

# STRIATAL GABA TRANSPORTER REGULATION OF DOPAMINE RELEASE AND DYSFUNCTION IN PARKINSONIAN MODELS



Bradley Mark Roberts

Department of Physiology, Anatomy and Genetics

Medical Sciences Division

Christ Church College, University of Oxford

Under the supervision of Professor Stephanie J. Cragg

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Dedicated to my grandfather

JACK ROBERTS

1933 - 2013

who modelled the importance of a strong work ethic,  
and inspired my pursuit to make an impact on Parkinson's disease

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Examination committee:  
Prof. Trevor Sharp, University of Oxford, UK  
Dr Jérôme Baufreton, Université de Bordeaux, France

Leave to supplicate for the degree of  
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## Statement of Authorship

This thesis contains no material that has been accepted for the award of any other degree in any University or other institution. All data represented in this thesis are wholly my own work, and where data has been contributed by other individuals, their contributions are noted below and within the main text.

The data represented in Chapter 3 are part of a wider study within the laboratory of Professor Stephanie Cragg, resulting in a joint first-authored publication:

Lopes EF\*, Roberts BM\*, Siddorn RE, Clements MA, Cragg SJ (2019) Inhibition of Nigrostriatal Dopamine Release by Striatal GABA<sub>A</sub> and GABA<sub>B</sub> Receptors. *Journal of Neuroscience* 39, 1058-1065 doi: <https://doi.org/10.1523/JNEUROSCI.2028-18.2018>

\* These authors contributed equally to this work

As such, some of the data represented in Chapter 3 have been collected by Mr Emanuel Lopes (DPhil student) and Ms Ruth Siddorn (MChem Student, under the supervision of Mr Bradley Roberts), and are provided with their full permissions. These data are identified in the corresponding figure legends.

The data represented in Chapters 4 and 5 are part of a wider study within the laboratory of Professor Stephanie Cragg, resulting in a first-authored manuscript:

Roberts BM, Doig NM, Brimblecombe KR, Lopes EF, Siddorn RE, Threlfell S, Connor-Robson N, Bengoa-Vergniory N, Wade-Martins R, Magill PJ, Cragg SJ (2019) Astrocytic striatal GABA transporter activity governs dopamine release and shows maladaptive downregulation in early parkinsonism. *BioRxiv*, 698274.

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As such, some of the data represented in Chapters 4 and 5 have been collected by Dr Natalie Doig (Postdoctoral researcher) and Dr Katherine Brimblecombe (Postdoctoral researcher) and are provided with their full permissions. These data are identified in the corresponding figure legends.

## Conference Abstracts and Manuscripts Arising

### *Conference Abstracts:*

**Roberts BM**, Siddorn RE, Doig NM, Magill PJ, Cragg SJ. Investigating the implications of GABA co-storage in dopamine axons on dopamine transmission. 17<sup>th</sup> International Monitoring Molecules in Neuroscience Meeting (2018; Oxford, UK; Invited Talk).

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**Roberts BM**, Doig NM, Brimblecombe KR, Lopes EL, Siddorn RE, Threlfell S, Wade-Martins R, Magill PJ, Cragg SJ. GABA transporter activity governs tonic GABA inhibition of striatal dopamine release and is dysfunctional in prodromal Parkinsonian mice. 13<sup>th</sup> International Basal Ganglia Society Meeting (2019; Biarritz, France; Poster).

**Roberts BM**, Doig NM, Brimblecombe KR, Lopes EL, Siddorn RE, Threlfell S, Wade-Martins R, Magill PJ, Cragg SJ. Striatal GABA transporter activity governs dopamine release: implications for Parkinson's disease. Department of Physiology, Anatomy and Genetics Away Day (2019; Oxford, UK; Invited Talk).

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Condon MD, Platt NJ, Zhang YF, **Roberts BM**, Clements MA, Vietti-Michelina S, Tseu MY, Brimblecombe KR, Threlfell S, Mann EO, Cragg SJ (2019) Short-term plasticity of striatal dopamine release is governed by release-independent depression and the dopamine transporter. Nature Communications 10: 4263

Bengoa-Vergniory N, Faggiani E, Ramos-Gonzalez P, Kirkiz E, Connor-Robson N, Vingill S, Cioroch M, Cavaliere F, Threlfell S, **Roberts BM**, Schrader T, Klärner FG, Cragg SJ, Dehay B, Bitan G, Matute C, Bezard E, Wade-Martins R. CLR01 protects dopaminergic neurons in vitro and in vivo in human neurons and mouse models of Parkinson's. Under review: Nature Communications.

Brimblecombe KR, Connor-Robson N, **Roberts BM**, Karthik G, Cragg SJ. L-type calcium channel control of dopamine release can be recruited by dopamine transporter and  $\alpha 2\delta$  auxiliary subunits, and silenced by calbindin-D28K and D2 receptors in different striatal dopamine axons. (*In preparation*).

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## Abstract

Mesostriatal dopamine (DA) signalling plays a central role in the functions of the basal ganglia, facilitating behavioural processes such as action selection and reinforcement learning. Consequently, dysregulation of DA signalling is implicated in a diversity of neurological and psychiatric disorders including Parkinson's disease and addictions. It is increasingly appreciated that DA output from DA axons in striatum is subject to major influences besides action potentials generated at the level of the cell body. Rather, local mechanisms in the striatum are able to drive and gate axonal DA release. Unravelling these mechanisms and understanding how they might be dysfunctional in diseases with DA dysregulation, is critical for identifying novel targets for disease-modifying therapies.

The striatum contains a high density of GABAergic projection neurons and interneurons, and in addition midbrain DA neurons also co-release GABA in striatum. Extrasynaptic striatal GABA can accumulate to exert tonic inhibition of principal striatal neurons as an ambient GABA tone, the level of which is determined by plasma membrane GABA uptake transporters (GATs). However, whether striatal GABA, and subsequently GATs, can modulate DA release are unknown. This thesis examines how striatal GABA signalling and GATs govern DA release in mouse striatum, and how they are dysfunctional in a mouse model of early parkinsonism.

The work presented in the first part of this thesis addressed whether striatal GABA and its receptors can directly modulate DA release using fast-scan cyclic voltammetry in mouse brain slices. DA release was found to be inhibited by the activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors. Furthermore, GABA receptor antagonists significantly enhanced DA release evoked by optogenetic stimulation, indicating that DA release is under tonic inhibition by striatal GABA. In testing whether striatal GATs determine DA output through limiting GABA tone onto DA axons, GAT-1 and GAT-3 are revealed to govern DA release in dorsolateral striatum, but not nucleus accumbens core, and exhibited correspondingly enriched expression in dorsolateral striatum. Further, GAT-3 and GAT-1 localised to striatal astrocytes were found to critically regulate the level of GABA inhibition of DA release, as astrocyte inactivation prevented the effects of GAT inhibition.

The second part of this thesis examined whether this exposed GABAergic regulation of DA release is dysfunctional in a human  $\alpha$ -synuclein overexpressing mouse model of early parkinsonism. GAT function and expression were revealed to be downregulated and tonic GABAergic inhibition of DA release correspondingly augmented in the dorsolateral striatum, but not accumbens core, despite attenuated GABA co-release from dopaminergic axons. Additionally, a multiple dose course of the gabapentinoid pregabalin was found to boost DA release specifically in dorsolateral striatum, potentially mediated by normalising dysregulated tonic GABAergic inhibition of DA release in this mouse model of early parkinsonism.

These data define previously unappreciated and important roles for GABA, GATs and astrocytes in sculpting DA release in striatum, and reveal that they underlie maladaptive plasticity in early parkinsonism that impairs DA output in vulnerable striatal regions.

## Abbreviations

|                   |                                                         |
|-------------------|---------------------------------------------------------|
| 3-MPA             | 3-Mercaptopropionic acid                                |
| 5-HT              | Serotonin                                               |
| AAV               | adeno-associated virus                                  |
| ACh               | Acetylcholine                                           |
| aCSF              | Artificial cerebrospinal fluid                          |
| ALDH1a1           | Aldehyde dehydrogenase type 1a1                         |
| ATP               | Adenosine triphosphate                                  |
| BGT-1             | Plasma membrane betaine-GABA transporter type 1         |
| cAMP              | Cyclic adenosine monophosphate                          |
| CFM               | Carbon fibre microelectrode                             |
| ChI               | Cholinergic interneuron                                 |
| ChR2              | Channelrhodopsin-2                                      |
| CPu               | Caudate-putamen                                         |
| D-APV             | D-(2R)-amino-5-phosphonopentanoate                      |
| DA                | Dopamine                                                |
| [DA] <sub>o</sub> | Extracellular concentration of dopamine                 |
| DAT               | Dopamine transporter                                    |
| DH $\beta$ E      | Dihydro- $\beta$ -erythroidine                          |
| DLS               | Dorsolateral striatum                                   |
| DOPAC             | 3,4-Dihydroxyphenylacetic acid                          |
| DOPAL             | 3,4-Dihydroxyphenylacetaldehyde                         |
| dSPN              | Direct-pathway spiny projection neuron                  |
| EPSC              | Excitatory postsynaptic current                         |
| eYFP              | Enhanced yellow fluorescent protein                     |
| FSCV              | Fast-scan cyclic voltammetry                            |
| FSI               | Fast spiking interneuron                                |
| GABA              | $\gamma$ -amino butyric acid                            |
| GABA <sub>A</sub> | GABA type-A receptor                                    |
| GABA <sub>B</sub> | GABA type-B receptor                                    |
| GAD               | Glutamic acid decarboxylase                             |
| GAT-1             | Plasma membrane GABA transporter type 1                 |
| GAT-2             | Plasma membrane GABA transporter type 2                 |
| GAT-3             | Plasma membrane GABA transporter type 3                 |
| GBP               | Gabapentin                                              |
| GIRK              | G protein-coupled inwardly-rectifying potassium channel |
| GPCR              | G Protein-coupled receptor                              |
| GPe               | External segment of the globus pallidus                 |
| GPi               | Internal segment of the globus pallidus                 |
| GWAS              | Genome-wide association studies                         |
| HPLC              | High-performance liquid chromatography                  |
| IPSC              | Inhibitory postsynaptic current                         |

|              |                                                                 |
|--------------|-----------------------------------------------------------------|
| IRES-Cre     | Internal ribosome entry site-linked Cre-recombinase             |
| iSPN         | Indirect-pathway spiny projection neuron                        |
| L-DOPA       | Levodopa                                                        |
| LED          | Light-emitting diode                                            |
| LTSI         | Low-threshold spiking interneuron                               |
| NAc          | Nucleus accumbens                                               |
| NAcC         | Nucleus accumbens core                                          |
| NAcS         | Nucleus accumbens shell                                         |
| nAChR        | Nicotinic acetylcholine receptor                                |
| NBQX         | 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione |
| NMDG         | N-Methyl-D-glucamine diatrizoate.                               |
| NPA          | (±)-Nipecotic acid                                              |
| NPY          | Neuropeptide Y                                                  |
| PBS          | Phosphate-buffered saline                                       |
| PD           | Parkinson's disease                                             |
| PGB          | Pregabalin                                                      |
| PPR or P2/P1 | Paired-pulse ratio                                              |
| PTX          | Picrotoxin                                                      |
| PV           | Parvalbumin                                                     |
| Rab11a       | Ras-related protein 11a                                         |
| SDS-PAGE     | Sodium dodecyl sulphate polyacrylamide gel electrophoresis      |
| SNc          | Substantia nigra pars compacta                                  |
| SNr          | Substantia nigra pars reticulata                                |
| SNCA         | $\alpha$ -synuclein gene                                        |
| SNCA-OVX     | Human $\alpha$ -synuclein overexpressing mouse model of PD      |
| SPN          | Spiny projection neuron                                         |
| SSRIs        | Serotonin reuptake inhibitors                                   |
| STN          | Subthalamic nucleus                                             |
| TH           | Tyrosine hydroxylase                                            |
| TTX          | Tetrodotoxin                                                    |
| UBE3A        | Ubiquitin protein ligase E3A                                    |
| VGAT         | Vesicular GABA transporter                                      |
| VGCC         | Voltage-gated calcium channel                                   |
| VGlut2       | Vesicular glutamate transporter type 2                          |
| VMAT2        | Vesicular monoamine transporter                                 |
| VTA          | Ventral tegmental area                                          |
| WT           | Wild-type                                                       |

# 1

## Introduction

## 1.1 Thesis overview

Dopamine (DA) release in the striatum can be dissociated from that of neuronal activity in the midbrain (Mohebi et al., 2019; Romo et al., 1986) and is gated locally by axonal mechanisms and striatal neuromodulators that regulate or even independently drive DA release (reviewed in Sulzer et al., 2016). Unravelling these local mechanisms which gate axonal DA release and understanding how they might be dysfunctional in diseases with DA dysregulation, like Parkinson's disease and addictions, is critical for identifying potential novel targets for developing disease-modifying therapies.

The striatum contains an exceptionally high density of projection neurons and interneurons that use the transmitter  $\gamma$ -aminobutyric acid (GABA) and is arguably the most GABAergic nuclei in the adult mammalian brain (Oorschot, 1996; Tepper et al., 2018). Extrasynaptic striatal GABA can accumulate to exert tonic inhibition of principal striatal neurons as an ambient GABA tone, the level of which is determined by plasma membrane GABA uptake transporters (GATs) (Ade et al., 2008; Kirmse et al., 2008, 2009). However, whether striatal GABA, and subsequently GATs, can modulate DA output has not been extensively studied. In this thesis, I explore the control of striatal DA release by local striatal GABA signalling (Chapter 3) and subsequently by striatal GATs (Chapter 4). Furthermore, I explore how this GABAergic control of striatal DA release might be dysregulated in a mouse model of early Parkinsonism (Chapter 5). In this chapter I will introduce background information that is relevant to the interpretation of this thesis as a whole.

## 1.2 Organisation and function of the striatum

The basal ganglia are a collection of subcortical nuclei which are known to be involved in a variety of behavioural processes including action selection, reinforcement learning and decision making (Balleine et al., 2007; Crittenden and Graybiel, 2011; Graybiel et al., 1994). Furthermore, dysfunction in basal ganglia signalling contributes to the aetiology of many neurological and psychiatric disorders, most notably Parkinson's disease, Huntington's

disease and addictions (Obeso et al., 2014). The basal ganglia are composed of five principal nuclei: caudate nucleus, putamen, globus pallidus, subthalamic nucleus, and substantia nigra. The function of the basal ganglia is to integrate information received by the striatum to allow execution of appropriate behaviours. The basal ganglia achieve action selection through inhibition of undesired actions: selected behaviours are promoted by disinhibition of specific pathways, whilst the default inhibition of non-selected pathways remains (Bolam et al., 2000; Gerfen and Bolam, 2010; Gerfen and Surmeier, 2011).

### 1.2.1 Structure and function of striatum

The striatum is the major input nucleus of the basal ganglia and receives excitatory and inhibitory inputs from a large network of brain areas, including various cortical regions, the thalamus, and from dopaminergic and GABAergic inputs from the ventral midbrain (Wichmann and DeLong, 2007). These inputs are integrated within striatum to activate downstream signalling pathways that project through the basal ganglia nuclei (**Figure 1.1**). The topographic arrangement of these inputs into striatum and maintenance of this organisation to downstream nuclei of the basal ganglia, forms a set of parallel loops that each support specific behavioural and cognitive functions (Alexander and Crutcher, 1990; Redgrave et al., 2011); however, these loops are not exclusively isolated as a degree of integration occurs at the level of the striatum and along other points in the signalling pathways (Averbeck et al., 2014).

The striatum receives its name due to its striated appearance in sagittal sections. The striatum can be divided based on connectivity, developmental and functional roles. The dorsal striatum, which includes the caudate nucleus and putamen, has traditionally been thought to subserve sensorimotor related functions and is necessary for action selection (Knowlton et al., 1996; Packard and McGaugh, 1996, 1999; Quinn et al., 2013; Yin et al., 2004, 2005). The ventral striatum includes the ventral junction of the caudate and putamen, the nucleus accumbens (NAc) and portions of the olfactory tubercle, and serves

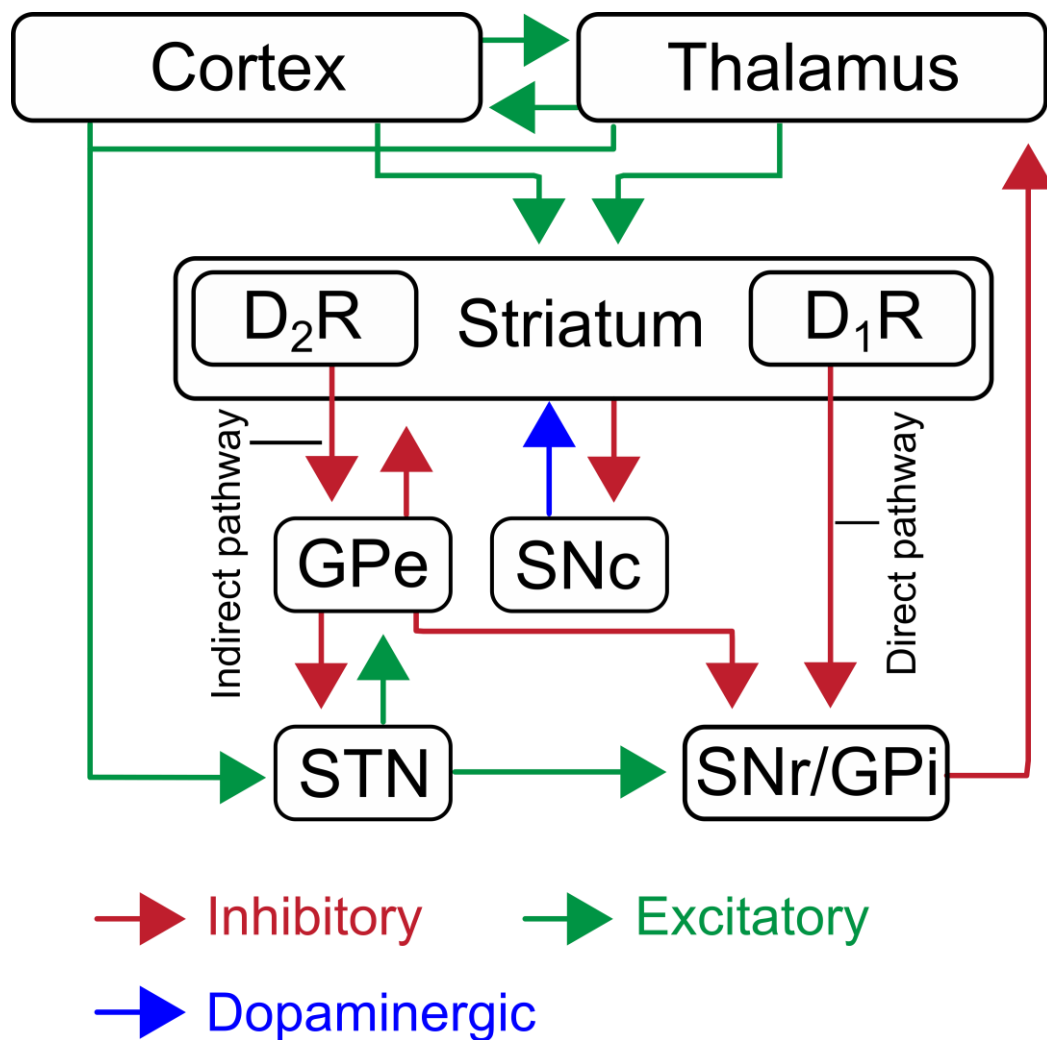


Figure 1.1 Striatal connectivity within the classical model of the basal ganglia.

A simplified representation of the major signalling pathways between the cortex, thalamus and basal ganglia nuclei. Indirect pathway represents D<sub>2</sub> DA receptor (D<sub>2</sub>R)-positive spiny projection neuron projections to GPe. Direct pathway represents D<sub>1</sub> DA receptor (D<sub>1</sub>R)-positive spiny

mainly as the limbic-motor interface and participates in reward-based learning and motivated behaviours (Carlezon et al., 1995; Ikemoto, 2007; Kalivas and McFarland, 2003; Rodd-Henricks et al., 2002; Sellings and Clarke, 2003). These regions both have unique innervation patterns and send projections out to distinct target structures. The dorsal striatum is a key integration centre of the basal ganglia loop, while the ventral stratum is important for integrating inputs in the limbic basal ganglia loop. The relative positioning of

striatal structures varies considerably between primates and rodents, although the cellular structure and function is largely conserved. In primates, the caudate nucleus and putamen are structurally and functionally distinct (Gerfen and Bolam, 2010). The caudate nucleus is found lateral of the anterior horn of the lateral ventricles, while the putamen sits more caudally, medial to the external capsule and lateral to the globus pallidus. The caudate and putamen are physically separated in primate by white matter of the internal capsule, with minor overlap in inputs to the two structures (**Figure 1.2**). The caudate nucleus primarily receives inputs from the prefrontal cortex, while putamen receives primary inputs from motor and somatosensory cortex. The NAc is adjacent to the two structures, although delineated by the ventromedial end of the internal capsule. In rodents, the striatum consists of one structure, positioned ventrally of the external capsule and lateral to the dorsal horns of the lateral ventricles. The caudate nucleus and putamen form one contiguous nucleus known as the caudate-putamen (CPu) and the anatomical boundaries between CPu and NAc are less evident (**Figure 1.3**). Despite less anatomically distinct divisions of striatal nuclei in rodent, the topographic pattern of afferent fibres is similar to that exhibited in primate.

The striatum can also be distinguished according to variable “patchy” expression of certain biochemical markers, which correspond to striosome and matrix regions (Gerfen et al., 1987). Striosomes are characterised by high levels of  $\mu$ -opioid receptor, D1-receptor, substance P and calretinin, whereas matrix is enriched with calbindin and acetylcholine (ACh) esterase (Besson et al., 1988; Crittenden and Graybiel, 2011; Graybiel and Ragsdale, 1978; Sakamoto et al., 1992). The matrix is thought to comprise approximately 85% of the striatal complex, while the striosome represents the remainder 15%. The functional implications of striosome-matrix divisions are not yet fully understood; however, they are known to differ in terms of their development, circuitry and disease susceptibility (Crittenden and Graybiel, 2011; Graybiel and Ragsdale, 1978).

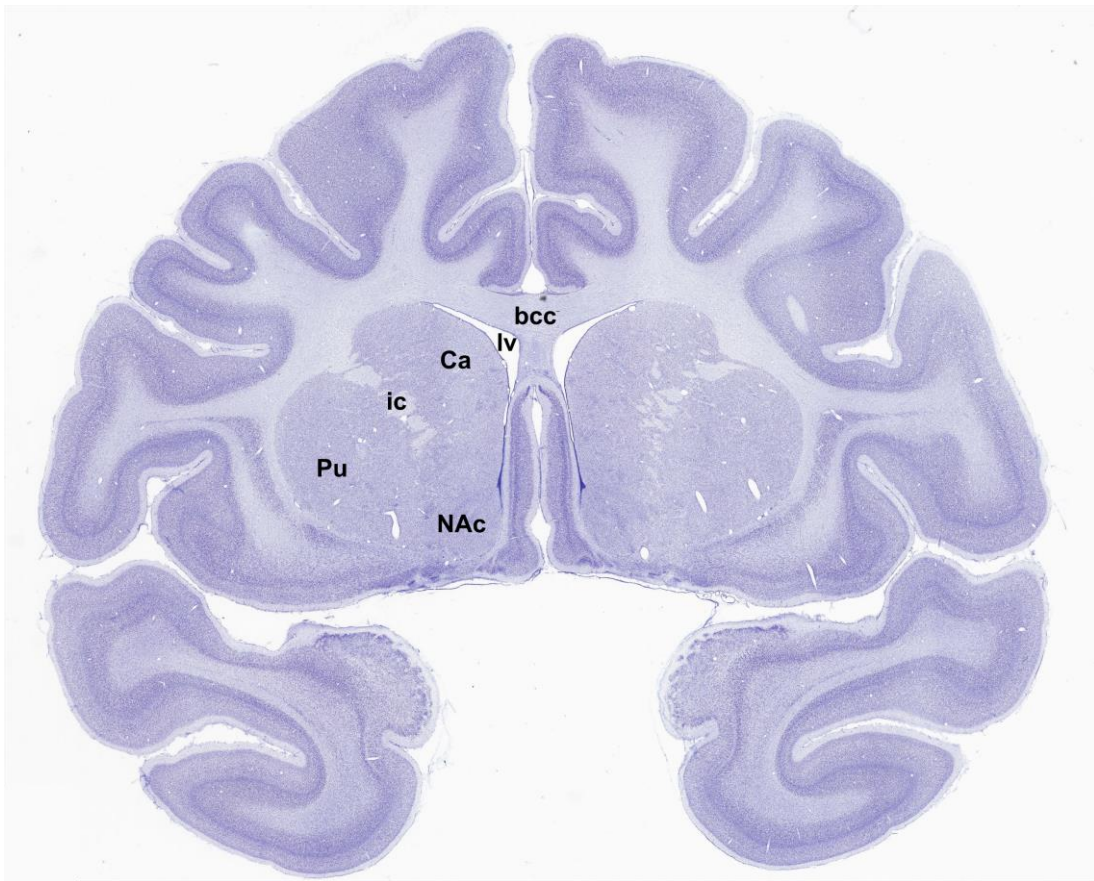


**Figure 1.2 Coronal brain section containing striatum from non-human primate.**

Coronal section of rhesus macaque (*macaca mulatta*, 24 months) brain with Nissl stain. Note caudate (Ca) and putamen (Pu) are physically separated by white matter of the internal capsule (ic), with minor overlap in inputs to the two structures. bcc, main body of the corpus callosum; lv, lateral ventricle; NAc, nucleus accumbens. Image from the NIH Blueprint Non-Human Primate (NHP) Atlas 2009.

### 1.2.2 Spiny projection neurons

The striatum is composed primarily of spiny projection neurons (SPNs), consisting of approximately 90-95% of all striatal cell counts (Bolam et al., 2000; Graveland and DiFiglia, 1985; Kemp and Powell, 1971a, 1971b; Smith and Bolam, 1990). Striatal neurons are homogeneously distributed, such that the striatum lacks a distinct cytoarchitectural organisation, unlike for example the laminar organisation of the cortex. SPNs integrate afferent inputs with local synaptic and neuromodulatory signalling and project to downstream basal ganglia nuclei, and as such are the primary input targets and the major



**Figure 1.3. Coronal brain section containing striatum from rodent.**

Coronal section of mouse (*mus musculus*, 4 months) brain with Nissl stain. Note caudate nucleus and putamen form one contiguous nucleus known as the caudate-putamen (CPu). bcc, main body of the corpus callosum; lv, lateral ventricle; ac, anterior commissure; NAc, nucleus accumbens core. Image from the Allen Mouse Brain Atlas 2004.

output neuron of the striatum (Figure 1.1). SPNs signal with GABA and contain the GABA synthesis enzyme glutamate decarboxylase (GAD) (Kita and Kitai, 1988). The primary axons of SPNs project out of the striatum to the globus pallidus and substantia nigra pars reticulata (SNr) (Kawaguchi et al., 1990). These neurons also feature a local axon collateral (Somogyi et al., 1981) which mediate a network of inter-inhibition, such that stimulation of a given SPN has the ability to inhibit surrounding SPNs (Guzmán et al., 2003; Tunstall et al., 2002).

SPNs are unique in that they have the ability to switch between periods of high and low excitability, known as 'up'- and 'down'-states, with corresponding resting membrane

potentials of approximately  $-60$  mV and  $-80$  mV respectively (Mahon et al., 2006; Plenz and Kitai, 1998; Wilson et al., 1996). The hyperpolarised resting down-state is maintained by inwardly-rectifying potassium channels (Nisenbaum and Wilson, 1995), which inactivate with depolarisation, such that concurrent activity in excitatory synaptic inputs will switch and maintain SPNs in the up-state (Plotkin et al., 2011; Wilson et al., 1996). Excitatory inputs to SPNs arise principally in the cortex and thalamus (Guo et al., 2015; Nauta et al., 1974; Somogyi et al., 1981). Both populations form asymmetric glutamatergic synapses onto SPNs (Doig et al., 2010), that differ in terms of their subcellular targets (Dubé et al., 1988; Smith et al., 1994). Corticostriatal projections form synapses principally onto dendritic spines on SPNs (Dubé et al., 1988; Xu et al., 1989) and form sparse connections with a large number of SPNs, such that the activation of a given SPN depends on synchronised activity in a specific set of corticostriatal projections (Yim et al., 2011; Zheng and Wilson, 2002). Projections from other basal ganglia nuclei also form inputs to SPNs, including GPe (Bevan et al., 1998; Guo et al., 2015; Saunders et al., 2016), and elsewhere in the brain, such as the hippocampus, amygdala and the dorsal raphe nuclei (Guo et al., 2015; Voorn et al., 2004; Wall et al., 2013). SPNs also receive inputs from local interneurons and neuromodulatory input from midbrain DA neurons. Dopaminergic modulation of SPN activity is discussed in Section 1.3.4.

Two major subpopulations of SPNs, of approximately even numbers, can be defined on the basis of their projection targets and expression of DA  $D_1$  or  $D_2$  receptor types (Albin et al., 1989; Gerfen et al., 1990; Surmeier et al., 1996). Direct-pathway SPNs (dSPNs), which express  $D_1$ -type DA receptors, send monosynaptic projections to the output nuclei of the basal ganglia, the internal segment of the GP (GPi) and the SNr. Activation of dSPNs therefore inhibits neurons in the GPi and SNr and consequently disinhibits the activity in the target regions of those outputs. Indirect-pathways SPNs (iSPNs), which express  $D_2$ -type DA receptors, project to the external segment of the GP (GPe), synapsing onto neurons that project to the GPi and SNr. Activation of iSPNs therefore inhibits neurons in the GPe,

disinhibiting GPi neurons, and consequently inhibiting activity in target regions of basal ganglia output (Figure 1.1).

According to this simplified model of basal ganglia function, the roles of the direct- and indirect-striatal output pathways are to promote particular actions by disinhibition of the relevant basal ganglia targets, and to inhibit possible ongoing actions by parallel inhibition of alternative targets (Alexander and Crutcher, 1990; Nelson and Kreitzer, 2014). In support of this model, the selective activation of dSPNs promotes the initiation and maintenance of movement, while activation of iSPNs results in freezing behaviour (Freeze et al., 2013; Kravitz et al., 2010). Furthermore, selective ablation of dSPNs or iSPNs leads to hypolocomotion and hyperlocomotion, respectively, and results in impaired motor learning in both cases (Durieux et al., 2012). However, it is largely accepted that this model described is largely oversimplified and does not account for all basal ganglia elements. As such, the intricacies and roles of the two striatal output pathways are still under investigation.

### 1.2.3 Striatal interneurons

In addition to SPNs, the striatum hosts a diversity of biochemically and physiologically distinct interneurons, which make up approximately 3-5% of the total neuronal count (Tepper and Bolam, 2004). All of these interneurons are GABAergic in nature, except for cholinergic interneurons which signal primarily through the release ACh.

The most extensively-characterised GABAergic striatal interneuron is the fast-spiking interneuron (FSI), which express the calcium-binding protein parvalbumin (PV) and demonstrate fast-firing frequencies (Kawaguchi, 1993). FSIs are unique in that they exhibit increasing expression along the medial-lateral striatal axis and therefore influence striatal output most significantly in dorsolateral striatum (Gerfen, 1985). FSIs receive input from sensory and motor cortices (Ramanathan et al., 2002) and inhibitory input from PV-expressing projection neurons in the GPe (Bevan et al., 1998; Saunders et al., 2016). FSIs provide powerful feed-forward inhibition onto SPNs (Koos et al., 2004; Tepper and Koós,

1999), mediated through their formation of “pericellular baskets” around the somata of both dSPNs and iSPNs (Gittis et al., 2010; Planert et al., 2010). FSIIs are also unique in that they form electrical synapses with neighbouring FSIIs via gap junctions, which are thought to promote synchrony throughout the FSII population (Hjorth et al., 2009; Kita et al., 1990; Tepper and Koós, 1999). FSIIs are hypothesised to play crucial roles in the control of action selection through their potent inhibitory control of SPNs (Gage et al., 2010; Gittis et al., 2011), and recent work indicates that FSIIs exhibit ensemble activity that encodes the speed of action sub-components (Roberts et al., 2019).

Another class of GABAergic striatal interneurons are the low-threshold spiking interneurons (LTSIs) which express somatostatin, neuropeptide Y (NPY) and synapse onto distal dendritic spines of SPNs (Kubota and Kawaguchi, 2000). LTSIs demonstrate prolonged plateau potentials in response to depolarisation, fire action potentials at low thresholds and exhibit autonomous firing in striatal slices (Beatty et al., 2012). Behaviourally, LTSIs are suggested to play an important role in goal-directed learning (Holly et al., 2019). A number of other minor populations of GABAergic interneurons have been identified in the striatum, including tyrosine hydroxylase (TH)-positive GABAergic interneurons, amongst many others (Tepper et al., 2018), but their physiological properties and roles within the striatum are less well-characterised.

In contrast to the GABAergic interneurons, cholinergic interneurons (ChIs) are not primarily GABAergic and release ACh. ChIs are identified by their characteristically large soma (20–50  $\mu\text{m}$ ) and lack of dendritic spines (Kawaguchi, 1993), and exhibit decreasing density along the anterior-posterior striatal axis (Matamales et al., 2016). Despite being so few in number, ChIs have an enormous influence throughout the striatum by virtue of their extended dendritic trees, dense axonal arbours, and the large number of vesicle containing varicosities (Hoover et al., 1978; Kawaguchi, 1993). ChIs signal by point-to-point transmission, but are also thought to partially operate through non-synaptic volume

transmission of ACh (Contant et al., 1996; Descarries et al., 1997). ACh exerts its postsynaptic effect at nicotinic ACh receptors (nAChRs), which are fast ligand-gated cation channels, and muscarinic ACh receptors, which are modulatory G-protein coupled receptors. In addition, recent evidence indicates that approximately half of all striatal ChIs are capable of GABA co-release, indicating a GABAergic role for these interneurons (Lozovaya et al., 2018).

In the absence of synaptic input, ChIs autonomously fire due to an intrinsic pacemaker mechanism and as such are tonically active in striatal slice preparations (Bennett and Wilson, 1999). ChIs synapse directly onto SPNs, with symmetric synapses distributed between the soma, dendrites and spines (Izzo and Bolam, 1988), and support corticostriatal plasticity here through activation of  $M_4$  muscarinic receptors expressed on SPNs (Pakhotin and Bracci, 2007; Pancani et al., 2014). Furthermore, cholinergic signalling also exerts powerful control over striatal DA release through action at nAChRs, which will be reviewed in **Section 1.3.3**. The behavioural role for striatal ChIs is still largely unknown, but involvement in reward-related signalling and learning is evident (Apicella, 2002; Morris et al., 2004; Schulz and Reynolds, 2013).

### 1.3 Mesostriatal dopaminergic system

Midbrain DA neurons send projections to various brain regions, including various cortical regions, amygdala, hippocampus, lateral habenula, septum, sub-thalamic nucleus, and most notably, to striatum (Yelnikoff et al., 2014). The striatum is densely innervated by two populations of mesostriatal dopaminergic neurons, which signal onto striatal DA receptors to facilitate a wide-range of striatal-dependent behaviours. This section will briefly review the anatomy of mesostriatal DA projections, the regulation of DA signalling in the striatum and the role of mesostriatal DA signalling in behaviour.

### 1.3.1 Mesostriatal dopamine projections

The cell bodies of the dopaminergic projections that innervate the striatum are located in the ventral midbrain, the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA), in groups numbered A8 to A10 from the caudolateral to rostromedial aspects (Andén et al., 1966), which send dopaminergic projections to the striatum (Björklund and Dunnett, 2007). Importantly, within these principal DA neurons contain a smaller population of GABAergic neurons that make local connections within the midbrain and also send projections to target structures including the striatum (Van Bockstaele and Pickel, 1995; Ciccarelli et al., 2012; Nair-Roberts et al., 2008).

The DA neurons within the SNc can be further subdivided into a dorsal tier, in which the DA neurons express the calcium buffering protein calbindin, and a calbindin-negative ventral tier more susceptible to neurodegeneration in Parkinson's disease (Yamada et al., 1990). DA neurons in the VTA are also positive for calbindin and less susceptible to neurodegeneration in Parkinson's disease (Lavoie and Parent, 1991). Several other biochemical markers can also be used to identify anatomical subdivisions of midbrain DA neurons. For example, DA neurons located in the ventrolateral tier of the SNc express the enzyme aldehyde dehydrogenase-1a1 (ALDH1a1), an enzyme important for oxidation of the neurotoxic DA intermediate DOPAL to DOPAC (Marchitti et al., 2007; Rees et al., 2009), and the synthesis of GABA in DA neurons (Kim et al., 2015), whereas DA neurons in the dorsomedial SNc are ALDH1a1-negative (McCaffery and Dräger, 1994). Like with calbindin expression, DA neurons which express ALDH1a1 appear to be less susceptible to neurodegeneration in Parkinson's disease (Liu et al., 2014).

Midbrain DA neurons generate relatively broad action potentials (>2ms) at frequencies up to 10 Hz and exhibit autonomous tonic firing at an average rate of approximately 4 Hz (Clark and Chiodo, 1988; Grace and Bunney, 1984a; Lacey et al., 1989). This autonomous tonic firing is driven by an intrinsic ionic pacemaking mechanism, in which sodium and calcium

channels gradually drive the membrane to a spiking threshold via slow sub-threshold oscillatory potentials (Bean, 2007; Chan et al., 2007; Puopolo et al., 2007). *In vivo*, DA neurons still exhibit low-frequency autonomous firing, interspersed with firing bursts of 2-5 spikes at 10-40 Hz (Grace and Bunney, 1984b, 1984a), with bursts up to 100 Hz also reported (Hyland et al., 2002). Burst firing is thought largely to be generated by NMDA receptor activation (Charlley et al., 1991) via excitatory synaptic inputs to the midbrain from the pedunculopontine nucleus (Floresco et al., 2003; Lokwan et al., 1999) and subthalamic nucleus (Chergui et al., 1994; Smith and Grace, 1992).

The axons of these dopaminergic neurons project differently throughout striatum, with topographic organisation. The dorsal striatum (or CPU) receives dense dopaminergic innervation primarily from the SNc (A9) and to a lesser degree from the VTA (A10), commonly known as the nigrostriatal DA system (Björklund and Dunnett, 2007). The ventral striatum (or NAc) receives dopaminergic innervation primarily from the VTA (A10) and to a lesser degree the from the SNc (A9), commonly known as the mesolimbic DA system (Björklund and Dunnett, 2007).

Each DA neuron projects a single, unmyelinated axon to the striatum that emanates from the shaft of a principle dendrite, rather than canonically from the soma (Tepper et al., 1987). DA neurons project their axons in the rostral direction to form the medial forebrain bundle, a loose collection of white matter projecting from the midbrain through the lateral hypothalamus to the olfactory tubercle, before reaching the final targets, i.e. striatum and the prefrontal cortex (Beckstead et al., 1979; Nauta et al., 1978; Ungerstedt, 1971). Within the striatum, DA axons branch excessively to form immense axonal arbours. It is estimated that each mesostriatal DA axon contains up to 25,000 branch points with a total membrane length up to 780 mm, reaches on average ~3% of striatum, and form  $\sim 10^5$  en passant varicosities in rat striatum (Matsuda et al., 2009; Pissadaki and Bolam, 2013). These large and highly complex mesostriatal DA axon arbours are a unique attribute to DA neurons,

and are major strategic sites for governing DA output through local mechanisms that are distinct from those governing action potential generation in the midbrain (Rice et al., 2011; Schmitz et al., 2003; Sulzer et al., 2016).

### 1.3.2 Mesostriatal dopamine release

Mesostriatal DA neuron form occasional symmetrical synapses which mainly contact the necks of dendritic spines of SPNs (Bolam et al., 2000). However, many axonal DA varicosities are not associated with postsynaptic densities (Descarries et al., 1996) and DA receptors are mostly localised outside of striatal synapses (Caillé et al., 1996; Uchigashima et al., 2016; Yung et al., 1995). Given these properties, and that DA receptors are G-protein coupled, whose signalling is more than two orders of magnitude slower than that of ionotropic receptors (Missale et al., 1998), it is thought that DA primarily exerts its effects through volume transmission (Cragg and Rice, 2004). Volume transmission of striatal DA release is further supported by the low transport rates and primarily extrasynaptic localisation of the DA transporter (Hersch et al., 1997; Povlock and Schenk, 2002), which facilitate diffusion of DA away from the release sites. This is in contrast to uptake transporters at fast point-to-point synapses, which limit spillover through fast rates of uptake and close spatial coupling to release sites (Danbolt, 2001).

Despite the extrasynaptic actions of DA release via volume transmission, the synaptic mechanisms which facilitate DA release are fundamentally thought to be equivalent to those at central synapses: DA is stored in small synaptic vesicles and it shows action potential- and calcium-dependent quantal release (Rice et al., 2011; Sulzer and Pothos, 2000). Furthermore, striatal DA axons contain active zone-like sites with protein assemblies consisting of bassoon, RIM, and ELKS in ~30% of DA varicosities, which support a high initial release probability with rapid kinetics (Liu et al., 2018).

### 1.3.3 Local striatal modulation of dopamine release

The firing of DA neurons at the level of the midbrain can be loosely correlated to DA release in the striatum, with burst firing in the midbrain suggested to increase extracellular DA levels in striatum by ~3-10 fold compared to tonic firing alone (Dreyer et al., 2010; Suaud-Chagny et al., 1992). However, it is increasingly appreciated that DA axons do not faithfully convert somatodendritic action potential activity to neurotransmitter output as DA release and DA axon activity in the striatum can be dissociated from that of neuronal activity in the midbrain (Mohebi et al., 2019) and DA release in striatum can still be observed *in vivo* following acute transection in front of substantia nigra to cut through all DA fibres (Romo et al., 1986). Rather, striatal DA release is gated by axonal mechanisms and striatal neuromodulators that regulate or even independently drive DA release (Rice et al., 2011; Schmitz et al., 2003; Sulzer et al., 2016), which might underlie the distinct striatal DA signals that can be dissociated from those of neuronal activity in the midbrain (Mohebi et al., 2019; Romo et al., 1986). Furthermore, the large number of branch points in striatal DA axons (Matsuda et al., 2009) might promote increased instances of branch point failure, which is observed in other highly branched structures (Grossman et al., 1979a, 1979b; Lüscher and Shiner, 1990). Branch point failure might therefore also contribute to DA neuronal activity in the midbrain that is dissociated from DA release in the striatum.

One of the most significant local neuromodulators of striatal DA release is ACh released from ChIs. Striatal DA axons express nicotinic ACh receptors (nAChRs) containing the  $\beta 2$  subunit (Jones et al., 2001) which, when activated, promote powerful short-term depression of DA release such that release is almost entirely insensitive to the frequency or duration of presynaptic stimulation (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Equally, when nAChRs are inhibited, evoked DA release becomes frequency-dependent. In addition to gating release, synchronous activation of ChIs can evoke DA release through a nAChR-dependent mechanisms, independent of presynaptic activity and action potential generation at the soma level (Cachope et al., 2012; Threlfell et al., 2012). Given ChIs

powerfully modulate and can independently drive DA release, they can also mediate the effects of other neuromodulators on DA release, including opioids, nitric oxide, glutamate, and insulin (Britt and McGehee, 2008; Hartung et al., 2011; Kosillo et al., 2016; Stouffer et al., 2015). For these reasons, it is necessary to occlude cholinergic signalling at nAChRs on DA axons in order to study the intrinsic properties of DA release and how other striatal neuromodulators might govern DA release through action directly on DA axons. All experiments in this thesis (unless otherwise noted) have been performed in the presence of an antagonist at  $\beta 2$ -subunit-containing nAChRs to remove cholinergic input at DA axons (see Section 2.2.5 for details).

Striatal DA release is also subject to multiple regulatory mechanisms outside the cholinergic axis. In addition to ACh, there are many other neuromodulators and neurotransmitters in the striatum including small molecules (e.g. ATP, nitric oxide, hydrogen peroxide), peptides (e.g. substance P), monoamines (e.g. serotonin) and excitatory/inhibitory neurotransmitters (e.g. glutamate, GABA). Many of these have been shown to modulate DA release (Avshalumov et al., 2003; Brimblecombe and Cragg, 2015; Hartung et al., 2011; Kosillo et al., 2016), however for others their potential effects on striatal DA release remain unexamined. Furthermore, it is important to note that these mechanisms which regulate striatal DA release can significantly diverge between dorsal and ventral striatal territories (Brimblecombe et al., 2015; Britt and McGehee, 2008; Janezic et al., 2013; Shin et al., 2017; Threlfell and Cragg, 2011; Threlfell et al., 2010), therefore novel regulators of DA release should be examined across striatal territories. Unravelling the local mechanisms which can modulate striatal DA release is critical for understanding striatal function and identifying novel targets for disease-modifying therapies of disorders with DA dysfunction. Evidence suggest that striatal GABA might directly regulate DA release (see Section 1.5.6) and this thesis investigates this potential GABA-DA interaction in Chapter 3.

### 1.3.4 Dopamine modulation of striatal processing

As discussed, mesostriatal DA signalling plays a central role in the functions of the basal ganglia. DA exerts its effects in striatum through binding and activating metabotropic G-protein-coupled DA receptors, of which there are five known types, D<sub>1</sub>-D<sub>5</sub>. These fall into two categories; D<sub>1</sub>-type, comprising D<sub>1</sub> and D<sub>5</sub>, and D<sub>2</sub>-type, comprising D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> (Beaulieu and Gainetdinov, 2011). D<sub>1</sub>-like receptors are G<sub>αs</sub> coupled, which activates adenylyl cyclase (AC) leading to increased cyclic adenosine monophosphate production (cAMP) production. D<sub>2</sub>-like receptors are G<sub>αi</sub> coupled, which inhibits AC and decreases cAMP production. D<sub>2</sub> receptors facilitate different effects of DA signalling depending on their splice variant; the D<sub>2S</sub> variant is expressed presynaptically and functions as an inhibitory autoreceptor, while the D<sub>2L</sub> variant mediates postsynaptic effects (Beaulieu and Gainetdinov, 2011; Usiello et al., 2000). D<sub>1</sub>-like receptors do not exhibit splice variants. In the striatum, D<sub>1</sub> and D<sub>2</sub> receptors are the most abundantly expressed receptor type (Levey et al., 1993).

DA neurons express D<sub>2</sub> autoreceptors at the level of the soma and at axon release sites. D<sub>2</sub> receptor activation at the soma induces a hyperpolarising outward current mediated by the opening of G-protein linked inwardly-rectifying potassium (GIRK) channels (Beckstead et al., 2004; Lacey et al., 1988). At axonal release sites, D<sub>2</sub> receptor activation decreases DA release facilitated by the coupling to voltage-gated potassium channels (Martel et al., 2011). D<sub>2</sub> receptor activation on DA neurons is also suggested to decrease DA synthesis through downstream phosphorylation of TH (Lindgren et al., 2001).

As described in **Section 1.2.2**, postsynaptic D<sub>1</sub> and D<sub>2</sub> receptors are differentially expressed on SPNs, with D<sub>1</sub> receptors expressed by direct pathway SPNs and D<sub>2</sub> receptors expressed by indirect pathway SPNs. Activation of D<sub>1</sub> receptors expressed by dSPNs increases their excitability and promotes firing during up-states by activations of L-type calcium channels (Hernández-López et al., 1997; Planert et al., 2013; Vergara et al., 2003). Conversely,

activation of D<sub>2</sub> receptors expressed by iSPNs decreases their excitability through reducing L-type calcium channel current and activating hyperpolarising potassium currents (Hernandez-Lopez et al., 2000; Perez et al., 2006; Planert et al., 2013). DA release in the striatum therefore differentially modulates the excitability of the two main output pathways of the striatum, through which striatal DA signalling consequentially functions to modulate the balance between these parallel processing systems.

DA release in striatum can also modulate the activity of local interneurons. D<sub>1</sub> and D<sub>5</sub> receptors are widely expressed by striatal GABAergic interneurons, including PV-positive fast-spiking interneurons and somatostatin/NPY-positive low-threshold spiking interneurons (Le Moine et al., 1991; Rivera et al., 2002), which upon activation induces depolarisation and increases input resistance to excite these GABAergic interneurons (Bracci et al., 2002). ChIs also express D<sub>2</sub> receptors (Alcantara et al., 2003), which upon activation decrease ACh release thorough limiting calcium entry (Stoof et al., 1987; Yan et al., 1997), an effect that is more pronounced in the dorsal striatum (Henselmans and Stoof, 1991).

DA signalling can additionally regulate striatal processing through facilitating corticostriatal plasticity, which plays a central role in the reinforcement of action selection in the striatum. Long-term plasticity at corticostriatal synapses onto SPNs changes the weight of excitatory inputs, which when targeted to specific populations of SPNs will bias striatal output towards the selection of specific actions, and allow simultaneous suppression of alternative actions (Cui et al., 2013; Freeze et al., 2013; Yin et al., 2009). Corticostriatal plasticity is largely dependent on striatal DA signalling (Calabresi et al., 2007; Kreitzer and Malenka, 2008; Reynolds and Wickens, 2002; Wang et al., 2006), and has been shown to be important for striatal reward-related learning, motor learning and general motor output (Calabresi et al., 2007; Fisher et al., 2017; Reynolds et al., 2001). DA signalling in the striatum is therefore well-positioned to modulate striatal processing, through modulating the activity of principal

striatal output neurons both directly and indirectly through modulating interneuron activity and corticostriatal plasticity.

### 1.3.5 Behavioural roles for mesostriatal dopamine release

Early work characterising the behavioural role of DA was guided by the discovery of DA depletion in Parkinson's disease (PD), which results in prominent motor dysfunction (Ehringer and Hornykiewicz, 1960). Indeed, DA plays an important role in the control of motor function in the basal ganglia and early studies report increased DA neuron firing on initiation of actions (Romo and Schultz, 1990; Schultz et al., 1983). A recent study has substantiated a role for striatal DA signalling in motor control as DA axons in the dorsal striatum signal the initiation of actions (Howe and Dombeck, 2016).

Other studies have indicated key roles for DA in reward-related learning. Recordings from midbrain DA neurons from awake behaving animals show phasic increases in firing upon the presentation of an unexpected reward, conversely if an expected reward is not presented firing activity decreases (Schultz and Romo, 1990). When the reward is paired with a predictive cue, with training, the timing of this phasic increase in firing becomes progressively time-locked to the presentation of the cue and not on the presentation of the reward itself (Mirenowicz and Schultz, 1994; Schultz and Romo, 1990; Schultz et al., 1997). Furthermore, the response to the reward is scaled on reward size (Mirenowicz and Schultz, 1996; Morris et al., 2004; Schultz, 2007) and corresponds with an increase in extracellular DA in the striatum (Day et al., 2007; Phillips et al., 2003; Roitman et al., 2004). Striatal DA therefore plays a key role in many fundamental behavioural including movement, motivation, and reward.

## 1.4 Co-release of dopamine and other primary neurotransmitters

In addition to the synthesis and release of DA, some dopaminergic neurons have the capacity to synthesise, co-store and co-release other primary neurotransmitters from axonal release sites in the striatum. These include the co-release of glutamate and DA (Adrover et

al., 2014; Chuhma et al., 2004; Stuber et al., 2010; Tecuapetla et al., 2010) and/or co-release of GABA and DA (Kim et al., 2015; Tritsch et al., 2012, 2014).

It has been established for many decades that exocytosis of one or more neuropeptides accompanies the release of classical small-molecule neurotransmitters across the majority of neuron types in the mammalian brain (Burnstock, 2004; Nusbaum et al., 2001). However, the 'one neuron, one neurotransmitter' doctrine (commonly known as Dale's principle)<sup>1</sup> has until recently dominated our understanding of neuronal function and communication, and states that communication between two neurons occurs through the release of one single chemical neurotransmitter (Strata and Harvey, 1999). With the advent of optogenetics, more recent findings suggest that neurons can communicate using more than one classical neurotransmitter (Vaaga et al., 2014), with neurotransmitter co-release and/or co-transmission becoming the norm and not the exception.

The term neurotransmitter co-release describes the phenomena whereby two (or more) neurotransmitters are released by a single neuron in response to an action potential and is restricted to release by vesicular exocytosis (Tritsch et al., 2016; Vaaga et al., 2014). Co-release can result from packaging of multiple neurotransmitter molecules into individual synaptic vesicles or from simultaneous membrane fusion of vesicles filled with distinct neurotransmitter molecules. The term co-transmission also refers to release of multiple molecules from the synapse of a single cell, but it does not require exocytosis and therefore is inclusive of gaseous neurotransmitter molecules. Co-transmission implies that both neurotransmitters contribute to synaptic transmission by evoking a postsynaptic response in the same postsynaptic cell (Burnstock, 2004). Therefore, co-release does not always imply

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<sup>1</sup>Sir Henry Dale (1875-1968) who earned the Nobel Prize in Physiology or Medicine in 1936 with Otto Loewi for establishing the chemical basis of neurotransmission, is often cited for proposing the 'one neuron, one neurotransmitter' doctrine. However, Dale never embraced the principle, but instead recognised the possibility that neurons might release more than one chemical transmitter (Strata and Harvey, 1999). 'Dale's principle' was actually introduced by John Eccles in 1954 in reference to Dale's hypothesis that neurons release the same neurotransmitter(s) across all of their synapses, which was largely misinterpreted as neurons release one, and only one, neurotransmitter type.

co-transmission e.g. if the postsynaptic cell does not express receptors for both neurotransmitters.

### 1.4.1 Mesostriatal glutamate and dopamine co-release

Glutamate and DA co-release from DA axons has been shown to occur in the striatum. Glutamate co-release was originally thought to be restricted to DA neurons projecting to the ventral striatum (Chuhma et al., 2004; Stuber et al., 2010; Tecuapetla et al., 2010), however more recent studies have reported monosynaptic EPSCs in SPNs and ChIs in the dorsal striatum in response to selective activation of DA axons (Chuhma et al., 2018; Tritsch et al., 2012). Glutamate is packaged into vesicles in DA axons through the vesicular glutamate transporter (VGLUT2), and has enriched expression in DA neurons in the VTA versus the SNc (Kawano et al., 2006; Mendez et al., 2008), which might help explain the apparent regional striatal heterogeneity in glutamate and DA co-release. Mesostriatal DA neurons which are positive for TH and VGLUT2 exhibit segregation of TH and VGLUT2 to distinct subcellular compartment of the DA axon in striatum: VGLUT2-positive axonal segments form asymmetric synapses onto SPN spines separate from the symmetric synapses formed by TH-positive DA release sites (Zhang et al., 2015), indicating that DA and glutamate can be released by the same axons, but are likely not released at the same site or from the same synaptic vesicle. However, follow up studies report that these microdomains are not as spatially exclusive as originally proposed (Zhang et al., 2015), as considerable co-localisation of VMAT2 and VGLUT2 are observed along mesoaccumbens DA axons (Silm et al., 2019).

The behavioural role of glutamate and DA co-release in striatum remains unclear, although emerging studies have explored the effects of preventing glutamate co-release from DA axons on striatal-dependent behaviours. Mice with conditional deletions of VGLUT2 in DA neurons have reduced DA release in NAc, resulting in reduced hyperlocomotion following cocaine administration (Hnasko et al., 2010), although these effects are thought to be

underpinned by resultant disruptions in vesicular DA storage (see Section 1.4.3), and not reduced glutamate signalling postsynaptically. Additionally, mice with the conditional heterozygous reduction of the glutamate recycling enzyme glutaminase in DA neurons show normal emotional and motor behaviours and show an unaffected response to acute amphetamine. These mice do exhibit reduced sensitisation to amphetamine and potentiated latent inhibition, suggesting that glutamate co-release might be important in attribution of motivational salience (Mingote et al., 2017). Importantly, unlike in mice with the selective VGluT2 knockout in DA neurons, these mice do not exhibit altered striatal DA transmission. In NAc dorsal medial shell, all DA neuron inputs are positive for TH and VGluT2 and therefore capable of glutamate co-release. These inputs make strong excitatory connections onto ChIs to drive burst firing, and mediate the switching of behavioural responses under circumstances of altered cue-reinforcer contingencies (Chuhma et al., 2018).

#### 1.4.2 Mesostriatal GABA and dopamine co-release

GABA co-release from DA axons in striatum is less well-described than glutamate co-release. Selective activation of DA axons in the dorsal and ventral striatum monosynaptically inhibits action potential firing in postsynaptic SPNs through the calcium-dependent release of a GABA<sub>A</sub> receptor agonist, which is thought to be mediated by GABA co-release from DA axons (Kim et al., 2015; Tritsch et al., 2012, 2014). GABA is thought to be co-stored and co-released from DA vesicles, as co-release of GABA is dependent on the activity of the vesicular monoamine transporter (VMAT2), which can be replaced by exogenous expression of the vesicular GABA transporter (VGAT) (Tritsch et al., 2012). Furthermore, ultrastructure data show the co-localisation of GABA to ~10-15% of VMAT-positive boutons in rodent striatum (Stensrud et al., 2014).

Synthesis of GABA in canonical GABAergic neurons is typically mediated by glutamate decarboxylase (GAD), but mouse midbrain DA neurons do not contain detectable levels of

mRNA or protein for GAD65, GAD67 or the VGAT (Kim et al., 2015; Tritsch et al., 2014). Instead, GABA synthesis in DA neurons is thought to be mediated by ALDH-1a1, an evolutionarily conserved GABA synthesis pathway used in plants (Figure 1.4), as pharmacological inhibition, global genetic deletion and midbrain knockdown of ALDH-1a1 in mice all reduced GABA co-release from DA axons in striatum by about half (Kim et al., 2015). Additionally, cytosolic accumulation of GABA in DA axons for GABA co-release is also thought to be mediated though uptake from the extracellular milieu by plasma membrane GABA transporters (GATs) presumably located on DA axons. This inference is based on the observations that mRNA for GAT-1 (and weakly for GAT-3) is present in the somata of DA neurons in SNc, and on the attenuation of GABA co-release from DA axons after the joint pharmacological inhibition of both GAT-1 and GAT-3 (Tritsch et al., 2014).

The functional role for GABA co-release in striatal circuits remains an open question. Electron microscopy studies have revealed that the majority of dopaminergic synapses terminate on the neck of the SPN spines that receive excitatory inputs from cortex and thalamus (Wickens and Arbuthnott, 2005). Although many axonal DA varicosities are not associated with postsynaptic densities (Descarries et al., 1996), and the actions of DA are

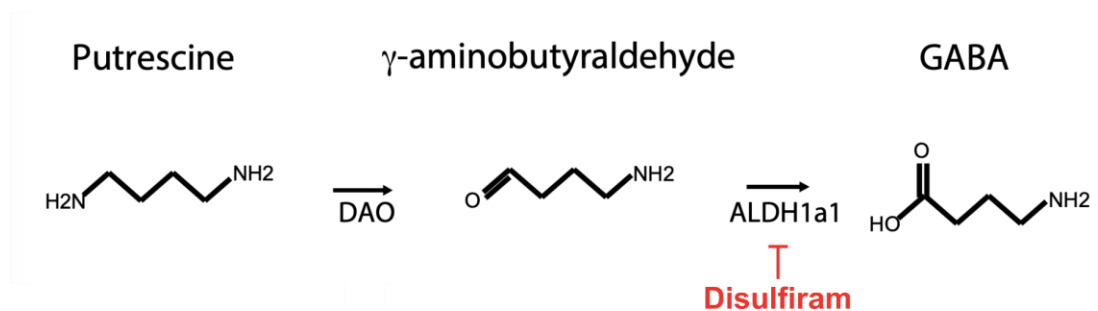


**Figure 1.4. Non-canonical GABA synthesis pathway from putrescine.**

GABA is synthesised from putrescine via a two-step conversion process catalysed by diamine oxidase (DAO) and aldehyde dehydrogenase (ALDH)-1a1. ALDH activity can be inhibited by disulfiram.

not believed to be spatially localised (Arbuthnott and Wickens, 2007), this unique synaptic arrangement might facilitate the point-to-point actions of GABA co-release. GABA co-release might function here to shunt excitatory cortical and thalamic inputs by activating GABA<sub>A</sub> receptors on SPNs, and therefore dampen ongoing excitatory drive onto SPNs to limit DA receptor-mediated plastic changes to inputs most strongly activated by salient stimuli.

Behaviourally, GABA co-release appears to play an important role in striatal-dependent reward-seeking behaviour. Diminished GABA co-release through conditional reductions in ALDH1a1 expression in DA neurons, leads to enhanced alcohol consumption and preference in mice, and intriguingly, GABA co-release is dampened by ethanol concentrations seen in blood alcohol after binge drinking (Kim et al., 2015). Furthermore, the conditional knockout of the E3-ubiquitin ligase, UBE3A, from DA neurons impairs GABA co-release from DA axons in striatum and enhances reward-seeking behaviour measured by optical self-stimulation (Berrios et al., 2016). The genetic ablation of ALDH1a1 (which will result in the attenuation of GABA co-release from DA axons) has also been reported to substantially increase DA release in striosomes, but not matrix in mouse striatum (Sgobio et al., 2017), which might underlie increased reward-seeking behaviour when GABA co-release is diminished.

### 1.4.3 Implications for mesostriatal co-release and co-storage

The potential roles that the co-release/co-transmission of neurotransmitters might play in the central nervous system are diverse. The co-release of a fast-acting neurotransmitter i.e. GABA or glutamate, with a slower-acting monoamine i.e. dopamine, allows for complex and flexible neuronal signalling that is dependent on the types of postsynaptic receptors for these neurotransmitters expressed (Vaaga et al., 2014). The synaptic effects of monoamines are achieved over hundreds of milliseconds to minutes since they signal almost exclusively through GPCRs, in stark contrast to the faster effects of transmitters acting at ionotropic

receptors i.e. GABA at GABA<sub>A</sub> receptors. Therefore, GABA co-release from DA axons in striatum might provide a temporally precise signal to time-lock sensory or motor events, not achievable by DA signalling alone (Tritsch et al., 2016).

Co-release might also provide a spatially targeted signal. Co-transmission, where it does occur, does not necessarily occur uniformly across all synapses, either because presynaptic neurons restrict the release of one of the neurotransmitters, or because postsynaptic targets do not express receptors for both neurotransmitter types. Thus, instead of broadcasting the same information to all postsynaptic targets, spatial heterogeneity in co-transmission might serve synergistic functions (Tritsch et al., 2016; Vaaga et al., 2014), which will be particularly important for systems that signal by volume transmission, i.e. DA release in striatum. This is indeed true for glutamate and DA co-release, which exhibit dopaminergic and glutamatergic subdomains across the DA axon (Zhang et al., 2015), but might also be relevant for GABA and DA co-release. GABA<sub>A</sub> receptor-mediated currents from selective activation of DA axons are not dependably recorded in all SPNs (Tritsch et al., 2012, 2014; recorded in ~75% of SPNs in dorsal striatum in this thesis), and might operate through point-to-point synaptic transmission, instead of volume transmission in the case of co-stored DA.

In addition to increasing the complexity and flexibility of synaptic transmission, neurotransmitter co-storage has been shown to have significant implications for vesicular neurotransmitter levels. The packaging of glutamate into DA vesicles can promote DA storage, through the effects of VGluT2 on the proton gradient that drives vesicular DA accumulation through VMAT2 (Hnasko et al., 2010). Similar synergistic effects are also reported for glutamate on vesicular ACh co-storage in striatal ChIs (Gras et al., 2008).

## 1.5 GABA receptor-mediated inhibition in striatum

GABA is the primary inhibitory neurotransmitter in the adult mammalian brain. GABA binds to and activates both fast-acting ionotropic GABA<sub>A</sub> receptors and slower metabotropic G-protein coupled GABA<sub>B</sub> receptors to mediate neuronal inhibition. Two forms of GABAergic

inhibition co-exist in the central nervous system to modulate the excitability of neuronal networks: phasic and tonic inhibition (Farrant and Nusser, 2005). Within the striatum, an ambient GABA tone provides tonic inhibition to principal striatal neurons, through action at GABA<sub>A</sub> and GABA<sub>B</sub> receptors. A collection of previous studies imply that DA release might similarly be under tonic inhibition by striatal ambient GABA, through direct action at GABA receptors on DA axons, which is expanded on in **Chapter 3** and **Chapter 4** of this thesis.

### 1.5.1 GABA<sub>A</sub> receptor-mediated inhibition

GABA<sub>A</sub> receptors are pentameric ligand-gated ion channels that undergo a conformational change upon binding of two agonist molecules, subsequently increasing membrane permeability to chloride and bicarbonate ions. In the large majority of mature neurons, this leads to a net inward flow of anions resulting in a hyperpolarising postsynaptic response and reduced neuronal activity. GABA<sub>A</sub> receptors can facilitate both phasic and tonic inhibition contingent on the biophysical properties of the subunits expressed and synaptic or extrasynaptic cellular location of their expression.

In phasic inhibition, quantal release of GABA from a presynaptic neuron induces a distinct high-amplitude, rapidly decaying, generally hyperpolarising event in the postsynaptic neuron, mediated by the near-synchronous activation of low-affinity synaptic GABA<sub>A</sub> receptors. This phasic mode of inhibition is defined by the short duration (< 1 ms) in which the receptors are exposed to high GABA concentrations in the millimolar range, facilitated by the rapid reuptake and diffusion away from the synapse (Mozrzymas et al., 2003; Nusser et al., 2001). Activation of phasic receptors induces inhibitory postsynaptic currents (IPSCs), characterized by high amplitudes and fast decay kinetics. The transient and specific point-to-point nature of phasic inhibition is critical in setting the temporal window for synaptic integration to precisely regulate action potential generation (Farrant and Nusser, 2005; Koos et al., 2004), and also synchronise neurons to generate rhythmic network activities (Cobb et al., 1995; Huntsman et al., 1999).

In tonic inhibition, low concentrations of extracellular ambient GABA (near  $\mu\text{M}$  levels) activate high-affinity extrasynaptic or perisynaptic GABA<sub>A</sub> receptors located outside of the active zone. The continuous, or tonic, activation of these receptors generate tonic inhibitory currents, which exhibit low amplitudes, long decay times and little or no desensitisation. Tonic currents are revealed in voltage-clamp electrophysiology recordings by their blockade with GABA receptor antagonists, which reduce the amount of current required to keep the membrane potential fixed (holding current) (Ade et al., 2008; Brickley and Mody, 2012; Brickley et al., 1996). Although tonic currents are small in amplitude relative to phasic currents, by increasing the cell's input conductance persistent tonic inhibition decreases the size and duration of excitatory postsynaptic potentials (EPSPs) while narrowing the temporal and spatial window in which synaptic integration can occur (Farrant and Nusser, 2005). Tonic GABA<sub>A</sub> receptor-mediated inhibition can therefore set the global level of neuronal excitability and powerfully regulate information processing (Scimemi, 2005; Semyanov et al., 2003).

### 1.5.2 GABA<sub>B</sub> receptor-mediated inhibition

GABA<sub>B</sub> receptors are chloride-independent receptors that mediate inhibition by heterotrimeric G-protein activation. GABA<sub>B</sub> receptors regulate neuron excitability through their locations both presynaptically and postsynaptically. Presynaptic GABA<sub>B</sub> receptors generally suppress neurotransmitter release by inhibiting calcium channels at the nerve terminal (Ulrich and Bettler, 2007), but second-messenger-mediated effects can also retard release by modulating synaptic vesicle priming (Sakaba and Neher, 2003). Postsynaptic GABA<sub>B</sub> receptors exert their effects through activating Kir3-type potassium channels, which hyperpolarise the membrane and shunts excitatory currents (Ulrich and Bettler, 2007). In addition to GABA<sub>A</sub> receptors, presynaptic GABA<sub>B</sub> receptors can also be under tonic activation by low concentration extrasynaptic GABA to tonically gate probability of neurotransmitter release (Dvorzhak et al., 2010; Le Feuvre et al., 1997; Kirmse and Kirischuk, 2006; Laviv et al., 2010; Mapelli et al., 2009).

### 1.5.3 Axonal GABA receptor-mediated neuromodulation

Axonal responses to GABA are not well-described in the central nervous system, but there is some existing evidence to indicate that GABA can play an important role in tonic modulation of axons through action at axonal high affinity GABA<sub>A</sub> receptors (Farrant and Nusser, 2005; Trigo et al., 2008). In spinal dorsal column tracts from young rats with incomplete myelination, tonic GABA<sub>A</sub> receptor activation reduces the amplitude of compound spikes, associated with a depolarisation, and pronounced slowing of conduction (Sakatani et al., 1991a, 1993). GABA sensitivity is still present in adult myelinated spinal dorsal column tracts, albeit less prominent, suggesting increased actions of tonic GABA on unmyelinated axons as well as a developmental role (Sakatani et al., 1991b). Furthermore in hippocampal mossy fibres, GABA spill-over from interneurons which tonically activate axonal GABA<sub>A</sub> receptors has significant effects on spike propagation and reduces excitability in adult rodents, the effects of which are reversed when intracellular chloride concentrations are artificially elevated (Alle and Geiger, 2007; Ruiz et al., 2003).

GABA<sub>A</sub>-mediated chloride conductance often results in a hyperpolarising potential at postsynaptic sites due to the relatively low intracellular chloride concentrations in postsynaptic compartments. This chloride-mediated increase in conductance can lead to shunting of spikes and therefore is generally functionally inhibitory (Farrant and Nusser, 2005). Intracellular chloride concentrations are relatively high in many axons, resulting in a depolarising response to axonal GABA<sub>A</sub> receptor activation (observed throughout development and into adulthood; Rocha-González et al., 2008; Sakatani et al., 1991a), however a GABA<sub>A</sub>-mediated shunting response has also been proposed for axonal segments (Verdier et al., 2003; Wall, 1995; Westberg et al., 2000). Furthermore, GABA-mediated depolarisation has been shown to affect intrinsic voltage-gated ion channels expressed in axonal segments and at nerve terminals, resulting in spike failures (Jackson and Zhang, 1995; Zhang and Jackson, 1993, 1995). Tonic activation of axonal GABA<sub>A</sub>

receptors are therefore largely inhibitory, promoting spike failure and the slowing of action potential conduction.

#### 1.5.4 Striatal extrasynaptic ambient GABA tone

As described, low concentrations of ambient GABA can have extrasynaptic actions on neuronal excitability in many brain nuclei through action at high-affinity extrasynaptic GABA<sub>A</sub> receptors to provide persistent tonic inhibition at presynaptic loci to gate the probability of neurotransmitter release. Within the striatum, where GABAergic neurons constitute the vast majority of the neuronal population (~98%; Oorschot, 1996), a sizeable ambient GABA tone provides tonic inhibition of SPNs at GABA<sub>A</sub> and GABA<sub>B</sub> receptors.

A tonic GABA<sub>A</sub> receptor-mediated inhibitory conductance has consistently been recorded in SPNs in dorsal striatum recorded from acute mouse brain slices as depolarising shifts in the baseline currents in response to GABA<sub>A</sub> receptor block (Ade et al., 2008; Cepeda et al., 2013; Janssen et al., 2009; Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014). In adult mice, SPNs express extrasynaptic and perisynaptic GABA<sub>A</sub> receptors containing  $\alpha 4$ ,  $\alpha 5$  and  $\delta$  subunits (Ade et al., 2008; Santhakumar et al., 2010), which are well-adapted for tonic signaling activated by ambient GABA tone due to their high affinity for GABA and low degree of desensitisation. In SPNs, extrasynaptic GABA<sub>A</sub> receptors mediate tonic inhibitory currents that are larger in iSPNs than in dSPNs during development (Ade et al., 2008; Janssen et al., 2009), but larger in dSPNs than in iSPNs in adult mice (Santhakumar et al., 2010), a developmental switch that is thought to occur due to changes in subunit composition (Luo et al., 2013).

This persistent tonic inhibition by striatal ambient GABA tone acting at GABA<sub>A</sub> receptors has also been demonstrated in primate striatum *in vivo*, reported to control spontaneous activity of striatal neurons (Darbin and Wichmann, 2008). Tonic activation of presynaptic GABA<sub>B</sub> receptors have also been demonstrated in SPNs recorded in mouse striatum through changes in vesicular transmitter release after the bath application of GABA<sub>B</sub>

receptor antagonists, at least under conditions with reduced GABA uptake (Kirmse et al., 2009).

Whether this striatal ambient GABA tone arises from action-potential dependent GABA release or action-potential independent i.e. due to “spontaneous” GABA release is still unclear. One study reported that TTX application abolished the GABA<sub>A</sub> receptor-mediated tonic current (Ade et al., 2008), while another indicted only a minor dependence of this inhibitory current on action-potential dependent GABA release (Wójtowicz et al., 2013). Therefore, striatal ambient GABA tone and tonic inhibition at GABA<sub>A</sub> receptors might arise from extrasynaptic spill over of both action-potential dependent and spontaneous GABA release. Given the density of GABAergic neurons within the striatum, even low rates of spontaneous vesicle release from a small fraction of these GABAergic neurons might summate sufficiently to provide a tone at GABA receptors.

### 1.5.5 Striatal GABA transporters

The concentration of ambient GABA tone, and therefore strength of tonic inhibition, is determined by the amount of GABA release and spillover into the extrasynaptic environment relative to the rate of GABA uptake by plasma membrane GATs (Brickley and Mody, 2012). GATs rapidly remove GABA from the extracellular space after presynaptic release to terminate inhibitory synaptic transmission, limit GABA spillover to neighbouring synapses or extrasynaptic sites, and maintain GABA homeostasis to prevent excessive tonic activation of synaptic and extrasynaptic GABA receptors (Borden, 1996). To date, four different isoforms of the GAT have been identified, GAT-1, GAT-2, GAT-3, and the betain/GABA transporter type 1 (BGT-1). These transporters are members of the solute carrier family of plasma membrane transporters which are 12-transmembrane spanning Na<sup>+</sup>/Cl<sup>-</sup> coupled transporters. Therefore, GATs exchange GABA for Na<sup>+</sup> and Cl<sup>-</sup>, and GABA transport is particularly dependent on the Na<sup>+</sup> gradient across the membrane. The proposed stoichiometry for GAT is 2 Na<sup>+</sup>:1 Cl<sup>-</sup>: 1 GABA (Borden, 1996; Jin et al., 2011).

There are two isoforms of the GAT in striatum: GAT-1 (Slc6a1), abundant in axons of GABAergic neurons (Augood et al., 1995; Durkin et al., 1995; Ng et al., 2000; Yasumi et al., 1997); and GAT-3 (Slc6a11), expressed moderately (Ficková et al., 1999; Ng et al., 2000; Yasumi et al., 1997) and seen particularly on astrocytes (Chai et al., 2017; Ng et al., 2000; Yu et al., 2018). However, whether GAT-1 and GAT-3 are evenly expressed across striatal territories has not yet been tested. Furthermore, this organisation of striatal GAT-1 to neuronal processes and GAT-3 to astrocytic ones might be oversimplified as emerging transcriptomic data indicate that striatal astrocytes express mRNA for both GAT-1 and GAT-3 (Chai et al., 2017; Gokce et al., 2016; Zhang et al., 2014). In addition, mRNA for GAT-1 and for GAT-3 has been found in midbrain DA neurons and these GATs have been suggested, but not confirmed to be, located on striatal DA axons to support GABA co-storage and co-release (Tritsch et al., 2014; **Section 1.4.2**).

Ambient GABA tone onto SPNs in the adult dorsal striatum in rodent is limited by the activity of GAT-1 and GAT-3: the pharmacological inhibition of striatal GATs dramatically increases the GABA<sub>A</sub>-mediated tonic inhibitory currents recorded in SPNs, even in *ex vivo* slice preparations (Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014; Wójtowicz et al., 2013). Recent evidence indicates that GATs on striatal astrocytes play a particularly important role, as increased GAT-3 expression on astrocytes results in decreased GABA<sub>A</sub>-mediated tonic inhibitory currents recorded in SPNs, resulting in profound changes to SPN activity and in striatal-dependent behaviour (Yu et al., 2018). Furthermore, striatal GATs are upregulated in transgenic mouse models of Huntington's disease resulting in decreased tonic inhibition in SPNs by ambient GABA (Cepeda et al., 2013; Wójtowicz et al., 2013).

### 1.5.6 Neuromodulation of mesostriatal dopamine release by striatal GABA signalling

Striatal GABA is in a key position to directly modulate DA output. The average arbour of a given rat nigrostriatal DA neuron, which reaches ~3% of the volume of striatum (Matsuda et

al., 2009), will encompass ~70,000 GABAergic neurons, calculated from rat striatal GAD-positive neuron counts (~2.8 million per hemisphere; Oorschot, 1996). Furthermore, ultrastructure studies in monkey and rat striatum report GABA<sub>B</sub> receptors on structures that resemble DA axons (Charara et al., 1999; Yung et al., 1999) and a recent unpublished immuno-electron microscopy study in mouse striatum shows that ~40% of striatal DA axons label positive for GABA<sub>A</sub> receptors containing  $\alpha 3$  subunits (Sherpa et al., 2019). Conversely, there is a paucity of GABAergic axoaxonic synapses on DA axons (Charara et al., 1999), but this does not preclude this potential GABA-DA interaction, as extrasynaptic ambient GABA might spill over onto DA axons.

There is an assortment of previous evidence indicating that striatal GABA<sub>A</sub> and GABA<sub>B</sub> receptors can modulate DA release, albeit yielding conflicting results. Early works arising in the late 1970s indicated that intrastriatal GABAergic neurons might both inhibit and potentiate DA output from striatal DA axons (Ch  ramy et al., 1978; Giorguieff et al., 1978; Reinmann et al., 1982, 1983; Starr, 1978, 1979; Stoof et al., 1979). Exogenous GABA perfusions into the caudate nucleus of anaesthetised cats was found to potentiate extracellular DA concentrations measured in superfusate from a push-pull cannula when  $10^{-5}$  M GABA was briefly introduced (~15 min) into the superfusing medium (Ch  ramy et al., 1978). This initial potentiation of extracellular DA was followed by an inhibition of extracellular DA, when GABA was introduced into the caudate nucleus on a longer (60 min) time scale (Ch  ramy et al., 1978). Other works measuring potassium-stimulated release of DA from striatum of *ex vivo* acute rodent brain slices and synaptosomes also demonstrated a potentiation in DA release upon bath application of exogenous GABA in the  $10^{-5}$  to  $10^{-3}$  M range (Giorguieff et al., 1978; Reinmann et al., 1982; Starr, 1978, 1979; Stoof et al., 1979). However, both *in vivo* and *ex vivo* GABA-induced potentiation of striatal DA levels was only mildly occluded (Starr, 1978; Giorguieff et al., 1978) or not at all occluded (Ch  ramy et al., 1978; Reinmann et al., 1982; Starr, 1979) in the presence of GABA receptor antagonists. It therefore remains uncertain whether the GABA-induced potentiation of DA release

observed is mediated via GABA receptors. Conversely, bath application of exogenous GABA at a higher concentration ( $10^{-1}$  M) and application of the GABA<sub>B</sub> receptor agonist baclofen inhibited potassium-stimulated release of DA from striatum of *ex vivo* acute rodent brain slices and synaptosomes (Giorguieff et al., 1978; Reinmann et al., 1983), suggesting an inhibitory role of GABA modulation of striatal DA release.

Subsequent studies arising from the early 1990s onwards and employing revolutionary electrochemical techniques further suggested an endogenous striatal GABA source might locally modulate DA output through action at GABA receptors on DA axons. Striatal administration of pregnanolone, a positive allosteric modulator of the GABA<sub>A</sub> receptor, or bicuculline, a GABA<sub>A</sub> antagonist, respectively decreased and increased extracellular DA levels in striatum of intact rats measured by microdialysis *in vivo* (Smolders et al., 1995). In corroboration, bicuculline has been shown to increase DA release from *ex vivo* striatal rat brain slices (Krebs et al., 1993) and muscimol, a GABA<sub>A</sub> agonist, has been shown to inhibit DA release from striatal synaptosomes prepared from rat striatum (Ronken et al., 1993). Other studies examining GABA<sub>A</sub> receptor control of DA release have revealed a GABA<sub>A</sub>-mediated enhancement of DA release in acute *ex vivo* striatal guinea pig slices during prolonged electrical pulse train stimulations, although this is hypothesised to arise indirectly via inhibition of H<sub>2</sub>O<sub>2</sub> release from SPNs during prolonged train stimulations (Avshalumov et al., 2003). Striatal administration of baclofen, a GABA<sub>B</sub> receptor agonist, or phaclofen, a GABA<sub>B</sub> receptor antagonist, respectively decreased and increased extracellular DA levels in intact rats measured by microdialysis *in vivo* (Smolders et al., 1995). Furthermore, striatal perfusions of baclofen decreased electrically evoked DA release in acute *ex vivo* slices of mouse caudate putamen (Schmitz et al., 2002) and nucleus accumbens (Pitman et al., 2014). These findings strongly suggest that an endogenous striatal GABA source might locally modulate DA output through action at GABA receptors on DA axons; however, definitive evidence is lacking: (1) Pharmacological agonists for GABA<sub>A</sub> and GABA<sub>B</sub> receptors might

induce nonphysiological attenuation in DA output and do not reveal a physiologically relevant role, like antagonists do. (2) Previous studies which use antagonists are conducted using purely an *in vivo* approach, which are confounded by potential effects on long loop circuits that could regulate striatal DA output via resultant changes in DA neuron firing in the midbrain. (3) These studies do not rule out indirect actions of GABA receptors to modulate DA release i.e. through action at ChIs which express both GABA<sub>A</sub> and GABA<sub>B</sub> receptors (Waldvogel et al., 1998; Yung et al., 1999) and can mediate effects of other neuromodulators on DA release, including opioids, nitric oxide, glutamate, and insulin (Britt and McGehee, 2008; Hartung et al., 2011; Kosillo et al., 2016; Stouffer et al., 2015). Whether striatal GABA signalling can modulate DA release through direct actions at putative GABA<sub>A</sub> and GABA<sub>B</sub> receptors on DA axons is tested in **Chapter 3**. Furthermore, whether striatal GATs are critical in governing the level of GABA receptor-mediated inhibition of DA release through limiting GABA spillover onto DA axons is tested in **Chapter 4**.

## 1.6 Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting ~6.1 million individuals globally and with a cumulative lifetime incidence estimated at 2% worldwide (Dorsey et al., 2018; Rocca, 2018). The worldwide burden of PD has more than doubled over 26 years, from 2.5 million patients in 1990 to 6.1 million patients in 2016 (Rocca, 2018). Furthermore, a relatively conservative doubling of the number of patients over the next 30 years would yield more than 12 million patients worldwide by 2050 (Dorsey et al., 2018). In our ageing population, PD represents a significant health and economic burden that requires scientific attention to understand better the aetiology and progression of PD to facilitate the development of novel treatment options.

PD is the result of the degeneration of a subpopulation of dopaminergic neurons residing in the midbrain, resulting in reduced DA signalling in the striatum and abnormal functioning of the basal ganglia. The pathological end-state of PD is well described, but there remains

a pressing need to define early functional changes to susceptible neurons and associated circuits. Unravelling the mechanisms which underlie DA dysfunction early on in the progression of PD is critical for identifying novel loci for developing new disease-modifying therapies.

### 1.6.1 Dopamine neuron degeneration in Parkinson's disease

PD is characterised by a triad of motor symptoms, namely resting tremor, bradykinesia and rigidity, in addition to a number of non-motor symptoms including constipation, loss of smell and sleep disturbances (Park and Stacy, 2009; Samii et al., 2004). The disease is most commonly an idiopathic disorder, although rare genetically inherited forms of the disease do exist (Chartier-Harlin et al., 2004; Singleton et al., 2003). The hallmark of both inherited and idiopathic forms of PD is the progressive and dramatic loss of striatal DA, due to the neurodegeneration of SNc DA neurons (Hornykiewicz, 1998). Specifically, degeneration of neuromelanin-positive and calbindin-negative ventral tier SNc DA neurons results in dopaminergic denervation of the dorsolateral striatum (Hornykiewicz, 1998; Iacopino et al., 1992; Surmeier and Sulzer, 2013). Consequentially, striatal DA denervation leads to downstream aberrant synaptic weighting of basal ganglia activity and the manifestation of motor symptoms (Albin et al., 1989; DeLong, 1990; Graybiel et al., 1994). The success of DA replacement therapies in alleviating motor impairments, such as the DA precursor L-DOPA, substantiates that dopaminergic deficits are central to the motor phenotype in PD (Connolly and Lang, 2014).

As outlined in Section 1.3.2, nigrostriatal DA axons in the dorsal striatum form extensive unmyelinated axonal arbours and provide an astonishing  $\sim 10^5$  DA release sites per rodent neuron (Arbuthnott and Wickens, 2007; Descarries et al., 1996; Matsuda et al., 2009). The metabolic demands and stress generated from supporting these immense axonal arbours, through maintaining resting membrane potential and propagation of action potentials across unmyelinated axons, is suggested to contribute to their preferential degeneration in

PD (Bolam and Pissadaki, 2012; Pissadaki and Bolam, 2013). The adjacent DA neurons of the VTA which innervate the ventral striatum are spared, and are significantly less branched and less complex (Arbuthnott and Wickens, 2007; Bolam and Pissadaki, 2012); as such a 'dying back' of nigrostriatal DA axons has been proposed as one of the earliest features of PD (Burke and O'Malley, 2013), making the striatum a critical site for targeting the disease. Indeed, the degree of terminal loss in striatum appears to be more pronounced than the magnitude of SNc DA neuron loss (Bernheimer et al., 1973).

### 1.6.2 Circuit-level dysfunction of basal ganglia function underlying motor symptoms in Parkinson's disease

The classical model of basal ganglia function (Figure 1.1) has critically shaped our understanding of how DA release in the striatum contributes to motor output, and how the loss of this nigrostriatal DA signalling leads to circuit-level dysfunction underlying the motor symptoms of PD. As previously described, the classical model segregates striatal SPNs into two populations, dSPNs and iSPNs, based on their projection targets. D<sub>1</sub> receptor-positive dSPNs project directly to basal ganglia output nuclei GPi/SNr (DeLong, 1990; Gerfen et al., 1990), and activation of this pathway is thought to promote movement by decreasing basal ganglia output and disinhibiting the thalamus. Conversely, D<sub>2</sub> receptor-positive iSPNs project indirectly to basal ganglia output nuclei through GPe and STN (DeLong, 1990; Gerfen et al., 1990), and activation of this pathway is thought to suppress movement. The net effect of nigrostriatal dopaminergic signalling is therefore to promote movement by suppressing basal ganglia output from the GPi, due to the opposing effects of DA on these two populations of SPNs. In PD, the loss of DA signalling in striatum causes an imbalance in the activity between these two pathways, resulting in excessive indirect-pathway activation and diminished direct-pathway activity. This imbalance is thought to drive GPi-mediated thalamic inhibition, and therefore suppress motor output (DeLong, 1990; Mcgregor and Nelson, 2019).

### 1.6.3 $\alpha$ -synuclein involvement in Parkinson's disease

A common feature of PD is the accumulation of cytoplasmic filamentous protein aggregates, known as Lewy bodies, which are principally comprised of  $\alpha$ -synuclein (Baba et al., 1998; Marques and Outeiro, 2012; Spillantini et al., 1998). Genetic and genome-wide association studies (GWAS) point to  $\alpha$ -synuclein involvement in both sporadic and familial PD. Identified point mutations, as well as duplication and triplication of the *SNCA*  $\alpha$ -synuclein gene locus have been identified in autosomal dominant PD cases (Bekris et al., 2010; Chartier-Harlin et al., 2004; Singleton et al., 2003). GWAS also support the involvement of  $\alpha$ -synuclein (*SNCA*) in sporadic cases (Satake et al., 2009; Simón-Sánchez et al., 2009; Venda et al., 2010). Furthermore, pathophysiology data from *SNCA* locus multiplication studies highlight a gene dosage effect, as triplication leads to earlier symptom onset and a more severe disease progression compared to gene duplication (Fuchs et al., 2007).

The function of  $\alpha$ -synuclein is still not fully understood, but it appears to interact with presynaptic function and bind to vesicles (Clayton and George, 1998), modulate transcription (Baptista et al., 2003; Yu et al., 2007), and modify striatal DA storage, synthesis and release in a dose-dependent manner (Anwar et al., 2011; Janezic et al., 2013; Senior et al., 2008). Studies suggest that both wild-type and mutant oligomeric  $\alpha$ -synuclein species show neurotoxic properties and provide the rudimentary structure for fibril formation (Feng et al., 2010; Kayed et al., 2003; Lashuel et al., 2002). Furthermore, aggregation of  $\alpha$ -synuclein into fibril formations promotes progressive neurotoxicity in adult rodent dopaminergic neurons *in vivo* (Paumier et al., 2015; Taschenberger et al., 2012). Post-mortem PD brains often present with  $\alpha$ -synuclein fibrillar tangles and increased  $\alpha$ -synuclein burden (Spillantini et al., 1997, 1998). However, it is still not clear whether dysfunction in DA physiology and cell death follow or precede the formation of these aggregates.

#### 1.6.4 Mouse models of early Parkinson's disease

Animal models recapitulating human disease enable examination of PD pathophysiology, with emerging genetic models allowing examination across different time stages. Therefore, such models are valuable for elucidating the earliest pathological and physiological processes underlying the final common pathway of DA neuron degeneration. These are in preference to neurotoxic lesion models, i.e. nigrostriatal 6-OHDA infusions, which result in extensive striatal DA denervation and recapitulate only the later stages of PD (Hisahara and Shimohama, 2010; Zeng et al., 2018).

Animal models which employ manipulations of  $\alpha$ -synuclein in rodent models are commonly employed for PD research. Models which used a vector-driven approach to overexpress human wild-type  $\alpha$ -synuclein in striatum or midbrain report a progressive insult to nigrostriatal DA transmission (Lo Bianco et al., 2002; Kirik et al., 2002; Lundblad et al., 2012). Furthermore, models which express mutant  $\alpha$ -synuclein A53T variant overexpression and A30P variant expression also present with striatal DA deficits (Gispert et al., 2003; Kurz et al., 2010; Platt et al., 2012; Taylor et al., 2014). These models importantly provide a causal link between the overexpression and/or dysfunction of  $\alpha$ -synuclein and a Parkinsonian-like nigrostriatal DA deficit. However, they employ general promoters to express these  $\alpha$ -synuclein variants that do not recapitulate the native expression profiles of the *SNCA* gene.

The human  $\alpha$ -synuclein overexpressing mouse model of PD (*SNCA-OVX*), developed at the Oxford Parkinson's Disease Centre, is a highly physiological, slowly progressive mouse model of parkinsonism, that captures a human disease-relevant genetic burden of  $\alpha$ -synuclein overexpression (Janezic et al., 2013). This model was generated using a BAC construct carrying the complete wild-type human *SNCA* locus, inclusive of the endogenous promoter, on a mouse *SNCA* knock-out background allowing native expression. The *SNCA-OVX* mouse expresses human wild-type  $\alpha$ -synuclein protein at 1.9 times endogenous levels, corresponding to PD patients with *SNCA* locus duplications.

These *SNCA-OVX* mice show early deficits in DA transmission (by 3 months of age) restricted to the dorsal striatum, but not observed in the ventral striatum, prior to late-stage degeneration of DA neurons, disturbed encoding of behaviour in surviving neurons, and manifestation of a motor phenotype (at 18 months of age; Dodson et al., 2016; Janezic et al., 2013).

This thesis explores maladaptive GAT regulation of striatal DA release during the prodromal early stages of PD in the *SNCA-OVX* genetic mouse model of early parkinsonism in **Chapter 5**. Herein, these mice were made 'optogenetic-capable', such that they allowed for the optical manipulations of DA axons (outlined in **Section 2.1.2** and **Section 5.2.1**).

## 1.7 Summary

The striatum is the input nucleus of the basal ganglia and functions to integrate excitatory, inhibitory and modulatory inputs from a diverse number of brain regions to facilitate the execution of appropriate behaviours. Midbrain DA neurons send projections to the striatum, where they form elaborate axonal arbours and volume-transmit DA, in addition to also co-releasing GABA and glutamate. This mesostriatal DA signalling is critical for a wide range of behaviours including, action selection and reinforcement learning. Furthermore, striatal DA release is dysregulated in a diversity of disorders, most notably PD.

It is increasingly appreciated that DA release in the striatum is subject to major influences besides action potentials generated at the level of the cell body. Rather, local mechanisms in the striatum are able to dynamically drive and gate DA release at the axonal release sites, most notably ACh. Understanding the diverse mechanisms through which DA release is locally regulated in striatum is critical for understanding its physiological function and will promote the development of novel treatment interventions in diseases where DA is dysregulated.

The striatum contains a high density of GABAergic projection neurons and interneurons, which are interspersed among DA axons and DA release sites. These neurons contribute to

an extrasynaptic ambient GABA tone in striatum, which exert tonic inhibition onto principal striatal neurons, a level which is determined by plasma membrane GATs. However, whether striatal GATs and GABA can modulate DA release has not yet been extensively studied.

In this thesis, I use fast-scan cyclic voltammetry (FSCV) in acute mouse brain slices to explore how presynaptic striatal DA release is controlled by striatal GABA signalling. In **Chapter 3**, I specifically test how local striatal GABA sources might inhibit DA release through actions on GABA<sub>A</sub> and GABA<sub>B</sub> receptors directly on DA axons. Subsequently in **Chapter 4**, I examine how the regulations of DA transmission by striatal GABA is governed by striatal GATs. Finally, in **Chapter 5**, I explore how maladaptive downregulation of striatal GATs contributes to dysregulated striatal DA release in a mouse model of early Parkinsonism. **Chapter 2** provides an overview of the methods utilised in the presented works.

# 2

## General Methods

In this chapter, I will describe the details of the methods and techniques used in the experimental chapters (Chapters 3-5) of this thesis. This will include a brief discussion of the animal models and experimental preparations used, in addition to an overview of and detailed methods for the detection of evoked DA release using fast-scan cyclic voltammetry (FSCV), patch-clamp electrophysiology and biochemical methods.

## 2.1 Mice as a model organism for investigating mesostriatal dopamine signalling

### 2.1.1 Ethical statement for the use of animals

This thesis explores the mechanisms through which GABA and subsequently, the GAT, regulate DA release within the mammalian adult striatum. It is essential then that DA neurons/axons and other striatal neurons/glia cells maintain as many features of the intact physiological scenario as possible, e.g. normal inputs/outputs and ratio of glia to neurons, throughout the experimental preparations used. There is therefore no substitute for real brain tissue. Accordingly, experiments are conducted in acutely living *ex vivo* sections of brain or post-mortem brain tissue taken from adult mammals. Mice are the least sentient species appropriate and are the species of choice for several reasons: (1) They are amenable to our genetic manipulation methods, and there are a large number of genetically-modified strains readily available, such as the Slc6a3<sup>IRE5-Cre</sup> mouse line used herein. (2) They enable the study of neural systems closely related to human, as the mesostriatal DA systems are conserved across mammalian species and there are strong similarities between mouse and human striatum. (3) The large majority of previous studies which examine the mechanisms which govern DA release are conducted in mouse striatum; therefore, the results presented herein can be directly compared with other studies.

All experimental procedures involving the use of animals were carried out according to institutional guidelines and conformed to UK Home Office regulations under the Animals (Scientific Procedures) Act of 1986. All mice were housed in the University of Oxford Biomedical Services Building. Mice were maintained on a C57BL/6J background, group

housed and had access to standard food and water *ad libitum*. Mouse holding rooms were maintained at 22°C and 60 to 70% humidity on a 12-hour light-dark cycle.

### 2.1.2 Animals

Adult male wild-type C57BL/6J (Charles River) were used for the majority of FSCV experiments involving electrical stimulation. The C57BL/6J strain is useful in the study of DA neurotransmission as it carries a wild-type gene for  $\alpha$ -synuclein, a protein important in the regulation of DA release (Abeliovich et al., 2000), unlike some C57BL/6 strains in which a spontaneous deletion of this gene has been observed (Specht and Schoepfer, 2001).

In experiments involving selective activation of DA axons using optogenetic strategies or labelling of DA axons, transgenic mice expressing Cre-recombinase under the control of the promoter for the DA transporter (Slc6a3) have been used. In these animals, an internal ribosome entry site-linked Cre-recombinase (IRES-Cre) gene has been inserted downstream of the endogenous Slc6a3 gene (Bäckman et al., 2006). Male heterozygous Slc6a3<sup>IRES-Cre</sup> mice, which were used in experiments, were locally bred from homozygous Slc6a3<sup>IRES-Cre</sup> mice on a C57BL/6J background (B6.SJL-Slc6a3<sup>tm1.1(cre)Bkmn</sup>/J; stock no. 006660, Jackson Laboratories). Male Pvalb<sup>Cre</sup> transgenic mice expressing Cre-recombinase under the control of the promoter for parvalbumin (Pvalb/PV) were used for labelling of striatal Pvalb-positive interneurons (B6;129P2-Pvalb<sup>tm1(cre)Arbr</sup>/J; stock no. 008069, Jackson Laboratories).

In experiments involving the human  $\alpha$ -synuclein overexpressing genetic mouse model of Parkinson's disease (OVX-SNCA), BAC-transgenic mice which overexpress human  $\alpha$ -synuclein (SNCA) at Parkinson's disease-relevant levels and show early deficits in DA release prior to cell loss in old age (Janezic et al., 2013) were back-crossed onto an  $\alpha$ -synuclein-null (Snca<sup>-/-</sup>) background (B6.Cg-Tg(SNCA)OVX37Rwm Snca<sup>tm1Rosl</sup>/J; Jackson Laboratories stock no. 023837). An 'optogenetic-capable' Parkinsonian mouse line was generated by crossing homozygous Slc6a3<sup>IRES-Cre</sup> mice devoid of mouse  $\alpha$ -synuclein (Snca<sup>-/-</sup>) with mice

heterozygous for human  $\alpha$ -synuclein overexpression (SNCA-OVX), also devoid of mouse  $\alpha$ -synuclein (Snca<sup>-/-</sup>), giving rise to Parkinsonian mice (SNCA+) and littermate control mice devoid of mouse  $\alpha$ -synuclein (Snca<sup>-/-</sup>) that each allow optogenetic manipulation of DA axons (see Section 5.2.1). Both male and female sex-matched SNCA+ and Snca<sup>-/-</sup> littermate pairs were used in experiments. The  $\alpha$ -synuclein-null mouse (Snca<sup>-/-</sup>) is a sufficient paired control to the human  $\alpha$ -synuclein overexpressing mouse (SNCA) as (1) it allows an experimental design with littermate pairs, and (2) mouse  $\alpha$ -synuclein knock out does not impact on evoked DA release or storage (Janezic et al., 2013). Alternatively, mice expressing native mouse  $\alpha$ -synuclein could be used as paired controls, however this would preclude pairings from the same litter.

### 2.1.3 Stereotaxic intracranial injections for viral transfection

Selective targeting of opsin expression to target neurons is commonly achieved with very high specificity using site-specific recombinases and relies on the specific expression of recombinases in transgenic animals driven by a cell-type specific genetic promoter in target cells (Branda and Dymecki, 2004; Dymecki et al., 2010). Here, stereotaxic intracranial injections were performed for viral transfection of an adeno associated virus (AAV) containing an inverted gene for channelrhodopsin-2 (ChR2; H134R variant) fused in-frame with a gene encoding enhanced yellow fluorescent protein (eYFP) in transgenic mice expressing Cre-recombinase under the control of cell type-specific promoters (Slc6a3<sup>IRE5-Cre</sup> and Pvalb<sup>Cre</sup> transgenic mice). The ChR2-eYFP gene is flanked in the construct by two inverted pairs of short palindromic *lox* sites, which are recognised and inverted by Cre-recombinase, resulting in forward orientation and therefore expression of the ChR2-eYFP gene only in neurons containing Cre-recombinase (Figure 2.1; Branda and Dymecki, 2004; Dymecki et al., 2010).

All surgical procedures were performed according to aseptic technique. Mice were deeply anaesthetised with isoflurane (induction at 4% w/o, maintenance 1-2% w/o). Prior to surgery,

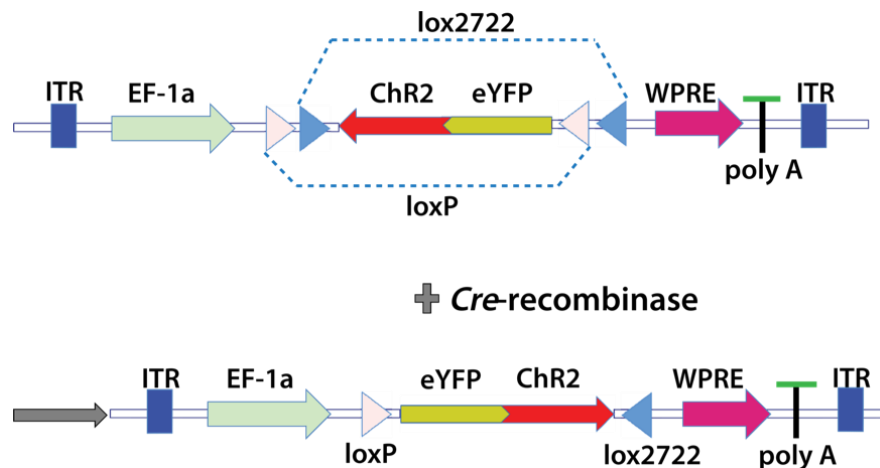


**Figure 2.1. Cre-dependent recombination of virally-delivered ChR2-eYFP construct.** Inverted ChR2-eYFP gene is flanked by two pairs of incompatible inverted lox sites (loxP and lox2722). In the presence of Cre-recombinase, i.e. in dopamine neurons of *Slc6a3<sup>IRE5-Cre</sup>* mice, recombination of lox sites results in forward, correct orientation of the ChR2-eYFP gene, such that expression can be driven by the EF-1 $\alpha$  promoter. The woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) is included to increase mRNA stability. Adapted from Threlfell et al., 2012.

meloxicam (Metacam, 5 mg/kg, subcutaneous) was administered for post-operative analgesia. The scalp was cleanly shaved, and the animal placed securely into a small animal stereotaxic frame (David Kopf Instruments). The incision site was then cleaned with chlorhexidine (HiBi scrub) and bupivacaine hydrochloride with epinephrine (Marcaine, 2 mg/kg, subcutaneous) was injected at the incision site for local anaesthesia. After exposing the skull, small craniotomies were made above the injection sites, and AAV ( $8 \times 10^{12}$  genome copies per ml; University of North Carolina Vector Core Facility) encoding Cre-dependent ChR2 was injected. Viral solutions were injected at an infusion rate of 100 nL/min with a 32-gauge Hamilton syringe (Hamilton Company) and withdrawn 5-10 min after the end of injection.

In *Slc6a3<sup>IRE5-Cre</sup>* mice (4-6 weeks) and *Slc6a3<sup>IRE5-Cre</sup>* x SNCA-OVX mice (11-12 weeks) a total volume of 1  $\mu$ L of AAV5-EF1 $\alpha$ -DIO-hChR2(H134R)-eYFP was injected bilaterally (500 nL per

hemisphere/injection) into substantia nigra pars compacta (SNc, AP -3.1 mm, ML  $\pm$ 1.2 mm from bregma, DV -4.25 mm from exposed dura mater). In Pvalb<sup>Cre</sup> mice (4-6 weeks), a total volume of 600 nL of AAV2-EF1 $\alpha$ -DIO-hChR2(H134R)-eYFP was injected bilaterally (300 nL per hemisphere/injection) into dorsolateral striatum (DLS, AP +0.65 mm, ML  $\pm$ 2.0 mm from bregma, DV -1.85 mm from exposed dura mater). Following wound closure with sterile monofilament suture (Ethicon), animals were allowed to recover in a temperature-controlled cage. Animals were closely monitored through postoperative days 0-7, and meloxicam (Metacam, 5 mg/kg, intraperitoneal) was administered, where needed, to alleviate postoperative discomfort. AAV-transfected mice were used for experiments 3-4 weeks following intracranial injection to allow for sufficient viral expression.

## 2.2 Investigating dopamine signalling in acute *ex vivo* slice preparations

Experimental approaches for investigating DA transmission range from recording DA neuron activity and DA release *in vivo* of freely-moving animals to studies of cultured DA neurons *in vitro*. The experiments described in this body of work have all been conducted in acute *ex vivo* coronal sections of mouse striatum, which are suitable for studying GABAergic factors which govern DA release and have several key advantages. Firstly, *ex vivo* striatal slices prepared from adult small mammals preserve typical striatal connectivity, including the unique axonal arborization in striatum from midbrain DA neurons (Matsuda et al., 2009), and maintain normal ratios and spatial organisation of DA neurons/axons in relation to other striatal neurons/glia cells. Secondly, measurement and experimental manipulation of extracellular DA in *ex vivo* slice preparations is more straightforward than that *in vivo*; DA release signals collected using FSCV in *ex vivo* slice preparations typically have much higher signal-to-noise ratios, allow control of extraneous variables, and allow ready administration of drugs and manipulation of the extracellular environment.

While acute *ex vivo* coronal sections of striatum do preserve local circuitry at the level of the DA axon within striatum, one major disadvantage of this preparation is the loss of the

somatodendritic control of DA release due to the severing of DA axons from their cell bodies in the midbrain. This can be overcome (although with limited success) through parasagittal slice preparations (not used herein) which partially preserve the mesostriatal pathways (Ammari et al., 2009). Coronal preparations do however offer the advantage of examining striatal DA signalling in the absence of somatodendritic influence, which has permitted the discovery that DA release can be driven by local striatal neurons, independent of ascending activity from DA cell bodies (Threlfell et al., 2012). However, *in vivo* experiments are necessary for corroborating key observations arising from experiments involving *ex vivo* slice preparations to understand if and how these observations are relevant in the intact CNS.

### 2.2.1 Electrochemical detection of dopamine release in real-time

In this body of work, evoked DA release was recorded using FSCV at carbon fibre microelectrodes inserted into acute *ex vivo* striatal brain slice preparations. This technique allows the detection of transient changes in extracellular DA concentrations ( $[DA]_o$ ) with high chemical selectivity and sub-second temporal resolution and remains the best optimised sub-second detection method for detecting endogenous DA release.

Electrochemical detection of monoamine neurotransmitters and their metabolites exploits a well-known property of catecholamines (e.g. DA, noradrenaline) and indoleamines (e.g. serotonin), which is that they are electroactive and undergo changes to their oxidation state. Electrochemical detection, such as voltammetry, uses the faradaic current produced by a change in the oxidation state (oxidation or reduction) to quantify the concentration of electroactive substrate. In voltammetric techniques, the potential difference (voltage) across a working electrode is varied to measure the faradaic current of applied voltage. This relationship between applied voltage and measured faradaic current can be plotted as a voltammogram.

FSCV employs rapid cycling of a potential across a range predicted to oxidise and subsequently reduce the compound of interest, using a triangular scan wave. On the forward sweep, from a negative potential to a positive potential, the substrate is oxidised, and subsequently reduced on the reverse sweep, producing measurable faradaic currents at both the oxidation and reduction potentials. The scan rates used can be very fast, typically 300 - 900 V/s, completed in ~5 ms, and thus are appropriate for monitoring neurotransmitter release events (Armstrong-James et al., 1980; Rice and Nicholson, 1989).

Dopamine has two readily-oxidised hydroxyl groups, making it suitable for electrochemical detection with FSCV: dopamine is oxidised to dopamine-o-quinone on the forward sweep, at approximately +650 mV, and reduced back to dopamine on the reverse sweep, at approximately -250 mV. Herein,  $[DA]_o$  was recorded using FSCV with a triangular scan waveform against Ag/AgCl reference electrode from -0.7 V to +1.3 V, at a scan rate of 800 V/s (~5 ms in duration) and sweep frequency of 8 Hz. This corresponds to a measurement of faradaic current (and therefore  $[DA]_o$ ) every 125 ms (Figure 2.2). Higher sweep frequencies can be employed, yielding more accurate representation of the kinetics of  $[DA]_o$  transients, but resulting in reduced signal amplitude.

It is important to note that there are other sources of current in the system, besides the expected faradaic current, which occur when the potential is rapidly altered. Background current arises due to the charging of the electrode (rearrangement of charged species in the electrode) and is known as the 'capacitive' or 'charging' current. In FSCV there is a comparably larger background charging current to typically measured faradaic current in our experimental preparations. Here therefore, the background charging current was digitally subtracted using the Millar Voltameter (Julian Millar, Barts and the London School of Medicine and Dentistry), which reveals only the faradaic voltammogram corresponding to an increase in  $[DA]_o$ , which can be monitored in real-time. Furthermore, current

attributable to the detections of the macromolecular environment are also detected, i.e. from pH shifts.

Several alternative methods are also available for the detection of extracellular DA release in acute *ex vivo* slice preparations in real-time, but none are currently able to surpass the advantages offered by FSCV. Constant-potential amperometry is a similar technique in which the applied potential is constantly maintained at the oxidation potential for the chemical species of interest (Michael and Wightman, 1999). As such, this approach offers greater temporal resolution when compared to FSCV but does not allow sufficient distinction of the faradaic currents produced by different oxidised species, which are readily distinguishable when using FSCV.

Microdialysis with high-performance liquid chromatography (HPLC) coupled to electrochemical detection offers greater chemical selectivity to FSCV, and furthermore can measure absolute baseline concentrations of neurotransmitters, which is not possible using FSCV methods. However, applications of microdialysis in *ex vivo* slice preparations for measurement of neurotransmitter release in real-time is disadvantageous. Microdialysis probes disrupt the tissue integrity through their relatively larger sizes to those used in FSCV, and through the inevitable removal of extracellular specimens for sampling and downstream detection (Clapp-Lilly et al., 1999; Yang and Michael, 2007). In FSCV, the detection of electroactive compounds happens *in situ*, and therefore ensuring normal homeostatic processes remain unaltered. Further, even modern microdialysis samples can only be collected at a maximum rate of ~1 sample/minute, a temporal resolution too low to study individual DA release events on a sub-second timescale. One major limitation of FSCV however is the poor ability to detect endogenous DA release outside DA rich regions. FSCV is therefore currently the most adequate method for the detection of endogenous DA release events in dopamine rich regions from acute *ex vivo* slice preparations, due to the high chemical selectivity coupled with sub-second temporal resolution of this method.

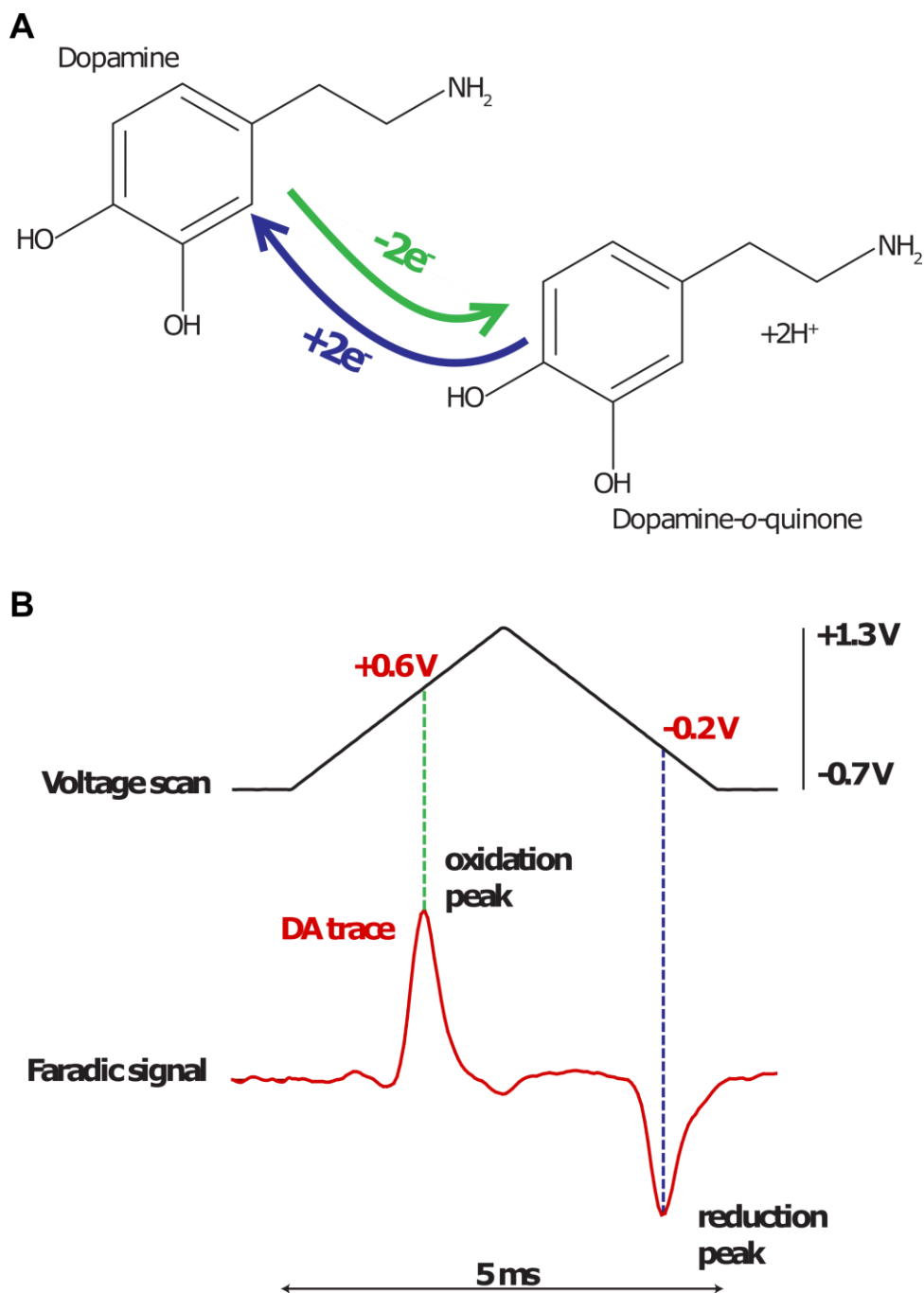


Figure 2.2. Oxidation of dopamine to dopamine-o-quinone generates faradic current recorded at carbon fibre microelectrodes with fast-scan cyclic voltammetry.

(A) Dopamine is electroactive and can undergo changes to its oxidation state.

(B) During the positive phase of the triangular waveform scan across the carbon fibre microelectrode (from -0.7 V to +1.3 V), dopamine is oxidised (*green*) to dopamine-o-quinone, and reduced back (*blue*) to dopamine during the negative phase of the scan (from +1.3 V to -0.7 V). The resulting faradaic current recorded produces a dopamine voltammogram showing typical oxidation peak at +0.6 V and reduction peak at -0.2 V. Scan rate of 800 V/s, 5 ms in duration.

Exciting novel methods for the measurement of DA release with high spatiotemporal precision have emerged throughout the course of this thesis work, namely the dLight and GRAB<sub>DA</sub> imaging methods (Patriarchi et al., 2018; Sun et al., 2018). In response to DA, these genetically encoded G protein-coupled receptor-activation-based-DA sensors exhibit large fluorescence increases with subcellular resolution, subsecond kinetics, nanomolar to submicromolar affinities, and excellent molecular specificity. Data indicate that these sensors can resolve a single electrically-evoked DA release event in acute ex vivo mouse brain slices and detect endogenous DA release in vivo in mice during complex behaviours (Sun et al., 2018). These methods were not explored herein, given their preliminary nature, but will need to be carefully evaluated in comparison to FSCV for consideration in future experimental investigations.

## 2.2.2 Fabrication of carbon fibre microelectrodes for fast-scan cyclic voltammetry

First invented in the late 1970s, carbon fibre microelectrodes (CFMs) revolutionised voltammetry applications in neuroscience and enabled development of FSCV in its current form (Armstrong-James and Millar, 1979; Armstrong-James et al., 1980; Gonon et al., 1980). CFMs commonly used have a diameter of  $\sim 7\ \mu\text{m}$ , too large a size to record from a single typical synaptic cleft ( $\sim 10\ \mu\text{m}$ ) but permits sampling of neurotransmitter which escapes the confines of the synapse. Therefore, CFMs are well suited to monitoring volume transmission, including DA transmission (Rice and Cragg, 2004). The length of a typical CFM used ( $\sim 75\ \mu\text{m}$ ) ensures sampling from a population of DA release sites, arguably providing a more accurate estimation of the typical transmitter release site, but still allowing adequate spatial resolution (i.e. many different sites within striatum can be discretely recorded from).

CFMs were produced in-house using epoxy-free carbon fibre  $7\ \mu\text{m}$  in diameter and each used for a single experiment, since changes in microelectrode kinetics and sensitivity can occur due to repeated exposure to DA and tissue fouling. Borosilicate capillary tubes (2.0

mm outside diameter, 1.16 mm inside diameter, Harvard Apparatus) were threaded with a single epoxy-free carbon fibre (7  $\mu\text{m}$  diameter, Goodfellow Cambridge Ltd.). The capillary tube was sealed around the carbon fibre using a PE-2 vertical microelectrode puller (Narishige) and an appropriately high heat setting was used to ensure a tight seal between the glass microelectrode and the carbon fibre, as this promotes a high signal-to-noise ratio. The resulting seal was visually inspected for damage under 100x magnification, and the exposed end of carbon fibre cut to a length of 50-100  $\mu\text{m}$  from the seal tip. This exposed carbon fibre length typically produces a sensitivity to DA within the desired range; shorter electrodes have a reduced sensitivity to DA, while longer electrodes have higher noise levels. A short length of insulated copper wire (0.1 mm diameter) was coated with silver conductance paint and inserted into the open end of the capillary tube, in contact with the carbon fibre close to the tip, and secured with cyanoacrylate glue.

### 2.2.3 Slice preparation for fast-scan cyclic voltammetry

Mice were decapitated after cervical dislocation and the brains were extracted and submerged into ice-cold cutting solution containing the following (in mM): 194 sucrose, 30 NaCl, 4.5 KCl, 1  $\text{MgCl}_2$ , 26  $\text{NaHCO}_3$ , 1.2  $\text{NaH}_2\text{PO}_4$  and 10 glucose. Acute 300  $\mu\text{m}$ -thick coronal slices containing CPu and NAc (spanning from AP +1.3 mm to +0.2 mm from bregma; Figure 2.3) were prepared from dissected brain tissue using a vibratome (VT1200S, Leica Microsystems) and transferred to a holding chamber containing a HEPES-based buffer solution maintained at room temperature (20-22°C) containing (in mM): 120 NaCl, 20  $\text{NaHCO}_3$ , 10 glucose, 6.7 HEPES acid, 5 KCl, 3.3 HEPES sodium salt, 2  $\text{CaCl}_2$ , 2  $\text{MgSO}_4$ , 1.2  $\text{KH}_2\text{PO}_4$ . All solutions were saturated with 95%  $\text{O}_2$  / 5%  $\text{CO}_2$ . Slices were allowed to recover for 1 hour before recording, and recordings were obtained within 6 hours of slicing.

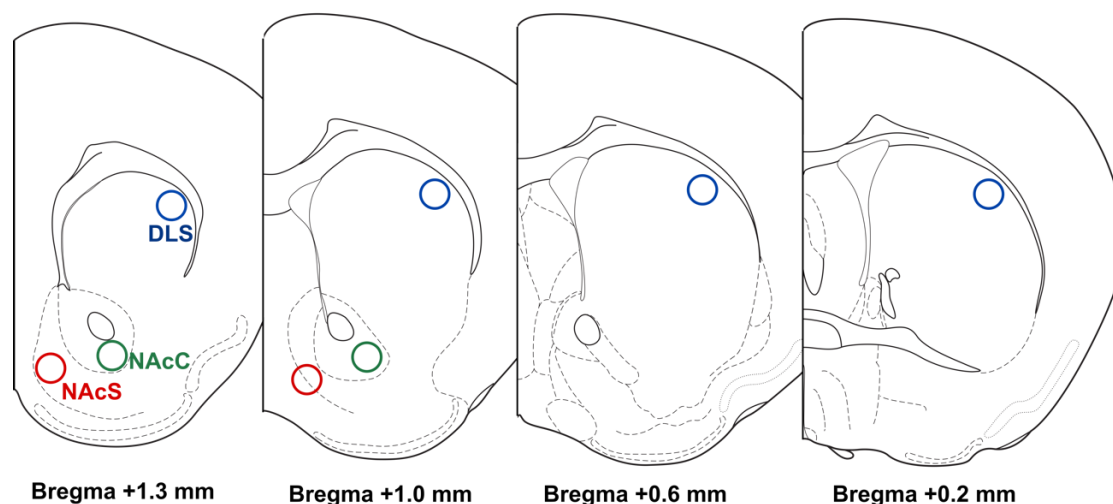


**Figure 2.3. Typical coronal slice preparations containing striatum and recording sites.** Acute ex vivo coronal slices containing striatum prepared from mouse brain spanning +1.3 mm to +0.2 mm anterior-posterior from bregma. Typical recording sites are indicated: dorsolateral striatum (DLS), nucleus accumbens core (NAcC) and nucleus accumbens shell (NAcS). Adapted from Paxinos and Franklin's *The Mouse in Stereotaxic Coordinates* 2019.

## 2.2.4 Fast-scan cyclic voltammetry

Individual slices were hemisected and transferred to a recording chamber and superfused at ~2.0 - 3.0 mL/min with carbogenated artificial cerebrospinal fluid (aCSF) containing (in mM): 130 NaCl, 2.5 KCl, 26 NaHCO<sub>3</sub>, 2.5 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub> and 10 glucose at 31-33 °C. A CFM was connected to the voltammeter headstage and inserted 100 µm into the tissue and slices were left to equilibrate and the CFM to charge for 30-60 min prior to recordings. These steps allow the electrode to equilibrate to the recording conditions and reduce subsequent baseline drift during the experiment.

Upon commencement of recording, the CFM was inserted at a depth of 100 µm into the recording site. Recordings sites included the dorsolateral quadrant of the striatum (DLS), the nucleus accumbens core (NAcC, within 100 µm of the anterior commissure), and the

nucleus accumbens shell (NAcS; see Figure 2.3). Following CFM insertion, the full current was inspected to determine whether the microelectrode seal remained intact, and full signal gain was set to provide a working range of 5 V. CFMs with a full signal gain in the range 3-10 mV/nA were used for recording. The full signal gain was monitored throughout the experiment, as CFMs typically become more sensitive during use, which can result in saturation if left uncorrected.

Evoked  $[DA]_o$  was measured via the CFM using FSCV. A biphasic triangular voltage waveform from -700 mV to +1300 mV vs an Ag/AgCl reference electrode (World Precision Instruments, USA) at a rate of 800 V/s was applied to the CFM using a Millar Voltammeter. The waveform was delivered at a frequency of 8 Hz, and between sweeps the electrode was held at 0 mV to limit adsorption (Heien et al., 2003). Background subtraction was performed on-line by the Millar Voltammeter, using a baseline recording of the total current stored before each stimulation was delivered. Background-subtracted currents were sampled at 50 kHz and digitised using a Digidata 1550A (Molecular Devices).

At a given site,  $[DA]_o$  was evoked by light or electrical stimulation (single pulses or trains) repeated every 2.5 min (Section 2.2.5). Initially, single-pulse stimulations were delivered until release was stable (less than 10% variation over 5 consecutive stimulations), after which release was typically sustained for the duration of the experiment. Data were only included for analysis where release remained stable for the duration of the experiment. Currents at the oxidation peak potential were measured from the baseline of each voltammogram and plotted against time to provide profiles of  $[DA]_o$  versus time. Evoked currents were confirmed as DA by comparison of the voltammogram with that produced during post hoc calibration with applied 2  $\mu$ M DA in aCSF (oxidation peak +500-600 mV and reduction peak -200 mV). CFM sensitivity to DA was between 10 and 40 nA/ $\mu$ M. Importantly, a linear relationship between DA concentration and faradaic oxidation current was observed (Figure 2.4c).

Data were collected using Axoscope 10.5 (Molecular Devices), and analysed using locally-written scripts in Visual Basic for Application (Microsoft). Each data set was collected from a minimum of three animals and threshold for statistical significance in all analyses was  $p < 0.05$ , adjusted for multiple comparisons where appropriate.

## 2.2.5 Electrical and light stimulation

Stimuli for evoking DA release were generated out-of-phase with the voltammetric scans to prevent stimulus artefacts from clashing with DA currents, using a Multi Channel Stimulus II generator (Multi Channel Systems) triggered by the Millar Voltammeter. In experiments where  $[DA]_o$  was evoked by electrical stimulation, a concentric bipolar Pt/Ir electrode (25  $\mu\text{m}$  tip diameter; FHC Inc.) was placed on the surface of the striatal tissue,  $\sim 100 \mu\text{m}$  from site of the recording electrode. In all experiments, individual pulses consisted of a square 0.6 mA current pulse of 200  $\mu\text{s}$  width (**Figure 2.4a**). Under drug-free conditions in *ex vivo* striatal slice preparations, a single pulse using these parameters evokes near-maximal  $[DA]_o$ , and as such is considered a perimaximal stimulation (Cragg, 2003). Local electrical stimulation is thought to indiscriminately activate all neurons within the spread of the stimulation that are successfully brought to threshold. In the striatum, this will include cholinergic interneurons (Melchior et al., 2015), which exert powerful control over DA release through cholinergic signalling at nicotinic ACh receptors (nAChRs) (Rice and Cragg, 2004; Threlfell et al., 2012). Therefore, to examine the mechanisms through which GABA regulates DA release through modulating the intrinsic properties of DA axons (and not indirectly through cholinergic interneurons), most experiments using electrical stimulation were carried out in the presence of dihydro- $\beta$ -erythroidine (DH $\beta$ E, 1  $\mu\text{M}$ ), an antagonist at  $\beta 2$ -subunit containing nAChRs. Electrically-evoked  $[DA]_o$  under these stimulation paradigms was sustained at a single recording site throughout repeated stimulations every 2.5 min by single pulses (1p) to 95% after the course of 40 min (**Figure 2.5**;  $t = 1.52$ ,  $p = 0.14$ , paired t-test of  $[DA]_o$  from 0-10 vs. 30-40 min,  $n = 12$  experiments/10 mice).

In experiments where  $[DA]_o$  was evoked by light stimulation in acute *ex vivo* slices prepared from Slc6a3<sup>IRES-Cre</sup> mice conditionally expressing ChR2-eYFP in DA axons, DA axons in striatum were activated by TTL-driven (Multi Channel Stimulus II, Multi Channel Systems) brief pulses (2 ms) of blue light (470 nm; ~5 mWmm<sup>-2</sup>; OptoLED; Cairn Research), which illuminated the field of view (2.2 mm, x10 water-immersion objective). Light stimulation of DA release was delivered at a perimaximal intensity that was determined at the beginning of each experiment to account for variability in the extent of ChR2 expression at the site of recording. DH $\beta$ E was not used, because selective activation of DA axons is not thought to evoke release of ACh from CHIs (Melchior et al., 2015). Epifluorescence used to visualize ChR2-eYFP expression was used sparingly to minimize ChR2 activation before recordings. Light-evoked  $[DA]_o$  under these stimulation paradigms was sustained at a single recording site throughout repeated stimulations every 2.5 min by single pulses (1p) to 90% after the course of 40 min ( $t = 2.00$ ,  $p = 0.09$ , paired t-test,  $n = 8$  experiments/6 mice; data not shown). No viral control experiment (e.g. GFP expression alone in DA neurons) was used in this study as (1) selective targeting of opsin expression to DA neurons has previously been used with high specificity using *Cre-lox* recombination in the Slc6a3<sup>IRES-Cre</sup> driver line by the Cragg Laboratory (Threlfell et al., 2012) and other (Tritsch et al., 2014; Melchior et al., 2015); (2) cyclic voltammograms of light-evoked  $[DA]_o$  recorded herein are comparable to those evoked by exogenous DA application (Figure 2.4).

### 2.3 Investigating GABA signalling in acute *ex vivo* slice preparations

Throughout this body of work, whole-cell patch-clamp electrophysiology recordings in voltage-clamp were used to measure the amplitude and kinetics of post-synaptic GABA<sub>A</sub> receptor-mediated currents in spiny projection neurons (SPNs). Two major types of GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic currents (IPSCs) were recorded in SPNs. The first were IPSCs resulting from synchronised and quantal release of GABA from a presynaptic neuron, which induces a distinct high-amplitude, rapidly decaying, generally hyperpolarising event in SPNs, and mediate phasic inhibition. In particular, these events

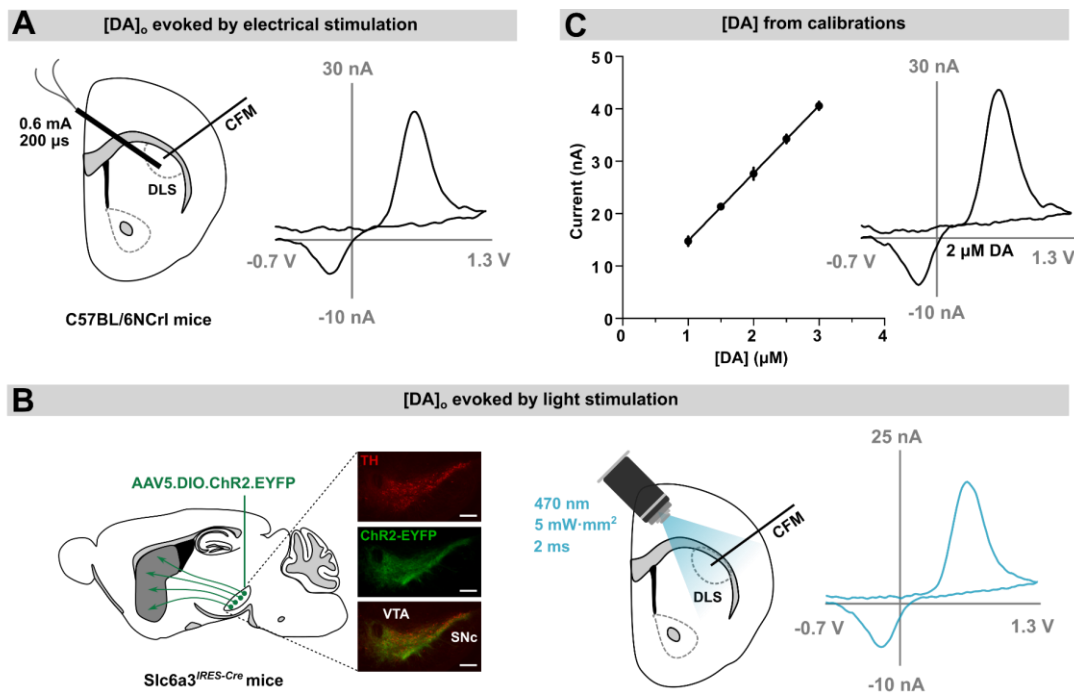


Figure 2.4. Representative cyclic voltammograms of [DA]<sub>o</sub> evoked with electrical or light stimulations, and from respective calibration.

(A-B) Schematics representing the experimental configuration and representative voltammograms for (A) electrically evoked [DA]<sub>o</sub> or (B) light-evoked [DA]<sub>o</sub> after injection and expression of viral ChR2-eYFP in VTA and SNc in *Slc6a3<sup>ires-Cre</sup>* mouse. TH (red), ChR2-eYFP (green). Scale bars in insets: 0.25 mm.

(C) There is a linear relationship between exogenous [DA] and faradaic oxidation current,  $R^2 = 0.99$  (left). Representative voltammogram for calibrating carbon fibre microelectrode with 2  $\mu$ M exogenous DA (right).

were recorded immediately following light activation of DA axons selectively expressing ChR2-eYFP, which is known to facilitate GABA co-release from presynaptic DA terminals onto SPNs (Kim et al., 2015; Tritsch et al., 2012, 2014). The second type were tonic GABA<sub>A</sub> receptor-mediated IPSC recorded in SPNs, which result from low concentrations of ambient GABA acting at high-affinity extrasynaptic GABA<sub>A</sub> receptors on SPNs. These currents, which provide tonic inhibition, have low amplitudes and longer decay times than synaptic receptor mediated IPSCs, and show little desensitization (Farrant and Nusser, 2005), recorded as depolarising shifts in the baseline currents in response to GABA<sub>A</sub> receptor

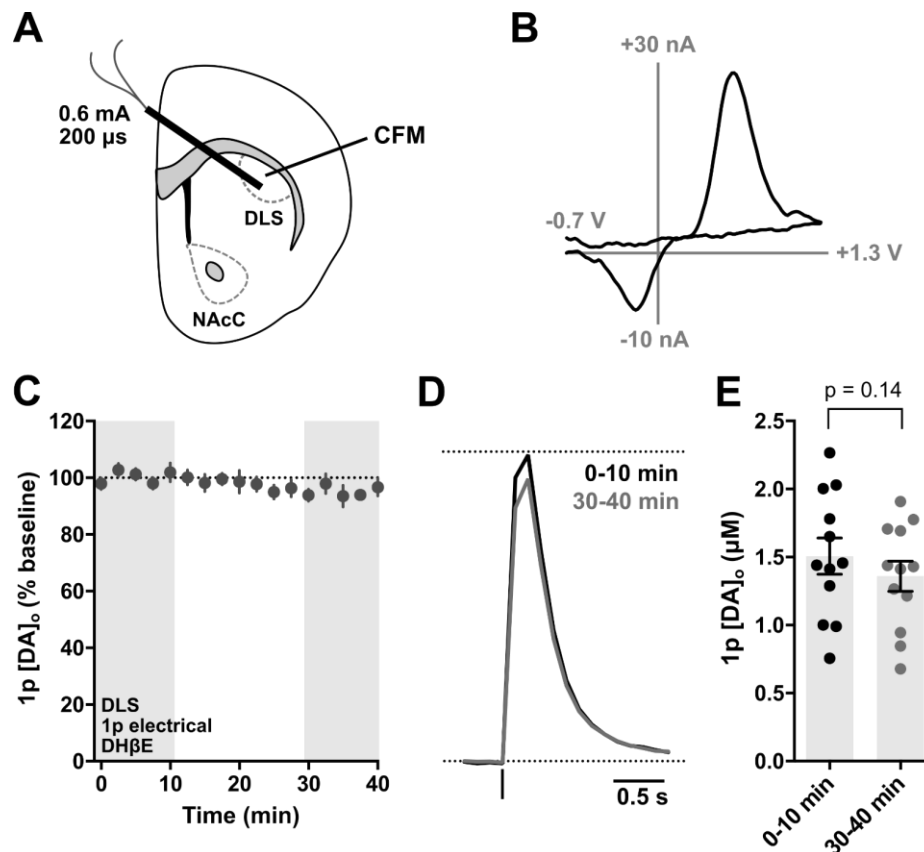


Figure 2.5. Evoked  $[DA]_o$  measured using FSCV is sustained across repeated stimulations.

(A-B) Schematics representing the experimental configuration (A) and representative voltammograms (B) for electrically evoked  $[DA]_o$ .

(C) Mean peak  $[DA]_o$  ( $\pm$  SEM) during consecutive recordings evoked by a single electrical pulse (1p) every 2.5 min in DLS for 40 min with the nAChR antagonist DH $\beta$ E (1  $\mu$ M) present throughout. Data are normalised to first 4 time points (0-10 min baseline, dotted line); and statistically compared to the last 4 time points (shaded regions).

(D-E) Mean ( $\pm$  SEM)  $[DA]_o$  transients over time normalised to baseline (D) and mean ( $\pm$  SEM)  $[DA]_o$  values in  $\mu$ M (E) averaged across 0-10 min baseline (black) and 30-40 min (grey). Paired t test.

### 2.3 Investigating GABA signalling in acute *ex vivo* slice preparations

Throughout this body of work, whole-cell patch-clamp electrophysiology recordings in voltage-clamp were used to measure the amplitude and kinetics of post-synaptic GABA $_A$  receptor-mediated currents in spiny projection neurons (SPNs). Two major types of GABA $_A$  receptor-mediated inhibitory postsynaptic currents (IPSCs) were recorded in SPNs. The first were IPSCs resulting from synchronised and quantal release of GABA from a presynaptic

neuron, which induces a distinct high-amplitude, rapidly decaying, generally hyperpolarising event in SPNs, and mediate phasic inhibition. In particular, these events were recorded immediately following light activation of DA axons selectively expressing Chr2-eYFP, which is known to facilitate GABA co-release from presynaptic DA terminals onto SPNs (Kim et al., 2015; Tritsch et al., 2012, 2014). The second type were tonic GABA<sub>A</sub> receptor-mediated IPSC recorded in SPNs, which result from low concentrations of ambient GABA acting at high-affinity extrasynaptic GABA<sub>A</sub> receptors on SPNs. These currents, which provide tonic inhibition, have low amplitudes and longer decay times than synaptic receptor mediated IPSCs, and show little desensitization (Farrant and Nusser, 2005), recorded as depolarising shifts in the baseline currents in response to GABA<sub>A</sub> receptor blockade.

### 2.3.1 Slice preparation for whole-cell patch-clamp electrophysiology

Mice were anaesthetised with pentobarbital and transcardially perfused with ice-cold calcium-free artificial cerebrospinal fluid (aCSF) containing (in mM): 130 NaCl, 2.5 KCl, 26 NaHCO<sub>3</sub>, 2 MgCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub> and 10 glucose. Acute 300 µm-thick coronal slices containing CPU and NAc (spanning from AP +1.3 mm to +0.2 mm from bregma; Figure 2.3) were prepared from dissected brain tissue using a vibratome (VT1200S, Leica Microsystems) in ice-cold cutting solution containing (in mM): 194 sucrose, 30 NaCl, 4.5 KCl, 1 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub> and 10 glucose, and transferred to a holding chamber containing aCSF with 2.5 mM CaCl<sub>2</sub> heated to 34°C for 15 min before returning to room temperature (20-22°C). All recordings were obtained within 6 hours of slicing. All solutions were saturated with 95% O<sub>2</sub> / 5% CO<sub>2</sub>.

For electrophysiology recordings from slices prepared from aged SNCA-OVX Parkinsonian mice, an alternate slice preparation method was performed. This method reduces swelling and damage in superficial layers of the slices and improves the success rate for targeted patch clamp recordings in aged animals (Ting et al., 2014). Mice were anaesthetised with

pentobarbital and transcardially perfused with 25–30 mL of room temperature NMDG aCSF containing (in mM): 92 NMDG, 2.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 30 NaHCO<sub>3</sub>, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 0.5 CaCl<sub>2</sub>·4H<sub>2</sub>O, and 10 MgSO<sub>4</sub>·7H<sub>2</sub>O (titrated to pH 7.3–7.4 with concentrated HCl). Acute 300 µm-thick coronal slices containing CPu and NAc (spanning from AP +1.35mm to +0.10 mm from bregma) were prepared from dissected brain tissue using a vibratome (VT1200S, Leica Microsystems) in room temperature NMDG aCSF, and transferred to a holding chamber containing NMDG aCSF heated to 34°C for 12 min. Directly following recovery heated recovery, slices were transferred into a new holding chamber containing room-temperature HEPES holding aCSF containing (in mM): 92 NaCl, 2.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 30 NaHCO<sub>3</sub>, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 2 CaCl<sub>2</sub>·4H<sub>2</sub>O, and 2 MgSO<sub>4</sub>·7H<sub>2</sub>O, taking care to ensure minimal carry-over of the NMDG aCSF into the new holding chamber. All recordings were obtained within 6 hours of slicing. All solutions were saturated with 95% O<sub>2</sub> / 5% CO<sub>2</sub>.

### 2.3.2 Whole-cell patch-clamp electrophysiology

Individual slices were hemisected and transferred to a recording chamber and superfused at ~3.0 mL/min with aCSF containing (in mM): 130 NaCl, 2.5 KCl, 26 NaHCO<sub>3</sub>, 2 MgCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub> and 10 glucose, heated to 31–33 °C. All whole-cell experiments were recorded using borosilicate glass pipettes with resistances in the 3 – 5 MΩ range and were pulled on a Flaming- Brown micropipette puller (P-1000, Sutter Instruments). Whole-cell voltage-clamp electrophysiology recordings were made from SPNs identified by their morphology and membrane properties (Gertler et al., 2008; Planert et al., 2013), as done previously (Patton et al., 2016, 2019), in the DLS or NAcC. SPNs were voltage-clamped at -70 mV using a MultiClamp 700B amplifier (Molecular Devices) and with pipettes filled with a CsCl-based internal solution (in mM 120 CsCl, 15 CsMeSO<sub>3</sub>, 8 NaCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP, 5 QX-314; pH 7.3 adjusted with CsOH; osmolarity ranging from 305 - 310 mOsmkg<sup>-1</sup>). Negative pressure was applied to form a seal with resistance > 1 GΩ, and negative pressure and current pulses were used to rupture the patch. During experiments,

access resistance was monitored by measurement of the initial current in response to a delivery of a 2 ms square pulse of +5 mV and cells were discarded from analysis if series resistance varied by more than 15% or increased over 25 M $\Omega$ . Errors due to the voltage drop across the series resistance (<20 M $\Omega$ ) were left uncompensated and membrane potentials were corrected for a ~5 mV liquid junction potential.

To record tonic GABA<sub>A</sub> receptor currents, SPNs voltage-clamped at -70 mV were recorded in gap-free mode to measure changes in the holding current. Cells were allowed to stabilize for 5-10 min before drug manipulations: GAT inhibitors were bath applied for 20 -25 min; picrotoxin (100  $\mu$ M) for an additional 3-5 min. Recordings of light-evoked GABA currents in SPNs from Chr2-expressing DA axons in slices from Slc6a3<sup>IRE5-Cre</sup> mice were taken 10 min after break-in, and at 30 s intervals for a duration of 10 min from SPNs voltage-clamped at -70 mV. Under these conditions, GABA<sub>A</sub> receptor-mediated currents appear inward as reported previously (Kim et al., 2015; Tritsch et al., 2012, 2014). TTL-driven (Multi Channel Stimulus II, Multi Channel Systems) brief pulses (2 ms) of blue light (470 nm; 5 mWmm<sup>-2</sup>; OptoLED; Cairn Research) illuminated the full field of view (2.2 mm, X10 water-immersion objective). All recordings were in the presence of NBQX (5  $\mu$ M) and APV (50  $\mu$ M) to block AMPA and NMDA receptor-mediated inward currents.

Recordings were made using a Multiclamp 700B amplifier (Molecular Devices), filtered at 5 kHz using a low-pass Bessel filter and digitised at 10-20 kHz using a Digidata 1550A acquisition board (Axon Instruments). Data were collected using Clampex and offline analysis performed using Clampfit (pClamp10). Each data set was collected from a minimum of three animals and threshold for statistical significance in all analyses was  $p < 0.05$ , adjusted for multiple comparisons where appropriate.

## 2.4 Biochemical methods

### 2.4.1 High-performance liquid chromatography with electrochemical detection of dopamine

HPLC with electrochemical detection was conducted throughout to assess for changes in DA content between specific conditions and between genotypes. Samples were taken from striatal slices used for FSCV experiments, routinely performed previously (Janezic et al., 2013; Sloan et al., 2016; Taylor et al., 2014). From each 300  $\mu$ m-thick coronal slice containing striatum, two tissue punches (2 mm in diameter) were taken from dorsal striatum (one from each hemisphere) and two tissue punches (1.2 mm in diameter) were taken from the nucleus accumbens (one from each hemisphere). Tissue samples were snap frozen and stored at  $-80^{\circ}\text{C}$  in 200  $\mu\text{L}$  0.1 M  $\text{HClO}_4$  until day of analysis. On the day of analysis, samples were thawed on ice, homogenised, and centrifuged at 15,000 g for 15 min at  $4^{\circ}\text{C}$ . The supernatant was analysed for DA content. Analytes were separated using a  $4.6 \times 250$  mm Microsorb C18 reverse-phase column (Varian or Agilent) and detected using a Decade II SDS electrochemical detector with a Glassy carbon working electrode (Antec Leyden) set at + 0.7 V with respect to a Ag/AgCl reference electrode. The mobile phase consisted of 13% methanol (vol/vol), 0.12 M  $\text{NaH}_2\text{PO}_4$ , 0.5–4.0 mM octenyl succinic anhydride (OSA), and 0.8 mM EDTA (pH 4.4–4.6), and the flow rate was fixed at 1 mL/min.

### 2.4.2 Sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed in Chapter 5 to assess for changes in striatal GAT expression levels in Parkinsonian mice. For SDS-PAGE, mouse brains were extracted and sliced using the procedures outlined above (see Section 2.2.3), albeit one 1200  $\mu$ m-thick coronal slice containing striatum was prepared from each brain and one tissue punch (2 mm in diameter) of dorsal striatum taken per hemisphere. Striatal tissue samples were snap-frozen and stored at  $-80^{\circ}\text{C}$ . For analysis, striatal tissue was defrosted on ice,

homogenised in RIPA Lysis and Extraction Buffer (Sigma-Aldrich) containing 150 mM NaCl, 1.0% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0, with Complete-Mini Protease Inhibitor and PhosStop (Roche), using a Tissue Tearor (Biospec Products, Inc), and soluble fraction isolated by microcentrifugation at 15,000 g for 15 min at 4°C. Total protein content was quantified using a BCA Protein Assay Kit (Thermo Scientific) and equal amounts of total protein were loaded onto 4 – 15% Tris-Glycine gels (BioRad). Note, GAT protein aggregates after boiling, making it necessary to run SDS-PAGE with non-boiled samples, therefore samples were not denatured by heating to 95 °C as regularly performed. Following electrophoresis (200 V for ~45 min), proteins were transferred onto polyvinylidene fluoride membranes (BioRad) using the BioRad Trans-Blot Turbo apparatus (BioRad). Blots were probed overnight at 4°C with 1:1,000 rabbit anti-GAT 1 (Synaptic Systems, 274102) or 1:1,000 rabbit anti-GAT 3 (Abcam, AB181783). Blots were incubated with HRP-conjugated secondary antibodies at 1:3,000 for 1 h at room temperature and bands developed using ECL Prime Western Blotting Detection Reagent (GE Healthcare). Blots were subsequently incubated with 1:20,000 HRP-conjugated  $\beta$ -actin (Abcam, AB49900) for 1 h at room temperature and bands developed as above. Visualization and imaging of blots was performed with a ChemiDoc Imaging System (BioRad) and bands quantified using Image Lab Software (BioRad). Protein concentration for GAT-1 and GAT-3 were normalized to  $\beta$ -actin.

### 2.4.3 Indirect immunofluorescence and confocal imaging

Immunohistochemistry was used throughout to confirm the localisation of the AAV construct to midbrain DA neurons and to examine heterogeneity of GAT expression across striatal territories and between striatal cell types. Mice were anaesthetised with an overdose of pentobarbital and transcardially perfused with 20-50 mL of phosphate-buffered saline (PBS), followed by 30-100 mL of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4. Brains were removed and post-fixed overnight in 4% PFA. Brains were embedded in agar (3-4%) and coronal sections (50  $\mu$ m) were cut on a vibrating microtome (Leica

VT1000S) and collected in a 1 in 4 series. Sections were stored in PBS with 0.05% sodium azide. Upon processing, sections were washed in PBS and then blocked for 1h in a solution of PBS TritonX (0.3%) with sodium azide (0.02%; PBS-Tx) containing 10% normal donkey serum (NDS). Sections were then incubated in primary antibodies overnight in PBS-Tx with 2% NDS at 4°C. Primary antibodies: rabbit anti-TH (1:2,000, Sigma-Aldrich, ab112); rabbit anti-GAT1 (1:1,000, Synaptic Systems, 274102); rabbit anti-GAT3 (1:250, Millipore/Chemicon, AB1574); guinea pig anti-S100 $\beta$  (1:2,000, Synaptic Systems, 287004); rat anti-GFP that also recognizes eYFP (1:1,000, Nacalai Tesque, 04404-84); and guinea-pig anti-parvalbumin (1:1,000, Synaptic Systems, 195004). Sections were then incubated in species-appropriate fluorescent secondary antibodies with minimal cross-reactivity overnight in PBS-Tx at room temperature (Donkey anti-Rabbit AlexaFluor 488, 1:1,000, Invitrogen, A21206; Donkey anti-Rabbit Cy3, 1:1,000, Jackson ImmunoResearch, 711-165-152; Donkey anti-Guinea Pig AlexaFluor 488, 1:1,000, Jackson ImmunoResearch, 706-545-148; Donkey anti-Rat AlexaFluor 488, 1:1,000, Jackson ImmunoResearch, 712-545-153). Sections were washed in PBS and then mounted on glass slides and cover-slipped using Vectashield (Vector Labs). Coverslips were sealed using nail varnish and stored at 4°C.

To verify the specificity of Chr2-eYFP expression in TH-positive structures in Slc6a3<sup>IRES-Cre</sup> mice, mounted sections were imaged with an Olympus BX41 microscope with Olympus UC30 camera and filters for appropriate excitation and emission wave lengths (Olympus Medical). For whole striatum analysis of GAT1 or GAT3, confocal images were acquired with an LSM880/Axio.Imager Z2 (Zeiss) and Image J was used for image analysis. The X10 (NA = 0.45) objective was used and all imaging settings (laser %, pinhole/optical section, pixel size, gain, and scanning speed) were kept constant between animals. For the quantification of fluorescence (mean grey values), 4 sections in the rostro-caudal plane were imaged at approximately the following distances rostral of Bregma; +1.3mm, +1.0mm, +0.6mm, and +0.25mm. A region of interest (ROI) of 300  $\mu$ m x 300  $\mu$ m was overlaid over the DLS; and an ROI of 200  $\mu$ m X 200  $\mu$ m was overlaid on NAcC, for both hemispheres. Values for NAcC

were taken from the 2 most rostral sections. Mean grey values from the areas of interest were normalised to the median grey value for each hemisphere ( $n = 12$  hemispheres from 6 animals). For examination of co-localisation a X63 objective was used ( $NA = 1.46$ ); Z-stacks were taken with the pinhole set to 1 Airy Unit (optical section =  $0.7\mu\text{m}$ ) with a z-stack interval of  $0.35\mu\text{m}$ . In order to assess co-localisation ZEN (blue edition v.2.3; Zeiss) software was used. For S100 $\beta$ , PV+ axons (eYFP in PV-Cre mice) or dopaminergic axons (eYFP in Slc6a3<sup>IRE5-Cre</sup> mice) and GAT1/3 co-localisation, stacks from 2 striatal regions and 2 NAcC regions in at least one section were examined per animal ( $n = 3$  per marker).

# 3

Tonic GABAergic  
inhibition of striatal  
dopamine release

### 3.1 Introduction and initial hypotheses

As described in **Chapter 1**, striatal DA signals can be dissociated from those of neuronal activity in the midbrain (Mohebi et al., 2019), likely due to striatal DA release being gated locally by axonal mechanisms and striatal neuromodulators that can regulate or even independently drive DA release (Rice et al., 2011; Schmitz et al., 2003; Sulzer et al., 2016). Of notable example are ChIs, which play a particularly powerful role in both gating and driving DA release through nAChRs on DA axons (Jones et al., 2001; Rice and Cragg, 2004; Threlfell et al., 2012; Zhou et al., 2001). In this chapter, I test how local striatal GABA sources might inhibit DA release through actions at GABA<sub>A</sub> and GABA<sub>B</sub> receptors directly on DA axons.

As previously outlined in **Section 1.5.5**, the striatum contains a high density of projection neurons and interneurons that use the transmitter GABA through canonical GAD-dependent GABA synthesis (Lozovaya et al., 2018; Oorschot, 1996; Tepper et al., 2018) and mesostriatal DA neurons themselves can synthesise, store and co-release GABA in striatum, through a non-canonical ALDH1a1-mediated GABA synthesis pathway (Kim et al., 2015; Tritsch et al., 2012). Furthermore, emerging evidence support GABA<sub>A</sub> and GABA<sub>B</sub> receptor expression on striatal DA axons (Charara et al., 1999; Sherpa et al., 2019; Yung et al., 1999). Striatal GABA is therefore in a key position to modulate DA output, although this interaction has not yet been extensively studied.

An assortment of previous evidence indicates that striatal GABA<sub>A</sub> and GABA<sub>B</sub> receptor activation inhibits DA release (Avshalumov et al., 2003a; Chéramy et al., 1978; Giorgiueff et al., 1978; Pitman et al., 2014; Reinmann et al., 1983; Ronken et al., 1993; Schmitz et al., 2002; Smolders et al., 1995; for extensive review see **Section 1.5.6**). These studies suggest that DA release might be under inhibition by an endogenous striatal GABA source, but this is yet to be confirmed. Studies which employ agonists for GABA<sub>A</sub> and GABA<sub>B</sub> receptors do not reveal a physiologically relevant role for these receptors, like antagonists do.

Furthermore, the only previous study which uses antagonists are conducted using purely an *in vivo* approach (Smolders et al., 1995), which is confounded by potential effects on long loop circuits that could regulate striatal DA output via resultant changes in DA neuron firing in the midbrain.

In addition to DA axons putatively expressing GABA<sub>A</sub> and GABA<sub>B</sub> receptors (Charara et al., 1999; Sherpa et al., 2019; Yung et al., 1999), ChIs also express both GABA<sub>A</sub> and GABA<sub>B</sub> receptors (Waldvogel et al., 1998; Yung et al., 1999). Considering that ChIs powerfully modulate DA release and can mediate effects of other neuromodulators on DA, including opioids, nitric oxide, glutamate, and insulin (Britt and McGehee, 2008; Hartung et al., 2011; Kosillo et al., 2016; Stouffer et al., 2015), ChIs emerge as un-examined alternate candidates for mediating GABA regulation of DA output. Furthermore, the source of striatal GABA, whether GAD- or ALDH1a1-dependent, which might provide inhibition of DA release remains unidentified.

In order to help resolve these open questions, we explored the control of DA release by GABA, GABA<sub>A</sub> receptors, and GABA<sub>B</sub> receptors in the absence of cholinergic signalling using FSCV in acute *ex vivo* striatal slices prepared from mice. We assessed GABA receptor regulation of DA release when evoked electrically in the presence of nAChR antagonist dihydro- $\beta$ -erythroidine (DH $\beta$ E) and also when DA release was evoked by targeted activation of DA axons using optogenetics when DA release is not under nAChR control (Melchior et al., 2015; Threlfell et al., 2012). Furthermore, we explored the possible inhibition of DA release by an endogenous striatal GABA source through testing the effects of GABA<sub>A</sub> and GABA<sub>B</sub> receptors antagonists, in addition to inhibiting ALDH- and GAD-dependent GABA synthesis.

### 3.1.1 Initial hypotheses

Overarching hypothesis - GABA signalling will inhibit dopamine output in striatum.

Hypothesis 1 - GABA<sub>A</sub> and GABA<sub>B</sub> receptor agonists will inhibit striatal dopamine output, independent of cholinergic signalling.

Hypothesis 2 - Striatal dopamine release is under inhibition by an endogenous striatal GABA source.

## 3.2 Methods

Experiments described in this chapter were carried out according to the general methods described in **Chapter 2**. Variations and further details are described below.

### 3.2.1 Slice preparation

In FSCV experiments where electrically-evoked [DA]<sub>o</sub> was recorded, adult 7- to 10-week-old wild-type male C57BL/6J mice (Charles River) were used. In FSCV experiments where light-evoked [DA]<sub>o</sub> was recorded through the selective activation of DA axons, adult 8- to 14-week-old male Slc6a3<sup>IRE5-Cre</sup> mice conditionally expressing ChR2-eYFP in DA axons after AAV intracranial injections ~3-4 weeks prior. Acute *ex vivo* coronal slices containing striatum were prepared for FSCV as described in **Section 2.2.3**. All experimental procedures involving the use of animals were carried out according to institutional guidelines and conformed to the UK Animals (Scientific Procedures) Act 1986.

### 3.2.2 Fast-scan cyclic voltammetry

Each FSCV experiment was performed at a single recording site in one brain slice as outlined in **Section 2.2**. Data are expressed as extracellular concentration of dopamine ([DA]<sub>o</sub>) or normalised to mean peak [DA]<sub>o</sub> evoked by single pulses in control conditions. FSCV data are normalised to pre-drug conditions for clarity and for comparisons between regions. Evoked [DA]<sub>o</sub> was recorded every 2.5 min and DHβE (1 μM) was present throughout all experiments where electrically-evoked [DA]<sub>o</sub> was recorded. In all cases, [DA]<sub>o</sub> displayed

typical kinetics to peak and decay, indicative of good slice quality and were obtained from recording sites that maintained sufficiently stable levels of release over time. No FSCV data were excluded after acquisition.

Drug washes were performed once stable release at the given recording site was stable: less than 10% variation over 5 consecutive stimulations. For drug effects, peak  $[DA]_o$  was averaged over 4 stimulations once peak  $[DA]_o$  had re-stabilised post-drug application (~10-20 min of application) and compared to time-matched data from drug-free controls and/or pre-drug baselines (averaged across 4 stimulations prior to drug wash-on). For experiments involving multiple pulse protocols, each stimulation type was repeated in triplicate, interspersed with single-pulse stimulations, and then averaged and normalised to single-pulse stimulations at each recording site, as previously (Lopes, Roberts et al., 2018; Threlfell et al., 2012).

### 3.2.3 Drugs

Drugs were dissolved in distilled water or dimethyl sulfoxide to make stock aliquots at 1,000 x final concentration and stored at -20°C until required. Stock aliquots were diluted with oxygenated aCSF to final concentrations immediately before use. The drugs used in this study, their final concentrations, and references to studies which clarified the choice of final concentration used are outlined in Table 3.1. Final concentrations used were determined from previous pharmacological studies using acute rodent brain slices (where possible in striatum), which often require concentrations significantly greater than the half maximal effective concentrations ( $EC_{50}$ ) for sufficient penetration of slices.

### 3.2.4 Data Analysis

Data are represented as means  $\pm$  standard error of the mean, and 'n' refers to the number of experiments. Each experiment was performed at a single recording site in one discrete brain slice. The number of animals in each dataset was  $\geq 3$ . Comparisons for statistical significance were assessed by one- or two-way ANOVA, paired t tests, or Mann-Whitney

tests where data were not normally distributed (assessed using D'Agostino-Pearson normality test) using Prism 7 (GraphPad). Threshold for statistical significance in all analyses was  $p < 0.05$ , adjusted for multiple comparisons.

Table 3.1. Drugs listed with method of action and references to studies which clarified the choice of concentrations used in Chapter 3.

| Drug                                          | Source                   | Action                                                                   | Final concentration     | Reference(s)                              |
|-----------------------------------------------|--------------------------|--------------------------------------------------------------------------|-------------------------|-------------------------------------------|
| 3-mercaptopropionic acid (3-MPA)              | Tocris Bioscience        | glutamate decarboxylase inhibitor                                        | 500 $\mu$ M             | Kim et al., 2015                          |
| Baclofen                                      | Sigma-Aldrich            | GABA <sub>B</sub> receptor agonist                                       | 100 $\mu$ M             | Schmitz et al., 2002; Pitman et al., 2014 |
| Bicuculline                                   | Tocris Bioscience        | GABA <sub>A</sub> receptor antagonist                                    | 10 $\mu$ M              | Cepeda et al., 2001; Brodnik et al., 2018 |
| CGP 55845                                     | Abcam                    | GABA <sub>B</sub> receptor antagonist                                    | 4 $\mu$ M               | Tritsch et al., 2014                      |
| Dihydro- $\beta$ -erythroidine (DH $\beta$ E) | Tocris Bioscience        | $\beta$ 2 subunit-containing nicotinic acetylcholine receptor antagonist | 1 $\mu$ M               | Lopes, Roberts et al., 2019               |
| Disulfiram                                    | Santa Cruz Biotechnology | aldehyde dehydrogenase inhibitor                                         | 10 $\mu$ M              | Kim et al., 2015                          |
| $\gamma$ -aminobutyric acid (GABA)            | Sigma-Aldrich            | exogenous GABA                                                           | 100 $\mu$ M, 2 mM, 5 mM | unpublished observations                  |
| Muscimol                                      | Abcam                    | GABA <sub>A</sub> receptor agonist                                       | 50 $\mu$ M              | Brodnik et al., 2018                      |
| Picrotoxin                                    | Sigma-Aldrich            | GABA <sub>A</sub> receptor antagonist                                    | 100 $\mu$ M             | Avshalumov et al., 2000                   |
| Saclofen                                      | Tocris Bioscience        | GABA <sub>B</sub> receptor antagonist                                    | 100 $\mu$ M             | Pitman et al., 2014                       |

### 3.3 Results

#### 3.3.1 GABA<sub>A</sub> and GABA<sub>B</sub> receptors inhibit striatal dopamine release

To explore whether GABA can modulate striatal DA release in the absence of cholinergic signalling at nAChRs, exogenous GABA was applied to striatal slices in the presence of the nAChR antagonist DH $\beta$ E (1  $\mu$ M). Bath application of GABA at 100  $\mu$ M, 2 mM and 5 mM concentration-dependently reduced DA release evoked by single electrical pulses by ~15 - 50% in DLS (Figure 3.1a-c;  $F_{2,13} = 18.4$ ,  $p = 0.0002$ , one-way ANOVA; 100  $\mu$ M GABA:  $t = 4.8$ ,  $p = 0.009$ ,  $n = 5$  experiments/4 mice; 2 mM GABA:  $t = 9.9$ ,  $p = 0.0002$ ,  $n = 6$  experiments/4 mice; 5 mM GABA:  $t = 15.8$ ,  $p < 0.0001$ ,  $n = 5$  experiments/4 mice; paired  $t$  tests from pre-drug baselines) and by ~5 - 60% in NAcC (Figure 3.1d-f;  $F_{2,14} = 39.7$ ,  $p < 0.0001$ , one-way ANOVA; 100  $\mu$ M GABA:  $t = 0.9$ ,  $p = 0.4$ ,  $n = 6$  experiments/4 mice; 2 mM GABA:  $t = 6.9$ ,  $p = 0.002$ ,  $n = 5$  experiments/4 mice; 5 mM GABA:  $t = 12.5$ ,  $p < 0.0001$ ,  $n = 6$  experiments/4 mice; paired  $t$  tests from pre-drug baselines), indicating that GABA is capable of inhibiting DA release in both dorsal and ventral striatum independently from any effects on ChI input to nAChRs.

To identify which GABA receptors can inhibit DA release, we tested the effect of GABA<sub>A</sub> and GABA<sub>B</sub> agonists in the DLS. To eliminate any effect due to coactivation of other striatal microcircuits during electrical stimulation, we used an optogenetic approach to selectively activate DA axons only. We expressed Chr2-eYFP in DA neurons and axons in *Slc6a3<sup>ires-Cre</sup>* mice using an established viral approach as previously described (Brimblecombe et al., 2015; Threlfell et al., 2012) and outlined in Section 2.1.3 and Section 2.2.5. Activation of GABA<sub>A</sub> receptors with muscimol (20  $\mu$ M) suppressed DA release evoked by single pulses of blue light by ~20% in DLS (Figure 3.2a;  $p = 0.008$ ,  $n = 5$  experiments/4 mice, Mann-Whitney test) and this effect was prevented by prior application of the GABA<sub>A</sub> channel blocker picrotoxin (100  $\mu$ M), confirming an effect via GABA<sub>A</sub> receptors (Figure 3.2b;  $p = 0.36$ ,  $n = 6$  experiments/4 mice, Mann-Whitney test). Activation of GABA<sub>B</sub> receptors with baclofen (10  $\mu$ M) also suppressed DA release evoked by single blue light pulses by ~30% in DLS (Figure

3.2c;  $p = 0.03$ ,  $n = 4$  experiments/3 mice, Mann-Whitney test) and this effect was confirmed to be due to GABA<sub>B</sub> receptors by prior application of the GABA<sub>B</sub> receptor antagonist saclofen (100  $\mu$ M), which prevented the effects of baclofen (Figure 3.2d;  $p = 0.13$ ,  $n = 5$  experiments/4 mice, Mann-Whitney test).

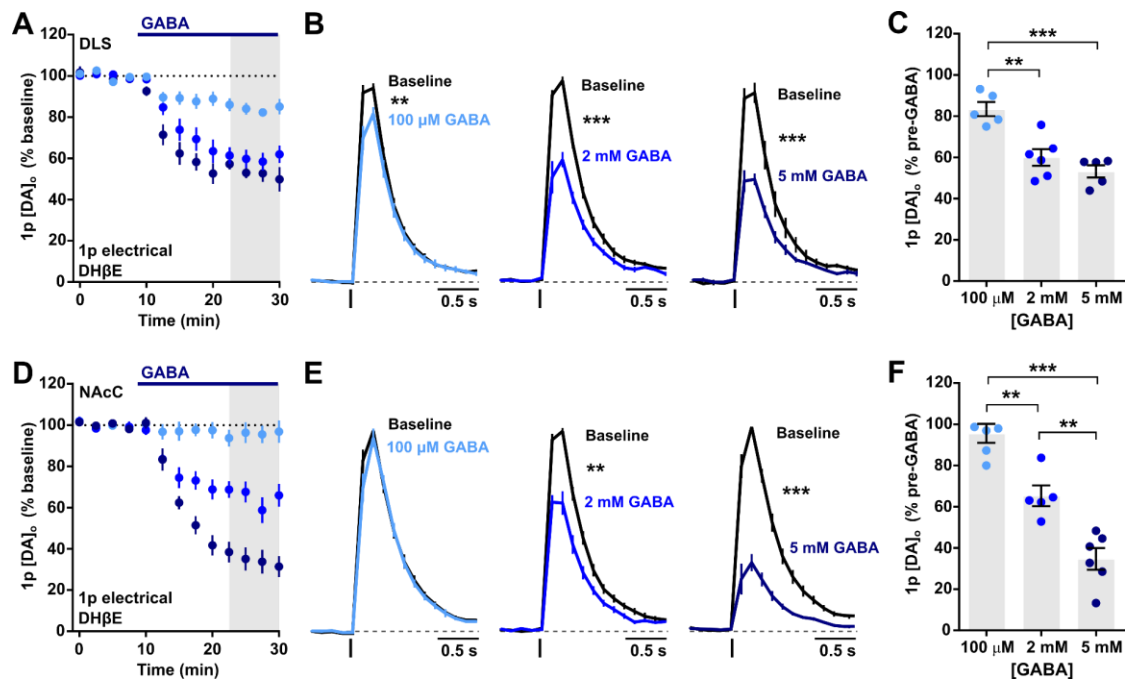


**Figure 3.1. Exogenous GABA concentration-dependently inhibits dopamine release.**

(A,D) Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) during consecutive recordings evoked by a single electrical pulse (1p) every 2.5 min in DLS (A) or NAcC (D) before and during bath application of exogenous GABA (100  $\mu$ M, 2 mM, 5 mM). Data are normalised to first 4 time points (drug-free baseline, dotted line); last 4 time points (shaded region) are used for statistical comparisons.

(B,E) Mean ( $\pm$  SEM) [DA]<sub>o</sub> transients over time before and after the application of exogenous GABA (from A,D and normalised to pre-drug baselines).

(C,F) Mean peak peak [DA]<sub>o</sub> ( $\pm$  SEM) evoked by 1p following application of exogenous GABA (from A,D) expressed as a percentage of pre-drug baseline.

Paired t-tests (B,E), one-way ANOVA and Bonferroni's multiple comparisons (C,F). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . The nAChR antagonist DH $\beta$ E (1  $\mu$ M) is present throughout. Data collected by B Roberts and R Siddorn.

We investigated whether GABA receptor activation attenuated DA release during trains of stimuli across a range of frequencies in the DLS. Muscimol significantly decreased  $[DA]_o$  evoked by 5-pulse trains of electrical stimulation at all frequencies tested (Figure 3.3a-b; two-way ANOVA: drug effect:  $F_{1,48} = 67.6$ ,  $p = 0.003$ , frequency effect:  $F_{3,48} = 259.6$ ,  $p < 0.0001$ ,  $n = 7$  experiments/5 mice) and, furthermore, there was a significant interaction between stimulation frequency and drug effect (Figure 3.3a; two-way ANOVA:  $F_{3,48} = 5.5$ ,  $p = 0.003$ ). This interaction was borne out by an increase in the 5p/1p ratio examined at 100 Hz (Figure 3.3c;  $t = 3.5$ ,  $p = 0.01$ , paired t-test), which indicates that GABA<sub>A</sub> receptor activation can marginally promote the contrast in DA signals released by different firing patterns or rates. Baclofen significantly decreased electrically evoked  $[DA]_o$  (Figure 3.3d-e; two-way ANOVA:  $F_{1,48} = 6.3$ , drug effect:  $p = 0.016$ ; frequency effect:  $F_{3,48} = 144.5$ ,  $p < 0.0001$ ,  $n = 7$  experiments/5 mice). Unlike muscimol, we did not detect a significant interaction between stimulation frequency and drug effect (Figure 3.3d; two-way ANOVA:  $F_{3,48} = 0.04$ ,  $p = 0.99$ ); however, the 5p/1p ratio for evoked  $[DA]_o$  examined at 100 Hz was slightly increased with baclofen (Figure 3.3f;  $t = 3.3$ ,  $p = 0.02$ , paired t-test), indicating that GABA<sub>B</sub> receptors can only marginally change the contrast in DA signals released by different activity. Altogether then, GABA<sub>A</sub> and GABA<sub>B</sub> activation though exogenous GABA and ligands for these receptors can attenuate evoked DA release through a mechanism which is independent of ChIs mediating these effects through cholinergic signalling at nAChRs on DA axons. Furthermore, GABA<sub>A</sub> and GABA<sub>B</sub> receptor activation can promote, albeit only marginally and primarily through GABA<sub>A</sub> receptors, the contrast in DA signals released across different firing patterns or rates. However, these data which investigate only the effect of receptor agonists do not test for physiologically relevant inhibition of DA release though an endogenous striatal GABA source acting at GABA<sub>A</sub> and GABA<sub>B</sub> receptors.

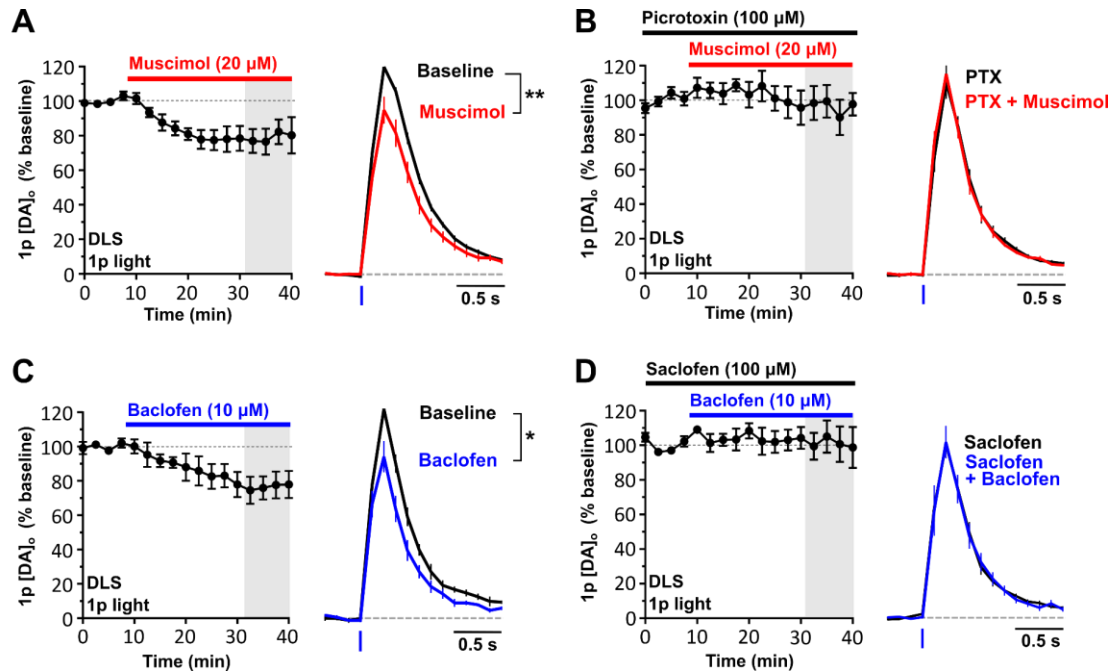


Figure 3.2. GABA<sub>A</sub> and GABA<sub>B</sub> receptor agonists inhibit optogenetically stimulated dopamine release.

(A,C) *Left*, Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) versus time recorded in DLS and evoked by 1 blue light pulse every 2.5 min before and during bath application of 20  $\mu$ M muscimol,  $n = 5$  (red, A) and 10  $\mu$ M baclofen,  $n = 4$  (blue, C). *Right*, Mean [DA]<sub>o</sub> ( $\pm$  SEM) transients over time.

(B,D) *Left*, Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) versus time recorded in DLS and evoked by 1 blue light pulse every 2.5 min before and during bath application of 20  $\mu$ M muscimol in the presence of 100  $\mu$ M picrotoxin,  $n = 5$  (red, B); and 10  $\mu$ M baclofen in the presence of 100  $\mu$ M saclofen,  $n = 6$  (blue, D). *Right*, Mean [DA]<sub>o</sub> ( $\pm$  SEM) transients over time.

Data are normalised to first 4 time points (drug-free baseline, dotted line); last 4 time points (shaded region) are used for statistical comparisons. \* $p < 0.05$ , \*\* $p < 0.01$ , Mann-Whitney tests. Data collected by E Lopes and stereotaxic surgeries performed by B Roberts.

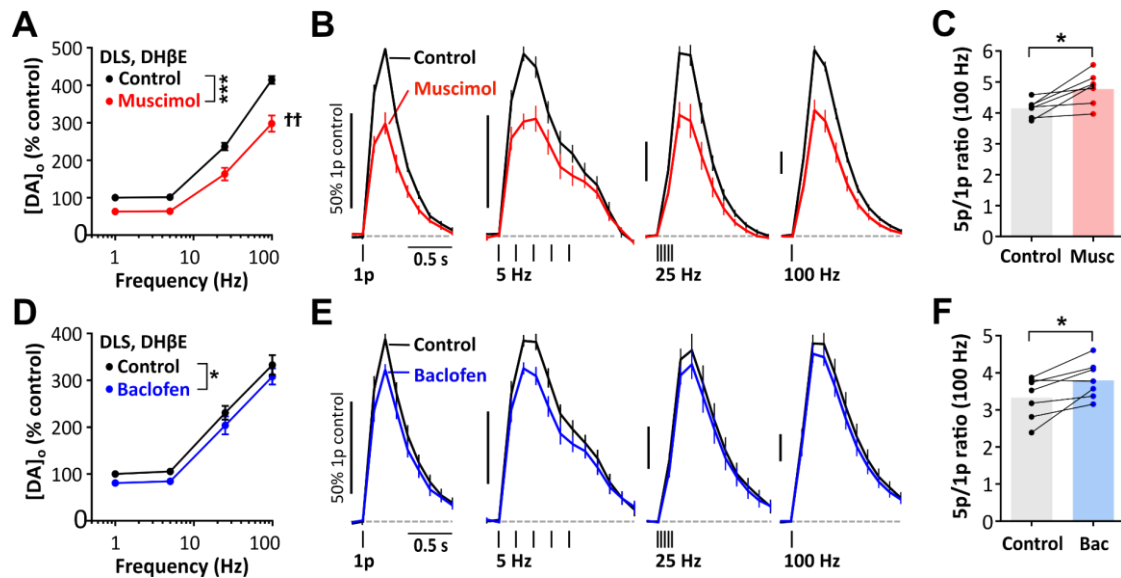


Figure 3.3. GABA $_A$  and GABA $_B$  receptor agonists modify DA release during pulse trains.

(A-B,D-E) Mean peak  $[DA]_o$  ( $\pm$  SEM) represented as a percentage of 1p in control drug-free conditions (A,D) and mean  $[DA]_o$  ( $\pm$  SEM) transients over time (B,E) for 1- or 5-pulse trains in control conditions (*black*); 20  $\mu$ M muscimol,  $n = 7$  (A,B) (*red*); or 10  $\mu$ M baclofen (D,E) (*blue*). Two-way ANOVA, effect of drug \*p < 0.05, \*\*\*p < 0.001, drug  $\times$  frequency interaction  $^{††}$ p < 0.01. Data are normalised to peak  $[DA]_o$  before drug application.

(C,F) Ratio of peak  $[DA]_o$  released by 5p versus 1p (100 Hz) in control conditions, muscimol (C), or baclofen (F). \*p < 0.05, paired t-tests versus control. The nAChR antagonist DH $\beta$ E (1  $\mu$ M) is present throughout. Data collected by E Lopes.

### 3.3.2 Striatal dopamine release is under tonic inhibition by a local, endogenous GABA source

There is a paucity of GABAergic axoaxonic synapses onto DA axons (Charara et al., 1999), which would suggest an absence of local striatal GABAergic inhibition of DA release. Nonetheless, GABA can spillover for extrasynaptic action in other nuclei (Farrant and Nusser, 2005) and in the dorsal striatum there is a well-documented and sizeable ‘ambient’ GABA tone, which provides a tonic GABA<sub>A</sub> receptor-mediated inhibitory conductance at SPNs (Ade et al., 2008; Cepeda et al., 2013; Kirmse et al., 2008, 2009; Santhakumar et al., 2010). Striatal DA release might therefore be under tonic inhibition by ‘ambient’ GABA, which we tested for by exploring the effects of GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists.

We first investigated which receptors could mediate inhibition of DA release by endogenous GABA by investigating the effect of GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists separately using electrical stimulation in DLS of wild-type mice (in the presence of DH $\beta$ E). GABA<sub>A</sub> antagonist bicuculline (10  $\mu$ M) slightly elevated 1p evoked [DA]<sub>o</sub> by ~10% (Figure 3.4a;  $p = 0.01$ ,  $n = 5$  experiments/4 mice, Mann-Whitney test) and GABA<sub>B</sub> antagonist saclofen (100  $\mu$ M) significantly increased 1p evoked [DA]<sub>o</sub> by ~20% (Figure 3.4b;  $p = 0.0079$ ,  $n = 5$  experiments/4 mice, Mann-Whitney test). Next we tested the effect of co-application of GABA<sub>A</sub> and GABA<sub>B</sub> antagonists bicuculline (10  $\mu$ M) and CGP55845 (4  $\mu$ M), respectively, which significantly enhanced 1p electrically evoked [DA]<sub>o</sub> by ~25% in DLS, when compared to drug-free time-matched controls (Figure 3.5a;  $p = 0.0004$ ,  $n = 6$  experiments/3 mice, Mann-Whitney test). Since mechanisms that regulate striatal DA release can diverge between dorsal and ventral striatum (Brimblecombe et al., 2015; Britt and McGehee, 2008; Janezic et al., 2013; Shin et al., 2017; Threlfell and Cragg, 2011; Threlfell et al., 2010), we determined whether DA release is also regulated by tonic GABA in ventral striatum. Co-application of bicuculline (10  $\mu$ M) and CGP55845 (4  $\mu$ M) significantly enhanced 1p electrically evoked [DA]<sub>o</sub> by ~25% in NAcC, when compared to drug-free time-matched controls (Figure 3.5b;  $p = 0.001$ ,  $n = 5$  experiments/3 mice, Mann-Whitney test), which was

a similar effect to that in DLS (Figure 3.5c;  $p > 0.9$ , Mann-Whitney test). We confirmed that inhibition of DA release by GABA<sub>A</sub> and GABA<sub>B</sub> receptors was independent of GABAergic microcircuits activated by the electrical stimulation paradigm through selectively activating DA axons with an optogenetic approach. Under these conditions, GABA receptor antagonism significantly enhanced  $[DA]_o$  evoked by single light pulses by ~25% in both the DLS and NAcC compared to time-matched drug-free control (Figure 3.5d-e; DLS:  $p < 0.0001$ ,  $n = 9$  experiments/5 mice; NAcC:  $p = 0.002$ ,  $n = 5$  experiments/4 mice, Mann-Whitney tests), and similarly so in DLS versus NAcC (Figure 3.5f;  $p > 0.9$ ; Mann-Whitney test). These results therefore collectively confirm that DA release is under tonic inhibition by an endogenous local GABA source acting through GABA<sub>A</sub> and GABA<sub>B</sub> in striatal regions spanning dorsal to ventral.

Striatal GABA sources can be separated into two categories based on their synthesis pathways. Mesostriatal DA neurons synthesise, co-store and co-release GABA (Tritsch et al., 2012), with GABA synthesis depending on aldehyde dehydrogenase (ALDH)-1a1 (Kim et al., 2015), unlike canonical synthesis by GABA neurons which requires glutamate decarboxylase (GAD). We therefore tested whether GABAergic inhibition of DA release arose from GABA co-released from DA axons (ALDH-dependent) or from a canonical striatal GABA neuron source (GAD-dependent). To do this, we inhibited GABA synthesis in DA axons by pre-treating slices with ALDH inhibitor disulfiram (10  $\mu$ M) for 2 to 4 hours, which depleted light-evoked GABA currents from DA axons onto SPNs by half (see Section 4.3.2), as reported previously (Kim et al., 2015). However, ALDH inhibition did not prevent GABA receptor antagonists from enhancing DA release: in the DLS, GABA receptor antagonists enhanced light-evoked  $[DA]_o$  by ~40% in the presence of disulfiram, which was a significantly larger effect than seen without disulfiram (Figure 3.6a,c; with disulfiram versus without,  $p = 0.005$ , Mann-Whitney test; disulfiram present:  $n = 6$  experiments/5 mice; disulfiram absent:  $n = 10$  experiments/7 mice). These data suggest that GABA co-released from DA axons does not directly inhibit DA release. They suggest conversely, that ALDH-

dependent GABA acts indirectly to limit tonic inhibition by a different, ALDH-independent source.

To assess whether tonic inhibition of DA release depended instead on a GAD-dependent GABA source, we pre-treated brain slices with GAD inhibitor 3-mercaptopropionic acid (3-MPA, 500  $\mu$ M) for 2 to 4 hours, which attenuates electrically evoked GABA transmission onto SPNs by more than half (Kim et al., 2015). After GAD inhibition, the disinhibition of DA release in the DLS by GABA receptor antagonists was attenuated (Figure 3.6b-c; with 3-MPA vs. without,  $p = 0.008$ , Mann-Whitney test; 3-MPA:  $n = 7$  experiments/5 mice; 3-MPA absent:  $n = 10$  experiments/7 mice), indicating that a GAD-dependent GABA source provides tonic inhibition of striatal DA release.

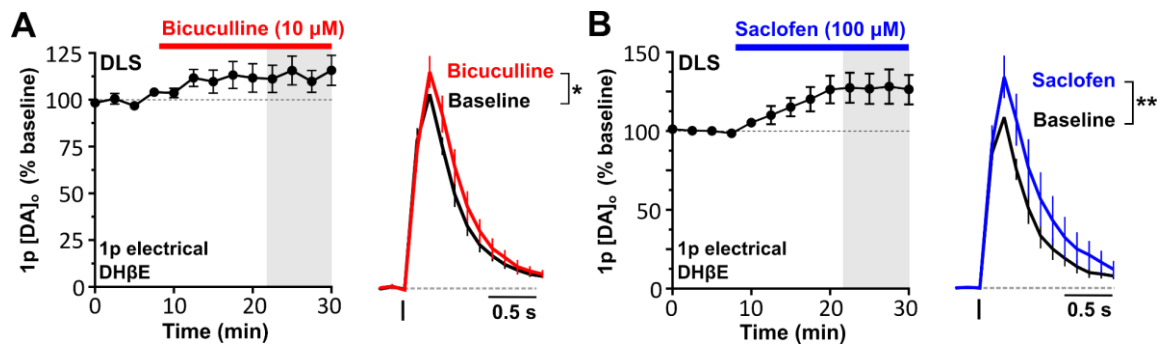


Figure 3.4. GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists increase electrically-evoked dopamine release.

(A,B) *Left*, Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) versus time recorded in DLS evoked by 1 electrical pulse every 2.5 min before and during bath application of 10  $\mu$ M bicuculline,  $n = 5$  (red, A) or 100  $\mu$ M saclofen,  $n = 5$  (blue, B). *Right*, Mean [DA]<sub>o</sub> ( $\pm$  SEM) transients over time.

Data are normalised to first 4 time points (drug-free baseline, dotted line); last 4 time points (shaded region) are used for statistical comparisons. \* $p < 0.05$ , \*\* $p < 0.01$ , Mann-Whitney tests. The nAChR antagonist DH $\beta$ E (1  $\mu$ M) is present throughout. Data collected by E Lopes and B Roberts.

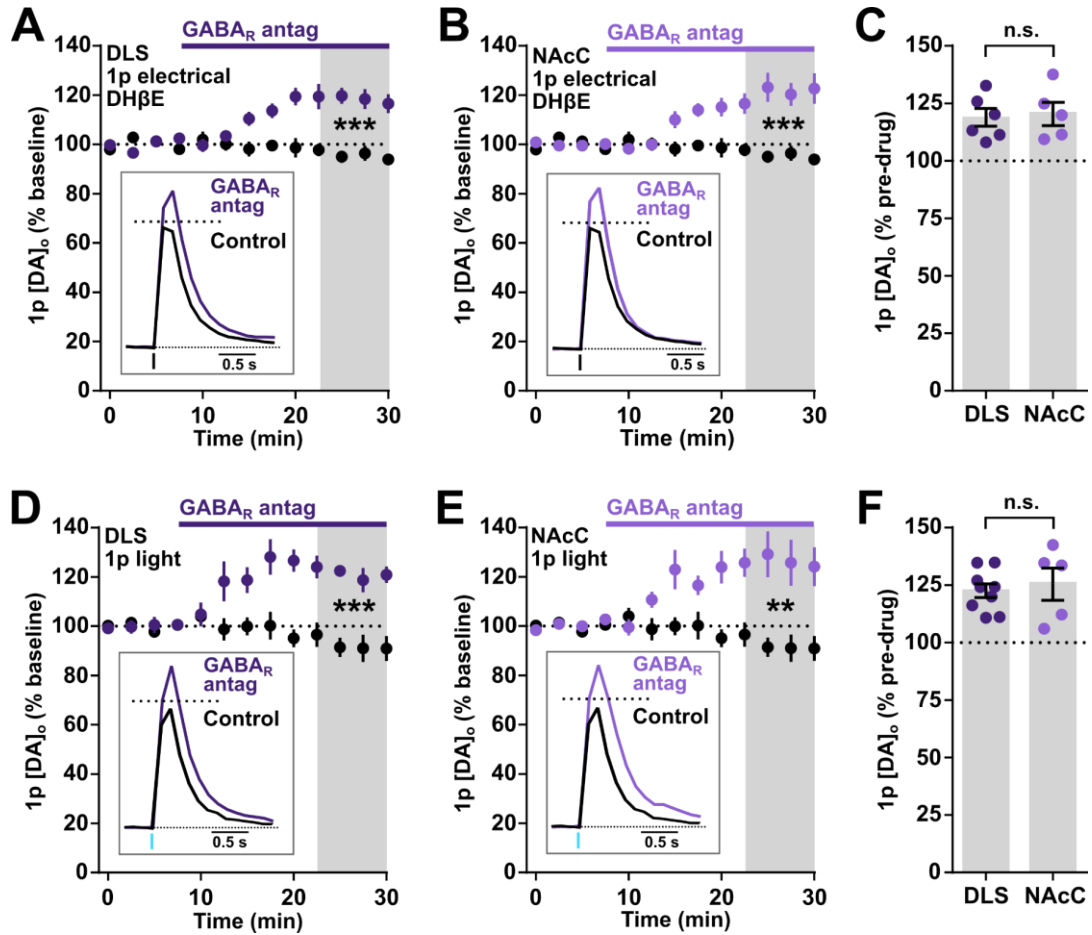


Figure 3.5. Dopamine release is under tonic inhibition across striatal territories.

(A-B, D-E) Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) during consecutive recordings evoked by a single electrical (A,B) or light pulse (D,E) (1p) in control drug-free conditions (*black*,  $n = 9$  for electrical,  $n = 8$  for light) and with GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists (*solid purple bar*, GABA<sub>R</sub> antag), (+)-bicuculline (10  $\mu$ M) and CGP 55845 (4  $\mu$ M), respectively, recorded in the DLS (A,D,  $n = 6$  for electrical,  $n = 9$  for light) or NAcC (B,E,  $n = 5$  for electrical,  $n = 5$  for light).

(C,F) Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) evoked by 1p following GABA<sub>R</sub> antagonism in DLS and NAcC (as % of pre-drug baseline).

Data are normalised to mean of 4 time points prior to GABA antagonist application (*dotted line*); last 4 time points (*shaded region*) are used for statistical comparisons. Significance was assessed by Mann-Whitney tests. \*\* $p < 0.01$ , \*\*\* $p < 0.0001$ , n.s. not significant. Inset, mean transients of DA<sub>o</sub> observed at each stimulus normalised to pre-drug baselines. DHβE (1  $\mu$ M) was present for experiments with electrical stimuli. Data collected by B Roberts.

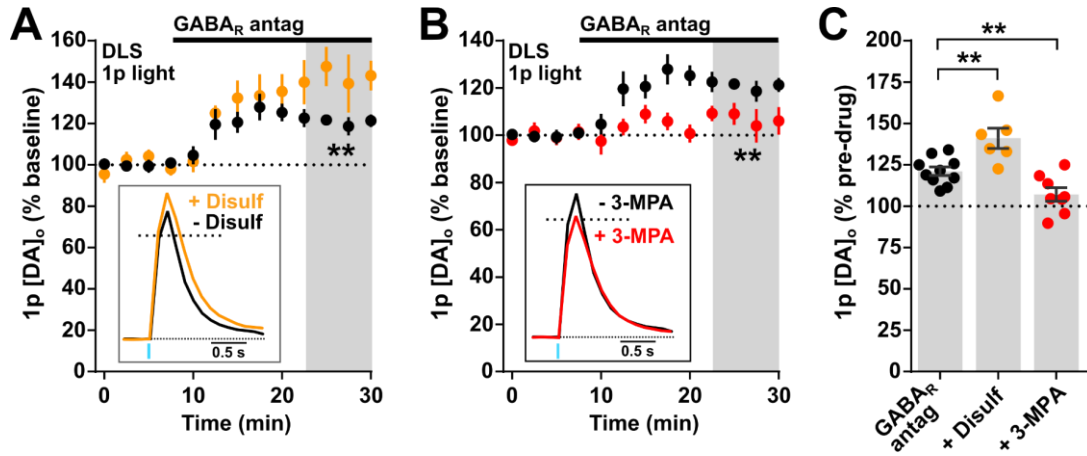


Figure 3.6. Striatal DA release is under tonic inhibition by a GAD-dependent GABA source. (A-B) Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) during consecutive recordings evoked by a single light pulse (1p) during application of GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists (*solid bar*, GABA<sub>R</sub> antag), (+)-bicuculline (10  $\mu$ M) and CGP 55845 (4  $\mu$ M), respectively, in the absence (*black*,  $n = 10$ ) or the presence of ALDH inhibitor disulfiram (10  $\mu$ M) (*orange*,  $n = 6$ ) or GAD inhibitor 3-MPA (5000  $\mu$ M) (*red*,  $n = 7$ ).

(C) Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) in DLS following GABA<sub>R</sub> antagonism in the absence or the presence of 3-MPA (*red*) or disulfiram (*orange*) (expressed as a % of pre-drug baseline). Data are normalised to mean of 4 time points prior to GABA antagonist application (*dotted line*); last 4 time points (*shaded region*) are used for statistical comparisons. Statistical significance was assessed by Mann-Whitney tests. \*\* $P < 0.01$ . Inset, mean transients of DA<sub>o</sub> observed at each stimulus normalised to pre-drug baselines. Data collected by B Roberts.

### 3.4 Discussion

Here we show that GABA<sub>A</sub> and GABA<sub>B</sub> receptor activation inhibits DA release across striatal territories, through a mechanism which is independent of striatal Chl signalling at nAChRs on DA axons. Through eliminating Chls as a site for mediating GABA inhibition of DA release, GABA<sub>A</sub> and GABA<sub>B</sub> receptors located directly on DA axons become a plausible site of action and the most parsimonious mechanism for GABA inhibition of DA release. Furthermore, we reveal that DA release is under tonic inhibition by endogenous GAD-dependent striatal GABA, as GABA<sub>A</sub> and GABA<sub>B</sub> antagonists disinhibit DA release evoked by single optogenetic stimuli, which was prevented in GAD-inhibited conditions.

#### 3.4.1 GABA receptor inhibition of dopamine release independent from striatal cholinergic signalling

We found that agonists for GABA<sub>A</sub> and GABA<sub>B</sub> receptors can inhibit DA release whether evoked by electrical stimulation in the presence of nAChR antagonists or by selective optogenetic activation of ChR2-expressing DA axons in *Slc6a3<sup>IRES-Cre</sup>* mice, where DA release is not under tonic control by striatal nAChRs (Melchior et al., 2015; Threlfell et al., 2012), indicating that GABA receptor inhibition of DA release is independent of GABA receptors on Chls. Our data are consistent with a study in ventral striatum which indicate GABA<sub>B</sub>-mediated inhibition of DA release independent of ACh input (Pitman et al., 2014), and with recent unpublished data indicating DA release evoked by optogenetic stimulation in dorsal and ventral striatum is inhibited by GABA<sub>A</sub> receptors (Sherpa et al., 2019).

To note, supraphysiological concentrations of exogenous GABA (2-5 mM) were required to significantly inhibit evoked [DA]<sub>o</sub> in both DLS and NAcC. The concentrations acting at target GABA receptors in striatum however might be considerably reduced from those bath applied due to several factors: (1) <100% penetration of exogenous GABA into acute brain slice preparations to target receptors at recording sites; (2) GABA transporter activity in the striatum actively transports GABA from the extracellular milieu and is known to strongly regulate ambient GABA tone in striatum (Kirmse et al., 2008, 2009; Santhakumar et al.,

2010; Tritsch et al., 2014; Wójtowicz et al., 2013); (3) GABA transaminase is known to participate in the degradation of GABA (Balazs et al., 1970), including in striatum (Overstreet and Westbrook, 2001). Therefore, the high concentrations of exogenous GABA required to inhibit evoked  $[DA]_o$  in striatum are not surprising. Importantly, we do show corroborative effects of GABA<sub>A</sub> and GABA<sub>B</sub> receptor specific ligands in inhibiting evoked  $[DA]_o$ , the effects of which are blocked by receptor-specific antagonists, and that GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists increase evoked  $[DA]_o$ .

We show that GABA receptor activation primarily limits the overall amplitude of DA output, while having limited effects on the frequency sensitivity of evoked DA release. GABA<sub>A</sub> activation marginally promoted the ratio of  $[DA]_o$  evoked by high-frequency trains over single pulse release, but no significant effect of GABA<sub>B</sub> activation was observed. Therefore, GABA receptor activation reduces DA output with only minor additional enhancement of frequency filtering. This is unlike ACh, which profoundly changes the frequency sensitivity of evoked DA release, changing the relationship between presynaptic activity and absolute DA output (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Further experiments exploring the effects of GABA receptor activation when cholinergic control of presynaptic dopamine release is fully intact (i.e. not in nAChR block) could potentially reveal an additional role for striatal GABA in modulating the frequency filtering of DA output mediated by upstream GABA<sub>A</sub> and GABA<sub>B</sub> receptors on ChIs to limit ACh output.

### 3.4.2 Direct versus indirect actions of GABA receptors

Midbrain DA neurons do express GABA<sub>A</sub> and GABA<sub>B</sub> receptors (Bowery et al., 1987; Boyes and Bolam, 2003; Nicholson et al., 1992), which are certainly functional in somatodendritic compartments. Activation of GABAergic afferents to substantia nigra DA neurons *in vivo* reduced, and in some cases completely inhibited, midbrain DA firing through an early inhibitory response (within 100 ms) mediated by GABA<sub>A</sub>, and a late inhibitory response (after 100 ms) that was mediated by GABA<sub>B</sub> (Brazhnik et al., 2008). Furthermore, local

application of GABA<sub>A</sub> and GABA<sub>B</sub> antagonists *in vivo* increases the spontaneous firing rate of substantia nigra DA neurons, principally through GABA<sub>A</sub> receptors (Brazhnik et al., 2008; Paladini and Tepper, 1999). Our data suggest a role for GABA<sub>A</sub> and GABA<sub>B</sub> in limiting DA output through direct action on DA axons themselves, however trafficking of GABA<sub>A</sub> and GABA<sub>B</sub> from somatodendritic compartments for localisation to DA axons has not yet been conclusively demonstrated, but is supported. GABA<sub>B</sub> receptors have been immunocytochemically detected in monkey and rat striatal neuropil (Charara et al., 1999; Yung et al., 1999), and GABA<sub>A</sub> receptors have been directly located on DA axons in a recent unpublished immunocytochemical study in mouse striatum (Sherpa et al., 2019). Additional experiments examining the physiological role of these receptors directly on DA axons though whole-cell patch-clamp recordings of DA axons might corroborate these findings and overcome the difficulty in verifying ultrastructural location of membrane bound receptors using classical immunocytochemical methods.

Here, we exclude the actions of GABA receptors on ChIs in attenuating DA release through downstream nAChRs. GABA receptors on GABAergic interneurons might alternatively play a role in modulating DA release, but is unsupported as activation of GABA receptors acting indirectly through an intermediary GABA circuit that modulates DA would be expected to inhibit the intermediary GABA circuit and therefore increase DA release, in disagreement to what is observed here. The remaining candidate locations for GABA receptors which regulate DA release are therefore either an undisclosed cell or neuron type that has the ability to directly modulate DA release, or more parsimoniously, GABA receptors directly located on DA axons themselves, which is supported by the emerging immunocytochemical findings.

Axonal GABA receptors have previously been described to play physiologically relevant roles in several other neuron types via activation by ambient GABA or GABA spillover to modulate axonal excitability and affect spike propagation (Bucher and Goillard, 2011;

Kullmann et al., 2005; Trigo et al., 2008). For example, activation of axonal GABA<sub>A</sub> can directly reduce action potential spike amplitude and propagation and promote spike failures (Ruiz et al., 2003; Verdier et al., 2003; Zhang and Jackson, 1995), whereas activation of axonal GABA<sub>B</sub> inhibits voltage-gated calcium channels (Sun and Chiu, 1999). Similar mechanisms are likely to account for GABA<sub>A</sub>- and GABA<sub>B</sub>-mediated inhibition of striatal DA release from the extensively arborized and unmyelinated DA axons.

### 3.4.3 Tonic inhibition of dopamine release

We found that GABA receptor antagonists can enhance DA release evoked by single and brief (2 ms) optogenetic stimulation of ChR2-expressing DA axons, indicating that DA release is under tonic inhibition by a striatal GABA source acting at GABA<sub>A</sub> and GABA<sub>B</sub> receptors. We found that tonic inhibition of DA release by GABA spans dorsal-ventral territories of striatum, and arises from a GAD-dependent source of GABA. The source of GABA was not a non-canonical ALDH-dependent source e.g. co-release from DA axons, as inhibition of ALDH did not attenuate the tonic inhibition of DA release by GABA, despite attenuating GABA co-release from DA axons, as seen previously (Kim et al., 2015). ALDH-inhibition even slightly boosted tonic inhibition of DA release, suggesting that GABA co-release acts to limit the tonic inhibition by the GAD-dependent GABA network. Although we did not directly test the effects of inhibiting GABA synthesis enzymes on raw evoked [DA]<sub>o</sub>, we predict that GAD inhibition would increase and ALDH inhibition would decrease evoked [DA]<sub>o</sub>, given a respective decrease and increase in tonic GABA inhibition of DA release. Interestingly, mutations of ALDH1a1 in humans (Liu et al., 2011; Sherva et al., 2009) and deletion in mice (Kim et al., 2015) lead to alcohol-consuming preferences which given the results presented here might plausibly be mediated through maladaptive striatal DA signalling.

The paucity of GABAergic synapses on DA axons (Charara et al., 1999) suggests that this tonic inhibition by GAD-dependent GABA might arise from a pre-existing extrasynaptic

ambient GABA tone, which is detected as a GABA<sub>A</sub> receptor-mediated tonic current in postsynaptic striatal neurons (SPNs in particular) even in *ex vivo* slice preparations (Ade et al., 2008; Cepeda et al., 2013; Kirmse et al., 2008, 2009; Santhakumar et al., 2010). Our findings here suggest that DA axons might also detect this striatal ambient GABA tone through both GABA<sub>A</sub> and GABA<sub>B</sub> receptors. An ambient GABA tone in striatum that can act on DA axons to limit DA output should not be entirely surprising when considering that the axonal arbour of a single nigrostriatal DA neuron (in rat) reaches ~3% of the volume of striatum on average (Matsuda et al., 2009; Oorschot, 1996), and that such volumes are expected to contain ~70,000 GABAergic neurons, assuming 2.8 million striatal neurons per hemisphere of which ~98% are GAD-immunoreactive (Oorschot, 1996). LTSIs, which are capable of autonomous firing in striatal slices (Beatty et al., 2012), and tonically active GAD-positive cholinergic interneurons that co-release GABA (Lozovaya et al., 2018) might contribute to this extracellular GABA tone. Furthermore, even low rates of spontaneous vesicle release from a small fraction of these ~98% GAD-positive neurons might summate sufficiently to provide a tone at GABA receptors on DA axons that limits DA output. An additional role of this tonic GABA tone, on top of modulating DA release, might be to regulate axonal membrane resistance to modify the impact of other inputs or limit the propagation of action potentials through the axonal arbour for a sparser coding.

Astrocytes are known to play significant roles in maintaining an extrasynaptic tonic GABA tone across the central nervous system, including in striatum, through several mechanisms (for extensive review see Ishibashi et al., 2019). It is well known that astrocytes actively transport GABA from the extracellular milieu via GABA transporters (GATs) located on the plasma membrane of astrocytes, to limit ambient GABA tone (Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014; Yu et al., 2018). Conversely, astrocytes in striatum are also proposed to release GABA and contribute to extracellular GABA tone through GABA release via the Best1 channel (Yoon et al., 2014) and/or reverse transport by astrocytic GATs (Wójtowicz et al., 2013). Furthermore, during intense periods of neuronal

activity, astrocytes can negatively regulate astrocytic GATs via activation of purinergic- and calcium-dependent signalling, reducing astrocyte GAT-mediated GABA uptake (Jacob et al., 2014; Yu et al., 2018), therefore contributing to extracellular GABA tone and tonic inhibition of neighbouring neuronal circuits. If and how astrocytes might contribute and/or regulate the tonic inhibition of striatal DA release is completely untested and unknown and explored in **Chapter 4**.

#### 3.4.4 Summary and implications

In conclusion, we show here that axonal DA release in striatum is under tonic inhibition by endogenous GABA through an apparent ambient GABA tone acting at GABA<sub>A</sub> and GABA<sub>B</sub> receptors presumably located directly on DA axons. Striatal GABA can therefore influence striatal output, not only through direct actions on output neurons and local interneurons, but also by governing DA transmission. These findings provide yet another example whereby DA release is gated locally in striatum, and provide further explanation for why DA release in striatum can be noticeably dissociated from neuronal activity in midbrain. Furthermore, GABAergic control of DA release provides a novel axis which might be dysfunctional in diseases with aberrant DA signalling. In **Chapter 4**, I test whether striatal GATs determine DA output through limiting GABA tone onto DA axons.

# 4

Astrocytic GABA  
transporter activity governs  
dopamine release

## 4.1 Introduction and initial hypotheses

The data outlined in Chapter 3 revealed that striatal DA release is inhibited by the activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors, and that striatal DA release is under tonic inhibition by a GAD-dependent, presumably ambient GABA tone. In this chapter, I test whether striatal GATs determine DA output through limiting GABA tone onto DA axons.

In the mammalian brain, two forms of GABAergic inhibition co-exist to modulate the excitability of neuronal networks: phasic and tonic inhibition (Farrant and Nusser, 2005). In phasic inhibition, quantal release of GABA from a presynaptic neuron induces a distinct high-amplitude, rapidly decaying, generally hyperpolarising event in the postsynaptic neuron, mediated by low-affinity synaptic GABA<sub>A</sub> receptors, which precisely regulate action potential generation and neuronal synchronization (Cobb et al., 1995; Koos et al., 2004). In tonic inhibition, low concentrations of GABA spill over for action at high-affinity extrasynaptic GABA<sub>A</sub> receptors to generate tonic inhibitory currents, which exhibit low amplitudes, long decay times and little or no desensitisation. This persistent tonic inhibition sets the global level of neuronal excitability and information processing (Scimemi, 2005; Semyanov et al., 2003). In addition, presynaptic GABA<sub>B</sub> receptors can also be under tonic activation by low concentration extrasynaptic GABA to tonically gate probability of neurotransmitter release (Dvorzhak et al., 2010; Le Feuvre et al., 1997; Kirmse and Kirischuk, 2006; Laviv et al., 2010; Mapelli et al., 2009).

Within the rodent striatum, where GABAergic neurons constitute the vast majority of the neuronal population (~98%; Oorschot, 1996), a sizeable ambient GABA tone provides tonic inhibition of SPNs at GABA<sub>A</sub> and GABA<sub>B</sub> receptors. A tonic GABA<sub>A</sub> receptor-mediated inhibitory conductance has consistently been recorded in SPNs as depolarising shifts in the baseline currents in response to GABA<sub>A</sub> receptor block (Ade et al., 2008; Cepeda et al., 2013; Janssen et al., 2009; Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014). Tonic activation of presynaptic GABA<sub>B</sub> receptors have also been demonstrated in

SPNs as changes in vesicular transmitter release after the bath application of GABA<sub>B</sub> receptor antagonists, at least under conditions with reduced GABA uptake (Kirmse et al., 2009). In **Chapter 3**, we revealed that DA release is under tonic inhibition by GAD-dependent striatal GABA, operating through action at GABA<sub>A</sub> and GABA<sub>B</sub> receptors presumably located on DA axons (Lopes, Roberts et al., 2018). Given the paucity of GABAergic axoaxonic synapses on DA axons (Charara et al., 1999), we hypothesised that tonic inhibition of DA release by GABA is mediated through extrasynaptic effects of striatal ambient GABA.

The concentration of ambient GABA tone, and therefore strength of tonic inhibition, is determined by the amount of GABA release and spill over into the extrasynaptic environment relative to the rate of GABA uptake by plasma membrane GATs (Brickley and Mody, 2012). There are two isoforms of the GAT in striatum: GAT-1 (Slc6a1), abundant in axons of GABAergic neurons (Augood et al., 1995; Durkin et al., 1995; Ng et al., 2000; Yasumi et al., 1997); and GAT-3 (Slc6a11), expressed moderately (Ficková et al., 1999; Ng et al., 2000; Yasumi et al., 1997) and seen particularly on astrocytes (Chai et al., 2017; Ng et al., 2000; Yu et al., 2018). This organisation of striatal GAT-1 to neuronal processes and GAT-3 to astrocytic ones might however be oversimplified as emerging transcriptomic data indicate that striatal astrocytes express mRNA for both GAT-1 and GAT-3 (Chai et al., 2017; Gokce et al., 2016; Zhang et al., 2014) and mouse midbrain DA neurons express mRNA for GAT-1 and for GAT-3 and are presumed, but not confirmed, to be located on striatal DA axons to support GABA co-storage and co-release from DA terminals (Tritsch et al., 2014).

Ambient GABA tone onto SPNs in the adult dorsal striatum in rodent is limited by the activity of GAT-1 and GAT-3: the pharmacological inhibition of striatal GATs dramatically increases the GABA<sub>A</sub>-mediated tonic inhibitory currents recorded in SPNs, even in *ex vivo* slice preparations (Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014; Wójtowicz et al., 2013). Recent evidence indicates that GATs on striatal astrocytes play a

particularly important role, as astrocytic GAT-3 dysregulation results in profound changes to SPN activity and in striatal-dependent behaviour (Yu et al., 2018). However, whether striatal GAT function is a critical factor in determining DA physiology and output though governing the level of tonic inhibition of DA release by striatal ambient GABA has not previously been examined. Furthermore, if and how GAT expression and function might vary across striatal territories remains unexamined, as all previous studies investigating the GAT-dependence of tonic inhibition of SPNs have been conducted in dorsal striatum alone (Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014; Wójtowicz et al., 2013).

In order to help resolve these open questions, we explored the control of DA release by striatal GAT-1 and GAT-3 using FSCV in acute *ex vivo* striatal slices prepared from mice. Since mechanisms that regulate striatal DA release can diverge between dorsal and ventral striatum (Brimblecombe et al., 2015; Britt and McGehee, 2008; Shin et al., 2017; Threlfell and Cragg, 2011; Threlfell et al., 2010), we examined striatal GAT regulation of DA release across striatal territories. In parallel, we assessed the impact of GAT inhibition on tonic GABA<sub>A</sub>-mediated currents recorded in SPNs using whole-cell voltage-clamp electrophysiology. Furthermore, we measured the immunoreactivity for GATs across striatal territories and on candidate cell types, namely astrocytes and DA axons, to determine which striatal cell types might critically limit ambient GABA levels through expression of GATs.

### 4.1.1 Initial hypotheses

Overarching hypothesis – Striatal GAT activity governs DA output.

Hypothesis 1 – GAT-1 and GAT-3 activity limit tonic inhibition of GABA<sub>A</sub> and GABA<sub>B</sub> receptors on DA axons.

Hypothesis 2 – GAT-1 and GAT-3 are expressed on striatal astrocytes and critically limit tonic inhibition of DA axons.

## 4.2 Methods

Experiments described in this chapter were carried out according to the general methods described in **Chapter 2**. Variations and further details are described below.

### 4.2.1 Slice preparation

In FSCV experiments where electrically-evoked  $[DA]_o$  was recorded, and in electrophysiology experiments where tonic  $GABA_A$ -mediated currents were recorded in SPNs, adult 7- to 10-week-old wild-type male C57BL/6J mice (Charles River) were used. In FSCV and electrophysiology recordings involving selective light activation of DA axons, adult 8- to 14-week-old male  $Slc6a3^{IRES-Cre}$  mice conditionally expressing ChR2-eYFP in DA axons after AAV intracranial injections ~3-4 weeks prior were used. Acute *ex vivo* coronal slices containing striatum were prepared for FSCV as described in Section 2.2.3, and for electrophysiology as described in **Section 2.3.1**. All experimental procedures involving the use of animals were carried out according to institutional guidelines and conformed to the UK Animals (Scientific Procedures) Act 1986.

### 4.2.2 Fast-scan cyclic voltammetry

Each FSCV experiment was performed at a single recording site in one brain slice as outlined in **Section 2.2**. Data are expressed as extracellular concentration of dopamine ( $[DA]_o$ ) or normalised to mean peak  $[DA]_o$  evoked by single pulses in control conditions. FSCV data are normalised to pre-drug conditions for clarity and for comparisons between regions. Evoked  $[DA]_o$  was recorded every 2.5 min and  $DH\beta E$  (1  $\mu M$ ) was present throughout all experiments where electrically-evoked  $[DA]_o$  was recorded. In all cases,  $[DA]_o$  displayed typical kinetics to peak and decay, indicative of good slice quality and were obtained from recording sites that maintained sufficiently stable levels of release over time. No FSCV data were excluded after acquisition.

Drug washes were performed once stable release at the given recording site was stable: less than 10% variation over 5 consecutive stimulations. For drug effects, peak  $[DA]_o$  was

averaged over 4 stimulations once peak  $[DA]_o$  had re-stabilised post-drug application (~10-20 min of application) and compared to time-matched data from drug-free controls and/or pre-drug baselines (averaged across 4 stimulations prior to drug wash-on). For experiments involving multiple pulse protocols, each stimulation type was repeated in triplicate, interspersed with single-pulse stimulations, and then averaged and normalised to single-pulse stimulations at each recording site, as previously (Lopes, Roberts et al., 2018; Threlfell et al., 2012).

### 4.2.3 Whole-cell patch-clamp electrophysiology

For all whole-cell electrophysiology recordings, SPNs were voltage-clamped at -70 mV and tonic GABA<sub>A</sub> receptor-mediated currents in slices from WT mice or light-evoked phasic GABA<sub>A</sub> receptor-mediated currents resulting from GABA co-release from Chr2-expressing DA axons in slices from *Slc6a3<sup>IRE5-Cre</sup>* mice were recorded as outlined in Section 2.3.2. All recordings were in the presence of NBQX (5  $\mu$ M) and D-APV (50  $\mu$ M) to block AMPA and NMDA receptor-mediated inward currents.

Peak amplitude, onset latency, peak latency, 10-90% rise time and decay time of light-evoked GABA currents in SPNs were measured from an average of 3 replicate traces per cell recorded before and after drug wash-on or incubation conditions. Tonic GABA<sub>A</sub> receptor-mediated currents in SPNs were recorded by averaging the holding current across 1 min sampling windows in each drug condition: cells were allowed to stabilise for 5-10 min before drug manipulations, GAT inhibitors were bath applied for 20-25 min, and then picrotoxin was applied for an additional 3-5 min. During all electrophysiology experiments, access resistance was monitored, and cells were discarded from analysis if series resistance varied by more than 15% or increased over 25 M $\Omega$ . All electrophysiology data were analysed in Clampfit 10.4.1.4 software (Molecular Devices).

#### 4.2.4 High-performance liquid chromatography

DA content from dorsal striatum tissue punches taken from acute sections prepared for FSCV were measured by HPLC with electrochemical detection as outlined in **Section 2.4.1**.

#### 4.2.5 Drugs

Drugs were dissolved in distilled water or dimethyl sulfoxide to make stock aliquots at 1,000 x final concentration and stored at -20°C until required. Stock aliquots were diluted with oxygenated aCSF to final concentrations immediately before use. The drugs used in this study, their final concentrations, and references to studies which clarified the choice of final concentration used are outlined in **Table 4.1**. Final concentrations used were determined from previous pharmacological studies using acute rodent brain slices (where possible in striatum), which often require concentrations significantly greater than the half maximal effective concentrations ( $EC_{50}$ ) for sufficient penetration of slices. Fluorocitrate was prepared as previously described (Paulsen et al., 1987). In brief, D,L-fluorocitric acid  $Ba_3$  salt was dissolved in 0.1 M HCl, the  $Ba^{2+}$  precipitated with 0.1 M  $Na_2SO_4$  and then centrifuged at 1,000 g for 5 min. Supernatant containing fluorocitrate was used for experimentation. Electrically evoked  $[DA]_o$  in slices pre-incubated in fluorocitrate (200  $\mu$ M) exhibited a slow ongoing rundown across repeated 1p stimulations ( $n = 5$  experiments/3 mice; data not shown) and therefore we used an alternative stimulation paradigm to compare a large number of dorsal striatal recording sites in slices pre-treated with fluorocitrate versus control conditions to minimise the effects of run-down (i.e. within subjects wash-on experimental design was precluded). There are previous reports of a fluorocitrate-dependent slow rundown of excitatory postsynaptic currents in the hippocampus (Boddum et al., 2016), possibly reflecting astroglia's role in stable synaptic neurotransmission.

Table 4.1. Drugs listed with method of action and references to studies which clarified the choice of concentrations used in Chapter 4.

| Drug                                          | Source                   | Action                                        | Final concentration | Reference(s)                              |
|-----------------------------------------------|--------------------------|-----------------------------------------------|---------------------|-------------------------------------------|
| Bicuculline                                   | Tocris Bioscience        | GABA <sub>A</sub> receptor antagonist         | 10 $\mu$ M          | Cepeda et al., 2001; Brodnik et al., 2018 |
| CGP 55845                                     | Abcam                    | GABAB receptor antagonist                     | 4 $\mu$ M           | Tritsch et al., 2014                      |
| D-APV                                         | Santa Cruz Biotechnology | NMDA receptor antagonist                      | 50 $\mu$ M          | Patton, Roberts et al., 2016              |
| Dihydro- $\beta$ -erythroidine (DH $\beta$ E) | Tocris Bioscience        | $\beta$ 2 subunit-containing nAChR antagonist | 1 $\mu$ M           | Lopes, Roberts et al., 2019               |
| Disulfiram                                    | Santa Cruz Biotechnology | Aldehyde dehydrogenase inhibitor              | 10 $\mu$ M          | Kim et al., 2015                          |
| Fluorocitrate                                 | Sigma-Aldrich            | Astrocyte metabolism inhibitor                | 200 $\mu$ M         | Boddum et al., 2016; Paulsen et al., 1987 |
| GBR 12395                                     | Sigma-Aldrich            | Selective dopamine transporter inhibitor      | 1 $\mu$ M           | Condon et al., 2019                       |
| L741,626                                      | Tocris Bioscience        | Selective D <sub>2</sub> receptor antagonist  | 2 $\mu$ M           | Condon et al., 2019                       |
| (RS)-MCPG                                     | Tocris Bioscience        | Non selective mGluR antagonist                | 200 $\mu$ M         | Kosillo et al., 2016                      |
| NBQX                                          | Abcam                    | AMPA receptor antagonist                      | 5 $\mu$ M           | Patton, Roberts et al., 2016              |
| ( $\pm$ )-Nipecotic acid                      | Sigma-Aldrich            | Broad-spectrum GAT inhibitor                  | 1.5 mM              | Goubard et al., 2011; Li et al., 2017     |
| Picrotoxin                                    | Sigma-Aldrich            | GABA <sub>A</sub> receptor antagonist         | 100 $\mu$ M         | Avshalumov et al., 2000                   |
| SKF-89976A                                    | Santa Cruz Biotechnology | Selective GAT-1 inhibitor                     | 20 $\mu$ M          | Tritsch et al., 2014                      |
| (S)-SNAP 5114                                 | Sigma-Aldrich            | Selective GAT-3 inhibitor                     | 50 $\mu$ M          | Tritsch et al., 2014                      |
| Tetrodotoxin (TTX)                            | Tocris Bioscience        | Na <sub>v</sub> blocker                       | 1 $\mu$ M           | Tritsch et al., 2012                      |

### 4.2.6 Indirect immunofluorescence and confocal imaging

Immunohistochemistry with confocal imaging was used to examine heterogeneity of GAT expression across striatal territories and to test for GAT-1 and GAT-3 localisation on DA axons, PV-positive interneurons and striatal astrocytes as outlined in Section 2.4.3. Antibodies used and their commercial source are outlined in **Table 4.2**.

For whole striatum analysis of GAT-1 or GAT-3, 4 sections in the rostro-caudal plane were imaged at approximately the following distances rostral of Bregma; +1.3mm, +1.0mm, +0.6mm, and +0.25mm. A region of interest (ROI) of 300  $\mu\text{m}$  x 300  $\mu\text{m}$  was overlaid over the DLS and an ROI of 200  $\mu\text{m}$  X 200  $\mu\text{m}$  was overlaid on NAcC for both hemispheres. Values for NAcC were taken from the 2 most rostral sections. Mean grey values from the areas of interest were normalised to the median grey value for each hemisphere ( $n = 12$  hemispheres from 6 animals). To assess co-localisation of GAT-1 or GAT-3 to DA axons, PV-positive axons or S100 $\beta$ -positive astrocytes, z-stacks from a minimum of 2 dorsal striatal regions in each animal ( $n = 3$  per marker) were examined using ZEN (blue edition v.2.3; Zeiss) software.

### 4.2.7 Data analysis

All data are represented as means  $\pm$  standard error of the mean, and 'n' refers to the number of experiments. Each experiment was performed at a single recording site in one discrete brain slice. The number of animals in each dataset was  $\geq 3$ . Comparisons for statistical significance were assessed by one- or two-way ANOVA with Sidak's multiple comparisons; or Kruskal-Wallis ANOVA with Dunn's multiple comparisons, Friedman's ANOVA on Ranks with Student-Newman-Keuls multiple comparisons, and Mann-Whitney U tests where data were not normally distributed (assessed using D'Agostino-Pearson normality test); and extra sum-of-squares comparison of fits for analysis of  $[\text{DA}]_o$  uptake kinetics, using Prism 7 (GraphPad). Threshold for statistical significance in all analyses was  $p < 0.05$ , adjusted for multiple comparisons.

Table 4.2. Drugs listed with method of action and references to studies which clarified the choice of concentrations used in Chapter 4.

| Primary antibody             | Concentration | Source                   | Secondary antibody                                                                                                                    |
|------------------------------|---------------|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| Rabbit anti-TH               | 1:1,000       | Sigma-Aldrich, ab112     | Donkey anti-Rabbit AlexaFluor488, 1:1,000, Invitrogen, A21206<br>Donkey anti-Rabbit Cy3, 1:1,000, Jackson ImmunoResearch, 711-165-152 |
| Rabbit anti-GAT1             | 1:1,000       | Synaptic Systems, 274102 |                                                                                                                                       |
| Rabbit anti-GAT3             | 1:250         | Millipore, AB1574        |                                                                                                                                       |
| Rat anti-GFP                 | 1:1,000       | Nacalai Tesque, 04404-84 | Donkey anti-Rat AlexaFluor488, 1:1,000<br>Jackson ImmunoResearch, 712-545-153                                                         |
| Guinea-pig anti-Parvalbumin  | 1:1,000       | Synaptic Systems, 195004 | Donkey anti-Guinea Pig AlexaFluor 488, 1:1,000,<br>Jackson ImmunoResearch, 706-545-148                                                |
| Guinea pig anti-S100 $\beta$ | 1:2,000       | Synaptic Systems, 287004 |                                                                                                                                       |

### 4.3 Results

#### 4.3.1 GAT-1 and GAT-3 inhibition attenuated dopamine release in dorsolateral striatum but not nucleus accumbens core

Given the paucity of GABAergic axoaxonic synapses identified on DA axons (Charara et al., 1999), tonic inhibition of DA release likely arises from extrasynaptic ambient GABA. We tested the hypothesis that GAT, through its ability to govern ambient GABA levels (Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Wójtowicz et al., 2013), will determine the level of tonic inhibition of DA release by using FSCV to detect  $[DA]_o$  at carbon-fibre microelectrodes evoked by single electrical pulses in DH $\beta$ E (1  $\mu$ M) in acute coronal slices containing mouse striatum. The non-selective GAT inhibitor ( $\pm$ )-nipecotic acid (NPA) (1 - 10 mM) inhibits all subtypes of GATs (Goubard et al., 2011; Li et al., 2017). Bath application of

NPA (1.5 mM) attenuated electrically evoked  $[DA]_o$  by 1p in the DLS to ~60% of time-matched controls (**Figure 4.1a**;  $F_{Drug(1,16)} = 69.7$ ,  $p < 0.0001$ , Two-Way RM ANOVA; NPA: ~60% of baseline,  $n = 9$  experiments/5 mice; time-matched controls: ~95% of baseline,  $n = 9$  experiments/7 mice;  $p < 0.0001$ , Mann-Whitney test). We confirmed that inhibition of DA release by NPA was independent of microcircuits activated by the electrical stimulation by activating DA axons selectively using an optogenetic strategy (see **Section 2.1.3** and **Section 2.2.5**) as NPA similarly attenuated light-evoked  $[DA]_o$  by 1p in DLS to ~50% of time-matched controls (**Figure 4.1b**;  $F_{Drug(1,15)} = 39.5$ ,  $p < 0.0001$ , Two-Way RM ANOVA; NPA: ~60% of baseline,  $n = 9$  experiments/5 mice; time-matched controls: ~90% of baseline,  $n = 8$  experiments/6 mice;  $p = 0.006$ , Mann-Whitney test).

Since mechanisms that regulate striatal DA release can diverge between dorsal and ventral striatal territories (Brimblecombe et al., 2015; Britt and McGehee, 2008; Janezic et al., 2013; Shin et al., 2017; Threlfell and Cragg, 2011; Threlfell et al., 2010), we determined whether DA release is similarly attenuated by GAT inhibition in ventral striatum, namely NAcC. NPA attenuated evoked  $[DA]_o$  to a greater extent in DLS than in NAcC (**Figure 4.1f**;  $p = 0.0008$ , Mann-Whitney test, DLS vs NAcC) where the effect of NPA on electrically evoked  $[DA]_o$  did not reach statistical significance compared to time-matched controls (**Figure 4.1c**;  $F_{Drug(1,13)} = 4.6$ ,  $p = 0.06$ ;  $F_{Interaction(16,208)} = 1.6$ ,  $p = 0.07$ , Two-Way RM ANOVA; NPA: ~90% of baseline,  $n = 6$  experiments/4 mice; time-matched controls: ~95% of baseline,  $n = 9$  experiments/7 mice;  $p = 0.06$ , Mann-Whitney test). These data indicate that the level of tonic inhibition of DA release by GABA is limited by GAT activity in the DLS, and to a greater degree than in NAcC.

Two main isoforms of the GAT are expressed in the basal ganglia: GAT-1 and GAT-3 (Jin et al., 2011). We identified which isoform(s) limits GABAergic inhibition of DA release in the DLS, using selective inhibitors. The GAT-1 selective inhibitor SKF89976A (20  $\mu$ M) significantly attenuated electrically evoked  $[DA]_o$  in DLS to ~75% of time-matched controls

(Figure 4.1d;  $F_{\text{Drug}(1,13)} = 28.4$ ,  $p = 0.0001$ , Two-Way RM ANOVA; SKF: ~25% of baseline,  $n = 6$  experiments/4 mice; time-matched controls: ~95% of baseline,  $n = 9$  experiments/7 mice;  $p = 0.0004$ , Mann-Whitney test). Combined inhibition of GAT-1 and GAT-3 with SKF89976A plus SNAP5114 (50  $\mu\text{M}$ ) significantly attenuated electrically evoked  $[\text{DA}]_o$  to ~60% of time-matched controls (Figure 4.1e;  $F_{\text{Drug}(1,16)} = 29.1$ ,  $p < 0.0001$ , Two-Way RM ANOVA; SKF+SNAP: ~60% of baseline,  $n = 9$  experiments/5 mice; time-matched controls: ~95% of baseline,  $n = 9$  experiments/7 mice;  $p < 0.0001$ , Mann-Whitney test, averaged across 30-40 min;  $n = 9$  experiments/5 mice), an additional effect compared to GAT-1 inhibition alone and equivalent to that seen with broad-spectrum GAT inhibitor NPA (Figure 4.1g;  $p = 0.003$ , Kruskal-Wallis ANOVA; SKF+SNAP vs SKF:  $p < 0.05$ , NPA vs SKF+SNAP:  $p > 0.5$ , NPA vs SKF:  $p < 0.05$ , Dunn's multiple comparison tests). These data therefore collectively indicate that both GAT-1 and GAT-3 limit the level of GABA inhibition of DA release in the DLS.

NPA did not affect the uptake kinetics of electrically evoked  $[\text{DA}]_o$  by 1p in DLS (Figure 4.2b;  $F = 0.92$ ,  $p = 0.34$ , concentration-matched comparison of fits; NPA:  $K = 3.99$ ,  $R^2 = 0.96$ ,  $n = 9$  experiments/5 mice, Control:  $K = 3.80$ ,  $R^2 = 0.96$ ,  $n = 9$  experiments/7 mice). Furthermore, NPA still significantly attenuated electrically evoked  $[\text{DA}]_o$  by 1p in DLS in the presence of  $D_2$  receptor antagonists (L741,626, 2  $\mu\text{M}$ ), in the presence of DA transporter (DAT) inhibitors (GBR 12395, 1  $\mu\text{M}$ ) and in the presence of ionotropic (D-APV, 50  $\mu\text{M}$ ; NBQX, 5  $\mu\text{M}$ ) and metabotropic glutamate receptor antagonists (MCPG, 200  $\mu\text{M}$ ) (Figure 4.2c;  $p = 0.0004$ , Kruskal-Wallis ANOVA; Control vs  $D_2$  antagonism:  $p < 0.05$ , vs DAT inhibition:  $p < 0.05$ , vs glutamate receptor antagonism:  $p < 0.05$ , Dunn's multiple comparison tests). These data indicate that the attenuation of evoked  $[\text{DA}]_o$  following GAT inhibition with NPA is not mediated through  $D_2$  or glutamate receptors, nor through interactions with the DAT.

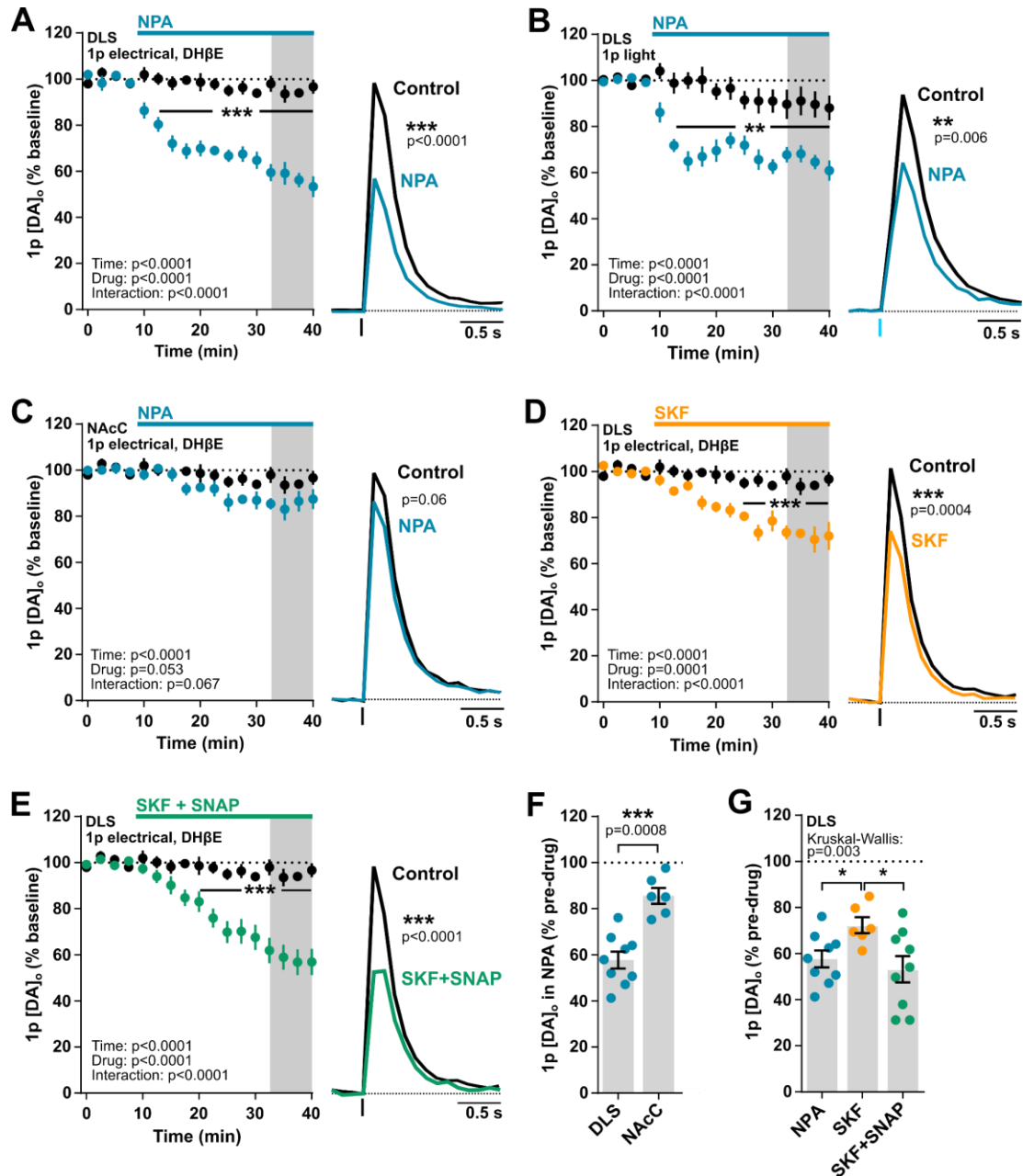


Figure 4.1. GAT-1 and GAT-3 inhibition attenuated DA release in DLS, but not NAcC.

(A-E) *Left*, Mean peak [DA]<sub>o</sub> (± SEM) during consecutive recordings evoked by a single electrical (A,C-E) or light pulse (B) in DLS (A-B,D-E) or NAcC (B) in control conditions (*black*, n = 9 experiments/7 mice for electrical, n = 8 experiments/6 mice for light) or with GAT inhibitor nipecotic acid (1.5 mM) (A, *blue*, n = 9 experiments/5 mice; B, n = 9 experiments/5 mice; C, n = 6 experiments/4 mice), the GAT-1 specific inhibitor SKF89976A (20 μM) (D, *orange*, n = 6 experiments/4 mice), or the combined bath application of SKF89976A and the GAT-3 specific inhibitor SNAP5114 (50 μM) (E, *green*, n = 9 experiments/5 mice). Data are normalised to first 4 time points (*dotted line*). Two-way RM ANOVA and Sidak's multiple comparisons (*asterisks in bars*). *Right*, Mean transients of [DA]<sub>o</sub> averaged across last 4 time points (*shaded region*, left) and normalised to pre drug baseline. Mann-Whitney tests.

(F-G) Mean peak [DA]<sub>o</sub> (± SEM) evoked by 1p following GAT inhibition (expressed as a % of pre-drug baseline) from (A-E). Mann-Whitney test (F) and Kruskal-Wallis test and Dunn's multiple comparisons (G). DHβE (1 μM) present throughout experiments with electrical stimuli.

Statistical significance: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

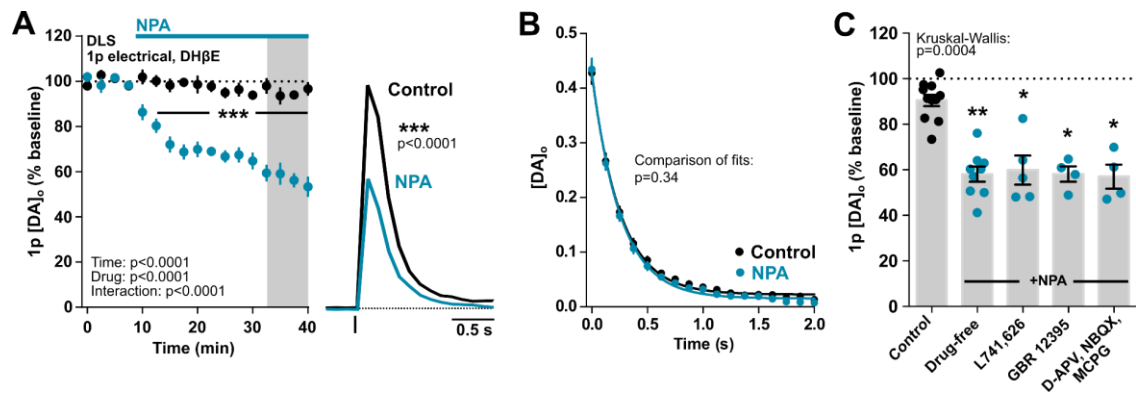


**Figure 4.2.** GAT-1 and GAT-3 inhibition attenuated dopamine release in DLS.

(A) *Left*, Mean peak [DA]<sub>0</sub> ( $\pm$  SEM) during consecutive recordings evoked by a single electrical pulse in DLS in control conditions (*black*,  $n = 9$  experiments/7 mice) or with GAT inhibitor nipecotic acid (NPA, 1.5 mM) (*blue*,  $n = 9$  experiments/5 mice). Data are normalised to first 4 time points (*dotted line*). Two-way RM ANOVA and Sidak's multiple comparisons (asterisks in bars). *Right*, Mean transients of [DA]<sub>0</sub> averaged across last 4 time points (*shaded region*, left) and normalised to pre drug baseline. Mann-Whitney tests.

(B) Concentration-matched [DA]<sub>0</sub> ( $\pm$  SEM) decay over time in control conditions (*black*,  $R^2 = 0.96$ ) or with the GAT inhibitor NPA (*blue*,  $R^2 = 0.96$ ) from (A). Extra sum-of-squares comparison of fits.

(C) Mean peak [DA]<sub>0</sub> ( $\pm$  SEM) evoked by 1p following GAT inhibition (expressed as a % of pre-drug baseline) in control conditions (*black*) or following GAT inhibition with NPA (*blue*) in the absence (drug-free) or presence of D<sub>2</sub> antagonist L741,626 (2  $\mu$ M); DA transporter inhibitor GBR 12395 (1  $\mu$ M); ionotropic and metabotropic glutamate receptor inhibitors D-APV (50  $\mu$ M), NBQX (5  $\mu$ M) and MCPG (200  $\mu$ M). Kruskal-Wallis test and Dunn's multiple comparisons to control conditions. DH $\beta$ E (1  $\mu$ M) present throughout. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

### 4.3.2 GAT inhibition attenuates striatal dopamine release independent of disrupting GABA and dopamine co-storage

GATs are presumed to be directly located on striatal DA axons to support GABA and DA co-storage within the same vesicle (Tritsch et al., 2014). Given this, we next evaluated if disrupting GABA and DA co-storage by preventing GABA uptake into DA axons following GAT inhibition with NPA facilitated the attenuation in evoked DA release. Co-storage has significant implications for other pairs of co-stored neurotransmitters, where packaging of glutamate promotes the storage and release of DA and ACh (Gras et al., 2008; Hnasko et al., 2010).

We began by replicating previous findings (and to observe GABA co-release in our own hands) by performing whole-cell voltage-clamp electrophysiology recordings from SPNs in acute mouse brain slices containing striatum prepared from *Slc6a3<sup>IRE5-Cre</sup>* mice conditionally expressing ChR2-EYFP in DA axons. Activation of DA axons with brief (2 ms) flashes of blue light (470 nm) reliably evoked inward inhibitory postsynaptic currents (IPSCs) in SPNs voltage clamped at -70 mV recorded in the dorsal striatum and in the presence of ionotropic glutamate receptors (NBQX, 5  $\mu$ M; D-AP5, 50  $\mu$ M; **Figure 4.3a**). Light-evoked IPSCs under control conditions ( $n = 8$  cells/5 mice) had a peak amplitude of  $1.3 \pm 0.1$  nA, onset latencies of  $2.27 \pm 0.09$  ms, thought to be consistent with monosynaptic transmission, and slow kinetics (10 - 90% rise time:  $4.39 \pm 0.9$  ms; 90 - 10 % decay time:  $149 \pm 13.8$  ms), and were blocked by the GABA<sub>A</sub> receptor antagonist bicuculline (10  $\mu$ M) (**Figure 4.3a-b**;  $p = 0.001$ , Mann-Whitney test vs. control,  $n = 6$  cells/4 mice), as described previously (Kim et al., 2015; Tritsch et al., 2012, 2014).

The non-canonical GABA synthesis enzyme ALDH-1a1 has been shown to contribute to GABA co-storage and co-release by synthesising GABA in DA axon (Kim et al., 2015). To corroborate this finding, we pre-treated slices with the ALDH inhibitor disulfiram (10  $\mu$ M) for 2 to 4 hours, a paradigm shown previously to deplete GABA co-release from DA axons by about half (Kim et al., 2015). In our hands, pre-treatment with disulfiram reduced the

amplitude of light-evoked IPSCs recorded in SPNs by ~50% (Figure 4.3a-b;  $p = 0.0006$ , Mann-Whitney test vs. control,  $n = 7$  cells/5 mice). Additionally, combined GAT and ALDH inhibition (1.5 mM NPA bath applied for 30 min onto slices pre-treated with disulfiram before recording) robustly reduced the amplitude of recorded light-evoked IPSCs (Figure 4.3a-b;  $p = 0.0006$ , Mann-Whitney test vs. control,  $n = 7$  cells/5 mice), also as observed previously (Kim et al., 2015). As previously outlined in Chapter 3, ALDH inhibition with disulfiram increased the tonic inhibition of DA axons, as the application of GABA receptor antagonists (bicuculline 10  $\mu$ M and CGP 55845 4  $\mu$ M) enhanced light-evoked  $[DA]_o$  by ~40%, which was a significantly larger effect than seen without disulfiram (Figure 4.3c;  $F_{Drug(1,14)} = 4.3$ ,  $p = 0.06$ ,  $F_{Interaction(12,168)} = 2.1$ ,  $p = 0.03$ , Two-Way RM ANOVA;  $p = 0.005$ , Mann-Whitney test, with disulfiram versus without; disulfiram present:  $n = 6$  experiments/5 mice; disulfiram absent:  $n = 10$  experiments/7 mice).

To assess if disruption in the availability of GABA co-storage in DA axons following GAT inhibition was contributing to the attenuation in evoked DA release, we pre-treated slices with disulfiram and then examined light-evoked DA release following bath application of NPA. We predicted that if disruptions to co-stored GABA in DA axons impacted on the attenuation of evoked DA release following GAT inhibition, then the effects of NPA would be occluded if GABA co-storage was already impaired by pre-treatment with disulfiram. Bath application of NPA similarly reduced light-evoked  $[DA]_o$  in the DLS by 1p in slices pre-incubated with and without disulfiram for 2 to 4 hours (Figure 4.3d;  $F_{Drug(1,14)} = 0.6$ ,  $p = 0.46$ ,  $F_{Interaction(16,224)} = 1.1$ ,  $p = 0.32$ , Two-Way RM ANOVA;  $p = 0.81$ , Mann-Whitney test with disulfiram versus without; disulfiram present:  $n = 6$  experiments/5 mice; disulfiram absent:  $n = 10$  experiments/7 mice). Taken together, these data indicate that the attenuation in DA release following GAT inhibition is not mediated through impeding on GABA co-storage in DA axons.

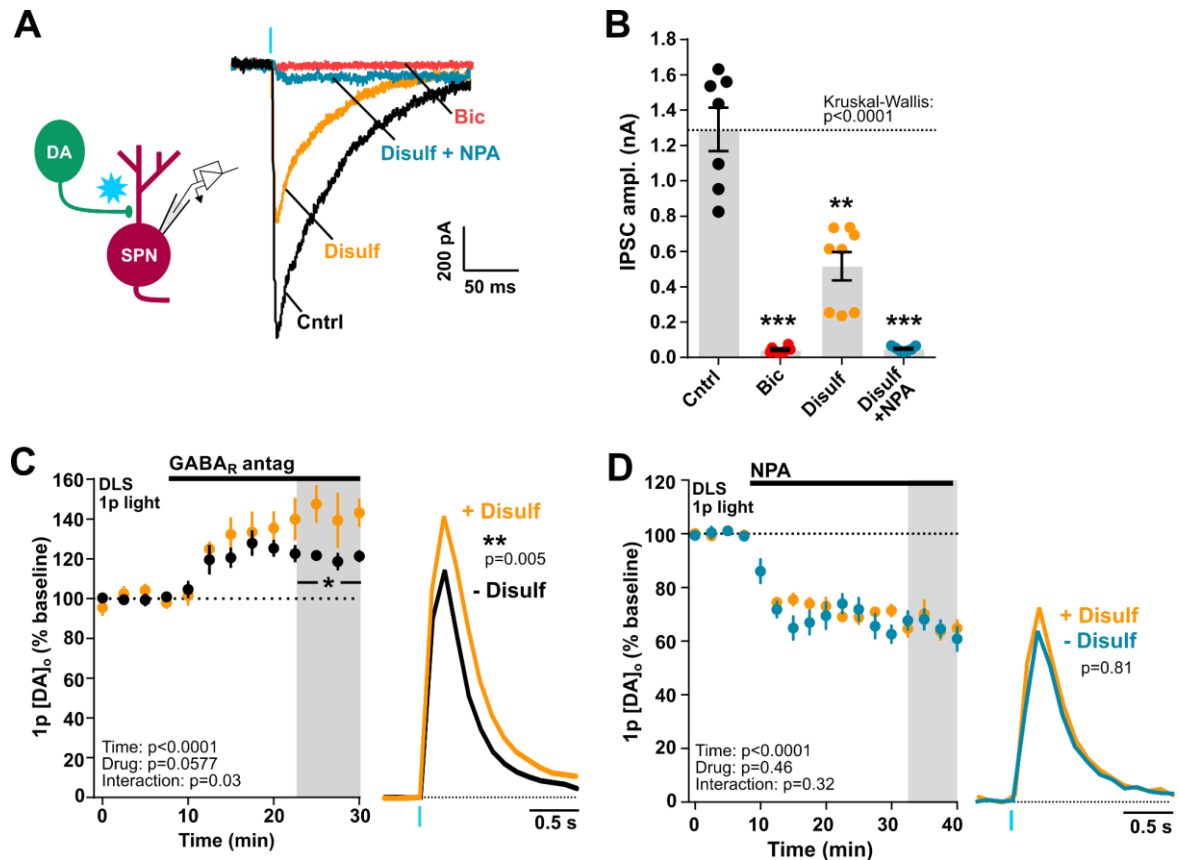


Figure 4.3. Disruptions to GABA co-storage and co-release in dopamine axons do not mediate NPA-induced attenuation of dopamine release.

(A) Representative light-evoked IPSCs recorded from spiny projection neurons (SPNs) in the DLS of *Slc6a3<sup>RES-Cre</sup>* mice expressing ChR2-eYFP in DA axons, voltage clamped at -70 mV and in the presence of ionotropic glutamate receptor antagonists (5  $\mu$ M NBQX, 50  $\mu$ M D-APV) in the perfusate. Light-evoked IPSCs were recorded in control conditions (black, n = 8 cells/5 mice) and in the presence of the GABA<sub>A</sub> receptor antagonist bicuculline (BIC, 10  $\mu$ M, red, n = 6 cells/4 mice), or the ALDH inhibitor disulfiram (Disulf, 10  $\mu$ M, orange, n = 8 cells/5 mice), or both disulfiram and the nonspecific GAT inhibitor NPA (1.5 mM, blue, n = 7 cells/5 mice).

(B) Mean ( $\pm$  SEM) peak amplitudes of IPSCs quantified from conditions in (A). Kruskal-Wallis test and Dunn's multiple comparisons to control conditions.

(C-D) Left, Mean peak  $[DA]_o$  ( $\pm$  SEM) during consecutive recordings evoked by 1p light during application of GABA<sub>R</sub> antagonists (bicuculline 10  $\mu$ M, CGP 55845 4  $\mu$ M; C) or NPA (1.5 mM; D) in the absence (black (C), n = 10 experiments/7 mice; blue (D), n = 9 experiments/5 mice) or the presence of ALDH inhibitor disulfiram (10  $\mu$ M) (orange (C), n = 6 experiments/5 mice; orange (D), n = 6 experiments/5 mice). Data are normalised to first 4 time points (dotted line). Two-way RM ANOVA and Sidak's multiple comparisons (asterisks in bars). Right, Mean transients of  $[DA]_o$  averaged across last 4 time points (shaded region, left) and normalised to pre drug baseline. Mann-Whitney tests. Statistical significance: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

### 4.3.3 GAT inhibition decreases probability of striatal dopamine release by increasing GABA receptor tone onto dopamine axons

We hypothesised that GAT inhibition would decrease the probability of DA release in a GABA receptor-dependent manner through increasing the ambient GABA tone onto DA axons. However, attenuation in evoked  $[DA]_o$  could alternatively be through mechanisms which involve diminished axonal DA storage. We ruled out diminished DA storage as a possible cause of the attenuation of DA release following GAT inhibition: striatal DA content measured using HPLC was unchanged after incubation in NPA compared to controls (Figure 4.4a;  $p = 0.60$ , Mann-Whitney test,  $n = 19$  experiments/5 mice per condition). Instead, we confirmed that GAT inhibition modified DA release in a GABA-receptor dependent manner. The acute effects of NPA on evoked  $[DA]_o$  were prevented in the presence of antagonists for GABA<sub>A</sub> and GABA<sub>B</sub> receptors combined (PTX, 100  $\mu$ M; CGP, 4  $\mu$ M) (Figure 4.4b;  $F_{Drug(1,12)} = 40.4$ ,  $p < 0.0001$ , Two-Way RM ANOVA; without GABA receptor antagonists: ~65% of baseline,  $n = 9$  experiments/5 mice; with GABA receptor antagonists: ~95% of baseline,  $n = 5$  experiments/4 mice;  $p = 0.001$ , Mann-Whitney test), consistent with the effects of GAT on DA release being mediated via extracellular GABA acting on GABA receptors.

In Chapter 3, we found that the activation of GABA receptors can slightly modify the activity-dependence of DA release during short stimulus trains (Lopes, Roberts et al., 2018; see Figure 3.3). Consistent with an increase in GABA receptor activation, GAT inhibitor NPA increased the dependence of  $[DA]_o$  on pulse number during 50 Hz pulse trains in DLS (Figure 4.4c;  $F_{3,48} = 434.7$ ,  $p < 0.0001$ , drug condition x pulse no. interaction, two-way RM ANOVA; 2p:  $p = 0.004$ , 5p:  $p < 0.0001$ , 10p:  $p < 0.0001$ , Sidak's multiple comparisons;  $n = 8$  experiments/5 mice each condition). NPA also increased the paired-pulse ratio of electrically evoked  $[DA]_o$  at short inter-pulse intervals, but not at long inter-pulse intervals (Figure 4.4d-e;  $F_{1,5} = 118.8$ ,  $p = 0.0001$ , drug condition x inter-pulse interval, two-way RM ANOVA; 10 ms:  $p < 0.0001$ , 100 ms:  $p = 0.99$ , Sidak's multiple comparisons;  $n = 6$  experiments/4 mice for each), consistent with a decrease in DA release probability

(Jennings et al., 2015). Together these data indicate that GAT inhibition attenuates DA release through increasing GABA receptor tone onto DA axons.

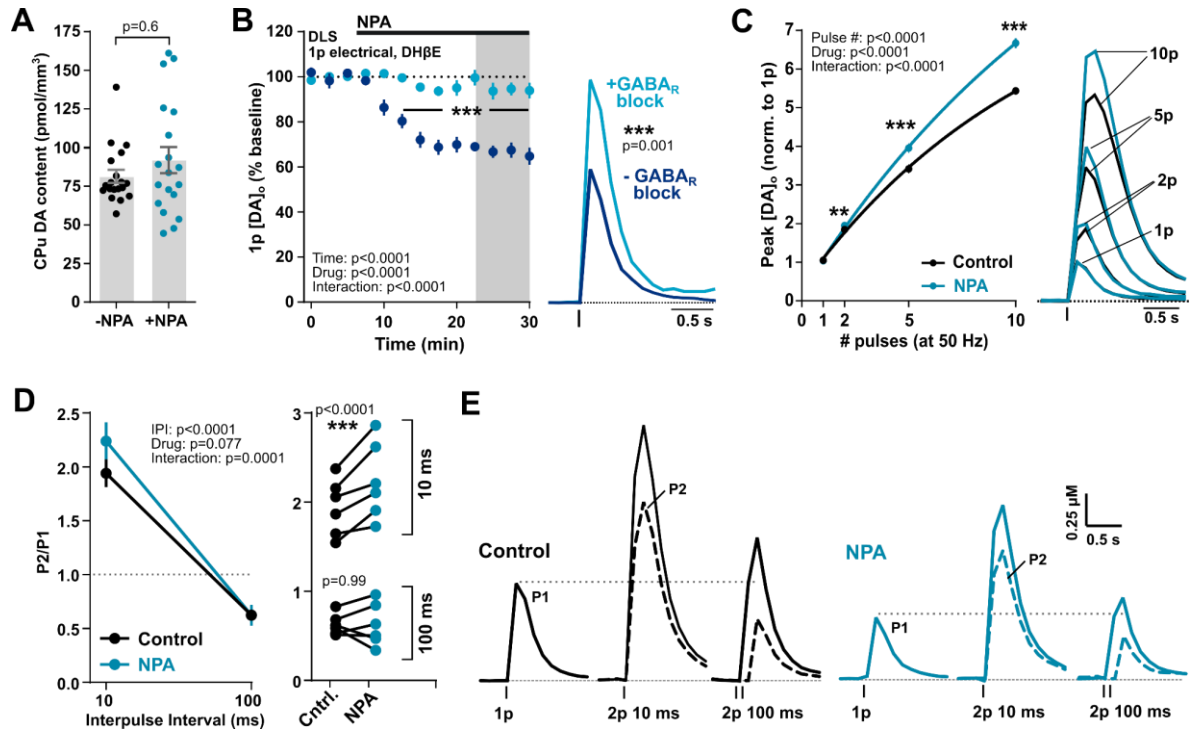


**Figure 4.4.** GAT inhibition modifies activity dependence and decreases probability of dopamine release, by increasing GABA receptor tone.

(A) Mean DA content ( $\pm$  SEM) of dorsal striatum (CPu) incubated in control conditions (black,  $n = 19$  punches/5 mice) or NPA (1.5 mM) (blue,  $n = 19$  punches/5 mice). Mann-Whitney test.

(B) *Left*, Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) during consecutive recordings evoked by a 1 electrical pulse in DLS during application of NPA (1.5 mM) in the absence (dark blue,  $n = 9$  experiments/5 mice) or presence (light blue,  $n = 5$  experiments/4 mice) of GABA<sub>A</sub> (picrotoxin, 100 μM) and GABA<sub>B</sub> (CGP 55845, 4 μM) receptor antagonists. Data are normalised to first 4 time points (dotted line). Two-way RM ANOVA and Sidak's multiple comparisons. *Right*, Mean transients of [DA]<sub>o</sub> averaged across last 4 time points (shaded region, left) and normalised to pre drug baseline. Mann-Whitney test.

(C) *Left*, Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) evoked by 50 Hz electrical pulses in DLS normalised to 1p in the absence (black,  $n = 8$  experiments/5 mice) or presence of NPA (1.5 mM) (blue,  $n = 8$  experiments/5 mice). Sigmoidal curve fits ( $R^2 = 0.98$ ). Two-way RM ANOVA and Sidak's multiple comparisons. *Right*, Mean [DA]<sub>o</sub> profiles.

(D) *Left*, Mean peak [DA]<sub>o</sub> ( $\pm$  SEM) evoked by paired-pulse electrical stimulation at inter-pulse intervals of 10 or 100 ms recorded in DLS in the absence (control, black,  $n = 6$  experiments/4 mice) or presence of NPA (1.5 mM, blue,  $n = 6$  experiments/ 4 mice). *Right*, Individual P2/P1 paired values. Two-way RM ANOVA and Sidak's multiple comparisons.

(E) Mean profiles of [DA]<sub>o</sub> transients from (D): solid traces exhibit [DA]<sub>o</sub> detected after stimulation (P1, P1 + P2), dotted traces show DA release attributed to the second pulse (P2) following subtraction of P1. DHβE (1 μM) present throughout. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

#### 4.3.4 GAT-1 and GAT-3 function and expression is enriched in dorsolateral striatum vs nucleus accumbens core

Given that we observed a greater effect of GAT inhibition in DLS than NAcC (Figure 4.1f), and that both DLS and NAcC are similarly under GABA receptor control (see Section 3.3.1), we hypothesised that GAT function might differ between DLS and NAcC. To identify whether GAT regulates tonic GABA inhibition to a greater degree in DLS than in NAcC, we recorded the tonic GABA<sub>A</sub> receptor-mediated currents in SPNs voltage-clamped at -70 mV and assessed the impact of GAT inhibition on holding current. We confirmed that changes in holding current were mediated by GABA<sub>A</sub> receptors by subsequently applying GABA<sub>A</sub> antagonist picrotoxin (PTX; 100  $\mu$ M). Consistent with the effects on DA release, GAT inhibition with NPA (1.5 mM) elicited a significantly greater increase in GABA<sub>A</sub>-mediated holding current in SPNs in the DLS than in the NAcC (Figure 4.5a-c; DLS vs NAcC:  $p = 0.008$ , Mann-Whitney test; DLS:  $p = 0.0003$ , Friedman's ANOVA on Ranks, NPA vs. drug-free baseline:  $p = 0.001$ , NPA + PTX vs. drug-free baseline:  $p = 0.16$ , NPA vs. NPA + PTX:  $p < 0.001$ , Student-Newman-Keuls tests,  $n = 7$  cells/5 mice; NAcC:  $p = 0.0001$ , Friedman's ANOVA on Ranks, NPA vs. drug-free baseline:  $p = 0.014$ , NPA + PTX vs. drug-free baseline:  $p = 0.014$ , NPA vs. NPA + PTX:  $p = 0.002$ , Student-Newman-Keuls tests,  $n = 6$  cells/3 mice). These data corroborate a greater role for GATs in limiting ambient GABA tone in DLS than in NAcC.

Furthermore, tonic GABA inhibition of SPNs in DLS, like DA release, was regulated by both GAT1 and GAT3. Inhibition of GAT-1 alone with SKF89976A (20  $\mu$ M) induced a small increase in the GABA<sub>A</sub>-mediated holding current (Figure 4.5d;  $p = 0.006$ , Friedman's ANOVA on Ranks, SKF vs. drug-free baseline:  $p = 0.001$ , SKF + PTX vs. drug-free baseline:  $p = 0.41$ , SKF vs. SKF + PTX:  $p = 0.01$ , Student-Newman-Keuls tests,  $n = 6$  cells/3 mice). Combined inhibition of GAT-1 and GAT-3 with SKF89976A (20  $\mu$ M) and SNAP5114 (50  $\mu$ M) induced a three-fold increase (Figure 4.5e;  $p = 0.006$ , Friedman's ANOVA on Ranks, SKF + SNAP vs. drug-free baseline:  $p = 0.001$ , SKF + SNAP + PTX vs. drug-free baseline:  $p = 0.41$ ,

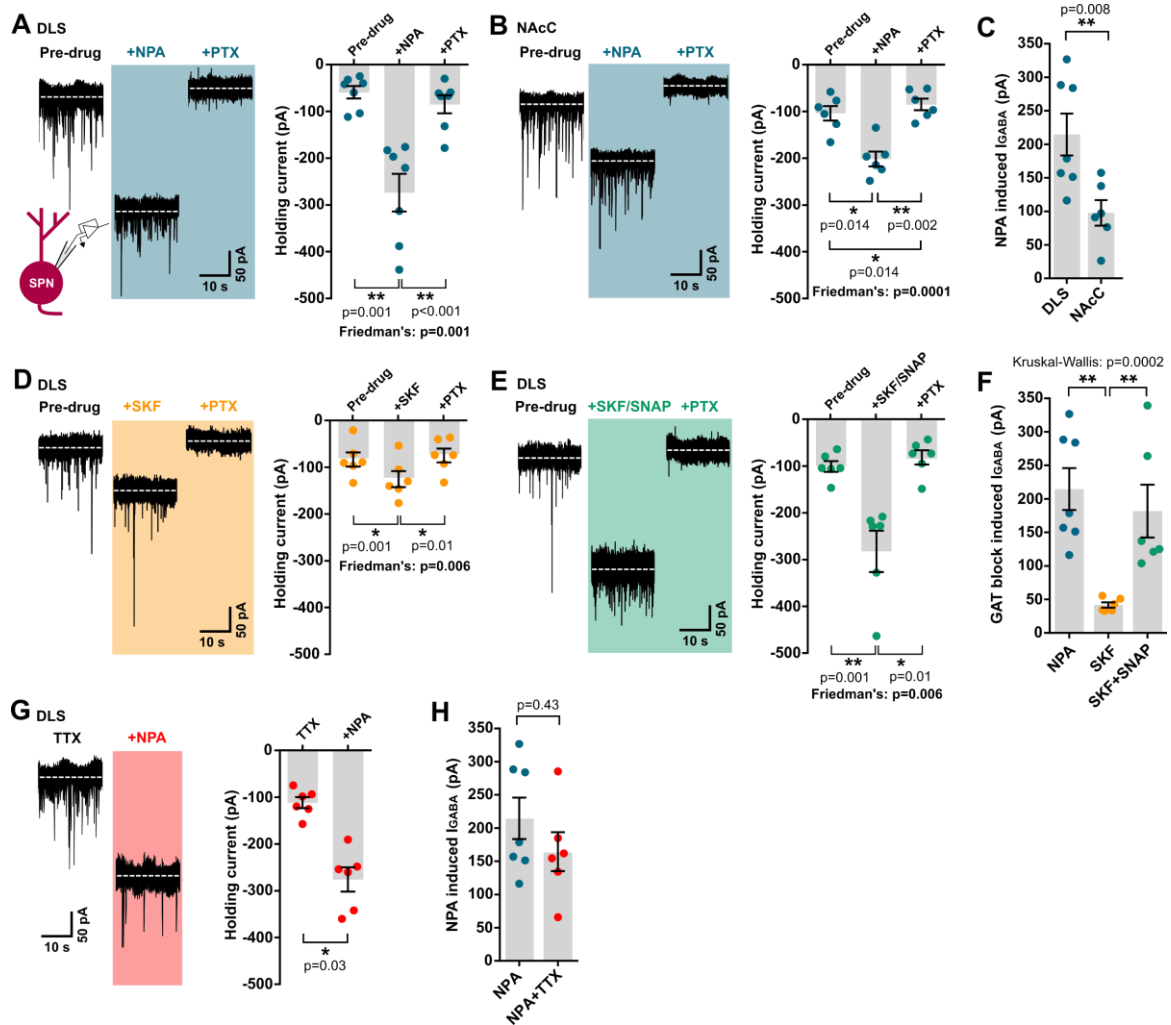


**Figure 4.5.** Tonic GABA currents in striatal spiny projection neurons (SPNs) are augmented by GAT inhibition.

(A-B, D-E, G) *Left*, Representative continuous whole-cell recordings from SPNs in DLS (A,D,E,G) or NAcC (B), voltage-clamped at -70 mV, before and during bath application of GAT inhibitor NPA (blue, 1.5 mM,  $n = 7$  cells/5 mice for DLS in A,  $n = 6$  cells/3 mice for NAcC in B), GAT-1 specific inhibitor SKF89976A (orange, 20  $\mu$ M,  $n = 6$  cells/3 mice in D), the combined application of SKF89976A and GAT-3 specific inhibitor SNAP5114 (green, 50  $\mu$ M,  $n = 6$  cells/4 mice in E), or NPA in the presence of TTX (1  $\mu$ M) (red,  $n = 6$  cells/3 mice in G). GAT inhibitors increase the extracellular GABA<sub>A</sub>-mediated inward current, revealed by a shift in the holding current, and is reversed upon application of GABA<sub>A</sub> receptor antagonist picrotoxin (PTX, 100  $\mu$ M). *Right*, mean ( $\pm$  SEM) holding current in pA recorded in SPNs in control conditions, upon addition of GAT inhibitors and then PTX. Friedman's ANOVA on Ranks and Student-Newman-Keuls multiple comparisons.

(C,F,H) Mean ( $\pm$  SEM) tonic GABA<sub>A</sub>-receptor-mediated currents induced by GAT inhibition recorded from SPNs, calculated by subtracting pre-drug holding current from GAT block-induced holding current. Mann-Whitney tests (C,H) and Kruskal-Wallis test and Dunn's multiple comparisons (F). Ionotropic glutamate receptor antagonists NBQX (5  $\mu$ M) and D-AP5 (50  $\mu$ M) present throughout. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$

SKF + SNAP vs. SKF + SNAP + PTX:  $p = 0.011$ , Student- Newman-Keuls tests,  $n = 6$  cells/4 mice), which was greater than after GAT-1 inhibition alone, but similar to that seen with broad-spectrum GAT inhibition (**Figure 4.5f**;  $p = 0.0002$ , Kruskal-Wallis analysis of variance; SKF + SNAP vs SKF:  $p < 0.01$ , NPA vs SKF + SNAP:  $p > 0.9$ , NPA vs SKF:  $p < 0.01$ ; Dunn's multiple comparison tests).

GABA tone in striatum has previously been reported to be action-potential independent i.e. due to spontaneous release (Wójtowicz et al., 2013). We assessed whether GATs were limiting an action potential-independent GABA tone. In the presence of  $\text{Na}_v$  blocker tetrodotoxin (TTX,  $1 \mu\text{M}$ ), NPA increased the  $\text{GABA}_A$ -mediated holding current in SPNs in the DLS (**Figure 4.5g**; NPA vs. baseline:  $p = 0.03$ , Wilcoxon signed-rank test;  $n = 6$  cells/3 mice) to a level not different to that induced by NPA in TTX-free conditions (**Figure 4.5h**;  $p = 0.43$ , Mann-Whitney test), confirming that GATs in DLS are limiting a spontaneous GABA tone. Collectively, these results show that GAT-1 and GAT-3 regulate an ambient GABA tone, which arises from a spontaneously active source, and do so to a greater degree in DLS compared to NAcC.

We explored an anatomical basis for the regional heterogeneity in GAT function across striatum. Striatal immunoreactivity to GAT-1 and GAT-3 in the DLS and NAcC revealed relative enrichment in the DLS for both GAT1 (**Figure 4.6a-b**;  $p = 0.009$ , Wilcoxon signed-rank test,  $n = 12$  hemispheres/6 mice) and GAT-3 (**Figure 4.6c-d**;  $p = 0.002$ , Wilcoxon signed-rank test,  $n = 12$  experiments/6 mice). We also noted enriched GAT-3 in the medial NAc shell (NAcS) contiguous with the medial septal nucleus (**Figure 4.6c**). This observation prompted us to identify the effects of GAT inhibition on DA release in NAcS. Correspondingly, we found that GAT inhibition diminished electrically evoked  $[\text{DA}]_o$  in the medial NAcS to ~80% of time-matched controls (**Figure 4.7a-c**;  $F_{\text{Drug}(1,13)} = 13.4$ ,  $p = 0.003$ , Two-Way RM ANOVA; NPA: ~80% of baseline,  $n = 6$  experiments/5 mice; time-matched controls: ~95% of baseline,  $n = 9$  experiments/7 mice;  $p = 0.002$ , Mann-Whitney test). This

effect was greater than in NAcC, where no significant effect of NPA was observed, and less than in DLS (Figure 4.7d;  $p < 0.0001$ , Kruskal-Wallis analysis of variance, DLS vs NAcS:  $p < 0.05$ , DLS vs NAcC:  $p < 0.0001$ , NAcC vs NAcS:  $p < 0.05$ , Dunn's multiple comparison tests), indicating further regional heterogeneity in the role of GAT in limiting tonic inhibition of DA release across striatum.

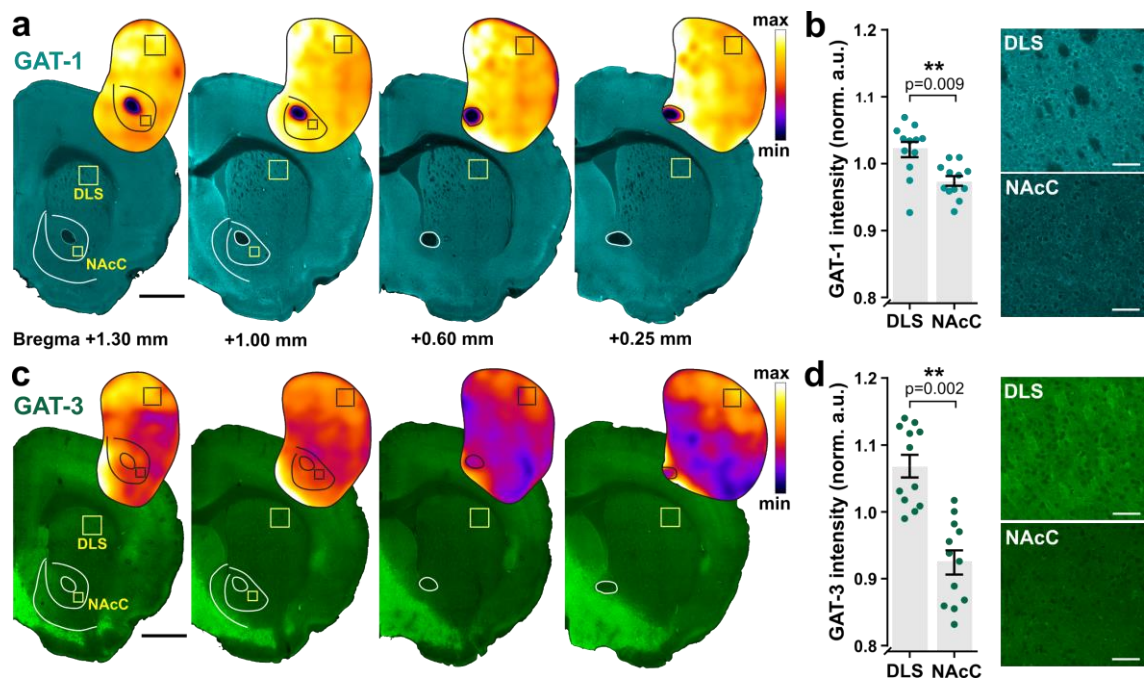


**Figure 4.6. Enrichment of GAT-1 and GAT-3 expression in the DLS versus NAcC.** (A,C) Representative immunofluorescence signals for GAT-1 (cyan, A) and GAT-3 (green, C) using confocal microscopy in coronal sections across the rostral-caudal limits containing striatum prepared from an individual C57BL/6J mouse with heat maps for striatal GAT intensity. Boxes indicate representative locations for GAT intensity measurements in the dorsolateral striatum (DLS) and nucleus accumbens core (NAcC). Scale bars: 1 mm. (B,D) *Left*, Mean (± SEM) GAT-1 (B) and GAT-3 (D) intensity in DLS and NAcC normalised to total striatum and averaged across rostral-caudal sites for each hemisphere (n = 12 hemispheres/6 mice for each GAT-1 and GAT-3). *Right*, Representative single plane images of GAT-1 (B) and GAT-3 (D) immunofluorescence from DLS and NAcC; imaging parameters were kept constant across regions. Scale bars: 50 µm. Mann-Whitney tests. Statistical significance: \*\*p<0.01. Confocal imaging performed by Dr Natalie Doig.

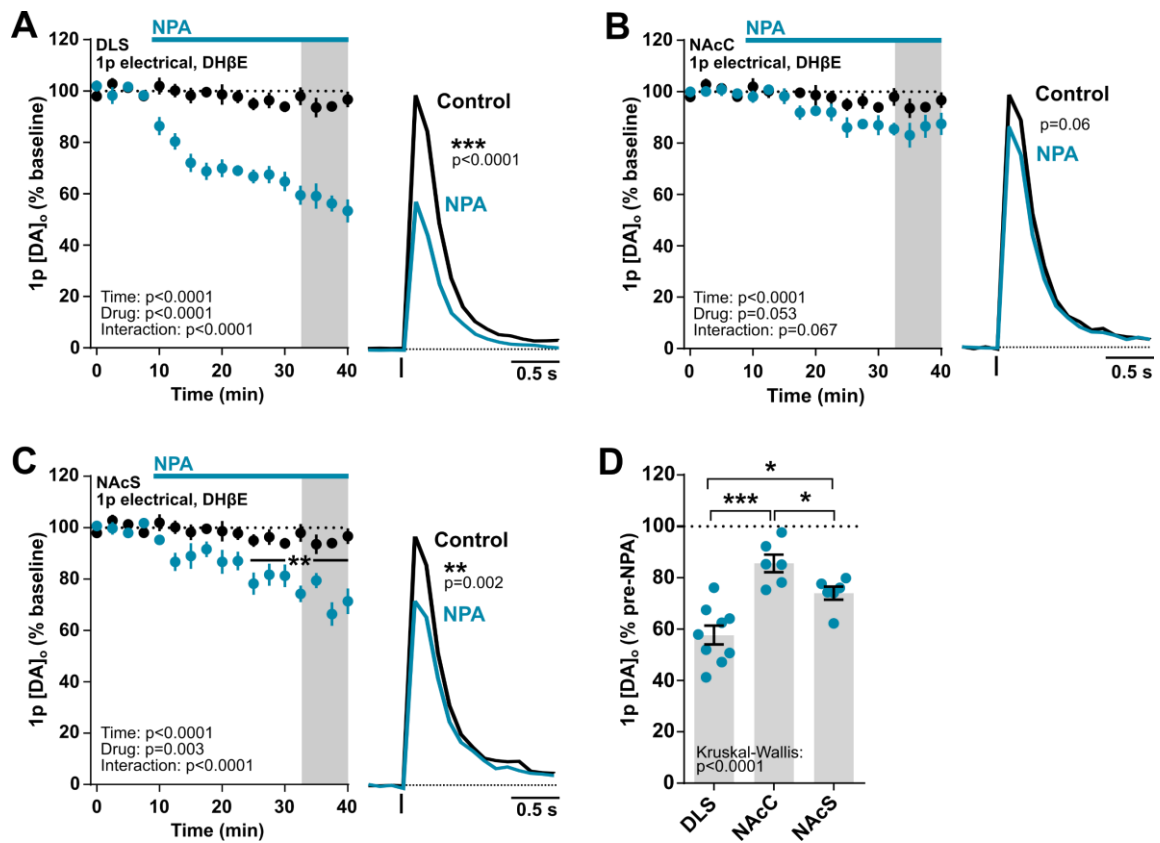


Figure 4.7. GAT inhibition differentially attenuates DA release across striatal territories.

(A-C) *Left*, Mean peak [DA]<sub>o</sub> (± SEM) during consecutive recordings evoked by a single electrical pulse in DLS (A), NAcC (B), or NAcS (C) in control conditions (*black*, n = 9 experiments/7 mice) or with GAT inhibitor nipecotic acid (1.5 mM) (*blue*; n = 9 experiments/5 mice in DLS; n = 6 experiments/4 mice in NAcC; n = 6 experiments/5 mice in NAcS). Data are normalised to first 4 time points (*dotted line*). Two-way RM ANOVA and Sidak's multiple comparisons (*asterisks in bars*). *Right*, Mean transients of [DA]<sub>o</sub> averaged across last 4 time points (*shaded region*, left) and normalised to pre-drug baseline. Mann-Whitney tests.

(D) Mean peak [DA]<sub>o</sub> (± SEM) evoked by 1p following GAT inhibition with NPA (expressed as a % of pre-drug baseline) from (A-C). Kruskal-Wallis test and Dunn's multiple comparisons.

DHβE (1 μM) present throughout experiments with electrical stimuli. Statistical significance: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

### 4.3.5 GAT-1 and GAT-3 on astrocytes are key regulators of ambient GABA inhibition of dopamine release

Striatal GATs are located on the plasma membranes of neuronal and non-neuronal structures that include GABAergic neurons (Augood et al., 1995; Durkin et al., 1995; Ng et al., 2000; Yasumi et al., 1997) and astrocytes (Chai et al., 2017; Ng et al., 2000; Yu et al., 2018) and they have also been presumed, but not confirmed, to reside on DA axons to support GABA co-storage and co-release (Tritsch et al., 2014). To better understand where GAT is located to regulate tonic GABAergic inhibition of DA, we probed two candidate locations, namely DA axons, and astrocytes. We explored whether GAT-1 or GAT-3 could be detected on DA axons using immunofluorescence and confocal microscopy, but we did not find robust evidence for localisation to eYFP-expressing DA axons (Figure 4.8a-b). To confirm that using this experimental approach was valid for detecting axonal GAT co-localisation, we found robust GAT-1 and eYFP co-localisation in the neurites of PV-expressing interneurons (Figure 4.9a-d), which are known to express GAT-1 (Augood et al., 1995). Our observations do not support the assumption that GATs are localised to DA axons (Tritsch et al., 2014) and argue against roles for GATs in regulating “spill-in” of a GABAergic tone and in supporting GABA uptake for co-storage and co-release.

Astrocytes are thought to regulate ambient GABA levels by uptake in many brain regions, including striatum (Yu et al., 2018). GAT-3 expression has been documented on striatal astrocytes (Chai et al., 2017; Ng et al., 2000; Yu et al., 2018), but GAT-1 is typically associated more with neuronal structures (Borden, 1996), despite transcriptomic data indicating RNA for both GAT-1 and GAT-3 in striatal astrocytes (Chai et al., 2017; Gokce et al., 2016; Zhang et al., 2014). To re-examine this we used immunofluorescence and confocal microscopy with antibodies directed against GAT-1 and GAT-3, as well as against the striatal astrocytic marker S100 $\beta$  (Chai et al., 2017) in the DLS and NAcC (Figure 4.10a-b). As expected, we found that GAT-3 was robustly co-localised on the plasma membrane of S100 $\beta$ -positive astrocytes (Figure 4.10d;  $n = 3$  mice), however we also found some instances

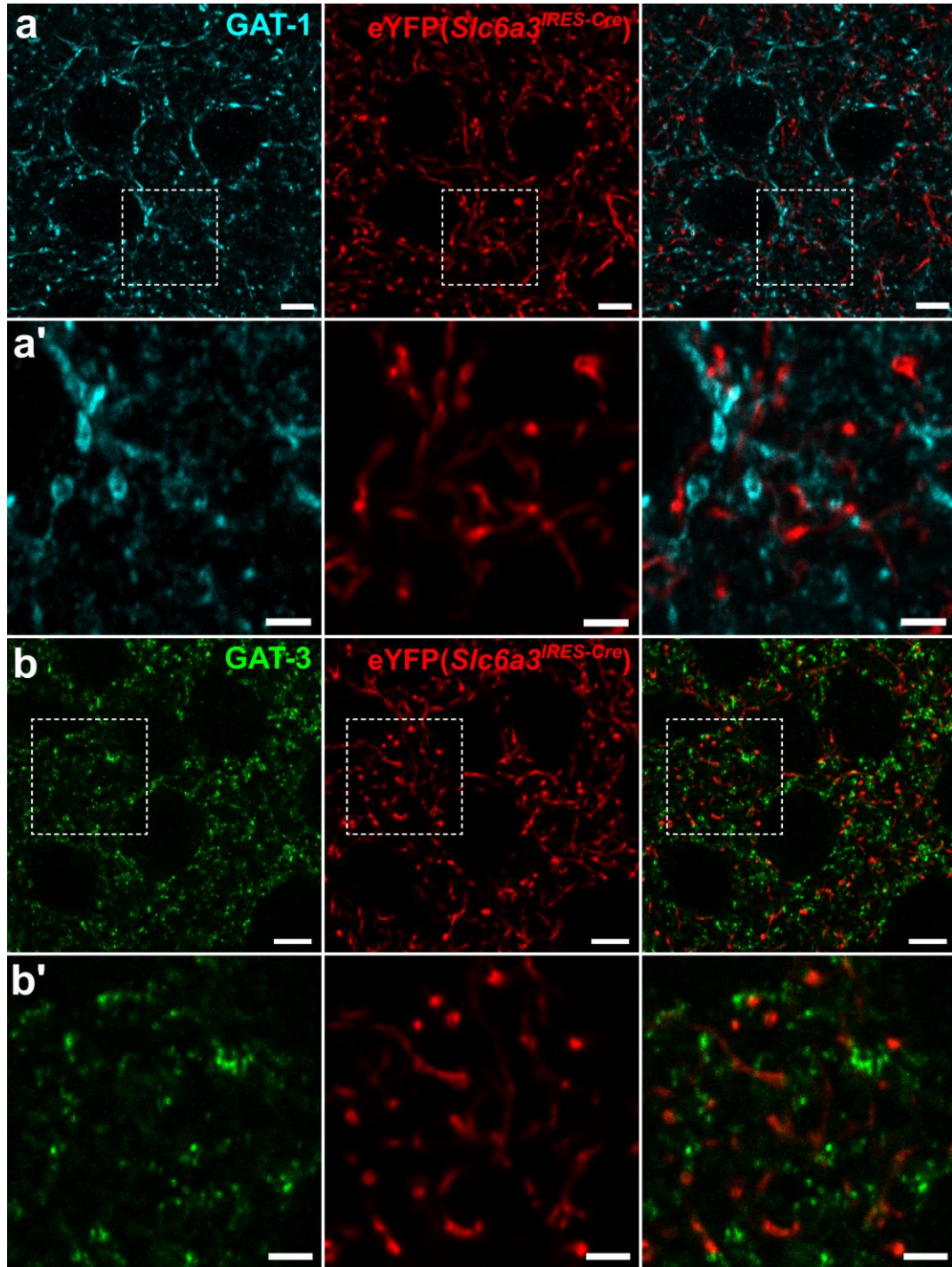


Figure 4.8. GAT-1 and GAT-3 are not typically co-localised to dopamine axons.

(A,B) Representative immunofluorescence signals in dorsal striatum for GAT-1 (cyan, A), GAT-3 (green, B), and conditional eYFP expression in DA axons from *Slc6a3<sup>IRES-Cre</sup>* injected mice (red). (A',B') Immunofluorescence signals with increased magnification from A and B as indicated. We did not observe robust co-localization of GAT-1 or GAT-3 on DA axons ( $n = 3$  mice), examined using ZEN (blue edition v.2.3; Zeiss) software. Scale bars: 10  $\mu\text{m}$  (A,B), 2  $\mu\text{m}$  (A',B'). Confocal imaging performed by Dr Natalie Doig.

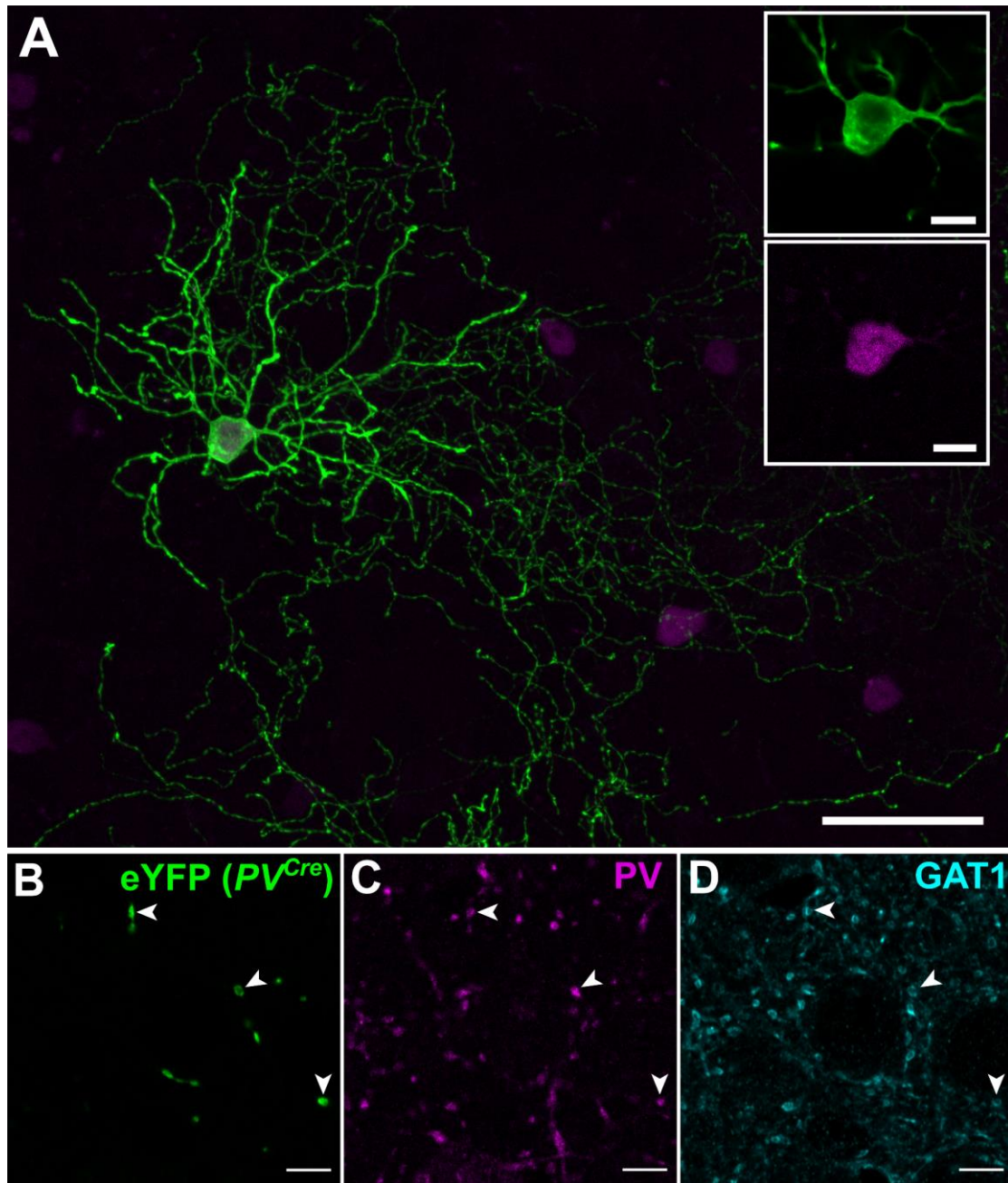


**Figure 4.9.** GAT-1 is co-localised to striatal parvalbumin-positive interneurons.

(A) Representative immunofluorescence signals for parvalbumin (PV, *purple*) and interneurons in the dorsal striatum conditionally expressing eYFP (*green*) in their processes after AAV striatal injection in a *PV<sup>Cre</sup>* mouse. Scale bars: 50 μm, 10 μm for insets.

(B-D) We observed robust GAT-1 (*cyan*) co-localisation in the neurites of parvalbumin-positive interneurons (*n* = 3 animals), indicated by white arrows. Scale bars: 5 μm.

Confocal imaging performed by Dr Natalie Doig.

of co-localisation of GAT-1 to the plasma membrane of S100 $\beta$ -positive astrocytes (Figure 4.10c; n = 3 mice). These data indicate that although GAT-1 is commonly co-localised with striatal GABAergic interneurons, GAT-1 can also be expressed by some astrocytes in striatum.

The impact of GATs expressed by astrocytes on DA transmission has previously been unknown. We next probed whether GATs on striatal astrocytes could act to govern tonic GABAergic inhibition of DA release. To address this, we pre-treated some striatal slices before FSCV recordings with fluorocitrate (200  $\mu$ M for >1hr), an established approach for metabolically inhibiting astrocytes through inhibition of the glial TCA cycle, thus rendering them inactive and preventing the effects of astrocytic GAT (Boddum et al., 2016; Bonansco et al., 2011). Electrically evoked [DA]<sub>o</sub> in slices pre-incubated in fluorocitrate exhibited a slow ongoing rundown across repeated 1p stimulations (n = 5 experiments/3 mice; data not shown) and therefore we used an alternative stimulation paradigm to compare a large number of dorsal striatal recording sites in slices pre-treated with fluorocitrate versus control conditions to minimise the effects of run-down (i.e. a within subjects wash-on experimental design was precluded). Therefore, as a control, other striatal slices were pre-treated in vehicle without fluorocitrate. DA release in both fluorocitrate-treated slices and vehicle-treated control slices was then recorded with/without the GAT inhibitor NPA. We first confirmed that we could detect the effects of GAT inhibition in DLS in control slices. Accordingly, [DA]<sub>o</sub> evoked by 1p electrical stimulation across a range of sites in the DLS from slices incubated in NPA (1.5 mM) for 30 min was significantly less than those incubated in NPA-free control conditions, as expected (Figure 4.11a; p = 0.0003, Mann-Whitney test; n = 24 observations/5 mice for each condition), and 4p/1p ratio (50 Hz) was appropriately enhanced (Figure 4.11b; p = 0.014, Mann-Whitney test; n = 8 experiments/5 mice for each condition), as exposed previously (see Figure 4.4). By contrast, when we performed these experiments in slices pre-treated with fluorocitrate to inactivate astrocytes, NPA did not significantly modify [DA]<sub>o</sub> evoked by 1p (Figure 4.11c; p = 0.10, Mann-Whitney test; n = 42

observations/7 mice for each condition), or the 4p/1p ratio (50 Hz), compared to without NPA (Figure 4.11d;  $p = 0.64$ , Mann-Whitney test;  $n = 13$  observations/7 mice for each condition). We noted also that evoked  $[DA]_o$  was lower in fluorocitrate-treated slices (Figure 4.11e;  $p = 0.0001$ , Mann-Whitney test). Additionally, the inhibition of  $[DA]_o$  by NPA was attenuated when astrocytes were inhibited compared to where astrocytes were not (Figure 4.11f;  $p = 0.004$ , Mann-Whitney test). Together, these data collectively reveal that GATs on striatal astrocytes regulate the level of inhibition of DA release by limiting the ambient GABA tone onto DA axons.

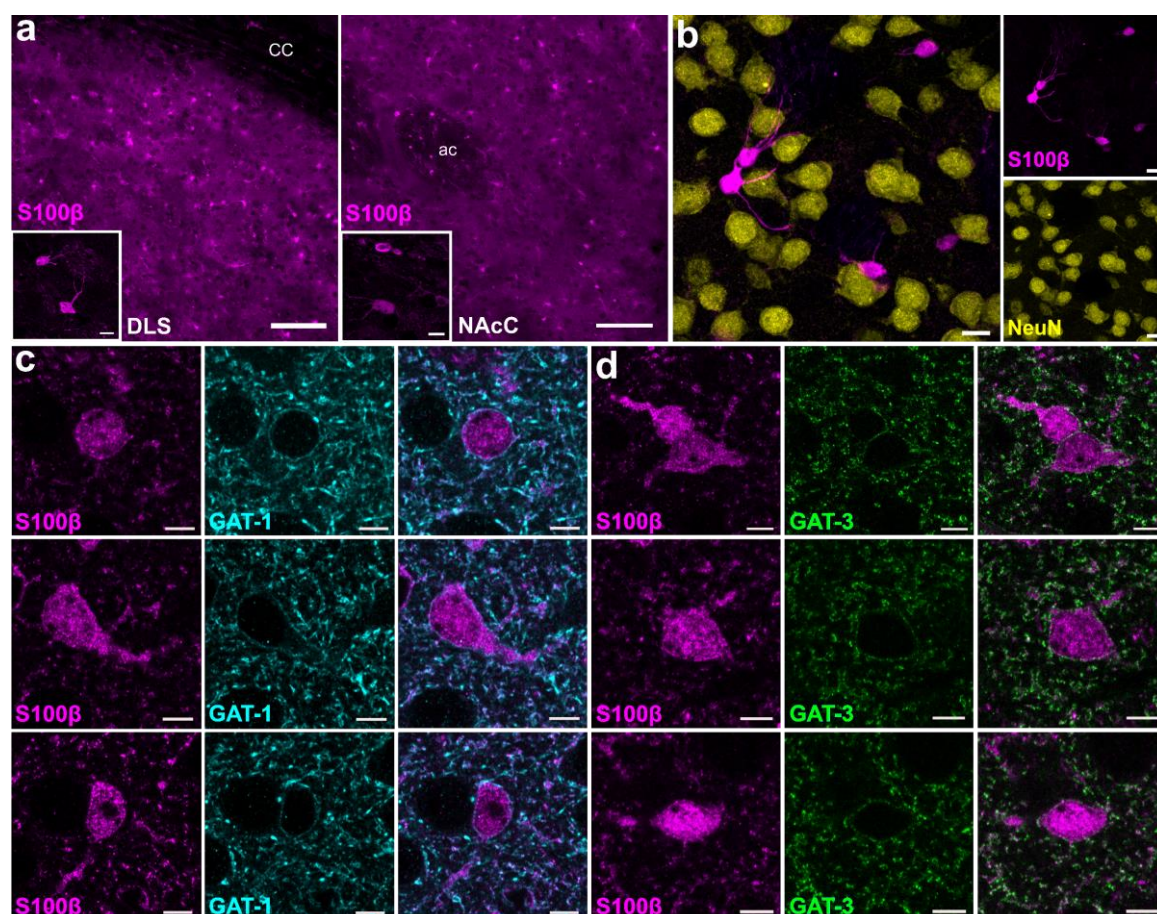


Figure 4.10. GAT-1 and GAT-3 are expressed on plasma membranes of striatal astrocytes. (A) Striatal immunofluorescence signals for astrocyte marker S100β (*magenta*) in dorsolateral striatum (DLS) and nucleus accumbens core (NAcC). Scale bars: 100 μm, for inset: 10 μm. cc: corpus callosum, ac: anterior commissure. (B) Immunofluorescence signals for S100β do not co-localize with immunofluorescence signals for neuronal marker NeuN. Scale bars: 10 μm. (C-D) GAT-3 (*green*, C) and GAT-1 (*cyan*, D) are expressed on plasma membranes of striatal S100β-expressing astrocytes imaged in DLS ( $n = 3$  animals). Immunoreactivity for *left*, S100β, *centre*, GAT, *right*, merged. Scale bars: 5 μm. Confocal imaging performed by Dr Natalie Doig.

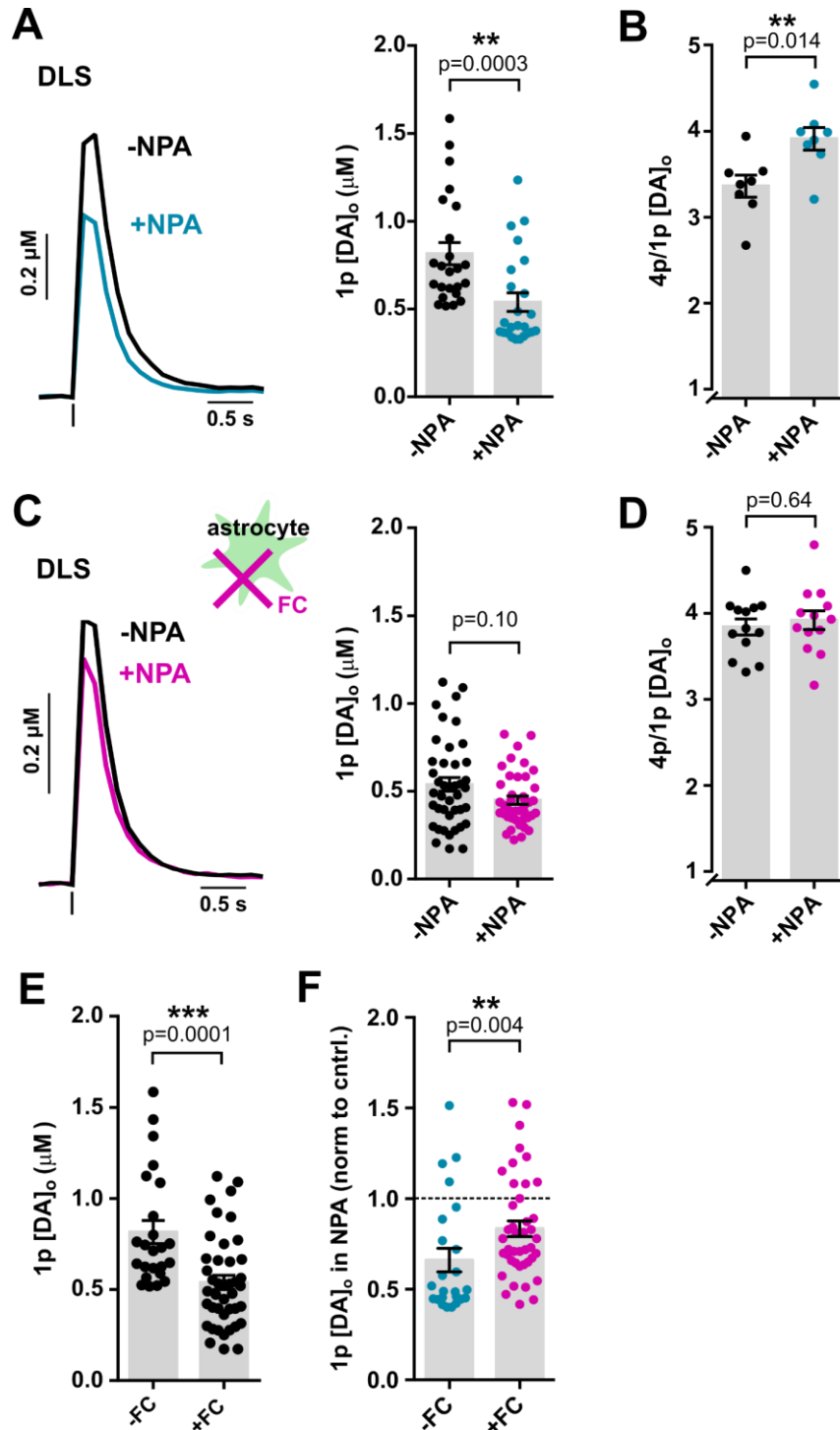


Figure 4.11. GAT-1 and GAT-3 expressed on striatal astrocytes regulated GABA inhibition of dopamine release.

(A-D) Mean profiles of  $[\text{DA}]_o$  and mean peak  $[\text{DA}]_o$  ( $\pm$  SEM) in DLS evoked by 1 electrical pulse (A,C) or 4 pulses normalised to 1p (B,D) in the absence (black) and presence of GAT inhibitor NPA (1.5 mM) in control conditions (blue,  $n = 5$  mice, A-B) or with the astrocyte inhibitor fluorocitrate (FC, 200  $\mu\text{M}$ , pink,  $n = 7$  mice, C-D). Mann-Whitney tests.

(E) Mean peak  $[\text{DA}]_o$  ( $\pm$  SEM) evoked by 1 electrical pulse in the absence (-FC) and presence (+FC) of FC. Mann-Whitney test.

(F) Mean peak  $[\text{DA}]_o$  ( $\pm$  SEM) evoked by 1 electrical pulse in the presence of NPA (1.5 mM) normalised to control conditions in controls (blue) or treated with FC (pink). Mann-Whitney test.

## 4.4 Discussion

We define here a major role for the GAT in governing DA output in the striatum. We show here that GAT-1 and GAT-3, located at least in part on striatal astrocytes, operate to limit tonic inhibition of DA release in DLS but not NAcC.

### 4.4.1 GAT limits tonic inhibition of dopamine release

The paucity of GABAergic synapses on DA axons (Charara et al., 1999) suggests that tonic inhibition of DA axons arises from the extrasynaptic ambient tone that can be detected in striatum (Ade et al., 2008; Cepeda et al., 2013; Kirmse et al., 2008, 2009; Santhakumar et al., 2010). We exposed previously that this tone is likely GAD-dependent (see Section 3.3), and confirmed here was mostly action potential-independent i.e. spontaneous source (Kaesler and Regehr, 2013), which is reported previously to mediate at least part of the tonic inhibition of SPNs (Wójtowicz et al., 2013). A spontaneous GABAergic regulation of DA release is not surprising when considering that the axonal arbour of a single nigrostriatal DA neuron (in rat) reaches on average 2.7% of the volume of striatum on average (Matsuda et al., 2009; Oorschot, 1996), and that such volumes are expected to contain ~74,000 GABAergic neurons (assuming 2.8 million striatal neurons per hemisphere (Oorschot, 1996) of which ~98% are GAD-immunoreactive). Even low rates of spontaneous vesicle release from a small fraction of these neurons might summate sufficiently to provide a tone at GABA receptors on DA axons that limits DA output. The potential functions of this spontaneous GABA tone are not known, but could be dissociated from those of synaptic events (Farrant and Nusser, 2005), and could include regulation of axonal membrane resistance to modify the impact of other inputs or limit the propagation of action potentials through the axonal arbour for a sparser coding.

These findings that striatal ambient GABA provides tonic inhibition of DA release that is governed by GAT activity are confounded by the fact that these experiments are conducted at a non-physiologically relevant temperature. Indeed, we conducted our electrophysiology

and FSCV experiments here at hypophysiological temperatures, i.e. at 32°C, typical practice for physiology experiments using acute *ex vivo* brain slices. Previous studies examining GATs role in limiting striatal ambient GABA tonic inhibition of SPNs have also been conducted in acute *ex vivo* slice preparations and at similar temperatures (Ade et al., 2008; Cepeda et al., 2013; Janssen et al., 2009; Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014). Central nervous system neurotransmitter transporters are temperature-dependent, and therefore tonic inhibition of DA release and SPNs by an ambient GABA tone in striatum could arise in *ex vivo* slice preparations through temperature-dependent dampened GAT activity. Nonetheless, ambient GABA tone in other rodent brain nuclei have been detected *in vivo* (Chadderton et al., 2004), and increased striatal GAT expression *in vivo* alters SPN activity and striatal-dependent behaviours through decreasing striatal ambient GABA tone (Yu et al., 2018). Therefore, it is likely that striatal ambient GABA tone detected at hypophysiological temperatures in acute brain slice preparations are also detectable in the intact environment *in vivo*, but future work should address whether an ambient GABA tone onto DA axons can be detected at more physiologically relevant temperatures, i.e. 37°C, or moreover in the intact brain *in vivo*.

We found that GAT-1 and GAT-3 both limit GABA action onto DA axons in DLS, and thereby indirectly support DA release. We found that this unprecedented role for the GAT in supporting DA output was heterogeneous. GATs limited tonic GABAergic inhibition of DA release in DLS, and also NAcS, but not NAcC, which corresponded with heterogeneity in GAT-1 and GAT-3 levels and with GAT control of tonic inhibition of SPNs. This heterogeneity in GAT function and expression across striatum has previously been unexplored, as other studies examining striatal GAT have all focused in dorsal striatum alone (Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Tritsch et al., 2014; Wójtowicz et al., 2013). Our findings therefore position GAT to modulate the excitability of neuronal networks preferentially in the dorsal striatum.

The positive relationship we find between GAT function and DA output in the dorsal striatum is paralleled by, and provides a candidate explanation for, some clinical effects of GAT inhibitors e.g. tiagabine. When used clinically as antiepileptics to increase extracellular GABA levels, these drugs can have Parkinsonian-like motor side effects (Zaccara et al., 2004). We propose future experiments which investigate the effects of GAT inhibition or striatal GAT knock-out on DA-dependent behaviours *in vivo*. Given that we observe a greater role for the GAT in supporting DA release in the dorsal versus ventral striatum, we predict that striatal GAT inhibition will impair dorsal-specific DA-dependent behaviours, i.e. locomotor activity and motor learning, while unaffected ventral-specific DA-dependent behaviours, i.e. reward-related types of behaviours.

#### 4.4.2 Implications for our mechanistic understanding of GABA co-release

GAT-1 and GAT-3 were suggested previously to be directly located on DA axons to support GABA co-storage and co-release (Tritsch et al., 2014). Under this presumption, GAT activity might influence DA release through disruption to the co-storage of GABA and DA, as co-storage can have significant implications for other pairs of co-stored neurotransmitters (Gras et al., 2008; Hnasko et al., 2010), and/or through physiologically relevant substrate induced currents of the GAT to directly modify axonal excitability, as shown previously in other types of neurons and/or with other transporter types (Bagley et al., 2011; Ingram et al., 2002; Veruki et al., 2006). However, we ruled out disruptions to GABA co-storage in attenuating DA release and did not find evidence for GAT-1 or GAT-3 localisation to DA axons in DLS.

Previous data suggesting that GATs reside on DA axons to support GABA uptake for co-release were based on mRNA for GAT-1 (and weakly for GAT-3) in the somata of DA neurons in substantia nigra, and on the attenuation of GABA co-release from DA axons after pharmacological inhibition of both GAT-1 and GAT-3 simultaneously (Tritsch et al., 2014). However, since our novel findings show that there is a tonic GABAergic inhibition of DA

release mediated by both GABA<sub>A</sub> and GABA<sub>B</sub> receptors (Lopes, Roberts et al., 2018; Chapter 3), that we show is profoundly limited by the GAT, then the observed dependence of GABA co-release on GAT is very likely a result of the GATs limiting tonic inhibition of GABA co-release, rather than being required for uptake of GABA for co-storage in DA axons. These findings therefore indicate that GABA co-storage and co-release from DA axons is likely dependent on GABA synthesis alone by ALDH-1a1 (Kim et al., 2015), but GATs supporting GABA uptake into DA axons will need to be completely ruled out through targeted knock-out studies.

#### 4.4.3 Astrocytes support dopamine release by limiting tonic GABA inhibition

We revealed that astrocytes play a critical role in limiting the tonic inhibition of DA release and therefore supporting DA output. We found that both GAT-3 and, to a lesser extent, GAT-1, could be identified on astrocytes, challenging the previous generalisation that GAT-1 is exclusively neuronal (Borden, 1996), and confirm emerging transcriptomic data that demonstrate striatal astrocytes express mRNA for both GAT-1 and GAT-3 (Chai et al., 2017; Gokce et al., 2016; Zhang et al., 2014). The role we find for astrocytes in supporting GABA uptake to minimise tonic inhibition of DA release, indicates a previously unappreciated role for astrocytes in determining the dynamic output of DA. We do note that fluorocitrate, which is thought to selectively inhibit astrocyte metabolism (Paulsen et al., 1987), might have unknown off-target action, but has previously been used successfully to prevent the action of astrocytic GAT (Boddum et al., 2016; Bonansco et al., 2011). Future work using selective astrocytic GAT knock-out strategies should be conducted to overcome the limitations of fluorocitrate and confirm the role for astrocytic GAT in governing DA output. Furthermore, molecular heterogeneity has been observed between dorsal and ventral striatal astrocytes (Chai et al., 2017), which should be further investigated in light of our observed greater regulation of DA release by astrocytic GAT in dorsal versus ventral striatum.

It has recently been found that astrocytes in the VTA can regulate DA neuron activity (Gomez et al., 2019), and that astrocytes in striatum can modulate the activity of other types of synapses and neurons (Martín et al., 2015; Yu et al., 2018) and can influence striatal-dependent reward and motivation to self-administer drugs of addiction (Bull et al., 2014; Scofield et al., 2015). Our findings here further show that astrocytes can govern striatal DA output through local interaction at axonal release sites, significantly revising our current understanding of the striatal mechanisms that can dynamically regulate DA transmission and warranting future investigations into the relationship between striatal astrocytes and DA.

#### 4.4.4 Summary and implications

In conclusion, we show here that the level of DA release in striatum is set by the activity of GAT-1 and GAT-3, located at least partly on astrocytes, through limiting the ambient GABA tone onto DA axons. Moreover, we show enriched GAT function and expression in the dorsal versus ventral striatum. These data define previously unappreciated and important roles for GATs and astrocytes in supporting DA release in striatum, and reveal a novel locus for potential dysfunction in disorders associated with dysregulated DA signalling.

# 5

Maladaptive downregulation of  
striatal GABA transporter levels in  
early Parkinsonian model

## 5.1 Introduction and initial hypotheses

The data outlined in **Chapter 4** provided moderate evidence that GATs regulate DA output in the DLS of neurologically-intact mice, through limiting GABA tone onto DA axons. Importantly, GAT dysregulation in the basal ganglia has been implicated in models of neurological disease. In a toxin-based mouse model of DA depletion in PD, astrocytes in the external globus pallidus downregulate GAT-3 through decreased DA signalling at D<sub>2</sub> receptors on astrocytes, and leading to persistent GABAergic tonic inhibition in globus pallidus neurons mediated through abnormally elevated extracellular GABA concentrations (Chazalon et al., 2018). In transgenic mouse models of Huntington's disease (R6/2 and FVB/N), GAT-3 expression in striatum is aberrantly increased resulting in decreased tonic inhibition in SPNs by ambient GABA (Cepeda et al., 2013; Wójtowicz et al., 2013; Yu et al., 2018), and the manifestation of excessive self-grooming behaviours (Yu et al., 2018). However, whether striatal GATs are implicated in PD remains completely unexamined. To probe further the significance of the regulation of striatal DA by striatal GATs, we explored GAT function in a mouse model of early parkinsonism.

Animal models recapitulating human disease enable examination of PD pathophysiology, with emerging genetic models allowing examination across different time stages. Therefore, such models are valuable for elucidating the earliest pathological and physiological processes underlying the final common pathway of DA neuron degeneration. A common feature of PD is the accumulation of cytoplasmic filamentous protein aggregates, known as Lewy bodies, which are principally comprised of  $\alpha$ -synuclein (Baba et al., 1998; Marques and Outeiro, 2012; Spillantini et al., 1998). Genetic and genome-wide association studies (GWAS) point to  $\alpha$ -synuclein involvement in both sporadic and familial PD as identified point mutations, as well as duplication and triplication of the *SNCA*  $\alpha$ -synuclein gene locus have been identified in autosomal dominant PD cases (Bekris et al., 2010; Chartier-Harlin et al., 2004; Singleton et al., 2003). GWAS also support the involvement of  $\alpha$ -synuclein (*SNCA*) in sporadic cases (Satake et al., 2009; Simón-Sánchez et

al., 2009; Venda et al., 2010). Furthermore, pathophysiology data from *SNCA* locus multiplication studies highlight a gene dosage effect, as triplication leads to earlier symptom onset and a more severe disease progression compared to gene duplication (Fuchs et al., 2007). The human  $\alpha$ -synuclein overexpressing mouse model of PD (*SNCA-OVX*), was developed at the Oxford Parkinson's Disease Centre to recapitulate a human disease-relevant genetic burden of  $\alpha$ -synuclein overexpression, resulting in a highly physiological, slowly progressive mouse model of Parkinsonism (Janezic et al., 2013). These *SNCA-OVX* mice show early deficits in DA transmission (by 3 months of age) restricted to the dorsal striatum, but not observed in the ventral striatum, prior to late-stage degeneration of DA neurons, disturbed encoding of behaviour in surviving neurons, and manifestation of a motor phenotype (at 18 months of age; Dodson et al., 2016; Janezic et al., 2013). Intriguingly, augmented  $\alpha$ -synuclein burden in astrocytes has also been observed in the *SNCA-OVX* mouse (unpublished observation from Wade-Martins Group, University of Oxford). Alternative genetic (e.g. *LRRK2*, *PRKN*) and neurotoxic lesion (e.g. 6-OHDA, MPTP) models of Parkinsonism exist (for extensive review see Konnova et al., 2018), which generally exhibit greater behavioural deficits and rapid nigrostriatal dopaminergic cell death, modelling the late stages of PD. Unlike these alternative models, the *SNCA-OVX* model allows investigation of the earliest pathological and physiological processes underlying the final common pathway of DA neuron degeneration (Janezic et al., 2013).

Given that astrocytes downregulate GAT-3 in GPe in response to DA depletion (Chazalon et al., 2018), we hypothesised that GATs would similarly downregulate in the dorsal striatum of Parkinsonian mice, which exhibit reduced DA signalling. Since striatal GATs play a principal role in limiting tonic inhibition of DA release by ambient GABA in the dorsal striatum (Section 4.3), we further hypothesised that DA release in dorsal Parkinsonian striatum would be under augmented tonic inhibition due to downregulated GAT(s). To test these hypotheses, we explored whether tonic inhibition of striatal DA release and its regulation by striatal GAT might be affected in the *SNCA-OVX* mouse model at its

prodromal stage (at 3-6 months of age), where there is a striatal DA deficit but not yet any observed degeneration of DA neurons or presentation of a motor phenotype.

DA release in the dorsal striatum is supported by calcium entry into DA axons through a diverse range of voltage-gated calcium channels (VGCCs), namely P/Q-, N-, L- and T-type VGCCs (Brimblecombe et al., 2015). The sources of calcium in nigrostriatal DA neurons are thought to contribute to their degeneration in PD, through generating a deleterious metabolic burden (Pissadaki and Bolam, 2013; Sulzer, 2007; Surmeier et al., 2010, 2017). As such, agents that might limit this deleterious calcium burden in nigrostriatal DA neurons, such as VGCC antagonists, are of recent great interest as potential neuroprotective agents in PD. Ongoing research within the Laboratory of Stephanie Cragg is currently examining the potential therapeutic effect of modifying VGCC function through targeting of auxiliary subunits, namely the  $\alpha_2\delta$  subunits, as a means to modify channel trafficking and location without directly blocking VGCCs which would significantly limit DA release and potentially worsen symptoms.

The  $\alpha_2\delta$  subunits facilitate correct trafficking of VGCCs to the axonal presynaptic membrane (Davies et al., 2010) and therefore critically regulate the presynaptic abundance, localisation and function of P/Q-, N- and L-type channels, and in turn, neurotransmitter release in other neuron types (Arikath and Campbell, 2003; Hoppa et al., 2012; Klugbauer et al., 1999). Gabapentinoids, namely gabapentin (GBP) and pregabalin (PGB), are known to bind to  $\alpha_2\delta$ -1 and -2 auxiliary subunits to alter VGCC function (Dolphin, 2012, 2016). Gabapentinoids show clinical efficacy in disorders associated with DA dysfunction, including restless-leg syndrome which is associated with hypodopaminergic signalling (Guo et al., 2017), and addictions (Fujishiro, 2014; Mason et al., 2018). Furthermore, previous work has found that prolonged incubation (>24 hours) of GBP or PGB caused a 2-fold increase in GABA uptake in hippocampal cultures, which was due to a redistribution of GAT protein from intracellular locations to the plasma membrane (Whitworth and Quick, 2001). We therefore

hypothesised that PGB treatment in *SNCA-OVX* mice might promote DA release in striatum through modulating VGCC function in DA axons and/or reducing GABAergic inhibition of DA release via upregulating striatal GATs.

Overarching hypothesis - GAT dysfunction results in dysregulated striatal dopamine signalling in the *SNCA-OVX* prodromal mouse model of early Parkinson's disease.

Hypothesis 1 - Downregulated GAT expression in striatum of *SNCA-OVX* mice leads to augmented tonic GABAergic inhibition of DA release.

Hypothesis 2 - PGB treatment in *SNCA-OVX* mice will boost DA release through normalising aberrant tonic GABAergic inhibition of DA release.

## 5.2 Methods

Experiments described in this chapter were carried out according to the general methods described in Chapter 2. Variations and further details are described below.

### 5.2.1 Parkinsonian mouse model

We chose to use *SNCA-OVX* mice, a model of early parkinsonism (Janezic et al., 2013). *SNCA-OVX* mice are devoid of mouse  $\alpha$ -synuclein but instead overexpress human wildtype  $\alpha$ -synuclein at disease-relevant levels and show early deficits in DA release prior to DA cell loss (Janezic et al., 2013). To address our aims, we made these *SNCA-OVX* mice 'optogenetics-capable', such that they allowed for optical manipulation of DA axons. We first crossed *Slc6a3<sup>IRE5-Cre</sup>* mice with  $\alpha$ -synuclein knockout mice to create *Slc6a3<sup>IRE5-Cre</sup>* mice devoid of mouse  $\alpha$ -synuclein. We then crossed this bespoke *Slc6a3<sup>IRE5-Cre</sup>* line with *SNCA-OVX* mice to generate two cohorts of mice, both devoid of mouse  $\alpha$ -synuclein: (1) "*SNCA+*" mice that express Cre recombinase in DA neurons and overexpress human  $\alpha$ -synuclein; and (2) as littermate controls, "*Snca*/-" mice that express Cre recombinase in DA neurons but no human transgene. Both male and female sex-matched *SNCA+* and *Snca*<sup>-/-</sup> littermate pairs were used in experiments involving use of these Parkinsonian mice. These mice were stereotactically injected with AAV-ChR2 at 3-5 months (as outlined in Section 2.1.3) and used

for FSCV and electrophysiology experiments at 4-6 months. SNCA-OVX mice (original line, not crossed with *Slc6a3<sup>IRE5-Cre</sup>* mice) were used at 11-13 months for Pregabalin dosing experiments. Acute *ex vivo* coronal slices containing striatum were prepared for FSCV experiments as described in Section 2.2.3. For the preparation of acute *ex vivo* slices for electrophysiology experiments the alternate method for aged animals outlined in Section 2.3.1 was used. All experimental procedures involving the use of animals were carried out according to institutional guidelines and conformed to the UK Animals (Scientific Procedures) Act 1986.

### 5.2.2 Fast-scan cyclic voltammetry

Evoked  $[DA]_o$  was recorded every 2.5 min using FSCV and the stimulation protocols outlined in Section 2.2. DH $\beta$ E was not used in this experimental chapter. Each FSCV experiment was performed at a single recording site in one brain slice. Data are expressed as extracellular concentration of dopamine ( $[DA]_o$ ) or normalised to mean peak  $[DA]_o$  evoked by single pulses in control conditions. In all cases,  $[DA]_o$  displayed typical kinetics to peak and decay, indicative of good slice quality and were obtained from recording sites that maintained sufficiently stable levels of release over time. No FSCV data were excluded after acquisition.

Drug washes were performed once stable release at the given recording site was stable: less than 10% variation over 5 consecutive stimulations. Data acquired were compared to pre-drug controls after drug effects had equilibrated after ~10-20 min of application (peak  $[DA]_o$  averaged over 4 stimulations in each condition). Drug wash on data are normalised to pre-drug conditions for clarity and for comparisons between regions and/or genotype.

### 5.2.3 Whole-cell patch-clamp electrophysiology

For all whole-cell electrophysiology recordings, SPNs were voltage-clamped at -70 mV and light-evoked GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic currents (IPSCs) resulting from GABA co-release from ChR2-expressing DA axons in slices from *Slc6a3<sup>IRE5-Cre</sup>*

Parkinsonian mice (*SNCA*+) and littermate controls (*Snca*-/-) were recorded as outlined in Section 2.3.2. All recordings were in the presence of NBQX (5  $\mu$ M) and APV (50  $\mu$ M) to block AMPA and NMDA receptor-mediated inward currents.

For electrophysiology experiments, peak amplitude, onset latency, peak latency, 10-90% rise time and decay time of light-evoked GABA currents in SPNs were measured from an average of 3 replicate traces per cell recorded before and after drug wash-on conditions. Access resistance was monitored, and cells were discarded from analysis if series resistance varied by more than 15% or increased over 25 M $\Omega$ .

#### 5.2.4 Drugs

Drugs were dissolved in distilled water or dimethyl sulfoxide to make stock aliquots at 1,000 x final concentration and stored at -20°C until required. Stock aliquots were diluted with oxygenated aCSF to final concentrations immediately before use. The drugs used in this study, their final concentrations, and references to studies which clarified the choice of final concentration used are outlined in Table 5.1. Final concentrations used were determined from previous pharmacological studies using acute rodent brain slices (where possible in striatum), which often require concentrations significantly greater than the half maximal effective concentrations (EC<sub>50</sub>) for sufficient penetration of slices.

#### 5.2.5 High-performance liquid chromatography

DA content from dorsal striatum and nucleus accumbens tissue punches taken from acute coronal sections prepared for FSCV were measured by HPLC with electrochemical detection as outlined in Section 2.4.1.

#### 5.2.6 SDS-PAGE and immunoblotting

GAT-1 and GAT-3 protein content from dorsal striatum tissue punches were measured by SDS-PAGE and immunoblotting as outlined in Section 2.4.2. Protein concentrations for GAT-1 and GAT-3 were normalised to  $\beta$ -actin.

### 5.2.7 Pregabalin dosing

The gabapentinoid pregabalin (marketed under the brand name Lyrica®) selectively binds to the auxiliary  $\alpha_2\delta$  -1 and -2 subunits of voltage-gated calcium channels (VGCC) and prevents their trafficking to the presynaptic membrane. Pregabalin (Sigma) was dissolved in sterile saline and subcutaneously injected twice daily for three consecutive days at 30 mg/kg into SNCA-OVX mice (11-13 months), equivalent of ~250 mg/day in patients. Acute *ex vivo* coronal slices containing striatum for FSCV experiments were prepared from these animals 1 hour following the final (sixth) injection as described in Section 2.2.3. This dosing scheme is consistent with published protocols used in rodents to mimic well-tolerated sub-sedative doses used in patients (Bauer et al., 2009; Field et al., 2006). PGB was chosen over GBP due to its improved bioavailability.

### 5.2.8 Data analysis

All data are represented as means  $\pm$  standard error of the mean, and 'n' refers to the number of experiments. Each experiment was performed at a single recording site in one discrete brain slice. The number of animals in each dataset was  $\geq 3$ . Comparisons for statistical significance were assessed by one- or two-way ANOVA with Sidak's multiple comparisons; or Kruskal-Wallis ANOVA with Dunn's multiple comparisons and Mann-Whitney U tests where data were not normally distributed (assessed using D'Agostino-Pearson normality test), using Prism 7 (GraphPad). Threshold for statistical significance in all analyses was  $p < 0.05$ , adjusted for multiple comparisons.

Table 5.1. Drugs listed with method of action and references to studies which clarified the choice of concentrations used in Chapter 5.

| Drug                     | Source                   | Action                                | Final concentration | Reference(s)                              |
|--------------------------|--------------------------|---------------------------------------|---------------------|-------------------------------------------|
| Bicuculline              | Tocris Bioscience        | GABA <sub>A</sub> receptor antagonist | 10 $\mu$ M          | Cepeda et al., 2001; Brodnik et al., 2018 |
| CGP 55845                | Abcam                    | GABAB receptor antagonist             | 4 $\mu$ M           | Tritsch et al., 2014                      |
| D-APV                    | Santa Cruz Biotechnology | NMDA receptor antagonist              | 50 $\mu$ M          | Patton, Roberts et al., 2016              |
| NBQX                     | Abcam                    | AMPA receptor antagonist              | 5 $\mu$ M           | Patton, Roberts et al., 2016              |
| ( $\pm$ )-Nipecotic acid | Sigma-Aldrich            | Broad-spectrum GAT inhibitor          | 1.5 mM              | Goubard et al., 2011; Li et al., 2017     |
| Picrotoxin               | Sigma-Aldrich            | GABA <sub>A</sub> receptor antagonist | 100 $\mu$ M         | Avshalumov et al., 2000                   |

## 5.3 Results

### 5.3.1 Attenuated co-release of dopamine and GABA from dopamine axons in dorsal striatum of mouse model of early parkinsonism

We confirmed that, as observed in the original *SNCA-OVX* mice (Janezic et al., 2013), the resulting *SNCA*<sup>+</sup> mice at 4 months exhibited a ~30% deficit in electrically evoked [DA]<sub>o</sub> when compared to littermate controls (*Snca*<sup>-/-</sup>) in the dorsal striatum (Figure 5.1a-b;  $t = 3.3$ ,  $p = 0.002$ , unpaired  $t$  test;  $n = 24$  observations/5 mice for each genotype) but not in the NAc (Figure 5.1a-b;  $t = 1.4$ ,  $p = 0.17$ , unpaired  $t$ -test;  $n = 21$  observations/5 mice for each genotype). The DA release deficit was also not attributable to any reduction in striatal DA content in *SNCA*<sup>+</sup> mice compared to *Snca*<sup>-/-</sup> mice (Figure 5.1c;  $t = 0.16$ ,  $p = 0.87$ , unpaired  $t$ -test, dorsal striatum;  $t = 0.74$ ,  $p = 0.47$ , unpaired  $t$ -test, NAc;  $n = 8$  experiments/5 mice for each genotype). We then established that [DA]<sub>o</sub> evoked optogenetically in DLS by single light pulses also showed a similar deficit in *SNCA*<sup>+</sup> compared to *Snca*<sup>-/-</sup> mice (Figure 5.2a-b;  $t = 2.44$ ,  $df = 29$ ,  $p = 0.02$ , unpaired- $t$  test; *SNCA*<sup>+</sup>:  $n = 16$  observations/3 mice; *Snca*<sup>-/-</sup>:  $n = 15$  observations/3 mice), and observed across single light pulse stimulations every

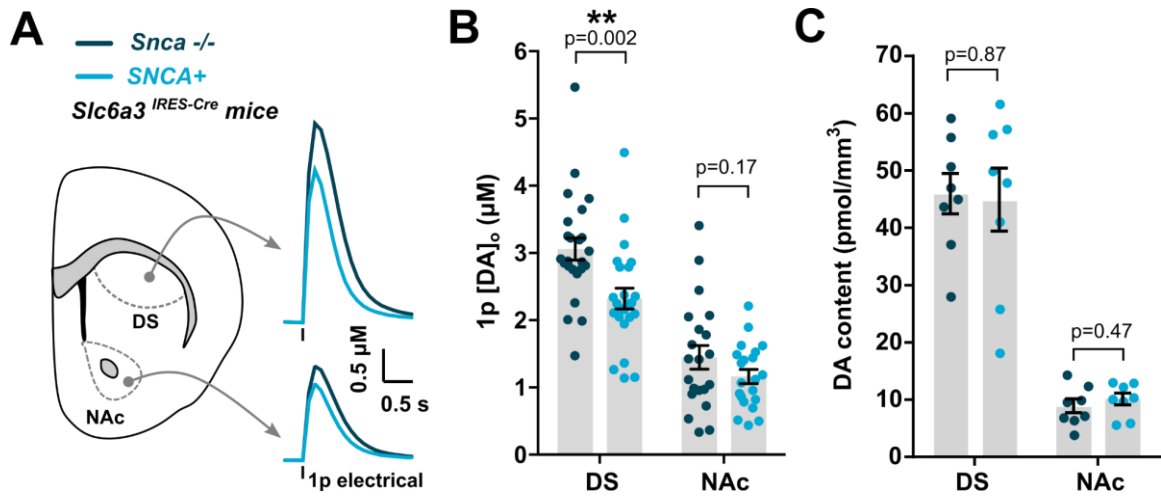


Figure 5.1. Attenuated electrical-evoked dopamine release in dorsal striatum of *Slc6a3*<sup>IRES-Cre</sup> mice overexpressing human alpha synuclein.

(A) Mean [DA]<sub>o</sub> profiles vs. time following 1-pulse electrical stimulation in dorsal striatum (DS) and nucleus accumbens (NAc) of *SNCA*<sup>+</sup> mice (light blue) and *Snca*<sup>-/-</sup> littermate controls (dark blue) at 3-4 months, backcrossed onto *Slc6a3*<sup>IRES-Cre</sup> background.

(B) Mean ( $\pm$  SEM) 1p-evoked [DA]<sub>o</sub> (in  $\mu$ M) from (A) (n = 24 observations/5 mice per genotype in DS, n = 21 observations/5 mice per genotype in NAc). Unpaired t-tests.

(C) Mean ( $\pm$  SEM) DA content in DS and NAc of *SNCA*<sup>+</sup> mice (light blue) and *Snca*<sup>-/-</sup> littermate controls (dark blue) (n = 8 experiments/5 mice per genotype in DS and NAc). Unpaired t-tests.

Statistical significance: \*\*p<0.01.

30 seconds (Figure 5.2c;  $F_{\text{Genotype}} = 6.38$ ,  $p = 0.025$ , Two-Way RM ANOVA; n = 7 observations/3 mice per genotype).

This new optogenetic-capable model of parkinsonism allowed us to address for the first time whether DA release deficits are accompanied by corresponding deficits in GABA co-release from DA axons. To provide a readout of GABA co-release, we used voltage-clamp recordings of currents evoked in SPNs by optical stimulation of DA axons (Figure 5.2d). We observed a significantly lower amplitude of light-evoked GABAergic co-release currents from DA axons detected in SPNs in DLS of *SNCA*<sup>+</sup> mice as compared to SPNs in *Snca*<sup>-/-</sup> mice (Figure 5.2d;  $t = 2.68$ ,  $p = 0.018$ , unpaired t-test; *SNCA*<sup>+</sup>: n = 9 cells/4 mice, *Snca*<sup>-/-</sup>: n = 7 cells/4 mice), observed across single light pulse stimulations every 30 seconds (Figure

**5.2e;**  $F_{\text{Genotype}(1,14)} = 7.28$ ,  $p = 0.017$ , Two-Way RM ANOVA; *SNCA*<sup>+</sup>:  $n = 9$  cells/4 mice, *Snca*<sup>-/-</sup>:  $n = 7$  cells/4 mice). These evoked currents were GABA<sub>A</sub> receptor-mediated as they were eliminated by picrotoxin (PTX, 100  $\mu\text{M}$ ) (Figure 5.2f;  $t = 4.55$ ,  $p = 0.001$ , paired t-test;  $n = 16$  cells/8 mice, genotypes combined). Importantly, this observed difference in GABAergic IPSC amplitudes was not due to differences in series resistance (Figure 5.2g;  $t = 0.23$ ,  $p = 0.82$ , unpaired t test; *SNCA*<sup>+</sup>:  $n = 9$  cells/4 mice, *Snca*<sup>-/-</sup>:  $n = 7$  cells/4 mice).

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**Figure 5.2. Attenuated light-evoked co-release of dopamine and GABA from dopamine axons in dorsal striatum of mouse model of early parkinsonism.** (Figure on next page, 134).

(A) Schematic representing the experimental configuration for light evoked DA and GABA co-release from DA axons in dorsolateral striatum (DLS) of *SNCA*<sup>+</sup> Parkinsonian mice and *Snca*<sup>-/-</sup> littermate controls at 4-6 months.

(B) *Left*, Mean ( $\pm$  SEM) peak  $[\text{DA}]_o$  (in  $\mu\text{M}$ ) evoked by 1-pulse light stimulation in DLS of *SNCA*<sup>+</sup> Parkinsonian mice (*light blue*) and *Snca*<sup>-/-</sup> littermate controls (*dark blue*). *Right*, Mean  $[\text{DA}]_o$  profiles vs. time. ( $n = 15$  observations/3 mice in *Snca*<sup>-/-</sup> mice;  $n = 16$  observations/3 mice in *SNCA*<sup>+</sup> mice). Unpaired t-test.

(C) Mean ( $\pm$ SEM) peak  $[\text{DA}]_o$  in  $\mu\text{M}$  evoked by 1-pulse light stimulation every 30 seconds in DLS of *SNCA*<sup>+</sup> Parkinsonian mice (*light blue*) and *Snca*<sup>-/-</sup> littermate controls (*dark blue*). ( $n = 7$  observations/3 mice for each genotype. Two-way RM ANOVA and Sidak's multiple comparisons.

(D) Mean amplitude ( $\pm$  SEM) of 1-pulse light-evoked inhibitory post synaptic currents (IPSCs) recorded from spiny projection neurons (SPNs) in the DLS of *SNCA*<sup>+</sup> Parkinsonian mice (*light blue*,  $n = 9$  cells/4 mice) and *Snca*<sup>-/-</sup> littermate controls (*dark blue*,  $n = 7$  cells/4 mice), voltage clamped at -70 mV and in the presence of ionotropic glutamate receptors (NBQX, 5  $\mu\text{M}$ ; D-APV, 50  $\mu\text{M}$ ). Unpaired t-test.

(E) Mean amplitude ( $\pm$  SEM) of 1-pulse light-evoked IPSCs recorded every 30s from (E). Two-way RM ANOVA and Sidak's multiple comparisons.

(F-G) IPSCs from (E) were abolished in the presence of GABA<sub>A</sub> receptor antagonist picrotoxin (PTX, 100  $\mu\text{M}$ , G) and differences in recorded amplitudes were not due to differences in series resistance ( $R_s$ ) between recordings (H). Paired (G) and unpaired (H) t-tests.

Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ .

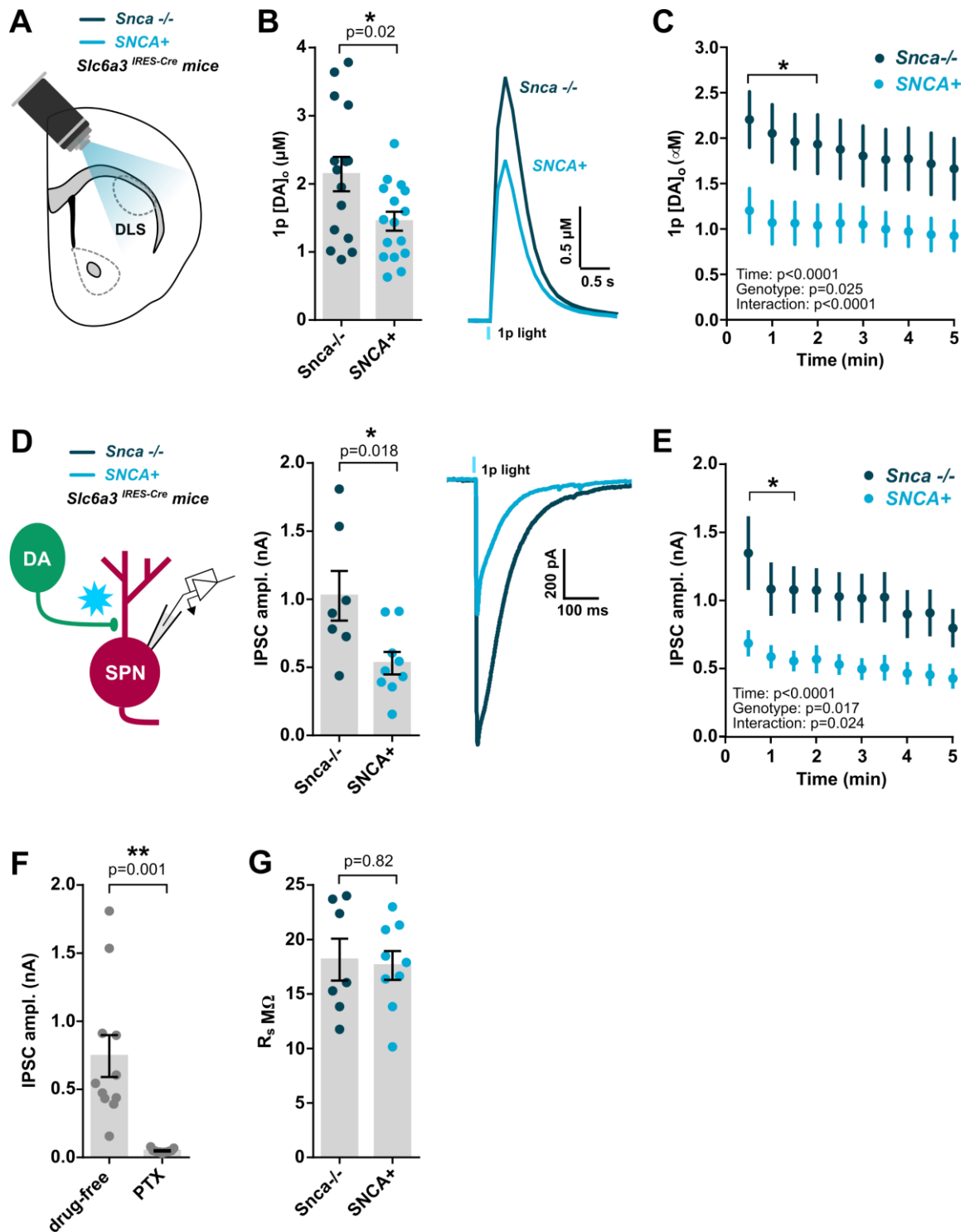


Figure 5.2. Attenuated light-evoked co-release of dopamine and GABA from dopamine axons in dorsal striatum of mouse model of early parkinsonism (see legend on previous page, 133).

### 5.3.2 Tonic inhibition of dopamine release in the DLS is augmented in mouse model of early parkinsonism

Next, we explored whether tonic GABA inhibition of DA release was modified by the genetic burden of  $\alpha$ -synuclein in *SNCA*<sup>+</sup> and *Snca*<sup>-/-</sup> littermate control mice during the prodromal stage (4-6 months of age). We found that GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonism, with bicuculline (10  $\mu$ M) and CGP 55845 (4  $\mu$ M) respectively, enhanced [DA]<sub>o</sub> evoked by single light pulses to a significantly greater degree in *SNCA*<sup>+</sup> mice than in *Snca*<sup>-/-</sup> controls in DLS (Figure 5.3a-b;  $F_{\text{Genotype}(1,13)} = 12.4$ ,  $p = 0.004$ , Two-Way RM ANOVA; *SNCA*<sup>+</sup>: ~170% of pre-drug baseline,  $n = 8$  experiments/5 mice; *Snca*<sup>-/-</sup>: ~120% of pre-drug baseline,  $n = 7$  experiments/5 mice;  $p = 0.0003$ , Mann-Whitney test). Conversely, GABA receptor antagonism similarly enhanced [DA]<sub>o</sub> evoked by single light pulses in *SNCA*<sup>+</sup> and *Snca*<sup>-/-</sup> control mice in NAcC (Figure 5.3c-d;  $F_{\text{Genotype}(1,15)} = 2.32$ ,  $p = 0.14$ , Two-Way RM ANOVA; *SNCA*<sup>+</sup>: ~130% of pre-drug baseline,  $n = 8$  experiments/5 mice; *Snca*<sup>-/-</sup>: ~125% of pre-drug baseline,  $n = 9$  experiments/5 mice;  $p = 0.09$ , Mann-Whitney test). Overall, disinhibition of evoked [DA]<sub>o</sub> by GABA receptor antagonism in *SNCA*<sup>+</sup> mice was significantly greater in DLS versus NAcC (Figure 5.3e-f;  $p = 0.02$ , Mann-Whitney test). These data indicate that the GABA tone onto DA axons is dysregulated in *SNCA*<sup>+</sup> Parkinsonian mice and in particular, that tonic GABAergic inhibition of DA release is augmented in DLS.

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**Figure 5.3. Enhanced tonic inhibition of dopamine release in DLS of mouse model of early parkinsonism.** (Figure on next page, 136).

(A,C) Mean ( $\pm$  SEM) peak [DA]<sub>o</sub> during consecutive recordings evoked by 1p light in DLS (A) or NAcC (C) during applications of antagonists for GABA<sub>A</sub> (bicuculline, 10  $\mu$ M) and GABA<sub>B</sub> receptors (CGP 55845, 4  $\mu$ M) in *SNCA*<sup>+</sup> Parkinsonian mice (*light blue*,  $n = 8$  experiments/5 mice in DLS,  $n = 8$  experiments/5 mice in NAcC) or *Snca*<sup>-/-</sup> littermate control mice (*dark blue*,  $n = 7$  experiments/5 mice in DLS,  $n = 9$  experiments/5 mice in NAcC) at 4-5 months. Data are normalised to first 4 time points (*dotted line*); last 4 time points (*grey shaded region*) are used for statistical comparisons (in B,D). Two-way RM ANOVA and Sidak's multiple comparisons.

(B,D) Mean transients showing release and uptake of [DA]<sub>o</sub> in drug condition (*dashed traces*) vs. pre-drug condition (*solid traces*), averaged across last 4 time points in A,C (*grey shaded regions*) and normalised to pre-drug baselines. Mann-Whitney tests.

(E-F) Cumulative frequency plots of individual data points (E) and mean ( $\pm$  SEM) per recording site (F) from A,C for *SNCA*<sup>+</sup> mice in DLS and NAcC recordings following GABA receptor antagonism. Komogorov-Smirnov test (E) and Mann-Whitney test (F).

Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

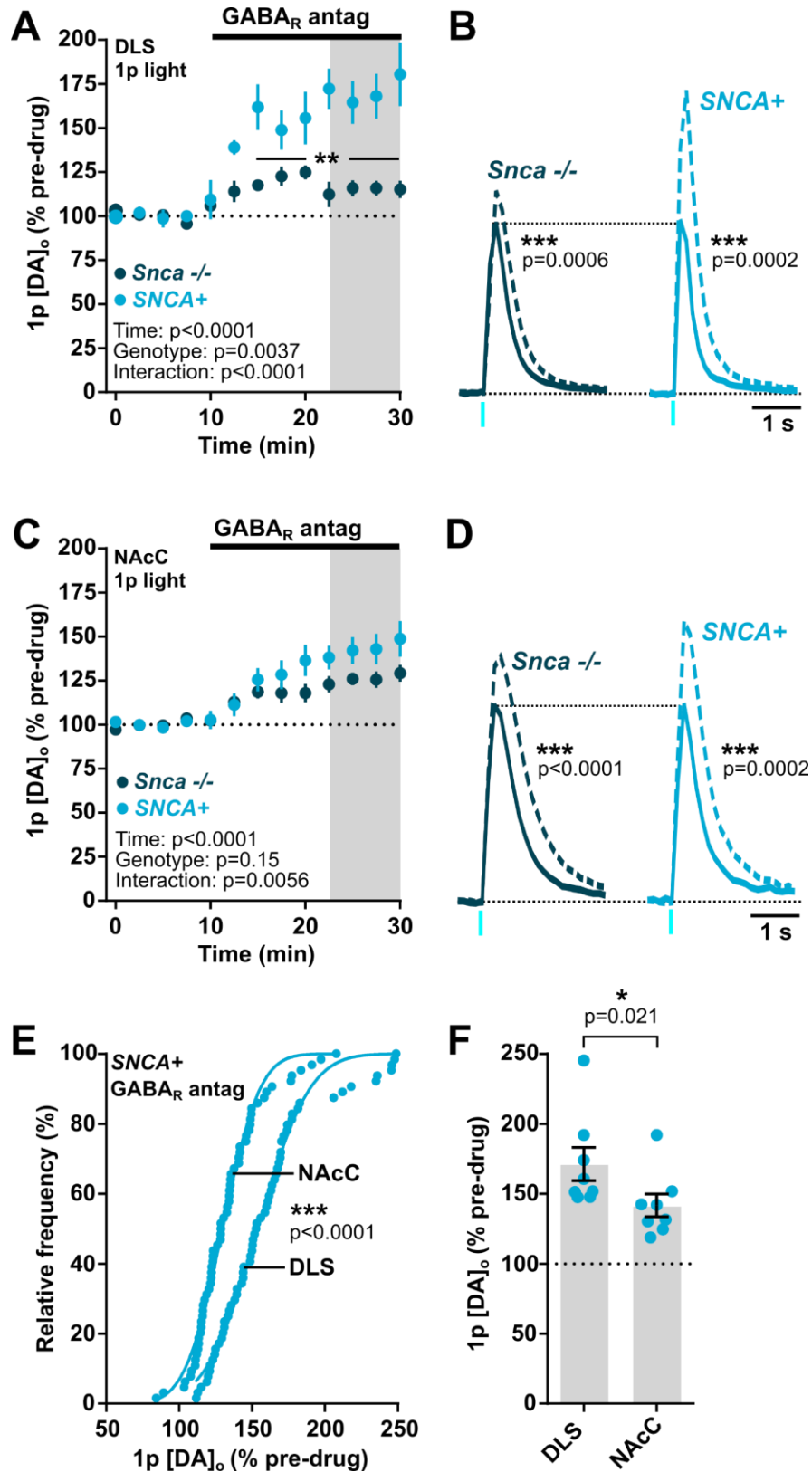


Figure 5.3. Enhanced tonic inhibition of dopamine release in DLS of mouse model of early parkinsonism (see legend on previous page, 135).

### 5.3.3 Attenuated striatal GAT function and expression in mouse model of early parkinsonism

We then tested the hypothesis that elevated tonic inhibition of DA release in the DLS of *SNCA*<sup>+</sup> mice might be due to impaired GAT function. We tested the effect of the non-selective GAT inhibitor NPA (1.5 mM) on [DA]<sub>o</sub> evoked by single electrical pulses in DLS and found a moderately attenuated effect of NPA in *SNCA*<sup>+</sup> versus *Snca*<sup>-/-</sup> controls (Figure 5.4a-d;  $F_{\text{Genotype}(1,8)} = 5.79$ ,  $p = 0.043$ , Two-Way RM ANOVA; *SNCA*<sup>+</sup>: ~60% of pre-drug baseline,  $n = 5$  experiments/4 mice; *Snca*<sup>-/-</sup>: ~75% of pre-drug baseline,  $n = 5$  experiments/4 mice;  $p = 0.03$ , Mann-Whitney test). Quantification of Western blots of dorsal striatal tissue showed ~40% decrease in the levels of both GAT-1 and GAT-3 proteins in *SNCA*<sup>+</sup> mice compared to *Snca*<sup>-/-</sup> controls (Figure 5.4e-f; GAT-1:  $p = 0.0004$ ; GAT-3:  $p = 0.01$ ; Mann-Whitney tests;  $n = 7$  *SNCA*<sup>+</sup> mice,  $n = 10$  *Snca*<sup>-/-</sup> mice). Taken together, these data suggest that tonic inhibition of DA release by ambient GABA is augmented in the dorsal striatum in early parkinsonism due to decreased GAT-1 and GAT-3 expression.

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**Figure 5.4. Attenuated striatal GAT function and expression in mouse model of early parkinsonism.** (Figure on next page, 138).

(A) Mean ( $\pm$  SEM) peak [DA]<sub>o</sub> during consecutive recordings evoked by 1p electrical stimulation in DLS during application of the nonspecific GAT inhibitor NPA (1.5 mM) in *SNCA*<sup>+</sup> Parkinsonian mice (*light blue*) or *Snca*<sup>-/-</sup> littermate control mice (*dark blue*) at 4-5 months. ( $n = 5$  experiments/4 mice for each genotype). Data are normalised to first 4 time points (*dotted line*); last 4 time points (*grey shaded region*) are used for statistical comparisons in B. Two-way RM ANOVA and Sidak's multiple comparisons (*asterisk in bar*).

(B) Mean transients showing release and uptake of [DA]<sub>o</sub> in drug condition (*dashed traces*) vs. pre-drug condition (*solid traces*), averaged across last 4 time points in A (*grey shaded regions*) and normalised to pre-drug baselines. Mann-Whitney tests.

(C-D) Cumulative frequency plots of individual data points (C) and mean ( $\pm$  SEM) per recording site (D) from A for *SNCA*<sup>+</sup> and *Snca*<sup>-/-</sup> mice in DLS following GAT inhibition with NPA. Komogorov-Smirnov test (C) and Mann-Whitney test (D).

(E-F) Representative Western blots and mean ( $\pm$  SEM) of GAT-1 and GAT-3 protein content of dorsal striatum tissue taken from *SNCA*<sup>+</sup> Parkinsonian mice (*light blue*,  $n = 7$  mice) or *Snca*<sup>-/-</sup> littermate control mice (*dark blue*,  $n = 10$  mice). Data are normalised to actin and littermate control expression. Mann-Whitney tests.

Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ .

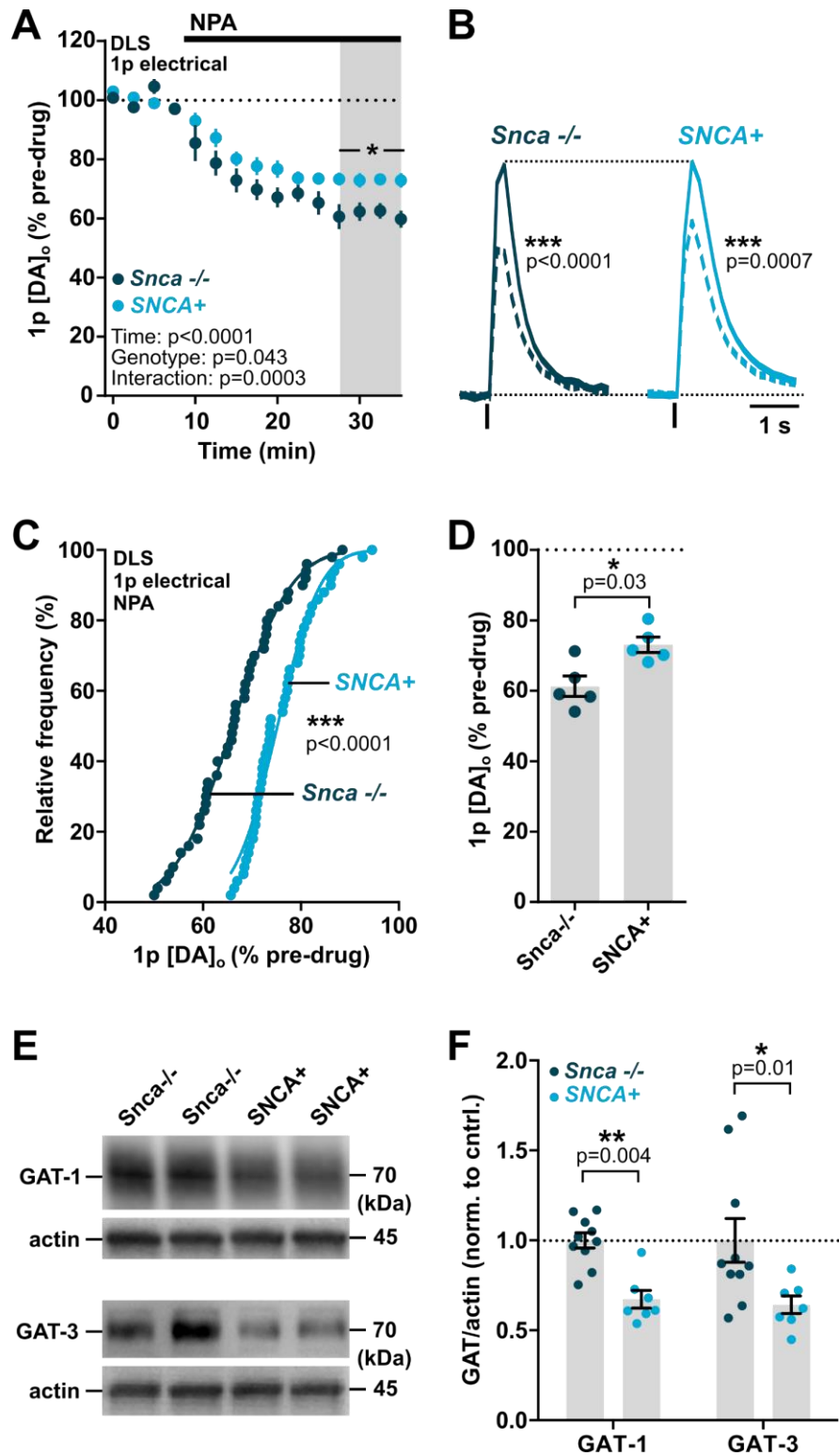


Figure 5.4. Attenuated striatal GAT function and expression in mouse model of early parkinsonism. (see legend on previous page, 137).

### 5.3.4 Pregabalin treatment boosts dopamine release selectively in the dorsal striatum in Parkinsonian mice

Given that, gabapentinoids show clinical efficacy in disorders associated with DA dysfunction, including restless-leg syndrome which is associated with hypodopaminergic signalling (Guo et al., 2017), and addictions (Fujishiro, 2014; Mason et al., 2018) we examined the effects of gabapentinoids on DA release in SNCA-OVX Parkinsonian mice. SNCA-OVX mice (11-13 months of age) were dosed twice daily for 3 consecutive days (6 doses total) with 30 mg/kg PGB dissolved in saline, the equivalent of ~250 mg/day in patients, or with saline as a control. PGB was chosen over GBP due to its improved bioavailability. We found that PGB boosted  $[DA]_o$  evoked by single electrical pulses by ~30% compared to saline controls across 2 out of the 3 sites we recorded in DLS (Figure 5.5a; DLS site 1:  $t = 2.62$ ,  $p = 0.013$ ; DLS site 2:  $t = 2.67$ ,  $p = 0.012$ ; DLS site 3:  $t = 0.09$ ,  $p = 0.93$ ; DLS combined:  $t = 2.64$ ,  $p = 0.0097$ ,  $n = 54$  recordings/8 mice in each PGB and saline condition). Conversely, PGB did not change  $[DA]_o$  evoked by single electrical pulses compared to saline controls in NAcC (Figure 5.5a;  $t = 0.06$ ,  $p = 0.96$ ,  $n = 10$  recordings/5 mice in each PGB and saline condition). Furthermore, unlike 6 doses of PGB across 3 days, a single dose of PGB did not enhance electrically-evoked  $[DA]_o$  in DLS compared to saline controls (Figure 5.5b;  $F_{(2,49)} = 6.57$ ,  $p = 0.003$ , one-way ANOVA; PGBx1 vs. Saline:  $p > 0.05$ , PGBx1 vs. PGBx6:  $p < 0.01$ , PGBx6 vs. Saline:  $p < 0.05$ , Sidak's multiple comparisons;  $n = 18$  recordings/4 mice in each condition), indicating that a multiple dose course of PGB is required to selectively boost DA signalling in the dorsal striatum of Parkinsonian mice.

### 5.3.5 Pregabalin treatment normalises aberrant tonic inhibition of dopamine release in Parkinsonian mice

PGB did not boost DA release in the dorsal striatum through altering the VGCC-dependence of DA release as the dependence of DA release in the dorsal striatum on N-, Q-, T- and L-type VGCCs was unaffected by this 3-day PGB dosing paradigm (assessed using selective blockers for each the N-, Q-, T- and L-type VGCC; data not shown). We

postulated whether PGB might normalise the enhanced tonic inhibition of DA release in dorsal striatum of these  $\alpha$ -synuclein overexpressing Parkinsonian mice. Previous work has found that prolonged incubation (>24 hours) of GBP or PGB caused a 2-fold increase in GABA uptake in hippocampal cultures, which was due to a redistribution of GAT-1 protein from intracellular locations to the plasma membrane (Whitworth and Quick, 2001). Furthermore, gabapentinoids have largely been shown to inhibit the release of many neurotransmitter types across many brain nuclei, including GABA (Bian et al., 2006; Uchitel et al., 2010), albeit these effects are generally observed even after acute exposures to GBP or PGB. We therefore hypothesised that PGB selectively boosts DA release in the dorsal striatum of *SNCA-OVX* mice through normalising the enhanced tonic inhibition by ambient GABA observed exclusively in the dorsal striatum of these Parkinsonian mice. We hypothesised that this might be mediated by selective boosting of striatal GAT levels and/or reduced GABA release.

To test this hypothesis, we explored whether tonic GABA inhibition of DA release was modified by PGB treatment (6 doses over three consecutive days) in *SNCA-OVX* mice (11-13 months of age). We found that GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonism, with bicuculline (10  $\mu$ M) and CGP 55845 (4  $\mu$ M) respectively, enhanced  $[DA]_o$  evoked by single electrical pulses recorded in DLS to a significantly lesser degree in PGB treated mice than saline treated mice (Figure 5.6a-d;  $F_{Drug\ Treatment(1,10)} = 7.53$ ,  $p = 0.021$ , Two-Way RM ANOVA; PGB: ~120% of pre-drug baseline,  $n = 6$  experiments/4 mice; Saline: ~140% of pre-drug baseline,  $n = 6$  experiments/4 mice;  $p = 0.015$ , Mann-Whitney test). Collectively, these data indicate that PGB can selectively boost DA signalling in the dorsal striatum of Parkinsonian mice, through a mechanism which reduces tonic inhibition of DA release.

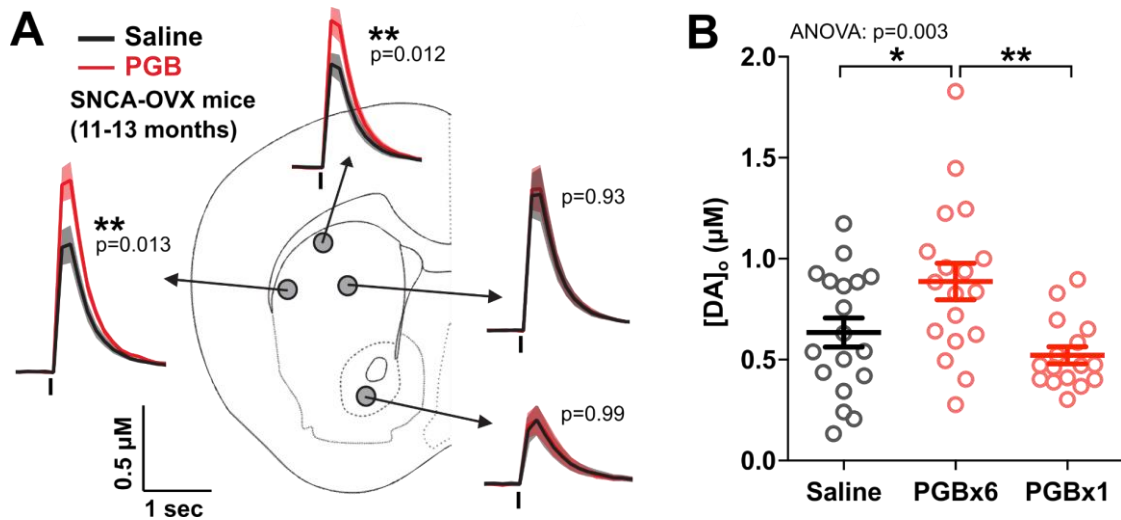


**Figure 5.5.** Pregabalin treatment boosts dopamine release selectively in the dorsolateral striatum in Parkinsonian mice.

(A) Mean ( $\pm$  SEM) transients showing release and uptake of [DA]<sub>o</sub> evoked by 1-pulse electrical stimulation in three dorsolateral sites of striatum (DLS,  $n = 8$  animal pairs) and in the nucleus accumbens core (NAcC,  $n = 5$  animal pairs) of SNCA-OVX Parkinsonian mice (11-13 months) dosed with 30 mg/kg pregabalin (PGB) twice daily for 3 days (red) or saline treated controls (black) ( $n = 48$  recording sites per condition/8 animal pairs). Unpaired t-tests.

(B) Mean ( $\pm$  SEM) peak [DA]<sub>o</sub> (in  $\mu$ M) in DLS from SNCA-OVX Parkinsonian mice treated with 1 single dose of PGB (red), 6 doses of PGB across 3 days (red) or saline treated controls (black). One-way ANOVA and Sidak's multiple comparisons ( $n = 18$  sites/3 animals for each condition).

Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ . Data collected by K. Brimblecombe.

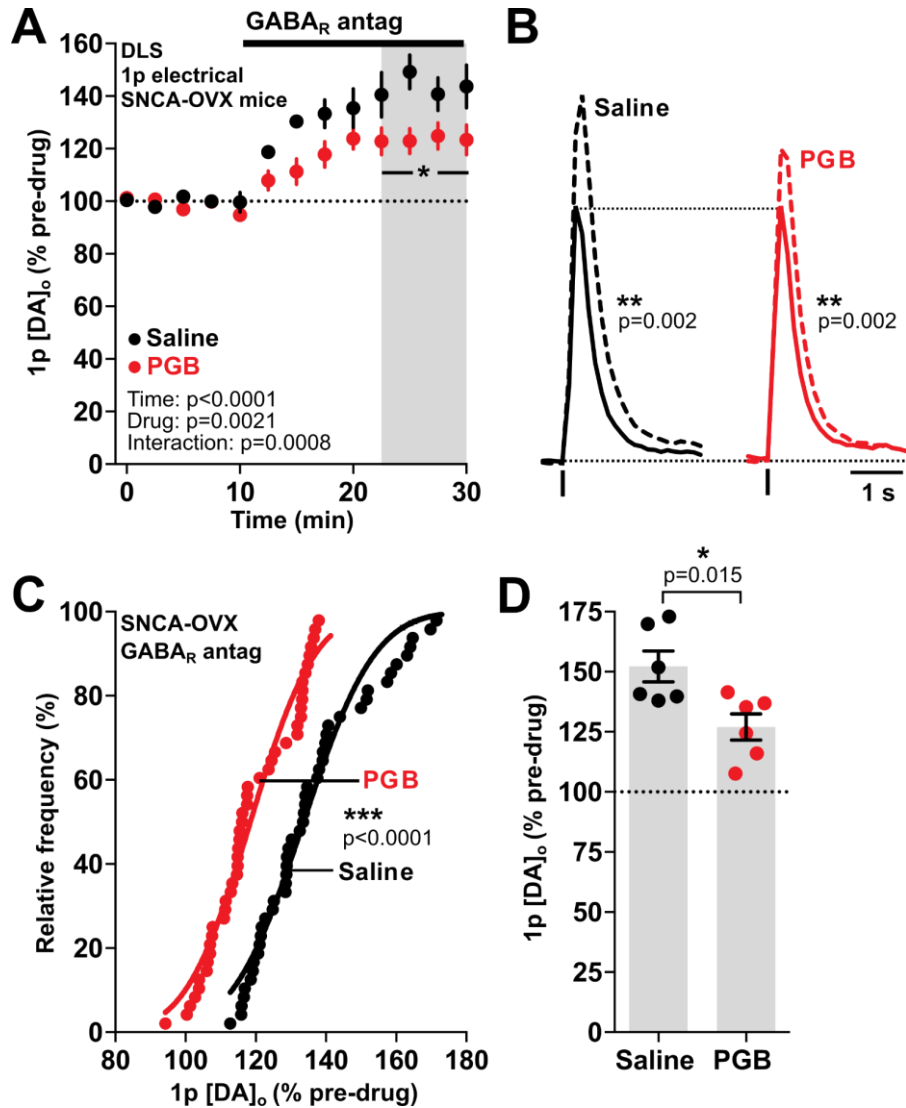


Figure 5.6. Pregabalin treatment normalises aberrant tonic inhibition of dopamine release in Parkinsonian mice.

(A) Mean ( $\pm$  SEM) peak [DA]<sub>o</sub> during consecutive recordings evoked by 1p electrical stimulation in DLS during applications of antagonists for GABA<sub>A</sub> (bicuculline, 10  $\mu$ M) and GABA<sub>B</sub> receptors (CGP 55845, 4  $\mu$ M) in SNCA-OVX Parkinsonian mice (11–13 months) dosed with 30 mg/kg PGB twice daily for 3 days (red) or saline treated controls (black). Data are normalised to first 4 time points (dotted line); last 4 time points (grey shaded region) are used for statistical comparisons (in D). Two-way RM ANOVA and Sidak's multiple comparisons (asterisk in bar) ( $n = 6$  experiments/4 animals each condition).

(B) Mean transients showing release and uptake of [DA]<sub>o</sub> in GABA<sub>R</sub> antagonist condition (dashed traces) vs. pre-drug condition (solid traces), averaged across last 4 time points in D (grey shaded regions) and normalised to pre-drug baselines. Mann-Whitney tests.

(C–D) Cumulative frequency plots of individual data points (C) and mean ( $\pm$  SEM) per recording site (D) from A following GABA receptor antagonism. Komogorov-Smirnov test (C) and Mann-Whitney test (D).

Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

## 5.4 Discussion

### 5.4.1 Striatal GAT dysfunction in a mouse model of Parkinson's disease

To probe further the significance of the regulation of striatal DA by striatal GATs, we explored GAT function in a mouse model of early parkinsonism. A recent study in GPe of DA-depleted rats found elevated extracellular GABA resulting from downregulation of GAT-3 on astrocytes, mediated through a loss of DA signalling at D<sub>2</sub> DA receptors (Chazalon et al., 2018). In addition, striatal GAT-3 levels have been shown conversely to be upregulated in mouse models of Huntington's disease (Wójtowicz et al., 2013; Yu et al., 2018). We explored potential adaptations to tonic GABA inhibition of DA release and GAT function in the striatum of the human  $\alpha$ -synuclein overexpressing mouse model of PD. This model is a physiological, slowly progressing mouse model of parkinsonism, that, in capturing a human disease-relevant genetic burden of  $\alpha$ -synuclein overexpression, shows early deficits in DA release restricted to dorsal striatum, prior to late-stage degeneration of DA neurons, disturbed encoding of behaviour of surviving DA neurons, and manifestation of a motor phenotype (Dodson et al., 2016; Janezic et al., 2013). We found an augmentation of tonic GABA inhibition of DA release in the DLS (and not NAcC) in early adulthood, which was likely due to downregulated GAT-1 and GAT-3 expression. Whether these adaptations in GAT are consequential to reduced DA signalling, as seen in the GP after substantial neurotoxin-induced depletion (6-OHDA) of DA (Chazalon et al., 2018), or directly underlie the DA release deficit due to a potential interaction between  $\alpha$ -synuclein biology and striatal GAT and/or astrocytes is not yet known. Regardless, this resulting enhanced tonic inhibition will diminish nigrostriatal DA release, compounding any release deficits underpinned by  $\alpha$ -synuclein actions e.g. tighter vesicle clustering at DA release sites (Janezic et al., 2013). As such, these changes in GATs and tonic GABA inhibition in early parkinsonism can be considered 'maladaptive'. Future work will need to ascertain whether this  $\alpha$ -synuclein overexpressing model accurately translates to human pathology and determine if striatal GATs are downregulated in human post-mortem PD brains. Furthermore, which striatal cell

types downregulate GATs e.g. striatal astrocytes alone or in parallel with other cell types, will need to be determined.

### 5.4.2 Attenuated GABA and dopamine co-release in early parkinsonism

Our optogenetic-capable model of parkinsonism allowed us to address for the first time whether DA release deficits were accompanied by corresponding deficits in GABA co-release from DA axons. We observed a robust attenuation in the amplitude of light-evoked GABA<sub>A</sub>-dependant IPSCs in SPNs in the dorsal striatum of  $\alpha$ -synuclein overexpressing mice in early adulthood, which corresponded with the light-evoked DA release deficit also observed in these animals. Our findings therefore reveal that malfunction in nigrostriatal DA in early parkinsonism is accompanied by malfunction in nigrostriatal GABA co-release. GABA and DA are thought to be packaged within the same vesicle by VMAT2 (Tritsch et al., 2012), and therefore a deficit in the release of both transmitters is not surprising. These findings therefore suggest that loss of striatal GABA co-release from DA axons in early parkinsonism may contribute to the symptoms of Parkinson's disease as well as to the alterations of striatal circuitry that accompany loss of nigrostriatal DA input, and should be factored in to future attempts to offset dysfunction in PD.

GABA synthesis in DA neurons depends on the noncanonical GABA synthesis enzyme, ALDH1a1 (Kim et al., 2015). Recent reports of reduced levels of expression of both ALDH1a1 mRNA and protein in the substantia nigra, but not in ventral tegmental area, of PD post-mortem brains suggest possible involvement of ALDH1a1 in PD disease progression (Galter et al., 2003). Additionally, recent studies suggest that ALDH1a1 plays a protective role in DA neuron degeneration and dysfunction by preventing the accumulation of DA metabolites, and formation of cytotoxic  $\alpha$ -synuclein oligomers, as well as supporting DA transporter and release functions (Anderson et al., 2011; Liu et al., 2014). Within striatum, we found that in neurologically-intact mice, ALDH inhibition slightly boosted tonic inhibition of DA release, suggesting that GABA co-release acts to limit the tonic inhibition

by the GAD-dependent GABA network (see **Section 3.3.2**). Correspondingly, in  $\alpha$ -synuclein-overexpressing mice, in which we found that GABA co-release from DA axons is attenuated, we also found that the level of tonic GABA inhibition on DA release was boosted. Therefore, attenuated GABA co-release might also contribute to aberrant tonic inhibition of DA release in early parkinsonism. These data therefore provide an additional supportive role ALDH1a1 might play in supporting nigrostriatal DA function.

### 5.4.3 Potential striatal astrocyte dysfunction in Parkinson's disease

The data outlined in **Chapter 4** provide compelling evidence that GATs on astrocytes regulate DA output in the DLS of neurologically-intact mice, through limiting GABA tone onto DA axons. We exposed in **Chapter 5** that in an  $\alpha$ -synuclein-overexpressing mouse model of early parkinsonism, there is enhanced GABA tone onto DA axons which corresponds with downregulated striatal GAT expression. This is, at least in part, due to the downregulation of GATs on striatal astrocytes since astrocytic GAT-3 expression is reduced. Astrocytes show dysfunction in Parkinson's disease and in other translational models. At the level of the substantia nigra, astrocytes in humans show conversion to an A1 reactive state, where they lose their ability to support neuronal function and gain neurotoxic function (Liddel et al., 2017). In the human striatum, astrocytes develop  $\alpha$ -synuclein reactivity (Braak et al., 2007), but other potential changes are less well understood. In the *SNCA-OVX* mouse, augmented  $\alpha$ -synuclein burden in astrocytes has been observed in parallel with increased markers for A1 reactive astrocyte conversion (unpublished observation from Wade-Martins Group, University of Oxford) and injections of monomeric human  $\alpha$ -synuclein into wild-type mouse striatum induce A1 reactive astrocyte conversion (Szejder-Pacholek et al., 2017). Additionally, in another area of the basal ganglia (GPe), a 6-OHDA-induced DA lesion in rats leads to downregulated GAT-3 on astrocytes (Chazalon et al., 2018). Further work should address whether this increased  $\alpha$ -synuclein burden and/or astrocyte conversion to an A1 reactive state results in the downregulation of astrocytic GAT in

striatum, or if GAT downregulates through other means, e.g. reduced DA signalling at astrocytic D<sub>2</sub> receptors.

Intriguingly, astrocytic GAT-3 can be bidirectionally modified. Within the striatum, genetically reducing astrocytic calcium signalling (through expression of a plasma membrane calcium pump) has been shown to increase GAT-3 expression, through Rab11a signalling (Yu et al., 2018), and decrease tonic inhibition of SPNs, with detectable consequences on behaviour. An upregulation of striatal GAT-3 has been observed in mouse models of Huntington's disease (Wójtowicz et al., 2013; Yu et al., 2018), which unlike hypokinetic PD, is a 'hyperkinetic' disorder treated by antidopaminergic drugs. We propose that GAT-3 upregulation will limit tonic inhibition of DA release in Huntington's disease models and thereby contribute to the disease symptomology. Thus, regulation of astrocytic GAT presents itself as a potential therapeutic treatment of PD, and Huntington's disease, which could be achieved by targeting Rab11a signalling, and/or upstream astrocyte calcium signalling.

#### 5.4.4 Pregabalin normalises aberrant tonic inhibition of dopamine release in Parkinsonian mice

We revealed that treatment with the gabapentinoid PGB in aged  $\alpha$ -synuclein overexpressing Parkinsonian mice selectively increased DA release by up to 50% in the dorsal striatum, thereby reversing the prior DA deficit in this Parkinsonian model. Furthermore, this boost to DA release was specific to dorsal striatum, leaving release in unaffected ventral striatum at normal levels. Unexpectedly, we found this effect to be independent of VGCC function in DA axons and also require prolonged PGB exposure, as a single dose had no effect on DA release. We further revealed that PGB normalises the increased tonic inhibition of DA release by GABA exhibited in this Parkinsonian model. Given we show this aberrant tonic inhibition of DA release by GABA in these  $\alpha$ -synuclein overexpressing Parkinsonian mice is underpinned by reduced striatal GAT, we propose PGB might upregulate striatal GAT expression in the dorsal striatum, where GAT function is

most influential in governing DA output (see **Section 4.3.3**). Whether these effects of PGB in selectively boosting DA release in the dorsal striatum are genotype-specific i.e. PGB operates to specifically normalise the DA release deficit in SNCA-OVX mice, or can also selectively boost DA release in the dorsal striatum of wildtype human  $\alpha$ -synuclein naïve mice has not yet been tested.

Striatal astrocytes express VGCCs and the  $\alpha_2\delta$ -1 and -2 auxiliary subunits (Chai et al., 2017). Gabapentinoids, including PGB, bind to the  $\alpha_2\delta$  subunits to limit the abundance, localisation and function of VGCCs, and therefore limit the influx of calcium (Arikkath and Campbell, 2003; Hoppa et al., 2012; Klugbauer et al., 1999). It has previously been demonstrated that limiting astrocytic calcium signalling in striatum (through expression of a plasma membrane calcium pump) results in increased astrocytic GAT-3 expression via a Rab11a signalling pathway (Yu et al., 2018). Furthermore, prolonged incubation (>24 hours) of gabapentinoids caused a 2-fold increase in GABA uptake in hippocampal cultures, which was due to a redistribution of GAT-1 protein from intracellular locations to the plasma membrane (Whitworth and Quick, 2001). Therefore, PGB might function to increase striatal GAT expression and function, thereby normalising the aberrant tonic inhibition of DA release in the dorsal striatum of  $\alpha$ -synuclein overexpressing Parkinsonian mice.

Alternatively, gabapentinoids are largely known to inhibit the release of many neurotransmitters including GABA (Bian et al., 2006; Uchitel et al., 2010), and therefore might decrease striatal GABA release and GABA tone onto DA axons. Indeed, in striatum gabapentinoids have been shown to prevent thrombospondin-dependent enhanced corticostriatal synaptic transmission, preventing an increase in SPN action potential firing from increased excitatory inputs to SPNs (Nagai et al., 2019). Therefore, PGB might additionally limit GABA release in the striatum through limiting corticostriatal excitatory synaptic transmission onto SPNs. However, we cannot yet definitively confirm that PGB increases DA release through a mechanism which modulates striatal GAT and/or GABA, as

PGB might boost DA release in the dorsal striatum through a mechanism entirely unrelated, which then in turn might normalise the aberrant tonic inhibition of DA release by striatal GABA.

Overall, these data suggest that gabapentinoids might have unappreciated ability to treat PD symptoms by boosting DA release, and selectively in parts of striatum that are most affected by the disease. Indeed, small scale studies in patients with advanced PD have suggested that gabapentinoids can improve a range of symptoms (Van Blercom et al., 2004; Olson et al., 1997). Furthermore, since the acute exposure of gabapentinoids can reorganise the spatiotemporal handling of calcium entry into DA axons without limiting DA release (Brimblecombe et al., manuscript under review), gabapentinoids also have neuroprotective potential, through limiting calcium-associated neurotoxicity without worsening symptoms.

#### 5.4.5 Summary and implications

In conclusion, we reveal maladaptive decreases in striatal GAT-1 and GAT-3 expression in a mouse model of early parkinsonism (prior to the overt loss of DA neurons), and consequently, considerable augmentation of tonic inhibition of DA release by GABA in the dorsal striatum. Moreover, we show that treatment of these Parkinsonian mice with the gabapentinoid PGB selectively boost DA release in the dorsal striatum and normalises the aberrant tonic inhibition of DA release, which we speculate might be mediated by increasing astrocytic GAT expression. The regulation of striatal DA release by GATs represent a loci for maladaptive plasticity in early parkinsonism, which could provide a novel therapeutic avenue for upregulating DA signalling in PD.

# 6

## General Discussion

DA release from mesostriatal DA axons in striatum is subject to major influences besides action potentials generated at the levels of the cell body, as local mechanisms in the striatum are able to drive and gate DA release. This local regulation of striatal DA release is important, because DA is the extracellular signal that mediates the transfer of information from mesostriatal DA neurons to postsynaptic striatal targets, and therefore influences striatal processing and downstream basal ganglia activity to consequently shape behaviour. Unravelling these mechanisms is therefore necessary to fully understand how striatal DA output is shaped to regulate and modify behaviour. Furthermore, understanding how these mechanisms which regulate striatal DA release might be dysfunctional in diseases with DA dysregulation, is critical for identifying novel targets for disease-modifying therapies.

The central aim of this thesis was to explore how striatal GABA and GATs locally regulate striatal DA release. **Chapter 3** identified that DA release is inhibited by the activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors presumably located on DA axons, and that striatal DA release is under tonic inhibition by a local GAD-dependent GABA tone. **Chapter 4** expanded upon these findings and revealed that the level of DA release in the striatum is set by the activity of GAT-1 and GAT-3, located at least in part on striatal astrocytes, through limiting the ambient GABA tone onto DA axons. **Chapter 5** probed further the significance of the regulation of striatal DA by striatal GATs and GABA, and found maladaptive decreases in GAT-1 and GAT-3 expression in a mouse model of early parkinsonism (prior to the overt loss of DA neurons), and consequently, considerable augmentation of tonic inhibition of DA release by GABA in the dorsal striatum. Here in this final chapter, I will consider these findings within the wider context of DA signalling, and their implications for the understanding of striatal function and Parkinson's disease.

## 6.1 Tonic inhibition of striatal dopamine release is regulated by GABA transporter activity

### 6.1.1 Tonic inhibition of dopamine release mediated through GABA<sub>A</sub> and GABA<sub>B</sub> receptor activation

The striatum contains a high density of projection neurons and interneurons that use the transmitter GABA through canonical GAD-dependent GABA synthesis (Oorschot, 1996; Tepper et al., 2018). The axon of a single nigrostriatal DA neuron (in rat) reaches ~3% of the volume of striatum (Matsuda et al., 2009), a volume expected to contain ~70,000 of these GAD-immunoreactive GABAergic neurons (Oorschot, 1996). Furthermore, evidence supports the axonal expression of GABA<sub>A</sub> and GABA<sub>B</sub> receptors by DA neurons (Charara et al., 1999; Sherpa et al., 2019; Yung et al., 1999). It was therefore unsurprising that we found DA release to be under tonic inhibition by a GAD-dependent striatal GABA source. Overall these findings revealed that GABA can influence striatal output, not only through direct actions on output neurons and local interneurons, but also by governing DA transmission.

These findings expand upon an assortment of previous evidence demonstrating attenuated striatal DA release following GABA<sub>A</sub> and/or GABA<sub>B</sub> receptor activation (Avshalumov et al., 2003; Pitman et al., 2014; Ronken et al., 1993; Schmitz et al., 2002; Smolders et al., 1995), as we revealed for the first time that DA release is under tonic inhibition, and by a GAD-dependent striatal GABA source (Lopes, Roberts et al., 2018). Through excluding the actions of GABA receptors on ChIs in mediating GABA attenuation of DA release, we were able to propose that GABA<sub>A</sub> and GABA<sub>B</sub> receptors located directly on DA axons mediate this tonic GABA inhibition of DA release (Lopes, Roberts et al., 2018). Furthermore, the paucity of GABAergic synapses on DA axons (Charara et al., 1999) further suggests that this tonic inhibition by GAD-dependent GABA arises from the extrasynaptic ambient GABA tone, which is detected in striatum by other striatal neurons (e.g. Santhakumar et al., 2010).

We found that the activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors primarily limits the overall amplitude of DA output, with only minor additional enhancement of frequency filtering,

unlike cholinergic signalling at nAChRs. An additional role of this tonic activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors on DA axons, on top of modulating DA release, might be to regulate axonal membrane resistance to modify the impact of other inputs or limit the propagation of action potentials through the axonal arbour for a sparser coding. Indeed, tonic activation of high-affinity axonal GABA<sub>A</sub> receptors in other neuron types can promote shunting inhibition (Verdier et al., 2003; Wall, 1995; Westberg et al., 2000) and promote branch-point/spike failure (Alle and Geiger, 2007; Jackson and Zhang, 1995; Ruiz et al., 2003; Zhang and Jackson, 1993, 1995).

In the central nervous system, tonic inhibition is mediated by GABA<sub>A</sub> receptors composed of specific subunits such as  $\delta$ ,  $\alpha 4$ ,  $\alpha 5$ , or  $\alpha 6$ , which give these receptors high affinity for GABA and little desensitisation (Brickley and Mody, 2012). Future works will need to initially revisit anatomical studies to confirm GABA<sub>A</sub> and GABA<sub>B</sub> receptor expression on DA axons, and determine which specific subunits are expressed, using electron microscopy and/or molecular strategies. Once GABA<sub>A</sub> and GABA<sub>B</sub> receptor expression on DA axons is established, future works should determine if tonic activation of these receptors promotes shunting inhibition or branch point failure in DA axons for a sparser coding across the intricate DA axonal arbour.

### 6.1.2 Astrocytic GABA transporter activity determines dopamine output through limiting GABA tone onto dopamine axons

Within a given nuclei of the central nervous system the concentration of ambient GABA tone, and therefore strength of tonic GABA receptor-mediated inhibition, is determined by the amount of GABA release and spillover into the extrasynaptic environment relative to the rate of GABA uptake by plasma membrane GATs (Brickley and Mody, 2012). In the striatum, GAT-1 and GAT-3 limit ambient GABA tone onto SPNs (Kirmse et al., 2008, 2009; Santhakumar et al., 2010), thereby regulating SPN excitability and striatal output. We extended the role GATs play in the regulation of striatal processing (e.g. Yu et al., 2018), as striatal GAT-1 and GAT-3 support DA output though limiting GABA tone onto DA axons

(Figure 6.1). We found that this previously unreported role for the GAT in supporting DA output was heterogenous across striatum. GATs limited tonic GABAergic inhibition of DA release in DLS, and also NAcS, but not NAcC, which corresponded with heterogeneity in GAT-1 and GAT-3 levels and with GAT control of tonic inhibition of SPNs. This heterogeneity in GAT function and expression across striatum was previously unexplored, but revealed that striatal GATs are in a position to modulate the excitability of neuronal networks preferentially in the dorsal striatum. This positive relationship we find between GAT function and DA output in the dorsal striatum might provide a candidate explanation for some of the clinical effects of antiepileptic GAT inhibitors e.g. tiagabine, which can present with Parkinsonian-like motor side effects (Zaccara et al., 2004). Furthermore, this dependence of GATs on determining the ambient GABA tone preferentially in dorsal striatum will likely have profound effects on the tonic inhibition of other striatal neuron types beyond that of SPNs and DA axons.

We found that GAT-3 and, to a lesser extent, GAT-1 could be identified on astrocytes, challenging the previous generalisation that GAT-1 is exclusively neuronal (Borden, 1996), and confirming emerging transcriptomic data that demonstrate striatal astrocytes express mRNA for both GAT-1 and GAT-3 (Chai et al., 2017; Gokce et al., 2016; Zhang et al., 2014). Inhibiting astrocyte function occluded the effects of GAT inhibitors, indicating a previously unappreciated role for astrocytes in determining the dynamic output of striatal DA through supporting GABA uptake to minimise tonic inhibition of DA release.

Increasing evidence indicates that astrocytes can dynamically regulate the function of neuronal synapses and circuits in and outside of the striatum. Astrocytes express receptors and transporters for many transmitters, and correspondingly can be activated in the form of rises in intracellular calcium (Halassa and Haydon, 2010). In turn, astrocytes can dynamically regulate extracellular levels of transmitters, and release gliotransmitters to regulate

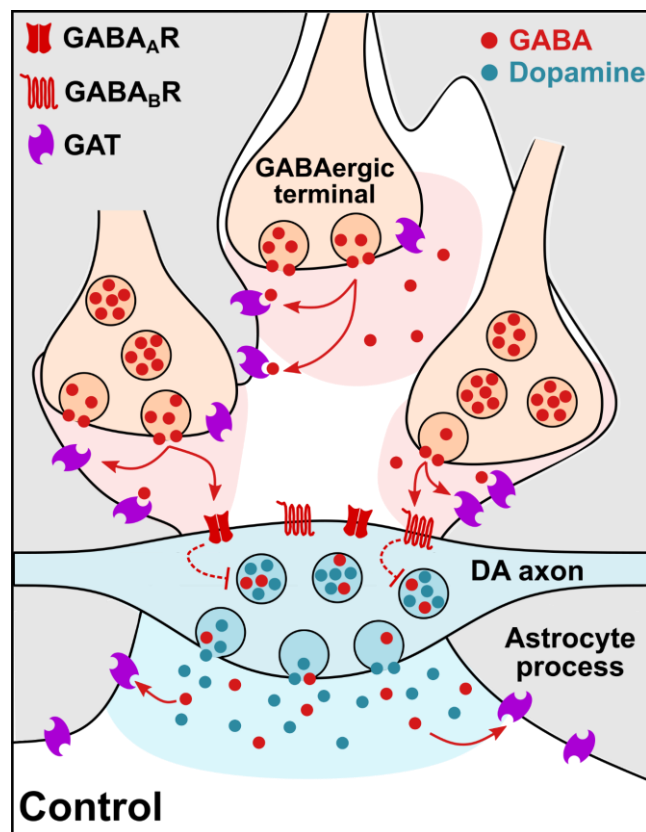


Figure 6.1. Astrocytic GAT activity limits tonic inhibition of striatal dopamine release by limiting ambient GABA tone onto dopamine axons.

GAD-synthesized GABA is released from GABAergic neurons in the striatum and can spillover to act at GABA receptors (GABA<sub>A</sub>R and GABA<sub>B</sub>R) located on DA axons, inhibiting (dashed red lines) DA and presumably GABA co-release. The level of GABA spillover and tonic inhibition of DA release is determined by the activity of GABA transporters (GATs) located on astrocytes (grey) and neurons, which remove GABA from the extracellular space.

neuronal circuits and synaptic transmission (Araque et al., 2014; Covelo and Araque, 2016; Halassa and Haydon, 2010). It has recently been found that astrocytes in the VTA can regulate DA neuron activity (Gomez et al., 2019), and that astrocytes in striatum can modulate the activity of other types of synapses and neurons (Martín et al., 2015; Yu et al., 2018) and can influence striatal-dependent reward and motivation to self-administer drugs of addiction (Bull et al., 2014; Scofield et al., 2015). Our findings that astrocytes can govern striatal DA output through local interaction at axonal release sites, significantly revises our current understanding of the neurocentric mechanisms that can dynamically regulate DA transmission and adds to the growing body of literature which points to astrocytes

functioning as an extension of neuronal circuits, both within and outside the striatum. These findings warrant further investigations into the relationship between striatal astrocytes and DA.

Effects of astrocyte manipulation on DA could also potentially be mediated through astrocytes acting to regulate levels of other striatal transmitters since astrocytes express transporters for many transmitters (e.g. GLT for glutamate). Furthermore, they could involve the release of gliotransmitters (Martín et al., 2015). Receptors and transporter currents on astrocytes can directly or indirectly (via  $\text{Na}^+$  entry and subsequent  $\text{Na}^+/\text{Ca}^{2+}$  exchange) drive changes in intracellular  $\text{Ca}^{2+}$  that “activate” astrocytes to release a range of potential gliotransmitters e.g. ATP, adenosine, glycine, D-serine and glutamate (Boddum et al., 2016; Covelo and Araque, 2018; Martin-Fernandez et al., 2017; Martín et al., 2015). Whether any gliotransmitters modulate striatal DA transmission remains undefined. Novel chemogenetic constructs (designer receptors exclusively activated by designer drugs; DREADDS) targeted to astrocytes (e.g. hM3D( $\text{G}_q$ ), rM3D( $\text{G}_s$ ), and hM4D( $\text{G}_i$ ) DREADDs directed to astrocytes through the use of the glial fibrillary acidic protein (GFAP) promoter embedded within the DREADD construct) have successfully been used to activate striatal astrocytes to modulate synaptic transmission and SPN activity in dorsal striatum (Chai et al., 2017; Dvorzhak et al., 2018). These technologies should be used to explore further whether astrocyte activity impacts on DA output through an additional role beyond that of GABA uptake.

Astrocytes exhibit molecular, functional and regional diversity within the central nervous system, proposed to facilitate circuit-specific roles through genetically tractable subpopulations (Khakh and Sofroniew, 2015; Zhang and Barres, 2010) and within the striatum, astrocyte heterogeneity has previously been observed (Chai et al., 2017; Martín et al., 2015). Given the molecular heterogeneity in astrocytes between the dorsal and ventral striatum reported previously (Chai et al., 2017), and in this body of work via regional

differences astrocytic GATs, the different roles for astrocytes across striatal territories should be further interrogated. Furthermore, future experiments should additionally assess whether the modulation of striatal astrocyte activity impacts on DA-dependent behaviours that are under the predominant control of dorsal or ventral striatum, e.g. movement (dorsal-dependent) versus reward-related (ventral-dependent) types of behaviours, using standard behavioural tests such as locomotor activity and conditioned place preference.

## 6.2 Implications for GABA co-release from striatal dopamine axons

### 6.2.1 GABA transporters are not directly located on dopamine axons

GAT-1 and GAT-3 were suggested previously to be directly located on DA axons to support GABA uptake for co-release, and were based on mRNA for GAT-1 (and weakly for GAT-3) in the somata of DA neurons in substantia nigra, and on the attenuation of GABA co-release from DA axons after pharmacological inhibition of both GAT isoforms together (no effect when blocked individually) (Tritsch et al., 2014). However, we did not observe GAT-1 or GAT-3 localisation to DA axons, despite observing robust GAT localisation to the neurites of parvalbumin-positive interneurons and on astrocytes. Given then the presented findings herein that GATs limit tonic GABA<sub>A</sub> and GABA<sub>B</sub> receptor-mediated inhibition of DA release, the observed dependence of GABA co-release on GAT is very likely a result of the GATs limiting tonic inhibition of GABA co-release, rather than being required for uptake of GABA for co-storage in DA axons. We therefore proposed that GABA co-storage and co-release from DA axons is dependent on GABA synthesis alone by ALDH-1a1 (Kim et al., 2015), and likely does not arise from GABA uptake from the extracellular milieu.

### 6.2.2 GABA co-release from dopamine axons indirectly supports dopamine release and is attenuated in early parkinsonism

We found tonic inhibition of DA release by GABA to arise from a GAD-dependent source of GABA, and not from non-canonical ALDH-dependent GABA source arising from co-release from DA axons. However, GABA co-release appeared to indirectly support DA output though limiting the tonic inhibition by a GAD-dependent GABA network, as ALDH-

inhibition counterintuitively boosted the tonic inhibition of DA release. Supporting this role for GABA co-release, in  $\alpha$ -synuclein-overexpressing Parkinsonian mice, in which we found that GABA co-release from DA axons is significantly attenuated, we also found that the level of tonic GABA inhibition on DA release was boosted. GABA co-release from DA axons might therefore limit GAD-dependent GABA release in striatum through its inhibitory actions onto SPNs (Tritsch et al., 2012). Further experiments will be required to dissect this potential circuit interaction, and to determine if attenuated GABA co-release directly impairs DA output. Interestingly, mutations of ALDH1a1 in humans (Liu et al., 2011; Sherva et al., 2009) and deletion in mice (Kim et al., 2015) lead to alcohol-consuming preferences, which given the proposed role herein for ALDH-dependent GABA to support DA release, might be mediated through maladaptive striatal DA signalling.

Our optogenetic-capable model of parkinsonism allowed us to address for the first time whether DA release deficits were accompanied by corresponding deficits in GABA co-release from DA axons. We observed a robust attenuation in the amplitude of light-evoked GABA<sub>A</sub>-dependant IPSCs in SPNs in the dorsal striatum of  $\alpha$ -synuclein overexpressing mice in early adulthood, which corresponded with the light-evoked DA release deficit also observed in these animals. Reduced levels of expression of mRNA and protein for the noncanonical GABA synthesis enzyme ALDH1a1 have been reported in DA neurons in the SNc, but not in VTA, of PD post-mortem brains (Galter et al., 2003), suggesting that loss of striatal GABA co-release from DA axons in early parkinsonism might translate to human PD. This GABA co-release deficit may contribute to PD symptoms, as well as to the alterations of striatal circuitry that accompany loss of nigrostriatal DA input, and should be factored in to future attempts to offset dysfunction in PD.

While the role nigrostriatal co-released GABA has within the neurologically-intact and Parkinsonian striatum is yet to be fully elucidated, GABA<sub>A</sub> receptor agonists have exhibited some therapeutic efficacy in PD. Clinical cases have reported significant improvement of

motor symptomology in PD patients following subhypnotic doses of zolpidem, an imidazopyridine hypnotic drug acting as a selective GABA<sub>A</sub> agonist (Chen et al., 2008; Daniele et al., 1997; Růzicka et al., 2000). Reported anti-Parkinsonian effects were similar to those of DA replacement therapy with L-DOPA, including substantial improvement in akinesia, bradykinesia and rigidity, but advantageously did not induce detectable dyskinesias (like with long-term L-DOPA therapy). The deficit in nigrostriatal GABA co-release observed herein provides rationale to re-examine GABA<sub>A</sub> receptor agonists as potential disease-modifying therapy in Parkinson's disease.

Intriguingly, attenuated GABA co-release has previously been observed from glutamatergic long-range basal ganglia inputs (entopeduncular nucleus) to the lateral habenula in a rodent model of depression; co-released glutamate was found not to be impaired, therefore resulting in a pathological aberration in the GABA to glutamate co-release ratio at this synapse (Shabel et al., 2014). Conventional antidepressant treatment with selective 5-HT reuptake inhibitors (SSRIs) was found to selectively increase GABA co-release at this synapse without impacting on co-released glutamate e.g. normalising the GABA:glutamate ratio at this synapse (Shabel et al., 2014). In light of these studies, the effects of 'gold-standard' therapeutics for Parkinson's disease (e.g. L-DOPA) on the ratio of co-released GABA:DA from DA axons in the striatum should be examined to determine if these therapeutic strategies result in an imbalance of GABA:DA co-release, which might result in aberrant striatal processing and motor dysfunction (e.g. L-DOPA-induced dyskinesia).

## 6.3 GABA transporter dysfunction in Parkinson's disease

### 6.3.1 Maladaptive downregulation of striatal GABA transporters in early parkinsonism

We explored the potential adaptations to GAT function and therefore tonic GABA inhibition of DA release in the striatum of a human  $\alpha$ -synuclein overexpressing mouse model of early parkinsonism. There remains a pressing need to define early functional changes to susceptible neurons and associated circuits in PD, for which this model is well suited. We

found a maladaptive augmentation of tonic GABAergic inhibition of DA release and correspondingly downregulated GAT-1 and GAT-3 expression in the disease-vulnerable dorsal striatum, but not the disease-spared NAc. These findings parallel recent observations in another region of the basal ganglia whereby astrocytic GAT-3 downregulation results in elevated extracellular GABA tone in DA-depleted rat GPe (Chazalon et al., 2018). These findings collectively implicate astrocytic GATs in the pathophysiology of parkinsonism across multiple basal ganglia nuclei, which imminently need to be substantiated in post-mortem PD brains.

This augmented tonic inhibition of striatal DA release is likely mediated through increased ambient GABA tone resulting from diminished striatal GAT expression (Figure 6.2). This maladaptive augmentation of ambient GABA tone will likely disturb normal striatal processing and output, not only through reduced DA signalling onto SPNs, but also likely due to augmented tonic inhibition by ambient GABA directly onto SPNs. SPNs express extrasynaptic and perisynaptic GABA<sub>A</sub> receptors containing  $\alpha 4$ ,  $\alpha 5$  and  $\delta$  subunits, which are well-adapted for tonic signalling activated by ambient GABA tone (Ade et al., 2008; Janssen et al., 2009; Santhakumar et al., 2010). Additionally, GABA<sub>A</sub>-receptor mediated tonic inhibitory currents recorded in adult mice are larger in D<sub>1</sub>-expressing dSPNs than in D<sub>2</sub>-expressing iSPNs (Santhakumar et al., 2010). Increased ambient GABA tone (resulting from reduced striatal GAT expression) might therefore contribute to the hypoactivity of direct-pathway dSPNs observed in PD (DeLong, 1990; McGregor and Nelson, 2019), through increased tonic inhibition preferentially targeted to dSPNs. Future work will need to establish if SPNs are under increased tonic inhibition in Parkinsonian models, and if this increased tonic inhibition contributes to the imbalance between the direct- and indirect-pathways observed in PD. In support of these hypotheses, increasing GAT-3 expression levels in striatum leads to diminished ambient GABA-mediated tonic inhibition of SPNs,

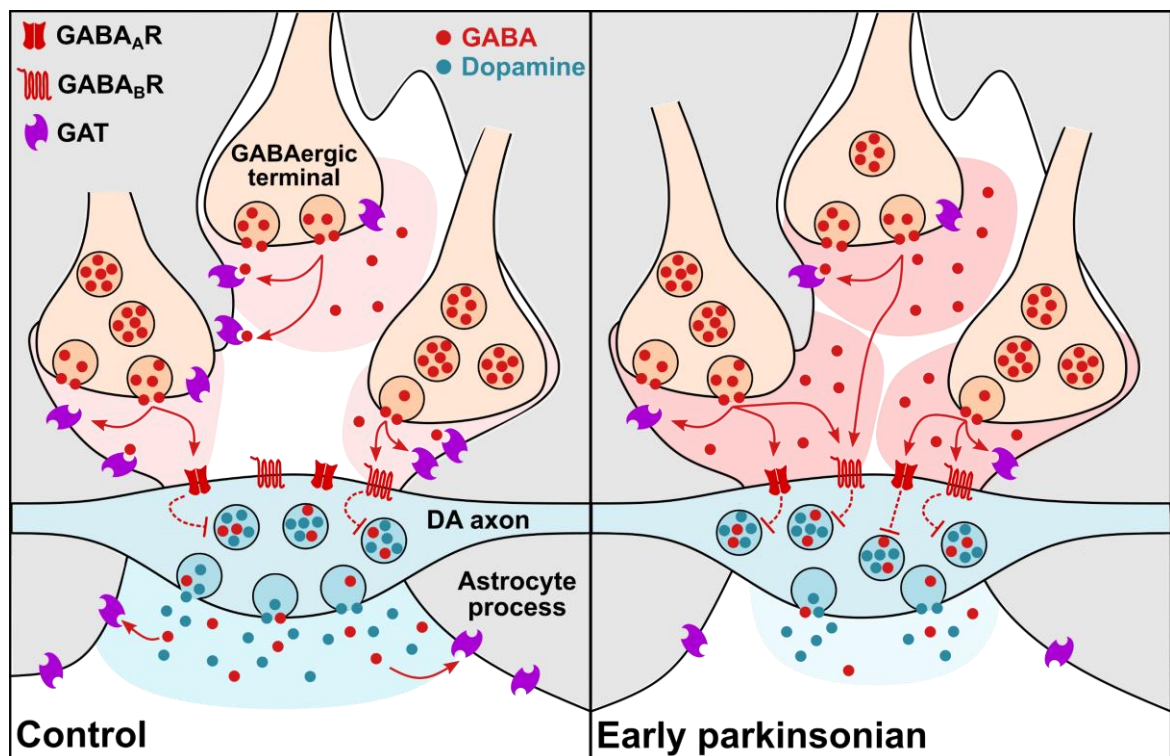


Figure 6.2. Augmented tonic inhibition of striatal dopamine release in dorsal striatum of Parkinsonian mice due to reduced striatal GAT expression.

Under normal circumstances (*left*), GAD-synthesised GABA is released from GABAergic striatal neurons can spillover to act at GABA receptors (GABA<sub>A</sub>R and GABA<sub>B</sub>R) located presumably on DA axons, inhibiting (*dashed red lines*) DA and GABA co-release. The level of GABA spillover and tonic inhibition of DA release is determined by the activity of GABA transporters (GATs) located on astrocytes (*grey*) and neurons, which remove GABA from the extracellular space. In a mouse model of early parkinsonism (*right*), striatal GAT expression is downregulated in dorsal striatum, resulting in augmented tonic inhibition of DA release by GABA. Co-release of GABA from DA axons is also reduced.

altered SPN activity, and results in increased striatal-dependent self-grooming behaviour (Yu et al., 2018).

An upregulation of striatal GAT-3 has been observed in mouse models of Huntington's disease (Wójtowicz et al., 2013; Yu et al., 2018), which unlike hypokinetic PD, is a 'hyperkinetic' disorder treated by antidopaminergic drugs. Given the profound ability for GATs to govern DA output in the dorsal striatum exposed in this body of work, GAT-3 upregulation in Huntington's disease models will likely diminish tonic inhibition of DA release and thereby contribute to Huntington's disease symptomology. The regulation of

astrocytic GAT therefore presents itself as a potential disease-modifying therapy in both PD and Huntington's disease. To date, there are no agonists or potentiators for GATs directly, but astrocytic GAT-3 levels can be modified within striatum. Reducing astrocytic calcium signalling in striatum results in increased GAT-3 expression mediated through a Rab11a signalling pathway (Yu et al., 2018) and therefore Rab11a presents as a novel target to potentially normalise striatal GAT levels in Huntington's disease and PD.

### 6.3.2 Therapeutic potential for the gabapentinoid pregabalin

We revealed that a six-dose course of treatment with the gabapentinoid PGB in aged  $\alpha$ -synuclein overexpressing Parkinsonian mice selectively increased DA release in the dorsal striatum and normalised the aberrant tonic inhibition of DA release presented in these mice. We proposed that PGB might upregulate striatal GAT expression in the dorsal striatum, where GAT function is most influential in governing DA output, as a prolonged course of gabapentinoids have previously been shown to boost GAT expression in other preparations (Whitworth and Quick, 2001). Alternatively, PGB might function to decrease striatal GABA release and therefore GABA tone onto DA axons by inhibiting striatal GABA release, as gabapentinoids have also been demonstrated to inhibit the release of many neurotransmitters, including GABA (Bian et al., 2006; Uchitel et al., 2010). The mechanisms through which PGB selectively boosts DA release in the dorsal striatum will need to be elucidated in future works. Regardless, PGB clearly has untapped potential to modify PD symptoms, and with adjunct neuroprotective potential by limiting calcium-associated neurotoxicity (Brimblecombe et al., manuscript under review). Several small-scale studies in patients have begun to validate PGB as a potential disease-modifying therapy in PD, as gabapentinoids administered to advanced PD patients can improve a range of symptoms (Van Blercom et al., 2004; Olson et al., 1997).

### 6.3.3 Astrocyte dysfunction in Parkinson's disease

As outlined previously, the metabolic demands and stress generated from supporting the extensively arborised nigrostriatal DA axons in the dorsal striatum is suggested to contribute to their preferential degeneration in PD, where the adjacent DA neurons of the VTA which innervate the ventral striatum are spared, and are less branched (Pissadaki and Bolam, 2013). A 'dying back' of nigrostriatal DA axons have been proposed as one of the earliest features of PD (Burke and O'Malley, 2013), making the striatum a critical site for targeting the disease process.

Emerging evidence implicate astrocytes as likely contributors to neurodegeneration and dysfunction in Parkinson's disease (Liddel et al., 2019; Liddel et al., 2017; McGeer and McGeer, 2008), which are expanded upon by the work presented in this thesis. Astrocytes have exhibited dysfunction in PD through loss of normal support functions and/or through gain of neurotoxic functions. At the level of the SNc, astrocytes in humans show conversion to an A1-type reactive state, where they lose their ability to support neuronal function and gain neurotoxic function (Liddel et al., 2017), and astrocyte dysregulation promotes DA neuron degeneration in mice and rats (Kuter et al., 2018; Yun et al., 2018). At the level of the striatum, astrocytes develop  $\alpha$ -synuclein reactivity in humans (Braak et al., 2007), and striatal infusions of  $\alpha$ -synuclein or 6-OHDA to induce a DA lesion lead to A1-type reactive astrogliosis in rodents (Morales et al., 2016; Szejda-Pacholuk et al., 2017). Further, striatal lipopolysaccharide injection induces reactive gliosis and astrogliosis accompanied by progressive degeneration of SNc neurons and Parkinsonian dysfunction (Choi et al., 2009; Hunter et al., 2009). In the GPe, a 6-OHDA-induced DA lesion in rats leads to modifications in astrocytic GAT-3 function and changes to neurotransmission (Chazalon et al., 2018). Collectively, these observations led us to speculate that maladaptive downregulation of GATs in striatum in our  $\alpha$ -synuclein overexpressing Parkinsonian mice might be underpinned by astrocyte dysfunction e.g. conversion to an A1-type reactive state.

It seems likely that there will be adaptations to astrocyte function in the Parkinsonian striatum beyond that of downregulated GAT expression, that will impact directly on DA transmission. With the advent of new molecular tools and transgenic mouse lines for the selective targeting of astrocytes e.g. ALDH1L1-Cre mouse line (Srinivasan et al., 2016), future works will allow us to investigate the impact astrocytes have on DA neuron physiology in the Parkinsonian striatum. For instance, to understand the fuller range of adaptations to astrocyte biology in PD (and to confirm downregulated astrocytic GATs) future unbiased analyses of the striatal astrocyte transcriptome could be performed in Parkinsonian mice using cell-type specific translating ribosome affinity purification (TRAP) technologies (Heiman et al., 2014).

## 6.4 Conclusions

The findings presented in this thesis define previously unappreciated and important roles for GABA, plasma membrane GABA transporters and astrocytes in sculpting dopamine output in striatum, and reveal that they underlie maladaptive plasticity in early parkinsonism that impairs dopamine output in disease-vulnerable striatal regions. Further work is required to understand the implications of these findings for downstream basal ganglia processing and on behaviour, and to validate the preclinical findings presented in human Parkinson's disease.

# Bibliography

- A -

- Abeliovich, A., Schmitz, Y., Fariñas, I., Choi-Lundberg, D., Ho, W.-H., Castillo, P.E., Shinsky, N., Verdugo, J.M.G., Armanini, M., Ryan, A., et al. (2000). Mice Lacking  $\alpha$ -Synuclein Display Functional Deficits in the Nigrostriatal Dopamine System. *Neuron* 25, 239-252.
- Ade, K.K., Janssen, M.J., Ortinski, P.I., and Vicini, S. (2008). Differential Tonic GABA Conductances in Striatal Medium Spiny Neurons. *Journal of Neuroscience* 28, 1185-1197.
- Adrover, M.F., Shin, J.H., and Alvarez, V.A. (2014). Glutamate and dopamine transmission from midbrain dopamine neurons share similar release properties but are differentially affected by cocaine. *Journal of Neurosciences* 34, 3183-3192.
- Albin, R.L., Young, A.B., and Penney, J.B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences* 12, 366-375.
- Alcantara, A.A., Chen, V., Herring, B.E., Mendenhall, J.M., and Berlanga, M.L. (2003). Localization of dopamine D2 receptors on cholinergic interneurons of the dorsal striatum and nucleus accumbens of the rat. *Brain Research* 986, 22-29.
- Alexander, G.E., and Crutcher, M.D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosciences* 13, 266-271.
- Alle, H., and Geiger, J.R.P. (2007). GABAergic Spill-Over Transmission onto Hippocampal Mossy Fiber Boutons. *Journal of Neuroscience* 27, 942-950.
- Ammari, R., Lopez, C., Fiorentino, H., Gonon, F., and Hammond, C. (2009). A mouse juvenile or adult slice with preserved functional nigro-striatal dopaminergic neurons. *Neuroscience* 159, 3-6.
- Andén, N.-E., Fuxe, K., Hamberger, B., and Hökfelt, T. (1966). A Quantitative Study on the Nigro-Neostriatal Dopamine Neuron System in the Rat. *Acta Physiologica Scandinavica* 67, 306-312.
- Anderson, D.W., Schray, R.C., Duester, G., and Schneider, J.S. (2011). Functional significance of aldehyde dehydrogenase ALDH1A1 to the nigrostriatal dopamine system. *Brain Research* 1408, 81-87.
- Anwar, S., Peters, O., Millership, S., Ninkina, N., Doig, N., Connor-Robson, N., Threlfell, S., Koener, G., Deacon, R.M., Bannerman, D.M., et al. (2011). Functional alterations to the nigrostriatal system in mice lacking all three members of the synuclein family. *Journal of Neuroscience* 31, 7264-7274.
- Apicella, P. (2002). Tonically active neurons in the primate striatum and their role in the processing of information about motivationally relevant events. *European Journal of Neuroscience* 16, 2017-2026.
- Araque, A., Carmignoto, G., Haydon, P.G., Oliet, S.H.R., Robitaille, R., and Volterra, A. (2014). Gliotransmitters Travel in Time and Space. *Neuron* 81, 728-739.
- Arbuthnott, G.W., and Wickens, J. (2007). Space, time and dopamine. *Trends in Neurosciences* 30, 62-69.
- Arikath, J., and Campbell, K.P. (2003). Auxiliary subunits: Essential components of the voltage-gated calcium channel complex. *Current Opinion in Neurobiology* 13, 298-307.

Armstrong-James, M., and Millar, J. (1979). Carbon fibre microelectrodes. *Journal of Neuroscience Methods* 1, 279-287.

Armstrong-James, M., Millar, J., and Kruk, Z.L. (1980). Quantification of noradrenaline iontophoresis. *Nature* 288, 181-183.

Augood, S.J., Herbison, A.E., and Emson, P.C. (1995). Localization of GAT-1 GABA transporter mRNA in rat striatum: cellular coexpression with GAD67 mRNA, GAD67 immunoreactivity, and parvalbumin mRNA. *Journal of Neuroscience* 15, 865-874.

Averbeck, B.B., Lehman, J., Jacobson, M., and Haber, S.N. (2014). Estimates of projection overlap and zones of convergence within frontal-striatal circuits. *Journal of Neuroscience* 34, 9497-9505.

Avshalumov, M. V., Chen, B.T., Marshall, S.P., Pena, D.M., and Rice, M.E. (2003). Glutamate-Dependent Inhibition of Dopamine Release in Striatum Is Mediated by a New Diffusible Messenger, H<sub>2</sub>O<sub>2</sub>. *Journal of Neuroscience* 23, 2744-2750.

- B -

Baba, M., Nakajo, S., Tu, P.H., Tomita, T., Nakaya, K., Lee, V.M., Trojanowski, J.Q., and Iwatsubo, T. (1998). Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *The American Journal of Pathology* 152, 879-884.

Bäckman, C.M., Malik, N., Zhang, Y., Shan, L., Grinberg, A., Hoffer, B.J., Westphal, H., and Tomac, A.C. (2006). Characterization of a mouse strain expressing Cre recombinase from the 3' untranslated region of the dopamine transporter locus. *Genesis* 44, 383-390.

Bagley, E.E., Hacker, J., Chefer, V.I., Mallet, C., McNally, G.P., Chieng, B.C.H., Perroud, J., Shippenberg, T.S., and Christie, M.J. (2011). Drug-induced GABA transporter currents enhance GABA release to induce opioid withdrawal behaviors. *Nature Neuroscience* 14, 1548-1554.

Balleine, B.W., Delgado, M.R., and Hikosaka, O. (2007). The Role of the Dorsal Striatum in Reward and Decision-Making. *Journal of Neuroscience* 27, 8161-8165.

Baptista, M.J., O'Farrell, C., Daya, S., Ahmad, R., Miller, D.W., Hardy, J., Farrer, M.J., and Cookson, M.R. (2003). Co-ordinate transcriptional regulation of dopamine synthesis genes by  $\alpha$ -synuclein in human neuroblastoma cell lines. *Journal of Neurochemistry* 85, 957-968.

Bauer, C.S., Nieto-Rostro, M., Rahman, W., Tran-Van-Minh, A., Ferron, L., Douglas, L., Kadurin, I., Sri Ranjan, Y., Fernandez-Alacid, L., Millar, N.S., Dickenson, A.H., Lujan, R., Dolphin, A.C. (2009). The increased trafficking of the calcium channel subunit  $\alpha_2\delta$ -1 to presynaptic terminals in neuropathic pain is inhibited by the  $\alpha_2\delta$  ligand pregabalin. *Journal of Neuroscience* 29, 4076-4088.

Bean, B.P. (2007). The action potential in mammalian central neurons. *Nature Reviews Neuroscience* 8, 451-465.

Beatty, J.A., Sullivan, M.A., Morikawa, H., and Wilson, C.J. (2012). Complex autonomous firing patterns of striatal low-threshold spike interneurons. *Journal of Neurophysiology* 108, 771-781.

Beaulieu, J.-M., and Gainetdinov, R.R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews* 63, 182-217.

- Beckstead, M.J., Grandy, D.K., Wickman, K., and Williams, J.T. (2004). Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron* 42, 939-946.
- Beckstead, R.M., Domesick, V.B., and Nauta, W.J.H. (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Research* 175, 191-217.
- Bekris, L.M., Mata, I.F., and Zabetian, C.P. (2010). The genetics of Parkinson disease. *Journal of Geriatric Psychiatry and Neurology* 23, 228-242.
- Bennett, B.D., and Wilson, C.J. (1999). Spontaneous activity of neostriatal cholinergic interneurons in vitro. *Journal of Neuroscience* 19, 5586-5596.
- Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., and Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of the Neurological Sciences* 20, 415-455.
- Berrios, J., Stamatakis, A.M., Kantak, P.A., McElligott, Z.A., Judson, M.C., Aita, M., Rougie, M., Stuber, G.D., and Philpot, B.D. (2016). Loss of UBE3A from TH-expressing neurons suppresses GABA co-release and enhances VTA-NAc optical self-stimulation. *Nature Communications* 7, 1-8.
- Besson, M.J., Graybiel, A.M., and Nastuk, M.A. (1988). [3H]SCH 23390 binding to D1 dopamine receptors in the basal ganglia of the cat and primate: delineation of striosomal compartments and pallidal and nigral subdivisions. *Neuroscience* 26, 101-119.
- Bevan, M.D., Booth, P.A., Eaton, S.A., and Bolam, J.P. (1998). Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. *Journal of Neuroscience* 18, 9438-9452.
- Bian, F., Li, Z., Offord, J., Davis, M.D., McCormick, J., Taylor, C.P., and Walker, L.C. (2006). Calcium channel alpha2-delta type 1 subunit is the major binding protein for pregabalin in neocortex, hippocampus, amygdala, and spinal cord: An ex vivo autoradiographic study in alpha2-delta type 1 genetically modified mice. *Brain Research* 1075, 68-80.
- Lo Bianco, C., Ridet, J.-L., Schneider, B.L., Deglon, N., and Aebischer, P. (2002). alpha - Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America* 99, 10813-10818.
- Björklund, A., and Dunnett, S.B. (2007). Dopamine neuron systems in the brain: an update. *Trends in Neurosciences* 30, 194-202.
- Van Blercom, N., Lasa, A., Verger, K., Masramón, X., Sastre, V.M., and Linazasoro, G. (2004). Effects of gabapentin on the motor response to levodopa: A double-blind, placebo-controlled, crossover study in patients with complicated Parkinson disease. *Clinical Neuropharmacology* 27, 124-128.
- Van Bockstaele, E.J., and Pickel, V.M. (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Research* 682, 215-221.
- Boddum, K., Jensen, T.P., Magloire, V., Kristiansen, U., Rusakov, D.A., Pavlov, I., and Walker, M.C. (2016). Astrocytic GABA transporter activity modulates excitatory neurotransmission. *Nature Communications* 7, 13572.
- Bolam, J.P., and Pissadaki, E.K. (2012). Living on the edge with too many mouths to feed: Why dopamine neurons die. *Movement Disorders* 27, 1478-1483.

- Bolam, J.P., Hanley, J.J., Booth, P.A.C., and Bevan, M.D. (2000). Synaptic organisation of the basal ganglia. *Journal of Anatomy* 196, 527-542.
- Bonansco, C., Couve, A., Perea, G., Ferradas, C.A., Roncagliolo, M., and Fuenzalida, M. (2011). Glutamate released spontaneously from astrocytes sets the threshold for synaptic plasticity. *European Journal of Neuroscience* 33, 1483-1492.
- Borden, L.A. (1996). GABA transporter heterogeneity: Pharmacology and cellular localization. *Neurochemistry International* 29, 335-356.
- Bowery, N.G., Hudson, A.L., and Price, G.W. (1987). GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* 20, 365-383.
- Boyes, J., and Bolam, J.P. (2003). The subcellular localization of GABAB receptor subunits in the rat substantia nigra. *European Journal of Neuroscience* 18, 3279-3293.
- Braak, H., Sastre, M., and Del Tredici, K. (2007). Development of  $\alpha$ -synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. *Acta Neuropathologica* 114, 231-241.
- Bracci, E., Centonze, D., Bernardi, G., and Calabresi, P. (2002). Dopamine excites fast-spiking interneurons in the striatum. *Journal of Neurophysiology* 87, 2190-2194.
- Branda, C.S., and Dymecki, S.M. (2004). Talking about a Revolution: The Impact of Site-Specific Recombinases on Genetic Analyses in Mice. *Developmental Cell* 6, 7-28.
- Brazhnik, E., Shah, F., and Tepper, J.M. (2008). GABAergic Afferents Activate Both GABAA and GABAB Receptors in Mouse Substantia Nigra Dopaminergic Neurons In Vivo. *Journal of Neuroscience* 28, 10386-10398.
- Brickley, S.G., and Mody, I. (2012). Extrasynaptic GABAA Receptors: Their Function in the CNS and Implications for Disease. *Neuron* 73, 23-34.
- Brickley, S.G., Cull-Candy, S.G., and Farrant, M. (1996). Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. *The Journal of Physiology* 497, 753-759.
- Brimblecombe, K.R., and Cragg, S.J. (2015). Substance P Weights Striatal Dopamine Transmission Differently within the Striosome-Matrix Axis. *Journal of Neuroscience* 35, 9017-9023.
- Brimblecombe, K.R., Gracie, C.J., Platt, N.J., and Cragg, S.J. (2015). Gating of dopamine transmission by calcium and axonal N-, Q-, T- and L-type voltage-gated calcium channels differs between striatal domains. *The Journal of Physiology* 593, 929-946.
- Britt, J.P., and McGehee, D.S. (2008). Presynaptic Opioid and Nicotinic Receptor Modulation of Dopamine Overflow in the Nucleus Accumbens. *Journal of Neuroscience* 28, 1672-1681.
- Bucher, D., and Goaillard, J.-M. (2011). Beyond faithful conduction: Short-term dynamics, neuromodulation, and long-term regulation of spike propagation in the axon. *Progress in Neurobiology* 94, 307-346.
- Bull, C., Freitas, K.C.C., Zou, S., Poland, R.S., Syed, W.A., Urban, D.J., Minter, S.C., Shelton, K.L., Hauser, K.F., Negus, S.S., et al. (2014). Rat nucleus accumbens core astrocytes modulate reward and the motivation to self-administer ethanol after abstinence. *Neuropsychopharmacology* 39, 2835-2845.

Burke, R.E., and O'Malley, K. (2013). Axon degeneration in Parkinson's disease. *Experimental Neurology* 246, 72-83.

Burnstock, G. (2004). Cotransmission. *Current Opinion in Pharmacology* 4, 47-52.

- C -

Cachope, R., Mateo, Y., Mathur, B.N., Irving, J., Wang, H.-L., Morales, M., Lovinger, D.M., and Cheer, J.F. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. *Cell Rep.* 2, 33-41.

Caillé, I., Dumartin, B., and Bloch, B. (1996). Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. *Brain Res.* 730, 17-31.

Calabresi, P., Picconi, B., Tozzi, A., and Di Filippo, M. (2007). Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci.* 30, 211-219.

Carlezon, W.A., Devine, D.P., and Wise, R.A. (1995). Habit-forming actions of nomifensine in nucleus accumbens. *Psychopharmacology* 122, 194-197.

Cepeda, C., Galvan, L., Holley, S.M., Rao, S.P., Andre, 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. *J. Neurosci.* 33, 7393-7406.

Chadderton, P., Margrie, T.W., and Häusser, M. (2004). Integration of quanta in cerebellar granule cells during sensory processing. *Nature* 428, 856-860.

Chai, H., Diaz-Castro, B., Shigetomi, E., Monte, E., Oceau, J.C., Yu, X., Cohn, W., Rajendran, P.S., Vondriska, T.M., Whitelegge, J.P., et al. (2017). Neural circuit-specialized astrocytes: transcriptomic, proteomic, morphological, and functional evidence. *Neuron* 95, 531-549.e9.

Chan, C.S., Guzman, J.N., Ilijic, E., Mercer, J.N., Rick, C., Tkatch, T., Meredith, G.E., and Surmeier, D.J. (2007). 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* 447, 1081-1086.

Charara, A., Heilman, C., Levey, A., and Smith, Y. (1999). Pre- and postsynaptic localization of GABAB receptors in the basal ganglia in monkeys. *Neuroscience* 95, 127-140.

Charlety, P.J., Grenhoff, J., Chergui, K., De la Chapelle, B., Buda, M., Svensson, T.H., and Chouvet, G. (1991). Burst firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurenate. *Acta Physiol. Scand.* 142, 105-112.

Chartier-Harlin, M.-C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., Levecque, C., Larvor, L., Andrieux, J., Hulihan, M., et al. (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 364, 1167-1169.

Chazalon, M., Paredes-Rodriguez, E., Morin, S., Martinez, A., Cristóvão-Ferreira, S., Vaz, S., Sebastiao, A., Panatier, A., Boué-Grabot, E., Miguelez, C., et al. (2018). GAT-3 dysfunction generates tonic inhibition in external globus pallidus neurons in parkinsonian rodents. *Cell Rep.* 23, 1678-1690.

Chen, Y.Y., Sy, H.N., and Wu, S.L. (2008). Zolpidem improves akinesia, dystonia and dyskinesia in advanced Parkinson's disease. *J. Clin. Neurosci.* 15, 955-956.

Chéramy, A., Nieoullon, A., Glowinski, J. (1978) Gabaergic processes involved in the control of dopamine Release from nigrostriatal dopaminergic neurons in the cat. *European Journal of Pharmacology*, 48, 281-295.

- Chergui, K., Akaoka, H., Charléty, P.J., Saunier, C.F., Buda, M., and Chouvet, G. (1994). Subthalamic nucleus modulates burst firing of nigral dopamine neurones via NMDA receptors. *Neuroreport* 5, 1185–1188.
- Choi, D.-Y., Liu, M., Hunter, R.L., Cass, W.A., Pandya, J.D., Sullivan, P.G., Shin, E.-J., Kim, H.-C., Gash, D.M., and Bing, G. (2009). Striatal neuroinflammation promotes parkinsonism in rats. *PLoS One* 4, e5482.
- Chuhma, N., Zhang, H., Masson, J., Zhuang, X., Sulzer, D., Hen, R., and Rayport, S. (2004). Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses. *Journal of Neuroscience* 24, 972–981.
- Chuhma, N., Mingote, S., Yetnikoff, L., Kalmbach, A., Ma, T., Ztaou, S., Sienna, A.-C., Tepler, S., Poulin, J.-F., Ansorge, M., et al. (2018). Dopamine neuron glutamate cotransmission evokes a delayed excitation in lateral dorsal striatal cholinergic interneurons. *Elife* 7, e39786.
- Ciccarelli, A., Calza, A., Panzanelli, P., Concas, A., Giustetto, M., and Sassoè-Pognetto, M. (2012). Organization of GABAergic synaptic circuits in the rat ventral tegmental area. *PLoS One* 7, e46250.
- Clapp-Lilly, K.L., Roberts, R.C., Duffy, L.K., Irons, K.P., Hu, Y., and Drew, K.L. (1999). An ultrastructural analysis of tissue surrounding a microdialysis probe. *J. Neurosci. Methods* 90, 129–142.
- Clark, D., and Chiodo, L.A. (1988). Electrophysiological and pharmacological characterization of identified nigrostriatal and mesoaccumbens dopamine neurons in the rat. *Synapse* 2, 474–485.
- Clayton, D.F., and George, J.M. (1998). The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci.* 21, 249–254.
- Cobb, S.R., Buhl, E.H., Halasy, K., Paulsen, O., and Somogyi, P. (1995). Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378, 75–78.
- Connolly, B.S., and Lang, A.E. (2014). Pharmacological treatment of parkinson disease: a review. *J. Am. Med. Assoc.* 311, 1670–1683.
- Contant, C., Umbriaco, D., Garcia, S., Watkins, K.C., and Descarries, L. (1996). Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. *Neuroscience* 71, 937–947.
- Covelo, A., and Araque, A. (2016). Lateral regulation of synaptic transmission by astrocytes. *Neuroscience* 323, 62–66.
- Covelo, A., and Araque, A. (2018). Neuronal activity determines distinct gliotransmitter release from a single astrocyte. *Elife* 7, e32237.
- Cragg, S.J. (2003). Variable dopamine release probability and short-term plasticity between functional domains of the primate striatum. *J. Neurosci.* 23, 4378–4385.
- Cragg, S.J., and Rice, M.E. (2004). DANCING past the DAT at a DA synapse. *Trends Neurosci.* 27, 270–277.
- Crittenden, J.R., and Graybiel, A.M. (2011). Basal ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Front. Neuroanat.* 5, 59.

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, 238–242.

- D -

Danbolt, N.C. (2001). Glutamate uptake. *Prog. Neurobiol.* 65, 1–105.

Daniele, A., Albanese, A., Gainotti, G., Gregori, B., and Bartolomeo, P. (1997). Zolpidem in Parkinson's disease. *Lancet* 349, 1222–1223.

Darbin, O., and Wichmann, T. (2008). Effects of striatal gaba a-receptor blockade on striatal and cortical activity in monkeys. *J Neurophysiol* 99, 1294–1305.

Davies, A., Kadurin, I., Alvarez-Laviada, A., Douglas, L., Nieto-Rostro, M., Bauer, C.S., Pratt, W.S., and Dolphin, A.C. (2010). The alpha2delta subunits of voltage-gated calcium channels form GPI-anchored proteins, a posttranslational modification essential for function. *Proc. Natl. Acad. Sci. U. S. A.* 107, 1654–1659.

Day, J.J., Roitman, M.F., Wightman, R.M., and Carelli, R.M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.* 10, 1020–1028.

DeLong, M.R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* 13, 281–285.

Descarries, L., Watkins, K.C., Garcia, S., Bosler, O., and Doucet, G. (1996). Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. *J. Comp. Neurol.* 375, 167–186.

Descarries, L., Gisiger, V., and Steriade, M. (1997). Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* 53, 603–625.

Dodson, P.D., Dreyer, J.K., Jennings, K.A., Syed, E.C.J., Wade-Martins, R., Cragg, S.J., Bolam, J.P., and Magill, P.J. (2016). Representation of spontaneous movement by dopaminergic neurons is cell-type selective and disrupted in parkinsonism. *Proc. Natl. Acad. Sci. U. S. A.* 113, 2180–2188.

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. *J. Neurosci.* 30, 14610–14618.

Dolphin, A.C. (2012). Calcium channel auxiliary  $\alpha 2\delta$  and  $\beta$  subunits: trafficking and one step beyond. *Nat. Rev. Neurosci.* 13, 542–555.

Dolphin, A.C. (2016). Voltage-gated calcium channels and their auxiliary subunits: physiology and pathophysiology and pharmacology. *J. Physiol.* 594, 5369–5390.

Dorsey, E.R., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J.C., Ansha, M.G., Brayne, C., Choi, J.Y.J., Collado-Mateo, D., et al. (2018). Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study. *Lancet Neurol.* 17, 939–953.

Dreyer, J.K., Herrik, K.F., Berg, R.W., and Hounsgaard, J.D. (2010). Influence of Phasic and Tonic Dopamine Release on Receptor Activation. *J. Neurosci.* 30, 14273–14283.

Dubé, L., Smith, A.D., and Bolam, J.P. (1988). Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neostriatum. *J. Comp. Neurol.* 267, 455-471.

Durieux, P.F., Schiffmann, S.N., and de Kerchove d'Exaerde, A. (2012). Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. *EMBO J.* 31, 640-653.

Durkin, M.M., Smith, K.E., Borden, L.A., Weinshank, R.L., Branchek, T.A., and Gustafson, E.L. (1995). Localization of messenger RNAs encoding three GABA transporters in rat brain: an *in situ* hybridization study. *Mol. Brain Res.* 33, 7-21.

Dvorzhak, A., Myakhar, O., Unichenko, P., Kirmse, K., and Kirischuk, S. (2010). Estimation of ambient GABA levels in layer I of the mouse neonatal cortex in brain slices. *J. Physiol.* 588, 2351-2360.

Dvorzhak, A., Melnick, I., and Grantyn, R. (2018). Astrocytes and presynaptic plasticity in the striatum: evidence and unanswered questions. *Brain Res. Bull.* 136, 17-25.

Dymecki, S.M., Ray, R.S., and Kim, J.C. (2010). Mapping Cell fate and function using recombinase-based intersectional strategies. *Methods Enzymol.* 477, 183-213.

- E -

Ehringer, H., and Hornykiewicz, O. (1960). Verteilung von noradrenalin und dopamin (3-hydroxytyramin) im gehirn des menschen und ihr verhalten bei erkrankungen des extrapyramidalen systems. *Klin. Wochenschr.* 38, 1236-1239.

- F -

Farrant, M., and Nusser, Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of GABA<sub>A</sub> receptors. *Nat. Rev. Neurosci.* 6, 215-229.

Feng, L.R., Federoff, H.J., Vicini, S., and Maguire-Zeiss, K.A. (2010).  $\alpha$ -synuclein mediates alterations in membrane conductance: a potential role for  $\alpha$ -synuclein oligomers in cell vulnerability. *Eur. J. Neurosci.* 32, 10-17.

Le Feuvre, Y., Fricker, D., and Leresche, N. (1997). GABA<sub>A</sub> receptor-mediated IPSCs in rat thalamic sensory nuclei: patterns of discharge and tonic modulation by GABA<sub>B</sub> autoreceptors. *J. Physiol.* 502, 91-104.

Ficková, M., Dahmen, N., Fehr, C., and Hiemke, C. (1999). Quantitation of GABA transporter 3 (GAT3) mRNA in rat brain by competitive RT-PCR. *Brain Res. Protoc.* 4, 341-350.

Field, M.J., Cox, P.J., Stott, E., Melrose, H., Offord, J., Su, T.Z., Bramwell, S., Corradini, L., England, S., Winks, J., Kinloch, R.A., et al. (2006). Identification of the  $\alpha$ 2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc. Natl. Acad. Sci. U. S. A.* 103, 17537-17542.

Fisher, S.D., Robertson, P.B., Black, M.J., Redgrave, P., Sagar, M.A., Abraham, W.C., and Reynolds, J.N.J. (2017). Reinforcement determines the timing dependence of corticostriatal synaptic plasticity *in vivo*. *Nat. Commun.* 8, 334.

Floresco, S.B., West, A.R., Ash, B., Moore, H., and Grace, A.A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.* 6, 968-973.

Freeze, B.S., Kravitz, A. V, Hammack, N., Berke, J.D., and Kreitzer, A.C. (2013). Control of basal ganglia output by direct and indirect pathway projection neurons. *J. Neurosci.* 33, 18531–18539.

Fuchs, J., Nilsson, C., Kachergus, J., Munz, M., Larsson, E.M., Schüle, B., Langston, J.W., Middleton, F.A., Ross, O.A., Hulihan, M., et al. (2007). Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. *Neurology* 68, 916–922.

Fujishiro, H. (2014). Effects of gabapentin enacarbil on restless legs syndrome and leg pain in dementia with Lewy bodies. *Psychogeriatrics* 14, 132–134.

- G -

Gage, G.J., Stoetznner, C.R., Wiltschko, A.B., and Berke, J.D. (2010). Selective activation of striatal fast-spiking interneurons during choice execution. *Neuron* 67, 466–479.

Galter, D., Buervenich, S., Carmine, A., Anvret, M., and Olson, L. (2003). ALDH1 mRNA: presence in human dopamine neurons and decreases in substantia nigra in Parkinson's disease and in the ventral tegmental area in schizophrenia. *Neurobiol. Dis.* 14, 637–647.

Gerfen, C.R. (1985). The neostriatal mosaic. I. compartmental organization of projections from the striatum to the substantia nigra in the rat. *J. Comp. Neurol.* 236, 454–476.

Gerfen, C.R., and Bolam, J.P. (2010). The neuroanatomical organization of the basal ganglia in *Handbook of Behavioral Neuroscience* 20, 3–28.

Gerfen, C.R., and Surmeier, D.J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34, 441–466.

Gerfen, C., Engber, T., Mahan, L., Susel, Z., Chase, T., Monsma, F., and Sibley, D. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250, 1429–1432.

Gerfen, C.R., Baimbridge, K.G., and Thibault, J. (1987). The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. *J. Neurosci.* 7, 3935–3944.

Gertler, T.S., Chan, C.S., and Surmeier, D.J. (2008). Dichotomous anatomical properties of adult striatal medium spiny neurons. *J. Neurosci.* 28, 10814–10824.

Giorguieff, M.F., Kemel, M.L., Glowinski, J., Besson, M.J. (1978) S stimulation of dopamine release by GABA in rat striatal slices. *Brain Research* 139, 115–130.

Gispert, S., Del Turco, D., Garrett, L., Chen, A., Bernard, D.J., Hamm-Clement, J., Korf, H.-W., Deller, T., Braak, H., Auburger, G., et al. (2003). Transgenic mice expressing mutant A53T human alpha-synuclein show neuronal dysfunction in the absence of aggregate formation. *Mol. Cell. Neurosci.* 24, 419–429.

Gittis, A.H., Nelson, A.B., Thwin, M.T., Palop, J.J., and Kreitzer, A.C. (2010). Distinct roles of gabaergic interneurons in the regulation of striatal output pathways. *J. Neurosci.* 30, 2223–2234.

Gittis, A.H., Leventhal, D.K., Fensterheim, B.A., Pettibone, J.R., Berke, J.D., and Kreitzer, A.C. (2011). Selective inhibition of striatal fast-spiking interneurons causes dyskinesias. *J. Neurosci.* 31, 15727–15731.

- Gokce, O., Neff, N.F., Fuccillo, M. V., Südhof, T.C., Treutlein, B., Quake, S.R., Malenka, R.C., Stanley, G.M., Rothwell, P.E., and Camp, J.G. (2016). Cellular taxonomy of the mouse striatum as revealed by single-cell rna-seq. *Cell Rep.* 16, 1126-1137.
- Gomez, J.A., Perkins, J.M., Beaudoin, G.M., Cook, N.B., Quraishi, S.A., Szoeki, E.A., Thangamani, K., Tschumi, C.W., Wanat, M.J., Maroof, A.M., et al. (2019). Ventral tegmental area astrocytes orchestrate avoidance and approach behavior. *Nat. Commun.* 10, 1455.
- Gonon, F., Buda, M., Cespuglio, R., Jouvet, M., and Pujol, J.-F. (1980). In vivo electrochemical detection of catechols in the neostriatum of anaesthetized rats: dopamine or DOPAC? *Nature* 286, 902-904.
- Goubard, V., Fino, E., and Venance, L. (2011). Contribution of astrocytic glutamate and GABA uptake to corticostriatal information processing. *J. Physiol.* 589, 2301-2319.
- Grace, A.A., and Bunney, B.S. (1984a). The control of firing pattern in nigral dopamine neurons: single spike firing. *J. Neurosci.* 4, 2866-2876.
- Grace, A.A., and Bunney, B.S. (1984b). The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.* 4, 2877-2890.
- Gras, C., Amilhon, B., Lepicard, È.M., Poirel, O., Vinatier, J., Herbin, M., Dumas, S., Tzavara, E.T., Wade, M.R., Nomikos, G.G., et al. (2008). The vesicular glutamate transporter VGLUT3 synergizes striatal acetylcholine tone. *Nat. Neurosci.* 11, 292-300.
- Graveland, G.A., and DiFiglia, M. (1985). The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum. *Brain Res.* 327, 307-311.
- Graybiel, A.M., and Ragsdale, C.W. (1978). Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. *Proc. Natl. Acad. Sci.* 75, 5723-5726.
- Graybiel, A.M., Aosaki, T., Flaherty, A.W., and Kimura, M. (1994). The basal ganglia and adaptive motor control. *Science* 265, 1826-1831.
- Grossman, Y., Parnas, I., and Spira, M.E. (1979a). Differential conduction block in branches of a bifurcating axon. *J. Physiol.* 295, 283-305.
- Grossman, Y., Parnas, I., and Spira, M.E. (1979b). Mechanisms involved in differential conduction of potentials at high frequency in a branching axon. *J. Physiol.* 295, 307-322.
- Guo, Q., Wang, D., He, X., Feng, Q., Lin, R., Xu, F., Fu, L., and Luo, M. (2015). Whole-brain mapping of inputs to projection neurons and cholinergic interneurons in the dorsal striatum. *PLoS One* 10, e0123381.
- Guo, S., Huang, J., Jiang, H., Han, C., Li, J., Xu, X., Zhang, G., Lin, Z., Xiong, N., and Wang, T. (2017). Restless legs syndrome: from pathophysiology to clinical diagnosis and management. *Front. Aging Neurosci.* 9, 171.
- Guzmán, J.N., Hernández, A., Galarraga, E., Tapia, D., Laville, A., Vergara, R., Aceves, J., and Vargas, J. (2003). Dopaminergic modulation of axon collaterals interconnecting spiny neurons of the rat striatum. *J. Neurosci.* 23, 8931-8940.

- H -

Halassa, M.M., and Haydon, P.G. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu. Rev. Physiol* 72, 335-355.

- Hartung, H., Threlfell, S., and Cragg, S.J. (2011). Nitric oxide donors enhance the frequency dependence of dopamine release in nucleus accumbens. *Neuropsychopharmacology* 36, 1811–1822.
- Heien, M.L.A.V., Phillips, P.E.M., Stuber, G.D., Seipel, A.T., and Wightman, R.M. (2003). Overoxidation of carbon-fiber microelectrodes enhances dopamine adsorption and increases sensitivity. *Analyst* 128, 1413–1419.
- Heiman, M., Kulicke, R., Fenster, R.J., Greengard, P., and Heintz, N. (2014). Cell type-specific mRNA purification by translating ribosome affinity purification (TRAP). *Nat. Protoc.* 9, 1282–1291.
- Henselmans, J.M.L., and Stoof, J.C. (1991). Regional differences in the regulation of acetylcholine release upon D2 dopamine and N-methyl-D-aspartate receptor activation in rat nucleus accumbens and neostriatum. *Brain Res.* 566, 1–7.
- Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., and Surmeier, D.J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type  $\text{Ca}^{2+}$  currents and excitability via a novel PLC[ $\beta$ ]1-IP3-calcineurin-signaling cascade. *J. Neurosci.* 20, 8987–8995.
- Hernández-López, S., Bargas, J., Surmeier, D.J., Reyes, A., and Galarraga, E. (1997). D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type  $\text{Ca}^{2+}$  conductance. *J. Neurosci.* 17, 3334–3342.
- Hersch, S.M., Yi, H., Heilman, C.J., Edwards, R.H., and Levey, A.I. (1997). Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J. Comp. Neurol.* 388, 211–227.
- Hisahara, S., and Shimohama, S. (2010). Toxin-induced and genetic animal models of Parkinson's disease. *Parkinsons. Dis.* 2011, 951709.
- Hjorth, J., Blackwell, K.T., and Kotaleski, J.H. (2009). Gap junctions between striatal fast-spiking interneurons regulate spiking activity and synchronization as a function of cortical activity. *J. Neurosci.* 29, 5276–5286.
- Hnasko, T.S., Chuhma, N., Zhang, H., Goh, G.Y., Sulzer, D., Palmiter, R.D., Rayport, S., and Edwards, R.H. (2010). Vesicular glutamate transport promotes dopamine storage and glutamate corelease in vivo. *Neuron* 65, 643–656.
- Holly, E.N., Davatolhagh, M.F., Choi, K., Alabi, O.O., Vargas Cifuentes, L., and Fuccillo, M. V. (2019). Striatal low-threshold spiking interneurons regulate goal-directed learning. *Neuron* 103, 92–101.
- Hoover, D.B., Muth, E.A., and Jacobowitz, D.M. (1978). A mapping of the distribution of acetylcholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. *Brain Res.* 153, 295–306.
- Hoppa, M.B., Lana, B., Margas, W., Dolphin, A.C., and Ryan, T.A. (2012).  $\alpha 2\delta$  expression sets presynaptic calcium channel abundance and release probability. *Nature* 486, 122–125.
- Hornykiewicz, O. (1998). Biochemical aspects of Parkinson's disease. *Neurology* 51, S2–9.
- Howe, M.W., and Dombeck, D.A. (2016). Rapid signalling in distinct dopaminergic axons during locomotion and reward. *Nature* 535, 505–510.

Hunter, R.L., Cheng, B., Choi, D.-Y., Liu, M., Liu, S., Cass, W.A., and Bing, G. (2009). Intrastriatal lipopolysaccharide injection induces parkinsonism in C57/B6 mice. *J. Neurosci. Res.* 87, 1913–1921.

Huntsman, M.M., Porcello, D.M., Homanics, G.E., DeLorey, T.M., and Huguenard, J.R. (1999). Reciprocal inhibitory connections and network synchrony in the mammalian thalamus. *Science* 283, 541–543.

Hyland, B., Reynolds, J.N., Hay, J., Perk, C., and Miller, R. (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114, 475–492.

- I -

Iacopino, A., Christakos, S., German, D., Sonsalla, P.K., and Altar, C.A. (1992). Calbindin-D28K-containing neurons in animal models of neurodegeneration: possible protection from excitotoxicity. *Mol. Brain Res.* 13, 251–261.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Rev.* 56, 27–78.

Ingram, S.L., Prasad, B.M., and Amara, S.G. (2002). Dopamine transporter-mediated conductances increase excitability of midbrain dopamine neurons. *Nat. Neurosci.* 5, 971–978.

Izzo, P.N., and Bolam, J.P. (1988). Cholinergic synaptic input to different parts of spiny striatonigral neurons in the rat. *J. Comp. Neurol.* 269, 219–234.

- J -

Jackson, M.B., and Zhang, S.J. (1995). Action potential propagation and propagation block by GABA in rat posterior pituitary nerve terminals. *J. Physiol.* 483, 597–611.

Jacob, P. F., Vaz, S. H., Ribeiro, J. A., & Sebastiao, A. M. (2014). P2Y1 receptor inhibits GABA transport through a calcium signalling-dependent mechanism in rat cortical astrocytes. *Glia* 62, 1211–1226.

Janezic, S., Threlfell, S., Dodson, P.D., Dowie, M.J., Taylor, T.N., Potgieter, D., Parkkinen, L., Senior, S.L., Anwar, S., Ryan, B., et al. (2013). Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model. *Proc. Natl. Acad. Sci.* 110, 4016–4025.

Janssen, M.J., Ade, K.K., Fu, Z., and Vicini, S. (2009). Dopamine modulation of GABA tonic conductance in striatal output neurons. *J. Neurosci.* 29, 5116–5126.

Jennings, K.A., Platt, N.J., and Cragg, S.J. (2015). The impact of a parkinsonian lesion on dynamic striatal dopamine transmission depends on nicotinic receptor activation. *Neurobiol. Dis.* 82, 262–268.

Jin, X.-T., Galvan, A., Wichmann, T., and Smith, Y. (2011). Localization and function of GABA transporters GAT-1 and GAT-3 in the basal ganglia. *Front. Syst. Neurosci.* 5, 1–10.

Jones, I.W., Bolam, J.P., and Wonnacott, S. (2001). Presynaptic localisation of the nicotinic acetylcholine receptor beta2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. *J. Comp. Neurol.* 439, 235–247.

- K -

Kaesler, P.S., and Regehr, W.G. (2013). Molecular Mechanisms for synchronous, asynchronous, and spontaneous neurotransmitter release. *Annu. Rev. Physiol.* 76, 333–363.

- Kalivas, P.W., and McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology (Berl)* 168, 44-56.
- Kawaguchi, Y. (1993). Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J. Neurosci.* 13, 4908-4923.
- Kawaguchi, Y., Wilson, C.J., and Emson, P.C. (1990). Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. *J. Neurosci.* 10, 3421-3438.
- Kawano, M., Kawasaki, A., Sakata-Haga, H., Fukui, Y., Kawano, H., Nogami, H., and Hisano, S. (2006). Particular subpopulations of midbrain and hypothalamic dopamine neurons express vesicular glutamate transporter 2 in the rat brain. *J. Comp. Neurol.* 498, 581-592.
- Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W., and Glabe, C.G. (2003). Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300, 486-489.
- Kemp, J.M., and Powell, T.P. (1971a). The structure of the caudate nucleus of the cat: light and electron microscopy. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 262, 383-401.
- Kemp, J.M., and Powell, T.P. (1971b). The synaptic organization of the caudate nucleus. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 262, 383-401.
- Khakh, B.S., and Sofroniew, M. V (2015). Diversity of astrocyte functions and phenotypes in neural circuits. *Nat. Neurosci.* 18, 942-952.
- Kim, J.I., Ganesan, S., Luo, S.X., Wu, Y.W., Park, E., Huang, E.J., Chen, L., and Ding, J.B. (2015). Aldehyde dehydrogenase 1a1 mediates a GABA synthesis pathway in midbrain dopaminergic neurons. *Science* 350, 102-106.
- Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T.E., Muzyczka, N., Mandel, R.J., and Björklund, A. (2002). Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *J. Neurosci.* 22, 2780-2791.
- Kirmse, K., and Kirischuk, S. (2006). Ambient gaba constrains the strength of gabaergic synapses at cajal-retzius cells in the developing visual cortex. *J. Neurosci.* 26, 4216-4227.
- Kirmse, K., Dvorchak, A., Kirischuk, S., and Grantyn, R. (2008). GABA transporter 1 tunes GABAergic synaptic transmission at output neurons of the mouse neostriatum. *J. Physiol.* 586, 5665-5678.
- Kirmse, K., Kirischuk, S., and Grantyn, R. (2009). Role of GABA transporter 3 in GABAergic synaptic transmission at striatal output neurons. *Synapse* 63, 921-929.
- Kita, H., and Kitai, S.T. (1988). Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. *Brain Res.* 447, 346-352.
- Kita, H., Kosaka, T., and Heizmann, C.W. (1990). Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. *Brain Res.* 536, 1-15.
- Klugbauer, N., Lacinová, L., Marais, E., Hobom, M., and Hofmann, F. (1999). Molecular diversity of the calcium channel alpha2delta subunit. *J. Neurosci.* 19, 684-691.
- Knowlton, B.J., Mangels, J.A., and Squire, L.R. (1996). A neostriatal habit learning system in humans. *Science* 273, 1399-1402.
- Konnova, E.A., and Swanberg, M. (2018). Animal models of Parkinson's disease in Parkinson's Disease: Pathogenesis and Clinical Aspects. 83-106.

- Koos, T., Tepper, J.M., and Wilson, C.J. (2004). Comparison of IPSCs evoked by spiny and fast-spiking neurons in the neostriatum. *J. Neurosci.* 24, 7916–7922.
- Kosillo, P., Zhang, Y.-F., Threlfell, S., and Cragg, S.J. (2016). Cortical control of striatal dopamine transmission via striatal cholinergic interneurons. *Cereb. Cortex* 26, 4160–4169.
- Kravitz, A. V., Freeze, B.S., Parker, P.R.L., 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, 622–626.
- Krebs, M.O., Kemel, M.L., Gauchy, C., Desban, M., Glowinski, J. (1993). Local GABAergic regulation of the N-methyl-D-aspartate-evoked release of dopamine is more prominent in striosomes than in matrix of the rat striatum. *Neuroscience* 57, 249–260.
- Kreitzer, A.C., and Malenka, R.C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron* 60, 543–554.
- Kubota, Y., and Kawaguchi, Y. (2000). Dependence of GABAergic synaptic areas on the interneuron type and target size. *J. Neurosci.* 20, 375–386.
- Kullmann, D.M., Ruiz, A., Rusakov, D.M., Scott, R., Semyanov, A., and Walker, M.C. (2005). Presynaptic, extrasynaptic and axonal GABAA receptors in the CNS: where and why? *Prog. Biophys. Mol. Biol.* 87, 33–46.
- Kurz, A., Double, K.L., Lastres-Becker, I., Tozzi, A., Tantucci, M., Bockhart, V., Bonin, M., García-Arencibia, M., Nuber, S., Schlaudraff, F., et al. (2010). A53T-alpha-synuclein overexpression impairs dopamine signaling and striatal synaptic plasticity in old mice. *PLoS One* 5, e11464.
- Kuter, K., Olech, L., and Głowacka, U. (2018). Prolonged dysfunction of astrocytes and activation of microglia accelerate degeneration of dopaminergic neurons in the rat substantia nigra and block compensation of early motor dysfunction induced by 6-OHDA. *Mol. Neurobiol.* 55, 3049–3066.

- L -

- Lacey, M.G., Mercuri, N.B., and North, R.A. (1988). On the potassium conductance increase activated by GABAB and dopamine D2 receptors in rat substantia nigra neurones. *J. Physiol.* 401, 437–453.
- Lacey, M.G., Mercuri, N.B., and North, R.A. (1989). Two Cell Types in Rat Substantia Nigra Zona Compacta Distinguished by Membrane Properties and the Actions of Dopamine and Opioids. *J. Neurosci.* 9, 1233–1241.
- Lashuel, H.A., Hartley, D., Petre, B.M., Walz, T., and Lansbury, P.T. (2002). Amyloid pores from pathogenic mutations. *Nature* 418, 291–291.
- Laviv, T., Riven, I., Dolev, I., Vertkin, I., Balana, B., Slesinger, P.A., and Slutsky, I. (2010). Basal GABA regulates GABABR conformation and release probability at single hippocampal synapses. *Neuron* 67, 253–267.
- Lavoie, B., and Parent, A. (1991). Dopaminergic neurons expressing calbindin in normal and parkinsonian monkeys. *Neuroreport* 2, 601–604.
- Levey, A.I., Hersch, S.M., Rye, D.B., Sunahara, R.K., Niznik, H.B., Kitt, C.A., Price, D.L., Maggio, R., Brann, M.R., and Ciliax, B.J. (1993). Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 90, 8861–8865.

- Li, G., Shao, C., Chen, Q., Wang, Q., and Yang, K. (2017). Accumulated GABA activates presynaptic GABAB receptors and inhibits both excitatory and inhibitory synaptic transmission in rat midbrain periaqueductal gray. *Neuroreport* 28, 313-318.
- Liddel, S.A., and Sofroniew, M. V. (2019). Astrocytes usurp neurons as a disease focus. *Nat. Neurosci.* 22, 512-513.
- Liddel, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.S., Peterson, T.C., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481-487.
- Lindgren, N., Xu, Z.Q.D., Herrera-Marschitz, M., Haycock, J., Hökfelt, T., and Fisone, G. (2001). Dopamine D 2 receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. *Eur. J. Neurosci.* 13, 773-780.
- Liu, C., Kershberg, L., Wang, J., Schneeberger, S., and Kaeser, P.S. (2018). Dopamine secretion is mediated by sparse active zone-like release sites. *Cell* 172, 706-718.
- Liu, G., Yu, J., Ding, J., Xie, C., Sun, L., Rudenko, I., Zheng, W., Sastry, N., Luo, J., Rudow, G., et al. (2014). Aldehyde dehydrogenase 1 defines and protects a nigrostriatal dopaminergic neuron subpopulation. *J. Clin. Invest.* 124, 3032-3046.
- Liu, J., Zhou, Z., Hodgkinson, C.A., Yuan, Q., Shen, P.-H., Mulligan, C.J., Wang, A., Gray, R.R., Roy, A., Virkkunen, M., et al. (2011). Haplotype-Based study of the association of alcohol-metabolizing genes with alcohol dependence in four independent populations. *alcohol. Clin. Exp. Res.* 35, 304-316.
- Lokwan, S.J.A., Overton, P.G., Berry, M.S., and Clark, D. (1999). Stimulation of the pedunculopontine tegmental nucleus in the rat produces burst firing in A9 dopaminergic neurons. *Neuroscience* 92, 245-254.
- Lopes, E.F., Roberts, B.M., Siddorn, R.E., Clements, M.A., and Cragg, S.J. (2018). Inhibition of nigrostriatal dopamine release by striatal GABA<sub>A</sub> and GABA<sub>B</sub> receptors. *J. Neurosci.* 39, 1058-1056.
- Lozovaya, N., Eftekhari, S., Cloarec, R., Gouty-Colomer, L.A., Dufour, A., Riffault, B., Billon-Grand, M., Pons-Bennaceur, A., Oumar, N., Burnashev, N., et al. (2018). GABAergic inhibition in dual-transmission cholinergic and GABAergic striatal interneurons is abolished in Parkinson disease. *Nat. Commun.* 9, 1422.
- Lundblad, M., Decressac, M., Mattsson, B., and Björklund, A. (2012). Impaired neurotransmission caused by overexpression of  $\alpha$ -synuclein in nigral dopamine neurons. *Proc. Natl. Acad. Sci. U. S. A.* 109, 3213-3219.
- Luo, R., Partridge, J.G., and Vicini, S. (2013). Distinct roles of synaptic and extrasynaptic GABA<sub>A</sub> receptors in striatal inhibition dynamics. *Front. Neural Circuits* 7, 186.
- Lüscher, H.R., and Shiner, J.S. (1990). Computation of action potential propagation and presynaptic bouton activation in terminal arborizations of different geometries. *Biophys. J.* 58, 1377-1388.
- M -
- Mahon, S., Vautrelle, N., Pezard, L., Slaght, S.J., Deniau, J.-M., Chouvet, G., and Charpier, S. (2006). Distinct patterns of striatal medium spiny neuron activity during the natural sleep-wake cycle. *J. Neurosci.* 26, 12587-12595.

- Mapelli, L., Rossi, P., Nieuws, T., and D'Angelo, E. (2009). Tonic activation of GABA<sub>B</sub> receptors reduces release probability at inhibitory connections in the cerebellar glomerulus. *J. Neurophysiol.* 101, 3089–3099.
- Marchitti, S.A., Deitrich, R.A., and Vasiliou, V. (2007). Neurotoxicity and metabolism of the catecholamine-derived 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde: the role of aldehyde dehydrogenase. *Pharmacol. Rev.* 59, 125–150.
- Marques, O., and Outeiro, T.F. (2012). Alpha-synuclein: from secretion to dysfunction and death. *Cell Death Dis.* 3, e350.
- Martel, P., Leo, D., Fulton, S., Bérard, M., and Trudeau, L.-E. (2011). Role of Kv1 potassium channels in regulating dopamine release and presynaptic D2 receptor function. *PLoS One* 6, e20402.
- Martin-Fernandez, M., Jamison, S., Robin, L.M., Zhao, Z., Martin, E.D., Aguilar, J., Benneyworth, M.A., Marsicano, G., and Araque, A. (2017). Synapse-specific astrocyte gating of amygdala-related behavior. *Nat. Neurosci.* 20, 1540–1548.
- Martín, R., Bajo-Grañeras, R., Moratalla, R., Perea, G., and Araque, A. (2015). Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. *Science* 349, 730–734.
- Mason, B.J., Quello, S., and Shadan, F. (2018). Gabapentin for the treatment of alcohol use disorder. *Expert Opin. Investig. Drugs* 27, 113–124.
- Matamalas, M., Götz, J., Bertran-Gonzalez, J., Graybiel, A., Parent, A., Hazrati, L., Gerfen, C., Surmeier, D., McGeer, P., McGeer, E., et al. (2016). Quantitative imaging of cholinergic interneurons reveals a distinctive spatial organization and a functional gradient across the mouse striatum. *PLoS One* 11, e0157682.
- Matsuda, W., Furuta, T., Nakamura, K.C., Hioki, H., Fujiyama, F., Arai, R., and Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J. Neurosci.* 29, 444–453.
- McCaffery, P., and Dräger, U.C. (1994). High levels of a retinoic acid-generating dehydrogenase in the meso-telencephalic dopamine system. *Proc. Natl. Acad. Sci. U. S. A.* 91, 7772–7776.
- McGeer, P.L., and McGeer, E.G. (2008). Glial reactions in Parkinson's disease. *Mov. Disord.* 23, 474–483.
- Mcgregor, M.M., and Nelson, A.B. (2019). Circuit mechanisms of Parkinson's disease. *Neuron* 101, 1042–1056.
- Melchior, J.R., Ferris, M.J., Stuber, G.D., Riddle, D.R., and Jones, S.R. (2015). Optogenetic versus electrical stimulation of dopamine terminals in the nucleus accumbens reveals local modulation of presynaptic release. *J. Neurochem.* 134, 833–844.
- Mendez, J.A., Bourque, M.-J., Bo, G.D., Bourdeau, M.L., Danik, M., Williams, S., Lacaille, J.-C., and Trudeau, L.-E. (2008). Developmental and target-dependent regulation of vesicular glutamate transporter expression by dopamine neurons. *J. Neurosci.* 28, 6309–6318.
- Michael, D.J., and Wightman, R.M. (1999). Electrochemical monitoring of biogenic amine neurotransmission in real time. *J. Pharm. Biomed. Anal.* 19, 33–46.

Mingote, S., Chuhma, N., Kalmbach, A., Thomsen, G.M., Wang, Y., Mihali, A., Sferrazza, C., Zucker-Scharff, I., Siena, A.-C., Welch, M.G., et al. (2017). Dopamine neuron dependent behaviors mediated by glutamate cotransmission. *Elife* 6, e27566.

Mirenowicz, J., and Schultz, W. (1994). Importance of unpredictability for reward responses in primate dopamine neurons. *J. Neurophysiol.* 72, 1024-1027.

Mirenowicz, J., and Schultz, W. (1996). Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* 379, 449-451.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., and Caron, M.G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189-225.

Mohebi, A., Pettibone, J.R., Hamid, A.A., Wong, J.-M.T., Vinson, L.T., Patriarchi, T., Tian, L., Kennedy, R.T., and Berke, J.D. (2019). Dissociable dopamine dynamics for learning and motivation. *Nature* 570, 65-70.

Le Moine, C., Normand, E., and Bloch, B. (1991). Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. *Proc. Natl. Acad. Sci. U. S. A.* 88, 4205-4209.

Morales, I., Sanchez, A., Rodriguez-Sabate, C., and Rodriguez, M. (2016). The astrocytic response to the dopaminergic denervation of the striatum. *J. Neurochem.* 139, 81-95.

Morris, G., Arkadir, D., Nevet, A., Vaadia, E., and Bergman, H. (2004). Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* 43, 133-143.

Mozrzymas, J.W., Zarnowska, E.D., Pytel, M., Mercik, K., and Zarnowska, E.D. (2003). Modulation of GABA(A) receptors by hydrogen ions reveals synaptic GABA transient and a crucial role of the desensitization process. *J. Neurosci.* 23, 7981-7992.

## - N -

Nagai, J., Rajbhandari, A.K., Gangwani, M.R., Hachisuka, A., Coppola, G., Masmanidis, S.C., Fanselow, M.S., and Khakh, B.S. (2019). Hyperactivity with disrupted attention by activation of an astrocyte synaptogenic cue. *Cell* 177, 1280-1292.

Nair-Roberts, R.G., Chatelain-Badie, S.D., Benson, E., White-Cooper, H., Bolam, J.P., and Ungless, M.A. (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience* 152, 1024-1031.

Nauta, H.J.W., Pritz, M.B., and Lasek, R.J. (1974). Afferents to the rat caudoputamen studied with horseradish peroxidase. An evaluation of a retrograde neuroanatomical research method. *Brain Res.* 67, 219-238.

Nauta, W.J.H., Smith, G.P., Faull, R.L.M., and Domesick, V.B. (1978). Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience* 3, 385-401.

Nelson, A.B., and Kreitzer, A.C. (2014). Reassessing models of basal ganglia function and dysfunction. *Annu. Rev. Neurosci.* 37, 117-135.

Ng, C.H., Wang, X.S., and Ong, W.Y. (2000). A light and electron microscopic study of the GABA transporter GAT-3 in the monkey basal ganglia and brainstem. *J. Neurocytol.* 29, 595-603.

Nicholson, L.F.B., Faull, R.L.M., Waldvogel, H.J., and Dragunow, M. (1992). The regional, cellular and subcellular localization of GABAA/benzodiazepine receptors in the substantia nigra of the rat. *Neuroscience* 50, 355-370.

Nisenbaum, E., and Wilson, C. (1995). Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *J. Neurosci.* 15, 4449-4463.

Nusbaum, M.P., Blitz, D.M., Swensen, A.M., Wood, D., and Marder, E. (2001). The roles of co-transmission in neural network modulation. *Trends Neurosci.* 24, 146-154.

Nusser, Z., Kay, L.M., Laurent, G., Homanics, G.E., and Mody, I. (2001). Disruption of GABA<sub>A</sub> receptors on GABAergic interneurons leads to increased oscillatory power in the olfactory bulb network. *J. Neurophysiol.* 86, 2823-2833.

- O -

Obeso, J.A., Rodriguez-Oroz, M.C., Stamelou, M., Bhatia, K.P., and Burn, D.J. (2014). The expanding universe of disorders of the basal ganglia. *Lancet* 384, 523-531.

Olson, W.L., Gruenthal, M., Mueller, M.E., and Olson, W.H. (1997). Gabapentin for Parkinsonism: A double-blind, placebo-controlled, crossover trial. *Am. J. Med.* 102, 60-66.

Oorschot, D.E. (1996). Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: A stereological study using the cavalieri and optical disector methods. *J. Comp. Neurol.* 366, 580-599.

- P -

Packard, M.G., and McGaugh, J.L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol. Learn. Mem.* 65, 65-72.

Packard, M.G., and McGaugh, J.L. (1999). Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning. *Proc. Natl. Acad. Sci.* 96, 12881-12886.

Pakhotin, P., and Bracci, E. (2007). Cholinergic interneurons control the excitatory input to the striatum. *J. Neurosci.* 27, 391-400.

Paladini, C.A., and Tepper, J.M. (1999). GABAA and GABAB antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. *Synapse* 32, 165-176.

Pancani, T., Bolarinwa, C., Smith, Y., Lindsley, C.W., Conn, P.J., and Xiang, Z. (2014). M4 mAChR-mediated modulation of glutamatergic transmission at corticostriatal synapses. *ACS Chem. Neurosci.* 5, 318-324.

Park, A., and Stacy, M. (2009). Non-motor symptoms in Parkinson's disease. *J. Neurol.* 256, 293-298.

Patriarchi, T., Cho, J.R., Merten, K., Howe, M.W., Marley, A., Xiong, W.-H., Folk, R.W., Broussard, G.J., Liang, R., Jang, M.J., et al. (2018). Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. *Science* 360, eaat4422.

Patton, M.H., Roberts, B.M., Lovinger, D.M., and Mathur, B.N. (2016). Ethanol disinhibits dorsolateral striatal medium spiny neurons through activation of a presynaptic delta opioid receptor. *Neuropsychopharmacology* 41, 1831-1840.

- Patton, M.H., Padgett, K.E., McKeon, P.N., Qadir, H., Patton, M.S., Mu, C., Roberts, B.M., and Mathur, B.N. (2019). TrkB-dependent disinhibition of the nucleus accumbens is enhanced by ethanol. *Neuropsychopharmacology* 44, 1114-1122.
- Paulsen, R.E., Contestabile, A., Villani, L., and Fonnum, F. (1987). An in vivo model for studying function of brain tissue temporarily devoid of glial cell metabolism: the use of fluorocitrate. *J. Neurochem.* 48, 1377-1385.
- Paumier, K.L., Luk, K.C., Manfredsson, F.P., Kanaan, N.M., Lipton, J.W., Collier, T.J., Steece-Collier, K., Kemp, C.J., Celano, S., Schulz, E., et al. (2015). Intrastriatal injection of pre-formed mouse  $\alpha$ -synuclein fibrils into rats triggers  $\alpha$ -synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiol. Dis.* 82, 185-199.
- Perez, M.F., White, F.J., and Hu, X.-T. (2006). Dopamine D2 receptor modulation of K<sup>+</sup> channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. *J. Neurophysiol.* 96, 2217-2228.
- Phillips, P.E.M., Stuber, G.D., Heien, M.L.A. V, Wightman, R.M., and Carelli, R.M. (2003). Subsecond dopamine release promotes cocaine seeking. *Nature* 422, 614-618.
- Pissadaki, E.K., and Bolam, J.P. (2013). The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. *Front. Comput. Neurosci.* 7, 13.
- Pitman, K.A., Puil, E., and Borgland, S.L. (2014). GABA<sub>B</sub> modulation of dopamine release in the nucleus accumbens core. *Eur. J. Neurosci.* 40, 3472-3480.
- Planert, H., Szydlowski, S.N., Hjorth, J.J.J., Grillner, S., and Silberberg, G. (2010). Dynamics of synaptic transmission between fast-spiking interneurons and striatal projection neurons of the direct and indirect pathways. *J. Neurosci.* 30, 3499-3507.
- Planert, H., Berger, T.K., and Silberberg, G. (2013). Membrane properties of striatal direct and indirect pathway neurons in mouse and rat slices and their modulation by dopamine. *PLoS One* 8, e57054.
- Platt, N.J., Gispert, S., Auburger, G., and Cragg, S.J. (2012). Striatal dopamine transmission is subtly modified in human A53T $\alpha$ -synuclein overexpressing mice. *PLoS One* 7, e36397.
- Plenz, D., and Kitai, S.T. (1998). Up and down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fast-spiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures. *J. Neurosci.* 18, 266-283.
- Plotkin, J.L., Day, M., and Surmeier, D.J. (2011). Synaptically driven state transitions in distal dendrites of striatal spiny neurons. *Nat. Neurosci.* 14, 881-888.
- Povlock, S.L., and Schenk, J.O. (2002). A Multisubstrate Kinetic Mechanism of Dopamine Transport in the Nucleus Accumbens and Its Inhibition by Cocaine. *J. Neurochem.* 69, 1093-1105.
- Puopolo, M., Raviola, E., and Bean, B.P. (2007). Roles of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. *J. Neurosci.* 27, 645-656.

- Q -

- Quinn, J.J., Pittenger, C., Lee, A.S., Pierson, J.L., and Taylor, J.R. (2013). Striatum-dependent habits are insensitive to both increases and decreases in reinforcer value in mice. *Eur. J. Neurosci.* 37, 1012-1021.

## - R -

- Ramanathan, S., Hanley, J.J., Deniau, J.-M., and Bolam, J.P. (2002). Synaptic convergence of motor and somatosensory cortical afferents onto GABAergic interneurons in the rat striatum. *J. Neurosci.* 22, 8158-8169.
- Redgrave, P., Vautrelle, N., and Reynolds, J.N.J. (2011). Functional properties of the basal ganglia's re-entrant loop architecture: selection and reinforcement. *Neuroscience* 198, 138-151.
- Rees, J.N., Florang, V.R., Eckert, L.L., and Doorn, J.A. (2009). Protein reactivity of 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite, is dependent on both the aldehyde and the catechol. *Chem. Res. Toxicol.* 22, 1256-1263.
- Reimann, W., Zumstein, A., Starke, K. (1982) Gamma-aminobutyric acid can both inhibit and facilitate dopamine release in the caudate nucleus of the rabbit. *J Neurochem.* 39, 961-969.
- Reimann, W. (1983). Inhibition by GABA, baclofen and gabapentin of dopamine release from rabbit caudate nucleus: are there common or different sites of action?. *Eur J Pharmacol.* 94, 341-344.
- Reynolds, J.N., and Wickens, J.R. (2002). Dopamine-dependent plasticity of corticostriatal synapses. *Neural Networks* 15, 507-521.
- Reynolds, J.N.J., Hyland, B.I., and Wickens, J.R. (2001). A cellular mechanism of reward-related learning. *Nature* 413, 67-70.
- Rice, M.E., and Cragg, S.J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat. Neurosci.* 7, 583-584.
- Rice, M.E., and Nicholson, C. (1989). Measurement of nanomolar dopamine diffusion using low-noise perfluorinated ionomer-coated carbon fiber microelectrodes and high-speed cyclic voltammetry. *Anal. Chem.* 61, 1805-1810.
- Rice, M.E., Patel, J.C., and Cragg, S.J. (2011). Dopamine release in the basal ganglia. *Neuroscience* 198, 112-137.
- Rivera, A., Alberti, I., Martín, A.B., Narváez, J.A., De La Calle, A., and Moratalla, R. (2002). Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. *Eur. J. Neurosci.* 16, 2049-2058.
- Roberts, B.M., White, M.G., Patton, M.H., Chen, R., and Mathur, B.N. (2019). Ensemble encoding of action speed by striatal fast-spiking interneurons. *Brain Struct. Funct.* 224, 2567-2576.
- Rocca, W.A. (2018). The burden of Parkinson's disease: a worldwide perspective. *Lancet Neurol.* 17, 928-929.
- Rocha-González, H.I., Mao, S., and Alvarez-Leefmans, F.J. (2008).  $\text{Na}^+$ ,  $\text{K}^+$ ,  $2\text{Cl}^-$  Cotransport and intracellular chloride regulation in rat primary sensory neurons: thermodynamic and kinetic aspects. *J. Neurophysiol.* 100, 169-184.
- Rodd-Henricks, Z.A., McKinzie, D.L., Li, T.-K., Murphy, J.M., and McBride, W.J. (2002). Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. *J. Pharmacol. Exp. Ther.* 303, 1216-1226.
- Roitman, M.F., Stuber, G.D., Phillips, P.E.M., Wightman, R.M., and Carelli, R.M. (2004). Dopamine operates as a subsecond modulator of food seeking. *J. Neurosci.* 24, 1265-1271.

Romo, R., Chéramy, A., Godeheu, G., Glowinski, J. (1986). In vivo presynaptic control of dopamine release in the cat caudate nucleus III. Further evidence for the implication of corticostriatal glutamatergic neurons. *Neuroscience* 19, 1091-1099.

Romo, R., and Schultz, W. (1990). Dopamine neurons of the monkey midbrain: contingencies of responses to active touch during self-initiated arm movements. *J. Neurophysiol.* 63, 592-606.

Ronken, E., Mulder, A.H., and Schoffelmeer, A.N.M. (1993). Interacting presynaptic k-opioid and GABA<sub>A</sub> receptors modulate dopamine release from rat striatal synaptosomes. *J. Neurochem.* 61, 1634-1639.

Ruiz, A., Fabian-Fine, R., Scott, R., Walker, M.C., Rusakov, D.A., and Kullmann, D.M. (2003). GABAA Receptors at Hippocampal Mossy Fibers. *Neuron* 39, 961-973.

Růžicka, E., Roth, J., Jech, R., and Busek, P. (2000). Subhypnotic doses of zolpidem oppose dopaminergic-induced dyskinesia in Parkinson's disease. *Mov. Disord.* 15, 734-735.

- S -

Sakaba, T., and Neher, E. (2003). Direct modulation of synaptic vesicle priming by GABAB receptor activation at a glutamatergic synapse. *Nature* 424, 775-778.

Sakamoto, S., Goto, S., Ito, H., and Hirano, A. (1992). Striosomal organization of substance P-like immunoreactivity in parkinsonian patients. *Neurology* 42, 1071-1075.

Sakatani, K., Chesler, M., and Hassan, A.Z. (1991a). GABAA receptors modulate axonal conduction in dorsal columns of neonatal rat spinal cord. *Brain Res.* 542, 273-279.

Sakatani, K., Hassan, A.Z., and Chesler, M. (1991b). GABA-sensitivity of dorsal column axons: an in vitro comparison between adult and neonatal rat spinal cords. *Dev. Brain Res.* 61, 139-142.

Sakatani, K., Chesler, M., Hassan, A.Z., Lee, M., and Young, W. (1993). Non-synaptic modulation of dorsal column conduction by endogenous GABA in neonatal rat spinal cord. *Brain Res.* 622, 43-50.

Samii, A., Nutt, J.G., and Ransom, B.R. (2004). Parkinson's disease. *Lancet* 363, 1783-1793.

Santhakumar, V., Jones, R.T., and Mody, I. (2010). Developmental regulation and neuroprotective effects of striatal tonic GABAA currents. *Neuroscience* 167, 644-655.

Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., Kawaguchi, T., Tsunoda, T., Watanabe, M., Takeda, A., et al. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat. Genet.* 41, 1303-1307.

Saunders, A., Huang, K.W., and Sabatini, B.L. (2016). Globus pallidus externus neurons expressing parvalbumin interconnect the subthalamic nucleus and striatal interneurons. *PLoS One* 11, e0149798.

Schmitz, Y., Schmauss, C., and Sulzer, D. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *J. Neurosci.* 22, 8002-8009.

Schmitz, Y., Benoit-Marand, M., Gonon, F., and Sulzer, D. (2003). Presynaptic regulation of dopaminergic neurotransmission. *J. Neurochem.* 87, 273-289.

Schultz, W. (2007). Behavioral dopamine signals. *Trends Neurosci.* 30, 203-210.

- Schultz, W., and Romo, R. (1990). Dopamine neurons of the monkey midbrain: contingencies of responses to stimuli eliciting immediate behavioral reactions. *J. Neurophysiol.* 63, 607-624.
- Schultz, W., Ruffieux, A., and Aebischer, P. (1983). The activity of pars compacta neurons of the monkey substantia nigra in relation to motor activation. *Exp. Brain Res.* 51, 377-387.
- Schultz, W., Dayan, P., and Montague, P.R. (1997). A neural substrate of prediction and reward. *Science* 275, 1593-1599.
- Schulz, J.M., and Reynolds, J.N.J. (2013). Pause and rebound: sensory control of cholinergic signaling in the striatum. *Trends Neurosci.* 36, 41-50.
- Scimemi, A. (2005). Multiple and plastic receptors mediate tonic GABAA receptor currents in the hippocampus. *J. Neurosci.* 25, 10016-10024.
- Scofield, M.D., Boger, H.A., Smith, R.J., Li, H., Haydon, P.G., and Kalivas, P.W. (2015). Gq-DREADD selectively initiates glial glutamate release and inhibits cue-induced cocaine seeking. *Biol. Psychiatry* 78, 441-451.
- Sellings, L.H.L., and Clarke, P.B.S. (2003). Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. *J. Neurosci.* 23, 6295-6303.
- Semyanov, A., Walker, M.C., and Kullmann, D.M. (2003). GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nat. Neurosci.* 6, 484-490.
- Senior, S.L., Ninkina, N., Deacon, R., Bannerman, D., Buchman, V.L., Cragg, S.J., and Wade-Martins, R. (2008). Increased striatal dopamine release and hyperdopaminergic-like behaviour in mice lacking both alpha-synuclein and gamma-synuclein. *Eur. J. Neurosci.* 27, 947-957.
- Sgobio, C., Wu, J., Zheng, W., Chen, X., Pan, J., Salinas, A.G., Davis, M.I., Lovinger, D.M., and Cai, H. (2017). Aldehyde dehydrogenase 1-positive nigrostriatal dopaminergic fibers exhibit distinct projection pattern and dopamine release dynamics at mouse dorsal striatum. *Sci. Rep.* 7, 1-13.
- Shabel, S.J., Proulx, C.D., Piriz, J., Malinow, R. (2014). GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment. *Science* 345, 1494-1498.
- Sherpa, A.D., Patel, J.C., O'Neill, B., Aoki, C., and Rice, M.E. (2019). Does GABA co-release from striatal dopamine axons autoregulate dopamine release? at 13th International Basal Ganglia Society Meeting, (Biarritz, France).
- Sherva, R., Rice, J.P., Neuman, R.J., Rochberg, N., Saccone, N.L., and Bierut, L.J. (2009). Associations and interactions between SNPs in the alcohol metabolizing genes and alcoholism phenotypes in european americans. *Alcohol. Clin. Exp. Res.* 33, 848-857.
- Shin, J.H., Adrover, M.F., and Alvarez, V.A. (2017). Distinctive modulation of dopamine release in the nucleus accumbens shell mediated by dopamine and acetylcholine receptors. *J. Neurosci.* 37, 11166-11180.
- Silm, K., Yang, J., Marcott, P.F., Asensio, C.S., Eriksen, J., Guthrie, D.A., Newman, A.H., Ford, C.P., and Edwards, R.H. (2019). Synaptic vesicle recycling pathway determines neurotransmitter content and release properties. *Neuron* 102, 786-800.
- Simón-Sánchez, J., Schulte, C., Bras, J.M., Sharma, M., Gibbs, J.R., Berg, D., Paisan-Ruiz, C., Lichtner, P., Scholz, S.W., Hernandez, D.G., et al. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* 41, 1308-1312.

- Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., et al. (2003). Alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302, 841.
- Sloan, M., Alegre-Abarategui, J., Potgieter, D., Kaufmann, A.-K., Exley, R., Deltheil, T., Threlfell, S., Connor-Robson, N., Brimblecombe, K., Wallings, R., et al. (2016). *LRK2* BAC transgenic rats develop progressive, L-DOPA-responsive motor impairment, and deficits in dopamine circuit function. *Hum. Mol. Genet.* 25, 951-963.
- Smith, A.D., and Bolam, J.P. (1990). The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci.* 13, 259-265.
- Smith, I.D., and Grace, A.A. (1992). Role of the subthalamic nucleus in the regulation of nigral dopamine neuron activity. *Synapse* 12, 287-303.
- Smith, Y., Bennett, B.D., Bolam, J.P., Parent, A., and Sadikot, A.F. (1994). Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. *J. Comp. Neurol.* 344, 1-19.
- Smolders, I., De Klippel, N., Sarre, S., Ebinger, G., and Michotte, Y. (1995). Tonic GABA-ergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat. *Eur. J. Pharmacol.* 284, 83-91.
- 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. *J. Comp. Neurol.* 195, 567-584.
- Specht, C.G., and Schoepfer, R. (2001). Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. *BMC Neurosci.* 2, 11.
- Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R., and Goedert, M. (1997).  $\alpha$ -synuclein in Lewy bodies. *Nature* 388, 839-840.
- Spillantini, M.G., Crowther, R.A., Jakes, R., Hasegawa, M., and Goedert, M. (1998). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6469-6473.
- Srinivasan, R., Lu, T.-Y., Chai, H., Xu, J., Huang, B.S., Golshani, P., Coppola, G., and Khakh, B.S. (2016). New transgenic mouse lines for selectively targeting astrocytes and studying calcium signals in astrocyte processes in situ and in vivo. *Neuron* 92, 1181-1195.
- Starr, M.S. (1978). GABA potentiates potassium-stimulated 3H-dopamine release from slices of rat substantia nigra and corpus striatum. *Eur J Pharmacol.* 48, 325-328.
- Starr, M.S. (1979). GABA-mediated potentiation of amine release from nigrostriatal dopamine neurones in vitro. *Eur J Pharmacol.* 53, 215-226.
- Stensrud, M.J., Puchades, M., and Gundersen, V. (2014). GABA is localized in dopaminergic synaptic vesicles in the rodent striatum. *Brain Struct. Funct.* 219, 1901-1912.
- Stoof, J.C., Verheijden, P.F.H.M., and Leysen, J.E. (1987). Stimulation of D2-receptors in rat nucleus accumbens slices inhibits dopamine and acetylcholine release but not cyclic AMP formation. *Brain Res.* 423, 364-368.
- Stoof, J.C., Den Breejen, E.J., Mulder, A.H. (1979). GABA modulates the release of dopamine and acetylcholine from rat caudate nucleus slices. *Eur J Pharmacol.* 57, 35-42.

- Stouffer, M.A., Woods, C.A., Patel, J.C., Lee, C.R., Witkovsky, P., Bao, L., Machold, R.P., Jones, K.T., De Vaca, S.C., Reith, M.E.A., et al. (2015). Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. *Nat. Commun.* 6, 8543.
- Strata, P., and Harvey, R. (1999). Dale's principle. *Brain Res. Bull.* 50, 349-350.
- Stuber, G.D., Hnasko, T.S., Britt, J.P., Edwards, R.H., and Bonci, A. (2010). Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J. Neurosci.* 30, 8229-8233.
- Suaud-Chagny, M.F., Chergui, K., Chouvet, G., and Gonon, F. (1992). Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. *Neuroscience* 49, 63-72.
- Sulzer, D. (2007). Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. *Trends Neurosci.* 30, 244-250.
- Sulzer, D., and Pothos, E.N. (2000). Regulation of quantal size by presynaptic mechanisms. *Rev. Neurosci.* 11, 159-212.
- Sulzer, D., Cragg, S.J., and Rice, M.E. (2016). Striatal dopamine neurotransmission: regulation of release and uptake in Basal Ganglia.
- Sun, B.B., and Chiu, S.Y. (1999). N-type calcium channels and their regulation by GABABReceptors in axons of neonatal rat optic nerve. *J. Neurosci.* 19, 5185-5194.
- Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S.F., Luo, Y., Li, F., Wang, H., Yamaguchi, T., et al. (2018). A Genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. *Cell* 174, 481-496.e19.
- Surmeier, D.J., and Sulzer, D. (2013). The pathology roadmap in Parkinson disease. *Prion* 7, 85-91.
- Surmeier, D.J., Song, W.J., and Yan, Z. (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.* 16, 6579-6591.
- Surmeier, D.J., Guzman, J.N., and Sanchez-Padilla, J. (2010). Calcium, cellular aging, and selective neuronal vulnerability in Parkinson's disease. *Cell Calcium* 47, 175-182.
- Surmeier, D.J., Obeso, J.A., and Halliday, G.M. (2017). Parkinson's disease is not simply a prion disorder. *J. Neurosci.* 37, 9799-9807.
- Sznejder-Pacholek, A., Joniec-Maciejak, I., Wawer, A., Ciesielska, A., and Mirowska-Guzel, D. (2017). The effect of  $\alpha$ -synuclein on gliosis and IL-1 $\alpha$ , TNF $\alpha$ , IFN $\gamma$ , TGF $\beta$  expression in murine brain. *Pharmacol. Reports* 69, 242-251.
- T -
- Taschenberger, G., Garrido, M., Tereshchenko, Y., Bähr, M., Zweckstetter, M., and Kügler, S. (2012). Aggregation of  $\alpha$ Synuclein promotes progressive in vivo neurotoxicity in adult rat dopaminergic neurons. *Acta Neuropathol.* 123, 671-683.
- Taylor, T.N., Potgieter, D., Anwar, S., Senior, S.L., Janezic, S., Threlfell, S., Ryan, B., Parkkinen, L., Deltheil, T., Cioroch, M., et al. (2014). Region-specific deficits in dopamine, but not norepinephrine, signaling in a novel A30P  $\alpha$ -synuclein BAC transgenic mouse. *Neurobiol. Dis.* 62, 193-207.

Tecuapetla, F., Patel, J.C., Xenias, H., English, D., Tadros, I., Shah, F., Berlin, J., Deisseroth, K., Rice, M.E., Tepper, J.M., et al. (2010). Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. *J. Neurosci.* 30, 7105–7110.

Tepper, J.M., and Bolam, J.P. (2004). Functional diversity and specificity of neostriatal interneurons. *Curr. Opin. Neurobiol.* 14, 685–692.

Tepper, J.M., and Koós, T. (1999). Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nat. Neurosci.* 2, 467–472.

Tepper, J., Sawyer, S., and Groves, P. (1987). Electrophysiologically identified nigral dopaminergic neurons intracellularly labeled with HRP: light-microscopic analysis. *J. Neurosci.* 7, 2794–2806.

Tepper, J.M., Koós, T., Ibanez-Sandoval, O., Tecuapetla, F., Faust, T.W., and Assous, M. (2018). Heterogeneity and diversity of striatal gabaergic interneurons. *Front. Neuroanat.* 7, 2794–2806.

Threlfell, S., and Cragg, S.J. (2011). Dopamine signaling in dorsal versus ventral striatum: the dynamic role of cholinergic interneurons. *Front. Syst. Neurosci.* 5, 11.

Threlfell, S., Clements, M.A., Khodai, T., Pienaar, I.S., Exley, R., Wess, J., and Cragg, S.J. (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J. Neurosci.* 30, 3398–3408.

Threlfell, S., Lalic, T., Platt, N.J., Jennings, K.A., Deisseroth, K., and Cragg, S.J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron* 75, 58–64.

Ting, J.T., Daigle, T.L., Chen, Q., and Feng, G. (2014). Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. (Humana Press, New York, NY), pp. 221–242.

Trigo, F.F., Marty, A., and Stell, B.M. (2008). Axonal GABA<sub>A</sub> receptors. *Eur. J. Neurosci.* 28, 841–848.

Tritsch, N.X., Ding, J.B., and Sabatini, B.L. (2012). Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature* 490, 262–266.

Tritsch, N.X., Oh, W.J., Gu, C., and Sabatini, B.L. (2014). Midbrain dopamine neurons sustain inhibitory transmission using plasma membrane uptake of GABA, not synthesis. *Elife* 3, e01936.

Tritsch, N.X., Granger, A.J., and Sabatini, B.L. (2016). Mechanisms and functions of GABA co-release. *Nat. Rev. Neurosci.* 17, 139–145.

Tunstall, M.J., Oorschot, D.E., Kean, A., and Wickens, J.R. (2002). Inhibitory interactions between spiny projection neurons in the rat striatum. *J. Neurophysiol.* 88.

- U -

Uchigashima, M., Ohtsuka, T., Kobayashi, K., and Watanabe, M. (2016). Dopamine synapse is a neuroligin-2-mediated contact between dopaminergic presynaptic and GABAergic postsynaptic structures. *Proc. Natl. Acad. Sci.* 113, 4206–4211.

Uchitel, O.D., Di Guilmi, M.N., Urbano, F.J., and Gonzalez-Inchauspe, C. (2010). Acute modulation of calcium currents and synaptic transmission by gabapentinoids. *Channels* 4, 490–496.

Ulrich, D., and Bettler, B. (2007). GABAB receptors: synaptic functions and mechanisms of diversity. *Curr. Opin. Neurobiol.* 17, 298-303.

Ungerstedt, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol. Scand.* 82, 1-48.

Usiello, A., Baik, J.-H., Rougé-Pont, F., Picetti, R., Dierich, A., LeMeur, M., Piazza, P.V., and Borrelli, E. (2000). Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* 408, 199-203.

- V -

Vaaga, C.E., Borisovska, M., and Westbrook, G.L. (2014). Dual-transmitter neurons: functional implications of co-release and co-transmission. *Curr. Opin. Neurobiol.* 29, 25-32.

Venda, L.L., Cragg, S.J., Buchman, V.L., and Wade-Martins, R. (2010).  $\alpha$ -Synuclein and dopamine at the crossroads of Parkinson's disease. *Trends Neurosci.* 33, 559-568.

Verdier, D., Lund, J.P., and Kolta, A. (2003). GABAergic control of action potential propagation along axonal branches of mammalian sensory neurons. *J. Neurosci.* 23, 2002-2007.

Vergara, R., Rick, C., Hernández-López, S., Laville, J.A., Guzman, J.N., Galarraga, E., Surmeier, D.J., and Bargas, J. (2003). Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. *J. Physiol.* 553, 169-182.

Veruki, M.L., Mørkve, S.H., and Hartveit, E. (2006). Activation of a presynaptic glutamate transporter regulates synaptic transmission through electrical signaling. *Nat. Neurosci.* 9, 1388-1396.

Voorn, P., Vanderschuren, L.J.M., Groenewegen, H.J., Robbins, T.W., and Pennartz, C.M.. (2004). Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci.* 27, 468-474.

- W -

Waldvogel, H.J., Fritschy, J.M., Mohler, H., and Faull, R.L.M. (1998). GABA(A) receptors in the primate basal ganglia: an autoradiographic and a light and electron microscopic immunohistochemical study of the  $\alpha 1$  and  $\beta 2,3$  subunits in the baboon brain. *J. Comp. Neurol.* 397, 297-325.

Wall, P.D. (1995). Do nerve impulses penetrate terminal arborizations? A pre-presynaptic control mechanism. *Trends Neurosci.* 18, 99-103.

Wall, N.R., De La Parra, M., Callaway, E.M., and Kreitzer, A.C. (2013). Differential Innervation of Direct- and Indirect-Pathway Striatal Projection Neurons. *Neuron* 79, 347-360.

Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H.H., Ding, J., Tkatch, T., Lovinger, D.M., and Surmeier, D.J. (2006). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* 50, 443-452.

Westberg, K.-G., Kolta, A., Clavelou, P., Sandström, G., and Lund, J.P. (2000). Evidence for functional compartmentalization of trigeminal muscle spindle afferents during fictive mastication in the rabbit. *Eur. J. Neurosci.* 12, 1145-1154.

Whitworth, T.L., and Quick, M.W. (2001). Upregulation of gamma-aminobutyric acid transporter expression: role of alkylated gamma-aminobutyric acid derivatives. *Biochem. Soc. Trans.* 29, 736-741.

Wichmann, T., and DeLong, M.R. (2007). Anatomy and physiology of the basal ganglia: relevance to Parkinson's disease and related disorders. *Handb. Clin. Neurol.* 83, 1-18.

Wickens, J.R., and Arbuthnott, G.W. (2005). Chapter IV Structural and functional interactions in the striatum at the receptor level. *Handb. Chem. Neuroanat.* 21, 199-236.

Wilson, C.J., Kawaguchi, Y., Masson, J., Zhuang, X., Sulzer, D., Hen, R., and Rayport, S. (1996). The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J. Neurosci.* 16, 2397-2410.

Wójtowicz, A.M., Dvorzhak, A., Semtner, M., and Grantyn, R. (2013). Reduced tonic inhibition in striatal output neurons from Huntington mice due to loss of astrocytic GABA release through GAT-3. *Front. Neural Circuits* 7, 188.

- X -

Xu, Z.C., Wilson, C.J., and Emson, P.C. (1989). Restoration of the corticostriatal projection in rat neostriatal grafts: electron microscopic analysis. *Neuroscience* 29, 539-550.

- Y -

Yamada, T., McGeer, P.L., Baimbridge, K.G., and McGeer, E.G. (1990). Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. *Brain Res.* 526, 303-307.

Yan, Z., Song, W.J., Surmeier, J., Ariano, M.A., Monsma, F.J., Barton, A.C., Kang, H.C., Haugland, R.P., Sibley, D.R., Baertschi, A.J., et al. (1997). D2 dopamine receptors reduce N-type Ca<sup>2+</sup> currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. *J. Neurophysiol.* 77, 1003-1015.

Yang, H., and Michael, A.C. (2007). In vivo fast-scan cyclic voltammetry of dopamine near microdialysis probes in *Electrochemical Methods for Neuroscience*.

Yasumi, M., Sato, K., Shimada, S., Nishimura, M., and Tohyama, M. (1997). Regional distribution of GABA transporter 1 (GAT1) mRNA in the rat brain: comparison with glutamic acid decarboxylase67 (GAD67) mRNA localization. *Brain Res. Mol. Brain Res.* 44, 205-218.

Yetnikoff, L., Lavezzi, H.N., Reichard, R.A., and Zahm, D.S. (2014). An update on the connections of the ventral mesencephalic dopaminergic complex. *Neuroscience* 282C, 23-48.

Yim, M.Y., Aertsen, A., Kumar, A., Calabresi, P., and Silberberg, G. (2011). Significance of input correlations in striatal function. *PLoS Comput. Biol.* 7, e1002254.

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. *Eur. J. Neurosci.* 19, 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. *Eur. J. Neurosci.* 22, 513-523.

Yin, H.H., Mulcare, S.P., Hilário, M.R.F., Clouse, E., Holloway, T., Davis, M.I., Hansson, A.C., Lovinger, D.M., Costa, R.M., Kennedy, P., et al. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat. Neurosci.* 12, 333-341.

Yoon, B. E., Woo, J., Chun, Y. E., Chun, H., Jo, S., Bae, J. Y., et al. (2014). Glial GABA, synthesized by monoamine oxidase B, mediates tonic inhibition. *J. Physiol.* 592, 4951-4968.

Yu, S., Li, X., Liu, G., Han, J., Zhang, C., Li, Y., Xu, S., Liu, C., Gao, Y., Yang, H., et al. (2007). Extensive nuclear localization of alpha-synuclein in normal rat brain neurons revealed by a novel monoclonal antibody. *Neuroscience* 145, 539-555.

Yu, X., Taylor, A.M.W., Nagai, J., Golshani, P., Evans, C.J., Coppola, G., and Khakh, B.S. (2018). Reducing astrocyte calcium signaling in vivo alters striatal microcircuits and causes repetitive behavior. *Neuron* 99, 1170-1187.

Yun, S.P., Kam, T.-I., Panicker, N., Kim, S., Oh, Y., Park, J.-S., Kwon, S.-H., Park, Y.J., Karuppagounder, S.S., Park, H., et al. (2018). Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat. Med.* 24, 931-938.

Yung, K.K.L., Bolam, J.P., Smith, A.D., Hersch, S.M., Ciliax, B.J., and Levey, A.I. (1995). Immunocytochemical localization of D 1 and D 2 dopamine receptors in the basal ganglia of the rat: Light and electron microscopy. *Neuroscience* 65, 709-730.

Yung, K.K.L., Ng, T.K.Y., and Wong, C.K.C. (1999). Subpopulations of neurons in the rat neostriatum display GABA(B)R1 receptor immunoreactivity. *Brain Res.* 830, 345-352.

- Z -

Zaccara, G., Cincotta, M., Borgheresi, A., and Balestrieri, F. (2004). Adverse motor effects induced by antiepileptic drugs. *Epileptic Disord* 6, 153-168.

Zeng, X.-S., Geng, W.-S., and Jia, J.-J. (2018). Neurotoxin-induced animal models of parkinson disease: pathogenic mechanism and assessment. *ASN Neuro* 10, 1759091418777438.

Zhang, H., and Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nat. Neurosci.* 7, 581-582.

Zhang, S.J., and Jackson, M.B. (1993). GABA-activated chloride channels in secretory nerve endings. *Science* 259, 531-534.

Zhang, S.J., and Jackson, M.B. (1995). GABAA receptor activation and the excitability of nerve terminals in the rat posterior pituitary. *J. Physiol.* 483, 583-595.

Zhang, Y., and Barres, B.A. (2010). Astrocyte heterogeneity: an underappreciated topic in neurobiology. *Curr. Opin. Neurobiol.* 20, 588-594.

Zhang, S., Qi, J., Li, X., Wang, H.-L., Britt, J.P., Hoffman, A.F., Bonci, A., Lupica, C.R., and Morales, M. (2015). Dopaminergic and glutamatergic microdomains in a subset of rodent mesoaccumbens axons. *Nat. Neurosci.* 18, 386-392.

Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929-11947.

Zheng, T., and Wilson, C.J. (2002). Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. *J. Neurophysiol.* 87, 1007-1017.

Zhou, F.-M., Liang, Y., and Dani, J.A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat. Neurosci.* 4, 1224-1229.