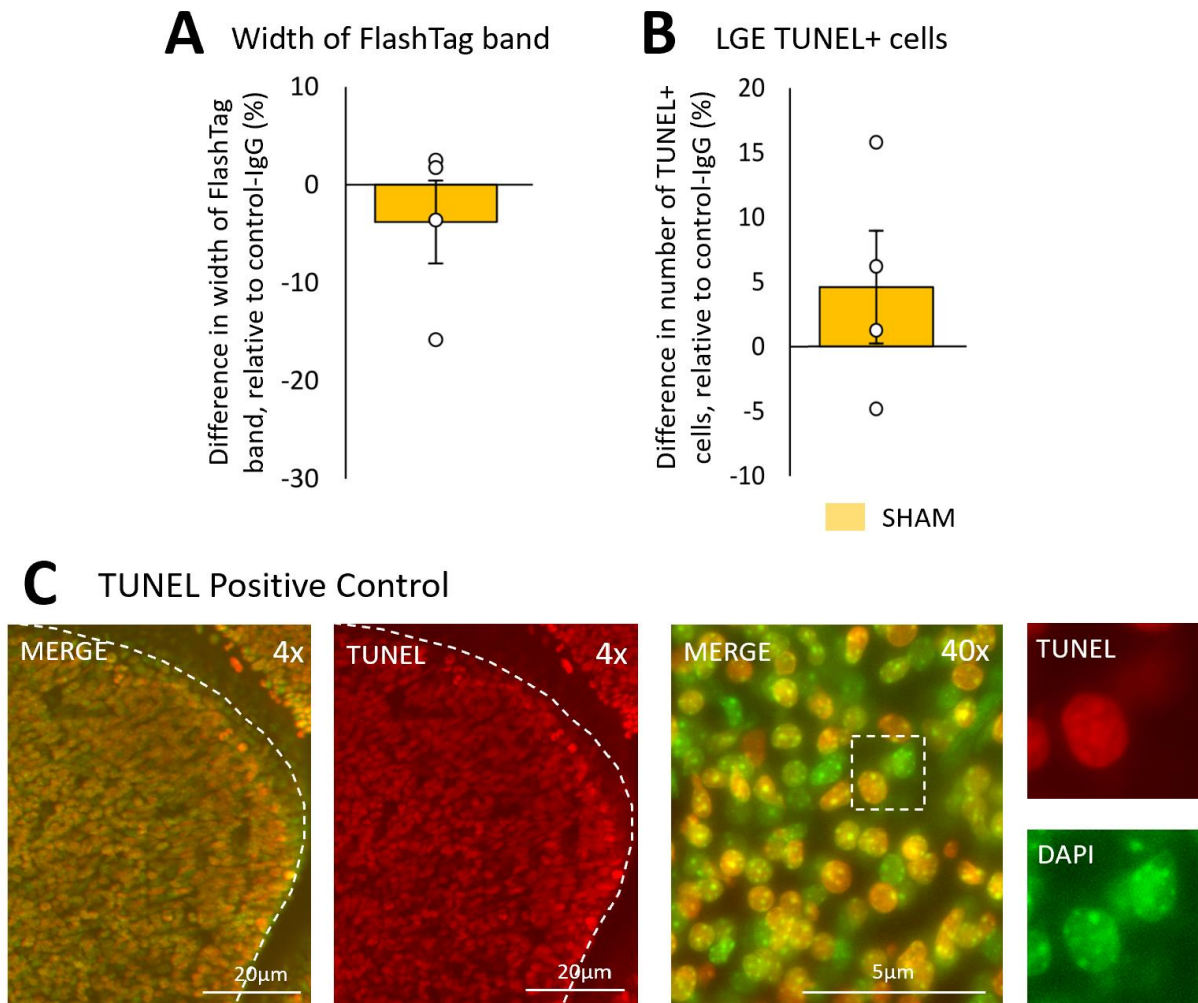
B LGE



Supplementary Figure S4.3. No significant correlations between area that PH3+ cells (Figure 4.3) were counted in and number of PH3+ cells. Graphs showing there were also no significant correlations between the area that PH3+ cells were counted in and number of PH3+ cells counted for either [A] the cortex or [B] the LGE, for any section level (rostral, medial, caudal). p-values given in main Figure 4.3.



Supplementary Figure S4.4. Supporting sham control data for progenitor proliferation-related IHC results (Figure 4.3). **[A]** Bar plot of the difference in PH3+ cell numbers in the cortex of sham control embryos, relative to control-IgG embryos (orange). PH3+ cell numbers in the cortex of sham control embryos showed no significant difference compared to control-IgG embryos (one-sample t-test: $p=0.99$). **[B]** Since the number of PH3+ cells, rather than density, was compared, it was important to control for area. The table states the mean cortical area PH3+ cells were counted in for control-IgG embryos (green) and sham control embryos (orange). There was no significant difference in the area PH3+ cells were counted in for any section type between the groups, and no strong correlations between number of PH3+ cells and area for sham control embryos. **[C]** Bar plot of the difference in PH3+ cell numbers in the cortex of sham control embryos, relative to control-IgG embryos (orange). PH3+ cell numbers in the LGE of sham control embryos showed no significant difference compared to control-IgG embryos (one-sample t-test: $p = 0.090$) (though a mild trend was observed). **[D]** Table of mean LGE area PH3+ cells were counted in in control-IgG embryos (green) and sham control embryos (orange). There was no significant difference in the area PH3+ cells were counted in for any section type between the groups, and no strong correlations between number of PH3+ cells and area for sham control embryos.



Supplementary Figure S4.5. Supporting sham control data for FlashTag and TUNEL assay results (Figures 4.7, 4.8). **[A]** Bar plot of the difference in FlashTag band width in sham control embryos, relative to control-IgG embryos (orange). The width of the FlashTag band in sham control embryos showed no significant difference compared to control-IgG embryos (one sample t-test: $p = 0.43$). **[B]** Bar plot of the difference in the number of apoptotic TUNEL+ cells in sham control embryos, relative to control-IgG embryos (orange). The number of apoptotic TUNEL+ cells in sham control embryos showed no significant difference compared to control-IgG embryos (one-sample t-test: $p=0.37$). **[C]** Representative epifluorescence images of the TUNEL assay positive control, using ezDNase enzyme (Thermo Fisher Scientific) to induce DNA strand breaks and apoptosis. High numbers of TUNEL+ cells confirm this assay worked.

	E16.5 microglia							
	Ctrl-IgG			NR1-IgG			NR1 % change relative to control	p value
Total number of branches	29.42	±	2.77	24.44	±	1.95	-12.95 ± 5.96	0.037
Number of primary branches	4.67	±	0.27	4.00	±	0.24	-12.38 ± 4.93	0.017
Number of end points	15.17	±	1.25	13.03	±	0.97	-11.31 ± 5.59	0.051
Skeleton length (μm)	202.96	±	16.84	177.46	±	11.67	-8.95 ± 5.61	0.12
Soma width (μm)	16.28	±	0.75	16.99	±	0.68	5.35 ± 4.27	0.53
Soma area (μm ²)	126.33	±	9.31	119.62	±	7.10	-4.11 ± 5.61	0.47
Soma perimeter (μm)	44.72	±	1.92	43.55	±	1.33	-2.17 ± 2.99	0.47
Soma circularity	0.79	±	0.01	0.77	±	0.01	-2.51 ± 1.53	0.22
Fractal dimension (D_B)	1.37	±	0.01	1.34	±	0.01	-1.86 ± 0.78	0.022
Lacunarity (λ)	0.31	±	0.01	0.33	±	0.01	5.82 ± 1.76	0.0020
Cell area (μm ²)	182.82	±	14.69	173.52	±	15.10	-2.04 ± 8.18	0.32
Hull area (μm ²)	1452.71	±	125.51	1357.82	±	114.19	-2.96 ± 8.46	0.73
Density (cell area/hull area)	0.39	±	0.02	0.37	±	0.02	-4.51 ± 5.54	0.033
Cell perimeter (μm)	761.05	±	53.38	659.74	±	43.02	-9.20 ± 6.18	0.15
Hull perimeter (μm)	151.52	±	7.01	146.60	±	5.97	-1.49 ± 4.04	0.71
Roughness (cell per./hull per.)	4.95	±	0.21	4.46	±	0.19	-7.61 ± 3.92	0.060
Hull span ratio	1.93	±	0.10	1.86	±	0.12	-3.55 ± 6.38	0.14
Cell circularity	0.28	±	0.01	0.27	±	0.02	-2.95 ± 6.59	0.037
Hull circularity	0.75	±	0.02	0.74	±	0.02	0.09 ± 2.41	0.26
Diameter of bounding circle (μm)	61.26	±	2.83	58.42	±	2.28	-2.59 ± 3.83	0.50
Maximum span across hull (μm)	60.84	±	2.84	58.11	±	2.28	-2.29 ± 3.87	0.56
Ratio of the max/min hull radii	5.69	±	0.79	6.72	±	0.95	30.34 ± 18.95	0.57
Mean radius (μm)	36.74	±	1.45	36.40	±	1.42	-0.89 ± 3.87	0.82

Supplementary Table S4.6. Embryonic microglial morphological parameters (Figure 4.11). There are significant differences in total number of branches, number of primary branches, fractal dimension, lacunarity, density and cell circularity in NR1-IgG exposed LGE microglia, all indicative of a more activated morphological state and/or maturational differences.

	p7-14						
	Ctrl-IgG			NR1-IgG			p value
Resting membrane potential (mV)	-67.0	±	0.5	-64.9	±	0.5	0.0010
Input resistance (MΩ)	409.6	±	35.2	603.4	±	61.2	0.012
Membrane time constant (ms)	15.9	±	1.4	22.3	±	2.0	0.026
Spike threshold (mV)	-40.7	±	0.8	-35.9	±	0.9	6.4 x 10 ⁻⁵
Rheobase (pA)	61.1	±	4.5	55.1	±	2.5	0.56
Time to first spike (ms)	922.4	±	15.3	930.3	±	13.0	0.63
First ISI (ms)	55.6	±	4.2	50.7	±	2.9	0.66
Second ISI (ms)	51.8	±	3.8	46.7	±	2.4	0.59
Third ISI (ms)	52.7	±	4.0	46.5	±	3.2	0.31
Fourth ISI (ms)	49.3	±	3.1	49.4	±	2.6	0.81
First spike amplitude (mV)	73.2	±	2.5	65.8	±	2.3	0.030
Second spike amplitude (mV)	65.6	±	2.5	56.9	±	2.3	0.013
First spike duration (ms)	2.9	±	0.2	3.0	±	0.2	0.62
Second spike duration (ms)	3.7	±	0.3	4.4	±	0.4	0.24
ANOVA							
Spike rate (100 pA) (Hz) #	19.5	±	1.4	21.1	±	1.2	F(1,94) = 0.13, p = 0.72
Spike rate (80 pA) (Hz) #	16.5	±	1.4	17.9	±	1.2	
Spike rate (60 pA) (Hz) #	11.5	±	1.4	11.6	±	1.1	
Spike rate (40 pA) (Hz)	5.5	±	0.9	5.4	±	0.8	
Spike rate (20 pA) (Hz)	0.9	±	0.3	0.8	±	0.3	
Voltage (-100pA) (mV) #	-41.9	±	4.0	-44.2	±	3.4	F(1,94) = 0.11, p = 0.75
Voltage (-80pA) (mV) #	-34.6	±	2.9	-36.2	±	2.3	
Voltage (-60pA) (mV) #	-28.3	±	2.2	-30.1	±	1.9	
Voltage (-40pA) (mV)	-22.3	±	1.7	-27.5	±	2.2	
Voltage (-20pA) (mV)	-12.7	±	1.1	-16.2	±	1.3	
Voltage (0pA) (mV)	0.0	±	0.1	-0.1	±	0.1	
Voltage (20pA) (mV)	12.7	±	1.0	13.8	±	0.8	
Voltage (40pA) (mV)	19.7	±	1.2	21.6	±	1.1	
Voltage (60pA) (mV) #	25.4	±	1.3	26.4	±	1.2	
Voltage (80pA) (mV) #	30.9	±	1.6	32.0	±	1.5	
Voltage (100pA) (mV) #	35.9	±	2.0	37.2	±	1.8	

Supplementary Table S5.1. Intrinsic properties of SPNs recorded from the p7-14 striatum (Figure 5.1). Table comparing intrinsic properties of SPNs from animals injected with NR1-IgG or control-IgG in utero showing significant differences in key intrinsic properties including resting membrane potential, input resistance, membrane time constant, spike threshold, first and second spike amplitude. All data was first assessed for normality and then significance assessed using either ANOVA, independent t-test (parametric data) or Mann-Whitney-U (MWU) test (non-parametric data), with Bonferroni post hoc corrections where appropriate. # = only includes data from p8+.

A

A

	p7-8						
	Ctrl-IgG			NR1-IgG		p value	
Resting membrane potential (mV)	-64.5	±	1.5	-62.9	±	1.0	0.38
Input resistance (MΩ)	616.3	±	147.8	1217.6	±	195.6	0.026
Membrane time constant (ms)	23.8	±	5.8	43.4	±	6.0	0.032
Spike threshold (mV)	-33.3	±	2.1	-30.4	±	1.3	0.27
Rheobase (pA)	50.0	±	6.3	52.9	±	5.4	0.84
Time to first spike (ms)	949.8	±	31.0	971.5	±	25.3	0.44
First ISI (ms)	56.2	±	11.3	55.7	±	4.4	0.97
Second ISI (ms)	58.5	±	13.6	61.0	±	5.1	0.96
Third ISI (ms)	53.2	±	13.6	62.1	±	13.8	0.65
Fourth ISI (ms)	34.6	±	2.4	57.5	±	8.9	0.038
First spike amplitude (mV)	67.9	±	8.3	56.7	±	3.2	0.25
Second spike amplitude (mV)	59.3	±	9.0	45.0	±	4.9	0.20
First spike duration (ms)	4.5	±	1.4	4.6	±	0.5	0.34
Second spike duration (ms)	5.6	±	1.6	7.8	±	1.1	0.21
ANOVA							
Spike rate (100 pA) (Hz) #	25.0	±	4.7	13.5	±	4.3	F(1,7) = 3.90, p = 0.089
Spike rate (80 pA) (Hz) #	22.5	±	5.1	11.5	±	3.0	
Spike rate (60 pA) (Hz) #	15.9	±	5.3	9.0	±	2.0	
Spike rate (40 pA) (Hz)	7.3	±	1.8	7.3	±	2.1	
Spike rate (20 pA) (Hz)	0.0	±	0.0	1.8	±	0.9	
Voltage (-100pA) (mV) #	-38.9	±	3.1	-81.1	±	19.9	F (1,7) = 4.73, p = 0.066
Voltage (-80pA) (mV) #	-34.0	±	3.3	-59.4	±	12.3	
Voltage (-60pA) (mV) #	-29.1	±	3.2	-50.5	±	8.5	
Voltage (-40pA) (mV)	-31.0	±	5.4	-49.9	±	6.1	
Voltage (-20pA) (mV)	-18.3	±	3.2	-28.6	±	3.0	
Voltage (0pA) (mV)	-0.4	±	0.2	-0.2	±	0.1	
Voltage (20pA) (mV)	18.4	±	1.9	16.5	±	1.2	
Voltage (40pA) (mV)	27.7	±	4.2	27.6	±	2.4	
Voltage (60pA) (mV) #	29.8	±	1.7	31.9	±	2.5	
Voltage (80pA) (mV) #	33.3	±	1.3	41.7	±	4.7	
Voltage (100pA) (mV) #	38.6	±	1.7	51.0	±	6.8	

= only includes data from p8+

B

B

	p9-10						
	Ctrl-IgG			NR1-IgG		p value	
Resting membrane potential (mV)	-66.7	±	1.0	-64.9	±	1.3	0.16
Input resistance (MΩ)	435.4	±	35.5	593.1	±	67.2	0.025
Membrane time constant (ms)	18.6	±	1.7	18.2	±	3.0	0.26
Spike threshold (mV)	-42.6	±	0.9	-34.7	±	1.9	0.0010
Rheobase (pA)	51.1	±	5.4	56.2	±	5.4	0.51
Time to first spike (ms)	887.9	±	24.2	923.6	±	30.5	0.37
First ISI (ms)	46.8	±	6.5	43.4	±	7.0	0.95
Second ISI (ms)	46.6	±	6.2	39.9	±	4.1	0.95
Third ISI (ms)	49.1	±	6.5	38.2	±	3.3	0.35
Fourth ISI (ms)	44.2	±	3.7	45.2	±	4.6	0.87
First spike amplitude (mV)	72.6	±	3.7	56.4	±	5.4	0.022
Second spike amplitude (mV)	65.6	±	3.9	53.0	±	4.8	0.051
First spike duration (ms)	2.1	±	0.1	3.1	±	0.7	0.13
Second spike duration (ms)	3.0	±	0.3	4.1	±	0.6	0.060
ANOVA							
Spike rate (100 pA) (Hz)	21.2	±	2.2	24.9	±	2.1	F (1,29) = 0.00021, p = 0.99
Spike rate (80 pA) (Hz)	18.8	±	2.1	20.0	±	2.4	
Spike rate (60 pA) (Hz)	14.3	±	2.0	12.4	±	2.6	
Spike rate (40 pA) (Hz)	7.8	±	1.6	6.0	±	2.0	
Spike rate (20 pA) (Hz)	1.9	±	0.7	0.6	±	0.4	
Voltage (-100pA) (mV)	-49.4	±	6.8	-52.7	±	7.3	F (1,29) = 2.56, p = 0.12
Voltage (-80pA) (mV)	-41.8	±	5.1	-42.5	±	4.8	
Voltage (-60pA) (mV)	-34.8	±	3.8	-34.6	±	3.9	
Voltage (-40pA) (mV)	-26.3	±	2.4	-26.5	±	3.1	
Voltage (-20pA) (mV)	-15.0	±	1.6	-16.0	±	2.4	
Voltage (0pA) (mV)	0.2	±	0.2	0.4	±	0.2	
Voltage (20pA) (mV)	12.8	±	1.5	16.2	±	2.4	
Voltage (40pA) (mV)	17.9	±	1.8	24.7	±	2.4	
Voltage (60pA) (mV)	22.2	±	2.2	32.2	±	2.7	
Voltage (80pA) (mV)	27.2	±	2.7	36.4	±	3.0	
Voltage (100pA) (mV)	31.3	±	3.1	41.8	±	3.5	

C

C

	p11-12						
	Ctrl-IgG			NR1-IgG			p value
Resting membrane potential (mV)	-68.3	±	0.4	-66.8	±	0.4	0.018
Input resistance (MΩ)	342.2	±	89.3	446.9	±	39.5	0.0070
Membrane time constant (ms)	11.1	±	1.3	15.6	±	1.5	0.039
Spike threshold (mV)	-42.6	±	1.2	-36.3	±	1.5	0.0040
Rheobase (pA)	65.6	±	6.7	55.9	±	3.7	0.22
Time to first spike (ms)	948.1	±	24.6	933.8	±	22.0	0.82
First ISI (ms)	56.5	±	8.0	53.9	±	5.8	0.71
Second ISI (ms)	50.2	±	6.4	43.4	±	3.5	0.26
Third ISI (ms)	49.6	±	5.2	44.9	±	4.0	0.24
Fourth ISI (ms)	54.6	±	5.5	45.5	±	3.0	0.17
First spike amplitude (mV)	82.4	±	4.4	67.8	±	3.6	0.020
Second spike amplitude (mV)	74.6	±	4.6	60.6	±	3.6	0.028
First spike duration (ms)	2.8	±	0.2	2.6	±	0.1	0.57
Second spike duration (ms)	3.5	±	0.3	3.7	±	0.3	0.92
ANOVA							
Spike rate (100 pA) (Hz)	18.6	±	2.6	20.7	±	1.9	F (1,24) = 0.81, p = 0.38
Spike rate (80 pA) (Hz)	15.0	±	2.6	18.0	±	1.9	
Spike rate (60 pA) (Hz)	9.4	±	2.5	11.9	±	1.8	
Spike rate (40 pA) (Hz)	2.8	±	1.4	3.5	±	1.3	
Spike rate (20 pA) (Hz)	0.0	±	0.0	0.0	±	0.0	F (1,24) = 0.38, p = 0.55
Voltage (-100pA) (mV)	-41.8	±	10.4	-39.4	±	3.5	
Voltage (-80pA) (mV)	-30.9	±	5.9	-33.7	±	2.8	
Voltage (-60pA) (mV)	-24.2	±	4.2	-28.0	±	2.3	
Voltage (-40pA) (mV)	-17.6	±	3.1	-21.5	±	1.9	
Voltage (-20pA) (mV)	-9.7	±	1.7	-13.0	±	1.3	
Voltage (0pA) (mV)	-0.1	±	0.1	-0.1	±	0.1	
Voltage (20pA) (mV)	10.6	±	1.2	13.7	±	0.8	
Voltage (40pA) (mV)	17.9	±	1.9	21.9	±	0.9	
Voltage (60pA) (mV)	24.9	±	2.9	28.6	±	1.1	
Voltage (80pA) (mV)	29.8	±	3.2	35.0	±	1.3	
Voltage (100pA) (mV)	34.3	±	3.9	40.6	±	1.9	

D	p13-14						
	Ctrl-IgG			NR1-IgG		p value	
Resting membrane potential (mV)	-67.7	±	0.5	-64.8	±	1.0	0.015
Input resistance (MΩ)	306.0	±	48.8	330.2	±	31.5	0.61
Membrane time constant (ms)	10.3	±	1.9	16.7	±	1.4	0.0070
Spike threshold (mV)	-40.3	±	1.6	-40.3	±	1.2	0.98
Rheobase (pA)	80.0	±	12.8	55.3	±	5.5	0.12
Time to first spike (ms)	946.4	±	39.1	900.1	±	25.4	0.34
First ISI (ms)	70.3	±	8.3	49.1	±	5.8	0.051
Second ISI (ms)	59.5	±	6.7	45.4	±	5.0	0.11
Third ISI (ms)	62.6	±	9.7	45.5	±	4.7	0.14
Fourth ISI (ms)	59.9	±	8.8	52.5	±	5.3	0.45
First spike amplitude (mV)	69.3	±	4.9	76.8	±	3.8	0.24
Second spike amplitude (mV)	61.4	±	4.6	64.3	±	4.3	0.65
First spike duration (ms)	3.2	±	0.3	2.2	±	0.1	0.0080
Second spike duration (ms)	4.0	±	0.4	2.8	±	0.2	0.012
ANOVA							
Spike rate (100 pA) (Hz)	15.6	±	2.8	20.9	±	2.3	F (1,28) = 2.92, p = 0.098
Spike rate (80 pA) (Hz)	11.7	±	2.6	18.0	±	2.4	
Spike rate (60 pA) (Hz)	6.9	±	2.3	11.5	±	2.0	
Spike rate (40 pA) (Hz)	3.2	±	1.7	5.4	±	1.4	
Spike rate (20 pA) (Hz)	0.2	±	0.2	1.1	±	0.6	
Voltage (-100pA) (mV)	-30.6	±	4.9	-33.0	±	3.2	F (1,28) = 15.02, p = 0.00059
Voltage (-80pA) (mV)	-25.9	±	4.2	-28.0	±	2.6	
Voltage (-60pA) (mV)	-20.5	±	3.3	-23.6	±	2.3	
Voltage (-40pA) (mV)	-14.9	±	2.4	-18.3	±	2.0	
Voltage (-20pA) (mV)	-8.3	±	1.4	-10.6	±	1.3	
Voltage (0pA) (mV)	-0.1	±	0.1	-0.4	±	0.4	
Voltage (20pA) (mV)	11.0	±	2.2	10.3	±	1.3	
Voltage (40pA) (mV)	19.7	±	2.2	14.9	±	1.6	
Voltage (60pA) (mV)	29.6	±	2.3	19.1	±	1.8	
Voltage (80pA) (mV)	37.2	±	2.5	23.7	±	2.2	
Voltage (100pA) (mV)	43.8	±	3.6	27.5	±	2.5	

Supplementary Table S5.2. Intrinsic properties of SPNs recorded from the [A] p7-8, [B] p9-10, [C] p11-12 and [D] p13-14 striatum (Figure 5.4). All data was first assessed for normality and then significance assessed using either ANOVA, independent t-test (parametric data) or Mann-Whitney-U (MWU) test (non-parametric data), with Bonferroni post hoc corrections where appropriate.

Intrinsic properties and morphology

A

TOTAL NUMBER OF BRANCHES – CONTROL			
Intrinsic property	R ²	p value	Direction
Resting membrane potential	0.0022	0.99	=
Threshold	0.0085	0.98	=
Input resistance	0.025	0.94	=
Membrane time constant	0.018	0.96	=
First spike amplitude	0.031	0.55	=
Second spike amplitude	8.0 x 10 ⁻⁵	0.98	=

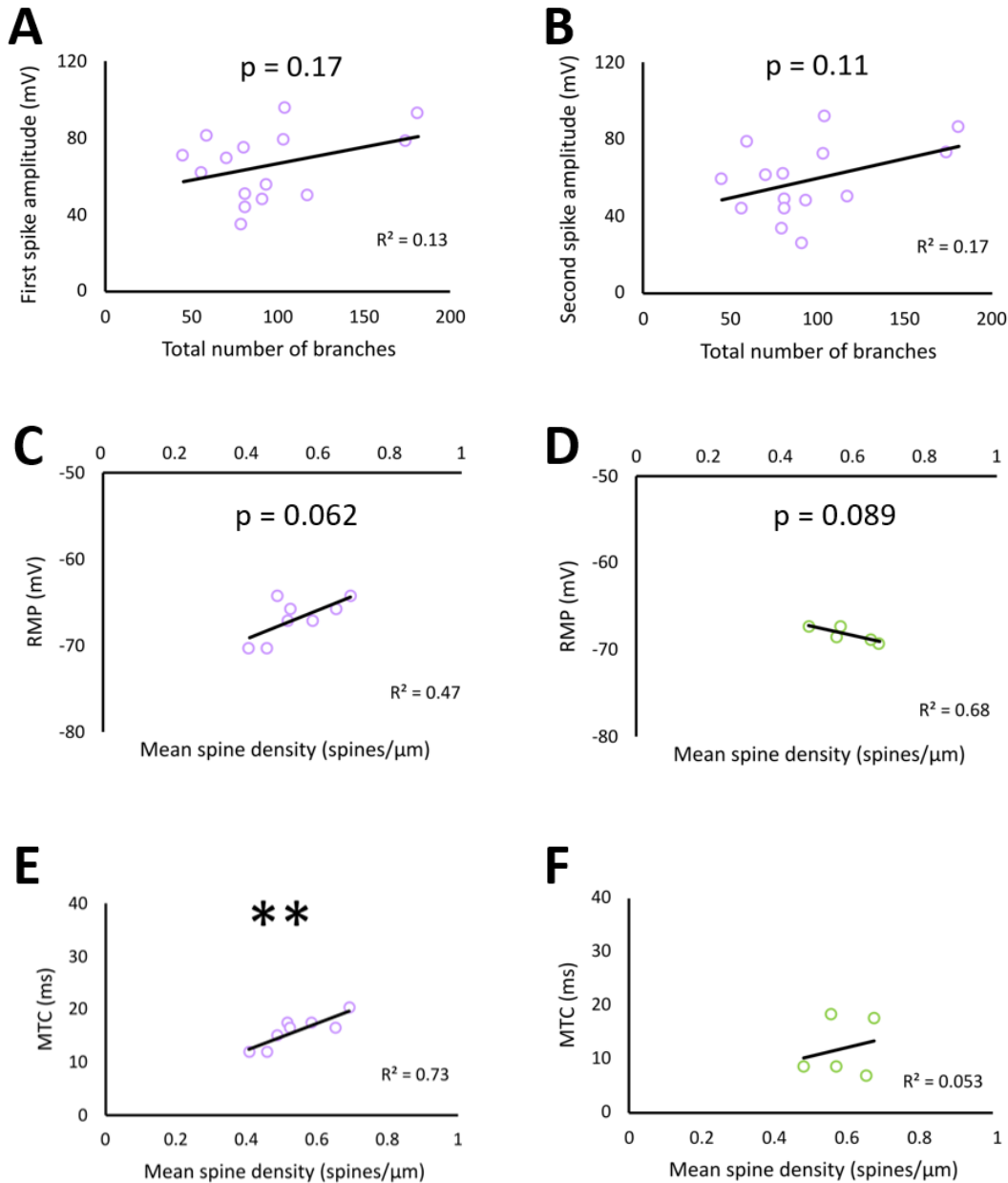
TOTAL NUMBER OF BRANCHES – NR1			
Intrinsic property	R ²	p value	Direction
Resting membrane potential	0.041	0.45	=
Threshold	0.0011	0.98	=
Input resistance	0.012	0.69	=
Membrane time constant	0.0015	0.89	=
First spike amplitude	0.13	0.17	+ (trend)
Second spike amplitude	0.17	0.11	+ (trend)

B

SPINE DENSITY – CONTROL			
Intrinsic property	R ²	p value	Direction
Resting membrane potential	0.68	0.089	- (trend)
Threshold	0.73	0.16	- (trend)
Input resistance	0.012	0.86	=
Membrane time constant	0.053	0.71	=
First spike amplitude	0.14	0.54	=
Second spike amplitude	0.31	0.33	=

SPINE DENSITY – NR1			
Intrinsic property	R ²	p value	Direction
Resting membrane potential	0.47	0.062	+ (trend)
Threshold	0.011	0.98	=
Input resistance	0.042	0.63	=
Membrane time constant	0.73	0.0067	+
First spike amplitude	0.021	0.73	=
Second spike amplitude	0.069	0.53	=

Supplementary Table S5.3. [A-B] Correlations of key intrinsic properties and morphological features.



Supplementary Figure S5.4. Key correlation graphs between intrinsic properties and morphological features. [A-B] Line plots of the relationship between total number of branches and first and second spike amplitude respectively, showed a trend towards a positive correlation in NR1-IgG exposed SPNs. There was no significant correlation in control-IgG SPNs for these characteristics (data in **Supplementary Table S5.3**). [C-D] Line plots showing how the intrinsic property resting membrane potential (RMP) correlated to spine density in NR1-IgG and control-IgG exposed SPNs respectively. In NR1-IgG exposed SPNs there was a trend towards a positive correlation, but in control-IgG exposed SPNs there was a trend towards a negative correlation. [E-F] Line plots showing the relationship between spine density and membrane time constant (MTC) for NR1-IgG and control-IgG exposed SPNs respectively. In NR1-IgG exposed SPNs there was a significant positive correlation whilst in control-IgG exposed SPNs there was no correlation.

Supplementary Table S5.3 and Supplementary Figure S5.4 Summary

Since intrinsic properties have been closely linked to morphology in previous literature (**Hou and Zhang 2017**), this study sought to identify if there was a relationship between the observed intrinsic property alterations and the morphology of the cells. Due to small sample size, only correlations in the full data set (p7-14) were analysed. Correlative analysis revealed no significant relationships between dendritic arbour properties (branch length, number of branches or number of primary branches) and intrinsic properties (resting membrane potential, threshold, input resistance, membrane time constant, first or second spike amplitude) in either the control-IgG cells or the NR1-IgG cells (**Supplementary Table S5.3**), although there were trends towards a significant positive correlation between first and second spike amplitude and total number of branches for NR1-IgG exposed SPNs (NR1-IgG: first spike amplitude vs total number of branches: $R^2 = 0.13$, $p = 0.17$, **Supplementary Figure S5.4 A**; second spike amplitude vs total number of branches: $R^2 = 0.17$, $p = 0.11$, **Supplementary Figure S5.4 B**) (but not control-IgG exposed SPNs) (**Supplementary Table S5.3**).

Of note, there was a trend towards a positive correlation between resting membrane potential and spine density (i.e. resting membrane potential decreases as spine density increases) in NR1-IgG exposed SPNs, but a trend towards a negative correlation in control-IgG exposed SPNs (NR1-IgG: RMP vs mean spine density: $R^2 = 0.46$, $p = 0.062$, **Supplementary Figure S5.4 C**; control-IgG: RMP vs mean spine density: $R^2 = 0.68$, $p = 0.089$, **Supplementary Figure S5.4 D**). There was also a significant positive correlation between membrane time constant and spine density for NR1-IgG exposed SPNs but no correlation in control-IgG exposed SPNs (NR1-IgG: MTC vs mean spine density: $R^2 = 0.73$, $p = 0.0067$, **Supplementary Figure S5.4 E**; control-IgG: MTC vs mean spine density: $R^2 = 0.053$, $p = 0.71$, **Supplementary Figure S5.4 F**).

		p7-14						
		Ctrl-IgG			NR1-IgG		p value	
Gabazine	EPSP amplitude (mV)	8.2	±	0.7	8.9	±	0.7	0.52
	EPSP duration (ms)	295.3	±	14.3	262.1	±	16.8	0.14
	EPSP rise time (ms)	6.7	±	0.3	6.9	±	0.3	0.77
	EPSP decay time (ms)	96.5	±	4.4	101.6	±	6.4	0.52
	Paired-pulse ratio	1.8	±	0.1	2.1	±	0.1	0.071
	ANOVA							
	Short-term plasticity (2 vs 1)	1.06	±	0.05	1.27	±	0.15	F(1,53) = 38.24, p = 9.0 x 10 ⁻⁸
	Short-term plasticity (3 vs 1)	0.91	±	0.06	1.18	±	0.12	
	Short-term plasticity (4 vs 1)	0.91	±	0.08	1.10	±	0.11	
	Short-term plasticity (5 vs 1)	0.85	±	0.08	1.16	±	0.14	
	Short-term plasticity (6 vs 1)	0.80	±	0.08	1.36	±	0.18	
p value								
Gabazine + DAP5	EPSP amplitude (mV)	5.3	±	0.6	6.4	±	0.6	0.09
	EPSP duration (ms)	190.7	±	15.8	176.4	±	14.0	0.57
	EPSP rise time (ms)	6.5	±	0.4	6.4	±	0.3	0.58
	EPSP decay time (ms)	87.0	±	7.1	80.9	±	7.0	0.54
	Normalised EPSP amplitude (%)	62.0	±	3.8	73.8	±	2.8	0.017
	Normalised EPSP duration (%)	65.5	±	4.0	68.2	±	3.8	0.64
	Normalised EPSP rise time (%)	94.8	±	3.6	95.3	±	3.4	0.78
	Normalised EPSP decay time (%)	88.0	±	5.9	79.4	±	5.2	0.28
	Paired-pulse ratio	1.7	±	0.1	1.8	±	0.2	0.89
	ANOVA							
	Short-term plasticity (2 vs 1)	1.01	±	0.05	1.18	±	0.14	F(1,48) = 1.23, p = 0.27
Short-term plasticity (3 vs 1)	0.97	±	0.09	1.11	±	0.11		
Short-term plasticity (4 vs 1)	0.96	±	0.10	1.08	±	0.11		
Short-term plasticity (5 vs 1)	0.88	±	0.09	0.97	±	0.11		
Short-term plasticity (6 vs 1)	0.84	±	0.09	1.03	±	0.12		
p value								
NMDA/AMPA ratio		0.79	±	0.14	0.44	±	0.07	0.022
NMDA component proportion		0.38	±	0.04	0.26	±	0.03	0.018

Supplementary Table S5.5. Corticostriatal excitatory input properties of SPNs recorded from the p7-14 striatum (Figures 5.9, 5.10). All data was first assessed for normality and then significance assessed using either ANOVA, independent *t*-test (parametric data) or Mann-Whitney-U (MWU) test (non-parametric data), with Bonferroni post hoc corrections where appropriate.

A

A

		p7-8						
		Ctrl-IgG			NR1-IgG		p value	
Gabazine	EPSP amplitude (mV)	8.1	±	0.3	6.7	±	1.0	0.16
	EPSP duration (ms)	320.7	±	32.9	299.7	±	39.3	0.69
	EPSP rise time (ms)	7.7	±	0.2	7.5	±	0.1	0.39
	EPSP decay time (ms)	86.2	±	11.7	111.8	±	13.9	0.19
	Paired-pulse ratio	1.5	±	0.0	2.4	±	0.2	0.0030
								ANOVA
	Short-term plasticity (2 vs 1)	0.99	±	0.18	1.63	±	0.41	F(1,11) = 7.40, p = 0.020
	Short-term plasticity (3 vs 1)	0.67	±	0.05	1.34	±	0.26	
	Short-term plasticity (4 vs 1)	0.51	±	0.05	1.28	±	0.22	
	Short-term plasticity (5 vs 1)	0.39	±	0.03	1.18	±	0.21	
	Short-term plasticity (6 vs 1)	0.43	±	0.07	1.82	±	0.38	
							p value	
Gabazine + DAP5	EPSP amplitude (mV)	6.2	±	0.5	5.5	±	1.0	0.35
	EPSP duration (ms)	188.7	±	53.5	209.0	±	29.9	0.79
	EPSP rise time (ms)	7.6	±	0.1	7.0	±	0.3	0.06
	EPSP decay time (ms)	60.9	±	17.6	81.3	±	15.8	0.42
	Normalised EPSP amplitude (%)	77.9	±	2.6	82.7	±	4.6	0.37
	Normalised EPSP duration (%)	61.6	±	12.5	69.9	±	7.6	0.60
	Normalised EPSP rise time (%)	101.0	±	0.4	94.0	±	4.3	0.10
	Normalised EPSP decay time (%)	65.2	±	10.5	70.2	±	8.9	0.78
	Paired-pulse ratio	1.2	±	0.2	1.8	±	0.2	0.0064
								ANOVA
	Short-term plasticity (2 vs 1)	0.83	±	0.18	1.40	±	0.39	F(1,10) = 14.9, p = 0.0030
Short-term plasticity (3 vs 1)	0.36	±	0.05	1.16	±	0.18		
Short-term plasticity (4 vs 1)	0.30	±	0.06	1.34	±	0.22		
Short-term plasticity (5 vs 1)	0.32	±	0.06	1.28	±	0.26		
Short-term plasticity (6 vs 1)	0.23	±	0.08	1.44	±	0.28		
							p value	
NMDA/AMPA ratio		0.29	±	0.04	0.25	±	0.09	0.37
NMDA component proportion		0.22	±	0.03	0.17	±	0.05	0.37

B

		p9-10						
		Ctrl-IgG			NR1-IgG			p value
Gabazine	EPSP amplitude (mV)	7.8	±	1.1	9.4	±	1.5	0.37
	EPSP duration (ms)	300.7	±	19.6	250.2	±	18.5	0.069
	EPSP rise time (ms)	6.3	±	0.7	6.8	±	0.5	0.69
	EPSP decay time (ms)	99.6	±	9.6	112.5	±	10.8	0.39
	Paired-pulse ratio	1.8	±	0.2	1.7	±	0.3	0.32
	ANOVA							
	Short-term plasticity (2 vs 1)	1.22	±	0.09	1.05	±	0.22	F(1,11) = 0.040, p = 0.85
	Short-term plasticity (3 vs 1)	1.02	±	0.10	1.04	±	0.23	
	Short-term plasticity (4 vs 1)	1.16	±	0.20	0.94	±	0.32	
	Short-term plasticity (5 vs 1)	0.99	±	0.16	1.37	±	0.48	
Short-term plasticity (6 vs 1)	0.92	±	0.12	1.26	±	0.46		
p value								
Gabazine + DAP5	EPSP amplitude (mV)	4.9	±	0.9	6.1	±	0.8	0.34
	EPSP duration (ms)	178.9	±	31.2	197.1	±	20.8	0.62
	EPSP rise time (ms)	6.8	±	0.9	6.11	±	0.7	0.55
	EPSP decay time (ms)	88.1	±	18.5	94.3	±	12.7	0.79
	Normalised EPSP amplitude (%)	65.8	±	8.9	68.2	±	5.1	0.82
	Normalised EPSP duration (%)	58.2	±	7.3	77.6	±	3.9	0.042
	Normalised EPSP rise time (%)	101.0	±	5.7	91.8	±	10.5	0.46
	Normalised EPSP decay time (%)	83.2	±	15.8	82.4	±	8.1	0.96
	Paired-pulse ratio	1.9	±	0.2	1.6	±	0.3	0.48
	ANOVA							
	Short-term plasticity (2 vs 1)	1.19	±	0.09	0.99	±	0.18	F(1,10) = 1.48, p = 0.25
	Short-term plasticity (3 vs 1)	1.27	±	0.15	1.13	±	0.29	
	Short-term plasticity (4 vs 1)	1.30	±	0.14	0.93	±	0.21	
	Short-term plasticity (5 vs 1)	1.09	±	0.11	0.82	±	0.15	
	Short-term plasticity (6 vs 1)	1.17	±	0.09	0.76	±	0.17	
p value								
NMDA/AMPA ratio		0.73	±	0.30	0.56	±	0.18	0.96
NMDA component proportion		0.34	±	0.09	0.32	±	0.05	0.82

C

C

		p11-12						
		Ctrl-IgG			NR1-IgG			p value
Gabazine	EPSP amplitude (mV)	7.6	±	1.7	9.2	±	1.1	0.45
	EPSP duration (ms)	259.7	±	34.1	255.8	±	33.2	0.76
	EPSP rise time (ms)	6.6	±	0.6	7.1	±	0.4	0.23
	EPSP decay time (ms)	98.2	±	6.9	93.9	±	11.7	0.75
	Paired-pulse ratio	1.8	±	0.1	1.8	±	0.2	0.81
	ANOVA							
	Short-term plasticity (2 vs 1)	1.03	±	0.06	0.92	±	0.14	F(1,13) = 0.25, p = 0.63
	Short-term plasticity (3 vs 1)	0.98	±	0.15	0.77	±	0.14	
	Short-term plasticity (4 vs 1)	0.79	±	0.12	0.82	±	0.11	
	Short-term plasticity (5 vs 1)	0.87	±	0.12	0.69	±	0.16	
	Short-term plasticity (6 vs 1)	0.66	±	0.16	0.69	±	0.17	
p value								
Gabazine + DAP5	EPSP amplitude (mV)	5.3	±	1.3	6.2	±	1.0	0.56
	EPSP duration (ms)	153.6	±	18.3	146.7	±	30.2	0.90
	EPSP rise time (ms)	6.0	±	0.8	6.4	±	0.7	0.23
	EPSP decay time (ms)	94.2	±	15.8	81.8	±	15.0	0.58
	Normalised EPSP amplitude (%)	60.1	±	3.8	69.6	±	5.3	0.17
	Normalised EPSP duration (%)	64.5	±	7.9	63.0	±	10.7	0.91
	Normalised EPSP rise time (%)	92.7	±	7.0	93.1	±	5.4	0.63
	Normalised EPSP decay time (%)	94.1	±	11.4	89.2	±	10.9	0.76
	Paired-pulse ratio	1.6	±	0.1	1.7	±	0.4	0.46
	ANOVA							
	Short-term plasticity (2 vs 1)	1.06	±	0.10	0.92	±	0.10	F(1,13) = 2.33, p = 0.15
Short-term plasticity (3 vs 1)	1.02	±	0.13	0.89	±	0.15		
Short-term plasticity (4 vs 1)	0.94	±	0.16	0.67	±	0.08		
Short-term plasticity (5 vs 1)	0.85	±	0.19	0.60	±	0.08		
Short-term plasticity (6 vs 1)	0.73	±	0.12	0.73	±	0.16		
p value								
NMDA/AMPA ratio		0.70	±	0.11	0.50	±	0.12	0.25
NMDA component proportion		0.40	±	0.04	0.30	±	0.05	0.18

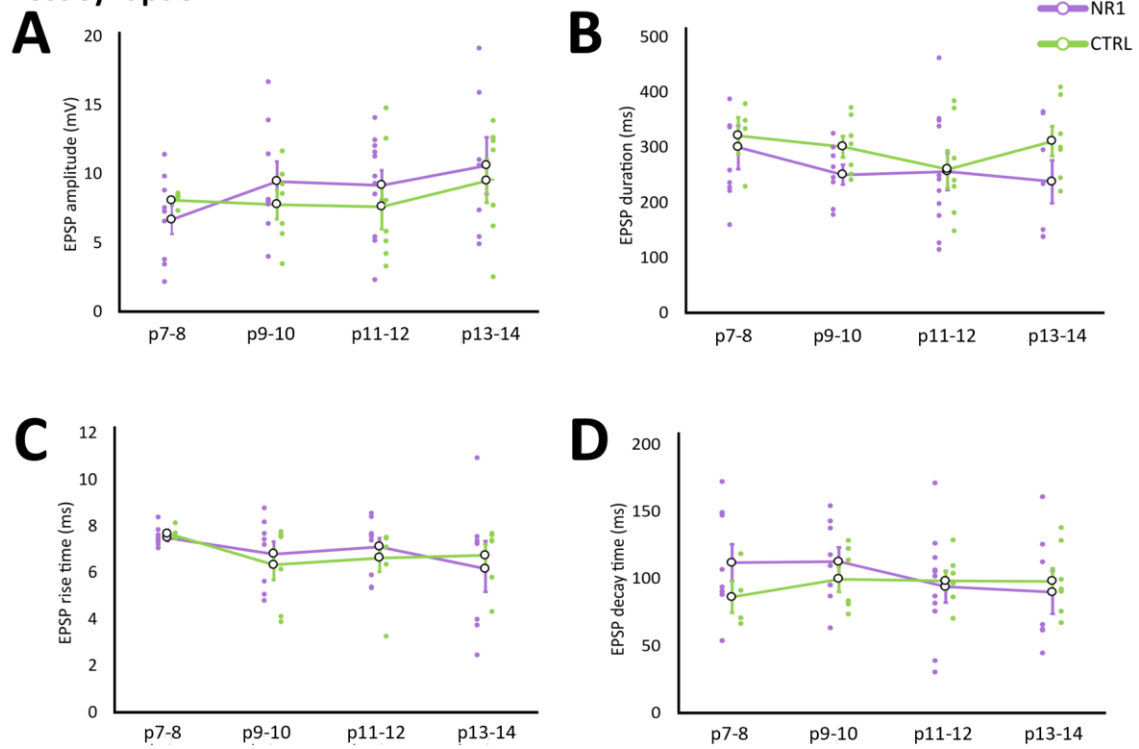
D

D

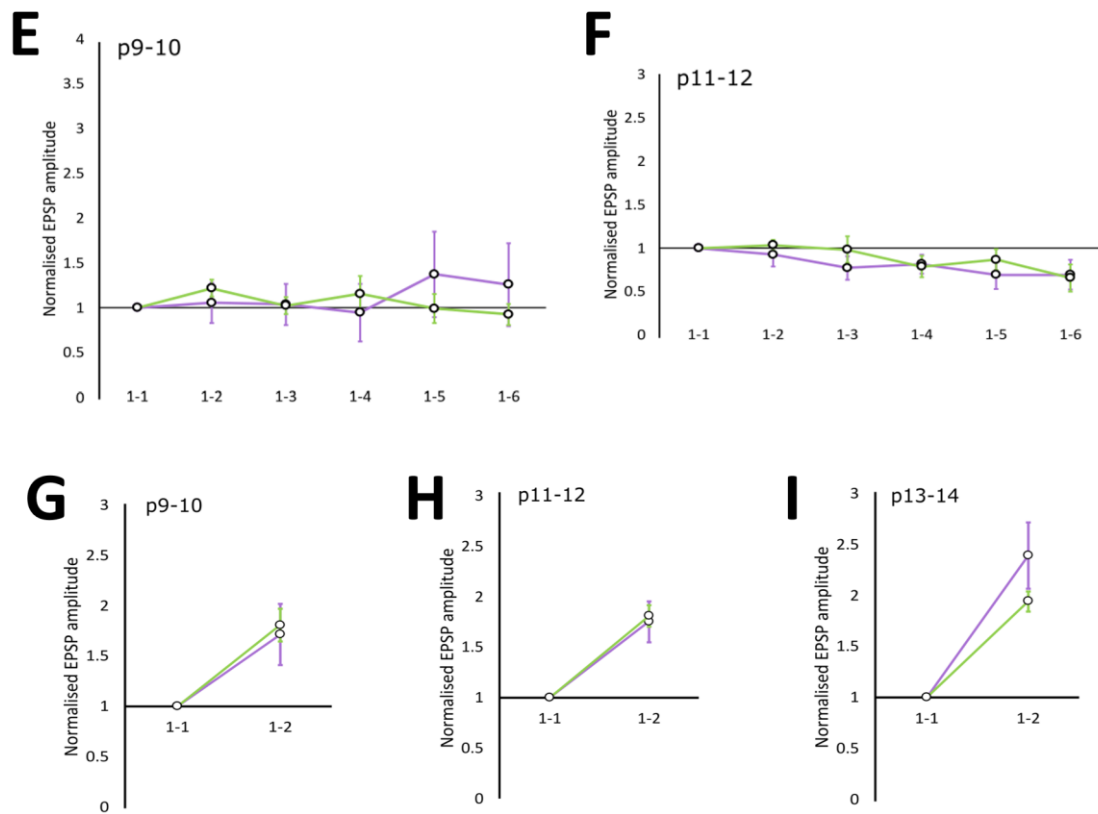
		p13-14						
		Ctrl-IgG			NR1-IgG		p value	
Gabazine	EPSP amplitude (mV)	9.5	±	1.6	10.6	±	2.1	0.68
	EPSP duration (ms)	311.0	±	26.9	237.3	±	38.8	0.50
	EPSP rise time (ms)	6.7	±	0.5	6.2	±	1.2	0.46
	EPSP decay time (ms)	98.0	±	9.7	89.8	±	16.0	0.67
	Paired-pulse ratio	1.9	±	0.1	2.34	±	0.3	0.23
	ANOVA							
	Short-term plasticity (2 vs 1)	0.99	±	0.07	1.52	±	0.23	F(1,11) = 10.81, p = 0.0070
	Short-term plasticity (3 vs 1)	0.88	±	0.08	1.71	±	0.25	
	Short-term plasticity (4 vs 1)	1.05	±	0.10	1.42	±	0.14	
	Short-term plasticity (5 vs 1)	0.98	±	0.15	1.59	±	0.21	
	Short-term plasticity (6 vs 1)	1.01	±	0.17	1.77	±	0.30	
p value								
Gabazine + DAP5	EPSP amplitude (mV)	5.3	±	1.2	7.9	±	1.8	0.24
	EPSP duration (ms)	235.0	±	28.8	145.0	±	25.8	0.040
	EPSP rise time (ms)	6.1	±	0.9	5.9	±	0.9	0.62
	EPSP decay time (ms)	91.2	±	7.1	64.2	±	13.1	0.10
	Normalised EPSP amplitude (%)	52.7	±	6.8	73.6	±	7.5	0.06
	Normalised EPSP duration (%)	75.4	±	6.5	60.9	±	6.1	0.09
	Normalised EPSP rise time (%)	88.5	±	8.1	104.5	±	4.0	0.07
	Normalised EPSP decay time (%)	95.9	±	8.00	75.4	±	14.4	0.24
	Paired-pulse ratio	1.7	±	0.2	1.9	±	0.5	0.65
	ANOVA							
	Short-term plasticity (2 vs 1)	0.93	±	0.06	1.43	±	0.18	F(1,10) = 2.05, p = 0.18
Short-term plasticity (3 vs 1)	0.98	±	0.13	1.33	±	0.28		
Short-term plasticity (4 vs 1)	1.01	±	0.13	1.50	±	0.30		
Short-term plasticity (5 vs 1)	1.00	±	0.13	1.20	±	0.27		
Short-term plasticity (6 vs 1)	0.96	±	0.14	1.14	±	0.24		
p value								
NMDA/AMPA ratio		1.15	±	0.33	0.46	±	0.17	0.13
NMDA component proportion		0.47	±	0.07	0.26	±	0.07	0.06

Supplementary Table S5.6. Corticostriatal excitatory input properties of SPNs recorded from the [A] p7-8, [B] p9-10, [C] p11-12 and [D] p13-14 striatum (Figure 5.11). All data was first assessed for normality and then significance assessed using either ANOVA, independent t-test (parametric data) or Mann-Whitney-U (MWU) test (non-parametric data), with Bonferroni post hoc corrections where appropriate.

Post-synaptic



Pre-synaptic



Supplementary Figure S5.7. Impacts on early postnatal SPN corticostriatal connections within specific two-day time windows (Figure 5.11). (Description on next page).

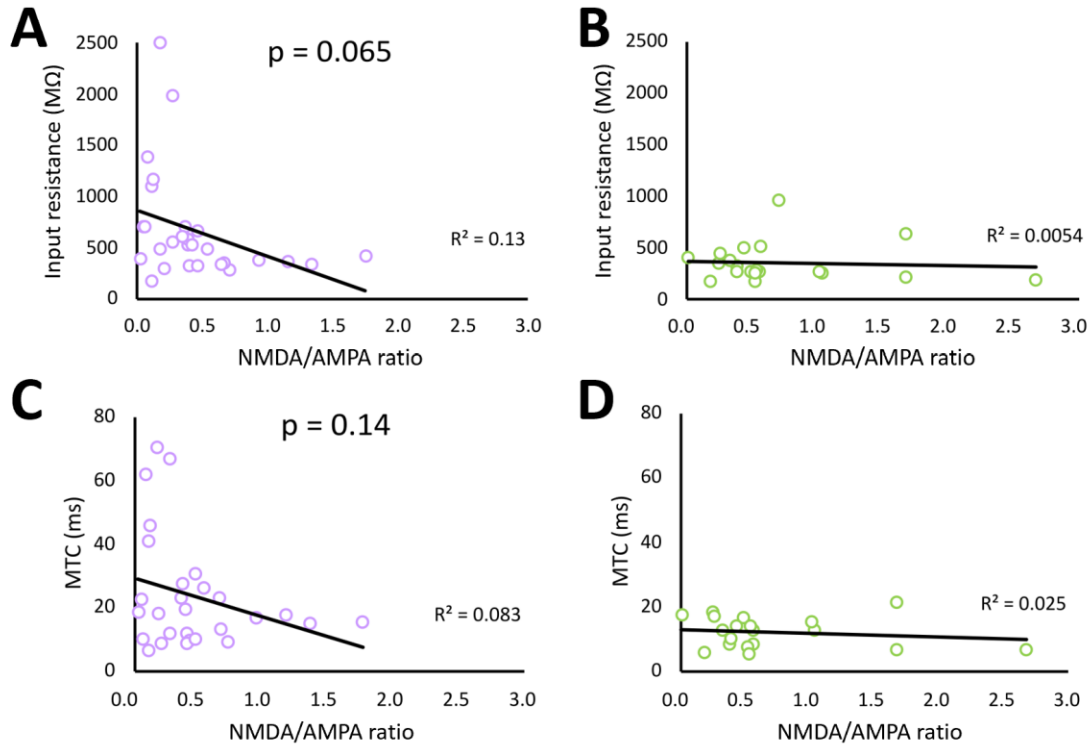
Supplementary Figure S5.7. Impacts on early postnatal SPN corticostriatal connections within specific two-day time windows (Figure 5.11) (cont.). **[A-D]** Graphs of **[A]** EPSP amplitude, **[B]** duration, **[C]** rise time and **[D]** decay time respectively of control-IgG and NR1-IgG in utero exposed early postnatal SPNs, split by two-day time windows (p7-8, 9-10, 11-12 and 13-14), which showed no significant differences in any of these post-synaptic properties between the groups. **[E-F]** Graphs of 20Hz train stimulation of **[E]** p9-10 and **[F]** p11-12 SPNs respectively, exposed to control-IgG or NR1-IgG in utero, exhibiting no significant differences. Significant differences were seen at both p7-8 and p13-14. **[G-I]** Graphs of the paired-pulse ratio at **[G]** p9-10, **[H]** p11-12 and **[I]** p13-14 respectively revealing no significant differences at any of these time points, although there was a trend towards a significantly higher ratio at p13-14. There was also a significant difference in paired-pulse ratio at p7-8.

NMDA/AMPA RATIO – CONTROL			
Intrinsic property	R ²	p value	Direction
Resting membrane potential	0.054	0.82	=
Threshold	0.12	0.61	=
Input resistance	0.0067	0.98	=
Membrane time constant	0.10	0.68	=
First spike amplitude	0.0082	0.97	=
Second spike amplitude	0.0023	0.99	=
Synaptic property	R ²	p value	Direction
sEPSC amplitude	3.0 x 10 ⁻⁵	1.0	=
sEPSC frequency	0.060	0.81	=

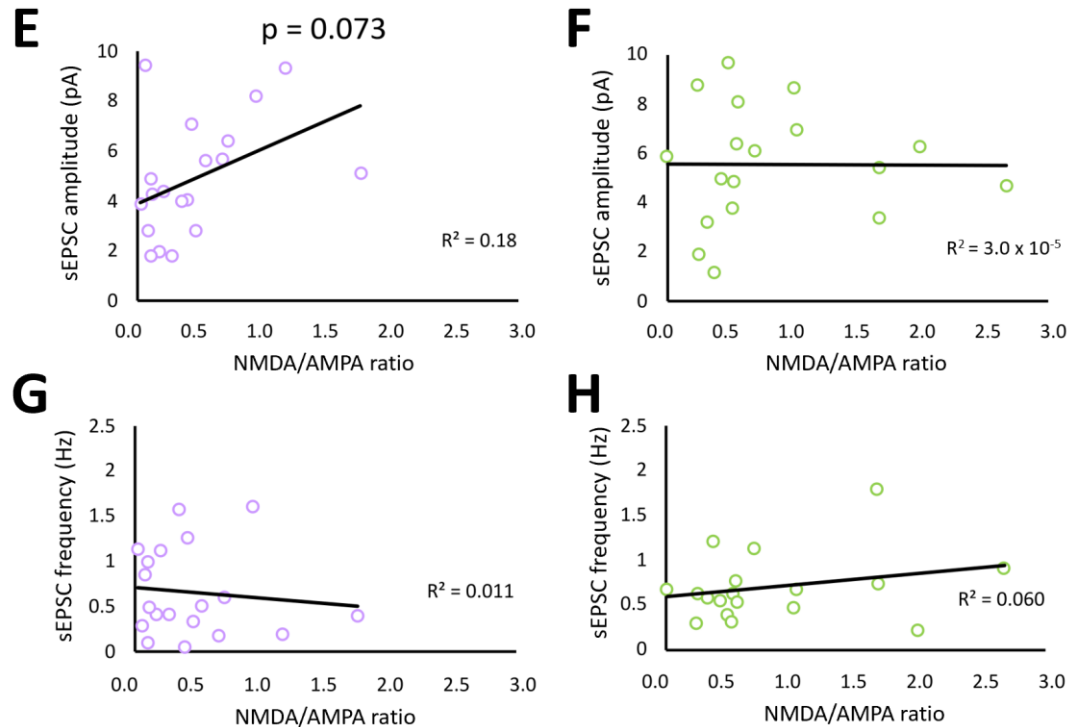
NMDA/AMPA RATIO – NR1			
Intrinsic property	R ²	p value	Direction
Resting membrane potential	0.021	0.92	=
Threshold	0.015	0.94	=
Input resistance	0.13	0.065	- (trend)
Membrane time constant	0.083	0.14	- (trend)
First spike amplitude	0.027	0.89	=
Second spike amplitude	0.048	0.81	=
Synaptic property	R ²	p value	Direction
sEPSC amplitude	0.18	0.073	+ (trend)
sEPSC frequency	0.011	0.96	=

Supplementary Table S5.8. Correlations of NMDA/AMPA ratio and key electrophysiological (intrinsic and synaptic) properties.

Intrinsic properties



Synaptic properties



Supplementary Figure S5.9. Key correlation graphs between NMDA/AMPA ratio and electrophysiological properties. (Description on next page).

Supplementary Figure S5.9. Key correlation graphs between NMDA/AMPA ratio and electrophysiological properties (cont.). [A-B] Line plots of correlations between NMDA/AMPA ratio and input resistance for [A] NR1-IgG and [B] control-IgG exposed SPNs respectively, revealing a trend towards a negative correlation in NR1-IgG exposed SPNs but no correlation in control-IgG exposed SPNs. [C-D] Line plots of correlations between NMDA/AMPA ratio and membrane time constant for [A] NR1-IgG and [B] control-IgG exposed SPNs respectively, revealing a trend towards a negative correlation in NR1-IgG exposed SPNs but no correlation in control-IgG exposed SPNs. [E-F] Line plots of correlations between NMDA/AMPA ratio and sEPSC amplitude for [E] NR1-IgG and [F] control-IgG exposed SPNs respectively, revealing a trend towards a positive correlation in NR1-IgG exposed SPNs but no correlation in control-IgG exposed SPNs. [G-H] Line plots of correlations between NMDA/AMPA ratio and sEPSC frequency for [G] NR1-IgG and [H] control-IgG exposed SPNs respectively, revealing no significant correlations for either NR1-IgG or control-IgG exposed SPNs.

Supplementary Table S5.8 and Supplementary Figure S5.9 Summary

Having observed alterations in several intrinsic and synaptic properties as well as a decrease in the NMDA/AMPA ratio, possible correlations between them were also briefly investigated. First, analysis of intrinsic properties against the NMDA/AMPA ratio revealed that there was a trend towards a negative correlation between NMDA/AMPA ratio and input resistance ($R^2 = 0.13$, $p = 0.065$, **Supplementary Figure S5.9 A**) for NR1-IgG exposed SPNs, but no significant correlation for control-IgG exposed SPNs ($R^2 = 0.0054$, $p = 0.75$, **Supplementary Figure S5.9 B**). Similarly, there was a trend towards a negative correlation between NMDA/AMPA ratio and membrane time constant for NR1-IgG exposed SPNs ($R^2 = 0.083$, $p = 0.14$, **Supplementary Figure S5.9 C**) but no significant correlation for control-IgG exposed SPNs ($R^2 = 0.025$, $p = 0.49$, **Supplementary Figure S5.9 D**). There were no significant correlations for any other intrinsic properties (**Supplementary Table S5.8**). For this analysis, $n(\text{NR1-IgG SPNs}) = 28$ cells, 12 brains, 4 litters, $n(\text{control-IgG SPNs}) = 21$ cells, 10 brains, 3 litters.

Next, comparison of synaptic properties against NMDA/AMPA ratio revealed a trend towards a positive correlation between NMDA/AMPA ratio and sEPSC amplitude in NR1-IgG exposed SPNs ($R^2 = 0.17$, $p = 0.072$, **Supplementary Figure S5.9 E**) but no significant difference in control-IgG exposed SPNs ($R^2 = 3.0$

$\times 10^{-5}$, $p = 0.98$, **Supplementary Figure S5.9 F**). There were no significant correlations between NMDA/AMPA ratio and sEPSC frequency for either NR1-IgG exposed SPNs ($R^2 = 0.11$, $p = 0.66$, **Supplementary Figure S5.9 G**) or control-IgG exposed SPNs ($R^2 = 0.060$, $p = 0.33$, **Supplementary Figure S5.9 H**). For this analysis, $n(\text{NR1-IgG SPNs}) = 19$ cells, 9 brains, 4 litters, $n(\text{control-IgG SPNs}) = 18$ cells, 9 brains, 3 litters.

Overall, these results reveal trending correlations between the NMDA/AMPA ratio and certain intrinsic and synaptic properties specifically for NR1-IgG exposed SPNs. In terms of intrinsic properties, they show trending correlations between the NMDA/AMPA ratio and input resistance as well as NMDA/AMPA ratio and membrane time constant. In terms of synaptic properties, they reveal a trending positive correlation between NMDA/AMPA ratio and sEPSC amplitude but not between NMDA/AMPA ratio and sEPSC frequency, suggesting a possible post-synaptic effect.

REPEATED MEASURES (RM) ANOVA	Main effect of day? (i.e does it change from day to day)	Individual day comparisons			Day x Group?	Main effect of group? (i.e. is there difference between NR1-IgG and control-IgG animals)
		1v2	1v3	2v3		
Centre circle frequency	F (2,24) = 1.73, p = 0.20	0.43	1.00	0.40	F(2,24) = 0.11, p = 0.90	F(1,12) = 6.22, p = 0.028
Periphery frequency	F (2,24) = 0.66, p = 0.53	0.73	0.83	0.98	F(2,24) = 0.27, p = 0.77	F(1,12) = 4.85, p = 0.048
Corners frequency	F(2,22) = 4.42, p = 0.024	0.044	0.15	1.00	F(2,22) = 0.65, p = 0.53	F(1,11) = 0.52, p = 0.49
Norm. duration in centre	F(2,26) = 0.53, p = 0.60	0.67	0.79	1.00	F(2,26) = 0.18, p = 0.84	F(1,13) = 1.78, p = 0.21
Norm. duration in corners	F(2,26) = 0.81, p = 0.46	0.78	0.55	0.98	F(2,26) = 2.12, p = 0.14	F(1,13) = 2.52, p = 0.14
Total distance moved	F(1.40, 18.22) = 16.91, p = 0.00023	0.0030	0.0020	0.12	F(2,26) = 0.12, p = 0.88	F(1,13) = 0.0080, p = 0.93
Norm. distance covered in centre	F(2,26) = 0.96, p = 0.40	0.60	0.61	1.00	F(2,26) = 0.063, p = 0.94	F(1,13) = 6.98, p = 0.020
Norm. distance covered in corners	F(2,26) = 0.065, p = 0.94	0.98	1.00	0.99	F(2,26) = 1.72, p = 0.20	F(1,13) = 2.20, p = 0.16
Mean velocity	F(1.40, 18.24) = 17.18, p = 0.00021	0.0030	0.0020	0.11	F(1.40, 18.24) = 0.13, p = 0.80	F(1,13) = 0.0050, p = 0.94
Mean velocity in centre	F(2,26) = 0.31, p = 0.74	0.96	0.79	0.99	F(2,26) = 0.21, p = 0.81	F(1,13) = 3.16, p = 0.099
Mean velocity in corners	F(2,26) = 14.45, p = 6.0 x 10⁻⁴	0.0030	0.0020	0.12	F(2,26) = 1.67, p = 0.21	F(1,13) = 0.0080, p = 0.93
Rotarod AVG. TRIALS	F(2,26) = 2.21, p = 0.13	0.16	0.32	0.97	F(2,26) = 0.97, p = 0.39	F(1,13) = 7.11, p = 0.019
	Main effect of trial?				Trial x Group	
Rotarod DAY 1	F(3,33) = 2.22, p = 0.10				F(3,33) = 1.26, p = 0.30	F(1,11) = 3.15, p = 0.10
Rotarod DAY 2	F(3,30) = 0.29, p = 0.83				F(3,30) = 0.060, p = 0.98	F(1,10) = 2.63, p = 0.14
Rotarod DAY 3	F(3,30) = 2.14, p = 0.12				F(3,30) = 1.97, p = 0.14	F(1,10) = 3.52, p = 0.090

Supplementary Table S6.1. Day, Group x Day and Group RM ANOVA statistics on p21-23 behaviour data (Figures 6.2, 6.3).

NON-RESCUE

Day	% population with righting reflex present	
	Ctrl IgG	NR1 IgG
p7	73.8 ± 5.0	27.0 ± 6.3
p8	84.9 ± 8.3	63.5 ± 3.2
p9	100.0 ± 0.0	85.7 ± 14.3
p10	100.0 ± 0.0	100.0 ± 0.0
p11	100.0 ± 0.0	100.0 ± 0.0
p12	100.0 ± 0.0	100.0 ± 0.0
p13	100.0 ± 0.0	100.0 ± 0.0
p14	100.0 ± 0.0	100.0 ± 0.0

Day	% population with negative geotaxis present	
	Ctrl IgG	NR1 IgG
p7	63.5 ± 10.1	26.2 ± 5.0
p8	80.2 ± 12.5	42.1 ± 4.8
p9	85.7 ± 14.3	69.0 ± 7.7
p10	94.4 ± 5.6	100.0 ± 0.0
p11	100.0 ± 0.0	100.0 ± 0.0
p12	100.0 ± 0.0	100.0 ± 0.0
p13	100.0 ± 0.0	100.0 ± 0.0
p14	100.0 ± 0.0	100.0 ± 0.0

Day	% population with cliff avoidance present	
	Ctrl IgG	NR1 IgG
p7	73.0 ± 6.3	26.2 ± 5.0
p8	73.0 ± 6.3	36.5 ± 3.2
p9	88.9 ± 5.6	42.1 ± 4.8
p10	88.9 ± 5.6	42.1 ± 4.8
p11	94.4 ± 5.6	53.2 ± 7.1
p12	100.0 ± 0.0	73.8 ± 5.0
p13	100.0 ± 0.0	100.0 ± 0.0
p14	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table S6.2. Data from testing of postnatal reflexes [A] righting reflex, [B] negative geotaxis and [C] cliff avoidance in NR1-IgG and control-IgG in utero exposed animals, tested daily from p7-14 (Figure 6.1).

RESCUE

Day	% population with righting reflex present	
	Ctrl IgG	NR1 IgG
p7	100.0 ± 0.0	83.0 ± 25.0
p8	100.0 ± 0.0	100.0 ± 0.0
p9	100.0 ± 0.0	100.0 ± 0.0
p10	100.0 ± 0.0	100.0 ± 0.0

Day	% population with negative geotaxis present	
	Ctrl IgG	NR1 IgG
p7	83.0 ± 0.0	75.0 ± 25.0
p8	83.0 ± 0.0	75.0 ± 25.0
p9	100.0 ± 0.0	100.0 ± 0.0
p10	100.0 ± 0.0	100.0 ± 0.0

Day	% population with cliff avoidance present	
	Ctrl IgG	NR1 IgG
p7	92.0 ± 0.0	67.0 ± 0.0
p8	100.0 ± 0.0	100.0 ± 0.0
p9	100.0 ± 0.0	100.0 ± 0.0
p10	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table S6.3. Data from testing of postnatal reflexes [A] righting reflex, [B] negative geotaxis and [C] cliff avoidance in NR1-IgG and control-IgG in utero exposed animals, tested daily from p7-10, following administration of 'rescue' injections of AMPAkin CX516 (Figure 6.4).

A

RESCUE NR1 vs NON-RESCUE NR1 REPEATED MEASURES (RM) ANOVA	Main effect of day? (i.e does it change from day to day)	Individual day comparisons			Day x Group?	Main effect of group? (i.e. is there difference between NR1-IgG and control-IgG animals)
		1v2	1v3	2v3		
Centre circle frequency	F(2,20) = 4.51, p = 0.024	0.56	0.037	0.25	F(2,20) = 1.36, p = 0.28	F(1,10) = 0.030, p = 0.87
Periphery frequency	F(2,22) = 2.10, p = 0.15	0.76	0.21	0.54	F(2,22) = 0.42, p = 0.67	F(1,11) = 0.38, p = 0.55
Corners frequency	F(2,20) = 8.94, p = 0.0020	0.0040	0.036	1.00	F(2,20) = 0.74, p = 0.49	F(1,10) = 2.67, p = 0.13
Norm. duration in centre	F(2,20) = 0.31, p = 0.74	1.00	0.93	0.89	F(2,20) = 1.17, p = 0.33	F(1,10) = 0.00049, p = 0.98
Norm. duration in corners	F(2,22) = 2.96, p = 0.073	0.28	0.16	0.87	F(2,22) = 0.30, p = 0.75	F(1,11) = 0.098, p = 0.76
Total distance moved	F(2,22) = 24.03, p = 2.9 x 10⁻⁶	0.0030	0.00042	0.031	F(2,22) = 1.29, p = 0.30	F(1,11) = 0.78, p = 0.40
Norm. distance covered in centre	F(2,20) = 0.045, p = 0.96	1.00	0.99	1.00	F(2,20) = 0.12, p = 0.89	F(1,10) = 2.35, p = 0.16
Norm. distance covered in corners	F(2,22) = 2.10, p = 0.15	0.52	0.26	0.78	F(2,22) = 0.049, p = 0.94	F(1,11) = 0.36, p = 0.56
Mean velocity	F(2,22) = 23.87, p = 3.1 x 10⁻⁶	0.0030	0.00044	0.030	F(2,22) = 1.26, p = 0.30	F(1,11) = 0.76, p = 0.40
Mean velocity in centre	F(2,20) = 0.12, p = 0.89	0.98	0.95	1.00	F(2,20) = 0.32, p = 0.73	F(1,10) = 0.42, p = 0.53
Mean velocity in corners	F(2,22) = 18.60, p = 1.9 x 10⁻⁵	0.0030	0.0010	0.44	F(2,22) = 0.080, p = 0.92	F(1,11) = 0.012, p = 0.92
Rotarod AVG. TRIALS	F(2,22) = 3.65, p = 0.043	0.35	0.15	0.39	F(2,22) = 0.18, p = 0.84	F(1,11) = 24.22, p = 0.11
	Main effect of trial?				Trial x Group	
Rotarod DAY 1	F(3,30) = 1.79, p = 0.17				F(3,30) = 0.92, p = 0.45	F(1,10) = 1.82, p = 0.21
Rotarod DAY 2	F(3,30) = 0.37, p = 0.78				F(3,30) = 0.91, p = 0.45	F(1,10) = 3.14, p = 0.11
Rotarod DAY 3	F(3,30) = 1.32, p = 0.29				F(3,30) = 4.00, p = 0.016	F(1,10) = 0.54, p = 0.48

B

RESCUE NR1 vs NON-RESCUE CTRL REPEATED MEASURES (RM) ANOVA	Main effect of day? (i.e does it change from day to day)	Individual day comparisons			Day x Group?	Main effect of group? (i.e. is there difference between NR1-IgG and control-IgG animals)
		1v2	1v3	2v3		
Centre circle frequency	F(2,12) = 8.24, p = 0.0060	0.39	0.013	0.082	F(2,12) = 4.58, p = 0.033	F(1,6) = 13.34, p = 0.011
Periphery frequency	F(2,14) = 2.95, p = 0.085	0.34	0.19	0.93	F(2,14) = 2.24, p = 0.14	F(1,7) = 2.32, p = 0.17
Corners frequency	F(2,14) = 6.82, p = 0.0090	0.087	0.040	0.73	F(2,14) = 0.40, p = 0.68	F(1,7) = 0.29, p = 0.61
Norm. duration in centre	F(2,14) = 0.39, p = 0.68	0.95	0.92	0.89	F(2,14) = 0.33, p = 0.72	F(1,7) = 1.00, p = 0.35
Norm. duration in corners	F(2,16) = 0.16, p = 0.86	0.95	0.95	1.00	F(2,16) = 0.79, p = 0.47	F(1,8) = 1.08, p = 0.33
Total distance moved	F(1.23, 9.84) = 18.79, p = 0.0010	0.0030	0.0070	0.19	F(1.23, 9.84) = 0.66, p = 0.47	F(1,8) = 0.43, p = 0.53
Norm. distance covered in centre	F(2,14) = 0.24, p = 0.79	0.89	0.90	1.00	F(2,14) = 0.66, p = 0.66	F(1,7) = 0.62, p = 0.46
Norm. distance covered in corners	F(2,16) = 0.14, p = 0.87	0.95	0.98	1.00	F(1,16) = 2.13, p = 0.15	F(1,8) = 0.55, p = 0.48
Mean velocity	F(1.23, 9.88) = 19.26, p = 0.00095	0.0030	0.0060	0.18	F(1.23, 9.88) = 0.64, p = 0.48	F(1,8) = 0.40, p = 0.54
Mean velocity in centre	F(2,14) = 0.080, p = 0.92	1.00	0.99	0.97	F(2,14) = 0.020, p = 0.98	F(1,7) = 0.50, p = 0.50
Mean velocity in corners	F(2,16) = 15.15, p = 0.00020	0.0020	0.012	0.57	F(2,16) = 1.60, p = 0.23	F(1,8) = 1.27, p = 0.29
Rotarod AVG. TRIALS	F(2,16) = 4.04, p = 0.038	0.10	0.19	0.82	F(2,16) = 1.80, p = 0.20	F(1,8) = 0.25, p = 0.63
	Main effect of trial?				Trial x Group	
Rotarod DAY 1	F(3,21) = 3.64, p = 0.030				F(3,21) = 0.73, p = 0.55	F(1,7) = 0.077, p = 0.79
Rotarod DAY 2	F(3,18) = 1.92, p = 0.16				F(3,18) = 1.83, p = 0.18	F(1,6) = 0.026, p = 0.88
Rotarod DAY 3	F(3,18) = 0.19, p = 0.90				F(3,18) = 0.78, p = 0.52	F(1,6) = 1.27, p = 0.30

C

RESCUE NR1 vs RESCUE CTRL REPEATED MEASURES (RM) ANOVA	Main effect of day? (i.e does it change from day to day)	Individual day comparisons			Day x Group?	Main effect of group? (i.e. is there difference between NR1-IgG and control-IgG animals)
		1v2	1v3	2v3		
Centre circle frequency	F(1.11, 7.77) = 8.81, p = 0.017	0.40	0.013	0.0080	F(1.11, 7.77) = 0.38, p = 0.58	F(1,7) = 0.35, p = 0.58
Periphery frequency	F(1.24, 9.95) = 8.73, p = 0.011	0.31	0.0090	0.035	F(1.24, 9.95) = 1.36, p = 0.28	F(1,8) = 0.0080, p = 0.93
Corners frequency	F(2,16) = 13.80, p = 0.00033	0.014	0.0040	1.00	F(2,16) = 0.93, p = 0.42	F(1,8) = 0.0050, p = 0.94
Norm. duration in centre	F(2,14) = 0.21, p = 0.82	0.92	0.90	1.00	F(2,14) = 1.22, p = 0.32	F(1,7) = 1.31, p = 0.29
Norm. duration in corners	F(2,16) = 5.85, p = 0.012	0.52	0.076	0.089	F(2,16) = 2.09, p = 0.16	F(1,8) = 0.25, p = 0.63
Total distance moved	F(2,16) = 32.60, p = 2.3 x 10⁻⁶	0.0050	0.00014	0.074	F(2,16) = 0.73, p = 0.50	F(1,8) = 0.00043, p = 0.98
Norm. distance covered in centre	F(2,14) = 2.03, p = 0.17	0.83	0.39	0.40	F(2,14) = 1.53, p = 0.25	F(1,7) = 0.23, p = 0.65
Norm. distance covered in corners	F(1.22, 9.79) = 7.17, p = 0.020	0.16	0.066	0.095	F(1.22, 9.79) = 0.97, p = 0.40	F(1,8) = 0.075, p = 0.79
Mean velocity	F(2,16) = 32.53, p = 2.3 x 10⁻⁶	0.0050	0.00013	0.075	F(2,16) = 0.73, p = 0.46	F(1,8) = 0.00023, p = 0.99
Mean velocity in centre	F(2,14) = 0.053, p = 0.95	1.00	1.00	0.99	F(2,14) = 0.21, 0.82	F(1,7) = 2.34, p = 0.17
Mean velocity in corners	F(2,16) = 27.67, p = 6.4 x 10⁻⁶	0.012	9.4 x 10⁻⁵	0.075	F(2,16) = 1.25, p = 0.31	F(1,8) = 0.22, p = 0.65
Rotarod AVG. TRIALS	F(2,16) = 3.98, p = 0.039	0.22	0.19	0.48	F(2,16) = 0.10, p = 0.90	F(1,8) = 0.072, p = 0.80
	Main effect of trial?				Trial x Group	
Rotarod DAY 1	F(3,18) = 1.51, p = 0.25				F(3,18) = 1.63, p = 0.22	F(1,6) = 0.010, p = 0.92
Rotarod DAY 2	F(3,21) = 2.00, p = 0.15				F(3,21) = 0.63, p = 0.61	F(1,7) = 1.48, p = 0.18
Rotarod DAY 3	F(3,21) = 0.36, p = 0.78				F(3,21) = 1.56, p = 0.23	F(1,7) = 0.0050, p = 0.94

D

RESCUE CTRL vs NON-RESCUE CTRL REPEATED MEASURES (RM) ANOVA	Main effect of day? (i.e does it change from day to day)	Individual day comparisons			Day x Group?	Main effect of group? (i.e. is there difference between NR1-IgG and control-IgG animals)
		1v2	1v3	2v3		
Centre circle frequency	F(1.12, 10.06) = 7.05, p = 0.0059	0.22	0.021	0.10	F(1.12, 10.06) = 3.76, p = 0.078	F(1,9) = 5.46, p = 0.044
Periphery frequency	F(1.23, 11.08) = 6.45, p = 0.023	0.23	0.020	0.38	F(1.23, 11.08) = 5.05, 0.040	F(1,9) = 5.27, 0.047
Corners frequency	F(2,18) = 8.19, p = 0.0030	0.039	0.020	1.00	F(2,18) = 1.89, p = 0.18	F(1,9) = 0.36, p = 0.56
Norm. duration in centre	F(2,20) = 0.28, p = 0.76	0.99	0.92	0.90	F(2,20) = 1.03, p = 0.38	F(1,10) = 0.00014, p = 0.99
Norm. duration in corners	F(2,20) = 2.11, p = 0.15	1.00	0.23	0.34	F(2,20) = 4.04, p = 0.034	F(1,10) = 0.85, p = 0.38
Total distance moved	F(2,20) = 26.66, p = 2.3 x 10⁻⁶	0.0020	0.00033	0.15	F(2,20) = 2.80, p = 0.085	F(1,10) = 0.66, p = 0.44
Norm. distance covered in centre	F(2,20) = 1.16, p = 0.33	1.00	0.57	0.42	F(2,20) = 4.85, p = 0.019	F(1,10) = 0.23, p = 0.65
Norm. distance covered in corners	F(2,20) = 1.07, p = 0.36	0.95	0.47	0.75	F(2,20) = 5.81, p = 0.010	F(1,10) = 1.18, p = 0.30
Mean velocity	F(2,20) = 27.04, p = 2.1 x 10⁻⁶	0.0020	0.00029	0.15	F(2,20) = 2.77, p = 0.087	F(1,10) = 0.62, p = 0.45
Mean velocity in centre	F(2,20) = 0.091, p = 0.91	0.97	1.00	1.00	F(2,20) = 0.30, p = 0.75	F(1,10) = 0.15, p = 0.71
Mean velocity in corners	F(2,20) = 21.35, p = 1.1 x 10⁻⁵	0.021	0.00014	0.041	F(2,20) = 4.26, p = 0.029	F(1,10) = 0.92, p = 0.36
Rotarod AVG. TRIALS	F(2,20) = 3.92, p = 0.037	0.069	0.25	1.00	F(2,20) = 1.07, p = 0.36	F(1,10) = 0.70, p = 0.42

Supplementary Table S6.4. Day, Group x Day and group RM ANOVA statistics on p21-23 behaviour data for [A] rescue NR1-IgG vs non-rescue NR1-IgG, [B] rescue NR1-IgG vs non-rescue control-IgG, [C] rescue NR1-IgG vs rescue control-IgG, and [D] rescue control-IgG vs non-rescue control-IgG datasets (Figures 6.5, 6.6).