The role of impaired synaptic plasticity in aberrant salience



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Abstract

The role of impaired synaptic plasticity in aberrant salience

Hippocampal network dysfunction has been recognised as a potential pathway to psychotic symptoms for many years. Reduced hippocampal synaptic plasticity is a core feature in a diverse range of conditions, from Alzheimer's Disease to depression and schizophrenia, and may explain overlap in symptom domains across these disorders.

This thesis has investigated cognitive impairments in mouse models relevant to schizophrenia, in the context of their known effects on synaptic plasticity; integrating these effects into a psychological framework for associative learning and attention. Hippocampal long-term potentiation requires finely balanced tuning of glutamatergic signalling via expression of NMDA and AMPA receptors at the synapse. We manipulated this balance by genetic knockout of the AMPA receptor subunit GluA1, or through pharmacological blockade of the NMDA receptor with MK-801. Additionally, we investigated the effect of increased dopamine transmission, also heavily implicated in schizophrenia, by acute administration of amphetamine.

Through extensive motor and cognitive profiling, we revealed divergent behavioural phenotypes dependent on the circuit manipulation. Glutamatergic dysfunction induced significant locomotor hyperactivity and impaired short-term habituation, but when investigated in more detail we saw increased responding specifically to repeatedly presented stimuli; indicative of stimulus-specific sensitisation. While amphetamine also induced hyperactivity, this was stimulus-independent and appeared to reflect general increases in arousal and attention regardless of environment.

We then went on to investigate how these deficits in habituation affect attentional processing in the 5-choice serial reaction time test. All treatment groups showed unique behavioural abnormalities in tests of attention. The increased arousal following amphetamine treatment was observed here again, to the point that animals were impaired in their ability to respond appropriately. MK-801 seemingly induced global deficits in attention that impaired an animals' ability to attend to the task, whereas GluA1 knockout left attending largely intact until conditions were made unpredictable.

Finally we investigated how impaired short-term habituation and attentional processes in GluA1$^{-/-}$ mice affect their ability to accurately form associations using sensory preconditioning. Mice lacking GluA1 showed enhanced performance in this task under simple conditions, indicating an increased propensity to form associations. When conditions were more complex, however, they were impaired. These data suggest maladaptive associative learning in GluA1$^{-/-}$ that can be beneficial or harmful to learning, depending on the circumstances.

These findings show that perturbations of glutamate transmission affect an individual's ability to accurately learn about their environment and suggest a mechanism through which this actually leads to sensitisation to recently familiar stimuli as a result of deficits in short-term habituation. The impact of this impairment on associative learning indicates a critical role for hippocampal long-term potentiation in this mechanism, and also suggests that GluA1$^{-/-}$ share construct validity with clinical psychosis.

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Abbreviations

AD	Alzheimer's Disease
A1	Focal attention
A2	Peripheral attention
Aβ	β-amyloid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
Amph	D-amphetamine
CA	*Cornu ammonis*
CaMKII	Calcium/calmodulin-dependent kinase II
CS	Conditioned stimulus
D-AP5	D-2-amino-5-phosphonopentanoic acid
DAT	Dopamine transporter
DG	Dentate gyrus
DSM-5	Diagnostic and statistical manual of mental disorders (Fifth edition)
EM	Estimated marginal
fEPSP	Field excitatory post-synaptic potential
GABA	Gamma-aminobutyric acid
GWAS	Genome-wide association study
HPA	Hypothalamic-pituitary-adrenocortical
I	Inactive attentional state
IA	Inhibitory avoidance
ITI	Inter-trial interval
LFP	Local field potential
LH	Limited hold
LI	Latent inhibition
LTD	Long-term depression
LTP	Long-term potentiation
MK-801	Dizocilpine
MRI	Magnetic resonance imaging
NCR	Novel context recognition
NMDAR	N-methyl-D-aspartic acid receptor
NOR	Novel object recognition
PCP	Phencyclidine
PET	Positron emission tomography
PIR	Passive infrared sensor
SD	Stimulus duration
SOP	Sometimes opponent processes
SPC	Sensory preconditioning
SSRI	Selective serotonin reuptake inhibitor
US	Unconditioned stimulus
VI	Variable interval
VTA	Ventral tegmental area
WT	Wild-type
5-CSRTT	5-choice serial reaction time task

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Chapter 1: Introduction

Mental health disorders pose a significant and growing problem in modern society. Between 1990 and 2016, global cases of schizophrenia increased from 13.1 to 20.9 million; an increase of more than 150% in cases (Charlson *et al.,* 2018). However, the aetiology of schizophrenia remains unclear, and current treatments work to silence the expression of symptoms without fixing the underlying cause. One of the defining features of schizophrenia is psychosis; the presence of at least one of delusions, hallucinations or thought disorders (collectively termed positive symptoms), is a prerequisite for a diagnosis under traditional criteria (American Psychiatric Association 2013), but psychotic symptoms are not unique to schizophrenia. They are relatively common across many psychiatric and neurodegenerative conditions.

Furthermore, whilst psychotic symptoms are required for a diagnosis, schizophrenia has a wide symptomatology of which these positive symptoms are just one component. Cognitive deficits are also prevalent, as are negative symptoms such as apathy and anhedonia. These symptom categories are largely unresponsive to available neuroleptic drugs, and have a big impact on patients quality of life. Both cognitive and negative symptoms are also found in depression and Alzheimer's Disease (AD); three distinctly different neurological disorders actually show considerable overlap of symptomatology.

It is beyond the scope of this thesis to discuss three diseases in great detail. Although the focus of this research has been schizophrenia, it is important to consider the similarity between symptoms prevalent in these conditions and whether mechanisms for disease onset or progression may overlap. In the next section we will discuss the symptomatology

of each of these disorders and discuss the evidence for a shared mechanism – reduced hippocampal plasticity – that may play a role in each of them.

Psychiatry and Synaptic Plasticity

Alzheimer's Disease

AD is a progressive neurodegenerative disease, associated with the prevalence of two neuropathological hallmarks; β-amyloid (Aβ) plaques and neurofibrillary tangles comprised of hyperphosphorylated tau. Post-mortem studies of AD brains have found increasing Aβ and tau burden throughout the course of the disease, and significant hippocampal and cortical neuronal loss in later stages (Braak and Braak 1991).

Cognitive decline is a defining feature of AD, particularly the loss of episodic memory, working memory impairments and executive dysfunction. However, patients can also present with psychotic symptoms. These have been linked with severity of cognitive impairments and predict more rapid cognitive decline (Ropacki and Jeste 2005; Stern *et al.,* 1994). Psychotic symptoms in AD are commonly persecutory, misidentification of people and places, or visual hallucinations (Rubin, Drevets, and Burke 1988).

There is considerable evidence of disrupted synaptic function in the early stages of the disease. Deficits in expression of glutamatergic AMPA receptors (AMPARs) at hippocampal synapses is one of the earliest molecular indicators of AD and has been attributed to detrimental effects of soluble Aβ oligomers (Hu *et al.,* 2018; Shankar *et al.,* 2008). Intact glutamatergic transmission is critical for induction and expression of hippocampal long-term potentiation (LTP), which is thought to play an important role in learning and memory. Both Aβ and tau have physiological roles at the synapse. The accumulation of toxic forms

of these proteins and subsequent synaptic dysfunction have long been thought central to disease onset and progression (Spires-Jones and Hyman 2014). Interaction with receptors critical to LTP at hippocampal synapses represents one possible mechanism by which behavioural symptoms may manifest.

In both patients and preclinical models of AD pathology, LTP-like plasticity has shown to be reduced (Battaglia *et al.,* 2007). *In vivo*, soluble Aβ oligomers have been shown to disrupt hippocampal LTP in anaesthetised rats (Walsh *et al.,* 2002). Mouse models of AD-like pathology exhibit cognitive decline with age that correlates with impaired synaptic plasticity, in the absence of gross structural changes (Chapman *et al.,* 1999).

In prodromal AD patients, volumetric reductions have been detected in hippocampus, further suggesting this formation has a central role in disease progression (Scahill *et al.,* 2002). Reduced hippocampal volume and impaired LTP contribute to the deficits in working memory associated with AD but may also induce psychotic symptoms. Memantine, one of the major treatment options for symptoms of AD normalises glutamatergic transmission to some extent by inhibiting extrasynaptic NMDA receptors (NMDARs) and has been shown to be beneficial not only for cognition, but for psychotic symptoms of the disease (Wilcock *et al.,* 2008).

In summary, there is strong evidence in the case of AD that cognitive deficits may result as a decline in LTP-like plasticity over the course of disease progression. Furthermore, psychotic symptoms also appear to develop as a result of this pathology indicating overlap in the mechanism through which different symptoms arise.

Depression

Major depressive disorder, or depression, is a psychiatric condition characterised by low mood and anhedonia, two symptoms central to its DSM-5 diagnosis (American Psychiatric Association 2013). However, cognitive and psychotic symptoms can also present in depression. Psychotic symptoms have been associated with more severe forms of the disorder and are linked with higher rates of suicide, but have also been shown to abate as the depressive episode remits (Rothschild 2018). Psychotic symptoms in depression tend to consist of "non-bizarre, nihilistic, somatic or guilty" delusional beliefs (Fleming, Blasey, and Schatzberg 2004), reflecting the internal state of low mood and feelings.

Depression is a highly complex disorder, with no single clear cause for its onset. Whilst genetic risk factors play a part in an individual's likelihood of developing depression in their lifetime, environmental stressors may more accurately predict the onset of depressive episodes. In animals, stressful and uncontrollable events such as inescapable footshock have been shown to produce "learned helplessness", where subjects acquire a belief that negative events are outside of their control (Seligman and Maier 1967). This belief then generalises to new contexts and situations where negative events are escapable. In this study in dogs, inescapable shock led to motivational, cognitive and emotional deficits, a phenotype similar to the depressed state in patients.

One potential pathway for this outcome is through the effect of stress on cortisol production, and the subsequent effect of cortisol on hippocampal LTP, structure and function. LTP is significantly reduced in rats exposed to unavoidable footshocks (Foy *et al.,* 1987; Shors *et al.,* 1989). Depression has previously been described as a vicious circle, whereby dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis and

increased cortisol production lead to morphological and functional deficits in the hippocampus (Liu *et al.,* 2017). As a result, hippocampal LTP is decreased and there is subsequently less regulation of the HPA axis, which becomes further disinhibited and drives more severe hippocampal deficits; including dendritic atrophy and neuronal loss. Specifically, cortisol has been shown to reduce neurogenesis and increase apoptosis in dentate gyrus (DG), and loss of dendrites in CA3 (de Kloet, Joëls, and Holsboer 2005).

Further evidence for hippocampal dysregulation in depression comes from imaging studies. Patients with psychotic major depression have been shown to be impaired in a task of verbal memory encoding and retrieval, with impairments associated with reduced hippocampal activity during encoding (Kelley *et al.,* 2013). During retrieval, whilst no differences were observed in hippocampus, prefrontal and parietal cortex activation was increased, potentially indicating a compensatory mechanism in other brain regions for impaired encoding. In a working memory *n*-back task, hippocampal activity was aberrantly increased in patients with psychotic major depression, particularly during a 1-back task with relatively low cognitive demand (Garrett *et al.,* 2011). These reports of either reduced, or increased hippocampal activity all indicate that dysregulation of this finely-tuned structure contributes to cognitive impairments seen in depression

Aberrant functional connectivity between networks appears to be implicated in depression. Eero Castrén (2005) has suggested that depression may primarily be a disorder of impaired network connectivity that disrupts information processing. Reductions in hippocampal plasticity, and including LTP, may be a causal factor in the onset of depressive symptoms. Traditional antidepressants, selective serotonin reuptake inhibitors (SSRIs) have targeted the serotonin system as a means to ameliorate symptoms, but with limited

success; approximately 30% of patients don't respond to available treatment (Al-Harbi 2012). The idea that depression may be primarily a disorder of neural plasticity, rather than reduced serotonin represents a potential turning point in our understanding of the disorder (Branchi 2011). Whilst SSRIs work to increase serotonin levels in the brain, they also enhance plasticity. This suggests a different potential mechanism for their therapeutic efficacy that would explain the relatively long period before patients see benefits from these treatments. It should be noted, however, that increased plasticity alone is unlikely to be sufficient to improve patient outcomes – this depends on environmental conditions being conducive to new learning that then leads to an improvement in mood (Branchi 2011). In mice, treatment with SSRI's coinciding with extinction training, had the biggest impact on eradicating conditioned fear memory than either treatment or extinction alone (Karpova *et al.,* 2011).

Recent advances in treating depression have looked at other mechanisms for boosting plasticity in patients. Both ketamine and psychedelic compounds, administered in assisted therapy sessions, have seen a resurgence in recent years in their potential to treat depression (Andersen *et al.,* 2021; Vollenweider and Kometer 2010). In psychedelics, the therapeutic benefits have also been linked specifically to their effects on enhancing synaptic plasticity, not just the psychedelic experience. Non-hallucinogenic analogues of psychedelic compounds have been shown to promote plasticity without generating psychedelic effects, and generate antidepressant-like effects on behaviour in rodents (Cameron *et al.,* 2020). Furthermore, in mice, pre-treatment with a 5HT-2A antagonist prior to psilocybin administration has also been shown to still enhance plasticity, despite blocking the mechanism of action of visual hallucinations (Hesselgrave *et al.,* 2021).

There is clear evidence of aberrant hippocampal activity, and functional connectivity, in depressed patients, that correlates with not only reduced mood and anhedonia, but also cognitive deficits and in severe cases, psychotic symptoms. Reduced hippocampal LTP has been implicated in the onset of depressive symptoms as a result of chronic stress. While conventional treatments have been limited in their efficacy, there is growing momentum in research in this field into drugs specifically targeting enhanced plasticity that is showing high therapeutic potential for patients.

Schizophrenia

There is a great deal of evidence for a causal role of impaired glutamate transmission and synaptic plasticity in schizophrenia. NMDAR antagonists can induce schizophrenia-like behavioural abnormalities in healthy subjects (Cheng *et al.,* 2018; Javitt and Zukin 1991; Krystal *et al.,* 1994) and exacerbate psychotic and cognitive symptoms in schizophrenic patients (Lahti *et al.,* 1995).

NMDAR-antibody encephalitis, an autoantibody-mediated disease targeting the glutamate system generates robust psychotic symptoms from the early stages in patients which are totally alleviated by immunotherapy treatment (Al-Diwani *et al.,* 2019; Titulaer *et al.,* 2013). Experiments applying patient antibodies to rat hippocampal neurons have shown them to promote NMDAR internalisation and reduce synaptic plasticity (Hughes *et al.,* 2010).

Further evidence for the role of glutamatergic disruption in schizophrenia comes from post-mortem findings. Hippocampal expression of the AMPAR subunits GluA1 and GluA2 are reduced in brains of schizophrenic patients (Eastwood *et al.,* 1995; Harrison, McLaughlin, and Kerwin 1991), while the NMDAR subunit GluNR2B appears to be

upregulated (Harrison, Law, and Eastwood 2003; Tamminga and Zukin 2015). More recently, genome-wide association studies (GWAS) investigating genetic risk factors for the disease have further implicated genes involved in glutamatergic signalling in schizophrenia. These GWAS studies have identified over 100 new loci of potential risk factor mutations for schizophrenia, including the *Gria1* gene encoding GluA1 AMPAR subunit, and *Grin2A* gene encoding the NR2A NMDAR subunit, both vital for effective glutamatergic transmission and for synaptic plasticity (Harrison 2015; Ripke *et al.,* 2013). Whilst the exact effect of the specific polymorphisms identified by GWAS is unclear, they add to a considerable body of evidence that implicates disrupted glutamatergic function and synaptic plasticity in the development of schizophrenia.

Furthermore, the hippocampal formation itself has been shown to be considerably affected by the disease. In magnetic resonance imaging (MRI) studies, schizophrenia has been linked with reduced hippocampal volume (Lawrie and Abukmeil 1998), even in the prodromal or early stages of the disease (Lawrie *et al.,* 2002; Mcintosh *et al.,* 2011). A recent study combining functional MRI (fMRI) with positron emission tomography (PET) imaging in patients at clinical high risk of schizophrenia found a relationship between adverse outcomes in patients, increased cerebral blood flow in hippocampus and increased dopamine synthesis capacity in striatum (Modinos *et al.,* 2021). Increased cerebral blood flow, a proxy of regional activity, indicates hippocampal hyperactivity in patients at risk of developing psychosis, and was linked to worsening of positive symptoms in patients.

Taken together, there is overwhelming evidence for disrupted hippocampal function in schizophrenia, and particularly for glutamatergic dysfunction and aberrant synaptic plasticity. In the next section, we will consider in more detail how disruption of glutamate

signalling could contribute to the onset of schizophrenia, how disturbances in synaptic plasticity may arise, and what this may mean at a behavioural level.

Aetiology of Schizophrenia

Hyperdopaminergia in Schizophrenia

While there is strong evidence of hippocampal and glutamatergic involvement in schizophrenia, some of the earliest work focused on the role of dopamine in the aetiology of the disease. Early research recognised the ability of dopamine-enhancing drugs like amphetamines to induce symptoms similar to that seen in psychosis (Bell 1965; Connell 1957; Griffith *et al.,* 1972), and conversely that drugs blocking dopamine receptors could alleviate positive symptoms of schizophrenia (Snyder 1976). Neuroleptic drugs have all been shown to have a high affinity for the D2 receptor (Seeman and Lee 1975). In PET imaging studies, schizophrenia has consistently been linked with an increased capacity for dopamine synthesis and release (Hietala *et al.,* 1995; Howes *et al.,* 2012).

Despite decades of research, the pathway by which alterations in glutamatergic and dopaminergic transmission lead to positive symptoms of psychosis is still not fully understood. Furthermore, the relationship between these two neurotransmitter pathways is far from clear. Because of the difficulty in combining MRI techniques to measure glutamatergic hippocampal activity, with PET imaging of dopamine synthesis and release, very few studies have attempted to measure simultaneously impairments in both pathways (but see Modinos *et al.*, 2021).

Dopamine has long been seen as central to the processing of reward and reinforcement, by signalling prediction errors when expectation of reward occurrence or size differs from

the real-world outcome (Schultz, Dayan, and Montague 1997). This has led some to consider schizophrenia as a disorder of impaired prediction error processing (Corlett, Honey, and Fletcher 2007). Additionally, dopamine has been shown to be critical for salience processing, modulating attending to motivationally neutral, sensory stimuli in one's environment (Takahashi *et al.,* 2017). It is possible that dopamine represents a prediction error signal, not just to rewarded events, but also to salient, unexpected events (Horvitz 2000). These events could be considered to generate a more general sensory prediction error. A link has been reported between patients with, or at risk of developing, psychosis and altered reward and sensory prediction errors, suggesting misattribution of salience in this population (McCutcheon *et al.,* 2018; Murray *et al.,* 2008; Winton-Brown *et al.,* 2014). Evidence for altered salience processing in psychosis comes from fMRI studies combining imaging with behaviour. When subjects were presented with images of faces that displayed either positive, negative or neutral/no emotion, individuals at high risk of developing psychosis showed higher levels of activation in areas of visual processing (Seiferth *et al.,* 2008).

The Aberrant Salience Framework of Psychosis

Whilst it is still unclear exactly how psychotic symptoms arise in patients, one of the most widely recognised frameworks was put forward by Shitij Kapur (Kapur 2003). This model builds on ideas of impaired salience processing in schizophrenic individuals, suggesting that delusions could be a top-down attempt to explain the increased motivational significance of physiologically neutral cues in an individual's environment. One explanation for this progression is an increased propensity for formation of maladaptive associations, simply

because environmental (or internal) events are aberrantly engaging and inappropriately assigned heightened meaning.

In the aberrant salience framework, increased dopaminergic transmission is suggested to impair salience attribution, such that rather than declining in attending to stimuli, individuals assign aberrantly high salience to environmental features. This leads to neutral environmental features becoming increasingly engaging and gaining motivational significance. Indeed, many prodromal accounts of schizophrenia describe how individuals have increased interest in their surroundings, or things taking on new significance, without a clear explanation for these feelings (Bowers and Freedman 1966). This prodromal period of increased, but inexplicable, salience for environmental stimuli can last from days to years before progressing to positive symptoms.

In tasks of visual responding, schizophrenic patients have been shown to spend longer fixating on specific features and shift attention less during free visual exploration, than healthy controls (Sprenger *et al.,* 2013). When repeatedly presented with the same image or stimulus, healthy individuals exhibit habituation; a decline in responding when this stimulus has no meaningful consequence (Rankin *et al.,* 2009). In fMRI studies, habituation (or repetition suppression) of neural responding is seen to repeated presentations of faces, in the hippocampus and visual cortex (Holt *et al.,* 2005; Williams *et al.,* 2013). However, schizophrenic patients failed to show fMRI habituation, and hippocampal discrimination between repeated or different images was lost.

Typical antipsychotics targeting hyperdopaminergia have been shown to reduce aberrant salience. However; rather than reduce only overactive salience in response to physiologically irrelevant events or stimuli, they dampen salience more generally. This lack

of specificity of typical antipsychotics, combined with their lack of efficacy on negative or cognitive deficits of schizophrenia, has been suggested as evidence that dopamine dysregulation is a “final common pathway”, rather than the causal factor of schizophrenia and its symptoms (Howes and Kapur 2009). This lack of specificity of current treatments highlights the urgent need for new therapeutics in this field.

Integrating the dopamine and glutamate hypotheses

While current treatments have focused on alleviating hyperdopaminergia, it is clear this is not the only pathway contributing to the development of schizophrenia. GWAS studies have implicated genes associated with synapses and synaptic plasticity, including glutamate receptors in schizophrenia. Furthermore, other lines of evidence such as post-mortem studies have suggested a role for the hippocampus in disease onset. One possibility is that glutamatergic dysfunction, specifically in the hippocampus, drives downstream hyperdopaminergia. In this next section we will consider the evidence for this possibility.

One explanation for this comes from a model first proposed by Lisman and Grace (2008). In this model the hippocampus acts to compute the relative novelty of a stimulus. Previous studies of single-unit recordings in hippocampus have suggested it acts as a comparator; assessing incoming information for its relative novelty compared to stored memories (Gray 1982; Gray and McNaughton 2000; Vinogradova 2001). Lisman and Grace posited that the hippocampus is then able to induce novelty-dependent firing of dopamine neurons in the ventral tegmental area (VTA) via the nucleus accumbens, both of which have been shown to respond to novel stimuli (Ihalainen, Riekkinen, and Feenstra 1999; Ljungberg, Apicella,

and Schultz 1992). This hippocampal-dependent increase in VTA dopamine activity likely drives an increase in salience in response to novelty.

One possibility is that VTA projections to the hippocampus drive hippocampal disinhibition, which could result in a positive feedback loop (Hammad and Wagner 2006; Lisman *et al.,* 2008). Hippocampal LTP has been shown to be highly dependent on dopamine; application of a D1 antagonist in rat hippocampal slices is sufficient to block LTP (Frey *et al.,* 1991; Morris *et al.,* 2003). Furthermore, exposure to novelty has been shown to generate increased dopamine release in the hippocampus (Ihalainen *et al.,* 1999). This hippocampal-VTA loop appears to ensure that LTP is only facilitated in response to novel experiences, and has been suggested to play a role in conjunctive encoding – the storage of memory inputs relating to multiple features of a stimulus (Tamminga, Stan, and Wagner 2010). This review by Tamminga *et al.* suggests that the hippocampus is responsible for the binding of many inputs from different neocortical inputs into a conjunctive representation of an event – or encoding of associations between components of a stimulus or event. Lisman and Grace (2008) suggested that in schizophrenia, NMDAR hypofunction alters the balance of inputs in CA1 from cortical vs. subcortical inputs, disrupting the comparator function of the hippocampus such that it becomes more difficult to separate novel inputs from those stored in long-term memory. Hippocampal disinhibition, in turn, drives dopaminergic VTA firing in response to perceived novelty, which feeds back to the hippocampus to produce further disinhibition. Over prolonged periods of time, this could contribute to the formation of maladaptive associations.

Additionally, loss of function of NMDARs on GABAergic interneurons could further drive disinhibition of hippocampal networks (Lisman *et al.,* 2008). Disruption of GABAergic

interneurons in the hippocampus, induced through NMDAR antagonists, has been shown to induce cognitive deficits relevant to schizophrenia (Carlén *et al.,* 2012; Nakazawa *et al.,* 2012). Whilst these studies indicate that NMDAR-related disruption of interneurons is sufficient to induce symptoms of schizophrenia, other studies indicate that this alone is not sufficient to drive the onset of symptoms (Bygrave *et al.,* 2016). It is possible that differences between acute pharmacological blockade and lifetime genetic ablation of these receptors are responsible for some of these discrepancies. It is also possible, given the multiple reported pathways that contribute to cognitive deficits relevant to schizophrenia, that these different mechanisms represent some of the heterogeneity of the clinical condition.

LTP and its mechanisms

Hippocampal LTP has classically been considered to be a key mechanism for encoding or formation of associative memories (Bliss and Collingridge 1993; Martin, Grimwood, and Morris 2003). Yet in schizophrenia, the outcome of reduced LTP is actually an increased propensity for association formation (albeit often maladaptive associations). If we are to reconcile these paradoxical statements, it is important to understand what LTP, particularly in the hippocampus, contributes to learning and memory, and how this mechanism is disrupted in psychosis.

LTP is an experimental model of a molecular process that regulates synaptic strengthening. This mechanism was first reported in 1973, when Bliss and Lømo discovered lasting increases in synaptic potentials in DG following high-frequency stimulation (Bliss and Lømo 1973). Since their discovery, LTP has been shown to occur all over the brain and through

many different mechanisms (Martin *et al.,* 2003). For the purposes of this thesis, the focus will be on hippocampal LTP.

LTP, and its opposing process long-term depression (LTD) work to promote either the up- or down-regulation of synaptic weights. LTP promotes synaptic strengthening and refinement of signal-to-noise ratios for efficient information processing. Synaptic connections within the brain are precisely balanced and highly dynamic, with synaptic weights typically increasing throughout the day and decreasing during sleep (Tononi and Cirelli 2006, 2014). Synaptic strengthening of connections across the brain during wakefulness ultimately saturates learning and increases sleep burden. Sleep has been shown to be a homeostatic mechanism for the global scaling-down of synaptic plasticity, regulated by proteins that accumulate during waking hours to increase sleep burden (Diering *et al.,* 2017; Vyazovskiy *et al.,* 2008). Furthermore, the plasticity of dendritic spines themselves – the loci of individual synapses – is equally dynamic. Two-photon imaging of synapses in CA1 showed a rapid turnover of dendritic spines, with a population of spines being replaced every 1-2 weeks (Attardo, Fitzgerald, and Schnitzer 2015).

Synaptic strengthening, governed by LTP, is recognised as the most likely molecular substrate for learning and memory. However, proving this experimentally has not been straightforward. Early experiments looked at changes in field excitatory post-synaptic potentials (fEPSPs) in hippocampus and changes in the population firing of postsynaptic neurons, in response to situations that should induce learning, like exposure to a novel environment. However, this was shown to be confounded by changes in brain temperature associated with exercise (Moser, Mathiesen, and Andersen 1993). Furthermore, changes in fEPSPs could be induced simply by passive warming of the animal on a heating blanket,

consistent with the idea that an increase in brain temperature could potentially drive fEPSP increases.

It wasn't until much later that LTP-like changes in hippocampus were convincingly linked to learning, when Whitlock *et al.,* (2006) used a multi-electrode array in CA1 to look for subtle changes, rather than the population dynamics that had previously been investigated. Animals were trained in an inhibitory avoidance (IA) task. They were placed in the light side of a chamber with light and dark components, and when the animals crossed over to the dark chamber they received a footshock. Latency to then return to the dark part of the box in IA-trained animals was much slower than in mice that did not receive a footshock.

They found very little population-wide change in IA-trained animals, although they did show more variance in the slopes of EPSPs. Using multielectrode arrays, they looked at fEPSPs in different locations and saw a substantial increase in a small subset of channels, but in the majority of neurons either a slight decrease or no change. Furthermore, after IA training, the authors were unable to artificially induce LTP with electrical stimulation, suggesting LTP at these channels had become saturated. They argued that these distributed changes in hippocampal synaptic plasticity, with upregulation of a small subset of neurons and downregulation of many others, supports a role for hippocampal LTP in associative memory formation.

This result cannot be explained by the temperature changes suggested by Moser *et al*. (1993), since rather than a global increase in fEPSPs they actually saw field potentials in most neurons decrease after learning. Only a small fraction of CA1 neurons increased their responding after IA training. To better understand the molecular processes of LTP and how

synaptic strengthening occurs, we need to look at glutamatergic transmission in more detail, and we need to understand the role of the two major ionotropic glutamate receptor classes that exist in the hippocampus; NMDA and AMPA receptors.

NMDARs

NMDARs have been known to play a pivotal role in LTP for many years; LTP induction can be blocked by application of NMDAR antagonists like D-AP5 (Collingridge, Herron, and Lester 1988; Collingridge, Kehl, and McLennan 1983). They were the first receptor to be linked to this mechanism and for many years were considered to be the only route through which LTP can occur. Whilst NMDAR-independent forms of LTP have been revealed (Grover and Teyler 1990), it is clear that NMDARs still play an important central role.

NMDARs are made up of four subunits, of which two subunits are GluN1, (Salussolia *et al.,* 2011). This subunit is obligatory, required for the generation of functional NMDARs; mice lacking this subunit die within hours after birth (Forrest *et al.,* 1994). Hypomorphs – animals with ~10% of the regular GluN1 expression – are viable, but are impaired on every behavioural paradigm on which they have been tested, indicating the importance of this receptor for learning (although this global impairment limits the utility of this model) (Barkus, Dawson, *et al.,* 2012). The other two subunits of the NMDAR are typically either GluNR2A or GluNR2B. As mentioned earlier, GluNR2A, encoded by the gene *Grin2A* has been implicated in schizophrenia by GWAS studies (Harrison 2015). GluNR2B has also been implicated; there is evidence of upregulated GluNR2B in CA3 of schizophrenic patients, from post-mortem studies (Tamminga and Zukin 2015).

These receptors are highly permeable to Ca^{2+} ions, and partially permeable to Na^{+}. The influx of Ca^{2+} following their potentiation is believed to trigger a cascade of events that

leads to plastic changes within the cell. However, at resting potentials, these receptors are resistant to depolarisation. Mg^{2+} ions occupy the ion channel, blocking Ca^{2+} influx into the cell following glutamate binding (NMDARs also require a second ligand to bind, glycine or D-serine, to sites on GluN1 subunits but this thesis will not cover this in detail). Additional to ligand binding, these channels also require a change in membrane potential to alleviate the Mg^{2+} block and allow Ca^{2+} ions into the cell. Therefore, they are both ligand- and voltage-gated. This gives the NMDAR properties of being relatively slow onset, typically requiring a strong tetanus to induce their activation, but also being slow to decay.

Evidence for the role of NMDARs in LTP came from early pharmacological studies investigating the effects of different compounds on hippocampal slices, following electrical stimulation of Schaffer collateral inputs to CA1 (Collingridge *et al.,* 1983). This study showed that the selective NMDAR antagonist D-AP5, prevented the induction of LTP, whilst having no effect on the initial increase in fEPSP amplitude, suggesting a different mechanism involved in the initial depolarisation of postsynaptic cells. Furthermore, D-AP5 had no effect on baseline field EPSPs or when applied after LTP had already been induced by a high-frequency electrical stimulation, indicating an important role in LTP induction but not in subsequent expression.

In a separate study, the role of NMDARs on LTP induction was directly linked to their function in allowing Ca^{2+} ions into the postsynaptic neuron (Lynch *et al.,* 1983). In the presence of a Ca^{2+} chelator, EGTA, LTP induction was blocked following a tetanus. These two studies suggest a role of NMDA-mediated transmission in inducing LTP, by nature of their permeability to Ca^{2+}, but argue that NMDARs are not important for the initial

depolarisation of the cell (or at least make only a small contribution), or the subsequent expression of LTP following induction.

Pharmacological blockade of the NMDAR has shed light on the role of hippocampal LTP in associative learning, though not without some controversy. Rats infused with D-AP5 were impaired in a spatial reference memory task; the Morris water maze (Morris *et al.,* 1986). Rats took longer to acquire the spatial location of a hidden escape platform, spent less time exploring this location in a transfer test where the platform was removed, and were less able to learn a reversal where the platform was moved to the opposite quadrant. Notably, latency to find the hidden platform did decrease over training, and on a reversal test D-AP5-treated rats showed longer latencies to find the new location, suggesting they had learned something during training, despite impairments in performance.

One explanation for this impairment is that sensorimotor performance was impaired more generally by D-AP5, however, it appears these impairments are not associated (Morris 1989). Furthermore, visual discrimination is intact in D-AP5-treated animals. In a non-spatial variant of the water maze, two visually distinct platforms were placed in the pool, above the surface of the water, but only one platform was rigid and reliably predicted escape. In this paradigm, D-AP5 had no effect on animals' acquisition of the escape platform location.

Impaired spatial learning following NMDAR blockade could instead be due to an inability to learn associations between a spatial location and reward (escape in this instance). In a different water maze study, rats were first pretrained in a different room, with different spatial cues, in the absence of NMDAR antagonist (Bannerman *et al.,* 1995). Following pretraining, these rats were then trained in a new room, with new spatial cues, with

hippocampal infusion of D-AP5. Surprisingly, these rats were unimpaired on acquisition of the escape platform location, and memory of this location in a transfer test, despite LTP induction being blocked whilst D-AP5 was administered. Hippocampal NMDAR-dependent LTP induction has long been thought a vital prerequisite for associative learning, but this may not be quite so straightforward (see also Saucier and Cain, 1995).

D-AP5 blocks NMDARs required for LTP in the perforant pathway (particularly DG to CA1) believed to be required for spatial learning, although LTP at other synapses was likely blocked as well. That these animals were able to learn the spatial location of an escape platform, despite LTP in this pathway being blocked, was a striking contradiction to the general consensus of learning models at the time. However, more recent studies have gone on to confirm this finding as we will discuss below. This study also highlights an important limitation of pharmacological interventions, particularly in the case of the NMDAR antagonists. These drugs have been shown to have a diverse range of effects that can affect sensorimotor behaviour and response inhibition, as well as cognitive processes (Cain *et al.,* 1996; Gilmour *et al.,* 2009). Because of the procedural simplicity of the water maze, deficits induced by D-AP5 were assumed to be cognitive. However, this task contains significant behavioural complexity, of which learning a memory representation of a spatial location associated with escape, is just a small component. It is possible that nonspecific effects of NMDAR antagonists have obscured or exaggerated learning impairments in pharmacological studies of associative learning.

Genetic manipulations represent another way of investigating the psychological effects of altered NMDAR function. Early genetic studies of GluNR2A subunit knockout indicated a role for this receptor subunit in associative learning in the water maze (Sakimura *et al.,*

1995). However, later work by the Bannerman group has revealed the contribution of GluNR2A on short-term and working memory during tasks, but no impairment in reference memory depending on association formation between a location and reward, including in the Morris water maze (Bannerman *et al.,* 2008). Bi-directional effects of GluNR2B expression have been shown; cognitive enhancement in its overexpression (Tang *et al.,* 1999) but impairments when it is ablated (von Engelhardt *et al.,* 2008). These impairments were primarily in spatial working memory when ablation was limited to the hippocampus, but subjects with GluNR2B knocked out from forebrain neurons revealed global deficits, making this manipulation difficult to interpret.

A vital role for NMDARs in LTP has also been indicated by studies where *Grin1* encoding the obligatory subunit GluN1 was knocked out from hippocampal subfields. However, these studies have also called into question the relationship between LTP and associative learning. In an early study where GluN1 was ablated in CA1, LTP was shown to be abolished, coinciding with impairments in the Morris water maze (Tsien, Huerta, and Tonegawa 1996). However, this paper also reported subtly delayed acquisition to a visible platform version of the water maze, a task that is hippocampus-independent, raising questions about the validity of this finding. Indeed, several years later this same group published findings that GluN1 was also missing in cortical regions in these mice (Fukaya *et al.,* 2003; Rondi-Reig *et al.,* 2006), indicating this water maze impairment may have been unrelated to hippocampal abnormalities. It is difficult to separate a memory impairment explanation for this deficit from sensorimotor or motivational accounts.

NMDAR ablation in other hippocampal subfields – CA3 and DG – have failed to induce impairments in spatial reference memory, in the water maze or similar tasks (McHugh *et*

al., 2007; Nakazawa *et al.,* 2002; Niewoehner *et al.,* 2007). They did, however find interesting impairments in associative memory, in CA3 knockouts, or in pattern separation following NMDAR ablation in the DG.

Following the finding from Tsien *et al*. the Bannerman group have profiled a mouse with GluN1 ablation specific to CA1 and DG (*Grin1*$^{\Delta DGCA1}$) (Bannerman *et al.,* 2012, 2014; Taylor *et al.,* 2014). Contrary to the earlier findings of impaired spatial reference memory, these studies showed mice to be unimpaired in the water maze, despite absent LTP in the CA3-CA1 pathway. These studies did, however, reveal deficits in *Grin1*$^{\Delta DGCA1}$ mice in a separate spatial reference memory task, the radial maze. In this assay mice were trained to run down specific arms of a 6-armed maze for food reward. The same three arms were always rewarded, and the other three were always unrewarded. *Grin1*$^{\Delta DGCA1}$ mice made more errors in this task, running down unrewarded arms more frequently than controls.

One potential reason for this dissociation between spatial reference memory tasks is that cues used to guide navigation in the water maze are less ambiguous, whereas in the radial maze there are prominent intramaze cues that are visually similar, which could lead to overlap of associations formed across rewarded and unrewarded arms. To test this idea, mice were tested on a variant of the water maze with added local ambiguous cues; a beacon water maze task. Animals were trained to find an escape platform as before, but two visible beacons (black spheres) were placed in the pool. One beacon (S+) represented the location of the escape platform, which was in a constant spatial location throughout training, whilst the other (S-) was a distracting or competing cue, with no escape platform.

In training, both *Grin1*$^{\Delta DGCA1}$ and control mice successfully learnt to swim to the correct beacon and escape the water. In a transfer test, with platform and beacons removed, both

groups spent more time searching the correct quadrant for escape. However, there was one interesting difference between groups. When looking at first-choice accuracy (i.e. where the animal initially swam to during training), based on starting position, *Grin1*$^{\Delta DGCA1}$ mice were more likely to swim to the rewarded S+ beacon if that was the closest beacon when they started that trial. If they started closer to the S- unrewarded beacon, they were more likely to swim to that initially. So despite learning an association between S+ and escape, these mice were more likely to initially swim to the closer beacon, and made more errors.

These studies suggest that hippocampal NMDARs are not essential for forming excitatory associations, required for paradigms of spatial reference memory. However, they do appear to play a role in disambiguating overlapping, or competing memories, such as visually similar rewarded or unrewarded arms of a radial maze, or two visually identical beacons in a water maze. This inability to distinguish between competing memories may have important ramifications for episodic memory retrieval, and indicates a potential pathway by which hippocampal dysfunction and specifically dysregulation of glutamatergic transmission and deficits in LTP, could lead to the memory deficits seen in AD.

Finally, it is worth briefly noting that whilst NMDARs have repeatedly been shown to be a vital component for LTP induction, NMDAR-independent forms of LTP do also exist. There are many different mechanisms for induction and expression of LTP (Martin *et al.,* 2003). This was first shown as far back as 1990, when LTP in CA1 was shown following electrical stimulation, despite the presence of D-AP5 (Grover and Teyler 1990). Interestingly, this study used a higher frequency of stimulation than previous experiments, 200Hz compared to 50-100Hz that was typically used (Bliss and Lømo 1973). They showed that NMDARs are

nonessential to the induction of LTP, instead showing the critical step to be the influx of Ca^{2+} into the postsynaptic cell, by blocking voltage-gated Ca^{2+} channels. More recently, the $Ca_V1.2$ L-type Ca^{2+} channel has specifically been implicated in NMDAR-independent LTP (Moosmang *et al.,* 2005). In mice lacking the gene encoding this Ca^{2+} channel, there was an absence of NMDAR-independent LTP, correlating with impairments in hippocampus-dependent spatial memory.

AMPARs

Whilst the importance of NMDARs to LTP has been known for a long time, comparatively little was understood about AMPARs, and their role in LTP, until much more recently. There were some early suggestions that these receptors may be important for the expression of LTP – maintenance of increased plasticity long after the initial induction – but how exactly this was regulated was unclear (Bliss and Collingridge 1993). The clearest indication of this had been an increased sensitivity to AMPA application to hippocampal slices, after an LTP induction by a tetanus (Davies *et al.,* 1989).

Two papers that came out at the end of the 1990's provided some clearer insight into the involvement of AMPARs in LTP expression. These papers found a rapid increase in the postsynaptic density of AMPARS following LTP induction, suggesting that LTP induces recruitment of AMPARs to the synapse, either from extrasynaptic sites or intracellular vesicles (Hayashi *et al.,* 2000; Zamanillo *et al.,* 1999). These two potential mechanisms for AMPAR incorporation into the membrane were confirmed more recently, but it was found that increased synaptic expression of AMPARS occurred through trafficking, and exocytosis was primarily a means to replenish extrasynaptic populations (Makino and Malinow 2009).

Compared to NMDARs, the channel conductance of AMPARs is very different, with AMPAR currents being much faster for onset and decay, mediating rapid synaptic transmission after glutamate release. Whilst NMDARs are highly permeable to Ca^{2+} ions, AMPARs mostly facilitate the influx of Na^{+} into the cell, driving depolarisation of the postsynaptic neuron. This rapid influx of Na^{+}, driven by AMPARs depolarises the cell and alleviates the Mg^{2+} block on the NMDAR during LTP induction.

AMPARs are heterotetrameric complexes, made up of four subunits, GluA1-4, with the different subunits conferring specific properties on the receptor. For example, receptors lacking GluA2 subunits have an increased permeability to Ca^{2+}, indicating a unique function in regulating synaptic plasticity for these receptor subtypes (Lalanne *et al.,* 2018). GluA4 receptors play a critical role in the developing hippocampus; whilst it is not found on mature excitatory pyramidal neurons (Zhu *et al.,* 2000), it is seen in parvalbumin-containing interneurons (Fuchs *et al.,* 2007). Adult hippocampal neurons typically contain heteromers consisting of GluA1/GluA2 or GluA2/GluA3.

One important difference between these subunits is in the length of their cytoplasmic carboxyl tails. Whilst GluA2 and GluA3 have short cytoplasmic tails, GluA1 and GluA4 carboxyl tail lengths are comparatively much longer (Shi *et al.,* 2001). This appears to confer specific properties on the trafficking of these specific subunit-containing receptors. GluA2/GluA3 heteromers, with short cytoplasmic tails, are generally regulated through vesicular exocytosis. GluA1-containing AMPARS, however, are anchored extrasynaptically in the membrane and recruited to the postsynaptic density following excitation. Shi *et al.* also showed that GluA2/GluA3 heteromers are continuously recycled at the synapse in an excitation-independent process, separately from processes regulating GluA1/GluA3

receptor subtype trafficking. So, upon stimulation, whilst these receptors continue to cycle in and out of the membrane, additional GluA1/GluA3 AMPARs are recruited to the synapse via extrasynaptic trafficking, increasing AMPAR density to enhance plasticity. This process increases the excitability of the synapse and indicates a possible mechanism for the expression of LTP via AMPARs, driven by GluA1-containing AMPARs.

As mentioned previously, Whitlock *et al.* demonstrated a clear link between learning in the one-trial inhibitory avoidance paradigm, and LTP-related changes in neuronal plasticity (Whitlock *et al.,* 2006). They went on to investigate this association further by looking at molecular changes at synapses post-training. They found phosphorylation of GluA1 following training, and increased presence of GluA1 and GluA2 at the postsynaptic density, highlighting the importance of GluA1-dependent upregulation of AMPARs in the expression of learning. However, this was a transient effect, AMPAR-related changes had declined back to baseline levels within two hours. Behavioural responding, the learned conditioned avoidance behaviour, was still present 24 hours after training however, long after AMPAR-related changes had returned to baseline. There is a clear disconnect therefore between changes in AMPAR upregulation, which is transient, and the associative memory trace, which is stored for much longer.

One explanation for the decline in post-synaptic GluA1 levels could be that AMPAR-related changes have been restored back to baseline levels during bouts of sleep, particularly since behavioural testing commonly occurs during the light cycle, when rodents are typically less active. The overall increase in synaptic weights throughout periods of wakefulness, and resetting of synaptic strengths during sleep is a finely-tuned homeostatic mechanism (Tononi and Cirelli 2006, 2014), and a similar equilibrium has been shown in GluA1 levels

(Vyazovskiy *et al.,* 2008). Levels of GluA1-containing AMPAR increase during wakefulness, and decline during sleep. Furthermore, Vyazovskiy *et al.* showed an increase in GluA1 phosphorylated at Ser831 – a modification associated with increased synaptic potentiation – in the waking group. Ser831 phosphorylation is associated with intracellular activation of calcium/calmodulin-dependent kinase II (CaMKII), which was also found in higher levels and increasingly phosphorylated, in the waking group. In animals with a high sleep pressure after a period of enforced wakefulness, it was harder to induce LTP through electrical stimulation. These findings provide strong evidence that synaptic strength is homeostatically maintained across the sleep/wake cycle, and that increasing synaptic strength throughout prolonged wakefulness not only increases sleep need but saturates synaptic plasticity preventing further induction of LTP. Not only this, but GluA1-containing AMPARs play a vital role in this process. However, these circadian changes in GluA1 levels do not explain the disconnect seen by Whitlock *et al.* (2006), where despite declining GluA1 levels after 2 hours, the conditioned avoidance behaviour was robust 24 hours later. It is possible that this memory is consolidated elsewhere in the brain. An alternative account of the changes in post-synaptic GluA1 levels, which we will return to later, is that changes in hippocampal AMPAR expression reflect other components of short-term memory.

Whilst GluA1 plays a central role in LTP through trafficking of AMPARs to the membrane, it's worth mentioning briefly that alternative GluA1-independent mechanisms for LTP expression exist. Early studies in mice lacking the GluA1 AMPAR subunit showed a complete lack of LTP (Zamanillo *et al.,* 1999). However, an alternative form of LTP has been shown where although the initial increase in activity is substantially reduced, an increasing amplitude of fEPSP slope was observed over time (Hoffman, Sprengel, and Sakmann 2002). This study used a theta-burst stimulation, resembling stimulation that would occur under

physiological conditions, and showed that by 50 minutes fEPSP amplitude in GluA1$^{-/-}$ slices matched that of wild-types (WTs). This process was blocked in the presence of the Ca^{2+} chelator EGTA, indicating that intracellular rises in Ca^{2+} following stimulation is critical to this form of LTP expression. A later study of LTP in these mice found GluA1-independent LTP to be highly dependent on GluNR2B-containing NMDARs and intracellular changes modulated by CaMKII (Romberg *et al.,* 2009). In WT mice an interaction between GluNR2B subunits and CaMKII has been shown to be important for LTP, indicating a possible pathway for LTP that is independent of GluA1-containing AMPARs (Barria and Malinow 2005).

In fact, it appears the composition of AMPARs at the synapse is relatively unimportant for the expression of LTP, so long as a reserve pool of AMPARs exists extrasynaptically that can be inserted into the synapse (Granger *et al.,* 2013). In the absence of AMPARs, even kainate-type glutamate receptors can serve this purpose. These findings revolutionised the idea that the finely-tuned regulation of synaptic AMPAR composition is essential for intact LTP. GluA1-dependent, GluA1-independent and even AMPAR-independent forms of LTP can occur, highlighting the remarkable resilience of LTP in response to deficits in functional receptors, but also reflecting the potential heterogeneity of this process. To be this well conserved across multiple mechanisms emphasises the importance of LTP to maintaining physiological function in the healthy brain. Whilst these alternative mechanisms can occur, it is likely that GluA1-dependent trafficking of AMPARs to the synapse is a key mechanism for rapidly induced LTP under normal conditions.

Critically, two dissociable processes appear to play a functional role under normal conditions; a rapid GluA1-dependent increase in AMPAR expression at the synapse following a stimulus that enhances plasticity (Whitlock *et al.,* 2006), but also a slower

GluA1-independent process that initiates intracellular Ca^{2+} influx, and potentially relies at least in part on the interaction between GluNR2B and CaMKII, to induce Ca^{2+}-dependent intracellular changes. It is possible that these dissociable plasticity processes have different contributions to learning and memory. Moreover, there is compelling evidence that GluA1-dependent process in particular are relevant for behavioural habituation, a major focus point of this thesis. To understand the physiological relevance of GluA1-dependent plasticity, we need to understand the contribution of GluA1 to behaviour.

GluA1 and behaviour

There is an extensive literature, from the Bannerman group and many others, exploring the relationship between GluA1, learning and behaviour. Much of this work has used manipulations to ablate or reduce GluA1 expression, such as the *Gria1* genetic knockout mouse model (Zamanillo *et al.,* 1999). In this initial study, there was no expression of LTP, and yet acquisition and retention of spatial reference memory in the water maze was unimpaired.

This intact spatial reference memory was later confirmed, not only in the water maze but also in an appetitive Y-maze task (Reisel *et al.,* 2002). Mice were trained to enter an arm associated with a food reward, based on its spatial location, starting from either of the other two arms of the Y-maze, something $GluA1^{-/-}$ were equally as able to learn as WT. However, $GluA1^{-/-}$ did show impaired learning on a spatial working memory T-maze task, where animals were required to retain a short-term memory trace of a previously visited location, but otherwise sensorimotor and motivational demands were similar to those of the Y-maze. In this non-match to place paradigm, they first entered either the left or right arms of the T-maze on a forced choice sample trial, where access to the alternate arm was

blocked. In a subsequent choice trial, mice were free to enter either arm but only the opposite arm was rewarded; they had to alternate their choice. Whilst control animals can do this, and innately prefer the novel arm over a previously visited arm where reward had already been consumed, GluA1$^{-/-}$ performed no better than chance, even after many trials. Importantly, both reference and working memory-related behaviours have been shown to be impaired by hippocampal lesion (McHugh *et al.,* 2008; Reisel *et al.,* 2002), indicating that whilst GluA1 was constitutively knocked out, hippocampal GluA1 likely plays a key role in these behaviours.

This dissociation between reference and working memory was investigated in a separate study, using a six-arm radial maze (Schmitt *et al.,* 2003). In this task, three of six arms were baited with food reward. In the first experiment animals were allowed to enter all arms repeatedly until all rewards were consumed. Entries into unrewarded arms were considered reference memory errors as this stayed constant between trials, whilst repeat entries into arms were working memory errors. Surprisingly, and in contrast to findings from the water maze and Y-maze, GluA1$^{-/-}$ mice repeatedly made both reference and working memory errors, failing to acquire this version of the task.

One argument for this finding is that impaired working memory performance subsequently disrupted reference memory, when tested simultaneously. In a second experiment, doors were closed after a first entry such that working memory errors could not be made. In this second reference memory-dependent version, GluA1$^{-/-}$ now learnt as rapidly as WTs. The working memory deficits were further confirmed to rely on GluA1 and its subsequent role in LTP, through partial genetic rescue of CA1 GluA1 levels, leading to improved performance on the working memory version of the radial maze (Schmitt *et al.,* 2005). As

described earlier, the radial maze consists of six visually similar arms, and requires the animals to distinguish between these locations based on their association with either reward, or lack of reward. NMDARs have previously been shown to be critical for dissociating between these visually similar cues (Bannerman *et al.,* 2012). It is possible that GluA1 is required for retaining a short-term memory trace of previously visited locations, a requirement for intact working memory, but has no effect on acquisition of reference memory acquired over multiple sessions (provided the opportunity for working memory errors is prevented), either in the radial maze or the Morris water maze.

GluA1 and Habituation

Additional to the effects on associative learning in tasks of reference and working memory, a range of other behaviours have been examined in GluA1$^{-/-}$ mice. They have been shown to exhibit hyperactivity in response to novelty during open-field exploration, but normal activity in the homecage (Bannerman *et al.,* 2004; Wiedholz *et al.,* 2008). Furthermore, locomotor hyperactivity was not further exacerbated by the administration of the NMDAR antagonist MK-801, suggesting some commonality in the pathway that drives this behaviour. The D2 antagonist haloperidol acted to reduce hyperactivity, also indicating that this behaviour is in some way mediated by the downstream effects disrupted of glutamatergic transmission on mesolimbic dopamine release (although haloperidol is relatively nonspecific and also acts at other receptors). One indication that this is indeed related to elevated striatal dopamine comes from recent fast-scan cyclic voltammetry studies showing hyperdopaminergia to emerge in GluA1$^{-/-}$ mice in response to repeated presentations of light cues (Panayi *et al.,* 2021), potentially supporting evidence of a hippocampal-VTA loop in regulating this behaviour (Lisman and Grace 2005).

One behaviour that has been studied extensively in the GluA1 knockout model is habituation; defined as the tendency to decline in behavioural responding following repeated presentations or exposure to a stimulus. This vital component of behavioural function is evolutionarily conserved across all animals that have been studied (Rankin *et al.,* 2009; Thompson 2009; Thompson and Spencer 1966). In a real-world situation, if an environmental feature is observed for the first time, it could potentially signal food availability, predators, or other physiologically relevant information and so attention should be paid to it. Additional to its potential valence, novel events with no prior expectation of their occurrence will be surprising. Following repeat or prolonged exposure to a stimulus, in the absence of physiological meaning, not only does the stimulus become less surprising, but the individual learns about its association, or lack of one, to current goals. As a result, habituation occurs. It is physiologically beneficial to attend to cues less if they are not immediately useful, in order to reserve attentional capacity for other events and environmental stimuli.

Impairments in habituation can account for the divergent behavioural responses to novel open-field and familiar homecage environments (Wiedholz *et al.,* 2008). GluA1$^{-/-}$ mice respond normally to their homecage after 48 hours of acclimation, arguing for the presence of a memory trace of this environment, or at least for one that develops over an extended period of time. On the other hand, the hyperactivity observed in response to novelty could be explained by an absence of short-term habituation; if mice are failing to recognise an environment as less novel over time they would be expected to continue to exert a high level of exploratory activity, instead of reducing their attending to their surroundings. Tasks such as the T-maze that are based on relative familiarity of recently visited arms rely on an innate drive to explore novelty over familiar locations. This win-shift behaviour has been

shown to be hippocampus (Deacon *et al.,* 2002) and GluA1-dependent (Bannerman *et al.,* 2004). A deficit in short-term habituation could account for cognitive findings in GluA1$^{-/-}$ mice, of intact reference memory on tasks acquired over many days, but impairments in working memory tasks that require a short-term memory of previously visited locations.

One task that can be adapted to assess both short- and long-term habituation is the spatial novelty preference Y-maze. In the short-term version of this assay, animals are initially exposed to two arms of a three-arm maze in a sample trial, with the third arm blocked off. After a brief inter-trial interval (ITI), animals are then exposed to the maze a second time with all three arms available. In this test trial the previously blocked arm will now be novel, while a recent memory trace should be present for the other two arms, such that they will be less engaging. WT animals show a preference for the novel arm during this test trial (Sanderson *et al.,* 2007). However, GluA1$^{-/-}$ fail to show a preference for the novel arm, indicating that they fail to recognise this arm as novel during the test. GluA1$^{-/-}$ were similar in their lack of preference to a complete hippocampal lesion animal, indicating the need for encoding of spatial information during the sample trial for accurate performance on this task. In contrast to intact reference memory in the water maze and radial maze which are trained over multiple days, this finding suggests an absence of a short-term memory trace in GluA1$^{-/-}$ mice, or at least a failure to appropriately express this memory in behavioural responding.

In a follow-up study Sanderson *et al*. then looked at how GluA1$^{-/-}$ perform if trials are spaced 24 hours apart; relying instead on long-term habituation (Sanderson *et al.,* 2009). Mice received multiple sample phase exposures to two arms, 24 hours apart, before a test phase with access to all three arms, a further 24 hours after the last exposure phase. Under

these conditions, GluA1$^{-/-}$ were unimpaired and in fact showed more robust novelty preference than WT. Taken together these results suggest a dissociation between GluA1-dependent short-term habituation, and GluA1-independent long-term habituation. One possibility is that these parallel memory processes are regulated through the GluA1-dependent/independent forms of LTP discussed previously. This could also explain the intact reference memory in these animals, which relies on the formation of associations between a spatial location and reward – food in the radial maze or escape in the water maze. Similarly, long-term habituation may depend on the formation of associations over days of training. In summary, GluA1 may be particularly important for a short-term memory trace that supports short-term habituation processes. But from the findings discussed so far, it is not clear whether this is restricted to the spatial domain.

Novel object recognition (NOR) has been used for many years as a means of assessing novelty discrimination and preference in rodents for non-spatial stimuli (Ennaceur and Delacour 1988). In this paradigm animals are first exposed to two copies of an identical object, in an open-field arena. After a few minutes of exploration, animals are removed from the arena, the objects are swapped for different ones and the animals are returned to the arena for a test phase. In the test phase animals are presented with a third copy of the sample object, and a different, novel object. Increased exploration of the novel, compared to the more familiar object, is considered to indicate intact novelty recognition, due to habituation to the previously experienced object.

It has been shown that GluA1$^{-/-}$ mice can respond normally to novelty in this task (Sanderson, Hindley, *et al.,* 2011). However, they also spent longer exploring objects during the sample phase than WT counterparts. When the total object exploration time during

the sample phase was matched between genotypes – by yoking GluA1$^{-/-}$ animals to WTs – GluA1$^{-/-}$ deficits in novelty discrimination were reported, suggesting a lack of habituation in the sample phase. This reveals an interesting insight into how these animals may be behaving at a psychological level. Increased attending to the objects in the sample phase (or at least a failure to decline in attending in this time) protected GluA1$^{-/-}$ from NOR deficits. This study indicates that the way in which GluA1$^{-/-}$ process novel stimuli may be different to their WT counterparts, showing either aberrantly high attending to novelty, or a failure to habituate in a timeframe in which habituation is seen in WT controls.

In a recency-dependent version of the NOR task, animals were first exposed to two copies of object A, as described before. They then received a second sample phase, where they were exposed to two copies of object B. The subsequent test consisted of a novel copy of objects A and B, but object B had been presented more recently and so should be more recently habituated. Thus wild-type mice show a preference for object A over object B during the test phase. When exposure exploration was yoked to WTs, GluA1$^{-/-}$ mice were again impaired in their ability to express a novelty preference for object B, implicating GluA1 in the expression of recency-dependent memory.

Finally, the authors also investigated NOR in a context-dependent task variant; animals were trained to multiple exposures of a sample object A, in a specific context X. They were also trained to a second sample object B, in a distinctly different context Y. In the test phase they received pairings of objects A and B, in either context X or Y, such that one object would be recognisable as familiar in that context and the second object would have never been experienced in that context before. This was run over several days of exposure, similar to the long-term Y-maze (Sanderson *et al.,* 2009), and as with that task, GluA1$^{-/-}$

mice were unimpaired. They showed a strong preference to explore the incongruent object in each of the contexts, reflecting their long-term habituation to the congruent object.

Taken together, this set of studies indicates that GluA1 is a requirement for intact non-associative recency-dependent memory (recency-dependent NOR and short-term spatial novelty preference Y-maze), but not for context-dependent memory that is based on associations formed over days of exposure (e.g., long-term Y-maze and context-dependent NOR). One explanation for this is that GluA1 impairs learning when a stimulus is primed into short-term memory via exposure to that stimulus, but not when the stimulus is primed via retrieval of an association of that stimulus with, for example, a context. This idea about how a stimulus enters short-term memory will be explored in more detail in the next section.

The aberrant salience framework posits that neutral or irrelevant stimuli gain increased significance over prolonged or repeated exposure. This can be expressed as either a failure to habituate, or in more extreme cases as sensitisation to stimuli – an increase rather than a decrease in attending to stimuli over repeated presentations. GluA1$^{-/-}$ and WT mice were also investigated for their responses to visual stimuli that were either novel, or repeat presentations (Sanderson, Sprengel, *et al.,* 2011). Mice were first trained on an operant variable interval (VI) schedule, to receive food reward on average every 120s upon nose-poking of a food magazine. After acquiring this learned behaviour, subjects were presented with pairs of light stimuli, repeated in sequence, 30s apart. Within a light-pair, the cues were either the same or different. The behavioural outcome of interest was time spent orienting to the light. Because mice were trained to nose-poke to a magazine, this orienting behaviour was measured indirectly as a reduction in nose-poking behaviour; the extent to

which the stimulus drew attention away from potential food reward. A novel stimulus on first presentation would be expected to be highly engaging, eliciting a big reduction in time spent in the magazine, whilst presenting this same stimulus again 30s later would be expected to induce less orienting.

This is exactly what was observed in WT mice. Using the first stimulus in each light-pair as the baseline of orienting responding, upon repeat presentation of the same stimulus, less orienting was seen. On the other hand, if a novel cue was presented 30s later, the orienting response was no different from the first presentation in the pair. In GluA1$^{-/-}$ mice, no difference was seen in responding to different visual stimuli. However, when the same light was presented twice in a row, these animals actually increased their responding. Rather than habituating to the repeated presentation, animals spent more time attending to the stimulus 30s later, indicating sensitisation.

Sensitisation, or increased attending, to stimuli, represents a mechanism by which individuals may show an increased propensity for formation of maladaptive associations – a key requisite of the aberrant salience framework in schizophrenia. If GluA1$^{-/-}$ mice have genuine construct validity for schizophrenia they would also be expected to exhibit this increased associative learning, something which will be investigated in detail in this thesis. But to understand how changes in habituation and attention could lead to maladaptive formation of associations, we must first consider the psychological mechanisms underpinning both basic habituation and higher cognitive function. Only by integrating these different aspects of cognition can we understand how animals may simultaneously express deficits in short-term memory, but enhanced long-term memory in other circumstances.

Wagner's SOP

Early Models of Associative Learning

In the early 1970's psychologists wanted to integrate what was known about learning at the time into a comprehensive model. Some of the earliest ideas of how learning occurs still influence our understanding of psychology today, and so are worth discussing briefly here.

At its most basic, learning – or conditioning – relies on the ability of a conditioned stimulus (CS) to accurately predict an unconditioned stimulus (US); If the CS-US representation is predictable, learning about this predictable relationship will occur throughout training. Robert Rescorla and Allan Wagner, in 1972, developed a model of conditioning to explain the relationship between the CS and US, and importantly, how much learning about this relationship would occur on a given trial, or presentation of those stimuli (Rescorla and Wagner 1972; Wagner and Rescorla 1972). This simple equation was able to explain nearly everything we understood to be true about conditioning at the time:

$$\Delta V = \alpha\beta(\lambda - \Sigma V)$$

ΔV represents the change in predictive value, V, or associative strength, on a given trial. The key to determining this value is the parentheses (λ – ΣV), a prediction error term describing the surprisingness of the US, λ, compared to what is known about the CS (the sum of all values, V, predicting the US). The final components, α and β, indicate the salience of the CS and US respectively and modulate the amount of learning that occurs.

The central concept this model was based around is surprise; if the US is surprising, as on trial 1 of a conditioning paradigm, then lots of learning will occur. On the second trial, the CS may be weakly able to predict the US, so $\lambda - \Sigma V$ will be slightly less than on trial 1. Learning will still occur, but less than on trial 1. Over many presentations of a stimulus, learning saturates as the CS comes to better predict the presentation of the US, and ΔV goes up by increasingly smaller increments. After enough trials ΣV equals λ, such that the parentheses cancel out and no new learning occurs.

This model explains some important early findings in psychology, such as blocking – if an animal is trained to learn a CS-US pairing such as presentation of a noise cue followed by an aversive footshock, to asymptote, it is impossible to then learn about a new CS (e.g., a light) when it is paired with the original CS-US pairing (Kamin 1968). ΣV represents the predictive value of all CSs. If the predictive value for the noise ($V_{N)}$ is at asymptote it will be equal to λ. Although the predictive value for the light ($V_{L)}$ is 0, since V_N is at asymptote, the summed value of all predictive values ($V_N + V_L$) is 1 and so ($\lambda - \Sigma V$) equates to 0; meaning no new learning occurs.

In a follow-up study, Kamin showed that subsequent unblocking of a stimulus can occur, if the intensity of the US is increased (Kamin 1969). A more intense US will be represented by a higher value for λ (e.g., increasing from 1 to 2). For example, in the case of a footshock US, if upon introduction of the novel CS the footshock intensity is also increased, then new learning will occur about the relationship between the footshock and both CSs. This alters the equation such that $\lambda - V_N + V_L$ now equals $2 - 1 + 0$. Because this equation now solves to a positive value, new learning can occur, a phenomenon termed unblocking.

One of the most important concepts to emerge from this model was the idea that learning occurs not only to the CS-US relationship, but also to the relationship between stimuli and the context; which in itself can act as a CS. Operant tasks don't take place in a vacuum devoid of interfering environmental features, but before the development of the Rescorla-Wagner model little consideration had been given to how other contextual stimuli might interact with conditioning.

Whilst this model is still considered relevant today, a few caveats were added in later versions that are worth mentioning. Nick Mackintosh introduced a new concept, reflecting the salience of the CS, α (Mackintosh 1975). He argued that α reflects not just the salience of the CS, but also the amount of attention paid to it. If a salient stimulus occurs, but the individual isn't paying attention to it, there will be no learning on that trial.

This new addition could explain latent inhibition (LI); an important feature of conditioning that the Rescorla-Wagner model couldn't account for. In LI, animals are first pre-exposed to many presentations of the CS, and subsequently fail to acquire, or are slower in acquisition of, a CS-US association when the CS is later paired with food or footshock. Mackintosh argued that the amount of attention paid to a stimulus would decrease if it repeatedly occurred without any meaningful consequence, i.e., habituation to the physiologically irrelevant stimulus would occur, such that α would go down during preexposure. Because of its modulatory effect on the parentheses of the Rescorla-Wagner model, if α is 0, no learning will occur.

Priming and Surprise

Throughout conditioning, the US becomes less surprising over many trials because through learning about the CS-US relationship, occurrence of the CS primes the US in short-term

memory. Priming refers to the presence of a memory trace in short-term memory, prior to that event happening, and if that memory is already primed in short-term memory it will therefore be less surprising if it subsequently happens.

In the late 1970's Allan Wagner reconceptualised the way we think about surprise (Wagner 1976, 1978). The idea that surprising events would get more processing than unsurprising events remained, but he considered there to be two ways in which surprise is mediated. Importantly, he also argued that the surprisingness of the CS was just as important for associative learning as the surprisingness of the US.

A stimulus is surprising if one isn't already thinking about it. Surprise is decreased in one of two ways; firstly a real-world occurrence of the stimulus, which generates a memory trace in short-term memory of that event. This is termed self-generated priming.

Alternatively, priming can occur when a memory trace is retrieved from long-term memory into short-term memory; termed retrieval-generated priming. For example, after many trials of a CS-US conditioning task, such that an excitatory association has formed between these two cues, the occurrence of the CS will trigger a memory trace of the US, which is retrieved or primed into short-term memory. Crucially, if an event is already primed in short-term memory when it occurs, Wagner considered that no (or at least less) learning would occur about it because the individual was already thinking about it, making it less surprising.

Early evidence for priming came from eyeblink conditioning experiments in rabbits using a puff of air on the eye to elicit a blink response (Terry 1976). If rabbits receive a presentation of the airpuff US immediately before the CS-US pairings are presented, less learning occurs to the CS, compared to pairings with no pre-exposure. This can be explained by self-

generated priming of the US into short-term memory. Because it was already primed, its subsequent occurrence was less surprising and less learning occurred. This priming could be disrupted by presenting a distractor – a clicking noise and a vibration – between the US and the CS-US pair. This was enough of a competing stimulus to knock the memory trace of the US out of short-term memory, such that it was surprising again in the CS-US presentation. This highlights another important feature of short-term memory that we will return to later; its relatively small capacity.

This model can also explain habituation. When a novel stimulus is presented for the first time, it is highly surprising and elicits a high attentional response. But over many repeated presentations, or prolonged exposure, the surprisingness of a stimulus decreases. If the cue bears no relevance to physiologically relevant goals, the animal will habituate to its presence.

A student of Wagner's, Whitlow, showed the effect of self-generated priming on habituation in a conditioning task in rabbits (Whitlow 1975). Rabbits were exposed to a series of tones, some high- and some low-pitched, 60s apart, and vasoconstriction responses in the ear were recorded. In half of these tone presentations, they were immediately preceded by a tone of the same pitch, whilst in the other half they were presented with tones of a different pitch. As predicted by the priming model (and later also shown by Sanderson *et al.* (2011) in their study on orienting to lights in mice), preceding a trial with the same tone caused animals to habituate, producing a weaker response on the second presentation. As seen in the eyeblink conditioning experiment by Terry (1976), a distractor between presentations was again able to reinstate a strong novelty response to

the tone, indicating the memory trace of the tone had been knocked out of short-term memory and was once again surprising.

Wagner's Model of SOP

Wagner combined all of these concepts in one framework, the sometimes opponent processes, or alternatively standard operating procedure (SOP) model (Wagner 1981). He considered that "nodes" of a memory exist in the brain, in a resting baseline "inactive" or "I" state. These nodes are made up of many individual elements, and when a stimulus is presented for the first time – and is therefore highly surprising – a subset of elements become activated into a state of focal attention (or the "forefront of attention"), termed "A1". Crucially, while the overall capacity for memory in the brain (in the I state) is nearly infinite, A1 has a very small capacity, as theorized by earlier conditioning studies (Terry 1976). But rather than decay immediately back to the inactive state, elements instead decay to a lower state of activation termed "A2", or peripheral attention. A2 has a larger capacity than A1, though still limited and less than I, but elements that decay to A2 are unavailable to be recruited back to A1 in Wagner's model. They can only leave the A2 state through further decay to I. This is a crucial feature of the SOP model, that explains habituation. If elements of a stimulus have recently been activated (and now in A2), they are unavailable to be activated again into A1 upon repeated presentation and so a smaller response will be seen on the second trial. Importantly, an element can only exist in one state at any given moment, although a specific stimulus is made up of multiple elements which can exist in different states. The summed activity of all elements making up a stimulus drives the behavioural response, however elements in A2 would be expected to elicit a much weaker response than those in A1.

This model also explains associative learning. For an excitatory association to be formed between stimuli, they must be activated at the same time, or in close temporal proximity, such that they are both active in A1 together. The study from Terry (1976) showed that no new learning occurred if the US is already primed in short-term memory, as a result of US presentation prior to the CS-US pairing. If elements of this memory trace were in A2 by the time of the CS-US pairing, new learning would be prevented.

There is one more critical feature of SOP that can explain the majority of conditioning experiments to date (in fact one of the major criticisms of this model is that it is so flexible it is difficult to disprove). We have so far described how self-generated priming can prime a real-world presentation of a stimulus into attention, such that rapid repeated presentations of a stimulus generate declining levels of attention (habituation). Retrieval-generated priming represents another mechanism through which habituation occurs that may explain habituation over longer timescales. When a memory is primed into short-term memory by retrieval of a memory from long-term storage (through learned associations with other stimuli or surroundings, e.g., context), a subset of elements of this memory are primed directly from the I state into peripheral attention, A2.

Following conditioning of a CS-US association, CS presentation will prime the memory of the US into short-term memory, into A2, through retrieval-generated priming. These elements are subsequently unavailable for activation to A1 upon presentation of the US. This can explain many core features of associative learning, such as the blocking result of Kamin (1968).

In order for excitatory associative learning to occur, cues must be presented contiguous with each other such that they are activated in A1 at the same time. However, it is possible

for some learning to occur, though only weakly, if cues are active together in the A2 state of peripheral attention, an idea argued in a more recent version of modified SOP (Aitken and Dickinson 2005). Furthermore, if elements of two stimuli occupy opposing states (i.e., CS in A1, US in A2) then inhibitory learning will occur. This can explain phenomena such as backward conditioning, where the US is presented before the CS, and the CS comes to predict the absence of the US thus acting as an inhibitor.

The Hippocampus and SOP

It should be noted that SOP is purely a psychological framework, as of yet there is no concrete evidence confirming its existence at a molecular level within the brain. That being said, it remains the most plausible account available for how associative learning could occur, whilst also integrating associative learning with lower levels of cognition like habituation and attention, which provides a useful framework for considering the relationship between these behaviours. Furthermore, hippocampal lesion studies have indicated that if this framework does exist biologically, it likely exists, at least in part, in the hippocampus (this doesn't exclude the chance of it also existing in other brain regions). This would make sense given the clear role of the hippocampus in habituation and associative learning, as described above.

In a study of orienting responses to a tone, habituation of the orienting response was seen in rats to presentations of the same tone when presented 10s, but not 70s, apart (Marshall *et al.,* 2004). In a choice presentation of both the tone that had been presented 70s previously, and the tone presented 10s previously, normal rats oriented more to the less recently presented tone. However, hippocampal-lesioned rats oriented more, rather than less, to a recently presented tone, and in a preference test, oriented more towards the

tone presented 10s previously. This finding suggests that the hippocampus is critical for habituation, through self-generated priming, decay of stimulus elements into a state of peripheral attention, and the subsequent decline in attending to that stimulus. Hippocampal lesions prevented that process from occurring, and instead appeared to induce stimulus-specific sensitisation to the recently presented stimulus. Importantly, this finding also argues for the existence of a memory trace for a stimulus outside of the hippocampus, and argues for a hippocampal role instead of modulating expression of that memory, potentially via SOP.

A separate lesion study has also implicated the hippocampus in retrieval-generated priming, as well as self-generated priming (Honey and Good 2000). In this study, rats were first trained to learn an association between two pairs of two stimuli; a tone followed by a constant light and separately, a click noise followed by a pulsing light. At the test stage, one auditory cue (e.g., tone) was presented, followed by both the constant and pulsing lights in a preference test. Because the constant light was paired with the tone during training, a memory trace of this light should be primed via retrieval-generated priming into short-term memory. The pulsing light was not paired with this stimulus and so was not primed into short-term memory, and should therefore elicit increased orienting in a preference test. This is what the authors saw in control rats. However, hippocampal lesions impaired this response; lesioned rats oriented more to the primed visual cue, again exhibiting stimulus-specific sensitisation, and confirming the role of the hippocampus in retrieval-generated priming.

These two studies together support the idea that the hippocampus is critical for both short- and long-term habituation, via two mechanisms of priming memories into short-term

memory, and could be a hub for SOP in the brain. The relationship between the hippocampus, habituation, and stimulus-specific sensitisation will be explored later in this thesis, as we first consider the role of GluA1 in more detail as well as pharmacological manipulations that have been shown to induce similar phenotypes.

GluA1 and SOP

To summarise our understanding of GluA1 so far, the receptor subunit has a clear role in certain forms of LTP, but is not required for others (Hoffman *et al.,* 2002). Furthermore, it has a role in short-term habituation, such as in the spatial novelty preference Y-maze, where GluA1$^{-/-}$ mice were impaired, but not in long-term habituation, where they were enhanced (Sanderson *et al.,* 2009). GluA1$^{-/-}$ mice have also been shown to be normal on reference memory tasks, trained over many days, that rely on the formation of long-term associations, like the water maze and radial maze (Reisel *et al.,* 2002; Schmitt *et al.,* 2003; Zamanillo *et al.,* 1999). GluA1$^{-/-}$ mice are able to discriminate between novel and familiar objects in a context-dependent object recognition test, indicating an intact ability to form associations between environmental stimuli, but crucially were impaired on the recency version which may rely on self-generated priming to modulate attending to the familiar (and less recently familiar) objects (Sanderson, Hindley, *et al.,* 2011). Finally, in a test of their orienting response to novel or familiar visual stimuli, GluA1$^{-/-}$ failed to habituate to repeat presentations of the same stimulus, instead showing sensitisation on the second presentation.

One account of these deficits could be that GluA1 deletion blocks elements from decaying from the A1 state to the A2 state (Barkus *et al.,* 2014). Thus, there is a deficit in self-generated priming in these mice leading to deficits in short-term habituation. If elements

are blocked from decaying to a lower state of activation, they could persist in focal attention for longer, and thus remain salient for longer than in WT mice. In fact, elements could even accumulate in A1 as more elements of a stimulus are gradually activated and this could lead to sensitisation, explaining the increased response to repeated presentations of a visual cue in the orienting to lights task (Sanderson, Sprengel, *et al.,* 2011). Additionally, this could explain an increased propensity to form long-term associations, due to elements staying in the forefront of attention for longer, an idea we will return to in later chapters.

Hippocampal lesion studies have shown an impairment in self-generated and retrieval-generated priming (Honey and Good 2000; Marshall *et al.,* 2004), revealing a potential role of the hippocampal formation in SOP. In GluA1$^{-/-}$ mice self-generated priming would be expected to be impaired, activating elements to A1 but preventing them from decaying, which could lead to sensitisation in a way similar to that seen in hippocampal lesioned animals. Retrieval-generated priming, however, is preserved; elements are still able to be activated from I to the A2 state and decay back to the I state. If anything, long-term habituation is enhanced because elements of a stimulus are active in focal attention for longer; more associative learning can occur, potentially accounting for the divergent short-term deficits but long-term enhancements seen in the Y-maze in these animals (Sanderson *et al.,* 2009).

One final indication that hippocampal GluA1, and its role in Wagner's SOP, are vital for appropriate habituation comes from a recent study using a viral vector to selectively restore GluA1 in the hippocampus of adult GluA1$^{-/-}$ (Bygrave *et al.,* 2019). This virus appeared to selectively affect CA2 and CA3 subfields of the hippocampus. However, this

was sufficient to rescue the GluA1$^{-/-}$ phenotype, reducing locomotor hyperactivity in a novel open-field and restoring short-term habituation as demonstrated in a spatial novelty preference Y-maze. Recordings of local field potentials (LFPs) in these animals also showed elevated theta power – believed to be a neural correlate of attention – in GluA1$^{-/-}$ mice in both hippocampus and prefrontal cortex that was rescued by restoration of GluA1 in the hippocampal CA3 subfield. Coherence – similarity in oscillations between regions – has been shown to indicate functional connectivity; regions that show high coherence have a high degree of functional connectivity. Hippocampus and prefrontal cortex have been shown to have high coherence during novelty exploration. Bygrave *et al.* showed increased exploratory behaviour in the novel open-field correlated with elevated hippocampal-prefrontal theta coherence. This neural correlate of attention mimics the phenotype of schizophrenic patients that similarly express increased theta oscillations and elevated hippocampal-prefrontal cortex theta coherence, correlating with positive symptom scores (Andreou *et al.,* 2015; Zheng *et al.,* 2015). This further suggests that GluA1$^{-/-}$ mice exhibit high construct validity as a tool for schizophrenia research.

Together these findings reveal an important role for GluA1-dependent synaptic plasticity in CA3 in self-generated priming, short-term habituation and attentional processing. SOP provides a potential explanation for this relationship at a psychological level, that this thesis will endeavour to explore further.

Aims

The focus of this work is a better understanding of the relationship between habituation, attention and associative learning, and the interaction of disrupted synaptic plasticity on these behaviours. In order to do this, we will use two models of glutamatergic

hypofunction; pharmacological intervention with the NMDAR antagonist MK-801 and genetic knockout of the GluA1 AMPAR subunit.

We will also investigate the effect of hyperdopaminergia via administration of amphetamine. Hyperdopaminergia is a consequence of glutamatergic hypofunction, shown preclinically in studies of GluA1$^{-/-}$ (Panayi *et al.,* 2021) but also a core feature of schizophrenia. Deficits will be investigated in the context of this disorder, as a disease of reduced hippocampal plasticity, but in the Discussion we will also consider the relevance of these deficits to other psychiatric and neurodegenerative diseases where hippocampal synaptic plasticity is affected.

In Chapter 2 we will describe an extensive behavioural phenotyping of the GluA1$^{-/-}$ mouse, looking at locomotor and cognitive deficits on both short- and long-term scales. Whilst some of these findings have been published previously, it is important to conduct a comprehensive battery of tests, to better understand the relationship between locomotor activity and habituation, and to highlight some of the flaws in these commonly used paradigms. Additionally it is important to replicate some of these findings in the literature. Particularly as we go on to study other models of interest, it is important that we can draw comparisons across these tests. We will detail a new behavioural test, developed in-house to overcome some of these flaws in other assays. Using this new paradigm, we will assess novelty recognition in different contexts and investigate aberrant behavioural responses to novel or familiar environments.

In Chapter 3 we will look at the effect of pharmacological manipulation of the glutamatergic and dopaminergic pathways on these same tests of locomotor activity and habituation. In doing so, we will generate an extensive phenotype of three models

commonly used in schizophrenia research that represent either disrupted synaptic plasticity (GluA1$^{-/-}$ and MK-801), or hyperdopaminergia in the absence of plasticity changes (amphetamine). By doing this, we can gain a better understanding of the contribution of specific LTP mechanisms to learning and memory, particularly in the context of priming, and to what extent aspects of behaviour are governed either by hippocampal alterations, or downstream consequences of these changes.

In Chapter 4 we will use a task of attentional processing – the 5-choice serial reaction time task (5-CSRTT) – to investigate attentional performance in these three models. We will detail the training of a cohort of inbred C57Bl/6j mice on this task, followed by pharmacological manipulation with either MK-801 or amphetamine and the effect of this on different measures of attentional processing. We will then go on to investigate these behaviours in GluA1$^{-/-}$ animals compared to their WT counterparts. We will also compare this data to some early pilot work done by a previous student in the Bannerman group to assess attentional performance on the 5-CSRTT in GluA1$^{-/-}$ mice.

In Chapter 5 we will then investigate the role of GluA1 in associative learning, through a series of sensory preconditioning studies. This paradigm allows us to assess how animals learn about the relationship between stimuli and events in their environment. By manipulating the complexity of this task we can test animals under conditions that are relatively simple, or more complex – representing something closer to a real-world environment where there are many different incoming stimuli to attend to. This task allows us to test predictions about the increased propensity of GluA1$^{-/-}$ mice to form associations, including maladaptive associations, integrating this with findings from previous chapters on how these mice respond in tasks of locomotor activity, habituation and attention.

Chapter 2: Behavioural phenotyping of locomotor and habituation deficits in GluA1$^{-/-}$ mice

Introduction

Locomotor activity is one of the simplest behavioural measures available, commonly used as a readout of neural dysfunction, and in particular for psychosis. Restoration of locomotor abnormalities, for a long time, has been used as a readout in drug discovery research, particularly in the context of psychosis in schizophrenia, although the utility of this rudimentary behavioural readout has been called into question more recently (Gilmour *et al.,* 2015; Pratt *et al.,* 2012). Under the face of a relatively simple behavioural readout, lies a great degree of psychological complexity that is rarely considered in detail. Determining the reasons an animal behaves abnormally and displays hyperactivity in a new environment requires a deeper understanding of how that phenotype develops which is often lacking when profiling a model, or using locomotor activity to probe efficacy for drug discovery.

Recent work from the Bannerman group has highlighted this disconnect between locomotor activity and cognitive function. Firstly, mice lacking NMDARs specifically in parvalbumin-containing interneurons (*Grin1*$^{\Delta PV}$*)* exhibit some locomotor hyperactivity, but no deficits in working memory or cognitive flexibility (Bygrave *et al.,* 2016). If NMDARs on GABAergic interneurons are responsible for triggering deficits relevant to schizophrenia with NMDAR antagonists (Carlén *et al.,* 2012; Nakazawa *et al.,* 2012), an NMDAR antagonist would be expected to have less of an effect in these mice. However, while Bygrave *et al.*

showed reduced hyperlocomotion in *Grin1*$^{\Delta PV}$ subjects in response to MK-801, this was actually due to an increased sensitivity, with mice displaying stereotypies and catalepsy associated with higher doses of the drug, rather than a reduced sensitivity. This highlights the importance of understanding a locomotor effect in the context of other behavioural changes occurring.

Additionally, agonists of the metabotropic glutamate receptor mGluR2 were shown to have promising therapeutic potential due to their reversal of PCP or amphetamine-induced hyperlocomotion in rodents (Fell *et al.,* 2008; Moghaddam and Adams 1998; Patil *et al.,* 2007). However, these benefits have failed to translate to the clinic. More recent preclinical work has shown that while mGluR2 agonists can ameliorate hyperactivity triggered through either enhanced dopamine transmission, or glutamatergic hypofunction, they have no impact on underlying cognitive deficits (Boerner *et al.,* 2017). These findings indicate that despite the early promise shown by mGluR2 agonists based on rescue of locomotor hyperactivity, they may not target the underlying cause of these deficits, potentially explaining their clinical failure.

Phenotyping of GluA1$^{-/-}$ mice

A major aim of this chapter is to develop a better understanding of the interaction between observed deficits in locomotor activity and habituation in GluA1$^{-/-}$ mice. GluA1$^{-/-}$ mice exhibit pronounced hyperactivity in a novel environment, but normal activity in their homecage (Bannerman *et al.,* 2004; Wiedholz *et al.,* 2008). This suggests hyperactivity could be due to a lack of habituation, and specifically a deficit in short-term habituation. Interestingly, Wiedholz *et al.* observed a rapid and persistent increase in activity upon introduction to a novel environment, with a much higher peak than that observed in WT

controls. This result indicates that rather than just a deficit in habituation, GluA1$^{-/-}$ mice may actually exhibit sensitisation. Indeed, sensitisation that was specific to repeated stimulus presentation, was observed in the orienting to lights task in operant chambers (Sanderson, Sprengel, *et al.,* 2011). The stimulus-specific sensitisation seen here is potentially consistent with a deficit in self-generated priming and an increased likelihood of elements being in the A1 state of focal attention following GluA1 ablation, in the SOP framework (Wagner 1981).

In the novelty preference Y-maze task assessing spatial memory, a dissociation between impaired short-term but normal long-term habituation has been reported (Sanderson *et al.,* 2009). Importantly, this paper went on to reveal that long-term habituation was actually enhanced in GluA1$^{-/-}$ mice, an early indication that increased attending to stimuli in these mice in the short-term may actually lead to an increased propensity for association formation in the long-term.

Development of a task to detect stimulus-specific sensitisation

There is an apparent disconnect between behavioural paradigms; GluA1$^{-/-}$ mice exhibit sensitisation in their locomotor activity when introduced to a novel environment, and orienting to visual stimuli, but not in the Y-maze spatial novelty preference task described above (nor do they show evidence of sensitisation in a non-spatial object recognition memory task, NOR (Sanderson, Hindley, *et al.,* 2011)), although they do nevertheless show deficits in habituation. We posit that the most likely explanation for this is a consequence of the way in which stimuli are presented. The most important difference between these tasks is that cues are presented in sequence in the orienting to lights task, but simultaneously in the Y-maze or NOR. In the orienting to visual stimuli task, sensitisation

to repeated cue presentation produces a stronger behavioural response to the same stimulus in GluA1$^{-/-}$ mice. It is possible that sensitisation also occurs to pre-exposed arms of a Y-maze, or to recently familiar objects, and elicits stronger responding to those cues over time. However, because cues are presented side-by-side (novel vs. familiar spatial locations or objects), in a choice preference test, this sensitisation may be obscured. In these tasks while the familiar cue becomes sensitised and increasingly engaging, the novel stimulus would also be expected to elicit a strong behavioural response. This phenomenon, termed response competition, between two highly salient cues, may obscure the expression of sensitisation in choice paradigms.

To try and circumvent this, we will here investigate an alternative version of the NOR task in GluA1$^{-/-}$ mice where objects are presented separately, in two different test sessions, rather than side-by-side in a choice preference paradigm. When objects are presented simultaneously, normal animals spend more time exploring the novel object than the familiar object during the test, and express a discrimination above 50% because they have habituated to the familiar object during the sample phase. Here, an impairment is typically observed as “chance” discrimination; approximately 50% of time spent exploring either object. But this cannot explain why animals express this lack of novelty preference.

By presenting objects separately, in a paradigm initially described by McTighe *et al.* (2010), we can explore behavioural responses specific to novel or familiar stimuli, to probe how animals respond specifically to these different cues. Normal animals show increased exploration during a test phase where the novel object is presented, and reduced exploration during the test phase where the familiar object is presented, such that in this between-trial comparison, exploratory activity is higher in response to novelty. McTighe *et*

al. used this test to determine whether rats with perirhinal lesions perceived the familiar object as novel, as had been widely predicted, or vice versa. Interestingly, they reported that rats actually perceived novel objects as familiar, attending less to a novel object during the test phase. In this chapter we will test GluA1$^{-/-}$ mice with this alternative paradigm to investigate whether we can see sensitisation effects to recently familiar objects across repeated exposures.

We will further investigate deficits in short-term habituation and sensitisation through the development of a new assay, aimed to test responding to spatial locations in the absence of response competition. Based on tasks where animals are presented with visual stimuli of varying novelty (Marshall *et al.,* 2004; Sanderson, Sprengel, *et al.,* 2011), the Novel Context Recognition (NCR) task will repeatedly introduce animals to an open-field arena with novel or familiar contextual stimuli to assess habituation to spatial locations. Sensitisation has previously been revealed in hippocampal-lesioned rats in a task of exploratory activity assessing novel vs. familiar locations (Honey *et al.,* 2007). Compared to tasks investigating orienting to lights, this has the advantage of not requiring extended training or dietary restriction. Furthermore, by investigating a relatively simple behavioural readout, locomotor activity across contexts, it may provide a more robust readout for stimulus-specific sensitisation than a suppression of magazine orienting. Critically, by measuring locomotor responses to contexts of differing novelty, we hope to explain how hyperlocomotion develops in GluA1$^{-/-}$ mice.

In the General Introduction we reviewed the existing literature on the phenotype of GluA1$^{-/-}$ mice and introduced a psychological framework – SOP (Wagner 1981) – that can potentially integrate some of the behavioural abnormalities described above into a

meaningful explanation. In this chapter we will look to expand on this by conducting a thorough behavioural phenotyping of these animals in tasks of habituation. By generating our own data on how these animals behave across novel and more familiar environments, we will consider how the SOP framework can account for behavioural abnormalities observed, in particular how these might be explained by a blockade of elements decaying from the forefront of attention, A1. We will then go on, in later chapters, to consider how the phenotype of GluA1$^{-/-}$ mice arises, in direct comparison to that observed in other, pharmacological manipulations of relevance to psychosis, and in particular their known effect on hippocampal LTP (see Chapter 3).

Aims

In this chapter we aim to confirm and expand on deficits reported previously in the GluA1 literature, with commonly used tasks of habituation, as well as novel approaches developed in-house. In doing so, we hope to explain how hyperactivity in this model develops and better understand the interaction between locomotor hyperactivity and habituation. In particular, this work will be framed in the context of deficits in self-generated priming, with an aim of understanding how disrupted synaptic plasticity and impaired LTP leads to disrupted habituation in the short-term, but can also produce enhanced habituation over longer timeframes (Sanderson *et al.,* 2009). We will consider how these findings fit with the SOP model of associative learning, and how impairments in plasticity affect these psychological processes.

2.1 GluA1$^{-/-}$ mice are hyperactive in a novel open-field

Introduction

To start, we wanted to look at one of the simplest components of behaviour; exploratory activity in a novel arena – a widely used assay of psychosis relevant to schizophrenia. Previous studies have shown GluA1$^{-/-}$ mice to be hyperactive in a novel open-field (Bannerman *et al.,* 2004; Wiedholz *et al.,* 2008). However, it is unclear how this hyperactivity arises over time and what psychological processes underlie this increase. Bannerman *et al.* only investigated locomotor activity, alongside many other behaviours, as a total over a prolonged period of time, with no consideration of how hyperactivity develops.

Wiedholz *et al.* confirmed this hyperactivity response to a single novel open-field exposure, but also showed that this persisted over several days of 5-minute exposures to a context. In a second experiment there was a strong suggestion that activity levels increase progressively across the first 10 minutes of exposure to a novel open field, potentially consistent with sensitisation in these animals. The peak of locomotor activity was far higher than anything exhibited by WT mice, but genotype differences in activity were not assessed in detail.

In this experiment, we aimed to confirm this hyperactivity that has been reported previously, and expand on these findings using a fine-grained analysis across time to investigate how this phenotype may develop in response to a novel environment.

Methods

Subjects

Male GluA1$^{-/-}$ and WT littermates (n = 16 per genotype) were used for this experiment. Animals were generated in-house at the University of Oxford Biomedical Services Building. GluA1$^{-/-}$ mice were generated as described previously (Zamanillo *et al.,* 1999), and for all studies using this genetic knockout model, WT littermates were used as healthy controls unless explicitly stated otherwise.

All subjects used throughout this thesis were housed on a 12-hour light:dark cycle (lights on at 09:00) with all testing taking place during the animals light phase. Animals were maintained on *ad libitum* food and water unless explicitly stated otherwise. All procedures in this thesis were conducted in accordance with the Animals (Scientific Procedures) Act 1986.

Apparatus

Experiments took place in a novel testing room. Clean homecages (48x35x21cm) made of clear perspex, with a layer of fresh sawdust were used as the novel arena. Infrared photocell sensors, arranged in a 5x4 arrangement around the base of the homecage, monitored animal activity via a PC running PAS Software (San Diego Instruments, USA).

Procedure

On the day of testing, animals were taken to the testing room and placed in clean homecages. Activity was recorded over a 60-minute period, after which mice were removed from the arena.

Analysis

Infrared beams from the photocell sensors were broken by movement of the animal. The primary outcome was therefore beam breaks as a proxy of locomotion, reported as both a session total and in 30s bins over time, allowing us to investigate the temporal profile of subjects throughout the session.

Results

As reported in earlier studies (Bannerman *et al.,* 2004; Wiedholz *et al.,* 2008), GluA1 knockout was associated with increased locomotor activity, seen as both an increase in total activity during the session, but also sustained elevated locomotion over the 1-hour period of recording in the GluA1$^{-/-}$ mice (Figure 2.1).

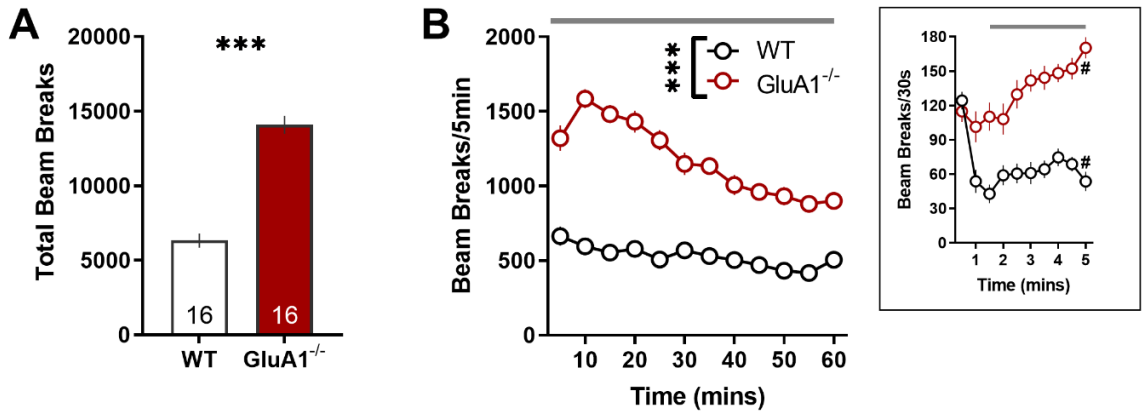

Figure 2.1 GluA1$^{-/-}$ mice are hyperactive in a novel open-field environment. GluA1$^{-/-}$ mice were significantly more active than WT littermates, observed as both total activity over 60 minutes (A), and activity broken down into 5-minute bins (B). Upon closer inspection of the first 5 minutes of activity, GluA1$^{-/-}$ started off comparable to WTs before becoming hyperactive after the first minute (inset). All data are presented as mean ± SEM. Numbers within bars indicate n. *** $p < 0.001$ vs. controls. # $p < 0.05$ vs. first 30s activity. Grey bar above figures indicates a significant difference in activity after Sidak corrections for multiple comparisons.

GluA1$^{-/-}$ mice were hyperactive compared to their WT counterparts, displaying a significantly higher number of total beam breaks, confirmed by the genotype effect from a repeated measures ANOVA (Figure 2.1A, $F_{(1,30)} = 104.771$, $p < 0.001$).

When activity was broken down into 5-minute bins, a clear hyperactive phenotype was observed in GluA1$^{-/-}$ mice across the entire session (Figure 2.1B). A repeated measures ANOVA of the 5-minute timebin data revealed a significant interaction of genotype*time ($F_{(11,330)}$ = 18.873, p < 0.001). *Post-hoc* comparisons investigated genotype differences in each 5-minute period from 0-60 minutes, with GluA1$^{-/-}$ significantly more active at every 5-minute period across the session (all p < 0.0043; significance threshold for 12 *post-hoc* tests after Sidak correction).

This reveals very little in terms of why these animals may be hyperactive, however. To better understand this, we looked more closely at the first 5 minutes of locomotor activity. Visual inspection of the raw data suggested that most of the variation in activity was occurring within the first few minutes of novel open-field exploration. We felt it was important to understand how animals reacted to this initial exposure to novelty, so looked at the first 5 minutes at a higher resolution of 30s bins. In this 30s data, we saw initially high exploratory activity in WT mice which rapidly declined within the first minute (Figure 2.1B inset). GluA1$^{-/-}$ mice started off at a comparable activity level to WT but instead of declining, increased their responding, reaching a much higher maximal value than that ever observed in WT mice. A *post-hoc* comparison of 30s timebin data revealed no difference between genotypes in the first 60s, but significantly elevated GluA1$^{-/-}$ activity at every 30s bin following that in the first 5 minutes compared to WT (0-30s: $t_{(30)}$ = 0.633, p = 0.433; 30-60s: $t_{(30)}$ = 7.940, p = 0.008; 60-300s all p < 0.0051, significance threshold for 10 *post-hoc* tests). Furthermore, both WT and GluA1$^{-/-}$ mice displayed a significant difference in activity in comparisons between the first and last timebin of the 30s analysis, for this first 5 min of exposure to the novel open field (both p < 0.001). Importantly, while this was driven by a

decline in activity in WT mice, it was due to an increase in locomotor responding in GluA1$^{-/-}$.

Discussion

This experiment found that in a novel environment, GluA1$^{-/-}$ mice were hyperactive for a prolonged period of time. This finding is not new; however, this experiment is still important for two reasons. Firstly, it provides a replication and validation that this model is behaving as expected, in line with existing literature. This lends support to arguments that will be made in experiments later in this chapter as we draw comparisons between deficits observed in our studies, and the wider GluA1$^{-/-}$ literature.

Secondly, the temporal profile of progressively increasing GluA1$^{-/-}$ locomotor activity over the first 5 minutes suggests that this is not an immediate response to novelty, or a general phenotype of increased motor activity/ poor motor control per se. Furthermore, this increased locomotor activity goes far in excess of anything seen in WT counterparts during the test session. Wiedholz *et al.* (2008) reported a similar pattern, but did not specifically assess this, nor formally test for differences in the pattern of activity between genotypes over time. Here we confirmed that GluA1$^{-/-}$ actually increase in responding to novelty over the first few minutes of open-field exposure, rather than just exhibiting a failure to habituate. This could potentially be consistent with stimulus-specific sensitisation, and may give insight into how and why GluA1-related deficits develop. This will be considered in the context of other behavioural impairments, in the discussion at the end of this chapter.

2.2 Normal homecage activity in GluA1$^{-/-}$ mice

Introduction

Having replicated hyperlocomotor activity in response to a novel environment in GluA1$^{-/-}$ mice, we next wanted to assess how these animals would respond in a highly familiar environment. For this purpose we chose to look at activity in the homecage. Previous studies have indicated that GluA1$^{-/-}$ mice behave normally in the homecage, with comparable activity patterns to WT mice (Fitzgerald *et al.,* 2010; Procaccini *et al.,* 2011; Wiedholz *et al.,* 2008).

For the following study, it was important to be able to draw comparisons, not only to the previous experiment, but also to similar tests that will be conducted alongside pharmacological manipulations in the next chapter. As well as assessing baseline, unperturbed locomotor activity, behaviour was also investigated in response to an arousal-boosting injection of saline, for comparison with drug studies in Chapter 3.

Methods

Subjects

Male GluA1$^{-/-}$ and WT littermates (n = 6 per genotype) were used for this experiment.

Apparatus

Homecage activity was measured in singly-housed animals, in a homecage (45x28x13cm) with a passive infrared (PIR) sensor placed above the cage, as has been described previously (Brown *et al.,* 2017). Cages were kept in light-tight chambers that were illuminated on a 12-hour light:dark cycle, with lights on at 8:00am. PIR sensors above each

homecage were triggered by activity, at a sampling rate of 10Hz, giving a maximal value of 100 activations per every 10s of time recorded.

Procedure

At the start of the experiment, animals were placed individually in novel, clean homecages, and moved to the light-tight chambers where they were allowed to acclimate for 8 days.

Following 8 days of acclimation, animals were injected intraperitoneally with saline at 6 hours after lights on (this corresponds to the lowest levels of activity in the 24 hr cycle), to generate a spike in arousal, and for consistency with drug studies (see Experiments 3.2 and 3.4). Following injection, animals were returned immediately to their homecage and activity recorded for a further 24 hours.

Analysis

Activity across days 2-8 was used to generate an average of activity across the light:dark cycle. Day 1, when animals were habituating to the novel homecage was not included but was consistent with that seen in Experiment 2.1. Days 2-8 were used for this measure, as this reflects a week of baseline activity in a familiar environment.

Numbers of PIR activations, as a proxy of locomotion, was the primary behavioural outcome measure recorded in this assay. This is reported here either as a percentage of total time active, or as a total number of activations in 5-minute timebins. The half-hour window immediately prior to saline injection is also reported here to indicate baseline activity in these animals.

Results

GluA1$^{-/-}$ mice displayed subtle fluctuations in circadian patterns but no overall differences in activity across the light:dark cycle (Figure 2.2A). An arousal-boosting injection of saline failed to produce any prolonged changes in exploratory activity in either genotype.

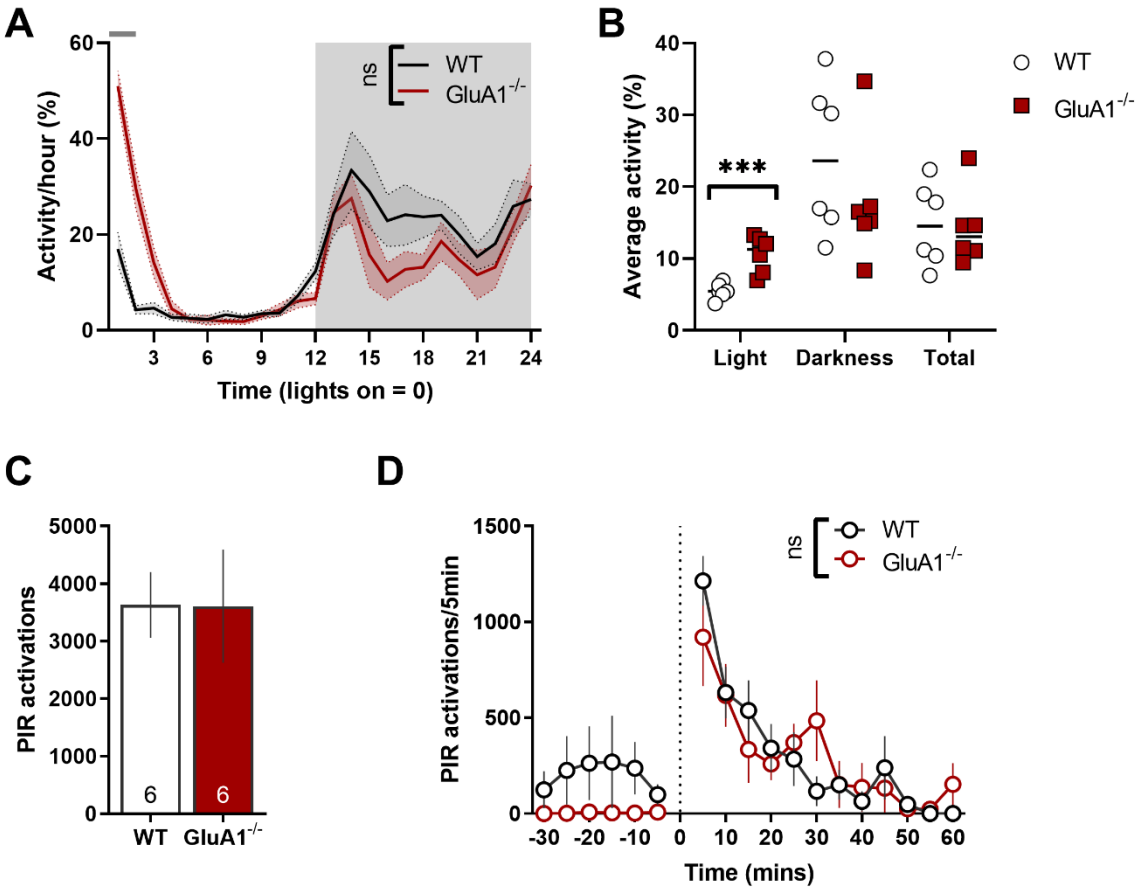

Figure 2.2. GluA1$^{-/-}$ mice show no overall hyperactivity in a familiar homecage. Subtle differences were observed in activity over the light:dark cycle (A). GluA1$^{-/-}$ mice were more active during the light phase, but overall no difference was seen in total activity across the 24-hour cycle (B). Following injection stress, GluA1$^{-/-}$ mice displayed similar levels of activity to WT controls (C). Over a 60-minute window following injection, both groups initially showed high levels of activity, which attenuated over 30 minutes post-injection (D). All data are presented as mean ± SEM. Numbers within bars indicate n. Dashed line indicates time of injection. *** $p <$ 0.001 vs. controls. Grey bar above (A) indicates a significant difference in activity, measured at each hour across the light:dark cycle ($p < 0.0021$ after Sidak correction).

Baseline Activity

No global differences were observed in locomotor activity across the light:dark cycle (Figure 2.2A and B). Despite there being no overall difference, subtle discrepancies were

observed between genotypes, confirmed by a lack of genotype effect but a significant genotype*time interaction (genotype: $F_{(1,10)} = 0.027$, $p = 0.872$; genotype*time: $F_{(23,230)} = 7.051$, $p < 0.001$). *Post-hoc* comparisons were used to determine the cause of this interaction, with a $p < 0.0021$ considered significant after Sidak correction for 24 tests across the circadian cycle. Significant differences were reported in the first 2 hours after lights on, with GluA1$^{-/-}$ mice more active than WT controls over this period(1 hour: $t_{(10)} = 52.545$, $p < 0.001$; 2 hours: $t_{(10)} = 35.047$, $p < 0.001$). A subtle decrease in GluA1$^{-/-}$ activity compared to WT was actually observed at the light:dark transition, though this did not survive Sidak correction ($t_{(1,10)} = 7.110$, $p = 0.0024$).

Overall, the intensity of activity during the light phase was higher in GluA1$^{-/-}$ mice than WT controls (Figure 2.2B, light: $t_{(10)} = 4.460$, $p < 0.001$). Despite not reaching statistical significance, GluA1$^{-/-}$ activity in the dark cycle was marginally lower than WT, resulting in similar levels of activity when compared across the whole circadian cycle (dark and total activity both $p > 0.3$). Other studies from the Bannerman group on GluA1$^{-/-}$ homecage activity have seen similar results in baseline activity (Ang *et al.,* 2021). While the lower activity in GluA1$^{-/-}$ mice seen here in the dark cycle was not significant, Ang *et al.* did report reduced activity that may reflect a rebound from the increased activity in the light phase.

Arousal-induced Activity

There was no difference in activity between genotypes in the one hour period following an acute injection of saline after 8 days of habituation to the testing context (Figure 2.2C and D, genotype: $F_{(1,10)} = 0.363$, $p = 0.985$). Both genotypes displayed an initial period of higher locomotor activity immediately post-injection which declined over the next 30 minutes. A lack of genotype*time interaction confirmed there was no difference in the profile of

activity following this arousal-inducing saline injection ($F_{(11,242)}$ = 1.151, p = 0.330). Post-injection locomotor activity was assessed for one hour in order to draw comparisons between this study and the previous experiment looking at novel open-field responding, but also for comparison with later drug studies (see Chapter 3). Pre-dosing baseline activity appeared to be higher in WT mice (median = 634.0) than in GluA1$^{-/-}$ mice (median = 10.50) (Figure 2.2D, -30 to 0 minutes). This was confirmed to be significant by a Mann-Whitney test (U = 0, p = 0.022). However, while activity was higher in all WT mice, this was driven mainly by sporadic bouts of activity in only a few of these animals. The baseline activity reported in Figure 2.2A is convincing that overall there are no genotype differences in activity. A few animals driving bouts of high baseline activity was also seen in later homecage studies in Chapter 3 but does not seem to be part of a larger trend. In this instance, the fact that WT activity was higher, if anything, than in GluA1$^{-/-}$ mice, further supports our argument that there was no hyperactivity in GluA1$^{-/-}$ subjects in the well habituated home cage.

Discussion

This experiment found that despite subtle differences in their wake/sleep cycle, activity overall is comparable between GluA1$^{-/-}$ mice and their WT counterparts in the familiar homecage. A saline injection temporarily increased activity in both groups but failed to stimulate any prolonged hyperactivity in GluA1$^{-/-}$ similar to that seen in response to novelty.

Previous published studies of GluA1$^{-/-}$ mice have found no difference in their light:dark activity compared to controls (Fitzgerald *et al.,* 2010; Procaccini *et al.,* 2011; Wiedholz *et al.,* 2008). However, some disruption to circadian rhythm, particularly in light phase

activity, has been reported previously in models of schizophrenia (Oliver *et al.,* 2012). Furthermore, this is not the first incidence of disruption in GluA1$^{-/-}$ light:dark activity observed in the Bannerman group (Ang *et al.,* 2021). In this paper, GluA1$^{-/-}$ mice exhibited elevated homecage activity in the first 2 hours after lights on, but reduced activity for several hours at lights off, with no difference in total activity across the day. Sleep and circadian disruption is a common symptom of schizophrenia, that has been previously implicated in the development of positive symptoms (Reeve, Sheaves, and Freeman 2015). While not the primary focus of the current experiment, it is an interesting, and unsurprising finding that some disturbances of circadian rhythm were seen in the GluA1$^{-/-}$ mouse, a mouse that has been considered a translationally relevant model for schizophrenia for over 20 years.

This experiment used an acute mild stressor, intraperitoneal injection of saline, as a means to stimulate arousal. This manipulation produced a robust increase in activity, but this had attenuated within 30 minutes in both groups. The locomotor hyperactivity that this model is associated with, as seen in Experiment 2.1, was not induced following this mild stressor in a familiar environment, indicating that locomotor hyperactivity is more than a generalised increase in arousal responses.

The normal activity levels in the familiar homecage environment (Wiedholz *et al.,* 2008), is in marked contrast to the pronounced locomotor hyperactivity that is seen in GluA1$^{-/-}$ mice when the environment is novel (Figure 2.1). As predicted by previous experiments in these animals (Sanderson *et al.,* 2009), this suggests dissociable mechanisms of short- and long-term habituation contribute to differential behavioural outcomes in novel and familiar environments. Furthermore, as shown in earlier studies (Sanderson *et al.,* 2009), long-term

habituation is seemingly preserved in GluA1$^{-/-}$ mice. This will be considered further in the rest of this chapter, as we continue to investigate GluA1$^{-/-}$ responding in novel or non-novel environments.

2.3 Novelty discrimination is impaired in GluA1$^{-/-}$ mice in a Y-maze spatial choice paradigm

Introduction

The first two experiments revealed clear differences in how GluA1$^{-/-}$ mice respond to their environment when it is either entirely novel, or highly familiar. GluA1$^{-/-}$ hyperlocomotion which is limited to novel stimuli, suggests a possible role for impairments in short-term habituation in their behavioural phenotype, something that has been reported previously in this model (Sanderson *et al.,* 2007; Sanderson, Hindley, *et al.,* 2011).

The spatial novelty preference Y-maze has been used for over 20 years to probe deficits in novelty recognition (Dellu *et al.,* 2000). One of the benefits of testing with this assay is the ability to test for stimulus-specific habituation to previously experienced stimuli. By comparing novel locations with less novel ones in a preference test, we can start to explore what may be driving aberrant responding to novelty. This allows us to rule out alternative explanations of hyperactivity such as general increases in arousal. GluA1$^{-/-}$ animals have been reported previously to show impaired spatial novelty preference, failing to discriminate between novel and familiar locations (Sanderson *et al.,* 2007, 2009). Importantly, this deficit does not reflect a general impairment in spatial learning and information processing (Reisel *et al.,* 2002; Zamanillo *et al.,* 1999). GluA1$^{-/-}$ mice are able to discriminate between arms of a maze when learning the locations of appetitive rewards as observed in the Y-maze and radial maze (Reisel *et al.,* 2002; Schmitt *et al.,* 2003), as well

as based on novelty vs. familiarity over longer timescales (Sanderson *et al.,* 2009). These findings point to a possible deficit in short-term habituation itself, rather than the formation of associations.

The aim of the current experiment was to confirm deficits in short-term habituation previously reported in GluA1$^{-/-}$ mice in the spatial novelty preference Y-maze, in our own cohorts. To validate our own behavioural phenotyping of this model it is important to compare how our cohorts behave in comparison to the wider literature, but by doing this it will also allow us to consider their relevance in relation to drug studies that will come in the next chapter, comparing manipulations in the same task, when run at the same time of day.

Methods

Subjects

Male GluA1$^{-/-}$ and WT littermates (n = 11 per genotype) were used for this experiment.

Apparatus

The apparatus consisted of a three-armed Y-maze, made of clear Perspex, with three identical arms (30x8x20cm), 120° apart. A layer of sawdust bedding was placed on the floor of the maze and extramaze spatial cues (e.g., computer, MED Associates chambers, testing room door) were situated around the room. A camera above the maze connected to a PC running Anymaze tracking software (Stoelting Europe, Dublin) recorded the animals' exploratory activity across each arm.

Procedure

The task consisted of two phases; the acquisition and test phases. During the acquisition phase, access to one arm was blocked with an opaque Perspex insert; this was the to-be-novel arm for the test phase. Access to the other two arms (start and to-be-familiar) was freely available. Arms were assigned as start, familiar, or novel to each animal in a counterbalanced way, such that they were evenly distributed and matched across both genotypes.

Animals were placed in the start arm in the acquisition phase and allowed to explore the two available arms for 5 minutes. They were then removed from the maze and returned to their homecage for a 1-minute ITI. During this ITI, the insert blocking the novel arm was removed and the sawdust was moved around all three arms of the maze. This prevented the animal from using local olfactory cues to guide navigation towards the novel area on the subsequent test trial. After the 1-minute ITI, the animal was once again placed in the start arm and allowed to explore, now across all three arms, for 5 minutes.

Analysis

The primary behavioural outcome of this experiment was time spent in each arm in the test phase, reported as both a raw value and a discrimination ratio (time spent in novel arm/ (time spent in novel + familiar arms)). The discrimination ratio was calculated only between the novel and familiar arms. The starting arm was not used to calculate this, as because the animal is manually placed in this location at the trial start, this can artificially inflate the amount of time spent in this location. Only the first 2 minutes of test activity were used to determine novelty preference; in other behavioural paradigms discriminative exploration of novelty declines after this time (Dix and Aggleton 1999).

Locomotor activity was recorded as a secondary outcome; reported here in 30s time bins across the full 5 minutes of both phases. This gives a useful frame of reference to how the animal is responding, for comparison with previous experiments where locomotor activity was the primary readout.

Results

Whilst WT mice spent more time exploring the novel arm than either start or familiar arms, GluA1$^{-/-}$ mice spent equal amounts of time across all three arms and failed to show any discrimination for novelty (Figure 2.3). Locomotor activity was also higher in GluA1$^{-/-}$ animals across the session, as seen earlier in Experiment 2.1.

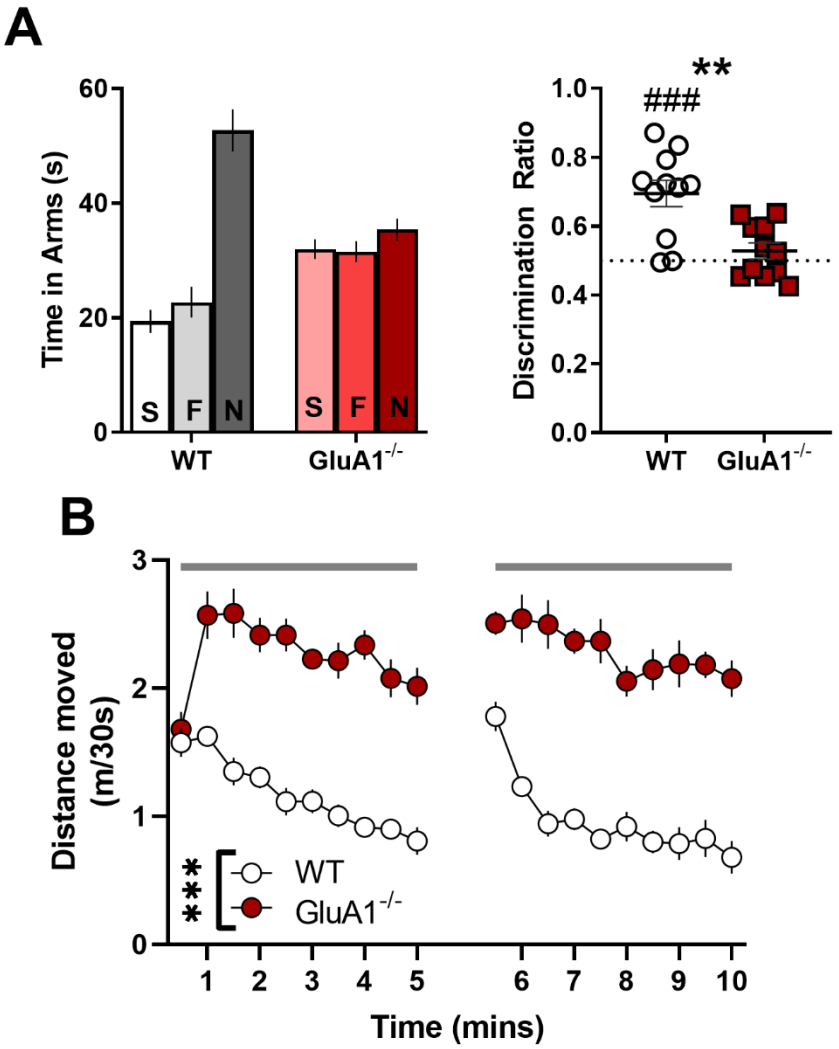

Figure 2.3 GluA1$^{-/-}$ mice fail to respond appropriately to novelty in a spatial novelty preference Y-maze. Whilst WT mice show increased time in the novel arm and a discrimination ratio above chance comparing activity in novel and familiar arms, GluA1$^{-/-}$ failed to discriminate and spent equal time in all arms of the maze (A). GluA1$^{-/-}$ were also hyperactive compared to WT across the entire session (B). All data presented as mean ± SEM. S, F, N within bar indicates start, familiar, and novel arms respectively. Dashed line indicates chance level. Distance over time presented during acquisition (minutes 1-5) and test (minutes 6-10) phases. ** p < 0.01, *** p < 0.001 vs. WT. ### p < 0.001 vs. chance discrimination. Grey bar above figures indicates significant difference in distance moved; measured at the first and last 30s of activity (p < 0.0253, corrected for 2 comparisons).

Spatial Novelty Preference

WT animals showed a robust preference for the novel arm, spending considerably more time exploring this arm than the others. The discrimination ratio of time spent in novel and familiar arms confirmed a significant preference for novelty (Figure 2.3A, WT vs. chance:

$t_{(10)}$ = 5.123, p < 0.001). However, GluA1$^{-/-}$ mice failed to discriminate between arms; discrimination ratio of time spent in novel and familiar arms was at chance levels ($t_{(10)}$ = 1.189, p = 0.262). Furthermore, GluA1$^{-/-}$ novelty discrimination was also significantly lower than that seen in WT ($t_{(20)}$ = 3.736, p = 0.001).During the sample phase, time spent in the to-be-familiar arm was no different between genotypes (WT time in familiar arm: 121.7s ± 5.5; GluA1$^{-/-}$ time in familiar arm: 133.7s ± 5.2; $t_{(20)}$ = 1.587, p = 0.128), indicating that these deficits are not simply due to a lack of exploration of this arm in the first exposure.

Locomotor Activity

Animals showed profiles of locomotor activity similar to that observed earlier in this chapter. Whilst WT quickly habituated to the novel environment, GluA1$^{-/-}$ animals showed rapid increases in activity (Figure 2.3B). Hyperactivity across both stages of the task was confirmed by significant effects of genotype and genotype*time interactions (genotype: $F_{(1,40)}$ = 66.378, p < 0.001; genotype*time: $F_{(9,360)}$ = 4.499, p < 0.011). The effect of task phase was also examined. No genotype*time*phase, or genotype*phase interaction was observed (genotype*phase: $F_{(1,40)}$ = 0.876, p = 0.355; genotype*time*phase: $F_{(9,360)}$ = 0.782, p = 0.633), likely reflecting that animals show a similar pattern of exploratory behaviour across both the acquisition and test phases of the task.

To confirm global differences in activity, without comparing at every 30s timebin of the session, *post-hoc* comparisons of the genotype*time interaction were used to investigate just the first and last 30s of activity – averaging across the sample and test phases due to the lack of any interaction with phase. This revealed significant hyperactivity across the session in GluA1$^{-/-}$ mice, with significant hyperactivity at both the start and end of the session (both p < 0.0253, Sidak-corrected for 2 comparisons). Although GluA1$^{-/-}$ mice

visually appeared similar to WT at the start of the sample phase, because there was no interaction of phase and this wasn't accounted for in the analysis, overall GluA1$^{-/-}$ mice were still shown to be hyperactive. This appears to be driven by robust hyperactivity in the test phase.

Discussion

The current experiment found deficits in spatial novelty preference in GluA1 knockouts, coinciding with locomotor hyperactivity. While WT mice showed a strong preference for novelty in the test phase, GluA1$^{-/-}$ mice seemingly failed to discriminate between the arms based on relative novelty/familiarity, suggesting short-term habituation is impaired in these animals.

This finding is not surprising; Sanderson *et al.* (2007, 2009) previously reported similar deficits in the spatial novelty preference Y-maze task. However, confirming this result is important for gaining a complete picture of the motor and cognitive deficits present in GluA1$^{-/-}$ animals. Furthermore, it will be important in later chapters to have a battery of tests of learning and memory that have been used across multiple models (e.g., MK-801 and amphetamine challenges), to draw comparisons between manipulations.

This experiment highlights the potential confound of response competition, described in the introduction to this chapter. GluA1$^{-/-}$ mice displayed locomotor activity levels increasing well above those seen in WT, replicating activity seen in the novel open-field, however we did not see evidence of sensitisation in their responding to the familiar arm using the choice measure.

There are multiple potential explanations for the impaired performance of GluA1$^{-/-}$ mice on this task, and this finding alone cannot parse these separate accounts, emphasising the

importance of extensive behavioural profiling to provide an understanding of how deficits arise at a psychological level. Impaired motor co-ordination or control could disrupt an animals' ability to navigate the three arms appropriately. Sensory disturbances could affect an animals' ability to visually discriminate between arms. Alternatively, GluA1$^{-/-}$ could generate a general lack of preference for novelty. However, other studies of this assay where GluA1$^{-/-}$ mice are unimpaired indicate this is not the case (Sanderson *et al.,* 2009). In this paper, GluA1$^{-/-}$ novelty recognition was intact when exposures to the familiar arms were multiple and spaced out in time, ruling out many of these confounds.

A third explanation could be a more general spatial impairment, but intact spatial reference memory in other tasks again indicates this is not what we are seeing in this study (Reisel *et al.,* 2002; Schmitt *et al.,* 2003; Zamanillo *et al.,* 1999).

Alternatively, this could be explained by impaired attentional processing in terms of Wagner's SOP. We argued in the Introduction that GluA1 deletion could block elements from decaying from A1 to A2, maintaining them in focal attention for longer than appropriate (Barkus *et al.,* 2014; Sanderson and Bannerman 2012). A deficit in self-generated priming, preventing the decay of elements out of A1 could explain the increase in activity seen here. If elements are still in A1 when mice are returned to the arena for the test phase, this may also explain the much more rapid onset of hyperlocomotion seen during the test. It is an open question whether elements could be retained in A1, after an animal has been removed from an environment, but not impossible that this memory trace could stay active over a 1-minute ITI. This is something we will consider in more detail later in this chapter.

The current experiment revealed an impairment in short-term habituation in GluA1$^{-/-}$ mice. However, it is not possible to say if this deficit in habituation produced stimulus-specific sensitisation under these circumstances. GluA1$^{-/-}$ mice spent equal time in the familiar and novel arms of the maze, seemingly displaying no preference for either. A task is required that separates the novel and familiar components of the task, eliminating response competition as a factor. This will allow us to investigate how animals are responding to specific features of the environment, and whether GluA1-related impairments could be due to disrupted self-generated priming.

2.4 Piloting task parameters in an assay of novel context recognition

Introduction

The previous experiment showed that GluA1$^{-/-}$ animals fail to respond appropriately to novelty in a choice paradigm. It is not clear, however, as to the underlying psychological explanation for this apparent memory deficit.

In this experiment, we describe a new behavioural paradigm that will later be used to further investigate deficits in short-term memory in GluA1$^{-/-}$ mice. The Novel Context Recognition (NCR) task uses contextual, environmental cues, repeated sequentially to test an animal's recognition of novel and recently presented, more familiar cues. Subjects are placed in an open-field arena with distinct floor and wall inserts for several trials, to assess habituation over time. On the last trial these inserts are replaced for novel ones, to probe dishabituation in response to novelty, and demonstrate the stimulus specificity of any change in exploratory behaviour.

This task builds on the principles of the paradigm described by Sanderson *et al.* (2011) but uses spontaneous locomotor exploratory responses to contextual environments rather than changes in magazine activity in response to punctate visual cues. Spontaneous locomotor behaviour may provide a more robust and sensitive behavioural outcome than the reduction in magazine activity used in the Sanderson study. Additionally, this task requires no pre-training or food restriction, making it a more rapid investigation of habituation and reducing the procedural burden placed on animals.

Before using this task to assess GluA1 knockout mice, it was important to optimise testing parameters used to assess habituation. This experiment describes efforts to do that, by manipulating trial length and ITI. The aim of this study was to validate task parameters and optimise the amount of exposure to stimuli required to induce strong habituation, and subsequent dishabituation on exposure to novelty.

Methods

Subjects

Animal information, including genotype, sex and number of animals per group is outlined in Table 2.1. One large cohort of WT GluA1$^{+/+}$ mice was used initially to test the original trial parameters of 300s trial length, and a 60s ITI. For subsequent manipulations of trial parameters, small sample sizes of heterozygous (GluA1$^{+/-}$) animals were used.

This experiment was intended to be exploratory, to test the potential for different trial lengths and ITIs to increase the effect size of changes in exploratory activity across trials. Specifically, we wanted to maximise the difference observed between exposures to same and different contexts. Heterozygous GluA1$^{+/-}$ animals have previously been shown to

behave similar to WT (Wiedholz *et al.,* 2008), and so small numbers of GluA1$^{+/-}$ mice were considered sufficient to test for any visually obvious effects. Following any interesting findings in this study, those experiments would be expanded to a full cohort.

The trial parameters of 300_60 were based on findings from other assays. In the Y-maze, after a sample phase of 5 minutes animals show a decline in responding to previously visited locations. In the novel open-field, WTs show significant habituation in the first 5 minutes, whereas GluA1$^{-/-}$ show sensitisation in the same timeframe. Other groups were tested on shorter trial lengths to see if this was sufficient to observe similar habituation (90_60, 120_60) or whether habituation was comparable when the amount of exposure was reduced, but overall experiment length was the same (90_270), which may indicate further information processing outside of the environment driving habituation.

Group	Trial Length (s)	ITI Length (s)	Animal info
300_60	300	60	WT (Male n = 10, Female n = 7)
90_60	90	60	Male GluA1$^{+/-}$ (n = 3)
120_60	120	60	Male GluA1$^{+/-}$ (n = 3)
90_270	90	270	Male GluA1$^{+/-}$ (n = 2)

Table 2.1 Trial parameters and sex, genotype and animal numbers of treatment groups.

Apparatus

All tests took place in a wooden 40x40x40cm open-field arena with walls and floor painted black. To provide different contexts, laminated sheets with printed patterns were attached to each wall of the arena, so that the same pattern would be used on all four walls but could be changed between contexts. Different materials were also laid on the base of the arena, giving distinct visual and textural stimuli in each context. Laminated wall inserts used included thick black and white horizontal stripes, thin diagonal black and white stripes, black crosses on white background and white dots on black background. Floor

inserts included tartan weave, duct tape, masking tape and laminate tiles with a pebbled pattern. Contextual cues used were counterbalanced between groups. A camera placed above the maze and connected to a PC running Anymaze (Stoelting Europe, Dublin) was used to record video and measure locomotor activity.

Procedure

Prior to testing, animals were habituated to a clean, empty homecage for two days, for five minutes at a time. On the day of testing, mice were transferred to this clean homecage and taken to the testing room. They were placed in the open-field arena and allowed to explore freely, with a specific set of contextual inserts (arbitrarily context A) on the walls and floor. At the end of a trial, mice were removed from the arena, and returned to the empty homecage for an ITI period (trial and ITI length determined by groups as set out in Table 2.1). The arena was cleaned and debris removed. This was repeated two more times, so that animals received three exposures to the first context, A.

During the ITI between trial 3 and 4, these inserts were replaced for novel ones, context B. They were then returned to the arena and allowed to explore this new environment in a final trial.

Analysis

Because the aim of this experiment was to assess habituation to contextual cues across trials, it was important to only measure the period in which exploratory activity tends to be highest in a novel environment. Previous experiments (see 2.1 and 2.3) have shown that exploratory activity is very high in a control animal for the first 30s of exposure to a novel environment, but almost immediately starts to decline. Analysis of WT activity in the

current experiment was found to be consistent with this (Figure 2.4), and so total locomotor activity during the first 30s of each trial was used as the primary behavioural outcome in this task. A decline in responding to the same environment across repeated exposures was considered to be indicative of habituation. A rebound of activity in the different context, B, was used as a measure of dishabituation to novelty, to detect the stimulus-specificity of any habituation.

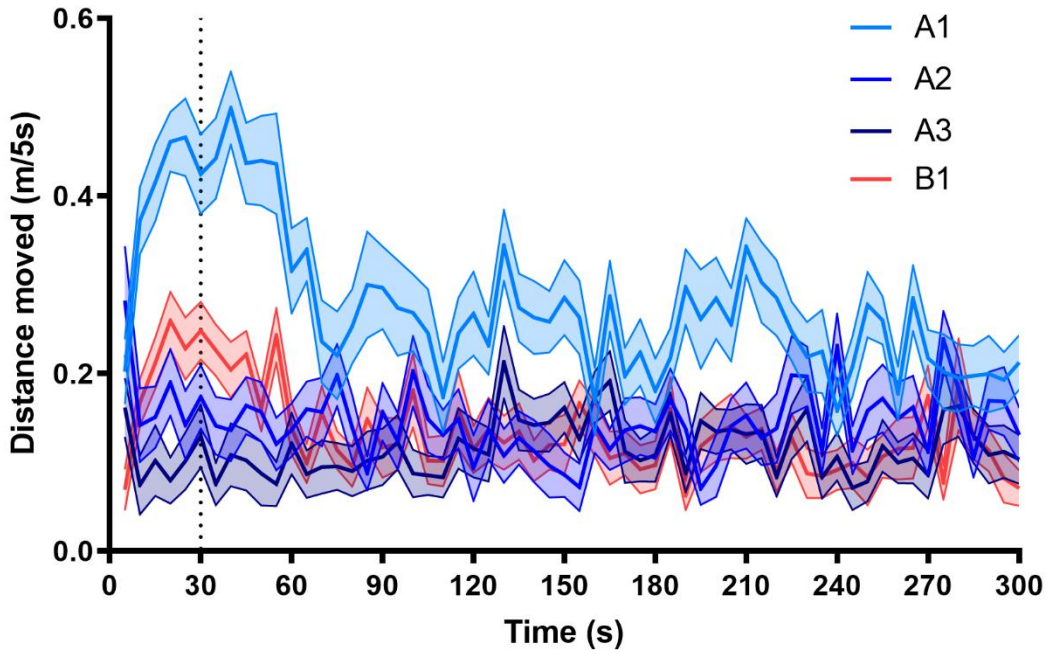

Figure 2.4 Activity of WT mice across trials in Novel Context Recognition. Wild-type activity over time showed an initial period of high locomotor activity when the context was novel (A1), peaking at approximately 30s. In the different context (B1) this burst of exploratory activity was reinstated, though not to the level seen on trial A1.

Results

Repeated exposures to the same environment, context A, for 300s at a time, produced a robust decline in responding in WT animals during the first 30s of a trial (Figure 2.5, 300_60). When the now familiar context was swapped for a novel one on trial 4 exploratory activity rebounded, though not to the initial levels seen in trial A1. This was confirmed by an overall effect of trial in the 300_60 group ($F_{(3,48)} = 36.677$, $p < 0.001$). To confirm an

effect of habituation to the same context, and dishabituation to the different context, *post-hoc* estimated marginal (EM) means comparisons were conducted between trials 1 and 3, and 3 and 4. Both tests confirmed a significant effect between trials (Trial 1-3: $t_{(14)} = 6.525$, $p < 0.001$; Trial 3-4: $t_{(14)} = 3.282$, $p = 0.0046$; $p < 0.025$ significant for two comparisons with Sidak correction).

Having demonstrated a robust effect in this group, we then looked at manipulations of varying trial and ITI length (summarised in Table 2.1). Each of these experiments exhibited similar behaviour, though observationally none were likely to alter significantly either habituation to context A, or dishabituation to context B on trial 4. Following the minimal effects of these pilot studies in small cohorts, we decided against pursuing these manipulations in fully powered studies, instead using the 300_60 design for future studies.

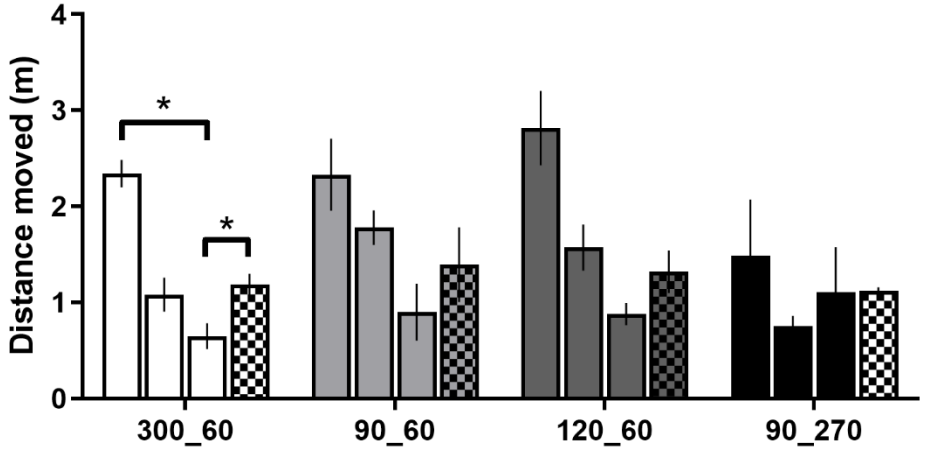

Figure 2.5 Little variation in habituation to familiar context, or dishabituation to novelty, with varying trial conditions. Decreasing trial length or increasing inter-trial interval had no obvious effect on habituation to repeated exposures to context A, or dishabituation upon reinstatement of novelty with context B. Solid bars represent exposures to context A; chequered bars represent context B on trial 4. All data presented as mean ± SEM. * $p < 0.025$ (significance threshold for two comparisons) in 300_60 group comparisons of effect across trial 1-3 and trial 3-4. No statistical analyses were conducted on other exploratory trial conditions.

Discussion

The current experiment confirms the utility of the NCR assay to test for intact short-term habituation and novelty discrimination. None of the variations of trial parameters had an observable impact on this behaviour; these pilot studies suggested there would be little value in following up with fully powered cohorts of animals.

Since control animals show habituation within the first few minutes in an open-field we looked at manipulating trial length to limit exposure to 90 or 120s per trial. We predicted that this may still produce robust habituation/dishabituation but increase throughput of the task. However, we didn't see convincing dishabituation (albeit with low power) to context B, a critical psychological component this task was developed to investigate.

In a final cohort, we looked at manipulating the ITI length, but with shorter trials, to make the time between context exposures consistent with the 300_60 group. If habituation reflects information processing offline, once animals are removed from the arena, it is possible that we would see a similar decline in responding across trials, despite this reduced trial length. No evidence was seen that this was happening in the pilot 90_270 cohort. That being said, trial 1 activity was lower in this small pilot group, before conditions had changed compared to other groups. This, combined with the low n of this exploratory pilot study, make it difficult to interpret this result.

Following the robust effect of habituation seen in the 300_60 group, we continued with these time parameters for subsequent studies.

2.5 Controlled feeding fails to enhance exploratory behaviour in a novel environment

Introduction

The norm in research utilising laboratory animals is to maintain them on an *ad libitum* diet unless dietary restriction is specifically required to conduct the research by motivating appetitive responding. However there is evidence that unrestricted food access throughout an animals lifetime can decrease the density of D2 receptors in the brain, and that this can be restored through food restriction (Thanos *et al.,* 2008). This could potentially facilitate increased exploration of a novel environment; restricted access to food has previously been shown to increase locomotor activity (Manning *et al.,* 1978; Paré 1975). Furthermore, in the orienting to lights assay that this contextual paradigm has been based on, mice were food restricted to perform the operant task (Sanderson, Hindley, *et al.,* 2011). By testing animals in the NCR assay in a food restricted state, it may be easier to draw comparisons between these separate studies.

The current experiment aimed to replicate the habituation findings of the previous experiment, but also assess whether dietary restriction can increase exploratory activity, and whether this has a beneficial effect on habituation.

Methods

Apparatus used and measures of analysis were the same as described in Experiment 2.4.

Subjects

Male GluA1$^{+/-}$ mice were used for this exploratory study; as described in the previous experiment they are behaviourally similar to a WT mouse. Animals were assigned to either

an *ad libitum* (AL, n = 8) or food restricted (FR, n = 8) group. Animals were weighed and handled for one week prior to testing. Animals in the FR group had food removed and were maintained on a controlled feeding diet at no less than 85% of their free-feeding body weight for the duration of the study.

Procedure

The procedure was the same as that reported for the 300_60 group in Experiment 2.4; all animals received three exposures to context A, followed by one exposure to context B.

Results

Food restriction failed to affect behavioural responding in the NCR task in a meaningful way. If anything, the locomotor response was slightly blunted in FR mice, declining less across trials, and showing a numerically smaller rebound upon novelty reinstatement compared to AL (Figure 2.6). Whilst there was a significant effect of trial, treatment had no effect on behaviour and there was no interaction (trial: $F_{(3,42)} = 9.678$, $p < 0.001$; treatment: $F_{(1,14)} = 0.317$, $p = 0.583$, treatment*trial: $F_{(3,42)} = 0.802$, $p = 0.500$). We had initially predicted that food restriction would increase exploratory behaviour. The lack of any treatment effect suggests that this was not the case, in fact if anything overall activity was marginally (though not significantly) lower in FR mice.

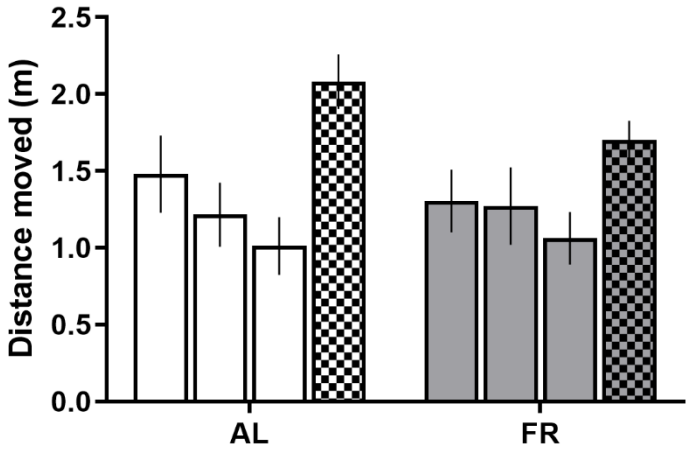

Figure 2.6 Food restriction fails to evoke a stronger novelty response or increase locomotor activity in the NCR task. Food restriction had no impact on the amount of habituation seen across repeated exposures to the same context. Solid bars represent exposures to context A; chequered bars represent context B on trial 4. All data presented as mean ± SEM.

Discussion

Despite plenty of evidence in the literature that controlled feeding diets can increase behavioural performance and spontaneous exploratory activity, the current experiment failed to see any effect in the NCR task. Activity of FR mice was no different to their AL counterparts; if anything habituation to repeated exposures of the same context was blunted, and there was a smaller dishabituation effect in response to novelty.

Whilst a robust effect of short-term habituation was seen in the previous experiment, it was predicted that controlled feeding would promote an increase in locomotor activity, similar to that reported elsewhere in the literature (Manning *et al.,* 1978; Paré 1975). Exploratory behaviour is typically increased in laboratory-housed rodents on a controlled feeding regimen, as they are motivated to forage for sustenance. Surprisingly this was not observed here. No difference in overall responding was seen between treatment groups, potentially due to the relatively short time animals were actually exposed to the arena. If anything, activity levels in FR mice were slightly blunted, staying higher across the three trials which may be a result of an increased drive to forage in a new environment. It may

be that the relatively short period of time assessed in the task here was not sufficient to see overt changes in behaviour as a result of food restriction; earlier studies investigating the effect of restricted feeding looked at activities in the homecage such as wheel-running, over many days (Manning *et al.,* 1978).

2.6 GluA1$^{-/-}$ mice exhibit stimulus-specific sensitisation to a recently experienced context

Introduction

Previous work has shown that GluA1$^{-/-}$ can recognise novelty, but the way that this is expressed is inappropriate; orienting more rather than less to repeated presentations of a light stimulus (Sanderson, Sprengel, *et al.,* 2011).

Whilst this study found a robust sensitisation effect in GluA1$^{-/-}$, compared to intact habituation in WT, this has not been seen in other tests of recognition memory (Sanderson *et al.,* 2007; Sanderson, Hindley, *et al.,* 2011). We posit several reasons why this may be the case. Firstly, the orienting to lights assay involves food-motivated magazine responding, rather than being a pure spontaneous behaviour, and so is potentially affected by the internal (appetitive) state of the animals. GluA1$^{-/-}$ mice have previously been shown to display a reduced preference for sucrose, suggesting they may exhibit some anhedonia (Austen, Sprengel, and Sanderson 2017). However, despite this reduced palatability, GluA1$^{-/-}$ consistently display increased responding at a magazine in operant tasks compared to WT mice (Sanderson *et al.,* 2017; Sanderson, Sprengel, *et al.,* 2011). This disparity of increased magazine activity despite reduced hedonic value of the reward may be a result of the increased propensity of these animals for habitual behaviours (Johnson *et al.,* 2005). The sensitisation effect in the orienting to lights task has been difficult to replicate in house

(Panayi *et al.,* 2021), possibly due in part to the altered motivational state of the animal obscuring some of the expression of behavioural responding to visual stimuli.

Additionally, other tasks of recognition memory have been confounded by presenting the opposing stimuli simultaneously in a choice preference paradigm. Response competition could obscure the expression of sensitisation, instead appearing as a general impairment in the ability to recognise novelty and a failure to habituate.

The aim of this experiment therefore, was to investigate the behavioural response in GluA1$^{-/-}$ mice to repeated presentation of the same stimulus, and to compare behavioural responding to same vs. different contexts in a between-trial comparison. In doing so, we can begin to explore in more detail some of the established deficits in short-term habituation resulting from GluA1 deletion.

Methods

Apparatus used was the same as described in Experiment 2.4.

Subjects

Male and female GluA1$^{-/-}$ and WT littermates were used for this experiment (WT: n = 26, 20 male and 6 female; GluA1$^{-/-}$: n = 25, 19 male and 6 female).

Procedure

The general procedure was the same as described for Experiment 2.4, except for one important difference. Rather than test animals across four trials, with a between-trials, within-subjects comparison of same and different contexts, there were only three trials. In the third trial, the context either remained the same (AAA) or was replaced for the different context (AAB, Figure 2.7A); comparisons made were now between-subjects tested on

different trial conditions. Cues to be used were pseudorandomly assigned to animals prior to testing, in a counterbalanced way, and such that the specific contextual cues used were split evenly between genotypes. Assignment of same or different condition was also pseudorandomly assigned and counterbalanced across genotype groups.

Analysis

Analysis was the same as described in the previous two NCR experiments. However, before conducting this analysis we confirmed the effect of habituation/dishabituation in WT animals by again plotting the activity over time across trials (Figure 2.7B). The peak of activity was again found to be around the first 30s of exploration.

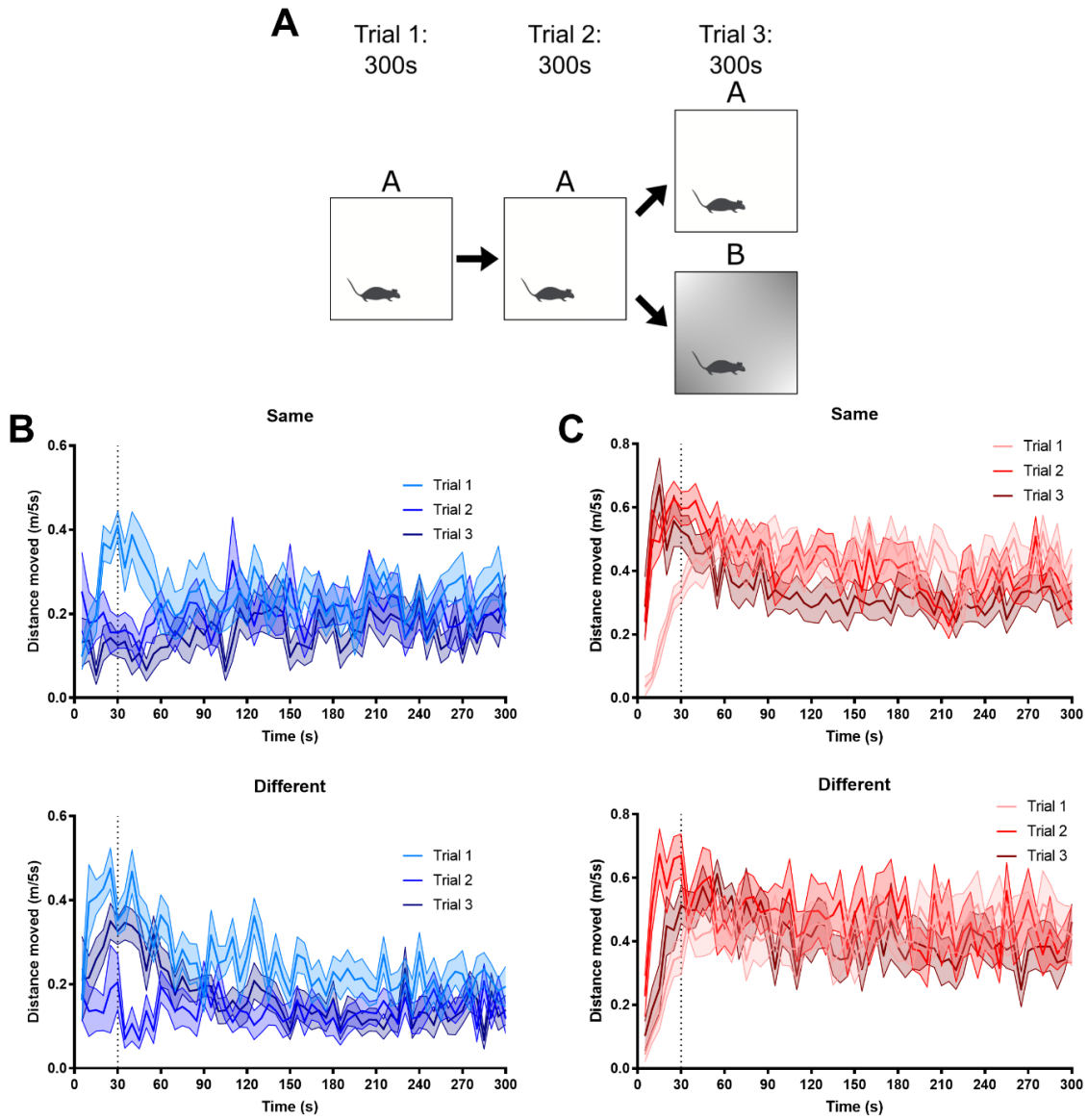

Figure 2.7 Schematic of the Novel Context Recognition task trial structure. Mice received two exposures, 300s each, to an open-field arena, decorated with contextual cues, A (A). On the third trial, these contextual cues were either kept the same, or switched for novel ones, B. Wild-type activity over time showed an initial period of high locomotor activity when the context was novel (Trial 1), peaking at approximately 30s (B). In the different condition (bottom panel) when a new context was introduced on Trial 3 this burst of exploratory activity was reinstated. GluA1$^{-/-}$ mice also showed a peak of activity at around 30s, but activity remained high throughout the session (C). Hyperactivity onset was much more rapid to repeated presentations of the same condition (top panel) but was reduced by introduction of novelty on trial 3 in the different condition (bottom panel).

Results

WT mice showed a decline in responding across trials when repeatedly exposed to the same context; a phenomenon which was reversed on trial 3 when a novel context was introduced (Figure 2.8, WT AAA and AAB). Rather than a decline in responding, GluA1$^{-/-}$

activity increased dramatically across repeated exposures to the same condition, but attenuated when a novel context was introduced on trial 3 (GluA1$^{-/-}$ AAA and AAB).

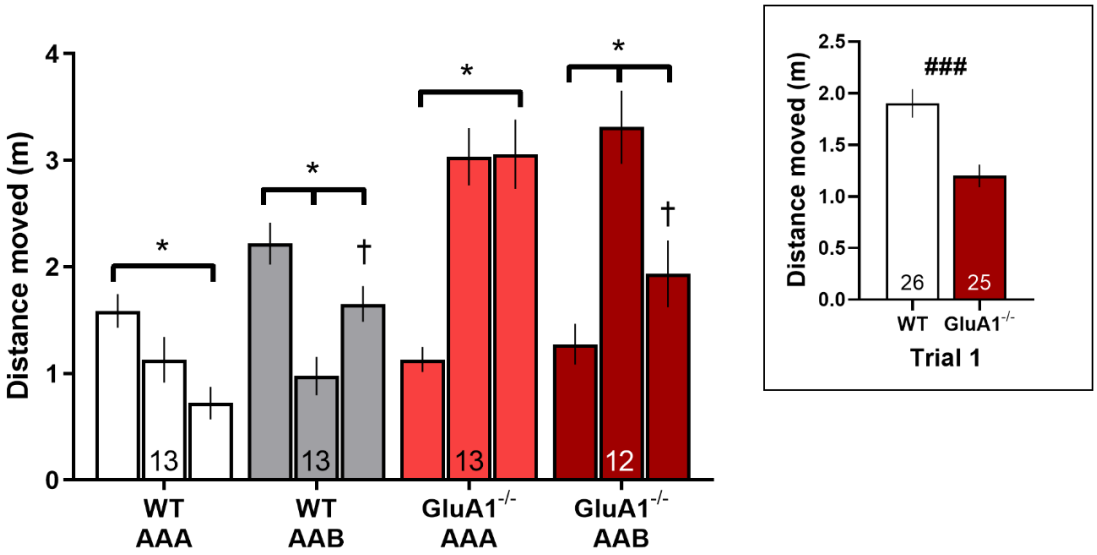

Figure 2.8 Stimulus-specific sensitisation in GluA1$^{-/-}$ mice in response to contextual stimuli. Activity in WT mice declined across repeat exposures to the same context (AAA), but rebounded when the familiar context was swapped for a novel one on trial 3 (AAB). GluA1$^{-/-}$ activity increased significantly across trials in response to the familiar context (AAA). This sensitisation to familiar contexts was abated when a novel context was introduced on trial 3 (AAB). On trial 1, GluA1$^{-/-}$ were initially hypoactive in response to the novel environment, compared to WT (inset). All data presented as mean ± SEM. Numbers within bars indicate n. * $p < 0.0170$ trial comparison (within subjects, adjusted for three tests), ### $p < 0.001$ vs. control, † $p < 0.05$ vs. AAA condition within-genotype.

In WT mice a significant interaction was observed on condition*trial ($F_{(2,48)} = 7.493$, $p < 0.001$), driven in part by a simple effect of condition on trial 3, indicating that WT mice dishabituated in response to novelty in the AAB condition ($t_{(24)} = 4.120$, $p < 0.001$). WT AAA mice showed a significant decline across trials 1-3 ($t_{(11)} = 3.896$, $p = 0.002$), whereas in the AAB condition, a significant decrease was seen across trials 1-2, but also between trials 2 and 3 (trial 1-2: $t_{(11)} = 8.232$, $p < 0.001$; trial 2-3: $t_{(11)} = 2.906$, $p = 0.013$). An effect of condition*trial was also seen in GluA1$^{-/-}$ mice ($F_{(2,46)} = 6.185$, $p = 0.004$), though they showed a very different profile of locomotor responding across trials. GluA1$^{-/-}$ mice tested in the same condition expressed a significant difference in activity across trials 1-3, but in the opposite direction to that seen in WTs and the magnitude of change in responding was

much greater ($t_{(11)} = 6.718$, $p < 0.001$). In the AAB condition, activity differed between trials 1 and 2, and 2 and 3, indicating an increase across the first two exposures to the same context, but a decline when novelty was introduced on trial 3 (trial 1-2: $t_{(10)} = 4.497$, $p < 0.001$; trial 2-3: $t_{(10)} = 4.716$, $p < 0.001$). These strikingly different activity profiles between genotypes were confirmed by a main effect of genotype*condition*trial ($F_{(2,94)} = 11.575$, $p < 0.001$).

To investigate the initial reaction to a novel context, trial 1 activity was pooled across conditions, since these conditions did not deviate until trial 3. Surprisingly, this revealed significant hypoactivity in GluA1$^{-/-}$ animals during the first 30 seconds of exposure on trial 1 (Figure 2.8 inset, $t_{(47)} = 17.436$, $p < 0.001$).

Discussion

The current experiment found GluA1$^{-/-}$ mice display higher levels of exploratory activity to a recently presented context than to a novel context, exhibiting sensitisation specifically to the more familiar context. Rather than being an amnestic effect per se, it appears these animals do in fact have a memory trace of a previously experienced context but express this behaviourally as an increase rather than a decrease in attending. This appears to support our hypothesis that the data from GluA1$^{-/-}$ mice is best explained as a deficit in self-generated priming in the context of SOP.

Whereas WT mice declined in exploratory locomotor responding across repeated exposures to the same context, GluA1$^{-/-}$ failed to reduce their activity, instead actually becoming more active on subsequent trials. In this chapter we have repeatedly shown that GluA1 knockouts become increasingly hyperactive in novel environments (Figure 2.1B, Figure 2.3B), ultimately reaching activity levels much higher than that of a normal novelty

response that would be seen in control animals. This experiment has shown this heightened exploratory response to be stimulus-specific, and therefore reliant on a memory trace in short-term memory. Locomotor hyperactivity was attenuated when the recent context was replaced for a novel one, indicating that stimulus-specific sensitisation does occur in GluA1 knockout animals.

In terms of SOP, WT habituation could be explained through self-generated priming of elements into A2 during prolonged exposure to context A, such that the overall behavioural response declines over time. When context B is introduced on trial 3, and novelty is reinstated, a higher behavioural response (dishabituation) was observed because of recruitment of a different set of elements representing this different context into A1.

We have suggested throughout this thesis that self-generated priming may be impaired in GluA1$^{-/-}$ mice, and that this could account for stimulus-specific sensitisation. This could be explained by blockade of decay from this focal state of attention into A2 and a resultant accumulation of elements in A1 across a single stimulus presentation. This could also explain the reduced response to context B in the different condition, that was closer to a novelty response for these animals (though activity was still higher than that seen in trial 1). To summarise, GluA1$^{-/-}$ mice displayed increased responding over repeated exposures to the same stimulus, but a "normal" novelty response when novel stimuli were introduced. This result replicates that observed by Sanderson *et al.* (2011) in responding to visual stimuli, but here in the spatial domain.

This does not explain what is happening when animals are then removed from the environment, but it would require A1 activity to persist even in the absence of the stimulus.

These results might suggest that aberrant A1 activation could persist after a 1-minute ITI without decaying. This is something we will explore in more detail in the next experiment.

Interestingly, GluA1$^{-/-}$ animals were actually hypoactive on first exposure to the novel environment on trial 1. This could be explained by a block of elements decaying from A1 to A2. If A1 is saturated at the start of a trial, with elements of a previous environment (i.e., the holding cage), there may be a delay in the onset of hyperactivity, requiring enough elements of a novel context to enter A1 to evoke this response. This does, however, raise the question as to why this wasn't seen in other tests of locomotor responding, a point we will consider in the discussion at the end of this chapter.

2.7 Stimulus-specific sensitisation in GluA1$^{-/-}$ mice persists over a 15-minute delay

Introduction

In Experiment 2.6 we found evidence of stimulus-specific sensitisation in GluA1$^{-/-}$ mice in response to repeated exposures to the same environment. We next wanted to investigate the persistence of this sensitisation effect over time. The aim of this next experiment was to explore how long a short-term memory trace can persist in attention in WT and GluA1$^{-/-}$ mice, by extending the length of time between trials 2 and 3 in the NCR assay.

Methods

Apparatus used and measures of analysis were the same as described in Experiment 2.4.

Subjects

Male and female GluA1$^{-/-}$ and WT littermates were used for this experiment (WT: n = 31, 16 male and 15 female; GluA1$^{-/-}$: n = 37, 18 male and 19 female).

Procedure

The procedure was the same as in Experiment 2.6 except for one key difference. Between trials 2 and 3 a 15-minute delay was introduced. Animals were removed from the arena at the end of trial 2, placed in the same clean homecage and kept there for 15 minutes, instead of 1 minute, before being returned to the testing environment and starting the third trial. Animals were kept in the testing room during these ITI periods, but the homecage was covered to eliminate any visual cues.

Results

Activity in WT mice declined across repeated exposures to the same context as described previously (Figure 2.9) and remained low on trial 3 in the same condition (AAA), showing no sign of dishabituation after a delay. In the AAB condition, WTs still showed increased activity in response to the novel context. GluA1$^{-/-}$ animals became hyperactive across the first two exposures to context A, as seen previously (Figure 2.8). On trial 3, this hyperactivity was still apparent in context A even after the longer delay of 15 minutes, but was attenuated in context B, declining to near-baseline levels of responding. These findings were supported by a significant genotype effect, and genotype*condition*trial interaction (genotype: $F_{(1,64)} = 39.078$, $p < 0.001$; genotype*condition*trial: $F_{(2,130)} = 3.803$, $p = 0.025$).

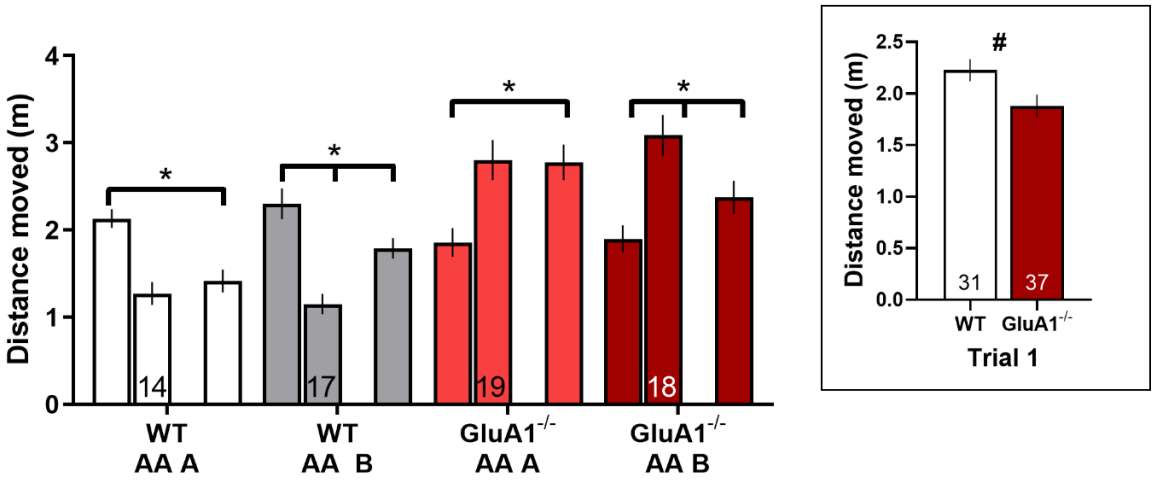

Figure 2.9 Stimulus-specific sensitisation in GluA1$^{-/-}$ mice persists over a 15-minute delay. When a delay of 15 minutes was introduced between trials 2 and 3 of the NCR task, WT mice in the same condition still showed robust habituation (AAA), but in the different condition this was attenuated (AAB). In GluA1$^{-/-}$ mice, hyperactivity in response to context A was still evident after the 15-minute delay (AAA). In GluA1$^{-/-}$ mice that were introduced to a novel context on trial 3 (AAB), activity was still high but lower than in the familiar context. GluA1$^{-/-}$ mice were hypoactive compared to controls on trial 1 (inset). All data presented as mean ± SEM. Numbers within bars indicate n. * $p < 0.0170$ (within subjects, adjusted for three tests).

Within each condition, a significant effect of genotype*trial was also observed (both $p <$ 0.001). We then looked at changes in activity over trials within each group separately. For WT mice, *post-hoc* comparisons confirmed a significant decline in activity across trials in the AAA condition, but an increase from trials 2 to 3 in AAB (AA A trial 1-3: $t_{(13)} = 5.506$, $p <$ 0.001; AA B trial 1-2: $t_{(16)} = 6.771$, $p < 0.001$; AA B trial 2-3: $t_{(16)} = 5.387$, $p < 0.001$). GluA1$^{-/-}$ mice displayed a significant increase in activity across trials in the AAA condition (trial 1-3: $t_{(18)} = 3.217$, $p = 0.005$). In AAB, GluA1$^{-/-}$ showed a significant increase from trials 1 to 2, but significant attenuation of this response from trials 2 to 3 (trial 1-2: $t_{(17)} = 7.352$, $p < 0.001$; trial 2-3: $t_{(17)} = 2.818$, $p = 0.012$).

There was no effect of condition*trial within each genotype (WT: $F_{(2,58)} = 2.292$, $p = 0.110$; GluA1$^{-/-}$: $F_{(2,70)} = 2.110$, $p = 0.129$) and so the difference in trial 3 responding between conditions was not formally investigated. While these interactions were not significant, WTs appeared to show some dishabituation in the AAB condition, but also did not decline

in attending as much on trial 3 of the AAA condition as seen previously. GluA1$^{-/-}$ mice declined in attending on trial 3 of the AAB condition to some extent, although not as much as before. This suggests a certain amount of general arousal in GluA1$^{-/-}$ mice over time following novelty exposure that may subtly exacerbate trial 3 responding in the different condition.

Finally, as in the previous experiment, we investigated trial 1 activity in more detail by pooling activity across conditions for each genotype (Figure 2.9 inset). This again revealed significant hypoactivity in GluA1$^{-/-}$ mice upon first exposure to the novel environment ($t_{(67)}$ = 2.422, p = 0.018).

Discussion

As seen previously, heightened exploratory behaviour in GluA1$^{-/-}$ mice was limited to recently experienced contexts, further indicating stimulus-specific sensitisation in this model. Here we also built on that finding, showing that this maladaptive locomotor response is still present 15 minutes after the last exposure to the same context. GluA1$^{-/-}$ mice that were instead exposed to a novel context on trial 3 showed a decline in responding compared to their levels on trial 2. That being said, there was still a subtle increase in activity suggesting some general hyperarousal over time in this task, or generalisation across contexts. Activity was higher than observed on trial 1 and not significantly different to trial 3 activity of their counterparts tested on the same condition.

It seems less likely that this behavioural response could arise through elements of a stimulus persisting in focal attention (A1) for 15 minutes when an animal is no longer in that specific environment. This argues against an account for GluA1$^{-/-}$ deficits as a blockade of elements decaying from A1 to A2 and thus remaining stuck in A1, but it is possible that

these instead decay back to the inactive state, bypassing A2. Under this alternative possibility, elements may then be available for associative priming into A2 (presuming associations have been formed during the previous exposure to the open-field). It is possible that the combined response of elements active in A1 and A2 generates the hyperactive phenotype seen in GluA1$^{-/-}$ mice, an idea we will explore in the discussion at the end of this chapter.

What is clear is that 15 minutes between exposures is not sufficient to restore baseline activity in GluA1$^{-/-}$ mice. However, these animals can exhibit long-term habituation (Sanderson *et al.,* 2009). To investigate this in more detail, we next wanted to explore the capacity of the NCR task to detect long-term habituation, in WTs and GluA1$^{-/-}$.

2.8 Normal long-term habituation in GluA1$^{-/-}$ mice in the novel context recognition task

Introduction

We have so far shown that the NCR assay is capable of detecting habituation in WTs, and subsequent dishabituation upon the introduction of novelty with a different context. This assay is sensitive to deficits in short-term habituation seen in GluA1$^{-/-}$ mice, and can furthermore detect not just impaired habituation, but also stimulus-specific sensitisation; an *increase* in responding over prolonged exposure to the same stimulus.

We next wanted to determine whether GluA1$^{-/-}$ mice could demonstrate a novelty preference, at least under some conditions, and exhibit long-term habituation in this task. Previous studies of long-term habituation have focused on the spatial novelty preference Y-maze, showing intact habituation over many spaced pre-exposure trials in both WT and

GluA1$^{-/-}$ mice (Sanderson *et al.,* 2009; Sanderson and Bannerman 2011). The aim of this experiment was to see if we could similarly detect intact long-term habituation in the NCR task.

Methods

Apparatus used was the same as that described in Experiment 2.4.

Subjects

11 WT and 11 GluA1$^{-/-}$ mice were used in this experiment. Within each genotype, there were 2 male mice and 9 females.

Procedure

Animals were first habituated to a clean homecage over two days, which was later used to transfer animals to the testing room. Animals were grouped in pairs – 1 WT and 1 GluA1$^{-/-}$ mouse – and each pair was assigned contextual patterns that would go on to be the same (context A) or different (context B) inserts used during testing.

After habituation to the transfer cage, animals were pre-exposed to context A over several days, with a single 5-minute trial per day. This was analogous to trials 1 and 2 in the previous experiments, but each trial was spaced 24 hours apart. We confirmed through visual inspection of the data and statistical analysis that both groups were expressing habituation after 5 exposures before going on to the test phase.

24 hours after the fifth pre-exposure animals were tested on one of two conditions; same or different. Animal pairs were randomly assigned to a condition, and then tested on the reverse a few days later, such that comparisons were within-subject. In the same condition, animals were again exposed to context A for 5 minutes, then removed and returned to the

homecage. In the different condition, however, the contextual cues were swapped for context B, and so differed from those used during pre-exposure. This switch to a novel context should in theory elicit a high behavioural response if animals appropriately recognise and respond to the novelty of the environment.

Following the first test, animals received two further pre-exposures to confirm expression of long-term habituation to the familiar environment was still intact. Finally, after a total of seven pre-exposures, animals were tested on the reverse condition to their previous test. Conditions were the same; animals were allowed to explore the arena for 5 minutes and then removed.

Analysis

During pre-exposure repeated measures ANOVAs of genotype*time were used to investigate the genotype effect and confirm the lack of genotype effect prior to testing.

At test, we compared exploratory activity in the first 30s of exposure to the arena, across genotype and condition (same or different context). Condition was tested within-subjects across the two test sessions. We also investigated how this behaviour changed over the course of the test session by looking at activity across 30s bins. This was statistically investigated through a genotype*condition*time repeated measures ANOVA.

Results

Pre-exposure Activity

Across the seven sessions of exposure, there was a significant effect of genotype and genotype*session interaction, indicating that GluA1$^{-/-}$ mice were significantly hyperactive compared to WT (Figure 2.10A, genotype: $F_{(1,20)} = 11.902$, $p = 0.003$; genotype*session:

$F_{(6,120)}$ = 11.493, p < 0.001). There was no overall interaction of genotype*session*time, likely reflecting that animals showed roughly the same pattern of within-trial activity across sessions ($F_{(54,1080)}$ = 1.261 p = 0.101). *Post-hoc* comparisons focused on the genotype*session interaction, comparing between WT and GluA1$^{-/-}$ across sessions. While GluA1$^{-/-}$ mice expressed significant hyperactivity to the pre-exposure context on the first three days of training, by the fourth session there was no difference between groups (sessions 1-3 all p < 0.001; session 4: $t_{(20)}$ = 1.858, p = 0.078; p < 0.0073 significance threshold for 7 comparisons).

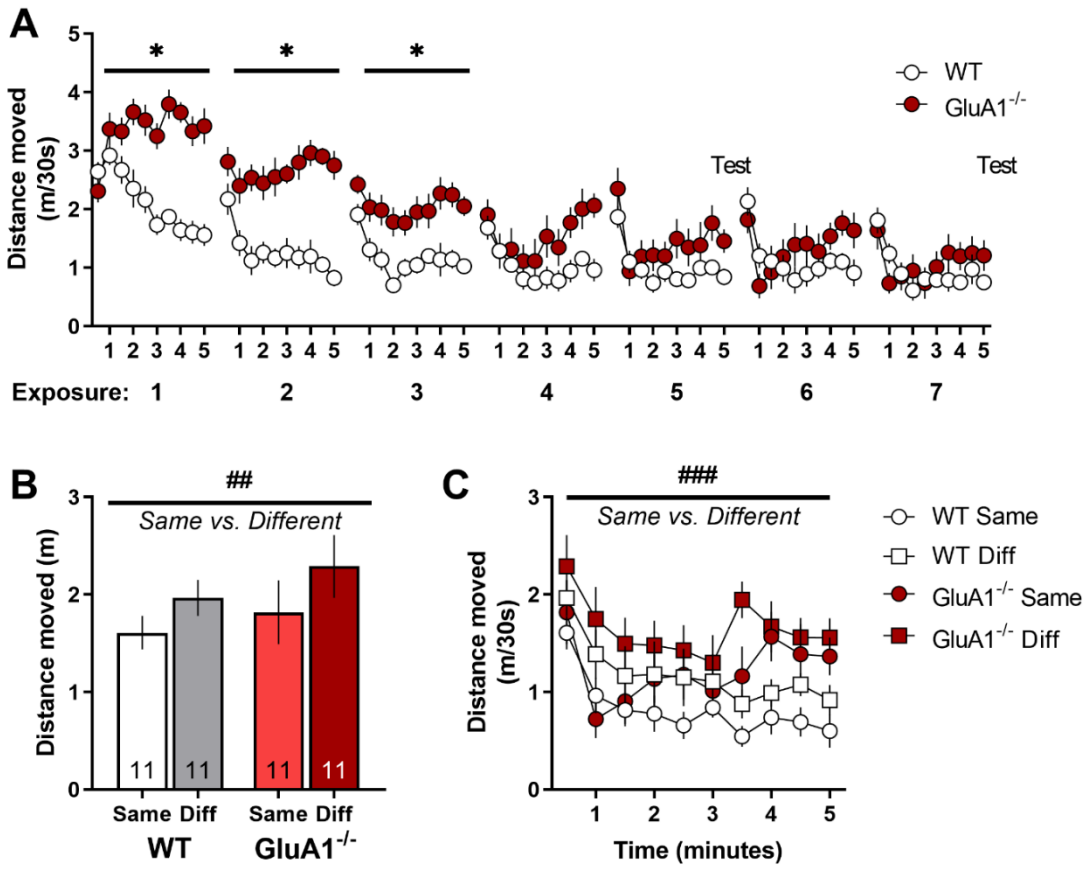

Figure 2.10 No effect of GluA1$^{-/-}$ on NCR performance after repeated pre-exposure to the familiar context. GluA1$^{-/-}$ were hyperactive when first introduced to a novel context but this response attenuated over time, with no genotype differences after 3 sessions of pre-exposure (A). Test sessions took place after 5 and 7 pre-exposure sessions. No effect of genotype but significantly higher responding to the novel context in both groups during the first 30s (B). Both groups expressed elevated responding to the different context across the full 5 minutes of the test sessions (C). All data presented as mean ± SEM. Numbers within bars indicate n. * p

< 0.0073 (corrected for 7 *post-hoc* comparisons). ## $p < 0.01$, ### $p < 0.001$ same vs. different context comparison.

Test Performance

We first investigated activity during the first 30s of the test, in line with previous experiments investigating NCR performance. Both groups displayed increased locomotor activity upon introduction to the novel, different context (Figure 2.10B, condition: $F_{(1,19)} = 6.863$, $p = 0.017$). There was no effect of genotype and no interaction of genotype*condition (genotype: $F_{(1,19)} = 0.912$, $p = 0.419$; genotype*condition: $F_{(2,19)} = 0.664$, $p = 0.526$). This finding indicates that long-term habituation is intact in GluA1$^{-/-}$ mice, with reduced responding to the same context used during pre-exposure, compared to novelty, that was comparable to WT activity levels.

We then investigated how animals respond throughout the test in response to the two contexts. Both groups showed an immediate decline in responding to the same context (Figure 2.10C). Activity also decreased over time in response to the different context, though more subtly. Interestingly, although overall GluA1$^{-/-}$ was higher than WT, there was no evidence of sensitisation, possibly reflecting generalisation to the task structure itself, or to components of the arena/testing room that were identical between contexts. These results were confirmed by a significant effect of condition, and an effect of genotype approaching significance (condition: $F_{(1,20)} = 14.890$, $p < 0.001$; genotype: $F_{(1,20)} = 4.134$, $p = 0.056$). There was no interaction of genotype*condition or genotype*condition*time (genotype*condition: $F_{(1,20)} = 0.097$, $p = 0.758$; genotype*condition*time: $F_{(9,180)} = 0.850$, $p = 0.571$).

Discussion

When GluA1$^{-/-}$ mice were pre-exposed to the familiar context over five to seven sessions, each spaced 24 hours apart, they demonstrated intact long-term habituation and an increase in responding to the introduction of novelty in the environment. This confirms previous reports that these animals are unimpaired (or even enhanced) in long-term habituation (Sanderson *et al.,* 2009) but also demonstrates that GluA1$^{-/-}$ mice can express a novelty preference on this task under different conditions.

Previous reports of long-term habituation in GluA1$^{-/-}$ revealed enhanced habituation when trials were spaced over days (Sanderson *et al.,* 2009). There was no indication that this behaviour was enhanced under the circumstances presented here. However, the confirmation that there is no impairment in these mice is still important. Increased exploration of the novel, or different, context was seen here both in the first 30s of open-field exploration, but also in the timecourse over the full five minutes of the trial.

Interestingly, GluA1$^{-/-}$ activity did seem to increase marginally towards the last 90-120s of the trial. While there was no observed effect of genotype after the third pre-exposure session, this trend of increasing activity late in the trial was fairly consistent in pre-exposure data. This suggests there may be some general increase in arousal over time in these animals, but it was not sufficient to significantly disrupt the expression of long-term habituation.

2.9 Impaired novelty preference but no sensitisation in a modified novel object recognition task

Introduction

In the final experiment of this chapter focusing on habituation in GluA1$^{-/-}$ mice, we wanted to further investigate how animals respond to their surroundings dependent on their novelty. Several studies have investigated the effect of GluA1 deletion on NOR behaviour; where time spent exploring a novel object, versus time spent exploring a less novel object, is used as an index of novelty discrimination (Sanderson, Hindley, *et al.,* 2011; Wiedholz *et al.,* 2008). These studies have shown that while GluA1$^{-/-}$ mice can display intact novelty recognition in this task, this may be due to increased attending (or reduced habituation) to the objects during the sample phase. When animals are yoked to WTs – matching their exploration time to WTs by removing them from the arena after exploring the object for as long as the paired WT mouse during their sample trial – GluA1$^{-/-}$ mice failed to discriminate. Furthermore, Sanderson *et al.* found GluA1 ablation impaired performance on a recency-dependent variant of the task that suggests an impairment specifically in self-generated priming in these mice. Despite this, there was no clear evidence of sensitisation in that study.

However, as with the spatial novelty preference Y-maze, interpretation of impaired novelty discrimination can be obscured by response competition between the two objects. In the paradigm developed by McTighe *et al.* (2010) presentation of the familiar and novel objects was decoupled, and as a result response competition is not a factor. By independently presenting the previously experienced object, or the novel object separately, it may be

possible to dissociate short-term habituation impairments from a potential sensitisation effect.

This experiment aims to more explicitly test for potential sensitisation effects to objects in GluA1$^{-/-}$ mice by adopting the protocol used by McTighe *et al*. to directly assess object preferences within-subject – but importantly between trials.

Methods

Subjects

Male and female GluA1$^{-/-}$ and WT littermates were used for this experiment (WT: n = 10, 4 male and 6 female; GluA1$^{-/-}$: n = 9, 3 male and 6 female).

Apparatus

Two Perspex boxes (25x25x25cm) boxes with white floors and wallpaper inserts were used as testing arenas. Both arenas were used, testing mice in pairs, to increase throughput. The testing order was counterbalanced such that even numbers of WT and GluA1$^{-/-}$ were tested in each arena. A camera placed above the boxes was connected to a PC running Anymaze tracking software (Stoelting Europe, Dublin) to record video files and track object exploration.

Procedure

All animals first received three sessions of context exposure to the arena; animals were placed in the arena for 10 minutes per session, 24 hours apart, in an attempt to minimise responding to the context itself on test days when the objects are presented (Besheer and Bevins 2000).

On the day of testing, animals were placed in the pre-exposed open-field arena. Two identical copies of an object (A1, A2) were present at East and West positions in the box (Figure 2.11A). Subjects were allowed to explore the objects for 10 minutes and then removed from the box for 1 minute. During this ITI the arena was cleaned, and objects replaced; either for two identical copies of the same objects used in the sample phase (same condition: A3, A4), or two novel objects (different condition: B1, B2). Subjects were returned to the arena and allowed to explore for a further 5 minutes in the choice phase. Objects used were either a small football, a scoop or a globe, pseudorandomly assigned and counterbalanced across animals.

One week later animals were tested a second time, receiving the reverse condition; i.e., if animals were tested on the same condition in the first session, they were tested on the different condition a week later.

Analysis

The primary behavioural outcome was object exploration, measured as time spent with the subject's nose within 2cm of the objects.

Results

Despite receiving three trials of pre-exposure, each spaced 24 hours apart, GluA1$^{-/-}$ were still more active on the third pre-exposure session, with higher locomotor responding than WTs across the pre-exposure session (WT trial 3 activity: 4.883m ± 0.715; GluA1$^{-/-}$ trial 3 activity: 13.136m ± 1.684; $t_{(17)} = 4.683$, $p < 0.001$).

WT mice showed a subtle preference for the novel object during the choice phase (Figure 2.11B and C), but no preference either way was seen in GluA1$^{-/-}$ mice. No genotype effect

was observed on the difference score of time spent exploring novel and familiar objects, though WT mice did show higher-than-chance levels of novelty exploration (genotype: $t_{(17)} = 0.498$, $p = 0.323$; WT vs. chance: $t_{(9)} = 2.586$, $p = 0.029$; GluA1$^{-/-}$ vs. chance: $t_{(8)} = 1.047$, $p = 0.326$).

Despite spending more time exploring the objects during both the sample and test phases (Figure 2.11D-F), GluA1$^{-/-}$ failed to show a preference for the novel object. Additionally, they did not exhibit any obvious sensitisation throughout the session either.

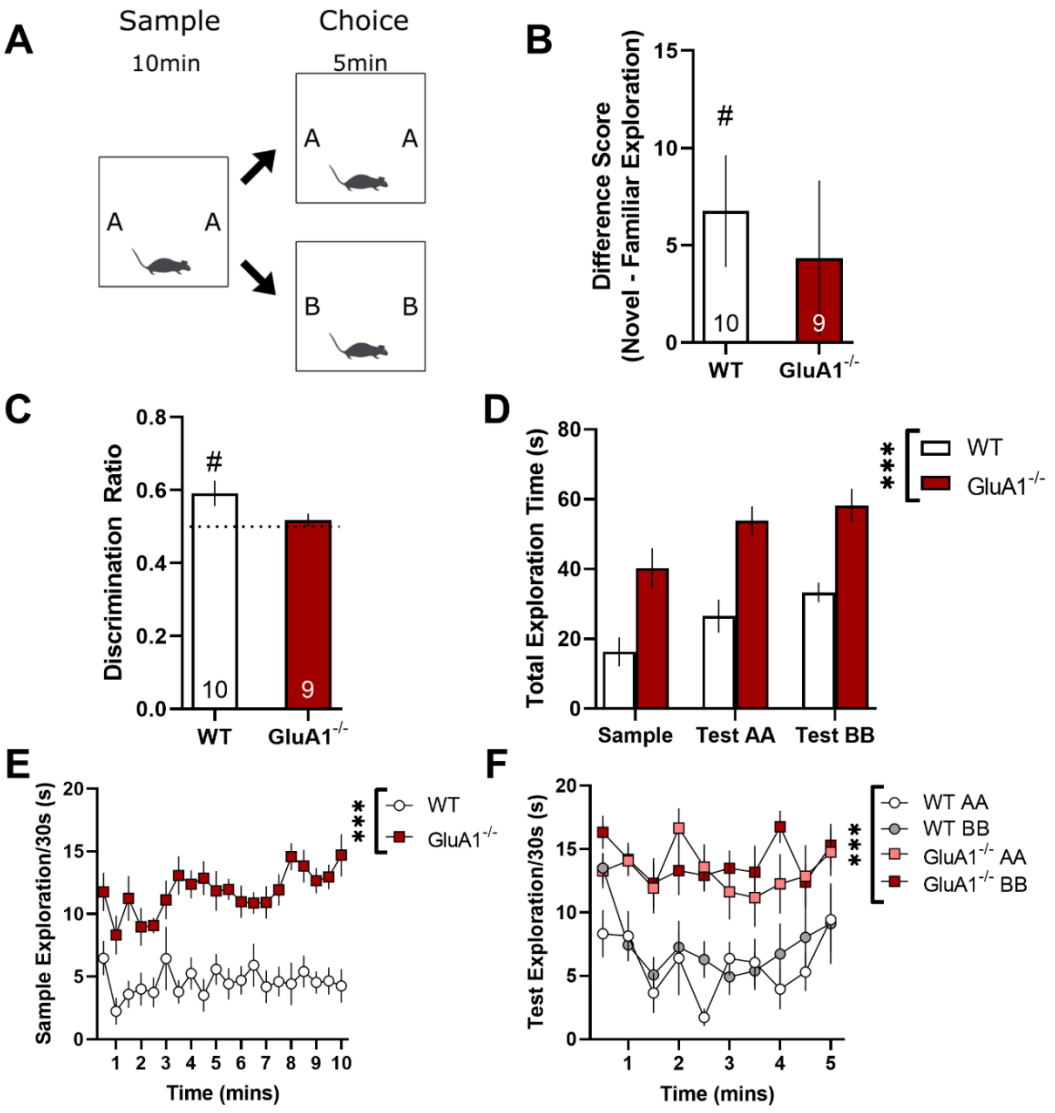

Figure 2.11 Increased object exploration but no novelty discrimination in GluA1$^{-/-}$ mice in the NOR task. Mice were presented with two copies of an object, A, in a sample phase (A). In the following choice phase animals mice were presented with either identical copies of the sample object (AA), or two copies of a novel

object (BB). WT mice spent more time exploring novel objects as observed in both a difference score (B) and discrimination ratio (C) of novel vs. familiar exploration. Total exploration time was higher in GluA1$^{-/-}$ across all stages of the task (D-F). All data presented as mean ± SEM. Numbers within bars indicate n. Dashed line indicates chance level. *** $p < 0.001$ vs. WT. # $p < 0.05$ vs. chance.

A similar finding was observed when comparing the discrimination ratio for novel vs. familiar exploration across the two sessions (Figure 2.11C, genotype: $t_{(17)} = 1.802$, $p = 0.089$; WT vs. chance: $t_{(9)} = 2.586$, $p = 0.029$; GluA1$^{-/-}$ vs. chance: $t_{(8)} = 1.047$, $p = 0.326$). Overall, it appears that GluA1$^{-/-}$ were equally drawn to novel and familiar cues.

Overall, GluA1$^{-/-}$ mice spent significantly more time exploring objects across all phases than WTs (Figure 2.11D). A significant genotype effect of exploration time was observed, $F_{(1,17)} = 25.582$, $p < 0.001$). A significant effect of phase was also observed, driven by increased object exploration across all animals during the test phases, but no genotype*phase interaction (phase: $F_{(2,34)} = 15.348$, $p < 0.001$; genotype*phase: $F_{(2,34)} = 0.143$, $p = 0.868$). This indicates that despite not appearing to fully habituate to the environment during pre-exposure, this did not affect subsequent object exploration in the test.

We then investigated changes in exploratory activity throughout the session. Again activity was higher in GluA1$^{-/-}$ mice across both the sample and test phases (Figure 2.11E,F). In the sample phase, a significant genotype effect but no genotype*time interaction was observed (genotype: $F_{(1,17)} = 49.645$, $p < 0.001$; genotype*time: $F_{(19,323)} = 1.130$, $p = 0.319$). There was no obvious change in exploratory behaviour over time to indicate a potential sensitisation effect.

Breaking down test phase exploratory activity into 30s bins revealed a similar profile; GluA1$^{-/-}$ mice were significantly more active, and a significant effect of condition (BB vs. AA) was also observed, indicating across both genotypes overall, animals spent more time exploring novel than familiar objects (genotype: $F_{(1,17)} = 30.089$, $p < 0.001$; condition: $F_{(1,17)}$

= 4.815, p = 0.042). However no interaction of this effect was found with time (genotype*condition*time: $F_{(9,153)}$ = 0.794, p = 0.622). In summary, this assay found no effect of sensitisation to previously experienced objects in GluA1$^{-/-}$ mice, but this may be due to a lack of sensitivity of the task itself.

Discussion

This study found that GluA1$^{-/-}$ mice failed to convincingly express a novelty preference when objects were presented separately, but additionally showed no evidence of sensitisation to recently presented objects.

We had predicted based on the sensitisation observed to recently presented stimuli in previous studies (Sanderson, Sprengel, *et al.,* 2011) and throughout this chapter (Figure 2.1 and Figure 2.8) that using this protocol developed by McTighe *et al.* (2010) would reveal the expression of stimulus-specific sensitisation in GluA1$^{-/-}$ mice. This does not seem to be the case. It is possible that animals were not sufficiently pre-exposed to the arena prior to testing, as activity levels in GluA1$^{-/-}$ mice were still well above that of WTs after three pre-exposure sessions. However, object exploration was not reduced as a result of this, in fact it was significantly higher at all stages of the test in GluA1$^{-/-}$.

It is also possible that the number of animals used here was not sufficient to confirm behavioural abnormalities. Genotype differences were close to significance and may have benefited from higher power. However, recency-dependent impairments in NOR have been shown elsewhere (Sanderson, Hindley, *et al.,* 2011) and this was not the main aim of the present experiment. We had predicted that this version of the NOR task may be able to detect stimulus-specific sensitisation and be a useful tool in our phenotyping of models

of psychosis. From these results it is clear that the utility of this task is limited under these circumstances.

Discussion

Impaired short-term habituation but preserved long-term habituation in GluA1$^{-/-}$ mice

The set of experiments described in this chapter reveal a phenotype of GluA1$^{-/-}$ in line with that observed in the wider literature. We have confirmed deficits in short-term habituation in tasks of locomotor activity, and novelty discrimination, similar to that reported elsewhere in the novel open-field (Bannerman *et al.,* 2004; Wiedholz *et al.,* 2008), spatial novelty preference Y-maze (Sanderson *et al.,* 2007), and novel object recognition (Sanderson, Hindley, *et al.,* 2011). In contrast, long-term habituation appears to be unaffected. Homecage activity was normal, as seen previously in the literature (Fitzgerald *et al.,* 2010; Procaccini *et al.,* 2011; Wiedholz *et al.,* 2008). In a long-term habituation variation of the NCR task, GluA1$^{-/-}$ and WT mice were more active in a different context than one they had been pre-exposed to, supporting other studies of intact long-term habituation in GluA1$^{-/-}$ mice (Sanderson *et al.,* 2009).

Across the assays of short-term habituation presented here, we have investigated locomotor responding at a high temporal resolution. Previous work has revealed hyperactivity to novelty in GluA1$^{-/-}$ mice but without investigating how this phenotype may arise (Bannerman *et al.,* 2004; Wiedholz *et al.,* 2008). Having confirmed a profile of prolonged hyperactivity in the novel open-field similar to these earlier reports, we went on to examine the first five minutes of exposure to this novel environment more closely. It is

clear from this analysis that this hyperactivity is not an immediate response to novelty, and is not simply a failure to habituate. GluA1$^{-/-}$ mice exhibited increasing responding to the environment over the first five minutes of exposure to novelty, indicative of sensitisation.

Deficits in GluA1$^{-/-}$ were expanded on in our findings on short-term habituation, we saw abnormalities in both the spatial novelty preference Y-maze and the NCR task. In NCR, WT mice displayed habituation over subsequent exposures to the familiar context A, but dishabituation, and an increase in locomotor responding, when novelty was introduced on trial 3. Rather than display habituation, or even just a failure to habituate, GluA1$^{-/-}$ subjects actually increased their responding across repeated trials in the familiar context, confirming sensitisation. Furthermore, this sensitisation was shown to be directly related to the stimulus presented. When animals were placed in context B on the third trial activity declined.

A similar profile was observed when the ITI between trials 2 and 3 was extended to 15 minutes, though habituation in WT animals was marginally attenuated, and in GluA1$^{-/-}$ mice there was a slight increase in trial 3 activity in the novel condition. While the trial 3 data was less robust after a 15-minute delay, this does appear to replicate the result observed in Experiment 2.6, and extend this by showing that the psychological mechanism driving this response persists for at least 15 minutes. Importantly, we have also shown that GluA1$^{-/-}$ mice can express appropriate novelty discrimination in the NCR task, attending more to the different context, when pre-exposure trials to context A are spaced apart by 24 hours. This normal behaviour under conditions that would not be expected to impair GluA1$^{-/-}$ performance provide further confidence that the short-term habituation impairments are

a genuine effect and not related to a general inability to discriminate between contexts in this task.

Impaired short-term habituation as a deficit in self-generated priming

In the General Introduction we described Wagner's model of SOP as a psychological framework capable of explaining habituation and related impairments (Wagner 1981). While the initial exposure to novelty generates a strong behavioural response via activation of elements into A1, in normal subjects these elements quickly decay, coinciding with a decline in the behavioural response. Reduced attending to an environment, either over continued exposure to a novel open-field, or through separate, repeated exposures to a context during the NCR task, could occur via self-generated priming of environmental cues into A2, a reduced state of attention. Since elements that are primed into A2 are unavailable for activation back to A1 (at least until decaying out of A2 back to the inactive state), subsequent exposure fails to generate a behavioural response of the same scale as that seen on the very first exposure. As well as environmental habituation, this can explain how animals habituate to punctate stimuli such as the lights used in the orienting task in operant chambers (Sanderson, Sprengel, *et al.,* 2011).

The importance of this process can be seen in how animals go on to respond to novelty in choice preference paradigms such as the Y-maze or NOR. While the familiar arms/objects evoke only a weak behavioural response due to self-generated priming of elements into A2, the distinct set of elements representing novel stimuli will be activated into A1 and generate a high level of exploration. This theoretical framework can therefore explain habituation and novelty discrimination in physiologically normal subjects.

It has been suggested that behavioural deficits seen in GluA1$^{-/-}$ mice could result from a block on the decay of elements out of A1 and into A2 (Sanderson and Bannerman 2012). This could explain a failure to habituate in the short-term, in tasks such as the spatial novelty preference Y-maze. If elements representing the to-be-familiar arm persist in A1 without appropriately decaying, when animals are then returned to the maze on the test phase, the familiar arm could still generate a strong attentional response. The behavioural outcome of this was hyperactivity throughout the session and equal time spent in the aberrantly salient familiar arm, and novel arm that would also be expected to elicit a strong novelty response.

However, this highlights a weakness in this Y-maze assay; by presenting cues simultaneously in this manner it is difficult to determine how animals are responding to specific components of their environment. We have seen suggestions that GluA1$^{-/-}$ mice express sensitisation, rather than just a failure to habituate. Activity in the novel open-field increased over the first five minutes of exposure (Figure 2.1B). This increase, and the much higher maximal level of activity than that seen in WT mice, may reflect an increased likelihood of elements being in A1, and potentially a block on elements decaying to A2 – which elicits a much weaker behavioural response. In order to determine the contribution of specific stimuli to behavioural responses, and whether an SOP account can explain these impairments, a task is needed that manipulates the novelty of stimuli, but presents them separately without the confound of response competition.

In the NCR task, GluA1$^{-/-}$ mice expressed sensitisation that was stimulus-specific. This appears to support the idea that this activity could be the result of elements representing the familiar context persisting in A1. In WT mice elements rapidly decay from A1 to the A2

state, resulting in a decline in behavioural responding, until the reintroduction of novelty on trial 3 in the AAB condition, where a strong response is again seen. Sensitisation to context A in GluA1$^{-/-}$ mice could represent the increased likelihood of elements being in the A1 state, which is then attenuated when novelty is reinstated on trial 3.

However, this raises the question as to what is happening to elements in A1 once an animal is removed from that environment. Animals were tested on three discrete trials, each 5 minutes long, but were removed from the environment for 1 minute after each trial. Trial 2 (and trial 3 in the AAA condition) activity was higher in GluA1$^{-/-}$ mice and appeared to develop more rapidly when looking at the timecourse of the session in 5s bins (Figure 2.7C). It is not impossible that these elements persist in A1 during the ITI, but this possibility is harder to conceive when the ITI was extended to 15 minutes.

One possibility is that elements that are stuck in A1 because of a blockade of decay to A2, instead get displaced by other elements and decay to I. A1 is believed to have a relatively small capacity, and could potentially get displaced by other elements of the same diffuse environment, or by other elements such as those relating to the transfer cage animals were kept in during the ITI. Animals were habituated to this cage prior to training, but not as extensively as they would be habituated to their homecage. In this event, at the start of subsequent trials, if elements are in I some may be associatively primed to A2, but the majority of available elements would then be recruited to the A1 state, as in trial 1. An interesting possibility is that this sensitisation effect is actually the cumulative effect of some elements that have decayed to I and been associatively primed to A2, and those elements that remain in A1 or are re-activated to A1. This could explain how sensitisation persists across discrete trials, even when the ITI is extended to 15 minutes. Furthermore,

it is not inconceivable that this could happen within a trial, in the NCR assay but also in continuous tasks like the novel open-field. If elements decay or are displaced to I, and some associative priming occurs, this could account for increasing activity over time if this behavioural response is the sum of A1 and A2 activation.

An alternative account could be that the behavioural response is a result of emotional components of the task. This possibility is not incompatible with SOP, but is difficult to parse from the contribution of contextual stimuli to behavioural outcomes. It is possible that as well as generating attentional changes to external surroundings, there is an emotional element to SOP, something suggested by Wagner in a later model of SOP – the affective extension of SOP (AESOP, Wagner and Brandon, 1989). Wagner suggested that these emotional elements relating to an individual's surroundings may also be activated to the focal state of attention, and persist for much longer than elements relating to external features. Either one of these accounts, or indeed a combination of the two, could potentially explain the sensitisation effect and how this persists over 15 minutes away from the context.

Interestingly, whilst the NCR task was able to demonstrate stimulus-specific sensitisation in GluA1$^{-/-}$ mice, trial 3 responding was typically higher than that seen on trial 1. There could be a few reasons for this. Firstly, there may be some generalisation across the stimuli used. The arena itself, testing room, and experimenter were all novel, and consistent throughout the session, potentially contributing partially to this novelty response. Additionally, there could be some general hyperarousal in response to the repeated handling involved in the testing process or indeed to the context over time, that is separate to the sensitisation effect but also contributes to the resulting hyperactivity. It is also

possible that some of the response seen was due to emotional components of the task, as suggested by Wagner's AESOP framework (Wagner and Brandon 1989). It is possible that any one, or a combination of these suggestions contribute to the increased activity seen in context B. While these represent potential confounds in this result, it is important to note that trial 3 activity in the AAB condition was still significantly reduced compared to both trial 2 within-condition, and to trial 3 of the opposite condition, AAA.

On trial 1 of the NCR task, GluA1$^{-/-}$ mice were significantly less active than their WT counterparts. This result is somewhat surprising, given that it was observed here, but not in the novel open-field or Y-maze. It may be that the beam-break software used for recording activity in the novel open-field was less sensitive to changes in activity than the motion tracking software used in later experiments. In the Y-maze, activity in both groups was closer to WT trial 1 activity of the NCR task, indicating that similarities in the Y-maze were due to GluA1$^{-/-}$ mice being more active here. The reasons for this are not clear, but it is possible that the Y-maze is slightly more anxiogenic, since the arms are transparent and animals can see the entire testing room, and mice typically run immediately to the other (to-be-familiar) arm at the start of the trial.

While it would be very difficult to prove experimentally, one intriguing possibility is that the hypoactivity seen in response to novelty on trial 1 of NCR, can also be accounted for by a blockade of elements decaying to A2. If elements of their previous environment (i.e., the transfer holding cage) are saturating A1 at the start of the session, it may be harder to get elements of a new stimulus into the A1 state. Hyperactivity may develop gradually because existing elements from a previous stimulus must first be displaced from A1 in order for the attention to new environmental features to occur.

Admittedly it is also possible that this hypoactivity was due to changes in arousal between genotypes, or a different emotional state in GluA1$^{-/-}$ mice. There are different predictions that we could make based on emotional states, where we could argue that GluA1$^{-/-}$ may show heightened emotionality that increases freezing behaviour. Alternatively, it could be argued that they show a reduced interest in their environment due to a blunted emotional state. It is difficult to argue definitively for any of the above explanations for hypoactivity observed in these animals, and further work would be required to explicitly test the nature of this deficit (which here was an interesting additional finding rather than the main aim of the test). Nevertheless, it is an interesting difference that appeared to be quite robust in the NCR assay and warrants further investigation, to determine if this effect is stimulus-specific and why it is seen in some circumstances but not others.

Importantly, no individual assay can provide a complete picture of the psychological processes governing behavioural impairments in a model. The spatial novelty preference Y-maze provided early indications that self-generated priming may be impaired in GluA1$^{-/-}$ mice, but is limited in the extent to which insights can be drawn because of response competition between novel and non-novel locations. The stimulus-specific effects on NCR suggest that there is a short-term memory trace in GluA1$^{-/-}$ mice, but how animals express this short-term memory trace of a familiar location is maladaptive. Rather than habituating to neutral, familiar locations, we believe these may become increasingly salient, causing GluA1 knockouts to sensitize to familiar cues. Misattribution of salience is a core feature of the aberrant salience framework of schizophrenia (Kapur 2003), and evidence from the clinical literature indicates that patients also exhibit sensitisation to familiar cues (Perry *et al.,* 2009; Williams *et al.,* 2013). This finding provides important construct validity for this

model as a tool for schizophrenia research, and highlights the importance of developing tasks sensitive to detecting this psychological phenomenon.

The Novel Context Recognition task is sensitive to stimulus-specific sensitisation

Previously, a task of orienting to visual light stimuli has been used to show stimulus-specific sensitisation in GluA1$^{-/-}$ mice (Sanderson, Sprengel, *et al.,* 2011). However, this task requires extensive operant training and food or water restriction that alters the motivational state of the animal. This makes it harder to draw comparisons between this task and other simple assays of behaviour which measure spontaneous activity in the absence of dietary restriction.

We felt it was important to design a simple behavioural assay that could be used to phenotype short-term habituation, and be sufficiently sensitive to changes in activity to detect sensitisation, in the models we have been working with. Developing such an assay could help to explain locomotor hyperactivity phenotypes seen in these models. Furthermore, in a task of basic behavioural responding to an open-field environment, it is easier to probe the timeframes at which these psychological processes operate, in comparison to operant tasks where animals are also highly reward-driven, potentially obscuring other behaviours. The aim was to develop a task that was both simple to run and could provide a high-throughput means of testing habituation, but also be able to manipulate parameters like ITI to investigate how rapidly habituation attenuates.

In this chapter we have shown the NCR assay to be capable of doing both of these things. We were able to replicate the stimulus-specific sensitisation effect reported previously in response to visual stimuli but now in the spatial domain. Additionally, there were clear

parallels to observations from other experiments that provide confidence that effects seen in this task were genuine.

Another important consideration in developing this task was the ability to manipulate parameters to investigate the temporal properties of deficits observed. Here we have demonstrated the ability to do this and shown divergent effects in GluA1$^{-/-}$ mice; with stimulus-specific impairments in the short-term but normal long-term habituation. Furthermore, the NCR assay has proven to be a valuable tool in detecting intact habituation/dishabituation in unimpaired subjects. This is something we will return to in the next chapter, where we continue to behaviourally phenotype models of relevance to psychosis research.

Chapter 3: Fractionating 'aberrant salience' in models of psychosis

Introduction

The previous chapter focused on the contribution of the GluA1 AMPAR subunit to short- and long-term habituation, its relevance to clinical schizophrenia, and the role of impaired self-generated priming in observed impairments. We next wanted to compare these findings with two of the most widely used pharmacological animal models of schizophrenia. The first of these, amphetamine, produces hyperdopaminergia to mimic symptoms of psychosis preclinically. The second, the NMDAR antagonist MK-801, disrupts synaptic plasticity by noncompetitively blocking NMDARs.

By investigating other mechanisms through which behavioural phenotypes relevant to psychosis manifest, we wanted to see what similarities these different phenotypes share, and how they diverge. Schizophrenia is a highly heterogeneous disorder, with diverse clinical manifestations, and its aetiology is almost certainly equally diverse. By comparing these different models preclinically, we hoped to gain insight into the contribution of different circuit-level disruption to behavioural outcomes. Furthermore, the focus of this thesis is the role of molecular alterations impairing synaptic plasticity in generating symptoms relevant to clinical schizophrenia. Particularly in the case of MK-801 which has a clear impact on plasticity by blocking NMDARs (Frankiewicz *et al.,* 1996), we might expect to see similar effects to the GluA1$^{-/-}$ model described in the previous chapter. Alternatively, we may see differences emerge through blockade of NMDAR-dependent, versus GluA1-

dependent mechanisms of LTP. Additionally, we may well see differences in the phenotype generated via acute blockade of a receptor versus a constitutive knockout.

Hyperdopaminergia following amphetamine administration

Drugs that facilitate dopamine transmission have long been recognised for their psychotomimetic properties; amphetamines have been shown to induce paranoid delusions and auditory and visual hallucinations in some individuals (Bell 1965; Connell 1957). Furthermore, all current neuroleptics abate symptoms by targeting the D2 receptor and reducing dopaminergic transmission (Seeman and Lee 1975). There is extensive evidence of elevated dopamine signalling in psychosis, coinciding with many reports on the vital role dopamine plays in salience processing (reviewed in Winton-Brown *et al.*, 2014). There is a growing consensus that while dopamine may not be the primary cause of schizophrenia, hyperdopaminergia is a key driver of clinical symptoms, and the aberrant salience framework offers a potential route for this symptom onset (Howes and Kapur 2009).

Preclinically, enhanced dopamine transmission, through pharmacological manipulation or knockdown of the dopamine transporter DAT produces a hyperactive phenotype comparable to that of unmedicated patients (Perry *et al.,* 2009). This hyperlocomotion has widely been used as a readout of aberrant salience in rodents.

While evidence of amphetamine-induced locomotor hyperactivity is compelling, dating back 50 years (Lindquist and Götestam 1977; Schiørring 1971), the effect of amphetamine on habituation is less clear. A recent paper from the Bannerman group, investigating acute administration of amphetamine on spatial novelty preference in the Y-maze, revealed no impairment (Wolff *et al.,* 2018). A test of recognition memory in human volunteers also

revealed no deficits after an acute dose of amphetamine, indicating intact habituation to stimuli throughout training (Zeeuws, Deroost, and Soetens 2010).

The literature on amphetamine research goes back over 50 years, and varies widely in terms of models used, doses, routes of administration and acute vs. chronic dosing regimens, and co-administration with other test compounds. This makes it very difficult to interpret results in the wider field with our own findings, within this and other manipulations. To understand the contribution of hyperdopaminergia to behavioural impairments relative to aberrant salience it is important to generate our own datasets, with behavioural paradigms and testing conditions comparable to those used in our tests with other rodent models. In this chapter, we have aimed to replicate the testing battery used in GluA1$^{-/-}$ mice, in animals administered with amphetamine, to investigate the contribution of hyperdopaminergia, to deficits in habituation.

Impaired synaptic plasticity via NMDAR blockade

In this chapter, we have also investigated the contribution of NMDAR-mediated changes in synaptic plasticity to habituation, learning and memory. Studies of NMDAR antagonists provided some of the earliest indications of a glutamatergic role in the development of schizophrenia, particularly of ketamine, that has repeatedly been shown to induce both positive and negative symptoms associated with the disorder (Cheng *et al.,* 2018; Fine and Finestone 1973; Krystal *et al.,* 1994). This presents an interesting dichotomy, of ketamine as a psychotomimetic compound in these studies, yet it has been touted as a promising rapid-acting antidepressant in recent years (Vollenweider and Kometer 2010). This may reflect the different effects of ketamine administration, acting as a psychotomimetic in the

short-term while the drug is present, but promoting neuroplasticity in the longer term after an acute dose.

As discussed in the General Introduction, there has been some dispute over the years into the effects of NMDAR antagonists on learning and memory. While early reports argued for a vital role of NMDARs in tasks of spatial navigation (Morris *et al.,* 1986), it has since proved to be more complicated; in certain conditions NMDARs were not required to learn the spatial location of an escape platform (Bannerman *et al.,* 1995). Hippocampal NMDARs instead may contribute to pattern separation of ambiguous or overlapping stimuli. *Grin1*$^{\Delta DGCA1}$ mice (lacking NMDARs in the DG and CA1 subfields of hippocampus) showed reduced first-choice accuracy on a visual beacon version of the water-maze, with visually identical cues representing rewarded and unrewarded spatial locations (Bannerman *et al.,* 2012, 2014).

These findings argue for an NMDAR-independent mechanism for associative long-term memory formation, since acquisition of an association between spatial location and reward (e.g., escape) is preserved, even when NMDARs are wholly ablated from the DG and CA1 subfields. Yet NMDAR antagonists have been consistently shown to produce locomotor hyperactivity in an open-field environment (Bygrave *et al.,* 2016; Gilmour *et al.,* 2009; Liljequist *et al.,* 1991). Acute administration of MK-801 also impairs recognition memory and novelty discrimination in the NOR task (King *et al.,* 2004; De Lima *et al.,* 2005; Torrisi *et al.,* 2017). Specifically, administration of MK-801 prior to the sample phase, but not the test phase, appeared to impair discrimination, indicating NMDAR activation was required for encoding, but not the consolidation or retrieval of memories (Nilsson *et al.,* 2007). This

appears to match our understanding of the role of NMDARs in LTP induction, but not in expression, as described in the General Introduction.

An earlier doctoral student in the Bannerman group has aimed to investigate the orienting response of MK-801-treated mice to novel or familiar visual stimuli – similar to the study from Sanderson *et al.* (2011) using GluA1$^{-/-}$ animals – in the hope of detecting stimulus-specific sensitisation in these animals (Boerner 2018). While behaviourally it was clear that MK-801 impaired habituation to repeated cue presentation, sensitisation was not evident under these circumstances (Figure 3.1). It should be noted, however, that this was at a sub-threshold dose for locomotor hyperactivity. Given our findings of the previous chapter, it is likely that this hyperactivity reflects a failure to habituate, but may also be a consequence of sensitisation.

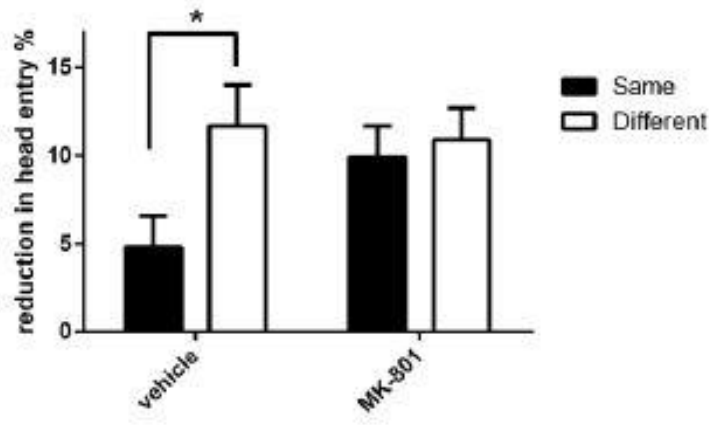

Figure 3.1 MK-801 induced impairments in habituation to repeated presentations of the same visual stimulus. C57Bl/6j mice treated with 0.1mg/kg MK-801 (i.p.) expressed a similar orienting response to visual stimuli, independent of whether the cue was the same, or different, to a stimulus presented 30s previously. Saline-treated vehicle controls expressed a greater head orienting response when the stimulus was different, than when the same stimulus was repeated twice (image taken from Boerner, 2018).

Further complicating this result, MK-801 may affect the motivational state of the animal. MK-801-treated mice spent significantly more time in the food magazine, affecting the baseline levels from which these responses were taken – head orienting to stimuli was measured as a suppression of magazine behaviour in comparison to a pre-stimulus

baseline. Given this unclear result, it is important to investigate the possibility of stimulus-specific impairments following MK-801 treatment in more detail, something we have done in this chapter using the NCR task.

Through replicating the behavioural profile conducted on GluA1$^{-/-}$ mice in the previous chapter, here we have aimed to investigate the phenotype of acute MK-801 administration in more detail. In doing so we hope to reveal insights into the hyperactivity observed following MK-801 treatment and whether this shares the same underlying basis as hyperactivity in other models used in schizophrenia research such as the GluA1 knockout mice.

Aims

We previously described an extensive behavioural phenotyping in the GluA1$^{-/-}$ mouse model in assays of short- and long-term habituation. In this chapter, we extend that phenotyping to two pharmacological manipulations commonly used in schizophrenia research, in order to draw comparisons across models and enhance our understanding of the contribution of specific receptors or neurotransmitters to cognitive deficits. Firstly, we will investigate the effects of hyperdopaminergia, via amphetamine administration, on locomotor activity, across novel and familiar environments and in assays of novelty discrimination. We will then go on to profile the effects of the NMDAR antagonist MK-801, on these assays of habituation. By doing so we aim to gain a better understanding of how habituation, and specifically priming mechanisms that contribute to behavioural habituation, are impaired or preserved across manipulations relevant to psychosis.

Experiments

3.1 Increased locomotor activity following acute amphetamine administration in a novel environment

Introduction

The hyperlocomotor properties of amphetamine have been well understood since the early 1970's. Amphetamine administration, and specifically in mesolimbic areas such as nucleus accumbens, has been shown to increase motor activity and induce stereotypical behaviours at higher doses in mice and rats (Kelly, Seviour, and Iversen 1975; Schiørring 1971). The aim of the current study was to both confirm and expand on these findings, looking at a high temporal resolution of activity to see how behavioural abnormalities develop in a novel environment.

Methods

Apparatus used, procedure, and measures of analysis were all the same as those described for Experiment 2.1.

Subjects

24 male C57Bl/6j mice were used for this experiment. All C57Bl/6j mice used in experiments throughout this thesis were generated in-house at the Biomedical Sciences Building, unless explicitly stated.

Drugs

D-Amphetamine sulfate (Tocris, UK) was dissolved in 0.9% saline, and administered intraperitoneally (i.p.) at a dose of 2.5mg/kg and volume of 5mL/kg.

Animals were weighed and dosed 30 minutes prior to behavioural testing, in this experiment and all others, unless explicitly stated otherwise. During this pre-treatment period, animals were kept separate from cagemates. After 30 minutes, animals were taken to the testing room for the behavioural procedure.

Results

An acute injection of amphetamine induced significant locomotor hyperactivity; amphetamine-treated mice had higher total beam breaks and exhibited increased numbers of beam breaks across the entire hour of testing (Figure 3.2).

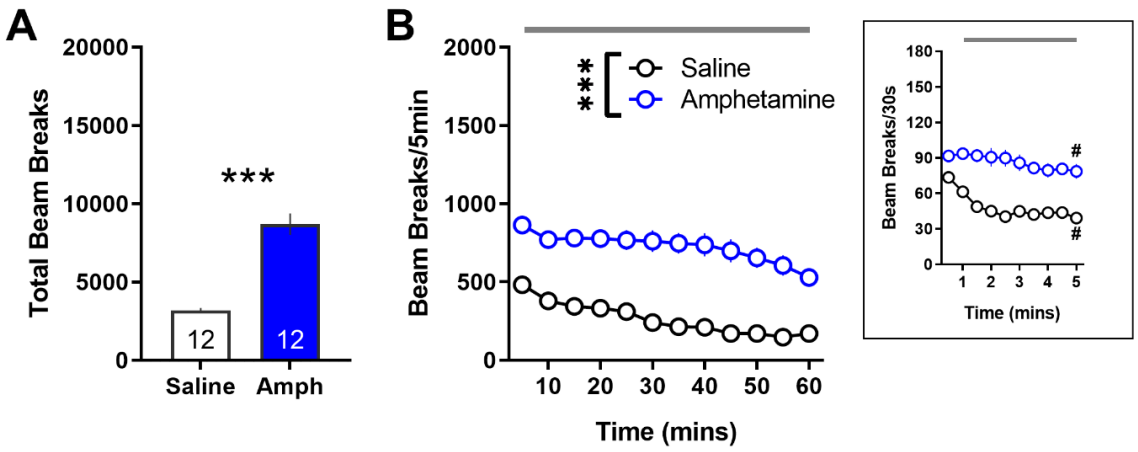

Figure 3.2 Amphetamine administration induced hyperactivity in a novel environment. Amphetamine induced significant hyperactivity in C57Bl/6j mice, observed as a total over 60 minutes (A) and when broken down into 5-minute bins (B). Analysis of the first 5 minutes in 30s bins revealed significant hyperactivity from 30s onwards (inset). Activity started higher than saline controls and remained higher across the entire session. All data are presented as mean ± SEM. Numbers within bars indicate n. *** $p < 0.001$ vs. controls. # $p < 0.05$ vs. first 30s activity. Grey bar above figures indicates a significant difference in activity, after Sidak correction.

Hyperactivity in response to amphetamine was observed as both a significant effect of treatment, and treatment*time interaction (treatment: $F_{(1,22)} = 60.220$, $p < 0.001$; treatment*time: $F_{(11,242)} = 4.202$, $p < 0.001$).

Post-hoc investigation of the treatment*time effect revealed significantly higher activity in the amphetamine group at every 5-minute timebin throughout the session (all $p < 0.0087$,

Sidak-corrected significance threshold for 12 comparisons across two families). To understand how this hyperactive phenotype arises, the first 5 minutes was examined at a higher temporal resolution (Figure 3.2B inset). Amphetamine induced significant hyperactivity at every timepoint after the first 30s. In the first 30s, although activity was still higher in the treatment group, this did not survive Sidak correction (first 30s: $t_{(22)}$ = 2.662, p = 0.014; all other timepoints: $p < 0.001$; $p < 0.0051$ significance threshold for 10 tests). Despite amphetamine activity appearing to stay very high throughout the first 5 minutes, both groups did show a decline in responding over this time, confirmed through *post-hoc* comparisons of the first and last 30s within each group (saline: $t_{(10)}$ = 6.169, $p <$ 0.001; amphetamine: $t_{(10)}$ = 3.024, p = 0.012).

Discussion

This experiment confirms findings from the historical literature that amphetamine induces locomotor hyperactivity in a novel environment (Schiørring 1971). While not apparent in the first 30s, amphetamine-induced hyperactivity was observed from 30s onwards, and appeared to stay relatively constant across the whole 60-minute session. During this first 30s the lack of effect appeared to be driven mainly by a high exploratory response in saline controls which then declined.

This provides some early indication that amphetamine administration may induce a different phenotype to that reported with GluA1 knockout in the previous chapter. Whereas GluA1$^{-/-}$ mice displayed progressively increasing activity during the first 5-10 minutes of the session, reaching much higher activity levels than controls, amphetamine treatment induced immediate hyperactivity that remained for the rest of the 1-hour session. Interestingly, amphetamine-induced hyperlocomotion did appear to decline over

time, and decreased in the first 5 minutes, despite staying well above control levels. It is possible that this reflects some habituation over the session, and the hyperarousal induced by amphetamine drives aberrant locomotor responding, but it is impossible to say with confidence this is the case from this result alone. Admittedly, a parsimonious explanation could also be that this more likely reflects metabolism or drug clearance over time.

3.2 Increased homecage activity in amphetamine-treated mice

Introduction

While the effect of amphetamine on novelty exploration is well documented, how amphetamine impacts homecage behaviour is less clear. Of the studies available in the literature, some report hyperactivity but in very high doses, up to 20mg/kg in mice (Khroyan *et al.,* 2012), or with unclear levels of prior habituation to the environment (Zhuang *et al.,* 2001), while others have looked at dose-response curves but for methamphetamine, and in rats (Dunne, O'Halloran, and Kelly 2007). While these studies all report homecage hyperactivity following drug administration, differences in protocol make it difficult to draw comparisons with other experiments conducted with amphetamine in this chapter.

To understand the relationship between amphetamine, and locomotor responding in familiar environments, it was therefore important to generate our own dataset, using a consistent drug dose and model. The aim of this experiment was to investigate homecage behaviour in amphetamine-treated mice in a highly familiar homecage environment.

Methods

Apparatus used was the same as in Experiment 2.2.

Subjects

6 male C57Bl/6j mice were used for this experiment; these were some of the saline controls (and therefore drug-naïve) from Experiment 3.1.

Procedure

The procedure was similar to that described in Experiment 2.2. Animals were first allowed to habituate for 8 days, prior to drug treatment. Compounds were then administered at approximately 6 hours after lights on, the lowest point of activity in the animals 24-hour cycle. Animals received both treatment conditions; saline and amphetamine, 7 days apart to allow adequate time for drug washout. Treatments were pseudorandomly assigned so that half of animals received saline, and half amphetamine in the first session.

Analysis

Analysis was conducted in the same way as in Experiment 2.2. Treatment of all animals was identical for the first 8 days whilst animals were acclimating to the homecages. These data have not been included but no differences were seen in the to-be-saline and to-be-amphetamine treatment groups during this period. Additionally, this experiment was performed within-subjects.

Results

Following an acute injection of amphetamine in the familiar homecage, significantly elevated activity was observed, recorded as a total of PIR activations in the 60 minutes immediately following injection (Figure 3.3A, treatment: $F_{(1,5)} = 11.211$, $p = 0.020$).

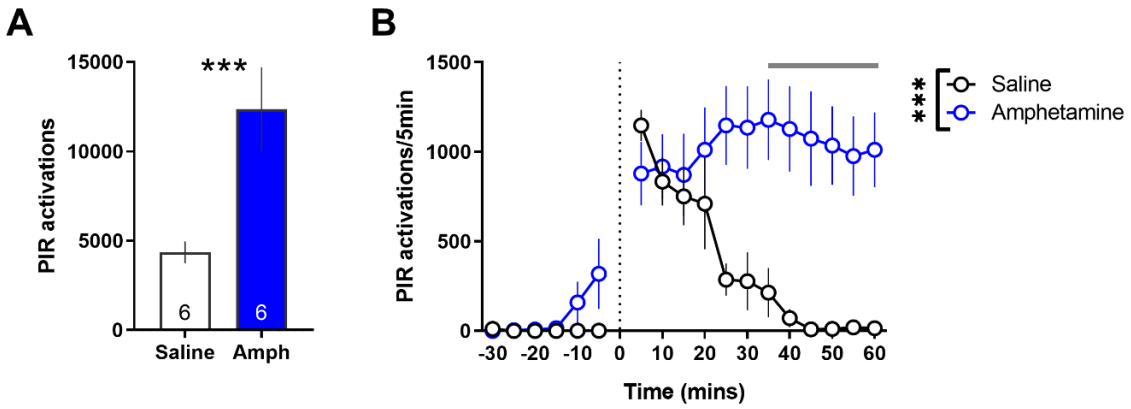

Figure 3.3 Amphetamine treatment induced pronounced hyperactivity in a familiar homecage environment. Amphetamine-treated C57Bl/6j mice displayed significant hyperactivity in the hour immediately following injection, reported as both a total (A) and in 5-minute bins, from 30 minutes prior, to 60 minutes after injection (B)**.** All data are presented as mean ± SEM. Numbers within bars indicate n. *** $p < 0.001$ vs. controls. Grey bar above indicates a Sidak-corrected significant difference in activity following drug injection.

A significant treatment*time interaction, when comparing 5-minute bins across the session between treatment groups, confirmed amphetamine induced hyperactivity that persisted for at least an hour post-injection (Figure 3.3B, $F_{(11,55)} = 7.516$, $p < 0.001$). *Post-hoc* tests of activity at each 5-minute period after injection revealed significantly different levels of activity between treatment groups from 45 minutes onwards (45-60 minutes, all $p < 0.0087$; significance threshold for 12 tests with an alpha of 0.1 when comparing across two families). However, being less conservative with correction for family-wise error rate, we saw significant effects at $p < 0.05$ from 20 minutes onwards.

Activity was slightly higher in the amphetamine-treated group immediately prior to dosing. However, this was driven by 2 animals, in the 5-10 minutes before injection, when the experimenter was in the room preparing for dosing.

Discussion

Amphetamine induced an increase in arousal within the homecage that persisted for much longer than in saline controls. Activity of saline-treated mice had declined back to baseline levels by 40 minutes post-dosing. However, amphetamine-treated mice expressed high

locomotor across the entire hour. In fact, whilst only the first hour after dosing is displayed here, amphetamine increased locomotor hyperactivity for at least 2 hours.

This result is supported by other reports in the literature, that have shown hyperactivity in the homecage, albeit in rats or with differing doses of amphetamine (Dunne *et al.,* 2007; Schiørring 1971). The importance of this finding will be discussed in more detail in the discussion at the end of this chapter, in the context of the wider behavioural phenotype of this manipulation. What is clear is that amphetamine treatment is generating a very different behavioural phenotype to that observed in GluA1$^{-/-}$ mice in the previous chapter.

3.3 MK-801 induces hypoactivity to novelty but hyperactivity over time in a novel open-field

Introduction

The hyperlocomotor effects of MK-801, and other NMDAR antagonists, are well documented, both in rats and mice, and at a range of doses (Bygrave *et al.,* 2016; Gilmour *et al.,* 2009; Tricklebank *et al.,* 1989). However, it is important for generating a complete profile of the animals' phenotype that we replicate these findings here. That is what we have aimed to do in the current experiment. Additionally, we wanted to investigate how this hyperactive phenotype develops using the same method of analysis described in previous locomotor experiments, looking at activity both across a session and during the initial exposure to the novel environment.

Methods

Apparatus used, procedure, and measures of analysis were all the same as those described for Experiment 2.1.

Subjects

31 male mice were used for this study (saline n = 15, MK-801 n = 16), procured from Envigo (UK), and shipped to the Biomedical Sciences Building at Oxford.

Drugs

(+)-MK-801 maleate (Tocris, UK) was dissolved in 0.9% saline and administered by i.p. injection at a dose of 0.2mg/kg and volume of 4mL/kg.

The dosing regimen was otherwise the same as that used for amphetamine and described in Experiment 3.1.

Results

MK-801 induced significant hyperactivity in a novel open-field environment, confirmed by a significant effect of treatment and treatment*time interaction when the session was split into 5-minute bins (Figure 3.4, treatment: $F_{(1,29)} = 69.167$, $p < 0.001$; treatment*time: $F_{(11,319)} = 27.640$, $p < 0.001$).

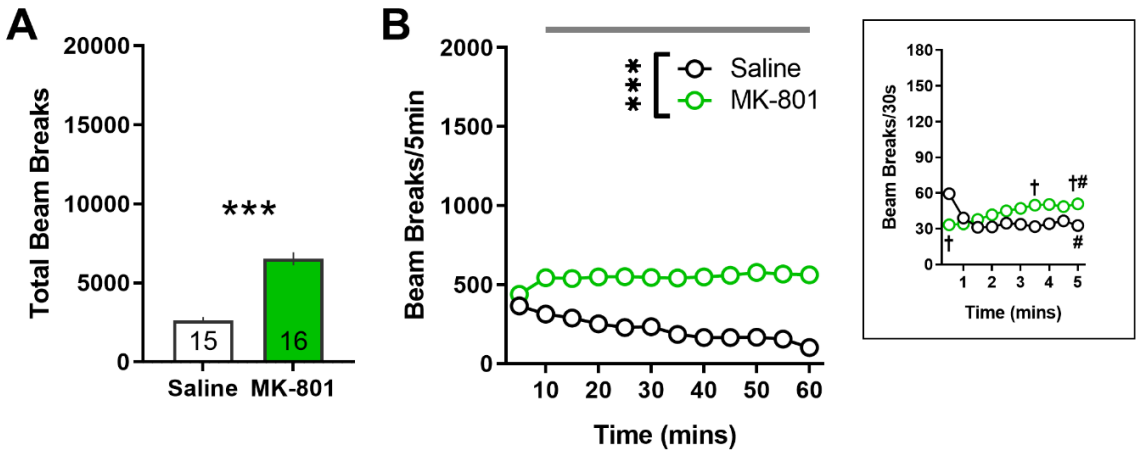

Figure 3.4 MK-801-treated mice are hyperactive in a novel environment. MK-801 induced significant hyperactivity in response to a novel open-field, reported here as a total over 60 minutes (A) and in 5-minute timebins across the session (B). MK-801 induced hyperactivity throughout the session, after the first 5 minutes. Looking at this early time bin more closely, MK-801 actually induced initial hypoactivity in the first 30s, but

mice became increasingly active, beginning to display hyperactivity relative to saline controls after around 3 minutes of exposure (inset). All data are presented as mean ± SEM. Numbers within bars indicate n. *** $p < 0.001$ vs. saline. # $p < 0.05$ vs. first 30s activity. Grey bar above figures indicates a significant difference in activity after Sidak correction. † $p < 0.051$ vs. saline in 30s bins during first 5 minutes (Sidak-corrected *p*-value).

Post-hoc EM means comparisons revealed significant hyperactivity following MK-801 treatment from 5 minutes onwards, in the novel open-field. In the first 5 minutes, no significant difference between genotypes was observed (first 5 minutes: $t_{(29)} = 3.112$, $p = 0.088$; all other timepoints: $p < 0.001$; significance threshold for 12 tests across two families of $p < 0.0087$).

To understand how MK-801 induces this hyperactive phenotype, we then investigated the first 5 minutes of activity at a higher temporal resolution of 30s bins (Figure 3.4B inset). This *post-hoc* comparison revealed MK-801-induced hyperactivity to develop gradually, with significant hyperactivity only observed at around 180-210s and 270-300s (both $p < 0.0051$, Sidak-corrected for 10 comparisons). Interestingly, a significant difference between treatments was also seen in the first 30s but in the opposite direction; with MK-801 inducing significant hypoactivity upon initial exposure to the novel open-field ($p < 0.0051$). Within-treatment comparisons revealed significant differences from the first and last timebin in the first 5 minutes in both groups (saline: $t_{(13)} = 9.013$, $p < 0.001$; MK-801: $t_{(14)} = 4.755$, $p < 0.001$). In saline, this reflected habituation over this time, whereas with MK-801 the difference was due to an increase in exploratory behaviour.

Discussion

This experiment found prolonged hyperactivity in mice treated with MK-801, with activity still far higher than control levels after 60 minutes of exploration of an open-field. This matches previous studies of MK-801 showing hyperactivity lasting for at least 2 hours post-dosing (Gilmour *et al.,* 2009; Tricklebank *et al.,* 1989; Whishaw and Auer 1989).

The current experiment expanded on this result by investigating the onset of this hyperactive phenotype. In the first five minutes of activity in the novel open-field, there were no significant differences between treatment groups on total activity. However, when we increased the temporal resolution to investigate activity over 30s bins upon first exposure to novelty, we actually saw hypoactivity in MK-801-treated animals compared to controls. It wasn't until four minutes into the session that we began to see evidence of hyperlocomotion.

This finding suggests that hyperactivity following MK-801 treatment is more than a general hyperarousal effect, like that seen with amphetamine, something we will investigate further in the experiments to come.

3.4 No homecage hyperactivity following acute MK-801 administration

Introduction

We next wanted to investigate the effect of MK-801 on locomotor activity when administered in a highly familiar homecage environment. To our knowledge, this has not been studied elsewhere in the literature; while many groups have reported on locomotor activity after some habituation, this tends to be between 30 minutes to a few hours spread over several days before dosing (Van Den Buuse *et al.,* 2009; Ueno *et al.,* 2019; Velardo, Simpson, and Zahniser 1998). This pre-treatment measurement of activity is commonly used to measure baseline behaviour, ensuring no clear differences between treatment groups prior to dosing, rather than to explicitly compare activity across novel or non-novel environments (for example, Gilmour *et al.*, 2009).

This experiment therefore aimed to generate locomotor activity data following treatment with MK-801, in a homecage location that animals were extensively habituated to before dosing. In doing so, this allows us to draw comparisons to results from both the previous experiment of MK-801 novelty responding and earlier studies of different manipulations such as with amphetamine and the GluA1$^{-/-}$ mice.

Methods

Apparatus, procedure used, and measures of analysis were the same as those described previously in Experiments 2.2 and 3.2.

Subjects

11 male C57Bl/6j mice were used for this experiment. Animals received both treatment conditions; saline and MK-801, 7 days apart to allow adequate time for drug washout. Treatments were pseudorandomly assigned so that half of animals received saline, and half MK-801 in the first session.

Results

While total PIR activations in the homecage were marginally higher in mice treated with MK-801, activity did not differ significantly between groups (Figure 3.5A, treatment: $F_{(1,11)} = 3.139$, $p = 0.104$).

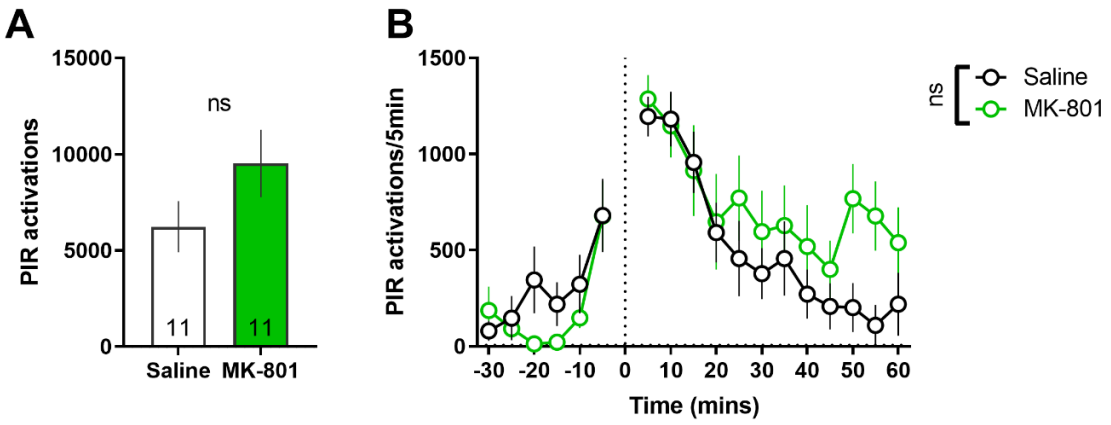

Figure 3.5 MK-801 treatment had no effect on locomotor activity in the familiar homecage. No difference in total locomotor activity was observed following MK-801 treatment, compared to saline (A). Over a 60-minute window following injection, both groups initially showed high levels of activity, which attenuated over the next hour, but there was no difference between treatment groups (B). All data are presented as mean ± SEM. Numbers within bars indicate n. Dashed line indicates time of injection.

While treatment had no effect (Figure 3.5B, $F_{(1,11)} = 3.139$, $p = 0.104$), there was a significant effect of time, with both treatment groups showing initially high locomotor activity immediately after injection but declining over the next hour ($F_{(11,121)} = 15.227$, $p < 0.001$). A lack of treatment*time interaction confirmed that MK-801 had no obvious impact on homecage locomotor activity ($F_{(11,121)} = 1.124$, $p = 0.348$), reported here in the first hour post-dosing, though no differences were seen in the 24-hour activity either. Although there were no significant effects of treatment, MK-801 did appear to subtly effect activity, particularly around 50 minutes post-dosing. Whilst it is not appropriate to conduct *post-hoc* comparisons on the treatment*time interaction, if we were to look at 45-50 and 50-55 minutes in isolation, they would have revealed some hyperactivity after MK-801 treatment. There was no difference in activity between treatment groups in the 1-2 hour post-dosing period (saline activity: 1396 ± 682; MK-801 activity: 2024 ± 608; $p > 0.5$).

Baseline activity between treatment groups was similar, though there are two things to note. Firstly, as seen in Experiment 3.2, there was an increase in activity across groups in the 5-10 minutes immediately prior to dosing as the experimenter entered the room.

Secondly, there was slightly higher activity in the saline group 10-20 minutes prior to dosing, but as in earlier experiments this was driven mainly by 3 animals out of 12 that showed bouts of higher activity over this time. Treatment was within-subjects and so groups should be similar.

Discussion

Despite displaying pronounced hyperactivity in the novel environment, MK-801-treated mice were mostly normal when dosed in a familiar homecage. Motor activity showed some subtle increases with MK-801, but overall was similar to that of controls. This contradicts other results reporting hyperlocomotion in a habituated environment, but likely reflects the extensive habituation over a week compared to 30 minutes, or several hours across days, used in other studies (Van Den Buuse *et al.,* 2009; Gilmour *et al.,* 2009; Velardo *et al.,* 1998). This finding indicates that MK-801 doesn't produce overt locomotor hyperactivity when tested in a familiar homecage environment. There was a marginal increase in activity in mice treated with MK-801 around 45 minutes after dosing, but this appeared to be transient and overall no significant treatment differences were observed. This may indicate some subtle effects unrelated to the effect of MK-801 on hippocampal plasticity, such as mild hyperarousal.

3.5 Y-maze novelty recognition impaired by MK-801 but not amphetamine administration

Introduction

In the first two experiments of this chapter, we found hyperactivity induced by amphetamine that was independent of the novelty of the environment. We next wanted to investigate how this affects the animals' ability to express short-term habituation, in the

spatial novelty preference Y-maze task. On the other hand, MK-801 appears to induce hyperactivity in a novel open-field, but not in a familiar environment. In this experiment we wanted to investigate the effect of these pharmacological manipulations on short-term habituation in more detail.

The novelty-independent locomotor abnormalities induced by amphetamine could be caused by a deficit of both short- and long-term habituation, or could reflect a general increase in arousal that is context-independent (Eban-Rothschild *et al.,* 2016). However, a recent paper investigating the effect of amphetamine on short-term habituation in the Y-maze revealed normal habituation, despite hyperactivity, at the same dose of amphetamine used in the first two experiments of this chapter (Wolff *et al.,* 2018). The next experiment aimed to confirm this finding.

Acute MK-801 treatment has previously been shown to disrupt novelty recognition and expression of preference in rodents in the NOR test (King *et al.,* 2004; De Lima *et al.,* 2005; Torrisi *et al.,* 2017). Hippocampal NMDARs are required for other spatial tasks of short-term memory, such as non-match to place tasks in the T-maze (McHugh *et al.,* 2008). To complete the cognitive profiling of our three models of interest, we next wanted to assess the impact of amphetamine and MK-801 treatment on spatial novelty preference, using the same method of testing as that described previously for the GluA1$^{-/-}$ mice.

Methods

Apparatus and procedures used, and measures of analysis were all the same as in Experiment 2.3.

Subjects

48 male C57Bl/6j mice were used across these experiments; 18 in the saline group, 11 in the amphetamine group and 19 in the MK-801 group.

Results

Both saline and amphetamine-treated groups spent more time in the novel arm of the maze than in either the start or familiar arms (Figure 3.6A). This intact preference for a novel location was seen in amphetamine-treated animals despite them displaying hyperactivity throughout the sample and choice phases (Figure 3.6B). MK-801 induced impairments in the expression of novelty preference behaviour, coinciding with locomotor abnormalities similar to those seen in the novel open-field (Figure 3.4B). MK-801 initially induced hypoactivity in animals both in the sample phase and then again in the test phase, with hyperactivity relative to saline controls then developing gradually across each phase

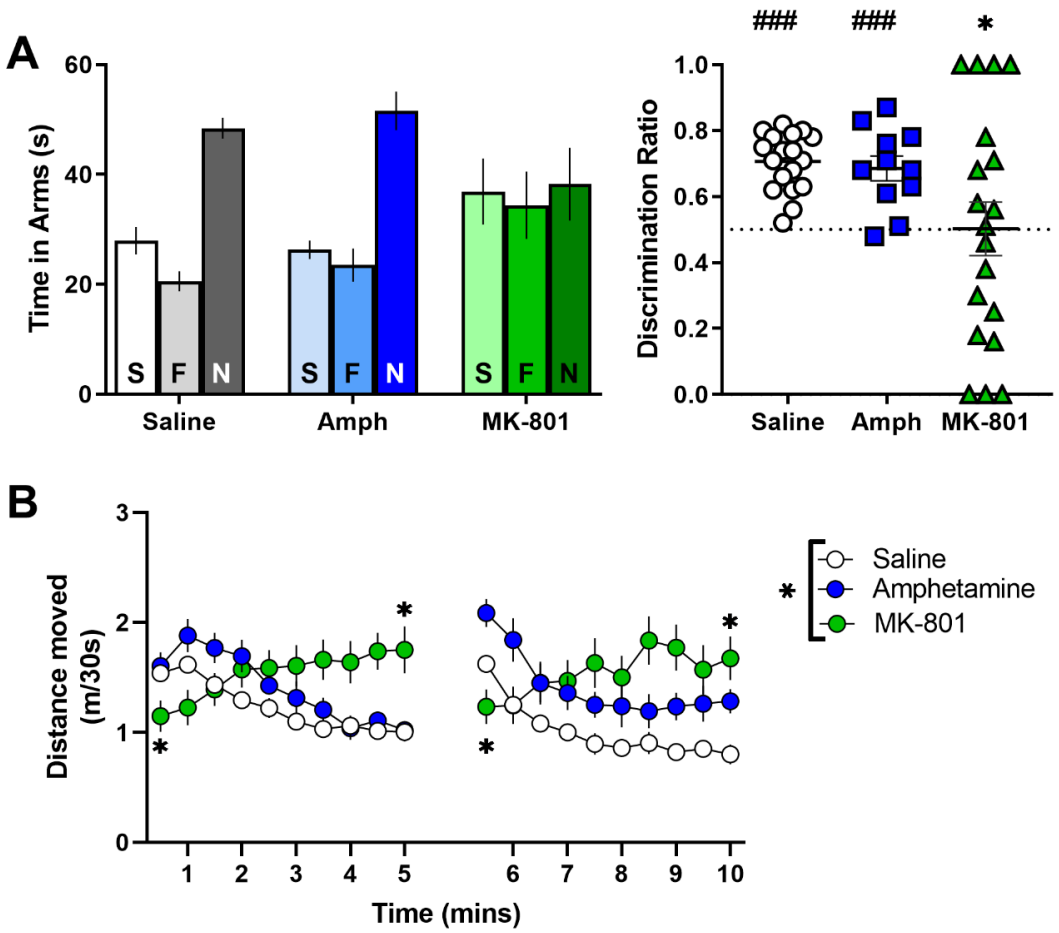

Figure 3.6 MK-801, but not amphetamine, impaired spatial novelty preference in the Y-maze. Amphetamine and saline groups spent more time exploring the novel arm, than the start or familiar arms, and displayed a significant discrimination for the novel arm compared to familiar (A). No novelty discrimination was observed in the MK-801 treatment group. Both drug treatment groups expressed significant hyperactivity throughout the session despite normal novelty recognition with amphetamine (B). All data presented as mean ± SEM. S, F, N within bar indicates start, familiar, and novel arms respectively. Dashed line indicates chance level. Distance over time presented during sample (minutes 1-5) and test (minutes 6-10) phases. * $p < 0.05$ vs. saline. ### $p < 0.001$ vs. chance discrimination.

Spatial Novelty Preference

Both saline controls and amphetamine-treated mice displayed a preference for the novel arm, spending significantly more time in the novel, than the familiar arm. This was confirmed by a discrimination ratio significantly above chance in both groups (Figure 3.6A right panel, saline vs. chance: $t_{(17)} = 9.946$, $p < 0.001$; amphetamine vs. chance: $t_{(10)} = 4.936$, $p < 0.001$).

MK-801-treated mice failed to discriminate between the novel and familiar arms (MK-801 vs. chance: $t_{(18)} = 0.042$, $p = 0.967$). This appears to be similar to deficits observed in GluA1$^{-/-}$ mice, but interestingly, these animals showed a striking bimodal distribution of choice preference that was not seen previously. Some animals showed a complete preference for novelty, not entering the familiar arm once, whilst some animals showed the reverse of this and failed to enter the novel arm. Furthermore, discrimination in the MK-801 treatment group was significantly lower than in saline controls, confirmed by an overall treatment effect and *post-hoc* comparison (treatment: $F_{(2,47)} = 3.925$, $p = 0.027$; MK-801 vs. saline: $t_{(35)} = 2.606$, $p = 0.013$). Amphetamine had no effect compared to controls ($p > 0.8$).

Locomotor Activity

Tracking for one animal in the amphetamine group failed to record properly and so one animal was excluded from these analyses. An overall effect of treatment and treatment*time interaction confirmed differences between groups ($F_{(2,44)} = 3.553$, $p = 0.037$; treatment*time: $F_{(18,396)} = 20.633$, $p < 0.001$). However, there was only a trend interaction of treatment*time*phase ($F_{(18,396)} = 1.613$, $p = 0.054$).

To investigate these effects in more detail, we looked at each treatment group separately compared to saline. A significant effect of treatment was seen with amphetamine, confirming hyperactivity similar to that seen in the novel open-field in Experiment 3.1 ($F_{(1,26)} = 15.429$, $p < 0.001$). This hyperactivity was shown to be generalised across the experiment, with no treatment*time*phase interaction ($F_{(9,234)} = 1.579$, $p = 0.122$). There was a treatment*phase interaction, however, although *post-hoc* comparisons confirmed a significant treatment effect across both exposure and test phases indicating amphetamine-induced hyperactivity throughout the experiment (treatment*phase: $F_{(1,26)} = 13.245$, $p <$

0.001; comparison of drug groups for exposure: $t_{(26)}$ = 2.769, p = 0.010; and test: $t_{(26)}$ = 4.562, p < 0.001).

Aberrant locomotor activity was also seen in animals following MK-801 treatment (Figure 3.6B). These animals appeared to initially be less active than controls, but became increasingly active across each phase, whereas controls displayed a decline in exploring the environment. This was confirmed by a significant treatment effect and treatment*time interaction (treatment: $F_{(1,35)}$ = 4.561, p = 0.040; treatment*time: $F_{(9,315)}$ = 29.988, p < 0.001). There was no main effect of phase, and no overall treatment*time*phase interaction, highlighting that both treatment groups showed a similar activity profile in both sample and choice phases of the task (phase: $F_{(1,35)}$ = 1.342, p = 02559; treatment*time*phase: $F_{(9,315)}$ = 1.020, p = 0.423).

To assess how hyperactivity developed, *post-hoc* comparisons were carried out on activity from the first and last 30s. Because there was no effect or interaction with phase, *post-hoc* tests were conducted on the pooled data from sample and choice phases. This revealed both hypo- and hyperactivity at early and late timepoints respectively, in mice treated with MK-801 (hypoactivity in the first 30s: $t_{(35)}$ =3.714, p < 0.001; hyperactivity in the last 30s: $t_{(35)}$ =3.853, p < 0.001; p < 0.025 significance threshold for 2 tests after Sidak correction).

Discussion

Amphetamine had no effect on short-term habituation or animals' ability to express a preference for the novel arm in the spatial novelty preference Y-maze, despite inducing locomotor hyperactivity. This could potentially be explained by a general hyperarousal effect of amphetamine, rather than a deficit in habituation. Importantly, this finding has

been reported previously, adding support to this hypothesis that normal dopamine modulation is not a prerequisite for intact short-term habituation (Wolff *et al.,* 2018).

MK-801 impaired animals' ability to express a preference for a novel spatial location, while inducing a locomotor profile of gradually increasing activity, similar to that observed previously in novel environments. This is in line with other reports in the literature that MK-801 is required for encoding of novel experiences, from the NOR task (Nilsson *et al.,* 2007). Replication of the locomotor profile first observed in Experiment 3.3, with initial hypoactivity, but gradual onset of hyperactivity, provides confidence that this somewhat striking activity profile is a genuine effect of MK-801.

3.6 MK-801 novelty discrimination is unimpaired in a long-term habituation variant of the Y-maze

Introduction

Our phenotyping of MK-801 treatment has so far shown behaviour consistent with GluA1$^{-/-}$ studies, albeit with a striking bimodal distribution of choice preference in the novelty Y-maze. We next wanted to see if these similarities extend to long-term habituation in the Y-maze. GluA1$^{-/-}$ mice have previously been shown to express divergent short- and long-term habituation, with impaired habituation on a short-term spatial novelty preference Y-maze, but enhanced habituation on a long-term variant (Sanderson *et al.,* 2009). The aim of this study was to investigate whether MK-801-treated mice display intact expression of long-term habituation.

In their 2009 paper, Sanderson *et al.* found that WT control mice failed to show convincing novelty discrimination when tested at intervals spaced 24 hours apart, however, in a

follow-up study, WT animals were given more extensive training to the sample phase (10 sample trials as opposed to just 5 sample trials), and novelty discrimination was robust and significant (Sanderson and Bannerman 2011). To ensure robust habituation in controls, animals received extensive exposure to the two sample arms prior to testing; trained over 10 days, with sessions 24 hours apart, to explore two arms of the Y-maze. On the 11th day of the task, animals then received either saline or MK-801 treatment before exploring all arms of the maze.

Methods

Subjects

24 male C57Bl/6j mice were used for this experiment (n = 12 per treatment).

Apparatus

Apparatus was the same as that first described in Experiment 2.3.

Procedure

The procedure was similar to that described in Experiment 2.3, except for a few important differences. Instead of receiving 1 acquisition phase exposure to the start and to-be-familiar arms, immediately before the choice phase, mice received 10 exposure sessions, each 24 hours apart (Figure 3.7A). The test phase, where all three arms were freely accessible, was conducted 24 hours after the final exposure phase.

Acquisition sessions were 2 minutes long, instead of 5 minutes in Experiment 2.3. The test session was still 5 minutes long. Animals were trained to the two arms during exposure

sessions in the absence of MK-801. On the test day, mice were treated with either saline or MK-801, 30 minutes prior to testing, as described previously.

Analysis

Animals were grouped in pairs, and at the start of training one animal in each pair was assigned to the to-be-saline or to-be-MK-801 group. After the last day of exposure, general exploratory activity, and time spent across the start and to-be-familiar arms were compared between groups, to ensure no differences emerged (exposure session 10 activity: to-be-saline: 1.322m ± 0.170; to-be-MK-801: 1.221m ± 0.256; $t_{(22)} = 0.309$, $p = 0.760$).

Novelty recognition was assessed as described in Experiment 2.3, reported as time spent in each arm and as a discrimination ratio of time spent in novel vs. familiar arms. Distance moved was also measured during the test phase as reported previously. We also measured distance travelled across each arm of the maze individually, reported here as distance moved over 30s bins of the test session.

Results

Following multiple exposure sessions to the start and to-be-familiar arms before the choice phase, MK-801 had no effect on novelty preference behaviour when all three arms were accessible (Figure 3.7B). No differences were observed in novelty discrimination, despite MK-801 inducing hyperactivity in the choice phase (Figure 3.7C).

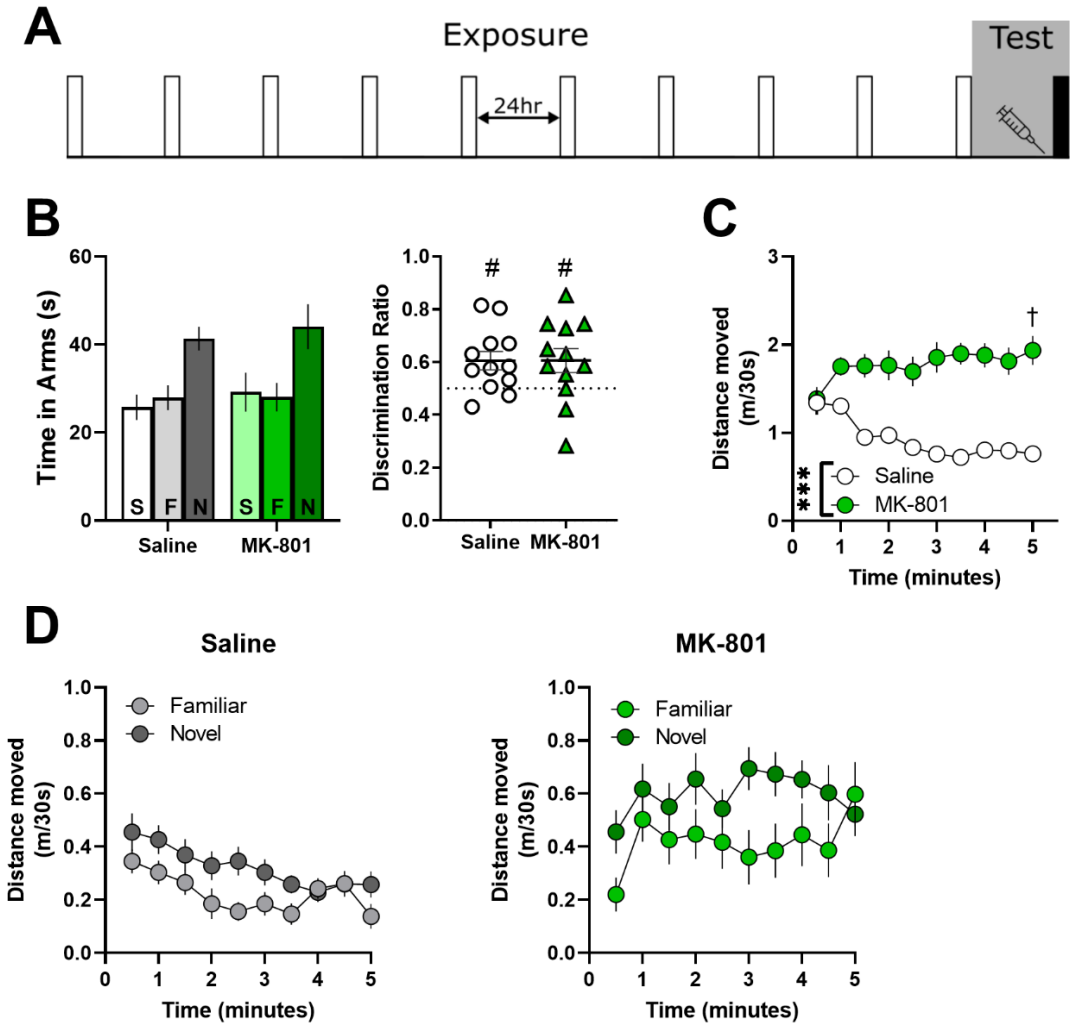

Figure 3.7 No impairment in a long-term habituation- dependent spatial novelty preference task in MK-801-treated mice. Mice were trained as normal animals over 10 exposure sessions, 24 hours apart, to explore two arms of a three-arm Y-maze (A). On the 11th session, mice were treated with either saline or MK-801 30 minutes before testing, and then allowed to explore all three arms of the Y-maze. Both saline- and MK-801-treated mice spent more time in the novel arm than start or familiar arms, and showed significant discrimination above chance between novel and familiar arms (B). MK-801 induced significant locomotor hyperactivity which increased across the 5-minute test session (C). While WT mice reduced exploration throughout the test session across all three arms, some increase in activity was seen across all arms in MK-801-treated mice (D). All data are presented as mean ± SEM. *** $p < 0.001$ vs. saline; # $p < 0.05$ vs. chance. † $p < 0.025$ vs. saline in 30s bins during first 5 minutes (Sidak-corrected p-value).

Spatial Novelty Preference

Both treatment groups spent more time in the novel arm, than either of the arms visited during exposure phases (Figure 3.7B). Discrimination ratios of time spent in novel vs. familiar arms were significantly above chance for both saline and MK-801 groups (saline

vs. chance: $t_{(11)} = 3.025$, $p = 0.012$; MK-801 vs. chance: $t_{(11)} = 2.330$, $p = 0.040$). Additionally confirming the lack of MK-801 effect on novelty discrimination, there was no difference between groups ($t_{(22)} = 0.012$, $p = 0.480$).

Locomotor Activity

Despite novelty preference remaining intact in this long-term variant of the Y-maze, MK-801 still induced significant hyperactivity on the test session (Figure 3.7C, treatment: $F_{(1,22)} = 45.005$, $p < 0.001$). This was further confirmed by a significant interaction of treatment*time ($F_{(9,198)} = 8.680$, $p < 0.001$). As with the short-term Y-maze experiments described previously, we then investigated the profile of hyperactivity through *post-hoc* comparisons of activity in the first and last 30s of the test. In contrast to the previous experiment where MK-801 induced hypoactivity upon first contact with the novel environment, there was no difference in the first 30s here, but we did see hyperactivity in the last 30s of the test (first 30s: $t_{(22)} = 0.233$, $p = 0.819$; last 30s: $t_{(22)} = 6.217$, $p < 0.001$; $p < 0.025$ significance threshold for 2 comparisons).

Experiments 3.3 and 3.4 revealed an apparent disparity in MK-801-induced activity in novel vs. familiar environments; whereby subjects became hyperactive over time in response to novelty, but little, if any, changes in locomotor responses were seen in the homecage. This long-term variant of the Y-maze simultaneously presents animals with locations that are novel, and locations with extensive previous exposure. We wanted to see whether it was possible to detect this dissociation between normal responding to familiar locations, but hyperactivity to novelty, in this choice preference test following MK-801 treatment.

Similar to the effect observed looking at total distance travelled, we saw an interaction of treatment*time comparing activity in the novel and familiar arms (Figure 3.7D, $F_{(9,198)} =$

6.867, p < 0.001). However, there was no interaction of treatment*arm or treatment*arm*time (treatment*arm: $F_{(1,22)}$ = 0.847, p = 0.367; treatment*arm*time: $F_{(9,198)}$ = 1.202, p = 0.296). Although there was no overall interaction, we wanted to understand if there were differences in how animals were responding to the novel and familiar locations and so split the data by treatment group for further *post-hoc* analysis. This did confirm a difference in activity across arms in controls, but only a trend effect in MK-801-treated mice (saline: $F_{(1,11)}$ = 22.950, p < 0.001; MK-801: $F_{(1,11)}$ = 4.670, p = 0.054). This hints at the possibility that MK-801-treated mice could be hyperactive specifically in response to the novel arm, but the results are underpowered. Admittedly this may also reflect the increased time spent in the novel arm compared to the familiar (Figure 3.7B, left panel).

Discussion

Despite impaired expression of novelty preference in the short-term variant of the Y-maze, MK-801 had no effect on animals' ability to appropriately respond to the novel arm of the maze when previously exposed over several days to the sample arms. MK-801 had no effect on an animals' ability to detect and choose the novel arm as novel, and attend more to this than the pre-exposed spatial locations. This suggests that while NMDAR modulation is required for short-term habituation, this is not a vital prerequisite for discrimination of novel vs. familiar stimuli or the expression of learning.

Both saline controls and MK-801-treated mice expressed a clear preference for the novel arm during testing. This supports earlier reports that MK-801 is required for the encoding of new memories, but not for consolidation or retrieval (Nilsson *et al.,* 2007). However, in hippocampal knockout studies of NMDARs such as the *Grin1*$^{\Delta DGCA1}$ mouse model

(Bannerman *et al.,* 2012, 2014), knockouts are still able to acquire the spatial location of a rewarded platform in the water maze, suggesting encoding does occur. An alternative account may be that self-generated priming is impaired, leading to a failure to habituate in the short-term (e.g., Figure 3.6), but not over longer timeframes. An interesting follow-up to the current experiment would be to administer MK-801 during exposure phases. However, the complications of prolonged repeated dosing of MK-801 and its long-term effects on the brain, as well as the need to control for state dependency, make this difficult to test and interpret in practice.

Locomotor activity in MK-801-treated animals was far higher than levels seen in control animals, despite previously being shown to express relatively normal locomotor responses to familiar locations (Figure 3.5). This suggests that this hyperactivity may have been driven by the reaction to novelty during the test, though we can not rule out a general hyperarousal effect particularly in response to experimenter handling across the session, which was less of a factor in the homecage experiment.

Interestingly, the hypoactivity to novelty seen previously (Figure 3.6) was not observed here. Hypoactivity may have been rescued in this experiment, through prior exposure to the two familiar arms of the maze, but novel components still induce hyperactivity over time. Alternatively, the increase in locomotor activity seen later in the session here could be a hyperarousal effect, or due to some impaired expression of associative priming through NMDAR blockade. We would need to test animals in a completely pre-exposed environment, but with repeated trials and experimenter handling, similar to this experiment, to rule these alternative possibilities out.

3.7 Amphetamine induces stimulus-nonspecific hyperactivity in Novel Context Recognition

Introduction

While the previous experiments have shown intact short-term habituation following amphetamine treatment, we wanted to further test this by investigating exploratory behaviour in NCR. In part, this allows us to further examine the functionality of NCR as a means of testing for deficits in short-term habituation, but also in separating impaired habituation from alternative explanations for behavioural abnormalities such as locomotor hyperactivity. But in doing so this also provides another measure which we can use to compare and differentiate between the three models of interest described throughout this thesis.

Methods

Apparatus and procedure used, and measures of analysis were the same as previously described in Experiment 2.6.

Subjects

23 male C57Bl/6j were used in this experiment. 12 mice were used in the saline group (saline AAA/AAB n = 6), and 11 in the amphetamine group (amphetamine AAA n = 6, amphetamine AAB n = 5).

Results

As seen with WT mice in the previous chapter, saline controls declined in attending to the same contextual stimuli across trials (Figure 3.8, Saline AAA) but increased exploratory behaviour on the third trial when the contextual cues were swapped for novel ones (Saline

AAB). Amphetamine induced an increase, rather than a decline in responding, which was independent of context cues (Amph AAA and AAB).

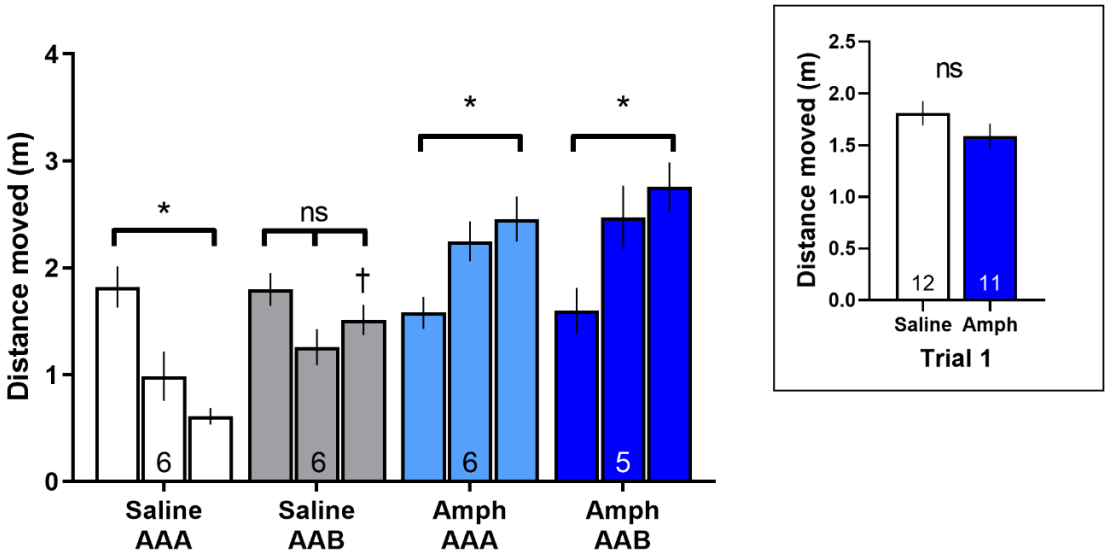

Figure 3.8 Amphetamine induced an increase in hyperlocomotion that was independent of contextual cues. Saline-treated mice displayed a decline in responding across repeated exposure to context A (AAA), but an increase in responding on the third trial when a novel context was introduced (AAB). Amphetamine-treated mice exhibited increasing hyperactivity across trials, but this was seen in both repeated exposures to the same context (AAA) and to novel contexts (AAB). Trial 1 data were no different between treatment conditions (inset). All data presented as mean ± SEM. Numbers within bars indicate n. * $p < 0.0170$ trial comparison (within subjects, adjusted for three tests), † $p < 0.05$ vs. AAA condition within-treatment.

Diverging locomotor activity profiles in the two treatment groups were confirmed by a significant main effect of treatment ($F_{(1,19)} = 30.592$, $p < 0.001$). While saline controls showed the same profile of habituation and/or dishabituation reported in the previous chapter, amphetamine induced increases in both conditions, that peaked on trial 3 independent of the novelty or relative familiarity of the animals' environment. This increase in amphetamine, compared to a decrease across repeated trials with saline, was confirmed by a significant treatment*trial interaction ($F_{(2,38)} = 47.979$, $p < 0.001$). However, presumably as a consequence of the context-independent increase seen with amphetamine, there was no interaction of treatment*condition*trial ($F_{(2,38)} = 1.626$, $p = 0.210$).

Post-hoc analysis focused on each treatment group separately to further investigate these divergent activity profiles. This confirmed the presence of a condition*trial interaction in controls, but a clear null effect in the amphetamine-treated group (saline: $F_{(2,20)} = 16.116$, $p < 0.001$; amphetamine: $F_{(2,18)} = 0.70$, $p = 0.516$). Comparisons of trial 3 activity in saline controls (A vs. B) confirmed a significant difference between contexts in these animals ($t_{(10)} = 31.206$, $p < 0.001$). The lack of condition effect with amphetamine was further confirmed by a null effect when investigating trial 3 activity separately ($t_{(9)} = 0.958$, $p = 0.363$).

Finally, as with the GluA1$^{-/-}$ cohort in Experiment 2.6, we looked at activity upon initial exposure to a novel environment, on trial 1 (Figure 3.8 inset). No difference between treatment groups was seen during the first 30s of novel context exposure ($t_{(21)} = 1.320$, $p = 0.201$).

Discussion

While in the Y-maze amphetamine had no effect on the expression of habituation, amphetamine-treated mice displayed deficits in NCR, increasing responding across repeated exposures to the same context. Control mice, meanwhile, demonstrated intact habituation and dishabituation upon reinstatement of novelty, replicating results reported in the previous chapter.

No difference between treatment groups was seen on trial 1 behaviour, but whereas controls declined in attending on trials 2 and 3 in response to the same context, the opposite effect was seen in the amphetamine group. One account for this is sensitisation, an increase in responding similar to that observed in the GluA1$^{-/-}$ mice. However, activity increased independent of stimulus, indicating that this hyperactivity was not related to the specific contextual cues. Furthermore, findings from the Y-maze in Experiment 3.3 show

that amphetamine can leave short-term habituation intact. This represents a potential limitation of the NCR assay; where using locomotor activity as the primary behavioural outcome can be confounded by other effects like general hyperarousal. This context-independent hyperactivity could be a result of a ceiling effect in terms of how much an animal can move in a 30s window. In the Y-maze, animals could express a novelty preference despite being hyperactive, but in the absence of a choice measure in this task, it may not be sensitive to intact novelty discrimination. This finding emphasises the importance of conducting a comprehensive battery of behavioural tests to fully understand how a given manipulation affects behaviour and eliminates alternative explanations for behavioural deficits.

3.8 Stimulus-specific deficits in habituation following MK-801 administration

Introduction

This final experiment looked to complete our behavioural profiling of habituation in manipulations of interest by investigating performance in the NCR task in animals treated with MK-801. While we have previously seen suggestions that stimulus-specific sensitisation may arise following MK-801 treatment in animals' orienting response to light stimuli, this finding was far from clear (Figure 3.1, from Boerner, 2018).

The aim of this experiment was therefore to determine whether MK-801 induces stimulus-specific deficits in short-term habituation, using the NCR assay developed in-house, which has previously been shown to parse out stimulus-specific abnormalities seen in GluA1$^{-/-}$ from general hyperarousal seen with amphetamine. If the GluA1$^{-/-}$ stimulus-specific

sensitisation is a result of impaired LTP, we may expect to see a similar outcome with MK-801.

Methods

Apparatus and procedure used, and measures of analysis were the same as previously described in Experiment 2.6.

Subjects

63 male C57Bl/6j mice were used for this study. 32 mice were procured from Envigo (UK), whilst the rest were generated in the Biomedical Sciences Building. Treatment groups were comprised of equal numbers of animals from each facility. In total 32 mice were assigned to the saline group (AAA/AAB n = 16), and 31 to the MK-801 group (AAA n = 16, AAB n = 15).

Results

Further replicating our findings in controls from Experiments 2.6 and 3.7, saline controls declined in responding across repeated exposures to the same context but increased on trial 3 in the different condition (Figure 3.9 saline AAA and AAB). In contrast, MK-801 induced increased responding across repeated trials with the same contextual cues (MK-801 AAA) which declined again back to levels seen on trial 1 when cues were switched to novel cues on trial 3 (AAB).

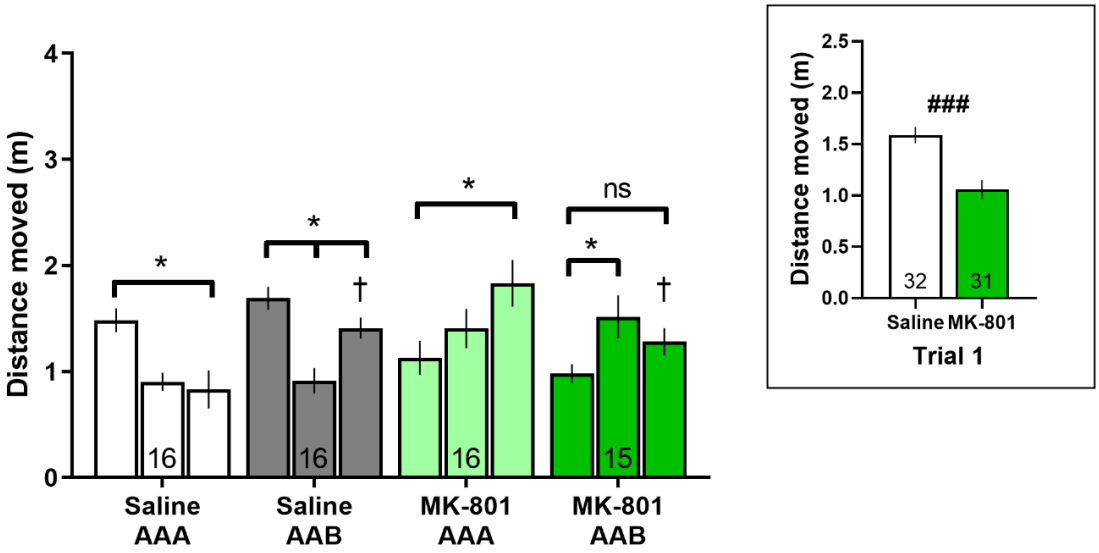

Figure 3.9 Stimulus-specific sensitisation to contextual stimuli in MK-801-treated mice. Activity in controls was comparable to previous experiments, declining to the same context (AAA) and rebounding on trial 3 to a new context (AAB). MK-801 induced increasing hyperlocomotion across trials in the same context (AAA) and in the AAB condition in the first two trials; but activity was diminished on trial 3 in this condition. On the first trial MK-801 induced significant hypoactivity (inset). All data presented as mean ± SEM. Numbers within bars indicate n. * $p < 0.0170$ trial comparison (within subjects, adjusted for three tests), ### $p < 0.001$ vs. control, † $p < 0.05$ vs. AAA condition within-treatment.

These effects of MK-801 on responding to novel or recently visited environments were confirmed by an overall interaction of treatment*condition*trial ($F_{(2,118)} = 8.684$, $p < 0.001$). To investigate these findings further the data was separated by treatment group, and a condition*trial interaction was confirmed in each group (saline: $F_{(2,60)} = 3.856$, $p = 0.027$; MK-801: $F_{(2,58)} = 4.846$, $p = 0.011$). *Post-hoc* comparisons revealed a significant effect of condition in controls on trial 3, due to increased novelty responding in the AAB condition ($t_{(30)} = 2.801$; $p = 0.009$). Activity in MK-801-treated mice was lower in a novel environment than a more familiar one. This was confirmed through a significant effect of condition on trial 3, due to a decline in trial 3 responding in the AAB condition ($t_{(29)} = 2.124$, $p = 0.042$).

Analysis of changing activity across trials, within each condition, confirmed declining responding across trials in saline AAA animals but an increase in trial 3 responding in the saline AAB condition (AAA trial 1-3: $t_{(14)} = 3.563$, $p = 0.003$; AAB trial 1-2: $t_{(14)} = 4.179$, $p <$

0.001; AAB trial 2-3: $t_{(14)}$ = 4.099, p < 0.001; p < 0.017 significance threshold for 3 comparisons).

In the MK-801 treatment groups, a significant increase in responding was seen in the AAA condition (AAA trial 1-3: $t_{(14)}$ = 4589, p < 0.001). In the AAB condition, a significant increase was observed across trials 1-2, but no difference between trials 1 and 3, or 2 and 3 (AAB trial 1-2: $t_{(13)}$ = 2.811, p = 0.014; trial 1-3: $t_{(13)}$ = 2.298, p = 0.038; trial 2-3: $t_{(13)}$ = 1.280, p = 0.221; p < 0.017 significance threshold for 3 comparisons). These findings confirm increasing hyperactivity in MK-801-treated animals, which is reduced on trial 3, relative to the AAA condition. However, there was no difference between trial 2 and 3 in the AAB condition, suggesting there may be some generalisation of the hyperactivity response across contexts.

Finally, as with previous NCR experiments, we investigated trial 1 activity, pooling conditions within each treatment group. MK-801 induced significant hypoactivity on trial 1 (Figure 3.9 inset, $t_{(59)}$ = 4.361, p < 0.001). This supports our earlier findings from novel open-field and short-term spatial novelty Y-maze testing that MK-801 initially induces hypo- rather than hyperactivity in a novel environment.

Discussion

MK-801 treatment induced stimulus-specific increases in locomotor responding to repeat exposures of the same context, indicating sensitisation is occurring over time. When the context was switched for novelty on the third trial, the increased responding over time was attenuated, decreasing slightly compared to trial 2. Furthermore, this activity in context B was lower than their counterparts tested in context A on trial 3. These context-dependent

findings on locomotor responding demonstrate the existence of a memory trace of previously visited locations in MK-801 treated mice that is inappropriately acted upon.

This finding is similar to that seen in the previous chapter with GluA1$^{-/-}$ mice. These findings taken together, seemingly confirm the importance of intact LTP for behavioural short-term habituation. The effects seen here are also similar to those reported in hippocampal lesioned rats by Honey *et al.* (2007) and point to a potential role specifically of hippocampal synaptic plasticity in the accurate representation of a location and subsequent habituation to environments recognised as familiar through self-generated priming. Furthermore, this finding supports similar results generated in-house where MK-801 induced stimulus-specific sensitisation to repeat presentations of the same light stimulus in operant chambers.

Discussion

Hyperarousal following amphetamine treatment but intact habituation

Across our studies of amphetamine, we saw excessive hyperactivity in all environments, both novel and highly familiar, that remained elevated for prolonged periods of time. Even in the homecage, amphetamine induced hyperactivity for over 2 hours post-dosing, an effect not seen with other manipulations. Despite this, amphetamine-treated animals expressed normal short-term memory in response to the sample arms of the Y-maze. This finding is supported by a recent study also showing unimpaired Y-maze novelty discrimination in mice (Wolff *et al.,* 2018), but also human studies of novelty recognition following amphetamine treatment (Zeeuws *et al.,* 2010). Based on the findings of this

chapter, it appears that short-term memory processes supporting short-term habituation are unaffected.

Despite this normal expression of short-term memory in the Y-maze, amphetamine-treated mice were apparently impaired on the NCR task. This highlights the importance of considering the limitations of individual behavioural assays prior to testing. Deficits seen in the NCR task may have been considered indicative of impaired habituation and a subsequent failure to discriminate between similar and different contexts. However, given the unimpaired Y-maze performance, it is more likely that this is instead revealing a number of limitations of the NCR assay. The primary readout – locomotor activity – could be confounded by a failure to inhibit locomotor responding and animals reaching a potential ceiling in terms of maximum activity within a 30s window. Additionally, the lack of an explicit choice measure in this task meant there was no capacity to express a preference for novelty or familiarity behaviourally.

Amphetamine appears to induce general hyperarousal in the absence of changes to short-term memory for an environment. In the NCR task this was expressed as a context-independent increase in responding that could be considered a failure to discriminate novelty, if interpreted as a standalone result. This emphasises the importance both of considering the most relevant behavioural tasks to measure a behaviour of interest, but also the need to conduct extensive behavioural phenotyping to fully understand the effects of a manipulation.

MK-801 impaired short-term habituation but long-term habituation was preserved

MK-801 induced impairments in short-term habituation, seen here as increased activity in open-field, and impaired novelty discrimination in both the Y-maze and NCR. Locomotor hyperactivity (Gilmour *et al.,* 2009; Liljequist *et al.,* 1991) and impaired novel object recognition (King *et al.,* 2004; De Lima *et al.,* 2005; Torrisi *et al.,* 2017) have been reported with MK-801 previously, but the experiments in this chapter have expanded on these studies with a few interesting findings.

Firstly, whilst habituation in the short-term was impaired following MK-801 treatment, the expression of long-term habituation was seemingly intact; little, if any hyperactivity was observed in the homecage, and novelty discrimination in the long-term Y-maze was normal, although there was some hyperactivity, even in this familiar environment. This would suggest that short-term memory deficits in the Y-maze are not a consequence of a general spatial or perceptual impairment, or an inability to express a choice preference per se. This intact expression of learning indicates that whilst self-generated priming may be impaired in these animals, disrupting short-term habituation, retrieval-generated priming appears to be (at least largely) preserved. This would suggest that the phenotype produced by MK-801 administration is similar to that seen in GluA1$^{-/-}$ mice, and that disruption of glutamatergic receptor-dependent synaptic plasticity generally may generate a similar behavioural outcome, although the differing distributions of Y-maze novelty preference indicate this may not be quite so straightforward.

Secondly, although MK-801-treated animals were hyperactive in novel environments, upon first exposure they actually exhibited hypoactivity, with activity increasing over the first 5-

10 minutes of exposure. Again, this was similar to the phenotype observed in GluA1$^{-/-}$ mice, though more robustly seen here across all assays of short-term habituation. If deficits are a result of similar impairments in self-generated priming in both manipulations, initial hypoactivity in MK-801 may also be due to a saturated A1 – given a potential blockade of decay from A1 to A2 it may be harder to displace elements in A1 and be slower for elements to accumulate in attention. The gradual onset of hyperactivity would then be related to the displacement of elements representing other previous events from A1. Interestingly, activity following MK-801 eventually plateaued at a level similar to that observed in controls in response to novelty, whereas in GluA1$^{-/-}$ animals the peak of activity was far in excess of the normal novelty response of WT mice. A parsimonious explanation of this result is that it reflects ataxia induced by MK-801 (Tricklebank *et al.,* 1989), or differences between acute blockade through pharmacology versus a constitutive genetic knockout. Whilst it could relate to differences in how these manipulations attend to stimuli, we have no definitive evidence to argue that is the case here.

Overall, the behavioural profile observed in NCR was very similar between MK-801 and GluA1$^{-/-}$ mice. Activity increased over repeated exposure to context A but was attenuated by the introduction of novelty in trial 3 of the AAB condition. This is the opposite of what was seen in controls across these studies, indicating instead that these manipulations can both generate stimulus-specific sensitisation to novel stimuli. Importantly, this argues against a purely amnestic effect of MK-801; there is a memory trace of a stimulus that is inappropriately acted upon. Early accounts of the role of NMDARs suggested a vital role in memory encoding, emphasising impaired spatial reference memory as evidence for this (Morris *et al.,* 1986). However, later studies from the Bannerman group have challenged this account, in studies of pharmacological NMDAR blockade (Bannerman *et al.,* 1995), but

also genetic ablation of hippocampal NMDARs (Bannerman *et al.,* 2012, 2014; Taylor *et al.,* 2014).

The stimulus-specific sensitisation seen here in tasks of short-term habituation suggests a role for impaired self-generated priming with MK-801, similar to that posited for GluA1$^{-/-}$. A key component of the SOP framework is the attention paid to a stimulus, and how this generates a behavioural response. This is also a critical feature of the aberrant salience framework; it is important to understand the relationship between attention and behavioural habituation if we are to draw conclusions on the construct validity of these manipulations. In the next chapter, we will go on to consider how impairments described so far in pharmacological and genetic models of psychosis could affect top-down attentional processing.

Novel Context Recognition as a sensitive measure of short-term habituation

Throughout this and the previous chapter, we have repeatedly shown intact habituation and dishabituation to subsequent novelty, in control animals, using the NCR assay. Furthermore, this task was able to detect impaired short-term habituation in GluA1$^{-/-}$ and MK-801 cohorts that led to stimulus-specific sensitisation. While amphetamine-treated mice appeared to show impairments in this assay, it was clear that the outcome in these animals was distinctly different to other manipulations that expressed stimulus-specific sensitisation. Amphetamine instead induced elevated locomotor responding independent of context that was indicative of a general hyperarousal effect; this was confirmed through testing of habituation in the Y-maze alongside this result.

This highlights the utility of this assay developed in-house to detect impairments in habituation, and specifically to test for sensitisation in models of interest, but also to parse out separate accounts for observed deficits.

Chapter 4: Attentional processing in preclinical manipulations relevant to psychosis

Introduction

So far in this thesis we have seen short-term habituation impairments in GluA1$^{-/-}$ and MK-801 cohorts that indicate that attentional processing is likely significantly impacted by these manipulations. Both of these manipulations induced deficits in short-term habituation leading to stimulus-specific sensitisation, although we did also see some interesting differences between groups – with increased maximal levels of locomotor responding in GluA1$^{-/-}$ mice, and a striking divergent profile of novelty preference in the Y-maze with MK-801.

Our studies of amphetamine have shown that short-term memory remains intact, although we observed a phenotype of general hyperarousal that may reflect increased salience of environmental stimuli similar to that reported with hyperdopaminergia in humans. This increased perceived salience of stimuli could also potentially affect how these animals attend to their environment.

The 5-Choice Serial Reaction Time Task as a measure of attentional performance

The 5-CSRTT was initially developed to provide a test of attentional performance, analogous to similar models that had been used in humans since the 1950's (Rosvold *et al.,* 1956). In rodents, and in particular in rats, this has widely been used to assess the effects of brain lesions or pharmacological manipulation for the last 40 years (reviewed in Robbins,

2002). In this task, animals are required to pay attention to a wall with five ports, with one of these ports briefly lighting up on each trial. Animals must nose-poke at the correct port to receive reward, providing a measure of sustained attention, defined by Robbins as "a continuous allocation of processing resources". Over extensive training, rodents are able to respond to a stimulus that appears for as little as 0.5s (though there is typically a period after stimulus offset where a correct choice can still be registered – the "limited hold" or LH). Choice accuracy and omissions (numbers of missed trials) are commonly reported as readouts of sustained attention.

One of the advantages of this task is that it provides information not just on sustained attention, but also on many related components of attentional performance. Impulsivity, indicating a lack of inhibitory control, can be measured through the number of responses an animal makes before the trial starts – premature responses, that lead to a punishing timeout period where no rewards can be earned. Compulsive responding – continuing to respond after making a correct choice or making redundant responses during a punishment period – represents another measure of response inhibitory control that the 5-CSRTT is sensitive to detect. Finally, it can also be used to measure response latencies, or latencies to collect reward, which could be affected by changes in motivational state – either lack of engagement with the task itself, or satiety may differentially affect latencies. These are all important behaviours to ideally rule out when investigating any model of interest – pharmacological or genetic – and so the ability to test many different components of behaviour alongside sustained attention is an advantage of this task.

Additional to generating this rich behavioural dataset, another advantage of this assay is that testing conditions can be varied extensively to manipulate attentional performance.

Although some training protocols train animals to a variable ITI (vITI), in most reported procedures the ITI is fixed across training. This allows researchers to introduce unpredictability about the ITI length at test, which can exacerbate mild attentional deficits, increasing impulsivity and rates of omissions. Making the trial start unpredictable can also impact choice accuracy, making animals more likely to respond inaccurately when they see a light. This has been shown previously with a low dose of amphetamine, where choice accuracy was largely unaffected, but was impaired when the ITI differed to that used during training (Balachandran *et al.,* 2018).

Another way of modulating task conditions, of particular relevance to our understanding of the role of self-generated priming in attention, is to introduce distracting auditory stimuli during the ITI, to distract subjects from the target stimulus. This has previously been shown to impair accuracy and increase impulsivity in animals administered with cholinergic antagonists, or with selective lesions of the serotonergic system (Harrison, Everitt, and Robbins 1997; Jones and Higgins 1995). Given our previous findings pointing to increased attending to novel stimuli in GluA1$^{-/-}$ and MK-801-treated cohorts, it is possible that these mice would be particularly susceptible to distracting tones.

Attentional processing in models of psychosis

In this chapter we will investigate attentional processing with manipulations of interest, assessing performance under normal conditions in the 5-CSRTT but also in the presence of distracting stimuli, or with a variable and unpredictable ITI length. Historically, most research to date has been done in rats, and with varying doses or testing protocols, complicating our interpretation of reported deficits.

There is no published literature reporting 5-CSRTT performance in GluA1$^{-/-}$ mice. However, there is some unpublished work investigating this model from a former postdoctoral researcher in the Bannerman group. There is some evidence that attentional performance in the 5-CSRTT is actually enhanced in this model, supporting our predictions of enhanced attending to stimuli in GluA1$^{-/-}$ mice, possibly due to impaired self-generated priming and increased likelihood of stimulus elements being in A1 (Figure 4.1). At a 1s stimulus duration (SD) there was a significant effect of genotype on choice accuracy, with GluA1$^{-/-}$ mice making more correct choices compared to WT counterparts (Figure 4.1A, $F_{(1,27)} = 6.251$, $p = 0.019$). There was also an effect of session, indicating that performance improved in both groups over time, though a lack of genotype*session interaction indicates that the genotype difference was stable across training (session: $F_{(6,162)} = 16.481$, $p < 0.001$; genotype*session: $F_{(6,162)} = 0.474$, $p = 0.827$). At a shorter SD of 0.5s this genotype effect on accuracy was lost, indicating no difference in attentional processing of very short stimuli (Figure 4.1B, $F_{(1,27)} = 1.028$, $p = 0.320$).

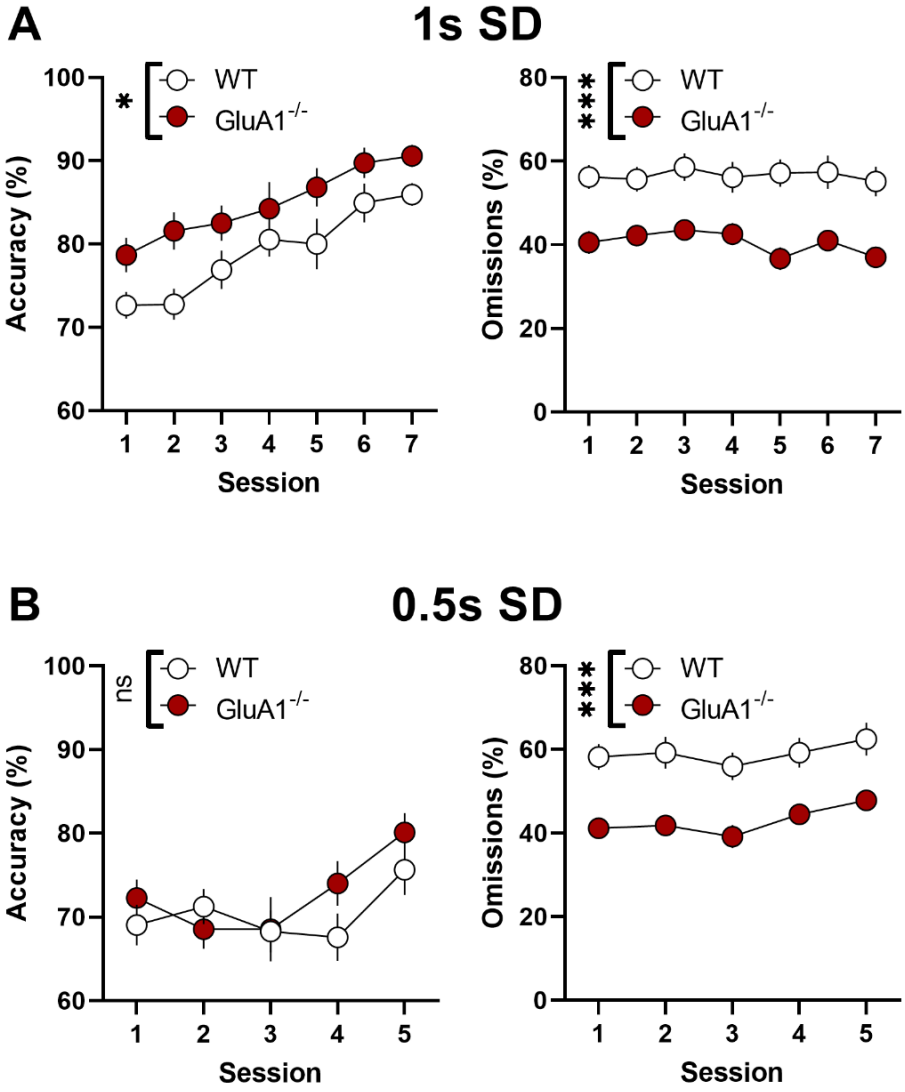

Figure 4.1 GluA1$^{-/-}$ mice display increased accuracy and fewer omissions compared to WT on the 5-CSRTT. At a 1s SD, GluA1$^{-/-}$ mice displayed enhanced choice accuracy, making more correct choices compared to WT, and had fewer omitted responses (A). At a shorter SD of 0.5s there was no difference between genotypes on choice accuracy (B), as GluA1$^{-/-}$ mice displayed lower accuracy compared to the previous stage. GluA1$^{-/-}$ mice still had fewer omissions than WT mice at 0.5s SD. All data are presented as mean ± SEM. * $p < 0.05$, *** $p < 0.001$ vs. WT.

Genotype differences in numbers of omitted responses were also observed at both 1s and 0.5s SD. GluA1$^{-/-}$ mice made significantly fewer omissions than WTs (1s SD: $F_{(1,27)} = 30.911$, $p < 0.001$; 0.5s SD: $F_{(1,27)} = 27.764$, $p < 0.001$). No interaction of genotype*session was observed at either SD (both $p > 0.25$). This finding appears to fit with predictions we have made regarding GluA1$^{-/-}$ mice and their impairments in behavioural habituation; aberrant salience to stimuli may actually benefit them in a sustained attentional task. However,

omissions were generally much higher than that commonly seen in rats or mice. We will consider this in contrast with future cohorts tested in this chapter, but other researchers typically see closer to 20% (private communications with Chris Barkus relating to Barkus *et al.*, 2016), compared to the 60% reported here in WT.

Interestingly, this result of enhanced performance in GluA1$^{-/-}$ mice is at odds with studies investigating the effect of NMDAR antagonists like MK-801. This is somewhat surprising, given the similarities observed between these models so far. Whilst most reported studies investigating the relationship between MK-801 and other NMDAR antagonists and attention have been conducted in rats, they have been shown to generate impairments on choice accuracy with 0.1mg/kg MK-801, alongside increased omissions and higher premature responding (Smith *et al.,* 2011), the opposite of what was observed in GluA1$^{-/-}$ mice. It should be noted that these effects did coincide with deficits suggesting potential sensorimotor impairments; namely longer latencies both to make a correct response and then to collect reward following a correct choice. Similar findings of impaired choice accuracy, with higher omissions and premature responding were reported in a recent dose-response investigation of MK-801 treatment in rats (Barlow, Dalley, and Pekcec 2018). Interestingly, a separate study found no impairments with MK-801 on either accuracy or omissions, in both the standard 5-CSRTT paradigm and a vITI session, at the same dose (0.1mg/kg) that others have seen impairments (Benn and Robinson 2014). However, on the whole, reports in the literature are relatively consistent in suggesting that MK-801 does impair sustained attention, but sensorimotor effects and a lack of inhibitory control may also contribute to deficits.

Similarly, there is some disagreement in the literature around the effects of amphetamine on attentional processing. As described earlier, some have found a mild impairment that only appears when conditions are unpredictable, such as when the ITI was unpredictable (Balachandran *et al.,* 2018). Others have reported a 10% reduction in choice accuracy in rats, even at a relatively low dose of amphetamine (0.6mg/kg) coinciding with robust impulsivity (Fletcher *et al.,* 2011). Most reports appear to indicate that amphetamine increases impulsivity, but has little other impact on performance (Cole and Robbins 1987; Harrison *et al.,* 1997), though there are reports of reduced accuracy in some studies without manipulating ITI length (Baarendse and Vanderschuren 2012; Fletcher *et al.,* 2011). In a study of mice, amphetamine was shown to dose-dependently reduce accuracy, and increase premature responding (Papaleo *et al.,* 2012), although the highest dose used here was only 0.75mg/kg, compared to 2.5mg/kg used throughout this thesis so far. Surprisingly, this group also saw a dose-dependent increase in omissions, something not seen with amphetamine in studies using rats. Some of these inconsistencies between studies likely reflect differences in doses used, as well as different metabolism between model organisms, but could also be impacted by specific parameters of the test that may not be immediately obvious. It is important to investigate manipulations that we are interested in here (i.e., amphetamine, MK-801 and GluA1$^{-/-}$) in equivalent conditions to be able to compare across models.

Arousal reflects the general state of physiological reactivity of the subject, whereas attentional processes primarily determine what to attend to when there are multiple incoming sensory inputs (selective attention), or the capacity to attend to stimuli for prolonged periods of time (sustained attention) (Coull 1998; Robbins 1984, 1997). Dopamine has been implicated in modulating arousal (Robbins 1984) and indicates a

potential pathway through which amphetamine may increase arousal, but leave selective or sustained attention intact.

On the other hand, there was evidence of increased attending, specifically to novel stimuli in both GluA1$^{-/-}$ and MK-801-treated mice. This reveals a potential disconnect (at least in the case of MK-801), of increased attending to incoming sensory stimuli, but impairments in sustained attention in the 5-CSRTT. One possible explanation for this is that attending to incoming sensory inputs represents a bottom-up mechanism of attention, whereas the 5-CSRTT requires a top-down input to modulate attending in well-trained animals, respond at the correct choice port at the appropriate time in the trial, and collect rewards. Whilst Wagner's SOP can explain how bottom-up attention to incoming sensory inputs is altered by their relative novelty or familiarity, it may be less applicable to the case of top-down attention. This could be considered as the weighting that is given to elements that enter A1 or A2 states of attention, to determine the level of behavioural responding that any stimulus elicits. This process may be independent of changes in self/retrieval generated priming, and is more akin to changes in arousal as described previously (Robbins 1984). Alterations in this mechanism and subsequent impairments in glutamatergic models, may be a consequence of hyperdopaminergia associated with changes in glutamatergic function (Panayi *et al.,* 2021). Whilst changes in arousal would be expected to influence the magnitude of behavioural responding, this mechanism is largely independent of Wagner's SOP and highlights a potential limitation of the model in that changes in top-down attentional processing cannot be described purely in terms of SOP.

Top-down attention as required for intact 5-CSRTT performance has been linked with alterations in connectivity between the hippocampus and prefrontal cortex. Theta

coherence between the hippocampus and prefrontal cortex correlates strongly with attentional performance (Tan *et al.,* 2018). It may be that top-down and bottom-up attentional processing is differentially affected by pharmacological or genetic manipulations under investigation here, an important consideration of this next chapter.

Aims

So far, the focus of this thesis has been on phenotyping of mouse models relevant to psychosis on tasks of behavioural habituation. In this chapter, we have extended that behavioural profiling into attentional processing, using the 5-CSRTT to investigate sustained attention, alongside other measures of impulsivity, compulsivity, as well as measures like response latency that may reveal sensorimotor effects.

In this chapter we have aimed to generate a comprehensive test of 5-CSRTT performance across all three models of interest, testing animals with a vITI length, or in the presence of auditory distractors during the ITI. The first half of this chapter details efforts to build and develop a novel system for assessing 5-CSRTT performance in mice, using a pilot cohort of C57Bl/6j mice. After training to acquisition, these mice were then tested for performance coinciding with administration of MK-801 or amphetamine. In the second half of the chapter we have expanded on interesting findings on attentional processing in GluA1$^{-/-}$ mice (Schneider, unpublished; Figure 4.1), training a new cohort of WT and GluA1$^{-/-}$ animals to acquisition before testing them on different task parameters to examine attention in these animals in more detail.

Experiments

4.1 Training C57Bl/6j mice to acquisition with a short stimulus on a 5-choice serial reaction time task

Introduction

In order to generate data from new cohorts of mice that we could use to compare pharmacological manipulation with GluA1$^{-/-}$, we wanted to develop a new testing apparatus for this paradigm with a number of advantages over existing Med Associates chambers. The new apparatus, using pyControl hardware (https://pycontrol.readthedocs.io/) used water rewards, rather than the liquid milkshake used in the existing chambers. By using small volumes of water as a reward, we hoped to increase the ceiling on the number of trials animals could complete in a session, also allowing us to increase the total session length and hopefully expedite the lengthy training schedule which typically takes 30-40 sessions (Bari, Dalley, and Robbins 2008).

The new setup, built in-house as part of this thesis, was operated via python and has the advantage of being more readily modifiable than the existing Med Associates apparatus. It is more straightforward to modify programs, change testing parameters in individual chambers in each session (e.g., for animals that are progressing through training more quickly), and troubleshoot problems in a session. Additionally, this equipment is open-source and readily available at a fraction of the cost of other operant chambers.

The hardware also offers more flexibility. Given our predictions around enhanced attention in GluA1$^{-/-}$ mice, we wanted more freedom in how we increase task difficulty, which with existing setups can only be done by decreasing SD. The pyControl hardware consisted of a

nine poke board, with four pokes obscured. One potential advantage of this system was having the option to make the task harder later in training by increasing the number of pokes animals had to attend to. In the case of seeing potentially enhanced sustained attention, this would give us another way in which we could manipulate task complexity (although we did not have the time, nor convincing evidence that this was a beneficial manipulation to investigate in studies carried out in this chapter, it is an important consideration for future work).

Finally, this equipment is also more readily compatible with existing hardware in the Bannerman/Walton group for future physiological recordings, an important consideration for later work investigating the neural basis of any interesting behavioural abnormalities discovered. This setup will be straightforward to integrate with fibre photometry hardware for recording real-time changes in dopamine transmission in response to stimuli with the dopamine sensor dLight (Cui *et al.,* 2013; Patriarchi *et al.,* 2018), or with electrophysiology (Tan *et al.,* 2018). While the current work has not investigated these effects, it was important to consider future plans and confirm that this equipment was sensitive to measures of attentional performance in both controls and models of psychosis.

The aim of the current experiment, therefore, was to train a naïve cohort of C57Bl/6j mice in the new 5-CSRTT operant chambers to criterion, where these animals could go on to be manipulated pharmacologically. In doing so, we hoped to confirm the suitability of the pyControl system for measuring attentional performance in the 5-CSRTT. While similar chambers have been used for other behavioural testing in Oxford, this is the first time we have used the pyControl system to assess attention.

Methods

Subjects

Male C57Bl/6j mice were used for this experiment (n = 12). The same cohort that was trained to acquisition in this experiment were then used to look at pharmacological manipulations on 5-CSRTT performance in Experiments 4.3-4.5. Of the 12 animals, only 1 failed to reach the end of training and so was excluded from analysis and future pharmacological experiments, giving a total n of 11.

Animals were generated in-house in the Biomedical Sciences Building at the University of Oxford. At 12 weeks of age animals were water-restricted to no less than 85% of their *ad libitum* body weight and maintained on water restriction for the duration of this study. Access to food was always freely available, both in their homecage, and during training. Providing food during training helped to sustain motivation during the task, as animals would sporadically eat the dry food, increasing thirst and leading to more trials. Furthermore, it may be beneficial from a welfare perspective as animals may eat less in the homecage when there is no access to water.

Apparatus

Experiments were conducted in a clear Perspex arena (13 x 14.5 x 17cm) inside a sound-attenuating chamber made of melamine MDF (40 x 30 x 40cm). 6 chambers were used in total, with the same animal always tested in the same chamber. 5-CSRTT training and subsequent manipulations were all conducted using python and pyControl software and hardware (https://pycontrol.readthedocs.io/) as described below.

A laptop running the pyControl GUI was connected to 6 breakout boards via USB. Each breakout board controlled a separate chamber, and was connected to all of the hardware components required to operate the 5-CSRTT task via an RJ45 connector ethernet Cat5 cable.

The left wall of the chamber housed a nine-poke board, with a poke cover that obscured four pokes. This left five pokes visible, split across two horizontal levels (Figure 4.2, left panel). Each poke on the nine-poke board housed an LED, and an IR emitter and photocell receiver diode to detect nose-poke activity. Above the nine-poke board, a speaker was fitted, connected to a pyControl audio board.

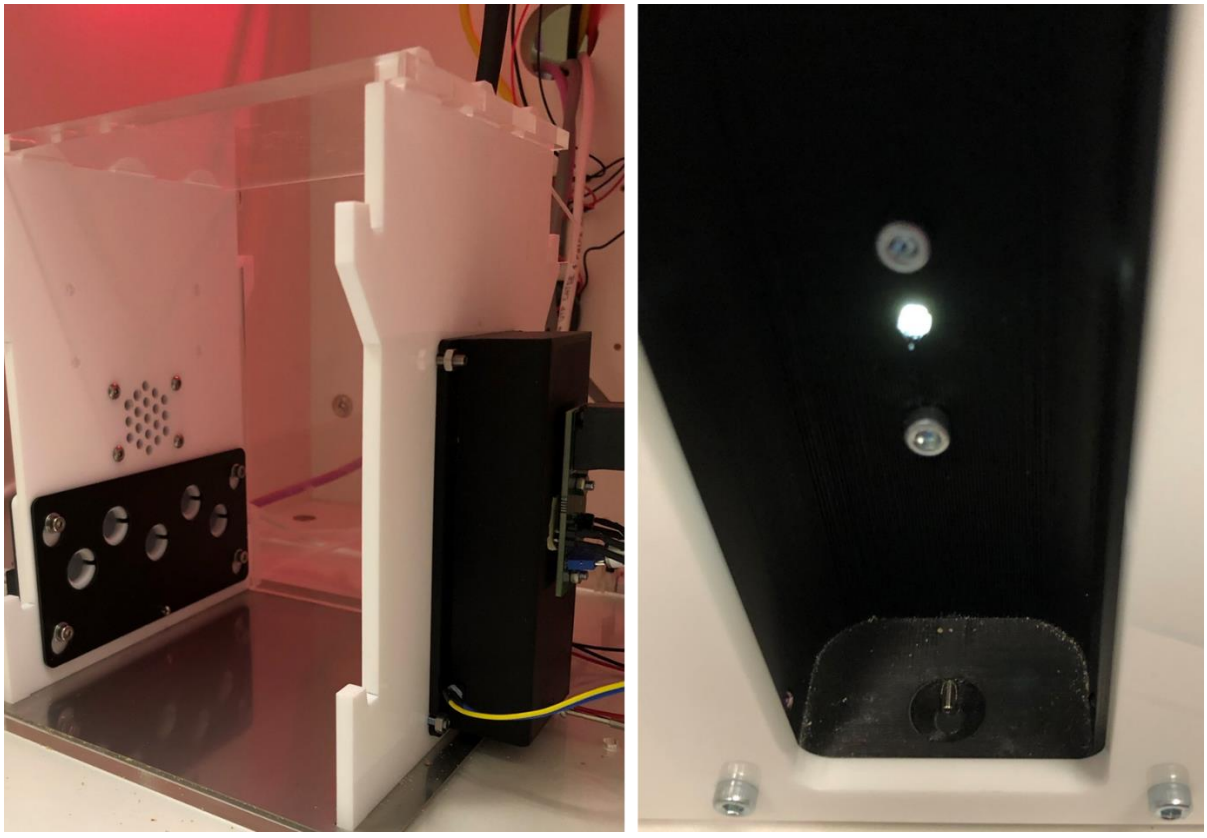

Figure 4.2 Design of pyControl 5-CSRTT testing boxes. Nine-poke board mounted to left wall of testing box with a cover obscuring the four pokes not used during training (left). Above the nine-poke board was a mounted speaker. On the opposite side of the box a magazine was mounted (right), with a recessed well for water delivery, LED to indicate trial initiation/reward availability, and IR emitter and receiver across the front of the magazine.

On the opposite, right-hand side of the chamber, a magazine was fitted where rewards were delivered and trials were initiated (Figure 4.2 right panel). An IR emitter and photocell receiver across the front of the magazine recorded animals head entries into and out of the magazine. At the base of the magazine was a small well where water rewards were delivered via a solenoid pump attached to a metal tube. At the top of the magazine, an LED was positioned to signal either reward availability or trial initiation.

At the back of the sound-attenuating chamber, a pyControl port adapter was connected to a fan, white LED "houselight", and red LED that illuminated the chamber in red light outside of the animals' visible spectrum. The fan and red LED were set to be always on, while the houselight was programmable. This allowed video recording of a session even during punishing timeouts where the houselight was switched off.

Procedure

Autoshaping

Animals were firstly trained to receive water rewards at the magazine. Magazine training sessions were 30 minutes long, with rewards available on a variable interval of approximately 30s (VI30). When the session started, the houselight came on and remained on for the duration of the session. Reward availability was signalled by illuminating the LED in the magazine.

The criteria to progress from this stage, was at least 40 complete trials in a session (collection of reward from the magazine) after a minimum of two sessions. For the first 6 animals in session 1, a computer error caused the session to crash after 13 minutes. All animals had completed at least 20 trials, and so were removed and tested again the next day. All of these animals then completed more than 40 trials in the second session.

Following this, animals were trained to nose-poke at the nine-poke wall in order to receive rewards at the magazine. These poke training sessions were also 30 minutes long but there was no longer an ITI; as soon as animals had collected reward the next trial began.

At the start of a trial, all five available nose-poke ports at the nine-poke wall were illuminated. A response at any port was registered as a correct choice, all five poke lights were turned off and the magazine light turned on to signal reward availability. After consuming reward and removing their head from the magazine, the next trial began immediately, and all five pokes were once again illuminated. Animals were moved on from this stage after ≥ 40 trials completed in a session for 2 consecutive sessions; all animals moved on in 3-5 sessions.

5-CSRTT Training

After being trained to poke at the choice wall and then collect reward, animals began 5-CSRTT training where they had to poke at a specific port to receive reward. Session length was increased to 60 minutes.

Animals were trained over several stages to make nose-poke responses at ports on the 5-choice wall. On a given trial, a light stimulus was presented at one port and only a poke at the illuminated poke was rewarded. After collecting reward, there was a short ITI of 2s (later extended to 5s) before the next trial began. At the start of training, the SD was 20s, plus a 2s LH period during which a response was still registered (Table 4.1) before being recorded as an omission.

If animals made an omission, an incorrect response, or a premature response they received a punishing timeout period of 5s. During a timeout the houselight turned off and animals had to wait until the end of the timeout period to start the next trial. At the start of training

the next trial began immediately following the timeout period, but later on, at stage 5.1, an extra step was introduced during which animals had to initiate the next trial themselves by poking at the magazine. During this trial initiation period, the houselight remained off until the animal nose-poked at the magazine, and the magazine light was then turned on to indicate when animals could initiate a trial. This helped to reduce omissions that were caused by the animal not engaging with the task, something we felt was necessary particularly due to the longer session length compared to other 5-CSRTT experiments.

SD gradually decreased from 20s during training until animals could reliably make a correct choice with a SD of 0.8s (Table 4.1). To progress through the training stages, animals had to reach criteria on correct choices, accuracy, and omissions for two consecutive days.

	Training parameters			**Progression criteria (2 consecutive days)**		
Stage	**SD (s)**	**LH (S)**	**ITI (s)**	**Correct trials (n)**	**Accuracy (%)**	**Omissions (%)**
1	20	2	2	≥ 45	≥ 30	-
2	8	2	2	≥ 60	≥ 50	-
3	8	2	5	≥ 60	≥ 80	≤ 50
4	4	2	5	≥ 60	≥ 80	≤ 50
5	2	2	5	≥ 60	≥ 80	≤ 50
5.1*	2	2	5	≥ 60	≥ 80	≤ 50
6	1	2	5	≥ 60	≥ 80	≤ 50
7	0.8	2	5	-		

Table 4.1 Training parameters and criteria to progress for 5-CSRTT training of a C57Bl/6j cohort. Stimulus duration (SD), limited hold (LH) and inter-trial interval (ITI) length across training, and criteria to progress to

the next stage. * An extra step to the task was added at stage 5.1, where animals had to manually initiate a trial after a punishment timeout by nose-poking at the magazine.

Analysis

To confirm learning across stages of training, we investigated averages across each stage on several measures of 5-CSRTT performance. We examined the average number of sessions taken to reach criterion across stages, and magazine behaviours; entries and time spent at the magazine. We looked at numbers of correct trials, and choice accuracy, across stages as a measure of focused attention on the task. We also looked at errors of omission, premature responding, perseverative responding, or responding during a penalty timeout. Choice accuracy was calculated as a percentage from the sum of correct and incorrect responses (excluding omissions). Total trials, and percent of trials omitted were calculated from the sum of all correct, incorrect, or omitted trials. Premature responses were calculated as a percentage of all trials, including trials that ended during the ITI due to a premature response.

We were looking for evidence of increased accuracy and decreasing errors across training. To assess this, we used one-way ANOVAs to investigate changes in average performance across training stages.

Finally, we looked at latency measures during training; latencies to make either a correct or incorrect choice, and to collect reward following a correct response, were extracted as the median for each animal per session. The mean of these values was then taken for each animal, at each training stage, and is reported here.

Results

5-CSRTT Performance

Animals took on average 11 sessions to reach criterion on the first stage of training, completing a minimum of 45 correct trials with 30% choice accuracy. After overcoming this first stage, the average number of sessions to reach criterion dropped dramatically until the final stage of training, which required a much faster response (Figure 4.3A, stage: $F_{(6,60)} = 35.841$, $p < 0.001$). Throughout training, a decrease in magazine entries, but increase in time spent in the magazine during a session, indicates that animals learnt the task structure, making fewer unnecessary head entries into the magazine but spending more time consuming reward (Figure 4.3B and C, entries: $F_{(7,70)} = 3.726$, $p = 0.002$; duration: $F_{(7,70)} = 5.998$, $p < 0.001$). An increase in magazine entries at stage 5.1 reflects the new step incorporated into the trial structure, of having to nose-poke to initiate a new trial after a punishment.

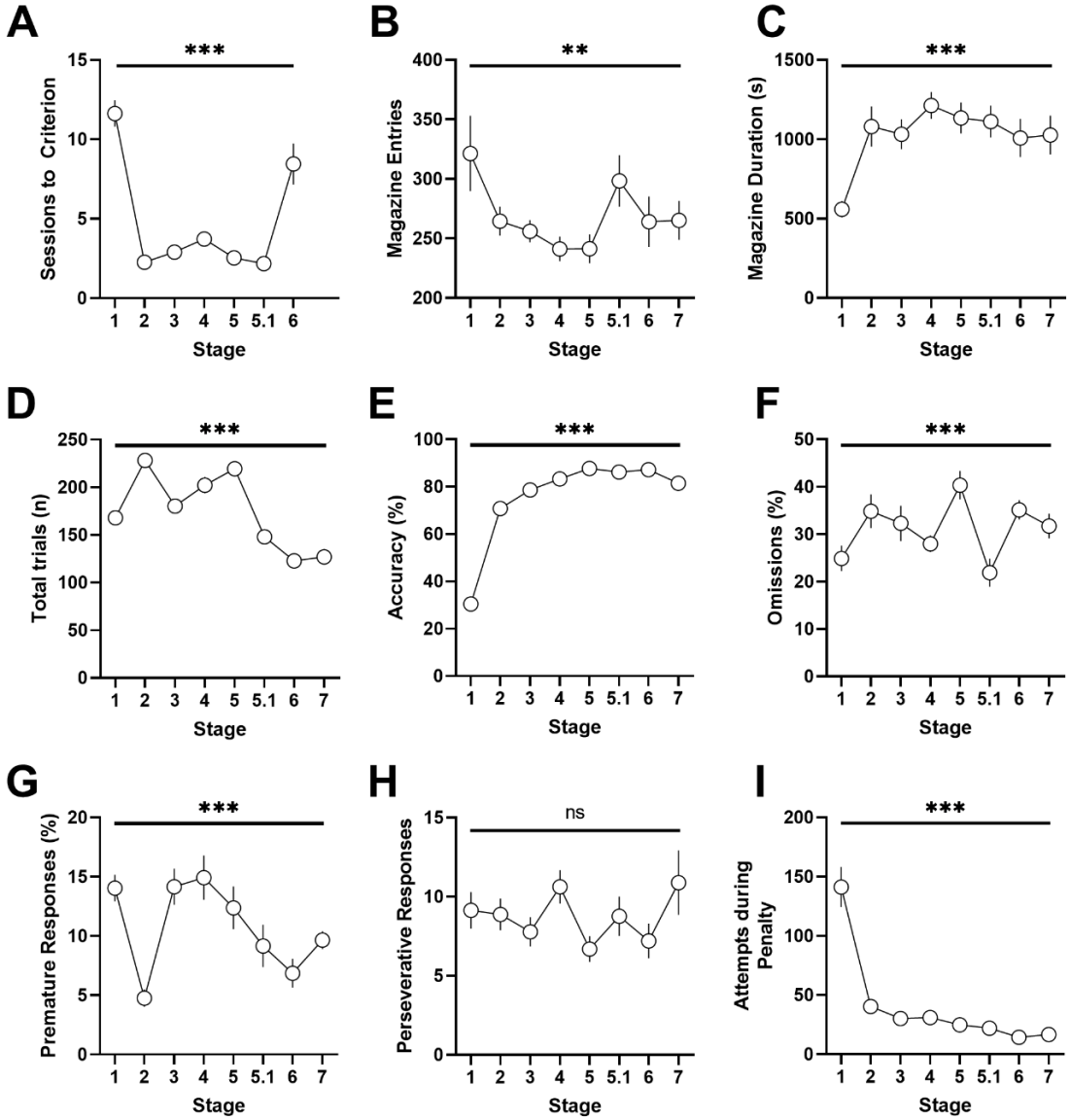

Figure 4.3 Improving performance across stages of training on a 5-CSRTT. After learning the initial stage of training, sessions to criterion on later stages decreased dramatically until later, more difficult stages of training (A). While magazine entries declined across training as animals learnt the task structure (B), duration in the magazine increased, likely reflecting increased time consuming rewards (C). C57Bl/6j mice displayed a decrease in total trials over time (D) but increasing choice accuracy indicating more efficient strategies (E). Omissions fluctuated over time, partially due to changes in the task structure at stage 5.1 but a slight increase was observed as SD decreased (F). Premature responding decreased over time as animals learnt the trial structure (G). No overall change was observed in perseverative responding after a correct trial (H), but animals did decrease perseverative behaviour after an incorrect response (I). All data are presented as mean ± SEM. ** $p < 0.01$, *** $p < 0.001$ measured as a one-way ANOVA across stages.

Animals completed fewer trials in a session towards the end of training, but expressed an increase in choice accuracy that indicates animals were successfully able to learn the task (Figure 4.3D and E, main effect of stage on total trials: $F_{(7,70)} = 20.257$, $p < 0.001$; accuracy:

$F_{(7,70)}$ = 179.065, p < 0.001). Trials completed increased across the early stages of training, but declined across stages 5.1-7, possibly reflecting the increased difficulty of these shorter SD trials and requirement to maintain a high level of cognitive effort across the 60-minute session. Furthermore, the decrease at stage 5.1 was likely an artefact of reduced omissions due to the different task design. Despite this decrease in trials completed at later stages, choice accuracy remained high, only decreasing very slightly at 0.8s SD.

A significant change in numbers of omissions was also seen across stages, though this is difficult to interpret as at stage 5.1 the way in which trials were initiated was changed (Figure 4.3F, effect of stage: $F_{(7,70)}$ = 4.932, p < 0.001). There was a suggestion that omissions increased as the task became more difficult. This is considered in more detail in a follow-up study with a consistent task structure across training (see Experiment 4.5).

Premature responding, a measure of impulsivity declined across training (Figure 4.3G, $F_{(7,70)}$ = 10.772, p < 0.001). An increase was observed at stage 3, due to the increase in ITI length from 2 to 5s after animals had started to acquire the task structure. Perseverative responses stayed relatively consistent, and were much lower than the number of attempts animals made during a penalty timeout period (Figure 4.3H and I). While no change was seen in perseverative responding over time, penalty attempts decreased dramatically after stage 1 (perseverative responses: $F_{(7,70)}$ = 1.801, p = 0.101; penalty attempts: $F_{(7,70)}$ = 44.781, p < 0.001).

Overall, these measures of attentional processing indicate that over 6-8 weeks of training, animals learnt to selectively respond to the illuminated nose-poke with 80% choice accuracy, and around 30% omissions. They reduced behaviours that either led to punishment, or wasted physical effort, over training. The rate of omissions observed here

was much lower than that reported in the previous study, and more consistent with that seen by other researchers in Oxford (Barkus *et al.,* 2016).

Latency

C57Bl/6j mice displayed a decline in latency across training, indicating faster responses over time as they learned the task structure and became more efficient at completing trials. Latency to make both correct and incorrect responses declined over training, as did latency to collect reward from the magazine opposite the 5-choice wall (Figure 4.4, main effect of stage on correct latency: $F_{(7,70)} = 97.427$, $p < 0.001$; incorrect latency: $F_{(7,70)} = 66.479$, $p < 0.001$; reward latency: $F_{(7,70)} = 31.581$, $p < 0.001$).

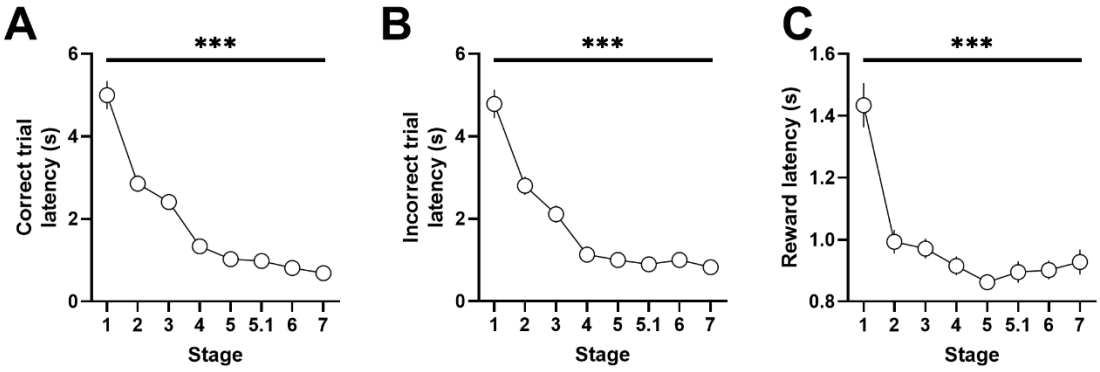

Figure 4.4 Changes in behaviour and response latencies over 5-CSRTT training. Response latencies decreased throughout training, reported as correct trial (A), incorrect trial (B), and reward latency (C). All data are presented as mean ± SEM. *** $p < 0.001$ measured as a one-way ANOVA across stages.

Performance by stimulus location

Because the task wall differs in setup from other 5-choice testing boxes (Figure 4.2, left panel), we wanted to investigate differences in sustained attention across ports at a late stage of training (stage 7). While overall accuracy was at around 80%, there was some variation in performance based on stimulus location (Figure 4.5A; $F_{(4,40)} = 5.051$, $p = 0.002$). This appeared to be driven by reduced accuracy at the ports on the higher plane; ports 2

and 4. It could be that these were harder to reach or attend to, or it could be that it was harder to visually discriminate between these and other ports as they were positioned between peripheral and central ports. Omissions also varied across pokes (Figure 4.5B; $F_{(4,40)} = 3.656$, $p = 0.012$). Interestingly, this did not follow the same pattern as choice accuracy, but instead appeared to indicate that animals were more likely to make an omission if the stimulus was at one of the two rightmost locations (pokes 4 and 5). This could be explained by animals hovering on the left side of the chamber, which was also closest to the food pellet magazine.

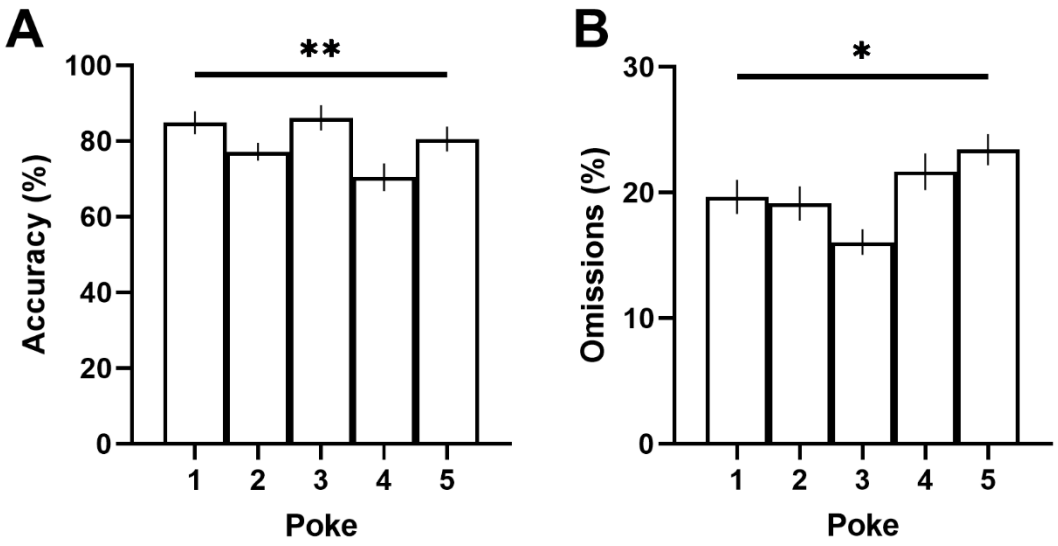

Figure 4.5 Reduced choice accuracy at pokes on the higher plane of 5-choice wall. C57Bl/6j mice expressed reduced choice accuracy at pokes 2 and 4, that were set slightly higher than other pokes on the 5-choice wall (A). Omissions also varied slightly by choice port, and appeared to be higher on one side of the chamber than the other (B). All data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ main effect of poke.

Discussion

Animals took on average 34 sessions to reach the end of training, in line with reports of 5-CSRTT training in rats using Med Associates operant chambers (Bari *et al.,* 2008). It was hoped that by using water rewards, which are slower to satiate, and increasing the session length to 60 minutes (while most training protocols suggest 30 minutes for food rewards), we may expedite the training process. This does not seem to have happened, maybe

highlighting a limitation of how long mice can work to sustain attention for reward. However, animals clearly displayed robust learning over time of the task structure and demonstrated high accuracy even at a relatively short SD of 0.8s.

The finding of reduced choice accuracy at ports 2 and 4 was somewhat unexpected. It may be that animals find it harder to detect and respond appropriately to these ports because they are positioned above the other three ports. Whatever the reasons for this difference in accuracy, it may reveal interesting insights into animals that have a mild attentional impairment; where a subtle discrepancy in performance may be exacerbated.

4.2 Effect of MK-801 and amphetamine on attentional processing in 5-CSRTT

Introduction

Having trained a cohort of C57Bl/6j mice to criterion on the 5-CSRTT paradigm using the pyControl system, we next wanted to assess the effect of pharmacological manipulation on task performance, when conditions were comparable to the last stage of training. Amphetamine has previously been shown to have only subtle effects on attentional performance in the basic version of the 5-CSRTT; most reports suggest it increases impulsivity without affecting accuracy (Cole and Robbins 1987; Harrison *et al.,* 1997), though it has been shown to reduce choice accuracy in some cases (Baarendse and Vanderschuren 2012; Fletcher *et al.,* 2011; Papaleo *et al.,* 2012). MK-801 and other NMDAR antagonists have been shown to affect sustained attention, decreasing accuracy and increasing omissions in rats (Barlow *et al.,* 2018; Smith *et al.,* 2011).

The aim of this experiment was to replicate these findings by testing the effects of these compounds in C57Bl/6j mice, in the pyControl 5-CSRTT testing chambers. By generating our own data on these manipulations we hoped to better understand some of the differences reported in the literature with amphetamine. In the case of MK-801, the aim was to confirm reports of impairments but also see in more detail how the drug affects all aspects of behaviour in the 5-CSRTT.

Methods

Subjects and apparatus used were the same as described in Experiment 4.1.

Drugs

Amphetamine (2.5mg/kg) administration followed the same protocol as described previously in Experiment 3.1. MK-801 (0.2mg/kg) was administered as described in Experiment 3.3. Saline was administered at a dose of 5mL/kg.

Procedure

After training to acquisition in the previous experiment, animals were then tested on 5-choice performance in the presence of either amphetamine, MK-801, or saline. Each animal received all three treatment conditions, over a 9-day period. On the first day, animals were trained on stage 7 as in Experiment 4.1 in a baseline session. The following day, animals received either one of the three treatments, pseudorandomly assigned such that a similar number of each treatment were in each group of six animals. 30 minutes after compound administration animals were then tested in the 5-CSRTT. Task parameters were the same as stage 7 of training, with a 0.8s SD and 5s ITI.

On day 3, a washout day, animals were not trained, instead receiving one hour of free access to water. This 3-day process was then repeated a further two times, with each animal receiving each treatment across the total 9 days, in a Latin-square design.

Analysis

We investigated measures of performance and latency, as described in Experiment 4.1, but in this experiment that analysis was restricted to the test sessions during which pharmacological compounds were administered, rather than examining many sessions of training. No lasting effects of compound administration were seen during baseline sessions. One-way ANOVAs of performance data were used to confirm effects of compound administration, with subsequent *post-hoc* comparisons used to investigate the nature of these effects following a significant finding. *Post-hoc* comparisons were Sidak-corrected for two comparisons; saline vs. amphetamine or MK-801 (significance threshold of $p < 0.025$ for two tests).

Results

5-CSRTT Performance

Following saline treatment, performance was largely similar to that seen at stage 7 of training in the previous experiment; if anything accuracy and trials completed were marginally higher, and errors of omission, premature responding, and perseverative responding decreased slightly (Figure 4.6). However, this was a snapshot from a single session, after extensive training at this stage, so comparisons to averages across earlier training are not necessarily useful. Overall, performance in saline animals appeared to

match what was seen during training, indicating there was no overt effect of injection stress on performance.

Pharmacological manipulation caused a decrease in the total number of trials completed (Figure 4.6A, $F_{(2,20)} = 3.643$, $p = 0.045$). Whilst amphetamine-treated mice appeared to complete fewer trials this was only approaching significance in *post-hoc* comparisons; whereas MK-801 did significantly reduce this measure (amphetamine vs. saline: $t_{(20)} = 2.023$, $p = 0.071$; MK-801 vs. saline: $t_{(20)} = 2.463$, $p = 0.031$).

Both compounds significantly impaired choice accuracy. Both amphetamine and MK-801 reduced accuracy compared to saline by over 20% (Figure 4.6B, $F_{(2,20)} = 37.458$, $p < 0.001$; amphetamine and MK-801 both $p < 0.001$ vs. saline).

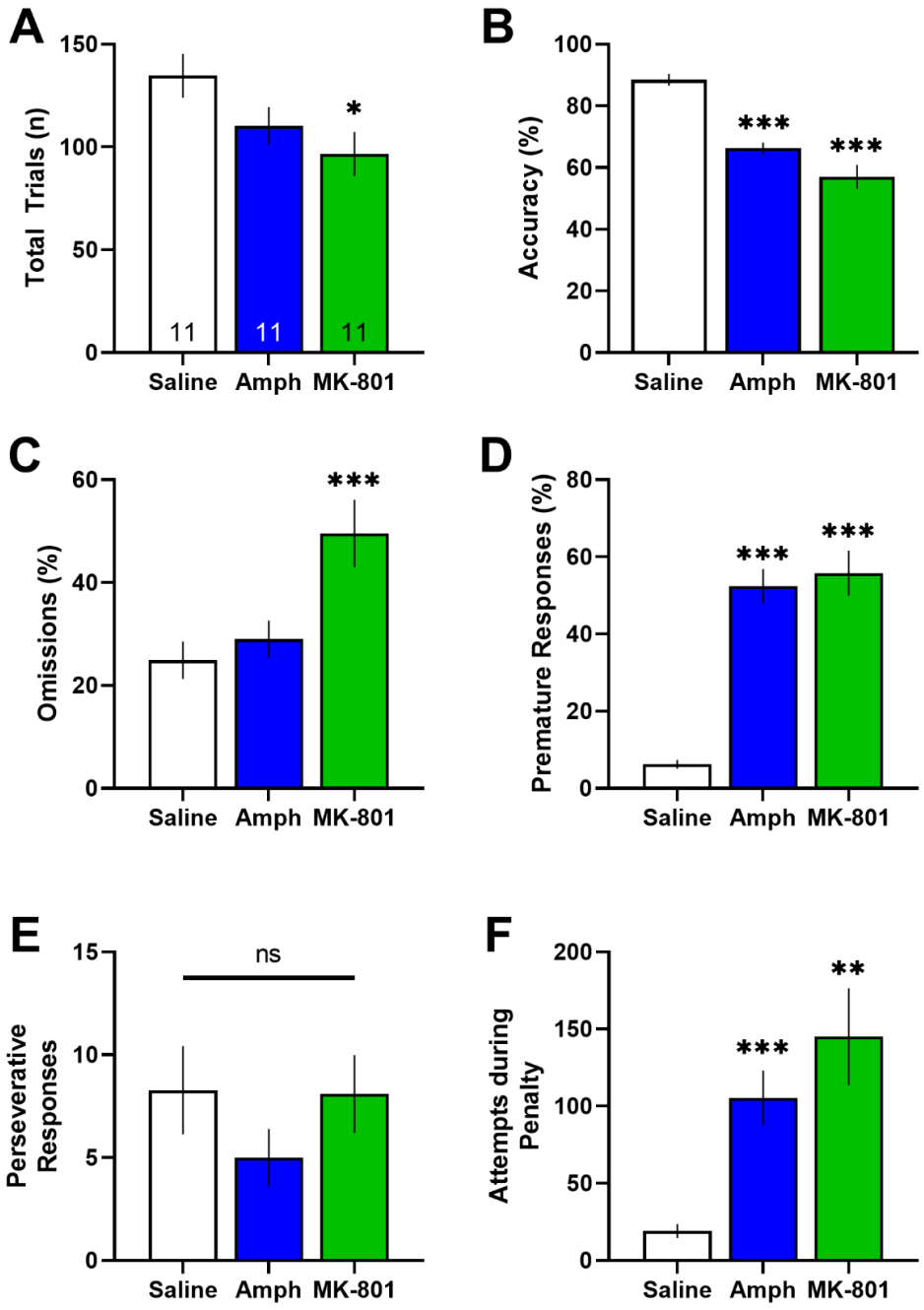

Figure 4.6 Task performance at a short stimulus duration in the 5-CSRTT with amphetamine or MK-801 manipulation. Amphetamine had no effect on trials completed (A), impaired choice accuracy (B), but did not affect omissions (C). MK-801 impaired both accuracy and trials completed, and increased omissions. Both compounds increased premature responding (D), without affecting perseverative responses after a correct response (E). Both compounds increased persistent responding at a poke during a penalty (F). All data are presented as mean ± SEM. Numbers within bars indicate n. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between treatments.

A significant treatment effect was also seen on omissions (Figure 4.6C, $F_{(2,20)} = 10.729$, $p < 0.001$). This effect was largely driven by the NMDAR antagonist MK-801, whereas

amphetamine had no effect (amphetamine vs. saline: $p > 0.3$; MK-801 vs. saline: $t_{(20)} = 5.112$, $p < 0.001$).

Both treatments increased impulsivity, increasing premature responding dramatically compared to controls, with both groups inappropriately making a response during the ITI on ~50% of trials (Figure 4.6D, $F_{(2,20)} = 33.489$, $p < 0.001$). This was confirmed by *post-hoc* comparisons in both treatment groups (both $p < 0.001$ vs. saline).

Finally, we looked at two measures of perseverative responding, either after a correct response (Figure 4.6E) or after an error leading to a timeout (Figure 4.6F). No difference was seen in perseverative responding after a correct choice ($F_{(2,20)} = 0.957$, $p = 0.401$). As this behaviour occurred during a period of time during which animals were supposed to collect reward following a correct choice, this indicates that neither treatment appeared to affect animals ability to complete the general pattern of behaviour required for the task, and were still reward-focused. However, differences between treatment groups were observed during a penalty ($F_{(2,20)} = 7.661$, $p = 0.003$). Both treatments led to increased responding during a penalty compared to saline (amphetamine vs. saline: $t_{(20)} = 5.727$, $p < 0.001$; MK-801 vs. saline: $t_{(20)} = 3.813$, $p = 0.003$). This redundant behaviour during a punishment may reflect frustration at being locked out from completing trials and earning rewards during a timeout.

Latency

We then looked at how these compounds affected response latencies, as a potential indicator of motoric effects. A significant effect of treatment was seen on correct and incorrect response latency (Figure 4.7A and B, correct latency: $F_{(2,20)} = 15.468$, $p < 0.001$; incorrect latency: $F_{(2,20)} = 4.044$, $p = 0.034$). Differences in correct response times were

driven by a longer latency to make a response in MK-801-treated animals, potentially reflecting a motoric effect of MK-801 (MK-801 vs. saline: $t_{(20)} = 4.920$, $p < 0.001$). In incorrect choice trials, while there was an overall effect of treatment, neither group were significantly different to saline controls (both $p > 0.1$). There was, however, a difference between amphetamine and MK-801 reflecting the faster latency with amphetamine but delayed responding with MK-801 compared to saline ($t_{(20)} = 3.046$, $p = 0.012$).

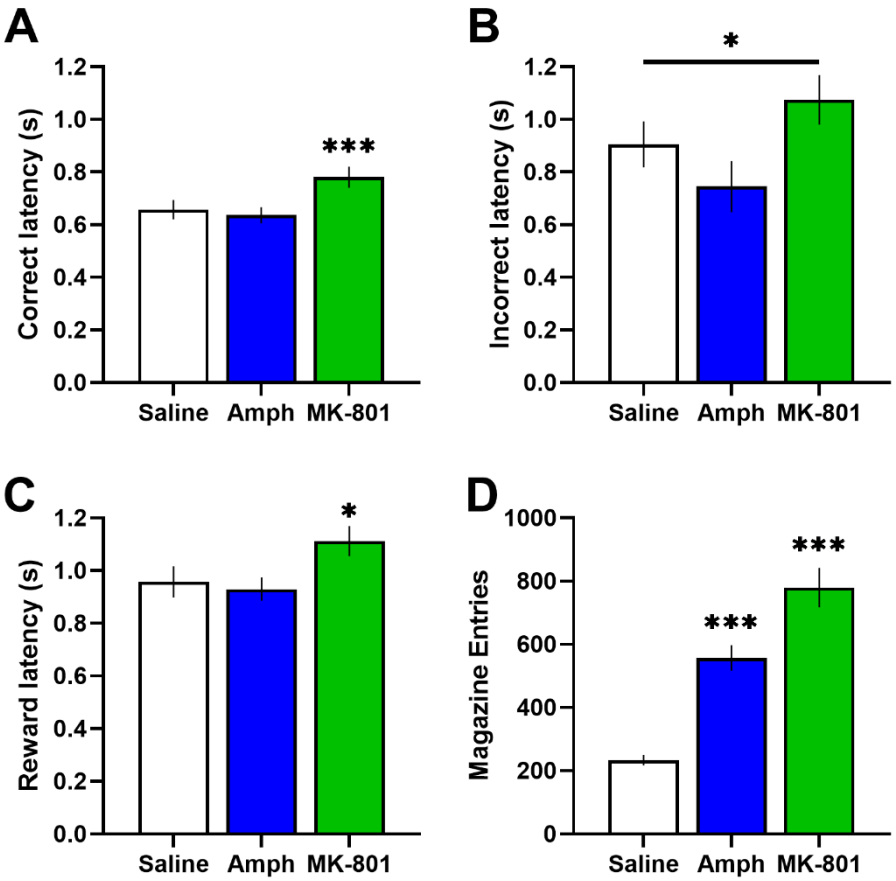

Figure 4.7 Latencies and magazine activity following amphetamine or MK-801 treatment. Amphetamine had no effect on correct choice (A), incorrect choice (B), or reward latencies (C) but significantly increased numbers of magazine entries. MK-801 induced subtle increases in latency, increasing correct choice latency and latency to collect reward, and increasing persistent behaviour at the magazine. All data are presented as mean ± SEM. * $p < 0.05$, *** $p < 0.001$ between treatments.

We also looked at reward-related behaviours following pharmacological manipulation. A significant effect of treatment was observed on both latency to collect reward, and total numbers of magazine head entries (Figure 4.7C and D, reward latency: $F_{(2,20)} = 6.595$, $p =$

0.006; magazine entries: $F_{(2,20)} = 39.441$, $p < 0.001$). MK-801-treated mice took significantly longer to collect reward (MK-801 vs. saline: $t_{(20)} = 2.534$, $p = 0.029$). Significant *post-hoc* effects were, also observed on magazine entries, with an increase in entries in both treatment groups (both $p < 0.001$). This further indicates that these compounds may make animals more impulsive, but also highly reward driven.

Performance by stimulus location

As in the previous experiment, we also wanted to look at how performance was affected by the position of the trial poke cue in the 5-choice wall. A significant main effect of poke and treatment*poke interaction was observed on choice accuracy, indicating that drug treatment had an impact on performance across individual pokes (Figure 4.8A, poke: $F_{(4,40)} = 15.129$, $p < 0.001$; treatment*poke: $F_{(8,80)} = 5.196$, $p < 0.001$). We next confirmed a significant effect of poke in each treatment group individually (all $p < 0.001$). Without reporting every *post-hoc* investigation of poke vs. poke comparisons, within each treatment group, on the whole amphetamine and MK-801 appeared to show significant performance deficits when comparing pokes 2 and 4 with the other locations. Interestingly, this suggests that pharmacological manipulation had the biggest effect at the pokes that were perceived as harder to attend to. In saline controls, these comparisons were only significant when comparing poke 4 vs. pokes 1 and 3 ($p = 0.023$ and $p = 0.006$ respectively). No interaction of treatment*poke was observed on omissions, though there was a main effect of poke (Figure 4.8B, poke: $F_{(4,40)} = 3.159$, $p = 0.024$; treatment*poke: $F(8,80) = 1.010$, $p = 0.435$).

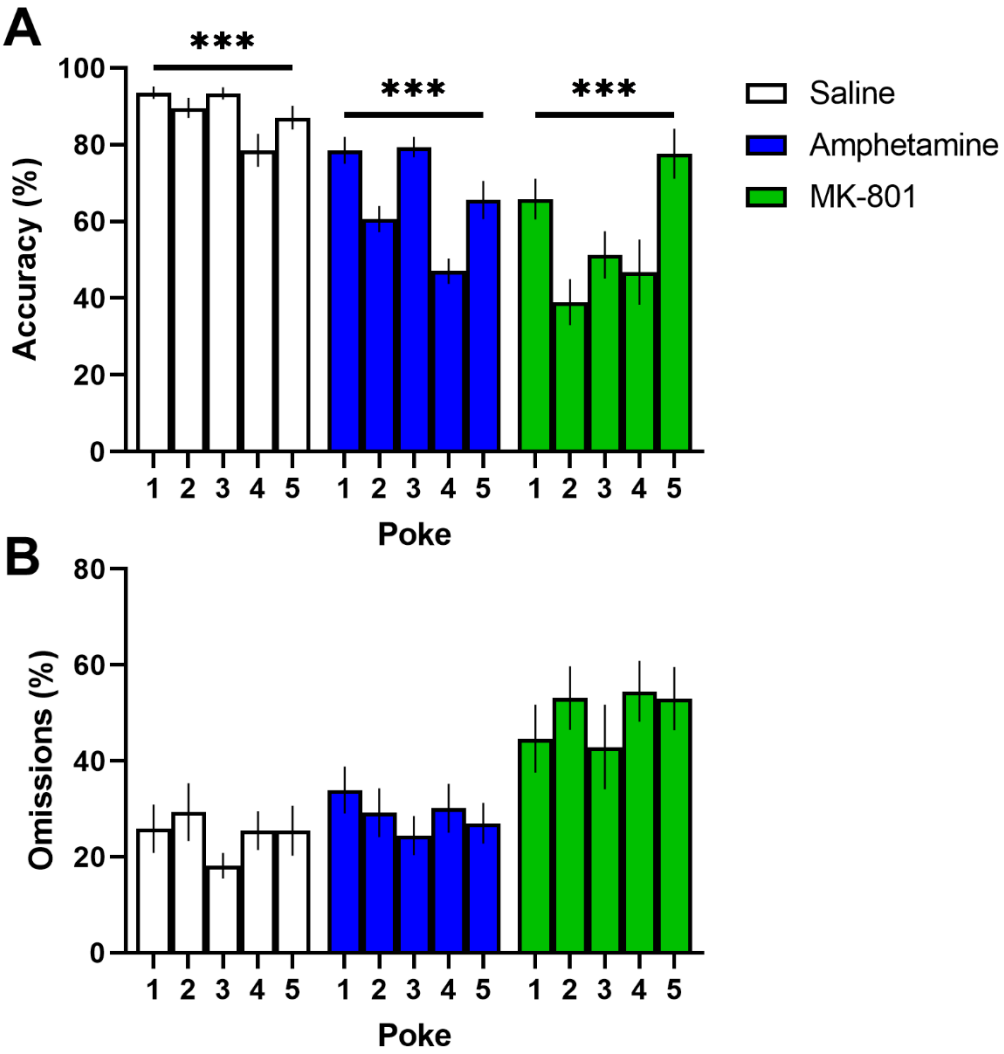

Figure 4.8 Exacerbated performance deficits at higher pokes following pharmacological manipulation. While accuracy was affected by trial location across all treatment groups, amphetamine and MK-801 performance appeared be worse on trials requiring animals to respond at ports 2 and 4 (A). There was no overall drug effect on rates of omissions at different pokes. All data are presented as mean ± SEM. *** $p <$ 0.001 within-treatment effect of poke (after confirming overall interaction).

Discussion

This experiment found a significant impairment in focused attention following both MK-801 and amphetamine treatment. MK-801 reduced accuracy and increased omissions, while also making animals more impulsive. This is in line with other reports of this compound in rats (Barlow *et al.,* 2018; Smith *et al.,* 2011), providing useful validation of the pyControl system for detecting impaired attentional processing, as well as confirming that mice are also sensitive to these pharmacological challenges. While MK-801 increased

magazine responding overall, latency to collect reward was increased, as were latencies to make a response during a trial. This could be an effect of sensorimotor impairments related to MK-801, but could also indicate that MK-801-treated mice were more distracted by other features of the environment during a session and so were slower to engage with the task.

The effects with amphetamine were a little more surprising; whilst there are reports in the literature of impaired choice accuracy (Baarendse and Vanderschuren 2012; Fletcher *et al.,* 2011; Papaleo *et al.,* 2012), most studies suggest amphetamine-induced changes in performance are mild under normal conditions and mainly affect impulsivity. We did also see this effect on impulsive responding, as well as a subtle decrease in response latencies. It is not impossible to reconcile these results with an account of hyperarousal in these mice. The reduced choice accuracy could also be a consequence of increased impulsivity, whereby mice in a heightened state of arousal poke indiscriminately at the 5-choice wall as soon as the trial starts. This would explain the slightly reduced latencies, particularly on incorrect choice trials, where a main effect of treatment was approaching significance but may have been underpowered to confirm a marginal effect.

4.3 Effect of MK-801 and amphetamine on 5-CSRTT performance with a variable ITI length

Introduction

One of the advantages of the 5-CSRTT as a behavioural task is that conditions can be manipulated to investigate attention. In the past, ITI length has been manipulated to exacerbate a mild attentional impairment in rats treated with amphetamine (Balachandran *et al.,* 2018). Initially, the aim of this experiment had been to investigate task performance

in animals that showed little change in sustained attention on the normal version of the task. However, we saw a significant reduction in choice accuracy with amphetamine even under predictable ITI conditions (Figure 4.6B). With that in mind, the current experiment set out to see whether an attentional impairment could be further exacerbated by unpredictable ITI lengths. This was also important for future comparison with a GluA1$^{-/-}$ cohort that were planned to be tested after this study concluded.

Methods

Subjects, compound administration and apparatus used were the same as that described in Experiment 4.1.

Procedure

Animals were tested over 9 days, with a baseline day, test day, and washout day, repeated 3 times as described in Experiment 4.2.

On washout days animals were rested and given free access to water for one hour. On baseline days animals were trained on stage 7, with a 0.8s SD and 5s ITI. However, on test days the program used was slightly different. The SD remained the same, but the ITI was manipulated across trials, with shorter or longer trials presented on a pseudorandom schedule. On 60% of trials animals received a 5s ITI, as in the later stages of training. On 20% of trials, a shorter ITI (3s) was used, and on 20% of trials a longer ITI (7s) was used. The rest of the task remained unchanged from previous training, and ITI length was only manipulated on test days such that shorter or longer ITIs remained surprising and could not reliably be predicted.

Analysis

Measures of performance and latency described in Experiment 4.1 were also investigated here, but to account for the different trial types with a varying ITI, two-way repeated measure ANOVAs were used to examine differences between treatment groups and ITI length. Some animals did not register any incorrect trials on a given ITI length and so were not included in analysis of the incorrect response latency measure, affecting the total degrees of freedom on this analysis.

Results

5-CSRTT Performance

While the previous experiment found a reduced number of total trials completed in drug treatment groups, this follow-up experiment found no main effect of drug on total trials (Figure 4.9A, $F_{(2,20)} = 0.902$, $p = 0.422$). However, there was an interaction of treatment*ITI ($F_{(4,40)} = 3.990$, $p = 0.008$). Pairwise comparisons revealed amphetamine-treated mice completed more trials at 3s compared to saline controls ($t_{(20)} = 9.050$, $p < 0.001$) but fewer trials at 7s ($t_{(20)} = 2.278$, $p = 0.046$). No differences were seen with MK-801.

Impaired choice accuracy was replicated, with MK-801- and amphetamine-treated mice both displaying significantly lower choice accuracy across all ITIs (Figure 4.9B, treatment: $F_{(2,20)} = 29.288$, $p < 0.001$). No effect of ITI was observed, and no interaction of treatment*ITI (ITI: $F_{(2,20)} = 0.552$, $p = 0.584$; treatment*ITI: $F_{(4,40)} = 0.441$, $p = 0.778$). *Post-hoc* comparisons of the treatment effect confirmed both amphetamine and MK-801 significantly reduce accuracy (both $p < 0.001$). This lack of treatment*ITI interaction was somewhat surprising, but it has historically been used to exacerbate a mild deficit in

performance. Given drug treatment groups were impaired in the standard version of the task, this lack of effect may be less surprising.

A significant effect of treatment was replicated on omissions, alongside an interaction with ITI length (Figure 4.9C, treatment: $F_{(2,20)} = 11.292$, $p < 0.001$; treatment*ITI: $F_{(4,40)} = 2.762$, $p = 0.041$). *Post-hoc* comparisons investigated the contribution of varying ITI, and of specific treatments to this effect. Saline controls showed slightly higher omissions at 3s, but this did not come out significant compared to 5s omission rates ($t_{(20)} = 2.067$, $p = 0.067$). This indicates that a reduced ITI marginally increases omissions in controls. If anything omissions were slightly lower at a short ITI with amphetamine, though this also didn't come out as significant (3s vs 5s: $t_{(20)} = 2.186$, $p = 0.054$). Amphetamine treatment did significantly lower omissions compared to saline at a 3s ITI ($t_{(20)} = 2.563$, $p = 0.028$). MK-801 increased omissions, as seen previously, but only at longer ITIs (5s: $t_{(20)} = 4.217$, $p = 0.002$; 7s: $t_{(20)} = 4.488$, $p < 0.001$).

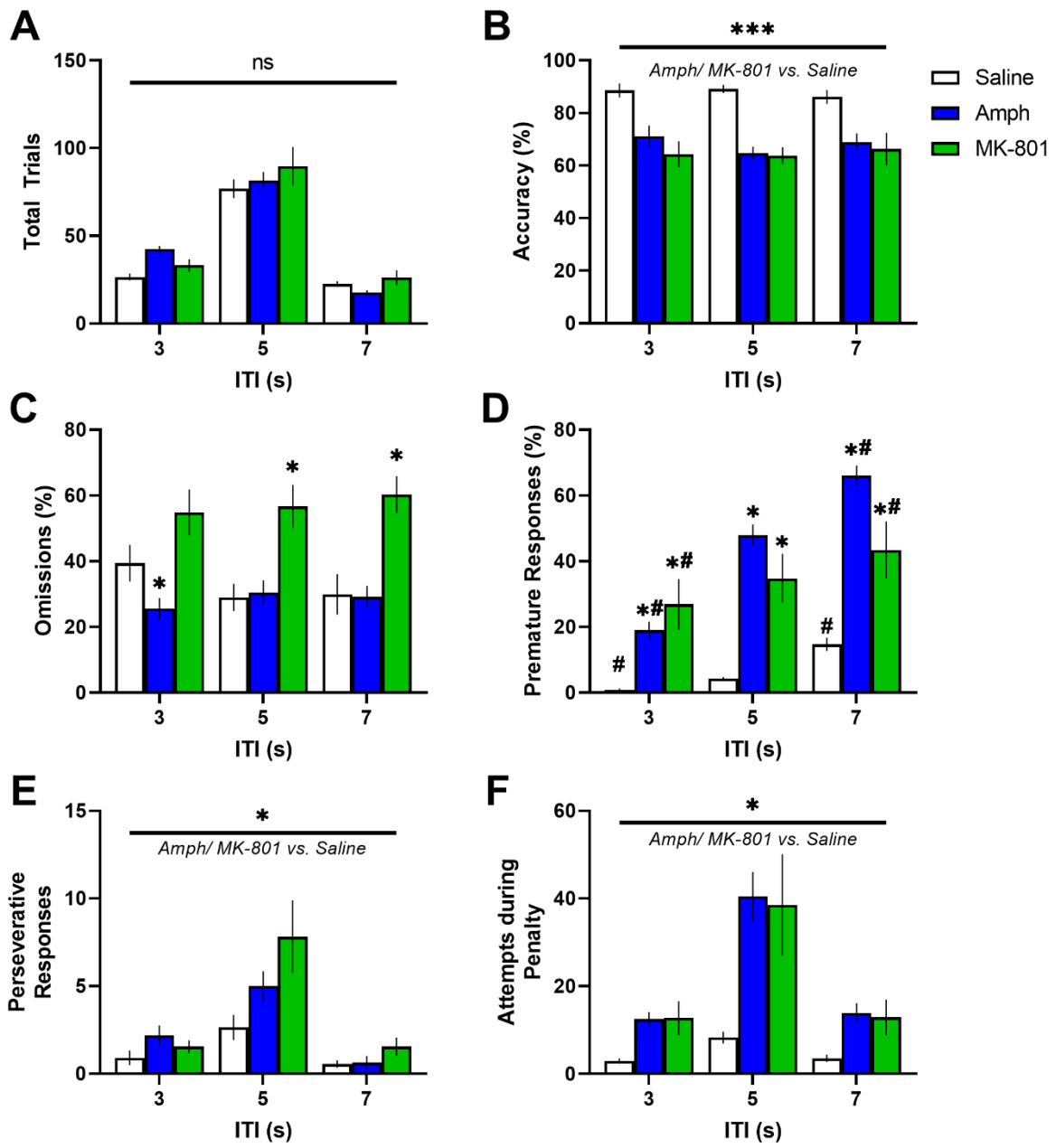

Figure 4.9 Reduced attentional performance following amphetamine or MK-801 treatment with a variable ITI. No difference was observed between treatment groups on trials completed (A) but both compounds reduced choice accuracy independent of ITI length (B). Saline controls showed higher omissions at 3s compared to 5s (C), and MK-801 was found to significantly increase omissions. Varying ITI had a profound effect on premature responding across all treatment groups (D), but this remained significantly higher following pharmacological manipulation compared to controls. MK-801 was found to increase perseverative responding (E), and responding in a penalty timeout (F), which was also increased following amphetamine. All data are presented as mean ± SEM. * $p < 0.05$, *** $p < 0.001$ between treatments. # $p < 0.05$ vs. 5s within-treatment groups.

Increases in impulsive premature responding were also replicated in the current experiment (Figure 4.9D). A significant effect of treatment, ITI, and interaction were reported (treatment: $F_{(2,20)} = 15.358$, $p < 0.001$; ITI: $F_{(2.20)} = 252.293$, $p < 0.001$;

treatment*ITI: $F_{(4,40)}$ = 24.000, p < 0.001). Reducing ITI length led to lower numbers of premature responses across all treatment groups (saline and amphetamine both p < 0.001; MK-801: $t_{(20)}$ = 3.695, p = 0.004). Increasing ITI length to 7s had the opposite effect; all treatment groups displayed a significant increase in premature responses comparing 7 and 5s (saline and amphetamine both p < 0.001; MK-801: $t_{(20)}$ = 2.849, p = 0.017).

Unlike the previous experiment, perseverative responding was affected by treatment in this ITI manipulation experiment (Figure 4.9E, treatment: $F_{(2,20)}$ = 6.926, p = 0.005). This wasn't investigated in relation to varying ITI as animals had more trials at 5s, but a *post-hoc* comparison of treatment revealed both amphetamine and MK-801 to increase perseverative responses (amphetamine: $t_{(20)}$ = 3.168, p = 0.010; MK-801: $t_{(20)}$ = 3.328, p = 0.008).

Finally, we also again observed a significant treatment effect on attempts made during a timeout period (Figure 4.9F, treatment: $F_{(2,20)}$ = 5.154, p = 0.016. *Post-hoc* comparisons confirmed this to be increased compared to controls in both amphetamine and MK-801 (amphetamine: $t_{(20)}$ = 6.251, p < 0.001; MK-801: $t_{(20)}$ = 2.624, p = 0.025).

Latency

We then looked at changes in latency in response to treatment and varying ITI length (Figure 4.10). A significant effect of treatment was observed on both correct and incorrect responses (correct: $F_{(2,20)}$ = 8.308, p = 0.02; incorrect: $F_{(2,12)}$ = 5.708, p = 0.018). A treatment*ITI interaction was observed on correct but not incorrect response latency (correct: $F_{(4,40)}$ = 4.030, p = 0.008; incorrect: $F_{(4,24)}$ = 2.001, p = 0.127). *Post-hoc* comparisons of correct responses revealed a longer latency in saline controls on a short ITI, but no other ITI effects (saline 3s vs. 5s: $t_{(20)}$ = 2.940, p = 0.014). This effect, in controls, may reflect the

surprise of a trial starting sooner than expected after a 3s ITI, causing animals to be delayed in responding but also more likely to miss the trial and register an omission. Amphetamine induced significantly shorter response latencies compared to saline at 3 and 5s (3s: $t_{(20)}$ = 3.689, p = 0.004; 5s: $t_{(20)}$ = 4.381, p < 0.001). MK-801 appeared to induce slightly longer latencies, though this was only observed statistically as a trend effect at 5 and 7s (5s: $t_{(20)}$ = 2.024, p = 0.068; 7s: $t_{(20)}$ = 2.119, p = 0.058). P*ost-hoc* comparisons of the treatment effect on incorrect response trials revealed marginally faster response times with amphetamine, though this was not significant (amphetamine vs. saline: $t_{(12)}$ = 2.180, p = 0.073). This main effect of treatment was seemingly driven by subtle decreases in response times with amphetamine, but increases with MK-801, confirmed by the significant difference between these two treatment groups ($t_{(12)}$ =3.45, p = 0.014).

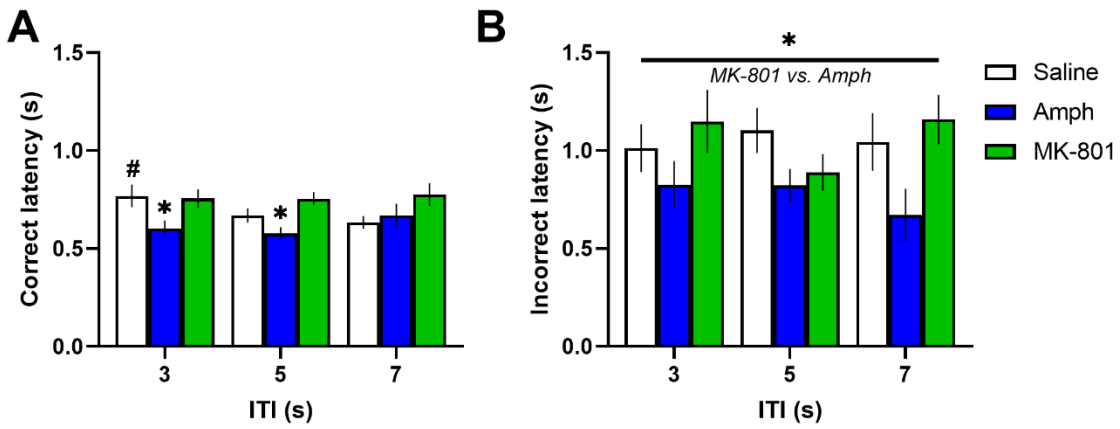

Figure 4.10 Effect of amphetamine and MK-801 on response latencies to a variable ITI 5-CSRTT. Saline controls took longer to make a correct response on a short-ITI trial (A) but no ITI-related differences in response times were seen in drug groups. Amphetamine induced shorter correct response latencies compared to saline. A subtle decrease in response time was also seen in amphetamine-treated mice specifically on incorrect choice trials but this was not significant compared to saline (B). All data are presented as mean ± SEM. * p < 0.05 between treatments. # p < 0.05 vs. 5s within-treatment groups.

Discussion

Impairments in 5-CSRTT performance observed in the previous experiment were replicated here, but on the whole the unpredictability of the ITI had little effect on this behaviour.

Both amphetamine and MK-801 reduced accuracy, coinciding with increased omissions in the MK-801 treatment group. Both treatment groups were more impulsive. Premature responses increased as ITI length increased, though this is perhaps unsurprising as there is a larger window to make a premature response with increasing duration. This is consistent with other literature suggesting amphetamine dose-dependently affects premature responding with increasing ITI length (Balachandran *et al.,* 2018). Other reports have indicated variable ITI length may also affect omissions, though this was not seen here (Bari *et al.,* 2008).

In the previous experiment there was a hint (albeit not significant) that amphetamine-treated mice responded more quickly when they went on to make an incorrect choice. This effect was apparent in the current experiment; response latency was marginally reduced across all ITI lengths for incorrect responses with amphetamine. This finding, alongside reduced accuracy and increased impulsivity suggest that amphetamine may be inducing hyperarousal and a lack of inhibitory control, contributing to the poor choice accuracy. In these last two experiments there has been no evidence of increased omissions with amphetamine, indicating that these animals are highly engaged with the task, perhaps supporting an argument for hyperarousal.

4.4 Effect of MK-801 and amphetamine on 5-CSRTT performance in the presence of auditory distractors

Introduction

Distracting stimuli, presented during the ITI, have previously been used in the 5-CSRTT to affect performance (Bari *et al.,* 2008), though changes may occur for a number of reasons. It could be, that by drawing attention away from the spatial location of the 5 choice ports

animals are more likely to miss trials or respond incorrectly. Alternatively, a surprising, loud auditory stimulus may trigger an inappropriate response at the 5-choice wall, causing faster responding and higher numbers of premature responses. Given that animals' attention is focused on the choice ports during the ITI, this suggests a distracting auditory stimulus elicits a behavioural response in the animals which is inappropriately directed at the task wall. However, an alternative possibility could be that by dividing attention away from the 5-choice wall, distracting stimuli could increase omissions and reduce premature responding.

This could be particularly relevant for animals with impairments in self-generated priming that result in an increased likelihood of stimulus elements being in A1, though it is difficult to predict what to expect here, and may be highly dependent on task parameters

The aim of this experiment was to assess distracting auditory stimuli to determine whether it was possible to see an effect of distractors on task performance. This will be important for a later planned study using GluA1$^{-/-}$ mice. But also, to complete our profile of each model of interest and to draw comparisons across the three, it is important to investigate the effects of these distracting tones on performance in animals treated with either amphetamine or MK-801.

Methods

Subjects, compound administration and apparatus used were the same as that described in Experiment 4.1.

Procedure

As described previously, animals were tested over 9 days, on a Latin-square design, with a baseline, test, and washout days, repeated 3 times. During baseline days animals were trained on stage 7, with a 0.8s SD and 5s ITI. On washout days animals were not trained.

The stage 7 parameters were also used on test days, with one important addition to the program. 25% of trials were no different to those in stage 7, but on the remaining 75% of trials (25% of trials each at either 1, 3, or 4.5s into the ITI) a distracting auditory cue was played, before a poke was illuminated at the start of a trial. The tone used was 0.5s of white noise at 80dB, from the speaker above the 5-choice wall (Figure 4.2). When presented at 4.5s into the ITI, the tone ended as the trial started, illuminating a choice port.

Analysis

Analysis was conducted in the same way as that described in Experiment 4.3, with two-way ANOVAs (distractor*treatment) used to investigate the effect of distractors on various measures of attentional performance and their interaction with pharmacological manipulation.

One saline-treated animal and one MK-801-treated animal were mis-dosed and had to be excluded from the analysis, leaving a total n of 9 in this experiment.

Results

5-CSRTT Performance

Following amphetamine or MK-801 treatment, animals completed significantly more trials compared to controls, driven by higher numbers of trials completed in both treatment groups (Figure 4.11A, treatment: $F_{(2,16)} = 20.811$, $p < 0.001$; amphetamine: $t_{(16)} = 3.184$, $p =$

0.013; MK-801: $t_{(16)} = 8.932$, $p < 0.001$). Experiments 4.2 and 4.3 revealed a decrease, and no difference, respectively, in trials completed in the drug groups. Taken together these three contradictory findings suggest a possible sensitisation (increased sensitivity to the drug) to repeated doses of amphetamine or MK-801 in the same subjects over time.

Choice accuracy was significantly reduced in both MK-801 and amphetamine treatment groups, confirmed by a main effect of treatment and *post-hoc* comparisons with saline (Figure 4.11B, treatment: $F_{(2,16)} = 10.917$, $p = 0.004$; amphetamine: $t_{(16)} = 6.458$, $p < 0.001$; MK-801: $t_{(16)} = 3.730$, $p = 0.006$). However, no effect was seen with auditory distractors, and no interaction of distractor*treatment, although this was approaching significance (distractor: $F_{(3,24)} = 2.257$, $p = 0.108$; treatment*distractor: $F_{(6,48)} = 2.049$, $p = 0.077$). While this interaction was not significant, it appeared that distractors subtly reduced accuracy in controls when presented later in the ITI.

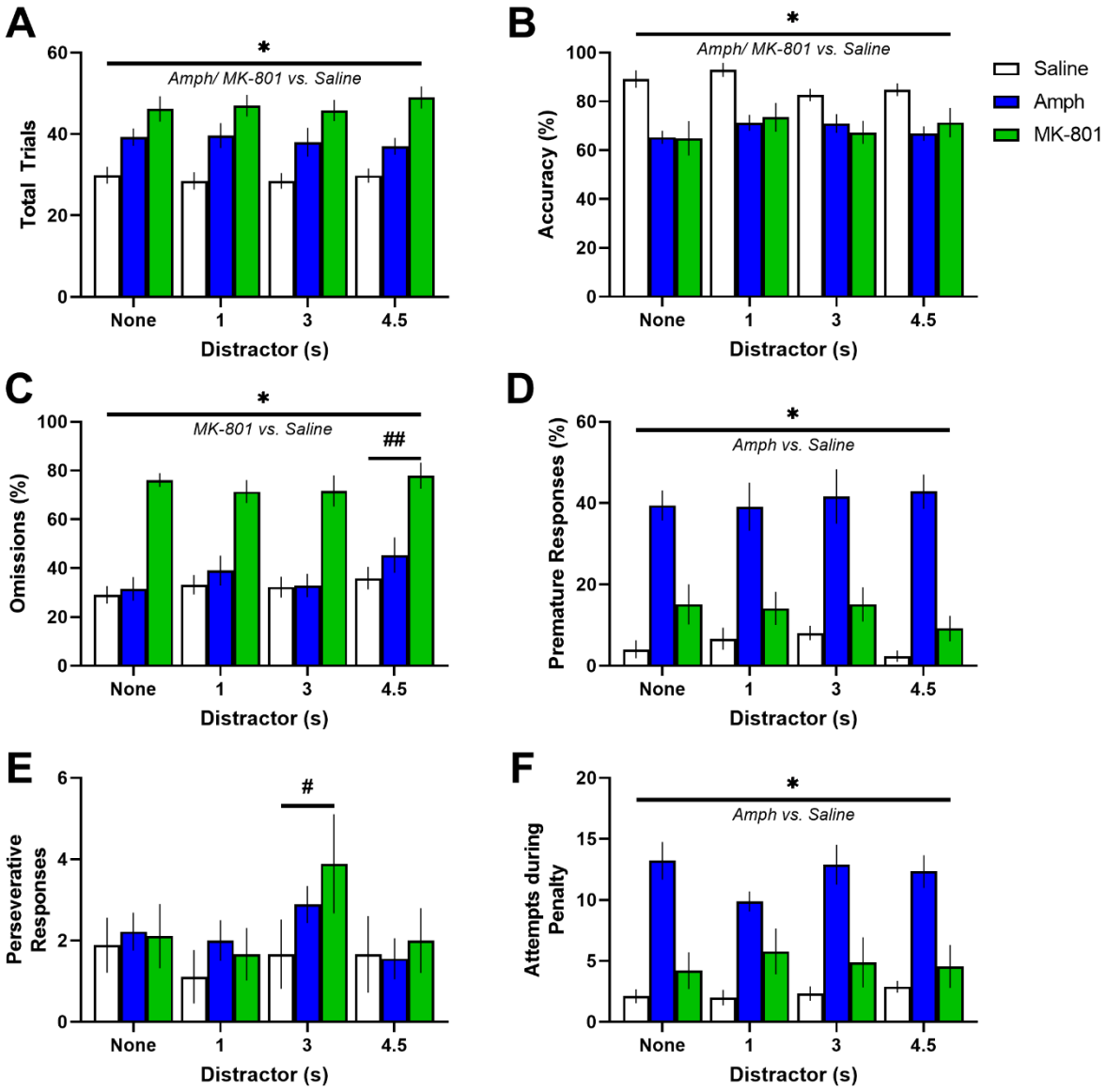

Figure 4.11 Effect of auditory distractors on attentional performance in the 5-CSRTT task following amphetamine or MK-801 treatment. Both compounds increased the number of trials completed by animals (A) whilst reducing choice accuracy (B) but distractors had no effect on these measures. Distractors increased the number of omissions animals made when presented immediately before the stimulus onset (C). Distractors did not affect premature responding (D), perseverative responding (E), or attempts made during a timeout (F). All data are presented as mean ± SEM. * $p < 0.05$ between treatments. # $p < 0.05$, ## $p < 0.01$ vs. no distractor condition.

Omissions were also different across treatment groups (Figure 4.11C, $F_{(2,16)} = 22.317$, $p < 0.001$). *Post-hoc* comparisons of this treatment effect revealed significantly elevated omissions in MK-801 animals but not amphetamine, compared to saline (MK-801: $t_{(16)} = 6.178$, $p < 0.001$; amphetamine: $t_{(16)} = 0.678$, $p = 0.517$). A significant effect of distractor condition was also observed, though there was no overall treatment*distractor interaction

(distractor: $F_{(3,24)}$ = 7.246, p < 0.001; treatment*distractor: $F_{(6,48)}$ = 1.024, p = 0.422). Investigation of the distractor effect revealed an effect at 4.5s, compared to no distractor, with this trial type significantly increasing omissions ($t_{(24)}$ = 4.382, p = 0.002). This suggests that a distractor immediately before trial start is sufficiently startling to cause animals to miss the cue, though this was not affected by treatment. Distractors earlier in the ITI period did not have an effect. Interestingly, the magnitude of increased omissions in MK-801-treated mice appeared higher across all trial conditions than in previous experiments. It is possible that the distracting auditory tones disrupted their ability to appropriately attend to the task, and that this extended across the whole session, even on trials when no distractor was played.

As seen in previous experiments, pharmacological manipulation also increased premature responding (Figure 4.11D, $F_{(2,16)}$ = 22.214, p < 0.001). However, no main effect was seen of distractors, and only a trend interaction was observed (distractor: $F_{(3,24)}$ = 1.825, p = 0.170; treatment*distractor: $F_{(6,48)}$ = 2.124, p = 0.068). *Post-hoc* comparisons of treatment revealed amphetamine to significantly increase premature responding ($t_{(16)}$ = 7.083, p < 0.001), but no effect in MK-801.

No effect of treatment, was reported here on perseverative responding after a correct choice, but there was an effect of distractor trial type (Figure 4.11E, treatment: $F_{(2,16)}$ = 0.417, p = 0.666; distractor: $F_{(3,24)}$ = 3.366, p = 0.035). No overall interaction was observed ($F_{(6,48)}$ = 1.603, p = 0.167). *Post-hoc* comparisons found increased perseverative responding when a distractor was presented at 3s ($t_{(24)}$ = 2.628, p = 0.030), though it is hard to see why this condition would affect this specific behaviour; it may just be a spurious result.

Finally, we compared responding during timeout periods across groups (Figure 4.11F). A treatment effect was again seen, though only amphetamine treatment came out as significantly higher than controls (treatment: $F_{(2,16)} = 23.876$, $p < 0.001$; amphetamine: $t_{(16)} = 12.218$, $p < 0.001$; MK-801: $t_{(16)} = 1.434$, $p = 0.189$). There was no effect of distractors at different points of the ITI, and no interaction between distractors and treatment (distractor: $F_{(3,24)} = 0.465$, $p = 0.709$; treatment*distractor: $F_{(6,48)} = 1.153$, $p = 0.347$).

Latency

As with previous experiments, we looked at how response times were affected by pharmacological compounds, and distractor cues (Figure 4.12). However, only 3 mice had no missing data on incorrect responses – all other animals registered no incorrect choices on at least 1 trial type, so this measure was not analysed statistically (Figure 4.12B).

An effect of treatment was seen on correct choice latency ($F_{(2,16)} = 8.649$, $p = 0.003$). However, there was no main effect of distractor, or interaction (distractor: $F_{(3,24)} = 1.256$, $p = 0.312$ distractor*treatment: $F_{(6,48)} = 1.467$, $p = 0.210$). *Post-hoc* comparisons showed no differences between treatment groups, though there was a trend effect of amphetamine vs saline controls, suggesting faster correct choice latencies ($t_{(16)} = 2.204$, $p = 0.060$). The main effect was driven by a difference between MK-801 and amphetamine ($p < 0.001$), again indicating the divergent effects these drugs have on response latencies as seen in previous experiments.

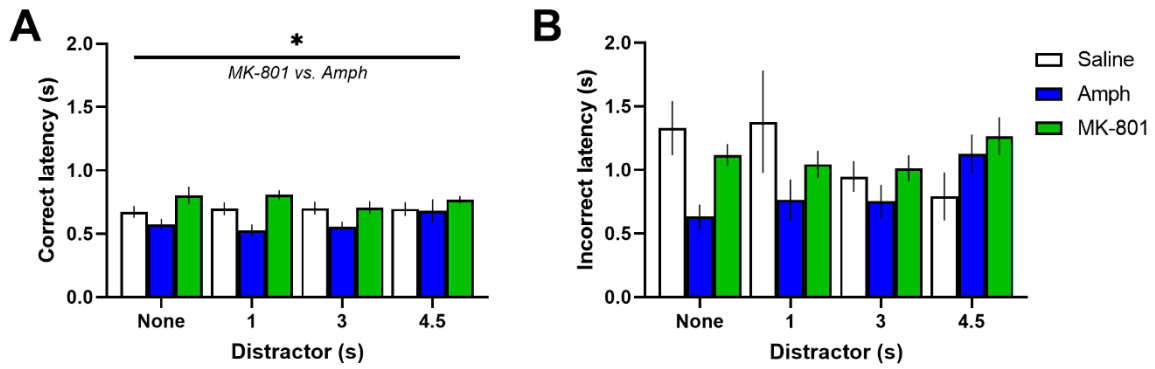

Figure 4.12 Effect of amphetamine or MK-801 treatment on response latencies in the presence of auditory distractors. A main effect of treatment, but not distractor cues, was seen on correct choice latency (A). Amphetamine appeared to reduce incorrect choice latency but was not analysed statistically because of missing data. All data are presented as mean ± SEM. * $p < 0.05$ between treatments.

Discussion

Previous reports of the use of distracting stimuli suggest that they can increase premature responding, and reduce response latency (Bari *et al.,* 2008). Neither of those findings were observed in the current experiment, either in saline controls or in drug treatment groups. In fact, there was only a very weak, if any, effect of distractors. Distracting auditory tones presented immediately before the trial start (Figure 4.11C, 4.5s) increased omissions suggesting that animals were attending to the tones and missed the cue stimulus. This effect was seen independent of drug treatment. There was no strong evidence for an effect of distractors on any other measure reported here.

Both MK-801 and amphetamine treatment led to animals completing more trials throughout the session in total compared to treatment with saline. This is interesting, as it contradicts the earlier results in Experiments 4.2 and 4.3 where drug treatment groups expressed fewer trials completed, and no change, respectively. These results together indicate that these compounds may be having a changing effect over time. This is a problem with repeated administration of these compounds, which have previously been shown to induce sensitisation over repeated doses – here meaning an increase in

sensitivity to the drug (Cui *et al.,* 2015; Paulson and Robinson 1995). Whilst this does represent a potential limitation of using pharmacology in these studies, all animals received each treatment three times throughout the study, and so effects of pharmacological sensitisation should have been consistent across animals. Furthermore, this was not entirely unexpected, and we made sure to complete experiments in an order that we felt would generate the most interesting data first. Furthermore, effects of drug treatment on measures of sustained attention – choice accuracy and omissions were highly consistent across three rounds of dosing in these last three experiments.

4.5 Training GluA1$^{-/-}$ mice to acquisition of a short stimulus on a 5-choice serial reaction time task

Introduction

We have previously seen enhanced performance in GluA1$^{-/-}$ mice in the 5-CSRTT at a 1s SD, though this effect disappears when the SD is reduced to 0.5s (Figure 4.1). At a 1s SD, GluA1$^{-/-}$ mice had higher choice accuracy and fewer omissions than their WT counterparts. This experiment was run by a former postdoctoral researcher in the Bannerman lab several years ago, with very different testing conditions. The aim of this experiment, and the remaining experiments in this chapter, was therefore to train a new cohort of GluA1$^{-/-}$ mice on the 5-CSRTT, using testing conditions similar to those used in our studies of pharmacological manipulations so that we can confidently compare across these studies. We also wanted to ensure GluA1$^{-/-}$ differences weren't simply due to them completing more trials, which was a possibility in the earlier experiment and could have facilitated learning, as seen with unyoked NOR performance (Sanderson, Hindley, *et al.,* 2011).

Methods

Apparatus used was the same as described previously in Experiment 4.1.

Subjects

A combination of male and female WT and GluA1$^{-/-}$ mice were used for this experiment. In total 12 WT and 12 GluA1$^{-/-}$ mice were used, with an even split of males and females in each group (n = 6 male, 6 female, per genotype). The same cohort of animals that were trained to acquire the 5-CSRTT in the current experiment were then used to investigate manipulating different task parameters in the remaining experiments in this chapter. Two male GluA1$^{-/-}$ mice failed to reach training criteria at the later stages of training and so were not included in any of the analysis shown here and were not used in later experiments. Total animals presented here are therefore 12 WT mice (6 male, 6 female), and 10 GluA1$^{-/-}$ (4 male, 6 female).

Animals were generated in-house in the Biomedical Sciences Building at the University of Oxford. At 9-12 weeks of age animals were water-restricted to no less than 85% of their *ad libitum* body weight and maintained on water restriction for the duration of this study. Access to food was always freely available, both in their homecage, and during training.

Procedure

Autoshaping

Animals were first trained to collect reward from the magazine, as described in Experiment 4.1. Whereas in the earlier study, animals were trained for at least 2 days, all animals were moved on after day 1 in this experiment, except for one run of 6 mice which were

accidentally run for a second session on this training stage. After 1 day of training all animals had collected at least 35 rewards from the magazine in a 30-minute session.

Animals were then trained to nose-poke at the 5-choice wall, again as described previously. Animals were moved on from this training stage after successfully completing a minimum of 40 trials per session for two consecutive sessions.

5-CSRTT Training

Training on the 5-CSRTT paradigm was similar to that described in Experiment 4.1; animals were trained to respond to an illuminated poke, starting at a long SD (20s) and gradually decreasing the SD as performance improved. However, whereas in the previous experiment for the first stages trials continued automatically after a punishing timeout, this experiment retained the additional step introduced towards the end of training previously. After a punishment following omission, premature or incorrect responding, the magazine LED illuminated, signalling trial initiation.

Some progression criteria were also adjusted. The biggest difference was a stricter progression criterion for omissions (now 30%) was required to progress after stage 3 (Table 4.2). At later stages with a shorter SD, some animals struggled to consistently attain 80% choice accuracy. From stage 5 this was relaxed to 75% to ensure animals reached the end of training.

As in Experiment 4.1, after stimulus presentation there was a 2s limited hold (LH) period where animals could still register a response before being punished for an omission. One key difference between training in this experiment, and the previous study (as well as the GluA1$^{-/-}$ experiment shown in Figure 4.1), was that we now imposed a ceiling on total number of trials animals could complete within a session. The session ended when animals

completed 150 trials, or after 60 minutes of training, in this experiment. This was designed to control for numbers of trials completed between genotype groups.

	Training parameters			Progression criteria (2 consecutive days)		
Stage	**SD (s)**	**LH (s)**	**ITI (s)**	**Correct trials (n)**	**Accuracy (%)**	**Omissions (%)**
1	20	2	2	≥ 45	≥ 30	-
2	8	2	2	≥ 60	≥ 50	-
3	8	2	5	≥ 60	≥ 80	≤ 30
4	4	2	5	≥ 60	≥ 80	≤ 30
5	2	2	5	≥ 60	≥ 75	≤ 30
6	1	2	5	≥ 60	≥ 75	≤ 30

Table 4.2 Training parameters and criteria to progress for 5-CSRTT training of a GluA1$^{-/-}$ cohort. Stimulus duration (SD), limited hold (LH) and inter-trial interval (ITI) length across training, and criteria to progress to the next stage.

Analysis

Performance and latency measures for statistical analysis were similar to those described in Experiment 4.1. However, we also investigated genotype effects throughout training through repeated measures ANOVA and relevant *post-hoc* comparisons. For *post-hoc* comparisons of significant genotype*stage interactions, a significance threshold was calculated based on 6 comparisons, across two families of interest (genotype and genotype*stage), with an alpha of 0.1. This gave a significance threshold of $p < 0.017$.

Results

5-CSRTT Performance

GluA1$^{-/-}$ mice required more sessions to reach training criteria than WT mice, especially at later stages when the SD was shorter (Figure 4.13A). On average WT mice completed training in 35 sessions, whilst GluA1$^{-/-}$ mice took 43 days on average. While there was no

effect of genotype, a genotype*stage interaction confirmed significant differences (genotype: $F_{(1,20)} = 3.127$, $p = 0.092$; genotype*stage: $F_{(5,100)} = 2.550$, $p = 0.032$). Both groups appeared to learn at a similar rate initially, taking around 10 sessions to progress through the first stage, then progressing through stages 2-4 relatively quickly. However, stages 5 and 6, with a 2s and a 1s SD respectively, took GluA1$^{-/-}$ mice more time to acquire, although this did not quite come out as significant in *post-hoc* comparisons compared to WT (stage 5: $t_{(20)} = 1.864$, $p = 0.077$; stage 6: $t_{(20)} = 1.815$, $p = 0.085$). The genotype*stage interaction instead seems to have been driven mainly by differences in sessions to criterion between stages, rather than between genotypes.

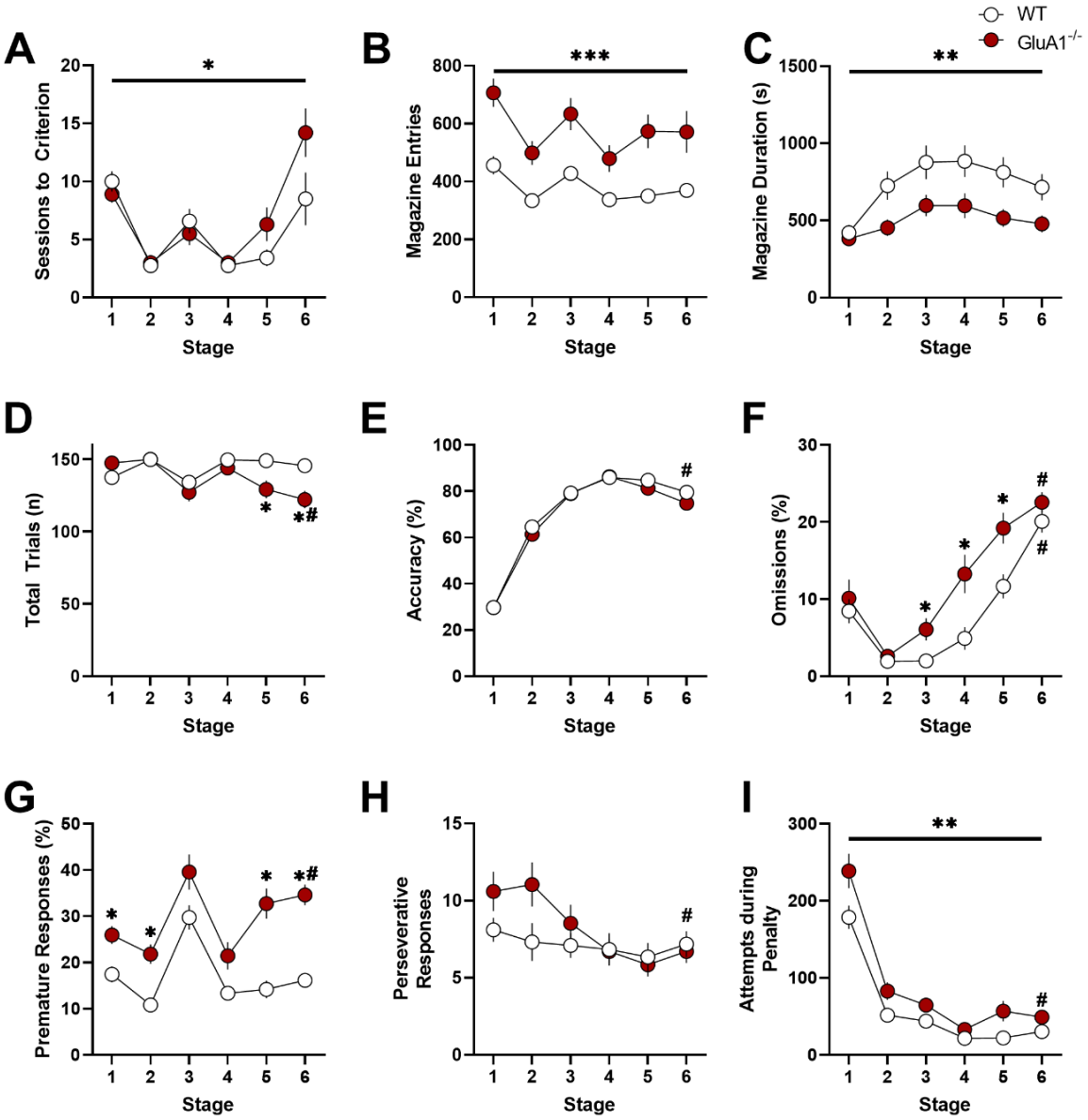

Figure 4.13 Delayed acquisition of a 5-CSRTT paradigm in GluA1$^{-/-}$ mice. GluA1$^{-/-}$ took more sessions overall to reach criteria for progression through training stages (A). They displayed significantly higher numbers of magazine entries (B) but reduced overall time in the magazine (C). WT mice completed more trials than GluA1$^{-/-}$ at later, training stages with a shorter SD (D), however, there was no difference between genotypes on choice accuracy (E). GluA1$^{-/-}$ mice had much higher levels of omissions (F), and increased premature responding (G). There was no difference between groups on perseverative responding (H), but increased responding during a timeout in GluA1$^{-/-}$ mice (I). All data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between genotypes (or Sidak-corrected value as *post-hoc* comparisons). # $p < 0.05$ stage 6 vs. stage 1.

Divergent results on magazine behaviours were seen, with GluA1$^{-/-}$ mice making significantly more magazine head entries but spending less overall time in the magazine, suggesting individual visits were little and often (Figure 4.13B and C). Genotype differences on magazine entries were significantly different but no interaction with stage was observed

(genotype: $F_{(1,20)}$ = 19.826, p < 0.001; genotype*stage: $F_{(5,100)}$ = 1.153, p = 0.338). While a significant main effect of genotype and genotype*stage interaction was found on magazine duration, no *post-hoc* comparisons of individual stage differences were significant after correction for 6 tests (genotype: $F_{(1,20)}$ = 5.219, p = 0.033; genotype*stage: $F_{(5,100)}$ = 3.242, p = 0.009; *post-hoc* comparisons all p > 0.017).

A significant genotype*stage interaction was seen on total trials completed per session, with GluA1$^{-/-}$ completing fewer trials as SD decreased (Figure 4.13D, $F_{(5,100)}$ = 6.654, p < 0.001). In particular, GluA1$^{-/-}$ completed significantly fewer trials at stages 5 and 6 (stage 5: $t_{(20)}$ = 3.530, p = 0.002; stage 6: $t_{(20)}$ = 4.145, p < 0.001). Furthermore, GluA1$^{-/-}$, but not WT mice, showed a significant decrease in stage 6 trials completed compared to stage 1, within-group (WT: $t_{(20)}$ = 1.736; p = 0.098; GluA1$^{-/-}$: $t_{(20)}$ = 4.862, p < 0.001).

Despite completing fewer trials, GluA1$^{-/-}$ were unimpaired on choice accuracy (Figure 4.13E). Whilst there was a significant effect of stage confirming acquisition across groups, there was no genotype effect and no genotype*stage interaction (stage: $F_{(5,100)}$ = 399.081, p < 0.001; genotype: $F_{(1,20)}$ = 1.296, p = 0.268; genotype*stage: $F_{(5,100)}$ = 1.025, p = 0.407). A significant *post-hoc* comparison of stage 6 vs. stage 1 confirmed acquisition across training ($t_{(20)}$ = 32.141, p < 0.001).

Whilst accuracy was unimpaired, GluA1$^{-/-}$ mice made more omissions than WTs, particularly as the SD got shorter in later stages of training (Figure 4.13F). This was confirmed by a significant main effect of genotype and genotype*stage interaction on omissions, and significant genotype differences from stages 3-5 (genotype: $F_{(1,20)}$ =10.481, p = 0.004; genotype*stage: $F_{(5,100)}$ = 2.536, p = 0.033; stage 3: $t_{(20)}$ = 2.984, p = 0.007; stage 4: $t_{(20)}$ = 2.975, p = 0.007; stage 5: $t_{(20)}$ = 3.006, p = 0.007). No effect was seen on stage 6,

during which WTs also began to make more omissions. Both groups displayed significantly elevated omissions compared to stage 1 when the SD was much longer (both $p < 0.001$). However, compared to the earlier study investigating GluA1$^{-/-}$ 5-CSRTT performance in Med Associates testing boxes (Figure 4.1), omissions here were much lower in both groups.

An effect of genotype and genotype*stage interaction was also seen on premature responding, with GluA1$^{-/-}$ mice being much more likely to make a premature response throughout training (Figure 4.13G; genotype: $F_{(1,20)} = 36.449$, $p < 0.001$; genotype*stage: $F_{(5,100)} = 3.095$, $p = 0.012$). Genotype differences were found across most stages of training, except for stage 3 when the ITI length was increased and both groups made more premature responses (stage 1: $t_{(20)} = 3.234$, $p = 0.004$; stages 2, 5 and 6 all $p < 0.001$). GluA1$^{-/-}$ premature responding at stage 3 was no different from WT (stage 3: $t_{(20)} = 2.192$, $p = 0.040$; significance threshold for 6 tests: $p < 0.017$). GluA1$^{-/-}$ premature responding increased as the SD was decreased, and was significantly higher at stage 6 compared to stage 1 ($t_{(20)} = 3.536$, $p = 0.002$). This was not seen in WT mice (WT stage 6 vs. stage 1: $p > 0.5$)

Perseverative responding did not differ between genotypes, but did decrease across training (Figure 4.13H, genotype: $F_{(1,20)} = 1.531$, $p = 0.230$; stage: $F_{(5,100)} = 4.682$, $p < 0.001$). No genotype*stage interaction was observed ($F_{(5,100)} = 2.036$, $p = 0.080$). *Post-hoc* comparison of the stage effect revealed reduced premature responding across both genotypes by stage 6, compared to stage 1 ($t_{(20)} = 2.553$, $p = 0.019$).

Finally, perseverative behaviour during penalty timeout periods declined over training (Figure 4.13I). Overall, perseverative responding was higher in GluA1$^{-/-}$ mice compared to WTs, confirmed by main effects of genotype and stage, and an interaction approaching

significance (genotype: $F_{(1,20)}$ = 13.150, p = 0.002; stage: $F_{(5,100)}$ = 124.211, p < 0.001; genotype*stage: $F_{(5,100)}$ = 1.981, p = 0.088). *Post-hoc* investigation of the stage effect revealed reduced penalty responding by stage 6 ($t_{(20)}$ = 12.948, p < 0.001).

Latency

Latencies to make a response at the 5-choice wall declined across training in both genotypes (Figure 4.14A and B). There was no effect of genotype on correct choice latency, or genotype*stage interaction, but a significant stage effect (genotype: $F_{(1,20)}$ = 1.513, p = 0.233; stage: $F_{(5,100)}$ = 56.450, p < 0.001; genotype*stage: $F_{(5,100)}$ = 0.650, p = 0.662). A similar finding was seen on responses when an incorrect choice was made (genotype: $F_{(1,20)}$ = 0.736, p = 0.401; stage: $F_{(5,100)}$ = 52.607, p < 0.001; genotype*stage: $F_{(5,100)}$ = 0.895, p = 0.488). Both measures revealed a significant reduction in response latency by stage 6 of training (both p < 0.001).

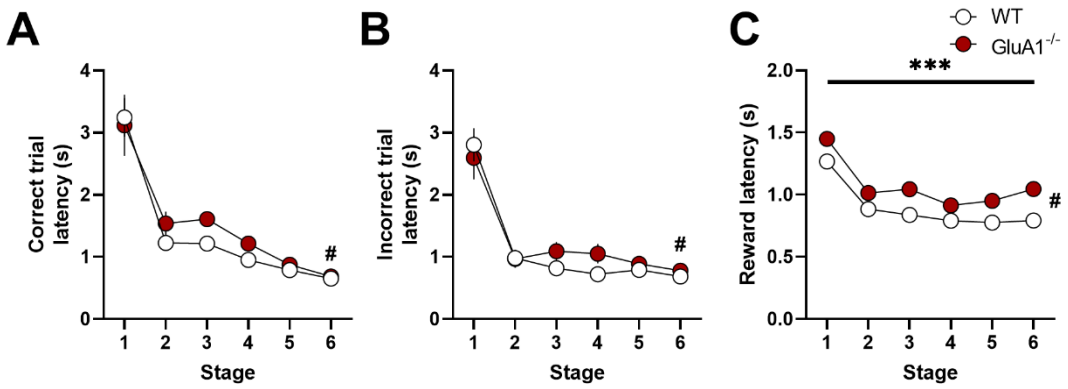

Figure 4.14 Decreasing response latencies across training in WT and GluA1$^{-/-}$ mice. Response times for both correct choice (A) and incorrect choice (B) trials decreased across both genotypes during training. Latency to collect reward also decreased significantly across training stages (C). All data are presented as mean ± SEM. # p < 0.05 stage 6 vs. stage 1.

Interestingly, there was a significant effect of genotype on latency to collect reward, with GluA1$^{-/-}$ mice being slower to collect reward ($F_{(1,20)}$ = 24.878, p < 0.001) There was also an effect of training stage; but no overall interaction (stage: $F_{(5,100)}$ = 95.909, p < 0.001;

genotype*stage: $F_{(5,100)}$ = 1.572, p = 0.175). A comparison of stage 6 vs. stage 1 reward response latency confirmed that response times decreased throughout training ($t_{(20)}$ = 11.282, $p < 0.001$).

Discussion

The findings presented in this experiment represent a stark contrast to that reported in the introduction to this chapter, where GluA1$^{-/-}$ mice displayed enhanced attentional performance. Here, GluA1$^{-/-}$ mice were shown to be impaired, or at least delayed in their acquisition of the 5-CSRTT task. It took GluA1$^{-/-}$ mice on average 8 days longer than WTs to reach the end of training and training criteria on stages 5 and 6 were eventually made slightly more lenient so that enough animals would reach the end of training in order to test them in later experiments. Although no differences in omissions were observed at the end of training, in earlier stages GluA1$^{-/-}$ omitted significantly more trials than their WT littermates, and the lack of difference on stage 6 omissions was due to WT levels increasing to match those of GluA1$^{-/-}$.

Perhaps one of the most striking differences observed between genotypes was the significantly elevated premature responding in GluA1$^{-/-}$ mice across training. Long-term associative learning is intact in these animals, and it is somewhat surprising that these animals still show performance deficits after ~43 sessions of training to inhibit responding during the ITI. This result also seems at odds with the finding of reduced latency to collect reward, following a correct response. It is clear that these animals are still highly reward driven; magazine entries overall were higher in this experiment, and increased magazine or nose-poke-directed behaviour has been reported elsewhere in these mice (Johnson *et al.,* 2007; Sanderson *et al.,* 2017). It is hard to explain this longer latency, specifically on

magazine behaviour, when other results point to animals that are highly motivated to earn reward. The consistently increased premature responding seen in GluA1$^{-/-}$ mice throughout training hints that whilst these animals are able to learn the task and generally behave appropriately, they remain highly impulsive with poor inhibitory control. An alternative account is that these mice may more readily form habits, an idea we will consider more in the General Discussion. It would be interesting to see how these animals behave in other variants of this task, such as the 5-choice continuous performance test where on some trials all pokes light up indicating animals should not respond in order to receive reward (Young *et al.,* 2009).

We will return to the seemingly impaired 5-CSRTT acquisition in GluA1$^{-/-}$ mice, and possible explanations for this, in the discussion later in this chapter. Importantly for the experiments still to come in this chapter, accuracy was not impaired, and while omissions overall were higher in GluA1$^{-/-}$ mice, they were not different to WT by stage 6.

4.6 5-CSRTT performance in GluA1$^{-/-}$ mice at a short stimulus duration

Introduction

Despite some surprising findings on 5-CSRTT performance, sustained attention was fairly well matched in GluA1$^{-/-}$ and WT mice at the end of training in Experiment 4.5. The aim of the next experiment was to train all animals over 5 sessions with a shorter SD, after reaching training criterion at stage 6. Up to this point animals were given as many training sessions as they required to meet criteria to progress. We now gave all animals an equal amount of training to compare differences in how they acquired a SD of 0.8s, having been trained to 1s SD previously.

Methods

Apparatus used was the same as described previously in Experiment 4.1. Subjects were the same as those described in Experiment 4.5.

Procedure

The procedure was similar to the final stage of training described in Experiment 4.5, however, the SD was reduced from 1s to 0.8s.

Analysis

Analysis was conducted in a similar manner to that described in Experiment 4.5.

Results

5-CSRTT Performance

Similar to the later stages of training in the previous experiment, GluA1$^{-/-}$ mice completed fewer trials in total than their WT counterparts, during training to a 0.8s SD (Figure 4.15A, $F_{(1,20)} = 10.669$, $p = 0.004$). There was no interaction with session as both groups remained stable over training ($F_{(4,80)} = 0.557$, $p = 0.694$).

Despite completing fewer trials GluA1$^{-/-}$ continued to show normal choice accuracy (Figure 4.15B, genotype: $F_{(1,20)} = 3.698$, $p = 0.069$). While this was approaching significance, it suggests that GluA1$^{-/-}$ are largely unimpaired. There was no effect of training, indicated by a lack of session effect or genotype*session interaction (session: $F_{(4,80)} = 0.893$, $p = 0.472$; genotype*session: $F_{(4,80)} = 1.103$, $p = 0.361$).

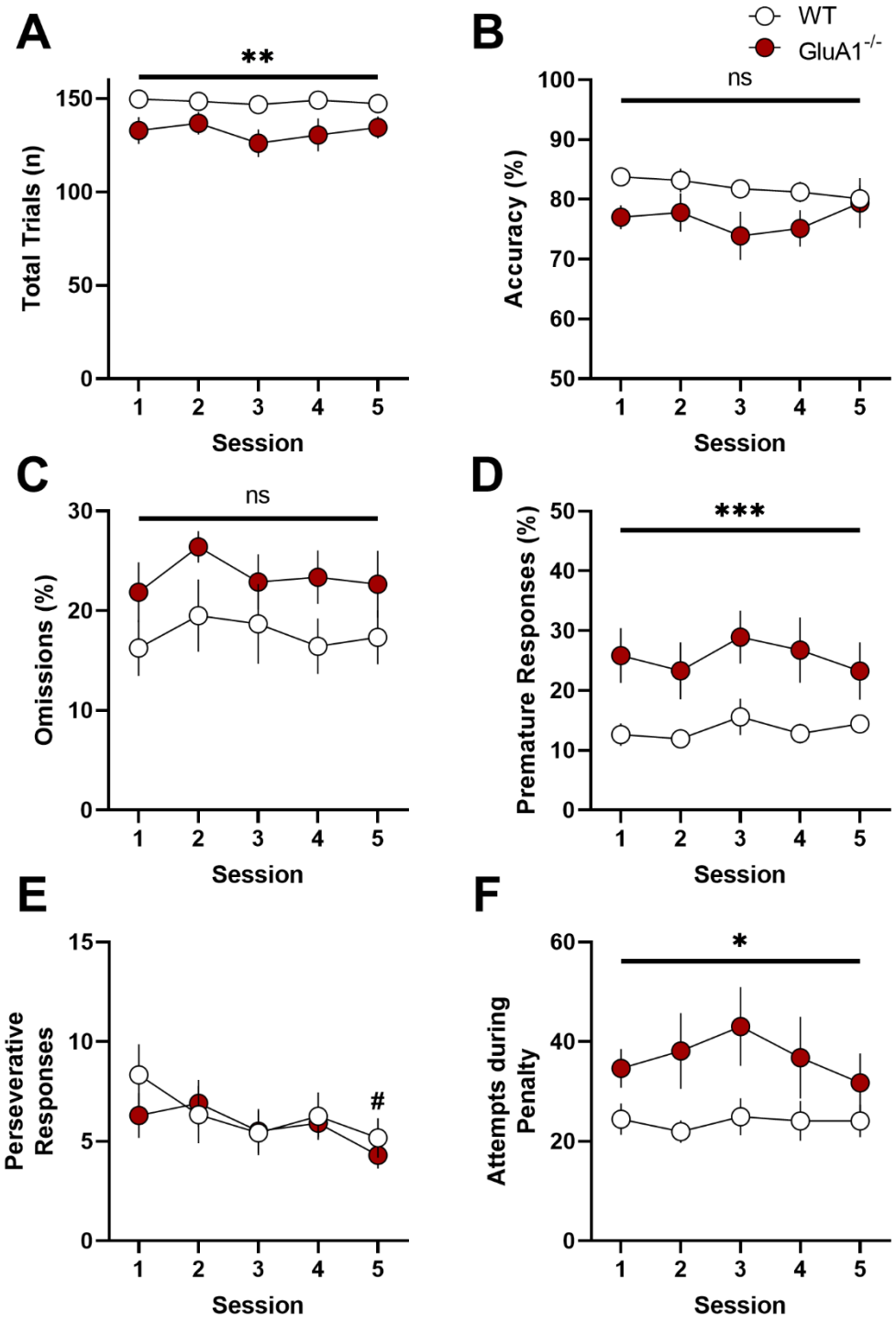

Figure 4.15 No difference in 5-CSRTT accuracy at a 0.8s SD in GluA1$^{-/-}$ mice but increased impulsivity. Across 5 sessions of acquisition to a 0.8s SD, GluA1$^{-/-}$ mice completed fewer trials (A) than WTs but choice accuracy was comparable between groups (B). No difference was seen in omissions (C) though GluA1$^{-/-}$ mice made more impulsive premature responses (D). GluA1 knockout had no effect on perseverative responding (E) though responding during a penalty was higher in these animals (F). All data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between genotypes. # $p < 0.05$ effect of training session.

The previous experiment found no change in omissions between groups in the last stage of training, as WT omissions increased to match levels seen in GluA1$^{-/-}$ mice. This trend continued here at a 0.8s SD, during which there was no effect of genotype on omissions, despite GluA1$^{-/-}$ mice appearing to be higher on this measure (Figure 4.15C, genotype: $F_{(1,20)}$ = 2.262, p = 0.148; genotype*session: $F_{(4,80)}$ = 0.257, p = 0.905). Premature responding remained higher in GluA1$^{-/-}$ mice, and stayed above WT levels across 5 training sessions, confirmed by a lack of session effect or interaction (Figure 4.15D, genotype: $F_{(1,20)}$ = 13.534, p < 0.001; session: $F_{(4,80)}$ = 0.731, p = 0.574; genotype*session: $F_{(4,80)}$ = 0.272, p = 0.895).

Perseverative responding after a correct choice continued to be indistinguishable between groups (Figure 4.15E, genotype: $F_{(1,20)}$ = 0.172, p = 0.682; genotype*session: $F_{(4,80)}$ = 0.666, p = 0.618). An effect of session, and significant *post-hoc* comparison of session 5 vs. session 1 of training suggests that animals became less persistent over continued training (session: $F_{(4,80)}$ = 2.681, p = 0.037; session 5 vs. session 1: $t_{(20)}$ = 3.286, p = 0.004). Responding during a punishment was higher in GluA1$^{-/-}$ mice (Figure 4.15F, $F_{(1,20)}$ = 6.012, p = 0.024). There was no session effect or interaction on penalty attempts (session: $F_{(4,80)}$ = 0.639, p = 0.636; genotype*session: $F_{(4,80)}$ = 0.586, p = 0.674).

Latency

In the previous experiment, correct and incorrect response latencies were indistinguishable between genotypes at the end of training. Here, GluA1$^{-/-}$ mice took longer to make a correct response, though incorrect latencies were unchanged (Figure 4.16A and B, correct: $F_{(1,20)}$ = 6.710, p = 0.017; incorrect: $F_{(1,20)}$ = 0.675, p = 0.421). An overall effect of session, but no genotype*session was seen on correct response times (session: $F_{(4,80)}$ = 3.550, p = 0.010; genotype*session: $F_{(4,80)}$ = 2.044, p = 0.096). A *post-hoc* comparison

investigating differences in correct latency between the first and last sessions of training indicate that animals actually became slower across 5 sessions of training ($t_{(20)}$ = 2.191, p = 0.042), mainly driven by slightly slower response times in GluA1$^{-/-}$ animals.

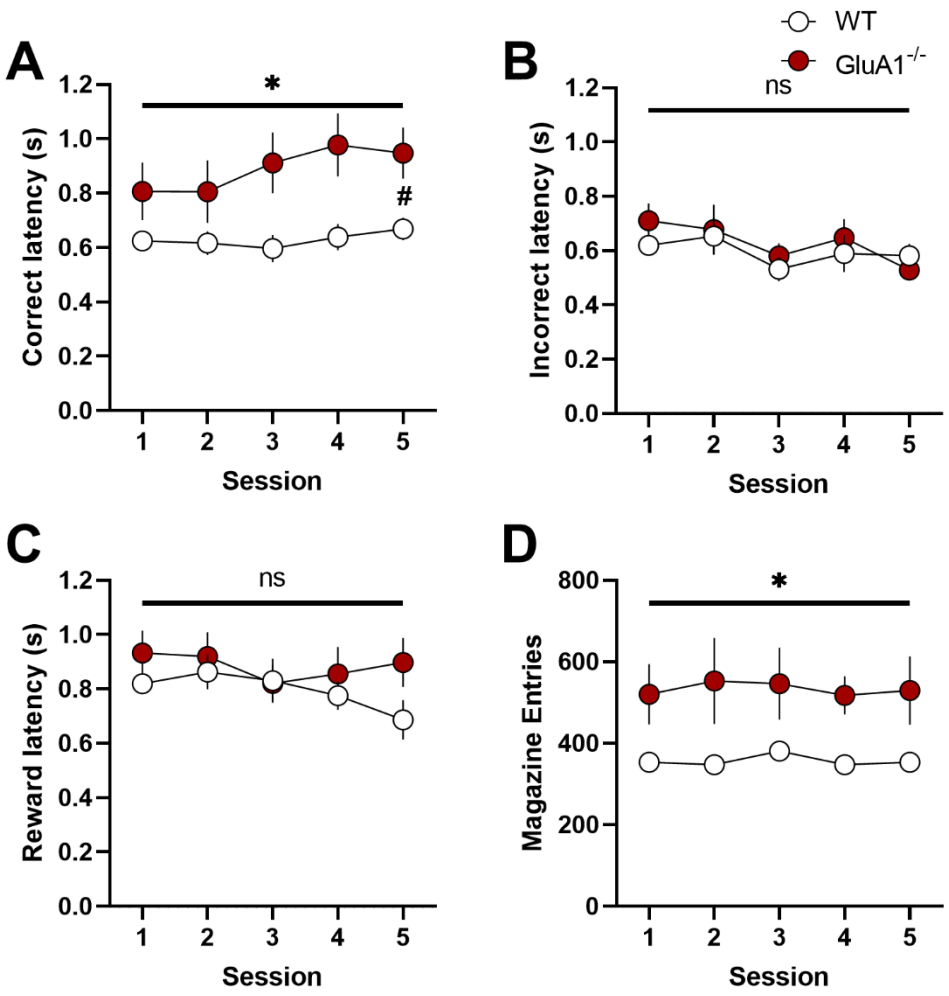

Figure 4.16 Delayed correct choice latency in GluA1$^{-/-}$ mice trained to a short SD. GluA1$^{-/-}$ mice took longer to respond when making a correct response (A) but not on trials where they responded incorrectly (B). Latencies to collect reward were similar between genotypes (C). Magazine entries remained much higher in GluA1$^{-/-}$ mice (D). All data are presented as mean ± SEM. * p < 0.05 between genotypes.

In contrast to the last experiment, where GluA1$^{-/-}$ mice were slower, no difference was seen between genotypes on reward latency (Figure 4.16C, genotype: $F_{(1,20)}$ = 1.888, p = 0.185; genotype*session: $F_{(4,80)}$ = 0.802, p = 0.527). Finally, as seen throughout training in

the previous experiment, magazine entries remained significantly higher in GluA1$^{-/-}$ mice (Figure 4.16D, genotype: $F_{(1,20)} = 7.731$, $p = 0.012$; genotype*session: $p > 0.9$).

Performance by stimulus location

As in our investigation of the previous cohort, we also compared performance on measures of sustained attention across the five choice ports (Figure 4.17). There was no interaction of genotype*poke on choice accuracy ($F_{(4,100)} = 0.0982$, $p = 0.983$). However, there was an overall main effect of poke suggesting animals were responding with higher accuracy at the centre port compared to other locations (poke: $F_{(4,100)} = 6.862$, $p < 0.001$), a slight difference to the behaviour observed in the previous cohort (Figure 4.8). There was no effect of poke, or any interaction of genotype*poke on omissions (Figure 4.17B, poke: $F_{(4,100)} = 1.886$, $p = 0.119$; genotype*poke: $F_{(4,100)} = 0.309$, $p = 0.871$).

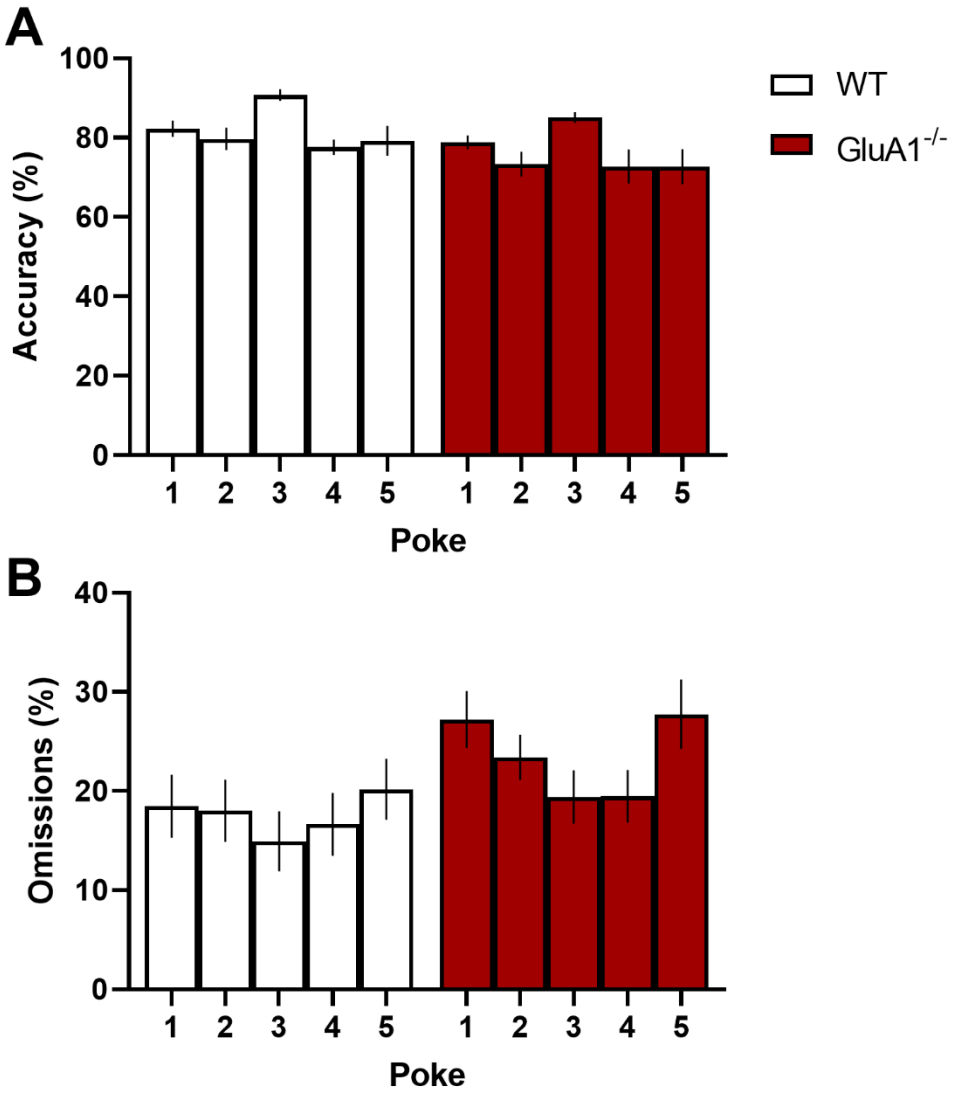

Figure 4.17 No effect of genotype on performance at individual stimulus locations. There was no effect of genotype on activity at different choice pokes (A). There was no effect of genotype on omissions at different locations although both groups seemed to miss fewer trials at pokes in the middle of the choice wall (B). All data are presented as mean ± SEM.

Discussion

In this experiment, we had aimed to see how responding changed during acquisition to the last stage of training; however, on most measures reported here performance stayed relatively stable over time. Overall, animals showed less compulsive responding, as seen with perseverative responses made after a correct choice, but this response measure was very low across training. It is difficult to make sense of what a small decrease in this measure late in training might mean at a psychological level. On the whole, the pattern of

behaviour seen in Experiment 4.5 was repeated here; GluA1$^{-/-}$ mice completed fewer trials overall and were more impulsive. There was a suggestion that these animals made more omissions, but this was not significant. GluA1$^{-/-}$ mice also appeared to be marginally lower in choice accuracy, but this was also not significant.

Whilst we have reported some mild genotype differences, basic attentional function in GluA1$^{-/-}$ mice was seemingly preserved. The findings so far suggest that top-down attention is relatively normal across the experiments described so far in GluA1$^{-/-}$ mice. We will continue to explore this over the next two experiments and discuss possible reasons for this effect in more detail at the end of this chapter.

4.7 Impaired 5-CSRTT attentional performance in GluA1$^{-/-}$ mice when a variable ITI is introduced

Introduction

In the introduction to this chapter we described how introducing unpredictability in the task, by modulating ITI length can exacerbate a mild attentional deficit and impair performance. This has previously been shown to reveal attentional deficits with amphetamine, where animals were not impaired on the standard version of the task (Balachandran *et al.,* 2018). We did not see the same result in our studies of amphetamine; however, performance was already significantly affected in amphetamine-treated mice even when task conditions were normal and predictable.

In Experiment 4.6, we saw subtle suggestions that GluA1 knockout can impair sustained attention in the 5-CSRTT. Omissions were higher, and choice accuracy was marginally lower, but neither of these effects were significant. In this next experiment, the aim was to

test whether introducing unpredictability into the task, by modulating ITI length, could exacerbate this seemingly mild attentional impairment in GluA1$^{-/-}$ mice.

Methods

Apparatus used was the same as described previously in Experiment 4.1. Subjects were the same as those described in Experiment 4.5.

Procedure

The task protocol for this experiment was similar to that described in Experiment 4.3, although the schedule for testing on this paradigm differed slightly due to the lack of pharmacological manipulation and subsequent requirement for washout days.

After baseline training, animals were tested for two sessions on a variable ITI; where 60% of trials remained at 5s ITI, 20% of trials were only 2s, and 20% of trials were 8s long. SD remained constant at 0.8s. To maximise the amount of data collected at each trial type, the limit of 150 trials was removed for these test sessions.

Analysis

Analysis was conducted in the same way as in Experiment 4.3 with two-way ANOVAs to compare the effect of varying ITI length between groups on measures of interest.

Results

5-CSRTT Performance

As seen in previous experiments with this cohort, total numbers of trials completed were significantly lower in GluA1$^{-/-}$ mice (Figure 4.18A, $F_{(1,20)} = 8.837$, $p = 0.008$). Contrary to earlier findings, however, when a variable ITI was introduced an impairment in choice

accuracy in GluA1$^{-/-}$ animals was revealed (Figure 4.18B, genotype: $F_{(1,20)} = 7.682$, $p = 0.012$). Changing the conditions of the task to make the ITI unpredictable appears to have exacerbated what appeared in the previous experiment to be a subtle deficit that was not statistically significant. An effect of ITI length was also seen, though this did not interact with the genotype effect (ITI: $F_{(2,40)} = 11.601$, $p < 0.001$; genotype*ITI: $F_{(2,40)} = 2.568$, $p = 0.089$). *Post-hoc* comparisons of short and long ITI lengths with a 5s ITI showed improved accuracy across both genotypes at 3s, but reduced accuracy when the ITI was extended to 7s (3s vs. 5s: $t_{(20)} = 4.105$, $p < 0.001$; 7s vs. 5s: $t_{(20)} = 2.468$, $p = 0.023$).

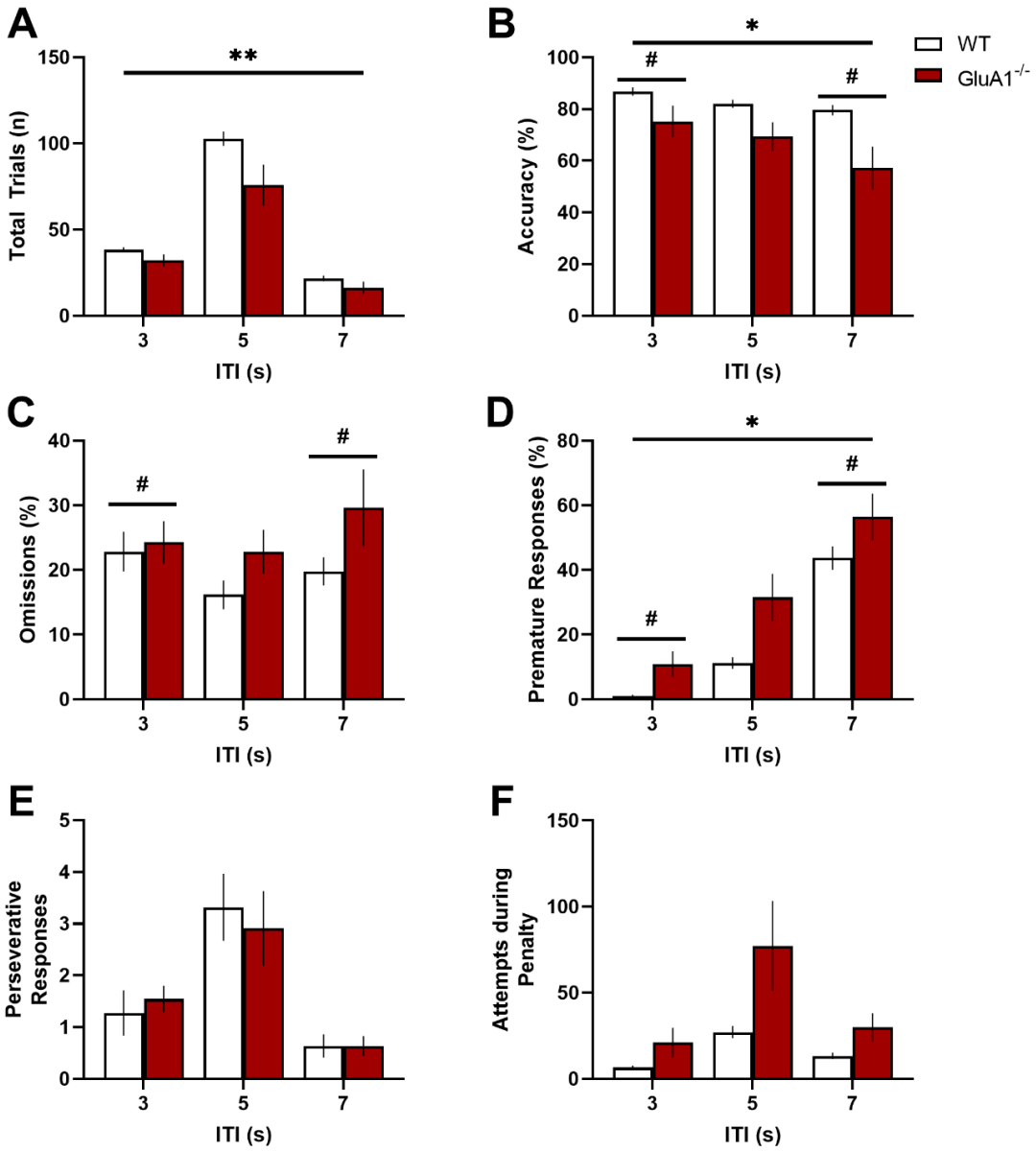

Figure 4.18 Manipulating ITI length reveals reduced decision accuracy in GluA1$^{-/-}$ mice in the 5-CSRTT. GluA1$^{-/-}$ animals completed fewer trials than WTs (A) and displayed reduced choice accuracy across all trial types (B). Both groups were more likely to omit a response when the ITI length was unpredictable (C). Premature responses increased with increasing ITI (D). GluA1 knockout had no effect on perseverative behaviours after reward (E) or during a punishment (F) at different ITI lengths. All data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ between genotypes. # $p < 0.025$ (Sidak-corrected for two tests) vs. 5s ITI.

Similar to the previous experiment using the same SD, no genotype difference was seen in number of omissions between groups, despite GluA1$^{-/-}$ mice appearing to omit more trials overall (Figure 4.18C, $F_{(1,20)} = 2.808$, $p = 0.109$). A significant effect of ITI length was observed, but no interaction (ITI: $F_{(2,40)} = 3.468$, $p = 0.041$; genotype*ITI: $F_{(2,40)} = 2.289$, $p = 0.114$). Animals were much more likely to miss a trial and register an omission, if the ITI

was different to the 5s they were trained to (3s vs. 5s: $t_{(20)} = 2.763$, $p = 0.012$; 7s vs. 5s: $t_{(20)} = 2.440$, $p = 0.024$). Importantly, given the lack of interaction, this was observed across both WTs and GluA1$^{-/-}$ mice, although it is possible that GluA1$^{-/-}$ mice were primarily driving the increased omissions seen with a 7s ITI.

Premature responding was, as before, much higher in GluA1$^{-/-}$ mice (Figure 4.18D, $F_{(1,20)} = 6.781$, $p = 0.017$). Premature responding was also significantly affected by ITI length, though no interaction was seen (ITI: $F_{(2,40)} = 143.387$, $p < 0.001$; genotype*ITI: $F_{(2,40)} = 2.196$, $p = 0.124$). *Post-hoc* investigation of the ITI effect revealed animals were significantly less likely to respond prematurely when the ITI length was reduced, but much more likely when the wait time between trials was increased to 7s (3s vs. 5s: $t_{(20)} = 7.207$, $p < 0.001$; 7s vs. 5s: $t_{(20)} = 11.928$, $p < 0.001$). This matches the result seen across treatment groups in Experiment 4.3 and likely reflects the decreased, or increased window to make a premature response with a 3s or 7s ITI respectively.

Perseverative responding was unaffected by genotype (Figure 4.18E, genotype: $F_{(1,20)} = 0.581$, $p = 0.455$; genotype*ITI: $F_{(2,40)} = 1.155$, $p = 0.325$). Genotype also had no impact on responding during timeout periods, although this result was close to significance (Figure 4.18F, genotype: $F_{(1,20)} = 3.654$, $p = 0.070$; genotype*ITI: $F(2,40) = 3.201$, $p = 0.051$). This seems to be driven by GluA1$^{-/-}$ mice expressing increased perseverative responding during a penalty. Because animals received different numbers of trials with short or long ITI lengths, the effect of ITI was not investigated here.

Latency

Latency to make a correct choice was unaffected by genotype (Figure 4.19A, $F_{(1,20)} = 1.811$, $p = 0.193$). ITI, however, did affect correct response time, independently of genotype (ITI:

$F_{(2,40)}$ = 8.233, p < 0.001; genotype*ITI: $F_{(2,40)}$ = 1.942, p = 0.157). *Post-hoc* tests confirmed an effect of short ITIs on response time but not a long ITI (3s vs. 5s: $t_{(20)}$ = 4.643, p < 0.001; 7s vs. 5s: $t_{(20)}$ = 1.273, p = 0.213). This longer latency to register a correct response on a short ITI may indicate the surprise of the trial starting earlier than animals have been trained to expect.

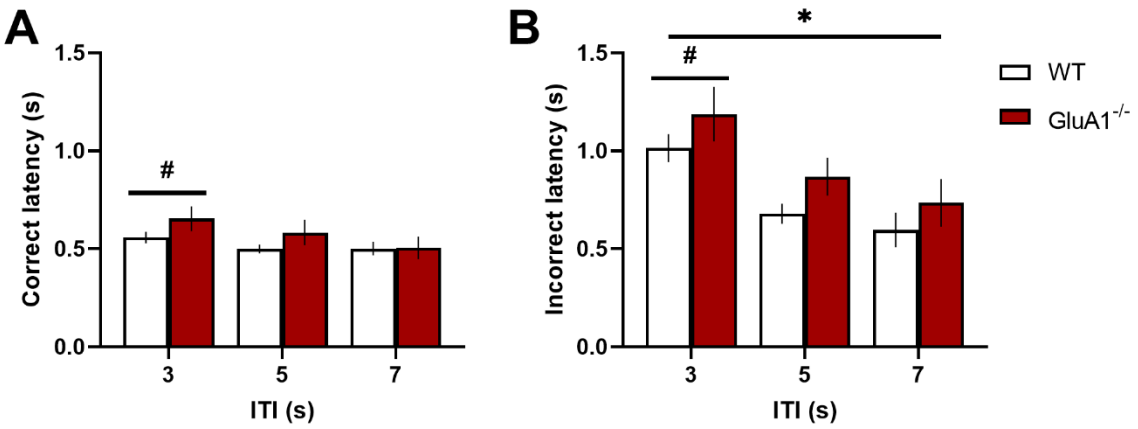

Figure 4.19 Delayed response latency in GluA1$^{-/-}$ mice when making incorrect responses. Correct choice response times were similar between genotypes but longer when the ITI was shorter than expected (A). Response times when an incorrect choice was made were also longer at a short ITI (B). Overall, GluA1$^{-/-}$ mice took longer to respond when they subsequently responded incorrectly. * p < 0.05 between genotypes. # p < 0.025 (Sidak-corrected for two tests) vs. 5s ITI.

Incorrect response times were affected by genotype, as well as ITI, though again there was no interaction (Figure 4.19B, genotype: $F_{(1,20)}$ = 6.182, p = 0.022; ITI: $F_{(2,40)}$ = 11.365, p < 0.001; genotype*ITI: $F_{(2,40)}$ = 0.005, p = 0.995). Animals took longer to respond on a trial preceded by a short ITI ($t_{(20)}$ = 5.094, p < 0.001), and on average these responses occurred after the stimulus light had turned off. This may indicate that in the case of an unexpectedly short ITI, animals sometimes fail to attend to the cue in time and nose-poke anywhere to try and avoid a punishment.

Discussion

Whilst the previous experiment found no, or at least only a mild, impairment in GluA1$^{-/-}$ mice under normal conditions, by manipulating ITI length here we have shown a deficit in focused attention in these animals. Accuracy was reduced across trials following all ITI lengths though this result appeared to be driven largely by unexpectedly long ITIs. Whilst omissions were unaffected significantly by ITI length, visually there was a subtle effect of genotype similar to that seen previously (Figure 4.15C).

These results so far point to an interesting divergence across studies. Whilst the historical dataset described in the chapter introduction showed potentially enhanced attentional processing in GluA1$^{-/-}$ mice, these last two experiments have shown a mild deficit, that is worsened when trial conditions are unpredictable. This behavioural test has a great degree of complexity, with a vast number of parameters or conditions that can be manipulated over 6-8 weeks of training and over multiple training stages. Differences in any one of these conditions of training could significantly impact how animals learn the task structure. We will consider possible reasons for this in the discussion at the end of this chapter.

4.8 5-CSRTT performance in GluA1$^{-/-}$ mice with a distracting auditory tone

Introduction

In the final experiment of this chapter, we wanted to investigate the effect of distracting auditory stimuli presented during the ITI. These distracting tones can disrupt attentional performance, dividing attention away from the task, and is something that we had predicted GluA1$^{-/-}$ mice may be particularly sensitive to, given the impaired self-generated priming discussed in Chapter 2. If GluA1$^{-/-}$ mice express sensitisation to these stimuli, they

may pay aberrantly high levels of attention to the auditory tones, disproportionately impacting task performance.

We previously showed very weak effects of distracting stimuli when used to investigate pharmacological manipulations (Figure 4.11). In part, this may have been due to splitting trials over four conditions, with the distractor being presented at 1, 3, 4.5s or not presented. Distractors have previously been said to be most effective when presented immediately prior to stimulus onset (Bari *et al.,* 2008), and we saw similar suggestions in our earlier study. While there is an argument, therefore, for limiting trials to no distractor, or distractors presented at 4.5s, we have not done that in this instance. An important aspect of this chapter was to draw comparisons across the models used throughout this work, and so conditions were matched as much as possible.

The aim of this experiment was to investigate the attentional deficit seen so far in GluA1$^{-/-}$ mice, and in particular to see whether these animals are particularly sensitive to unexpected auditory stimuli presented in the ITI. By completing this attentional profiling study of GluA1$^{-/-}$ mice, we can also compare the phenotype observed with GluA1 knockout to that reported with pharmacological manipulation described earlier in the chapter.

Methods

Apparatus used was the same as described previously in Experiment 4.1. Subjects were the same as detailed in Experiment 4.5.

Procedure

As described in the previous experiment, animals received at least 1 baseline session on stage 6 of training in between tests of different task parameter manipulations.

The task design for this experiment was the same as that described in Experiment 4.4. There were four trial types; no distractor, or distractors at 1, 3 or 4.5s into the ITI. The distractor used was 0.5s white noise at 80dB. There was an even distribution of trial types; 25% of each across a session. Animals were tested on this task over two sessions. No differences were seen in performance when only looking at the first session of testing with distractors. To maximise the amount of data collected at each trial type, the limit of 150 trials was removed for these test sessions.

Analysis

Analysis was conducted in the same way as in Experiment 4.4.

Results

5-CSRTT Performance

As seen throughout the previous experiments with these animals, GluA1$^{-/-}$ mice completed fewer trials than WTs (Figure 4.20A, $F_{(1,20)} = 14.917$, $p < 0.001$). Additionally, a deficit in choice accuracy was again observed in GluA1 knockouts (Figure 4.20B, $F_{(1,20)} = 27.989$, $p < 0.001$). This effect was independent of the presence of distractors (distractor: $F_{(3,60)} = 1.346$, $p = 0.268$; genotype*distractor: $F_{(3,60)} = 1.446$, $p = 0.238$).

No effect of genotype, or distractor cues, was observed on omitted responses (Figure 4.20C, genotype: $F_{(1,20)} = 0.822$, $p = 0.375$; distractor: $F_{(3,60)} = 0.644$, $p = 0.590$; genotype*distractor: $F_{(3,60)} = 0.193$, $p = 0.901$). There was an effect, as seen in earlier experiments, of premature responses (Figure 4.20D). Significantly higher premature responding was seen in GluA1$^{-/-}$ mice, but we also saw an interaction with distractors on this measure (genotype: $F_{(1,20)} = 23.941$, $p < 0.001$; distractor: $F_{(3,60)} = 2.496$, $p = 0.068$;

genotype*distractor: $F_{(3,60)} = 5.197$, $p = 0.003$). *Post-hoc* comparisons of this effect revealed higher premature responding selectively in GluA1$^{-/-}$ mice when the distractor was presented immediately before stimulus onset (GluA1$^{-/-}$ 4.5s vs. no distractor: $t_{(20)} = 2.986$, $p = 0.007$). When investigating differences between genotypes, for each trial type, GluA1$^{-/-}$ mice expressed increased premature responding across all conditions (all $p < 0.001$).

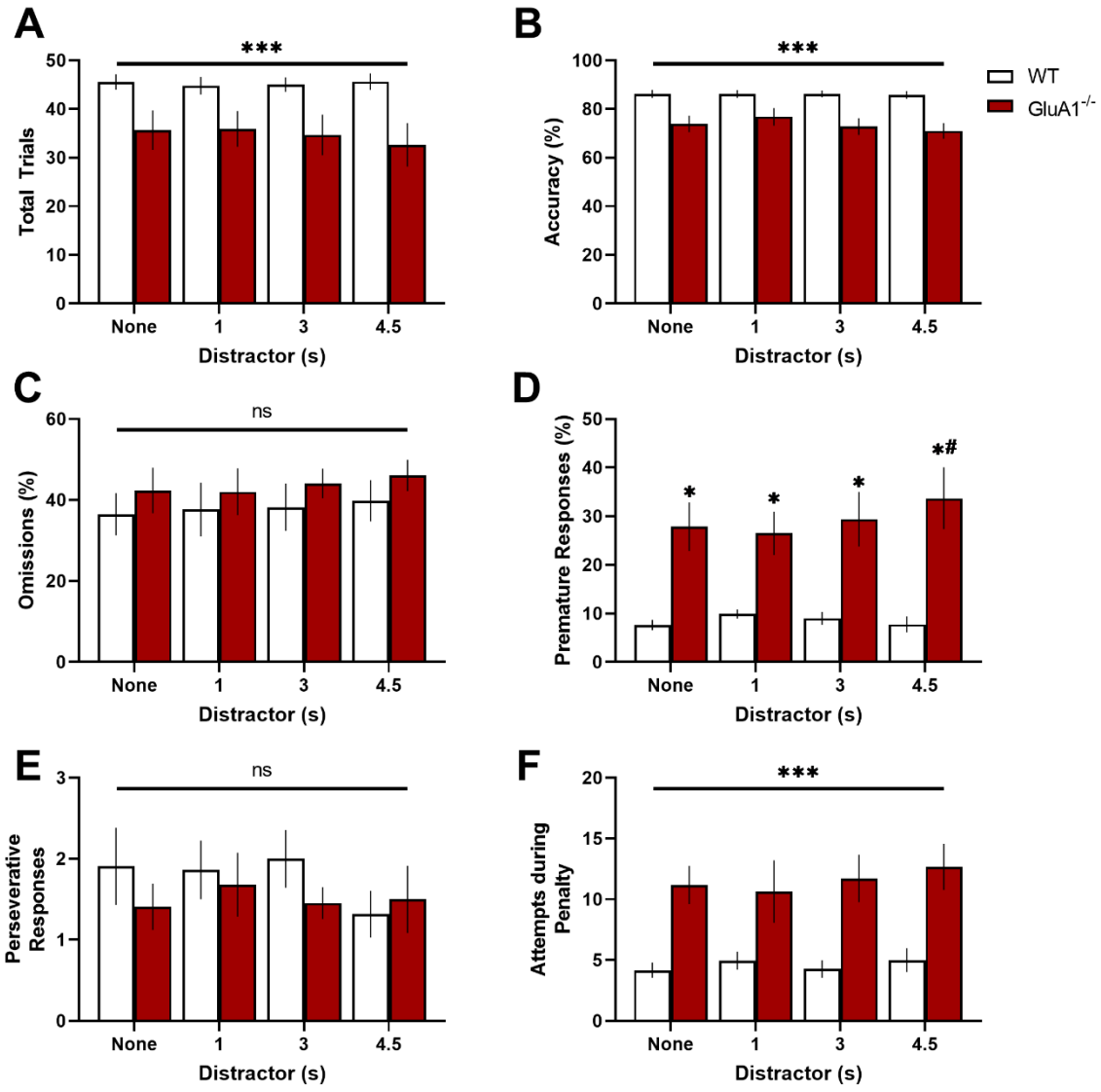

Figure 4.20 Distracting auditory cues impaired attentional performance and increased impulsivity in GluA1$^{-/-}$ mice. GluA1$^{-/-}$ mice completed fewer trials than WTs (A). There was no effect of distractors on choice accuracy (B) or numbers of omissions (C), though accuracy was reduced overall. Premature responding was higher in GluA1$^{-/-}$ mice when a distractor was presented late in the ITI period (D). There were no differences between genotypes or distractor trials on perseverative responding (E), but an increase in penalty responses in GluA1$^{-/-}$ mice (F). All data are presented as mean ± SEM. * $p < 0.05$ (or Sidak-corrected value), *** $p < 0.001$ between genotypes. # $p < 0.0085$ (Sidak-corrected for six tests) vs. no distractor.

There was no effect of genotype, or distractors, on perseverative responding after a correct choice (Figure 4.20E, genotype: $F_{(1,20)} = 1.426$, $p = 0.246$; distractor: $F_{(3,60)} = 0.632$, $p = 0.597$; genotype*distractor: $F_{(3,60)} = 0.268$, $p = 0.848$). There was, however, a genotype effect on penalty responses, but once again distractor had no effect on this behaviour (Figure 4.20F, genotype: $F_{(1,20)} = 15.809$, $p < 0.001$; distractor: $F_{(3,60)} = 1.211$, $p = 0.314$; genotype*distractor: $F_{(3,60)} = 0.851$, $p = 0.472$).

Latency

There was no observable effect of either genotype or distractor on latency to make a correct response (Figure 4.21A, genotype: $F_{(1,20)} = 0.509$, $p = 0.484$; distractor: $F_{(3,60)} = 0.990$, $p = 0.404$; genotype*distractor: $F_{(3,60)} = 1.366$, $p = 0.262$).

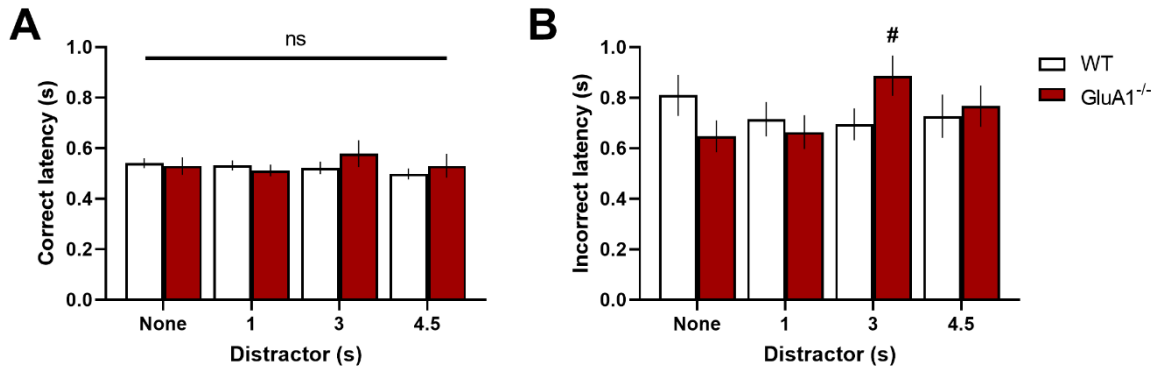

Figure 4.21 Subtle effect of distracting cues on response latency in GluA1$^{-/-}$ mice. There was no effect of genotype or distractors on correct choice latency (A). Incorrect choice latency was increased in GluA1$^{-/-}$ mice but only when a distractor was presented 3s into the ITI (B). All data are presented as mean ± SEM. # $p < 0.0085$ (Sidak-corrected for six tests) vs. no distractor.

While there was also no main effect of genotype or effect of distracting tones on incorrect response latency, there was an interaction across these effects (Figure 4.21B, genotype: $F_{(1,20)} = 0.153$, $p = 0.700$; distractor: $F_{(3,60)} = 1.533$, $p = 0.215$; genotype*distractor: $F_{(3,60)} = 4.871$, $p = 0.004$). This interaction was driven by an effect of a distractor presented at 3s

into the ITI, increasing GluA1$^{-/-}$ incorrect response latency compared to trials where there was no distractor ($t_{(20)} = 3.470$, $p = 0.002$).

Discussion

Similar to the findings of pharmacological manipulations in Experiment 4.4, the effects of distractors on GluA1$^{-/-}$ performance were somewhat unclear. While we observed a reduction in choice accuracy similar to that observed with a variable ITI, there was no interaction with distractors, suggesting these auditory stimuli may have had a more general effect on behavioural performance over the entire session. The most obvious effect here was an increase in premature responding in GluA1$^{-/-}$ subjects when a stimulus was presented just before the trial started. It may be that distracting stimuli generate a burst of arousal, particularly in GluA1$^{-/-}$ mice that induced increased impulsivity, or hindered inhibitory control of responding.

These studies on distracting stimuli have shown some promise at revealing interesting effects, in GluA1$^{-/-}$ mice but also with amphetamine and MK-801. However, for future studies it would be sensible to limit tones to those presented at 4.5s into the ITI, when they appeared to have the most significant impact on performance.

Discussion

Impaired 5-CSRTT performance following amphetamine treatment

Previous studies of amphetamine on sustained attention in the 5-CSRTT have reported mixed results; while some suggest there is little, if any, effect of amphetamine (Cole and Robbins 1987; Harrison *et al.,* 1997), others have shown a dose-dependent decline in choice accuracy (Baarendse and Vanderschuren 2012; Fletcher *et al.,* 2011). Others have

suggested there is little effect on choice accuracy under predictable conditions, but impairments can be exacerbated by introducing variability into the task, by manipulating ITI length (Balachandran *et al.,* 2018). In this chapter, we wanted to generate our own data, at a dose of amphetamine used throughout this thesis (2.5mg/kg) to compare alongside other manipulations of interest. This was important to do, given previous studies have generally been in rats and at a range of different doses of amphetamine.

Somewhat surprisingly, under normal task conditions we saw a striking decrease in choice accuracy when treated with amphetamine; over 20% lower correct responses than when treated with saline. Subsequent testing when the ITI was manipulated, or in the presence of auditory distractors failed to further impact the performance deficit induced by amphetamine on baseline performance. The lack of interaction with ITI suggests that amphetamine-induced impairments on normal task performance could not be further exacerbated through introducing unpredictable testing conditions. There were also no convincing interactions with distractors, indicating that auditory stimuli had very little effect on sustained attention following amphetamine treatment. This suggests that novel events during testing had little effect on top-down control of sustained attention after amphetamine treatment, potentially indicative of a floor effect of treatment.

Deficits in performance seen here may have been caused by severely affected impulsivity, and reduction of inhibitory control, rather than an inability to discriminate or attend appropriately to stimuli. On average, amphetamine-treated mice exhibited much higher premature responding, were faster to make a response, but were comparable on rates of omissions to controls. While in some circumstances increasing arousal can be beneficial to cognition and attention, this was not the case here (Yerkes and Dodson 1908). It may be

that hyperarousal following amphetamine increased impulsivity and impaired inhibitory control to the point that animals could not appropriately express sustained attending to the choice ports during a trial.

Mixed effects of impaired self-generated priming on sustained attention

Overall, reports in the literature on the effect of MK-801 on sustained attention suggest it leads to reduced choice accuracy, increased omissions and increased premature responding (Barlow *et al.,* 2018; Smith *et al.,* 2011). The results of this chapter are entirely consistent with these findings, an important replication given that most reported studies are in rats and all experiments here used mice. MK-801 also had subtle effects on response latencies (though not on overall magazine entries suggesting they were still food-motivated). This could be a result of a mild ataxia, but could also be because they were more easily distracted, or engaged with other components of the box and therefore further from the 5-choice wall when a trial started, an explanation that could also account for the increased omissions. It is possible then, that MK-801 affected top-down control of attention, though whether this is due to the effects of hippocampal NMDAR blockade, or extrahippocampal receptors is impossible to say from these results alone. Coherence between the hippocampus and prefrontal cortex has been shown to play an important role in modulating attention (Tan *et al.,* 2018), indicating one possible pathway through which NMDAR antagonism may impair 5-CSRTT performance.

GluA1$^{-/-}$ performance was fairly well matched with WTs across training, there were no overt differences in omissions or choice accuracy by the end of training, although GluA1$^{-/-}$ mice were consistently more impulsive than controls. However, differences between genotype groups were observed when the task was made unpredictable, through the addition of a

vITI. Across two testing sessions on a vITI GluA1$^{-/-}$ mice had reduced choice accuracy, and were slower to make a response when they went on to respond incorrectly (no difference was seen on correct trials). These results suggest that the ability to focus attention on the 5-CSRTT is relatively well preserved in GluA1$^{-/-}$ mice, and that top-down control of behavioural responding is intact, but that added unpredictability can disrupt attentional processes. Interestingly, choice accuracy in GluA1$^{-/-}$ mice was lower across all ITI lengths, including 5s (which animals were previously trained to expect as the ITI length), suggesting that this unpredictability on some trials affects performance more globally across the entire session.

A similar result was seen on the effect of distractors, during which GluA1$^{-/-}$ performance was worse across all trial conditions, even now when there was no distractor present. Whilst there was little interaction of distractors with measures of performance, they did appear to also make GluA1$^{-/-}$ mice more likely to respond prematurely, specifically when white noise was presented just before cue onset. Given the relatively small testing chamber size, and limited capacity for expression of different behavioural repertoires, GluA1$^{-/-}$ mice may have been more likely to respond to the distracting stimulus by responding at the task wall as they had been trained to.

The results of our GluA1$^{-/-}$ study presented across Experiments 4.5-4.8 are very different to the findings of a previous study conducted by a postdoctoral researcher in the Bannerman group (Schneider, unpublished). However, this task required months of training, and has a wide array of parameters that can be manipulated throughout training that could affect learning and performance. Furthermore, the two experiments were conducted in entirely different testing environments with a different reward type. Omissions in the earlier

Schneider study were much higher than those reported in our own cohort, and higher than those seen in other studies conducted with the same testing equipment at the University of Oxford (Barkus *et al.,* 2016). Omissions were also higher than in other 5-CSRTT studies in the literature using mice (Oliver, Ripley, and Stephens 2009; Wrenn *et al.,* 2006). Differences between the two studies described in this chapter could in part reflect the trial initiation step that we incorporated to minimise omissions, but this does not explain why other researchers in Oxford have also seen fewer omissions. It could be that animals were older, and fatter, and quicker to satiate in this earlier GluA1$^{-/-}$ experiment. The high rate of omissions seemed to be particularly driven by males in the WT group, where omissions were on average 10% higher at later stages of training than in females. Whilst it was surprising to some extent that we did not replicate the enhanced performance in GluA1$^{-/-}$ mice in our own experiments, there are a number of potential differences in the way that these studies were run that could have affected this, that we will consider below.

Another key aim of this chapter was to compare altered attentional processing across manipulations of interest that we have been investigating throughout this thesis. Seemingly GluA1$^{-/-}$ performance was largely intact, at least on the baseline level of performance, whilst a different model of impaired self-generated priming – MK-801 – exhibited profoundly impaired choice accuracy and high rates of omissions. However, this highlights the difficulty in comparing an acute pharmacological manipulation in previously well-trained normal animals to a constitutive knockout of a receptor subunit across an animals' entire lifetime, when animals required months of training to complete training on this task. GluA1 knockout could have profound effects on the learning experience during any of the different stages of acquisition, yet MK-801 was only administered at test, highlighting an obvious difference in the way these studies were run. This could potentially

be overcome through hippocampal administration of an antibody transiently blocking GluA1-dependent AMPAR function, in otherwise normal control animals, only at the point of testing (Farhy-Tselnicker *et al.,* 2017). This manipulation has been shown to impair short-term memory performance in C57Bl/6j mice (Lee, unpublished). This is an interesting consideration for future experiments where it would be important to investigate the effects of reduced GluA1-containing AMPAR-dependent function specifically during test stages. Furthermore, if implanting hippocampal cannulae for the administration of a GluA1-Fab antibody it would also be interesting to investigate the effect of hippocampal-specific NMDAR blockade, through infusion of MK-801. We may expect to see different effects of MK-801 if the effect of NMDAR blockade in extrahippocampal regions like prefrontal cortex were absent (when compared to systemic drug administration).

Other measures of 5-CSRTT performance

One interesting finding of the current set of experiments was that performance seemingly varied by choice port, and while there was some variation between cohorts/manipulations, accuracy was marginally lower at ports 2 and 4 – which were raised slightly above the other ports (Figure 4.2). Whilst it could be argued we would want consistent performance across all ports, it is not inherently a bad thing if certain ports are perceived as more difficult to attend and respond to than others. This could have exacerbated some of the deficits seen in these studies; both amphetamine- and MK-801-treated mice were impaired in choice accuracy but this impairment was more extreme at these ports positioned higher on the choice wall (Figure 4.8).

In the final test conducted with both cohorts, we wanted to investigate the effect of auditory distractors on task performance, previously shown to increase response latencies

and incidences of premature responding (Bari *et al.,* 2008). That is largely consistent with what we saw in this chapter, though we did not see any obvious interaction of distractors with treatment groups. Distractors presented at 1s or 3s into the ITI period seemingly had limited impact on performance. In future studies it would be interesting to investigate distractors when presented much more sporadically throughout the session such that they remain surprising, but also limit them to just before cue onset when they seemed to be most effective.

Limitations of the 5-CSRTT experiments

Due to the time commitment required to train and test a cohort of animals on the 5-CSRTT – 3-4 months after all testing manipulations were completed – and the need for consistency both across and between studies in which we want to draw comparisons, there was limited scope for re-evaluation of testing conditions after we had started training. Testing parameters could have been optimised in a number of ways that may have facilitated acquisition, particularly for GluA1$^{-/-}$ mice. However, we did not want to change testing parameters mid-training without a strong scientific reason, and after completion of the C57Bl/6j 5-CSRTT study, we wanted to keep parameters as consistent as possible in the GluA1$^{-/-}$ cohort in order to compare these animals with pharmacological manipulations. Ideally we would train new cohorts of animals with a number of revisions to the methods, but the experiments in this chapter took over 6 months to complete and a second round of testing was sadly not possible here.

During training of the C57Bl/6j cohort (Experiment 4.1), we decided at stage 5 to incorporate an extra step into the training protocol – such that animals had to reinitiate the next trial after a punishing timeout. This was important because animals were trained

over 60-minute sessions and would naturally have periods of inactivity in that time. Adding this measure helped to reduce the number of omissions, that were artificially inflated by animals not being able to sustain attention solely on the task, or not remaining highly motivated, over this timeframe.

However, there were a number of other measures that we considered including, particularly as we commenced training of the GluA1$^{-/-}$ cohort, but decided against as it would have made it harder to compare GluA1$^{-/-}$ performance to that of MK-801- or amphetamine-treated mice.

Firstly, animals would sometimes move their head in the magazine whilst consuming rewards such that it restored the IR beam and this was then recorded as a head exit, initiating the ITI, despite animals still being engaged with the magazine. As such, animals would occasionally miss trials because they were still consuming reward. For future studies, it would be sensible to incorporate a small hold period, before the ITI is triggered, after a head exit event, to ensure that this is a genuine behaviour.

On the opposite side of the chamber, animals would sometimes accidentally register a response at the task wall, something that could have been minimised by introducing a hold period during which animals have to stay in a port for 50-100ms before it is registered as a choice. When anticipating a trial to start, animals would approach the ports, and because the IR beam is proximal to the port entrance, a very subtle, transient, entry could be registered as a choice. This could seem an unfair punishment to an animal that was exploring but not trying to respond at a port, and it would likely have been unclear as to why they were not able to obtain rewards at that time, potentially slowing acquisition of the task. Adding a noise to confirm a response had been made – like a click – may have

further reduced ambiguity for the animal around whether a response had been made (though this would have to be subtle enough to not be comparable to a distracting stimulus). These changes may also rescue the impaired choice accuracy seen following amphetamine treatment, by reducing ambiguity around when a response has been made and punishing anticipatory approaches to the 5-choice wall less strictly.

Conclusions

Overall, our studies of pharmacological manipulations appear to be broadly consistent with that seen in the literature. Deficits in amphetamine accuracy may reflect aberrantly high arousal and increased impulsivity, rather than a deficit in directed attention. Studies investigating GluA1$^{-/-}$ mice found attentional performance to be relatively well preserved, but mild deficits were worsened by the introduction of unpredictability through vITI length, as well as through the addition of auditory distractors, although effects extend throughout entire sessions independent of the nature of individual trials.

Chapter 5: Effect of GluA1 knockout on associative learning processes

Introduction

Attention and associative learning in GluA1$^{-/-}$ mice

We have so far described the GluA1$^{-/-}$ model as one of impaired self-generated priming, in which there is an increased likelihood that elements representing a stimulus will be in A1 compared to WT counterparts, and this leads to inappropriate behavioural responses, at least in a novel environment. This has been confirmed in tasks of short-term habituation in Chapter 2, during which animals became sensitised to repeated or prolonged exposures to novel stimuli. This leads to potentially important predictions about how these animals go on to learn about stimuli and form associations when environmental events or features are aberrantly salient. We will now explore this in more detail in this final experimental chapter.

The importance of attention paid to a stimulus was emphasised in one of the earliest models of associative learning, conceptualised by Nick Mackintosh (1975). He incorporated attention into a model of associative learning by arguing that the amount of learning on any given trial depends not just on the salience of the CS, but the amount of attention paid to it. Given our results so far on studies of the GluA1$^{-/-}$ mouse model, it seems likely that these animals could exhibit an increased propensity for the formation of associations between stimuli due to aberrantly high attending to events in their environment.

This could be particularly important when considering the construct validity of this model as a research tool for studying psychosis. In the aberrant salience framework of psychosis, delusions have been suggested to arise as a top-down cognitive attempt to explain maladaptively formed associations between events or features of an individual's environment that have become aberrantly salient (Kapur 2003). While it would be very difficult to prove the existence of positive symptoms of psychosis in GluA1$^{-/-}$ mice, evidence of aberrant salience and altered, maladaptive associative learning would go a long way to provide support for this model as a tool to study psychosis. There is some evidence that GluA1$^{-/-}$ form associations more readily than WT mice from earlier studies, looking at long-term habituation in the spatial novelty preference Y-maze (Sanderson *et al.,* 2009).

We can think about how aberrant salience may affect associative learning by considering what would happen in Wagner's model of SOP (Wagner 1981) during conditioning when self-generated priming is impaired. Under normal circumstances, excitatory associations are formed by elements of stimuli that are active together in A1 at the same time (and weakly from elements co-occurring in A2 (Aitken and Dickinson 2005)). This is a vital feature of associative learning used to explain how one learns about a CS-US representation. When the CS and US occur together in close temporal proximity, elements of each stimulus are activated to A1 and excitatory associative learning occurs. However, if elements have an increased likelihood of being active in A1 due to impaired synaptic plasticity blocking self-generated priming, this could lead to maladaptive learning about stimuli that persist in attention. For example, physiologically irrelevant stimuli that would normally be habituated to, may take on meaning simply because they persist in attention for longer than appropriate. This meaning may arise through top-down attempts to explain these salient events.

Our results so far in GluA1$^{-/-}$ mice, of increased attending to novel stimuli which can lead to stimulus-specific sensitisation hint at a possible route to maladaptive formation of associations in this model. This is something we have investigated in this chapter by manipulating task conditions in a recognised assay of associative learning.

Sensory preconditioning as a test of associative learning

Sensory preconditioning (SPC) has been used for decades as a measure of stimulus-stimulus associative learning about neutral stimuli, before they go on to be linked with meaningful outcomes (Brogden 1939; Sharpe, Batchelor, and Schoenbaum 2017; Thompson 1972). In this paradigm, two stimuli are initially paired together in a preconditioning phase, where they are presented over multiple or prolonged exposures, in the absence of a rewarding or aversive outcome. This is similar to the initial pre-exposure that occurs during LI, but exposure is much lower in SPC such that exposure doesn't completely extinguish any engagement with preconditioned stimuli.

Following preconditioning, one of these stimuli are then paired with either reward (e.g., sucrose pellets) or alternatively, with an aversive stimulus (e.g., lithium chloride injection) in a conditioning stage, such that this preconditioned stimulus takes on the properties of a CS. In this chapter we have focused on sequentially presented auditory tones (e.g., A → B) as the preconditioned stimuli, later paired with reward (e.g., B+).

In the test phase of this paradigm, animals are tested on their conditioned responding to the preconditioned stimulus (tone A in this example) that has never been paired with the US. Since A has never been conditioned as a CS, it should elicit no behavioural response. However, because of the association formed between A and B during preconditioning, and

subsequent associative learning about the B-US relationship in the second stage, tone A takes on the properties of the CS and anticipatory reward-related responding is observed.

One of the main advantages of this test over other forms of conditioning is that it assesses the formation of associations in the absence of any motivational valence that may alter top-down processes driving attention and learning. Here we have looked at SPC performance in GluA1$^{-/-}$ mice, with the prediction that increased attending to stimuli during the preconditioning phase may change association formation between tone-pairs. GluA1$^{-/-}$ mice have been shown to acquire a conditioned discrimination between a CS+ and CS- at a similar rate to WTs (Sanderson *et al.,* 2017). Interestingly, in this study, Sanderson *et al.* found impaired inhibitory associative learning when a delay was introduced between CS and US presentations. However, it is still not entirely clear how these animals learn about their environment and form excitatory associations in the absence of meaningful outcomes, such as in the preconditioning stage of SPC.

To test associative learning in GluA1$^{-/-}$ mice, we have looked at manipulating task parameters of SPC, using more “simple” or “complex” conditions across the first two stages of the task (Table 5.1). By this we mean the number of cues that must be attended to in a given stage.

	Preconditioning	Conditioning	Probe Test
Simple-Simple	A → B	B → ●●	A C
Simple-Complex	A → B	B → ●● D	A C
Complex-Simple	A → B C → D	B → ●●	A C
Complex-Complex	A → B C → D	B → ●● D	A C

Table 5.1 Experimental design for different versions of the sensory preconditioning task. ●● indicates delivery of two fruit punch flavoured sucrose pellets.

In the "simple" paradigm, there is just one stimulus-pair during preconditioning; A → B. Animals were then conditioned to a single cue, paired with reward, B → ●●. In the probe test they were then tested on their conditioned responding to the preconditioned cue A, versus a novel cue, C, that had never been experienced before and should, in theory, elicit no behavioural response.

In the "complex" version of SPC, there were multiple cue-pairs to learn about. During preconditioning, animals were preconditioned to two separate auditory cue-pairs (A → B, C → D). Animals were then conditioned to a discrimination between rewarded and unrewarded stimuli (B → ●●, D). In the probe test animals were tested on their responding to the two preconditioned stimuli; one that had been preconditioned to a cue later paired with reward, and one that hadn't (A vs. C). Separate groups of animals were also tested on the two intermediary versions, with simple conditions in one stage of the task, and complex in the other, to complete this profile of associative learning and determine the contribution of each stage to maladaptive associative learning.

Aims

In this chapter we have described efforts to investigate differences in associative learning between GluA1$^{-/-}$ and WT mice, with the prediction, based on earlier findings of this thesis, that GluA1$^{-/-}$ mice may elicit an increased propensity for association formation. To test this we have investigated SPC performance across a range of conditions, manipulating the task complexity and therefore the number of different events in an animals' environment that they must attend to. By testing animals on their capacity to form associations, we can integrate these results with earlier findings on habituation and attention to determine the translational value of GluA1$^{-/-}$ mice as a model relevant to understanding clinical psychosis.

Experiments

5.1 Validation of an appetitive sensory preconditioning protocol in control mice

Introduction

We first wanted to pilot testing parameters for a complex SPC task in-house, using control mice, to confirm that we could detect an effect of SPC during the probe test. There is a high degree of psychological complexity involved in this paradigm and so this represents an important validation step prior to conducting any experiments with GluA1$^{-/-}$ mice. For example, in the preconditioning stage of the task, animals must be sufficiently pre-exposed to tone-pairs in order to form an association between them, but not overly exposed to the extent that they no longer engage an animals attention, which may instead lead to LI. Additionally, during conditioning, it is important that animals acquire the conditioned response to the CS+, or discrimination between rewarded and unrewarded CSs, but if the

period of time between preconditioning and the probe test is too long, or the amount of exposure to B in the absence of A is too high, the effects of SPC may dissipate.

The aim of this experiment was therefore to confirm the effect of SPC in a cohort of control mice under complex conditions; using two tone-pairs during preconditioning and training animals to discriminate between a CS+ and CS- during conditioning. If these animals were able to express SPC during the probe test, we would then go on to test GluA1$^{-/-}$ mice against controls, in the full 2x2 factorial experimental design outlined in Table 5.1. To do this, we have used heterozygous GluA1$^{+/-}$ mice in this pilot study, shown previously to behave similarly to WT controls (Wiedholz *et al.,* 2008).

Methods

Subjects

14 male heterozygous GluA1$^{+/-}$ mice were used in this experiment.

Apparatus

Experiments were conducted in eight identical operant testing chambers (ENV-307A, Med Associates), contained in sound-attenuating chambers (ENV-022V, Med Associates). Each chamber was equipped with a pellet dispenser, dispensing 14mg fruit punch flavoured sucrose pellets (TestDiet). Pellets were dispensed to a recessed magazine in the wall of the operant chamber, fitted with an IR emitter and photocell receiver to measure entries and duration spent in the magazine. Auditory cues were presented during testing via a speaker and a clicker on the wall opposite the food magazine, at approximately 80dB. The stimuli used were 2Hz clicker, white noise, 3kHz steady tone, and a 5Hz siren, counterbalanced

across animals (Table 5.2). A houselight in each operant box, and fan in each sound-attenuating chamber was turned on during sessions.

Counterbalancing Groups	Tone A	Tone C	Tone B	Tone D
A	Clicker	White Noise	Steady Tone	Siren
B	Clicker	White Noise	Siren	Steady Tone
C	White Noise	Clicker	Steady Tone	Siren
D	White Noise	Clicker	Siren	Steady Tone

Table 5.2 Auditory stimuli assigned to counterbalancing groups. Cues were counterbalanced across two stimuli used for the first half of a pair (A and C) and two stimuli used for the second half (B and D).

Procedure

Magazine Training

Subjects were first given one day of magazine training. Over a 60-minute session, pellets were dispensed at a variable interval (VI) of 120s for a total of 30 rewards delivered (VI120 range: 75-165s).

Preconditioning

Subjects received two sessions of preconditioning, one per day, starting 24 hours after magazine training. Preconditioning sessions lasted for 70 minutes and were carried out in the absence of reward. During preconditioning sessions, animals were exposed to pairs of auditory stimuli (A→B; C→D); each stimulus lasting for 10s and onset of the second stimulus (e.g., B or D) following immediately after the offset of the first. Animals received six presentations of one pair, followed by six presentations of the second pair, with the reverse order during the second session. Cue presentation was scheduled on a VI of 300s (VI300 range: 150-450s).

Conditioning

Animals received eight conditioning sessions; two per day for four days. Conditioning sessions lasted 70 minutes. Subjects received six presentations of cue B, and six presentations of cue D, both for 10s duration, but only B was paired with delivery of 2 fruit punch pellets (B+, D-). Auditory stimuli were presented in a random order on a VI300 schedule.

Probe Test

Following conditioning, mice received two probe tests; the first conducted 24 hours after conditioning ended and the second a further four days later. Probe sessions lasted 70 minutes and were carried out in the absence of reward. Animals were exposed to 6 presentations each of cues A and C, each lasting 10s, on a VI300 schedule. Cues were played in a fixed order, with either A or C coming first in each pair of presentations; the order was reversed in the second session. Order of presentation was counterbalanced across groups such that half the animals received A first in session 1 and half the animals received C first.

Analysis

Conditioned responding was measured as number of magazine entries made during the second 5s of cue presentation, minus the number of entries made during the 5s immediately prior to stimulus onset (CS – PreCS). The second 5s of CS presentation was used as this was when anticipatory responding in expectation of reward was highest. Analyses of probe test performance were carried out on the combined responding across both probe sessions. Probe test performance was measured as responding to the second 5s of CS presentation compared to 5s of PreCS baseline, as reported previously. But we also investigated the first 5s of the postCS period where animals may expect either reward,

or the conditioned stimulus in the cue-pair to follow, depending on the nature of associations formed.

Conditioned discrimination to B+ and D- was analysed as a two-way ANOVA of cue*session. Following a significant interaction, *post-hoc* comparisons were conducted to examine the simple effect of cues across sessions. Because we were looking at main effects across two families; session and cue*session interaction, a nominal alpha of 0.1 was considered statistically significant, Sidak-corrected for 8 comparisons to provide a significance threshold of $p < 0.013$. To investigate the interaction of cue*session, we also used a simple contrast comparing discrimination between cues on the last session of conditioning, to discrimination in the first session.

In the probe test a two-way ANOVA of cue*time was used to test for discrimination of preconditioned cues, where time relates to whether we are looking at activity during either the CS, or later in the postCS period.

Results

In GluA1$^{+/-}$ mice, conditioned responding was seen to A, but not C in the probe test, indicating learning about the relationship between the two stimulus-pairs during preconditioning (Figure 5.1). Whereas responding to C, which was initially paired with a cue that was never rewarded remained low, responding to A, previously paired with cue B that was later conditioned as a CS+, was higher during the probe test during both the CS and postCS periods.

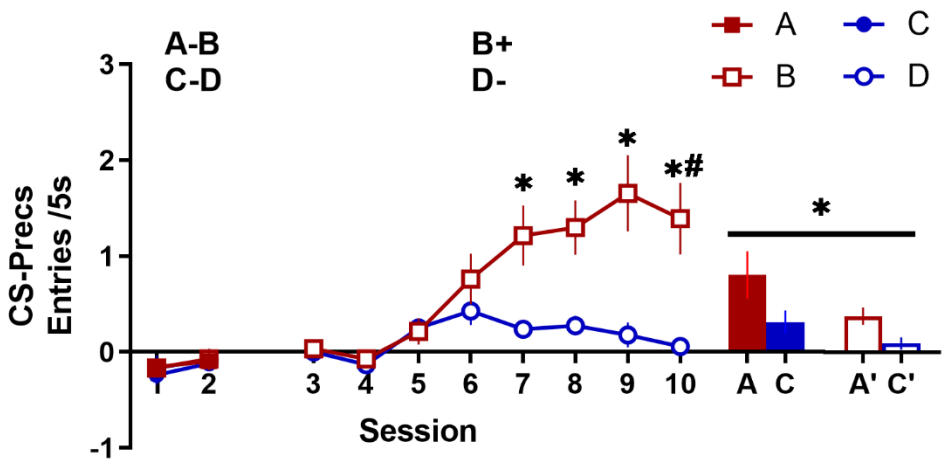

Figure 5.1 GluA1$^{+/-}$ mice discriminate between preconditioned cues after conditioning. During preconditioning, no difference was seen in animals' magazine responses to cues in either cue-pair of A-B or C-D. Over 8 conditioning sessions, GluA1$^{+/-}$ mice learnt to discriminate between rewarded (B+) and unrewarded (D-) stimuli, increasing responding to B+ over time. At probe test, responding to A, was higher than responding to C across both the CS (filled bars) and postCS (hollow bars), indicating sensory preconditioning. All data are presented as mean ± SEM. * $p < 0.05$ (or Sidak-corrected value of 0.013 during conditioning) between cues, # $p < 0.05$ cue*session interaction vs. session 3 (first conditioning session).

During preconditioning, there was no difference in responding to any cue above preCS levels, and no effect of counterbalancing group (both F > 0.5). Prior to preconditioning animals had been trained to receive sucrose pellet rewards at the magazine. During CS presentation head entry behaviour at the magazine was slightly, though not significantly, lower than in the preCS baseline period. This reduction in head entries potentially indicates attending to stimuli during preconditioning.

Animals displayed a significant increase in responding to the rewarded stimulus, B+, but not to the unrewarded D- stimulus across 8 sessions of conditioning (cue: $F_{(1,13)} = 14.168$, $p = 0.002$; session: $F_{(7, 91)} = 12.487$, $p < 0.001$; cue*session: $F_{(7,91)} = 8.951$, $p < 0.001$). *Post-hoc* comparisons of cue across conditioning sessions found significant differences from session 7 onwards (session 7: $t_{(13)} = 3.655$, $p = 0.003$; session 8: $t_{(13)} = 4.376$, $p < 0.001$; session 9: $t_{(13)} = 3.681$, $p = 0.003$; session 10: $t_{(13)} = 3.151$, $p = 0.008$). A simple contrast comparing

discrimination between stimuli in the last session vs. the first session of conditioning also confirmed significant discrimination over time ($F_{(1,13)} = 9.812$, $p = 0.008$).

Investigation of conditioned responding during the probe test revealed increased responding to A, but not to C (cue: $F_{(1,13)} = 9.483$, $p = 0.009$). This discrimination was observed during both the CS and postCS period, resulting in no overall interaction, though responding was lower in the postCS (time: $F_{(1,13)} = 5.896$, $p = 0.03$; cue*time: $F_{(1,13)} = 2.336$, $p = 0.15$).

Discussion

Under the conditions used here, heterozygous GluA1 mice displayed robust discrimination across conditioning sessions, and higher anticipatory magazine responding during presentations of A than C at test, indicating intact SPC. This provides important validation of these testing parameters, in control mice in a pilot study, before we go on to investigate the effect of GluA1 knockout on associative learning in SPC.

There was no evidence of abnormal responses to any particular tone used during preconditioning. In the session prior to this, animals had been trained to collect sucrose pellet rewards from the magazine. The slight decrease in magazine responding during cue presentations seen here compared to baseline activity likely reflects attending to these novel stimuli.

This experiment aimed to confirm that 8 conditioning sessions, over 4 days, was sufficient for animals to acquire a conditioned discrimination between cues. This has been shown to be the case. Additionally, we aimed to show that after 4 days between pre-exposure sessions and the probe test, animals would retain a memory of the association between cue-pairs presented in preconditioning, and that this memory wasn't extinguished through

later conditioning. A clear preference was seen for the cue preconditioned with the to-be-rewarded stimulus, indicating that this was the case here.

5.2 Impairments in sensory preconditioning in GluA1$^{-/-}$ mice

Introduction

Having confirmed that the conditions tested in the previous experiment are capable of generating and detecting an effect of SPC, we next wanted to assess the capacity of GluA1$^{-/-}$ mice to form associations by testing them under these same conditions. The aim of the current experiment was therefore to investigate differences in associative learning between WT and GluA1$^{-/-}$ mice under complex conditions, with multiple stimuli to attend to during both the preconditioning and conditioning phases (Table 5.1, complex-complex).

Methods

Apparatus used and testing procedure were the same as described in Experiment 5.1.

Subjects

12 WT and 12 GluA1$^{-/-}$ mice were used in this experiment. Within each genotype, 6 mice were male and 6 female.

Analysis

Analysis was conducted as described in the previous experiment. However, in this experiment there was an extra factor in our analysis as we also compared the effect of genotype across each stage of training. Sex was included in initial comparisons but was found not to have any effect on performance at any stage, and so was removed as a factor.

Results

GluA1$^{-/-}$ mice were slower to acquire the conditioned discrimination to B+ compared to WT mice, showing less robust discrimination between B+ and D- after 8 conditioning sessions (Figure 5.2). Furthermore, sensory preconditioning in these animals was also impaired, with similar levels of responding to both cues in a probe test.

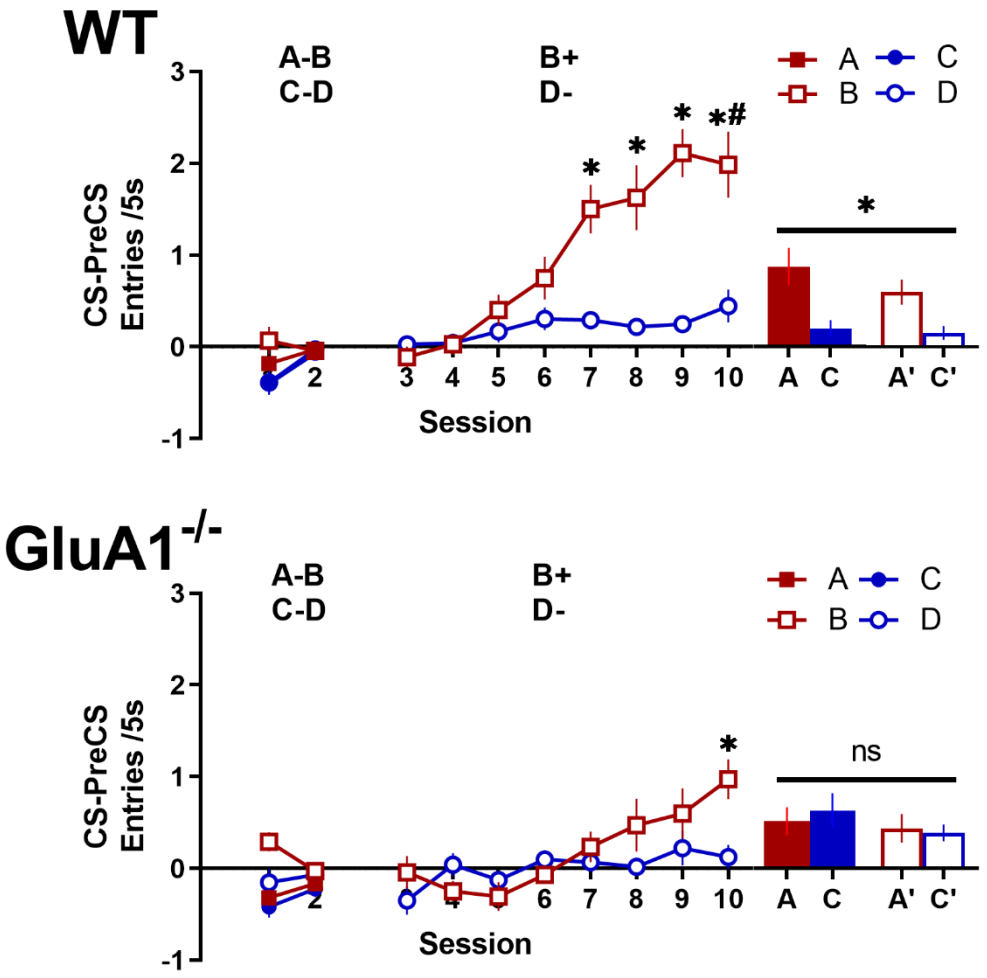

Figure 5.2 GluA1$^{-/-}$ mice are impaired in conditioned discrimination between tones and sensory preconditioning in a complex-complex task design. WT mice exhibited increased responding to the rewarded stimulus, B+, across conditioning (top panel). During a probe test responding was higher to A, preconditioned with B, than to C that was preconditioned to the unrewarded cue D. GluA1$^{-/-}$ mice displayed less robust conditioning to the rewarded stimulus. At test, they did not discriminate between A and C. All data are presented as mean ± SEM. * $p < 0.05$ (or Sidak-corrected value of 0.013 during conditioning) between cues, # $p < 0.05$ cue*session interaction vs. session 3 (first conditioning session).

Whereas in Experiment 5.1 there were no differences in responding to cues during preconditioning, an effect of cue was observed here ($F_{(3,66)} = 10.591$, $p < 0.001$). This was driven by increased magazine entries during cue B presentations compared to other cues, seemingly largely driven by GluA1$^{-/-}$ responses, though there was no effect of genotype or genotype*cue interaction (genotype: $F_{(1,22)} = 0.001$, $p = 0.980$; genotype*cue: $F_{(3,66)} = 1.999$, $p = 0.123$).

Conditioning in WT mice was similar to that observed in GluA1$^{+/-}$ mice in the previous experiment. However, GluA1$^{-/-}$ mice were slower to discriminate between the stimuli, as confirmed by a significant genotype*cue*session interaction ($F_{(7,154)} = 3.783$, $p < 0.001$). To investigate differences in acquisition in each genotype we split the data by genotype for *post-hoc* comparisons to look at when discrimination emerges in each group. WT mice displayed evidence of discrimination between rewarded and unrewarded stimuli from session 7 onwards (session 7-10: all $p < 0.013$). Furthermore, a simple contrast comparing discrimination during the last conditioning session to the first, revealed an increase in conditioning in WT mice ($F_{(1,11)} = 16.841$, $p = 0.002$). GluA1$^{-/-}$ mice displayed weaker conditioning; only showing significant discrimination in *post-hoc* comparisons when looking at the last conditioning session (session 10 B vs. D: $t_{(11)} = 3.921$, $p = 0.002$). There was no indication from a simple contrast analysis that GluA1$^{-/-}$ discrimination had increased over training ($F_{(1,11)} = 2.765$, $p = 0.125$).

During the probe test WT, but not GluA1$^{-/-}$ mice showed evidence of sensory preconditioning, confirmed by a genotype*cue interaction ($F_{(1,22)} = 8.852$, $p = 0.007$). There was no overall interaction of genotype*cue*time indicating that responding within each genotype was stable across both the CS and PostCS period ($F_{(1,22)} = 0.025$, $p = 0.875$). *Post-*

hoc tests revealed elevated responding to cue A vs. C in WT mice, confirmed by an effect of cue but no cue*time interaction (cue: $F_{(1,11)} = 9.573$, $p = 0.010$; cue*time: $F_{(1,11)} = 1.262$, $p = 0.285$). In GluA1$^{-/-}$ mice, there was no evidence of discrimination between cues A and C; there was no main effect of cue or cue*time interaction (cue: $F_{(1,11)} = 0.595$, $p = 0.457$; cue*time: $F_{(1,11)} = 2.196$, $p = 0.166$). In summary, there was no evidence for intact sensory preconditioning in GluA1$^{-/-}$ subjects; however, they were also slower to acquire the conditioned discrimination which may account for weaker responding to the preconditioned cue A at test.

Discussion

While WT mice expressed intact SPC replicating the previous experiment, GluA1$^{-/-}$ mice failed to express a discrimination between cues during the probe test. Furthermore, GluA1$^{-/-}$ mice also failed to acquire the conditioned discrimination between rewarded and unrewarded CSs during conditioning. This is somewhat surprising, given our predictions that associative learning may be enhanced in GluA1$^{-/-}$ mice (Sanderson *et al.,* 2009; Schmitt *et al.,* 2003), we may have expected them to more rapidly and robustly form associations in this task.

The failure to acquire conditioned discrimination to B vs. D makes the result somewhat difficult to interpret. It is hard to say with confidence that GluA1$^{-/-}$ deficits at test were due to maladaptive learning during preconditioning, because they also failed to acquire the conditioning rule; a failure to express SPC could reflect a lack of learning about the CS+. However, previous studies of conditioning have shown no difference in the rate of conditioning between WT and GluA1$^{-/-}$ mice, in a paradigm very similar to the conditioning stage here (Sanderson *et al.,* 2017). Given our results in Chapter 2, it is possible that GluA1^{-}

$^{-/-}$ mice attend more to stimuli during preconditioning, or that they remain in attention for longer. It is then possible that faster or more robust associative learning in GluA1$^{-/-}$ mice led to impairments in SPC; overtraining on the preconditioning stage can lead to reduced or impaired expression of SPC at test (Hoffeld *et al.,* 1960; Sharpe *et al.,* 2020).

Another possibility is that because these stimuli are presented repeatedly across preconditioning sessions, within a relatively short space of time, there is some overlap of learning across stimulus-pairs. Specifically, if elements representing individual cues are persisting in attention beyond the normal timeframe, when the second stimulus-pair is presented, they may maladaptively form associations across all four stimuli. This suggestion is not straightforward, however. During preconditioning, animals were presented with 6 presentations of the first cue-pair in series, followed by 6 presentations of the second cue-pair, rather than interspersing the two sets of stimuli (this was counterbalanced such that on the second preconditioning session cue-pairs were presented in the opposite order). For associations to form across all stimuli, this first cue-pair would have to stay in attention for a long time after the last stimulus presentation. There is some precedent of stimuli persisting in focal attention in GluA1$^{-/-}$ mice; this can potentially explain enhanced associative learning in long-term habituation paradigms such as the Y-maze (Sanderson *et al.,* 2009), although in this study spatial cues were present all the time during training. It is possible that the rate of decay from A1 to A2 is reduced in GluA1$^{-/-}$ mice, contributing to the impaired self-generated priming described previously with this model (Barkus *et al.,* 2014; Sanderson and Bannerman 2012). As a result of this slower decay from focal attention, there is an increased window of time for associative learning about elements of stimuli that co-exist in A1. It could be that stimuli persist for longer in A1, and so more learning occurs during this time before elements decay back to

the inactive state (bypassing A2) as we suggested may occur in Chapter 2. However, if more learning has occurred about associations between the context and auditory cues during this time, there may be increased retrieval-generated priming of cues based on context-cue associations. It could therefore be, that there is some mixed learning about cues across cue-pairs from associations formed while these cues are in A2, and so there is also enhanced mediated conditioning in these animals (Aitken and Dickinson 2005).

It is hard to say from the current experiment whether maladaptive associative learning is occurring during preconditioning, or appetitive conditioning, or in both of these stages to impair expression of SPC in GluA1$^{-/-}$ mice. Whilst there may be overlap of stimuli in attentional states during preconditioning, this could also happen during conditioning, and may explain the delayed acquisition of conditioned discrimination in GluA1$^{-/-}$. However, intact discriminative conditioning has also been reported previously in these mice (Sanderson *et al.,* 2017). This result seemingly argues against a contribution of impaired conditioning to the SPC effect seen here. This is something we will continue to investigate in the experiments to follow.

5.3 GluA1$^{-/-}$ mice show enhanced sensory preconditioning under simplified conditions

Introduction

The previous experiment showed that GluA1$^{-/-}$ mice fail to express SPC under complex conditions. However, they were also impaired on the conditioned discrimination between a CS+ and CS-. This result alone is hard to interpret and could be due to a multitude of reasons. To better understand what may be driving impairments in expression of SPC, we next looked at performance under simplified conditions. By removing a stimulus-pair

during preconditioning, and the CS- during conditioning, there were fewer stimuli present at each stage of learning. The aim of this experiment was to determine whether GluA1$^{-/-}$ mice were able to express SPC under these simple conditions, to better understand how associative learning is affected in this model.

Methods

Apparatus used was the same as described in Experiment 5.1.

Subjects

16 WT and 16 GluA1$^{-/-}$ were tested in this experiment. Within each genotype there were 8 male and 8 female mice.

Procedure

The procedure was the same as that described in Experiment 5.1 except for a few key differences. The testing parameters are summarised in Table 5.1.

In the preconditioning stage, only one pair of cues was presented (A-B). To keep session length the same, the ITI between tone presentations was increased to a VI600s (range: 325-875s).

During conditioning, animals were trained to acquisition of the rewarded stimulus, B+, in the absence of a CS-. Because there were fewer stimulus presentations overall, again the ITI was increased to a VI600, and session length remained the same at 70 minutes.

At probe test, conditions were the same as before. Animals were presented with the preconditioned stimulus, A, or a novel stimulus, C, both in the absence of reward.

Analysis

Analysis was conducted in a similar manner to that described in Experiment 5.1 and 5.2. However, as there was only one cue during conditioning, a two-way ANOVA of genotype*session was used for this stage, differing from previous analyses. Sex was included in initial comparisons but was found not to have any effect on performance at any stage, and so was removed as a factor.

Results

When conditions were simplified genotype differences on conditioning were eliminated, with both groups showing increased responding to the CS across training (Figure 5.3). Interestingly, during the probe test, now only GluA1$^{-/-}$ showed evidence of sensory preconditioning.

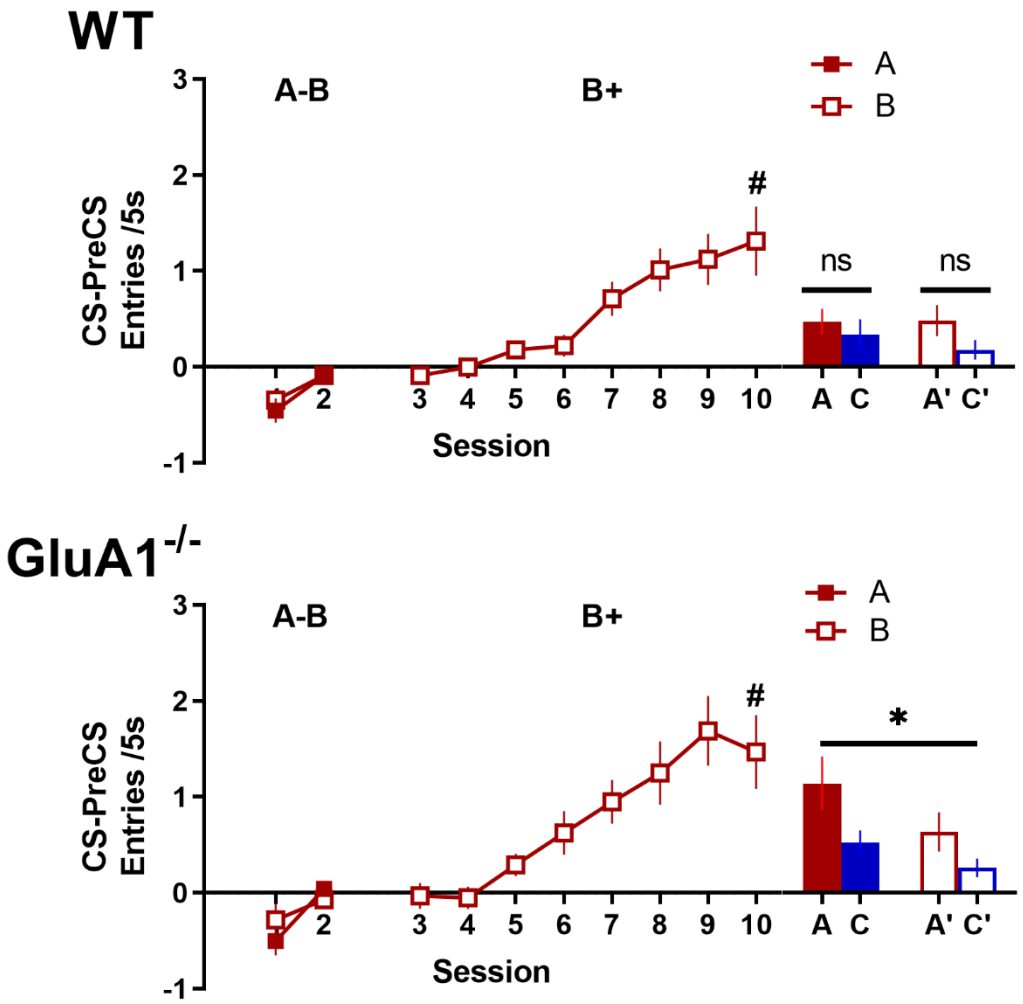

Figure 5.3 Enhanced sensory preconditioning in GluA1$^{-/-}$ mice under simple conditions. No differences between genotypes were observed during preconditioning or conditioning; both groups acquired the conditioned response to B+ across 8 conditioning sessions. In a probe test, GluA1$^{-/-}$ mice exhibited increased responding to the preconditioned cue A, compared to C. No discrimination was observed in WT mice. All data are presented as mean ± SEM. * $p < 0.05$ between cues, # $p < 0.05$ cue*session interaction vs. session 3 (first conditioning session).

During preconditioning, although there was no effect of genotype and no interactions (genotype, genotype*cue and genotype*cue*session all $p > 0.6$), a significant effect of cue was reported, with less suppression of baseline magazine entries in response to B compared to A (cue: $F_{(1,29)} = 6.987$, $p = 0.013$). A similar effect was observed in the previous experiment and may reflect declining attending to the auditory stimuli over time, across the 20s presentation of a stimulus-pair, since there is seemingly less suppression to stimuli presented second in the pair.

Both groups successfully acquired the conditioned response to increase magazine behaviour in anticipation of reward delivery across conditioning (session: $F_{(7,203)} = 24.080$, $p < 0.001$). There was no effect of genotype, and no genotype*session effect, confirming the similar rate of acquisition in both groups (genotype: $F_{(1,29)} = 0.833$, $p = 0.369$; genotype*session: $F_{(7,203)} = 0.631$, $p = 0.730$). Due to the lack of an interaction, data from both genotypes were pooled to compare session effects. A significant simple contrast was observed comparing sessions 10 and 3, confirming an increase in conditioned responding from the start to the end of training ($F_{(1,29)} = 30.140$, $p < 0.001$). Importantly, the same effect would have been observed if we had analysed each genotype separately, both groups expressed robust conditioning.

No main effects of genotype were observed at the probe test, or genotype*cue, but importantly, an interaction of genotype*cue*time was seen reflecting differences across both stages of the CS between groups (genotype: $F_{(1,29)} = 2.284$, $p = 0.142$; genotype*cue: $F_{(1,29)} = 1.049$, $p = 0.314$; genotype*cue*time: $F_{(1,29)} = 4.621$, $p = 0.040$). To investigate the nature of this interaction, we then looked at each genotype separately. WT mice showed no effect of cue, or cue*time interaction (cue: $F_{(1,14)} = 1.670$, $p = 0.217$; cue*time: $F_{(1,14)} = 2.626$, $p = 0.127$). However, GluA1$^{-/-}$ mice did significantly discriminate between cues, showing a main effect of cue but no cue*time interaction (cue: $F_{(1,15)} = 5.949$, $p = 0.028$; cue*time: $F_{(1,15)} = 2.396$, $p = 0.143$). These findings indicate that in GluA1$^{-/-}$ mice, but not in WTs, responding to A was higher than C across both periods of the CS.

Intriguingly, and in contrast to the previous experiment, these findings suggest that GluA1$^{-/-}$ mice actually exhibit enhanced associative learning compared to WT during the preconditioning stage of training, but this effect is dependent on task conditions.

Discussion

In contrast to the previous experiment, not only were GluA1$^{-/-}$ mice unimpaired here, they actually seemed to show enhanced SPC. While WT mice had no problem acquiring conditioned responding to B+, they expressed little preference for cue A during a probe test, suggesting little, if any SPC. GluA1$^{-/-}$ mice also successfully acquired the conditioning rule, but interestingly exhibited much higher anticipatory responding to cue A during a probe test. This was evident both during the CS itself, and in the postCS period during which animals may expect either stimulus B, or the reward to follow, depending on the nature of the associations they have formed.

The fact that GluA1$^{-/-}$ mice demonstrate SPC in a situation where WT mice do not, could be viewed as cognitive enhancement in these mice. However, it could also suggest maladaptive associative learning in GluA1$^{-/-}$, and whilst this could be viewed as beneficial under these circumstances, it still represents a divergence from normal behaviour expressed by controls. Under the conditions tested here, it is seemingly advantageous to respond to stimuli that may be associated with reward. However, doing so under conditions where control animals do not express this association, suggests an increased propensity for the formation of associations in GluA1$^{-/-}$ mice. Forming associations in a situation where WT mice do not could also be considered harmful, or maladaptive.

The experimental conditions tested here represent a very unique, simplified scenario, manufactured specifically to test aspects of behaviour in as simple a way as possible. Real-world conditions are typically much more complicated than this. The complex rules of the previous experiment, while still a simplification, more accurately reflect a real-world

scenario where there are many environmental events to attend to, and potentially respond to.

This result of enhanced associative learning in GluA1$^{-/-}$ mice, under certain conditions, indicates potential translational validity of this model as a tool for studying clinical psychosis. Psychotic symptoms are posited to be the consequence of aberrantly high levels of attending to stimuli and an increased propensity for the formation of associations (Kapur 2003), it is possible that a similar framework exists in this model. This is something we will consider in further detail, in the context of all the behavioural data generated on this model, in the discussion at the end of this chapter.

5.4 Deficits in GluA1$^{-/-}$ mice are driven by increased complexity during both preconditioning and conditioning

Introduction

The previous two experiments revealed an interesting divergence in associative learning in GluA1$^{-/-}$ mice. While these animals were impaired under complex conditions, when the task was simplified they actually showed enhanced SPC compared to WT counterparts, who did not express SPC under simple conditions. This suggests that reducing the complexity of cues presented in either the preconditioning or conditioning stage has facilitated associative learning in GluA1$^{-/-}$ mice. But from the results so far it is unclear whether complexity during the preconditioning, or conditioning stage is driving impaired associative learning.

Previous work investigating GluA1$^{-/-}$ mice revealed normal conditioned discrimination to tones in these animals when there was no pre-exposure (Sanderson *et al.,* 2017). A

reasonable presumption may therefore be that it is this pre-exposure that is contributing to deficits seen during the test. But in order to fully understand the contribution of each stage of training to the observed maladaptive associative learning, we should separately test the effect of simple or complex conditions across both of the first two stages. The aim of this current experiment was to complete the 2x2 factorial design outlined in Table 5.1, by completing the two remaining conditions where there is only increased complexity during one task stage. In doing so we hoped to better understand whether it is complexity during preconditioning, or conditioning, or a combination of both, that leads to impairments in SPC performance in GluA1$^{-/-}$ mice.

Methods

Apparatus used and measures of analysis were the same as those described in the previous experiments of this chapter. Where there was only one set of cues (simple), analysis was as conducted in Experiment 5.3, where there were two sets of cues (complex), analysis was as described in Experiment 5.2.

Subjects

A total of 48 mice were used in this experiment; 24 WT and 24 GluA1$^{-/-}$. Within each genotype, half the animals were tested on a complex design during preconditioning with simple task parameters during conditioning, and half the animals received the reverse conditions (n = 12 per condition). There were even numbers of males and females within each genotype and condition.

Procedure

The testing procedure was similar to that described in previous experiments; however, animals received a hybrid of simple and complex parameters (see Table 5.1). Simple cue testing procedures were as described in Experiment 5.3, and complex procedures were as described in Experiment 5.2. The test stage was identical to that described in these two previous experiments.

Results

Complex Preconditioning/ Simple Conditioning

Both WT and GluA1$^{-/-}$ mice displayed robust conditioning under simple conditions, increasing conditioned responding at the magazine over time (Figure 5.4). Furthermore, sensory preconditioning was evident in both groups during the probe test, with increased responding to the preconditioned stimulus A vs. C that had never been associated, either directly or indirectly, with reward.

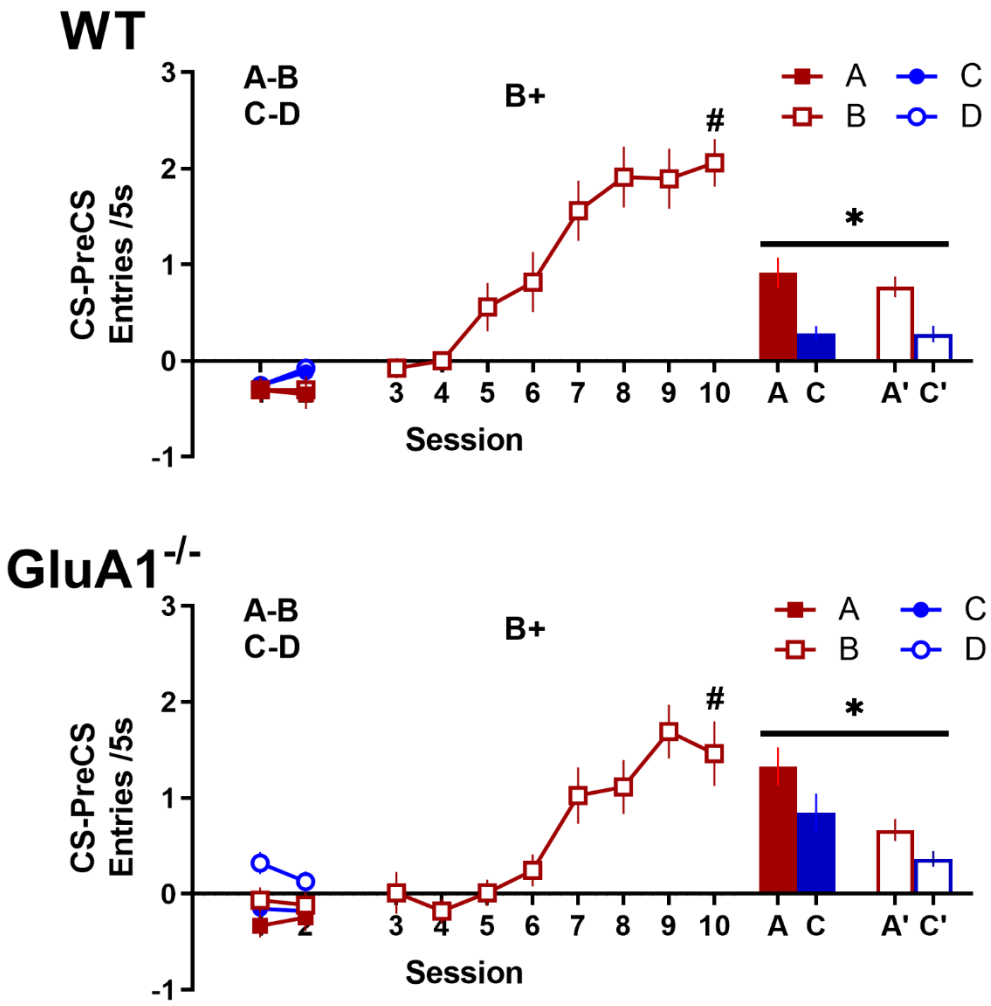

Figure 5.4 No effect of GluA1 deletion on sensory preconditioning when conditioning is simplified. No difference was seen between WT and GluA1$^{-/-}$ on conditioning, both groups acquired a conditioned response to presentations of B paired with reward. At test, responding was higher to A, initially preconditioned alongside B, than to C. All data are presented as mean ± SEM. * $p < 0.05$ between cues, # $p < 0.05$ cue*session interaction vs. session 3 (first conditioning session).

During preconditioning, although there was no effect of genotype, a cue effect was seen, similar to previous experiments (genotype: $F_{(1,22)} = 0.839$, $p = 0.370$; cue: $F_{(3,66)} = 4.588$, $p = 0.006$). This effect appeared to be driven by reduced suppression of magazine responding in the second half of the cue-pair presentation, indicating temporal order of cue presentation rather than cue identity is driving this behaviour.

There was no effect of genotype on conditioning (genotype: $F_{(1,22)} = 2.886$, $p = 0.103$; genotype*session: $F_{(7,154)} = 1.114$, $p = 0.357$). Both groups displayed significantly elevated

responding to B across conditioning sessions, confirmed by a main effect of session and simple contrast comparing responding at session 10 vs. session 3, pooled across genotypes (session: $F_{(7,154)} = 33.473$, $p < 0.001$; session 10 vs. session 3: $F_{(1,22)} = 56.035$, $p < 0.001$).

During the probe test, there was no difference between genotypes, confirmed by a lack of genotype*cue or genotype*cue*time interaction, although there was a trend effect of genotype (genotype: $F_{(1,22)} = 3.610$, $p = 0.071$; genotype*cue: $F_{(1,22)} = 0.955$, $p = 0.339$; genotype*cue*time: $F_{(1,22)} = 0.014$, $p = 0.907$). The trend effect of genotype likely reflects slightly elevated responding across both cues and timepoints in GluA1$^{-/-}$ mice. Importantly there was a main effect of cue confirming increased responding to cue A vs. C ($F_{(1,22)} = 30.071$, $p < 0.001$). To confirm that this effect was present in both groups, we did check the effect of cue in each genotype separately (WT: $F_{(1,10)} = 33.482$, $p < 0.001$; GluA1$^{-/-}$: $F_{(1,12)} =8.183$, $p = 0.014$). In summary, increasing the complexity of cues presented during preconditioning resulted in normal sensory preconditioning in both WT and GluA1$^{-/-}$ mice.

Simple Preconditioning/ Complex Conditioning

We then assessed the effect of increased complexity specifically during conditioning, while only presenting one tone-pair during preconditioning. This also resulted in normal performance in GluA1$^{-/-}$ mice, with both groups able to both acquire the conditioned discrimination, and exhibit increased responding to tone A during a probe test (Figure 5.5).

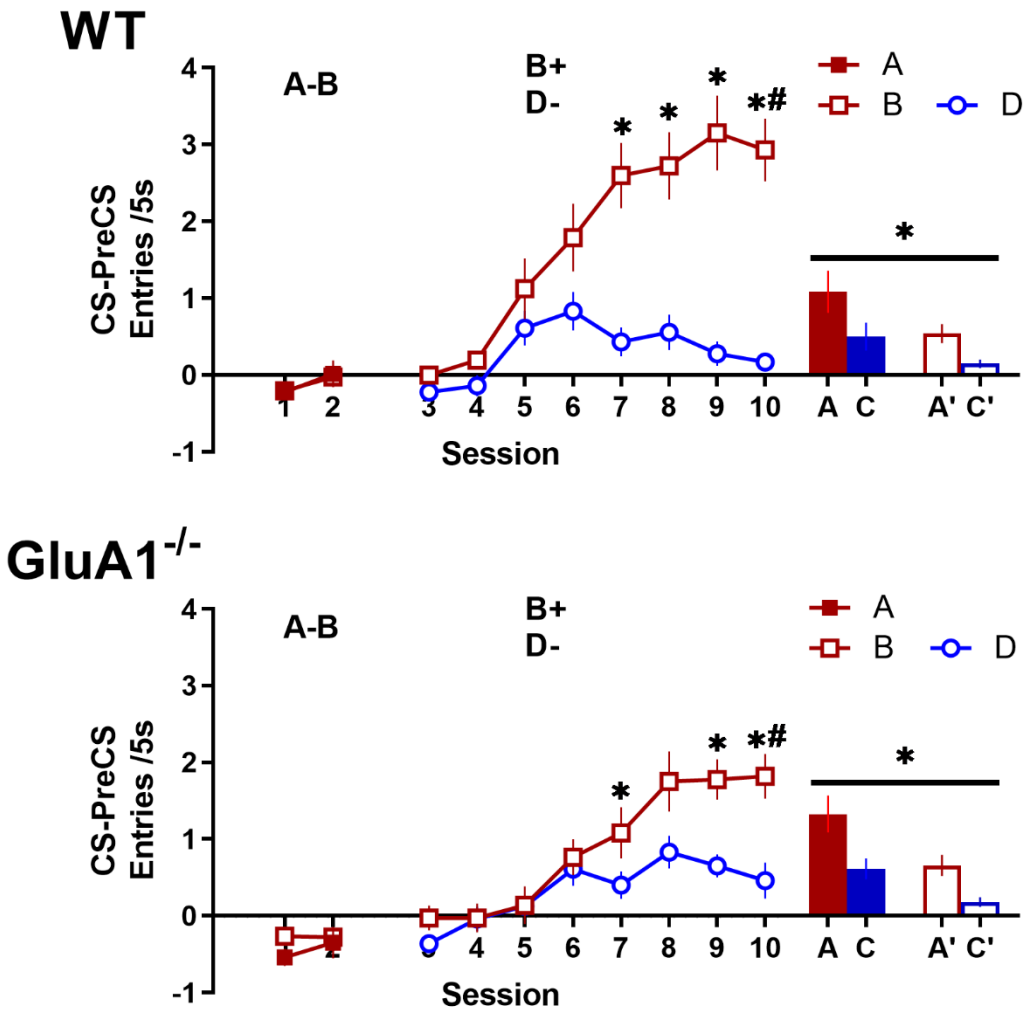

Figure 5.5 No impairments in GluA1$^{-/-}$ mice on conditioning or sensory preconditioning when simple cues are presented during preconditioning. Both WT and GluA1$^{-/-}$ successfully learnt to discriminate between rewarded and unrewarded stimuli during conditioning. At test there was no difference between genotypes, with both groups showing elevated responding to the preconditioned tone A compared to C. All data are presented as mean ± SEM. * $p < 0.05$ between cues, # $p < 0.05$ cue*session interaction vs. session 3 (first conditioning session).

GluA1$^{-/-}$ mice displayed less suppression of responding during tone B presentations than tone A, confirmed by a genotype*cue interaction during the preconditioning stage ($F_{(1,22)}$ = 6.649, p = 0.017). *Post-hoc* tests confirmed a main effect of cue in GluA1$^{-/-}$ but not in WT mice (WT: $F_{(1,11)}$ = 0.788, p = 0.394; GluA1$^{-/-}$: $F_{(1,11)}$ = 10.336, p = 0.008).

However, both groups exhibited normal conditioning, acquiring a conditioned response to tone B, but not tone D, over time, despite GluA1$^{-/-}$ displaying impaired conditioning in

Experiment 5.2. A trend effect of genotype was seen here, coinciding with a significant interaction of genotype*cue*session (genotype: $F_{(1,22)}$ = 3.928, p = 0.060; genotype*cue*session: $F_{(7,154)}$ = 3.028, p = 0.005). For *post-hoc* comparisons, we therefore split the data by genotype to investigate each group separately.

WT mice displayed significantly higher responding to the rewarded tone B vs. D, from session 7 onwards (all $p < 0.001$). GluA1$^{-/-}$ mice also began to show discrimination between cues from session 7, though the effect size was smaller on sessions 7 and 8 (comparisons of B vs. D in GluA1$^{-/-}$; session 7: $t_{(11)}$ = 3.000, p = 0.012; session 8: $t_{(11)}$ = 2.754, p = 0.019; session 9: $t_{(11)}$ = 4.344, $p < 0.001$; session 10: $t_{(11)}$ = 4.419, $p < 0.001$; Sidak-corrected significance threshold $p < 0.013$). Importantly, a simple contrast investigating the cue*session interaction revealed significantly elevated discrimination between cues in the last conditioning session, compared to the first, in both genotypes (WT: $F_{(1,11)}$ = 28.845, $p <$ 0.001; GluA1$^{-/-}$: $F_{(1,11)}$ = 9.336, p = 0.011). It is possible that these results reflect slower conditioning in GluA1$^{-/-}$ mice, although importantly they did still acquire this conditioning after 8 sessions.

No differences were seen between groups at test, confirmed by a lack of genotype main effect, genotype*cue or genotype*cue*time interaction, indicating similar levels of discrimination across both timepoints of the CS (genotype: $F_{(1,22)}$ = 0.541, p = 0.470; genotype*cue: $F_{(1,22)}$ = 0.196, p = 0.662; genotype*cue*time: $F_{(1,22)}$ = 0.043, p = 0.838). However, a main effect of cue was observed indicating that both groups displayed elevated responding towards tone A ($F_{(1,22)}$ = 19,979, $p < 0.001$). Similar to the opposite conditions of complex preconditioning but simple conditioning (Figure 5.4), this experiment appears

to confirm that GluA1$^{-/-}$ mice display normal sensory preconditioning under conditions where the study design is partially simplified.

Discussion

Simplifying task complexity in the experiments reported here appeared to lead to similar levels of SPC at test across genotypes, independent of which task stage was manipulated. Both GluA1$^{-/-}$ and WT mice expressed increased responding to tone A during a probe test when there were fewer stimuli to attend to during preconditioning, but also when conditioning was measured as acquisition to a single stimulus, with no CS-.

Interestingly, and in contrast to Experiment 5.2, under simple-complex conditions GluA1$^{-/-}$ mice were able to acquire the conditioned discrimination to B as a CS+ and D as a CS-. Testing conditions were identical with the exception of the stimuli presented during preconditioning, during which only one tone-pair was presented in this experiment. This seems to suggest that the addition of a second tone-pair during preconditioning is sufficient to impair conditioning. One possible explanation for this, as suggested earlier, is that there may be some overlap across pairs of cues in attentional states in GluA1$^{-/-}$ mice, due to impaired self-generated priming and a subsequent increase in the time that elements remain active. This leads to an interesting question around whether separating tone-pairs during preconditioning (i.e., pre-exposing animals to each tone-pair separately across separate sessions), would allow GluA1$^{-/-}$ mice to overcome this impairment.

While conditioning was intact in GluA1$^{-/-}$ mice, there was a trend effect compared to WT that suggests they may still show delayed acquisition. Average responding during CS+ presentation was lower at the end of training in GluA1$^{-/-}$ mice than WTs, which may indicate they had not acquired the conditioned discrimination as robustly. However, performance

in GluA1$^{-/-}$ mice was stable over the last three sessions of conditioning, with very little fluctuation in CS+ anticipatory responding. Furthermore, there was no difference between genotypes in expression of SPC during the test stage.

Another possibility is that this subtle difference between genotypes is a consequence of different baseline activity; overall GluA1$^{-/-}$ mice make more magazine entries than WTs and there may be a ceiling effect, when comparing CS to preCS activity, in terms of how much further responding can increase. This could explain the effect seen in the simple-complex condition, where discrimination was intact but less robust than discrimination seen in WTs. However, this does not explain differences seen in the complex-complex condition where there was little evidence of discrimination, and SPC was impaired. Furthermore, we can not rule out that differences in conditioning in the simple-complex condition were a consequence of preconditioning in the first stage of the task, without directly assessing conditioned discrimination in the absence of pre-exposure to stimuli.

5.5 No impairment in acquisition of a conditioned discrimination in GluA1$^{-/-}$ mice without any pre-exposure

Introduction

The previous experiments in this chapter have shown different outcomes on SPC as a consequence of modulating conditions during preconditioning and conditioning stages. Interestingly, particularly in Experiment 5.2 we saw delayed or impaired conditioning in GluA1$^{-/-}$ mice, with poor discrimination between rewarded and unrewarded stimuli after 8 sessions. This was surprising, particularly as previous studies in GluA1$^{-/-}$ mice showed normal performance on a conditioned discrimination, when there was no delay between CS and reward (Sanderson *et al.,* 2017).

In this final experiment, we wanted to confirm the lack of conditioned discrimination impairment in GluA1$^{-/-}$ mice in our own hands, to add support to earlier findings in this chapter of reduced conditioning, dependent on stimulus-pair pre-exposure.

Methods

Apparatus used was the same as that described in Experiment 5.1. Testing procedure and analysis were the same as that described for conditioning in Experiment 5.1, with the exception that animals were conditioned across more sessions here (12 instead of 8 in previous experiments). Stimuli were nominally named “B” and “D” for ease of drawing comparisons between this experiment and previous sensory preconditioning experiments.

Subjects

7 WT and 7 GluA1$^{-/-}$ mice were used in this experiment. Within each genotype there were 4 male and 3 female mice. No effect of sex was seen in initial analysis of these data.

Results

Both WT and GluA1$^{-/-}$ mice were able to acquire a conditioned discrimination between tones when only one was positively rewarded (Figure 5.6). GluA1 knockout appears to have no effect on conditioning; there was no main effect of genotype, nor was there a genotype*cue or genotype*cue*session interaction (genotype: $F_{(1,12)} = 0.157$, $p = 0.699$; genotype*cue: $F_{(1,12)} = 0.572$, $p = 0.464$; genotype*cue*session: $F_{(11,132)} = 1.311$, $p = 0.225$).

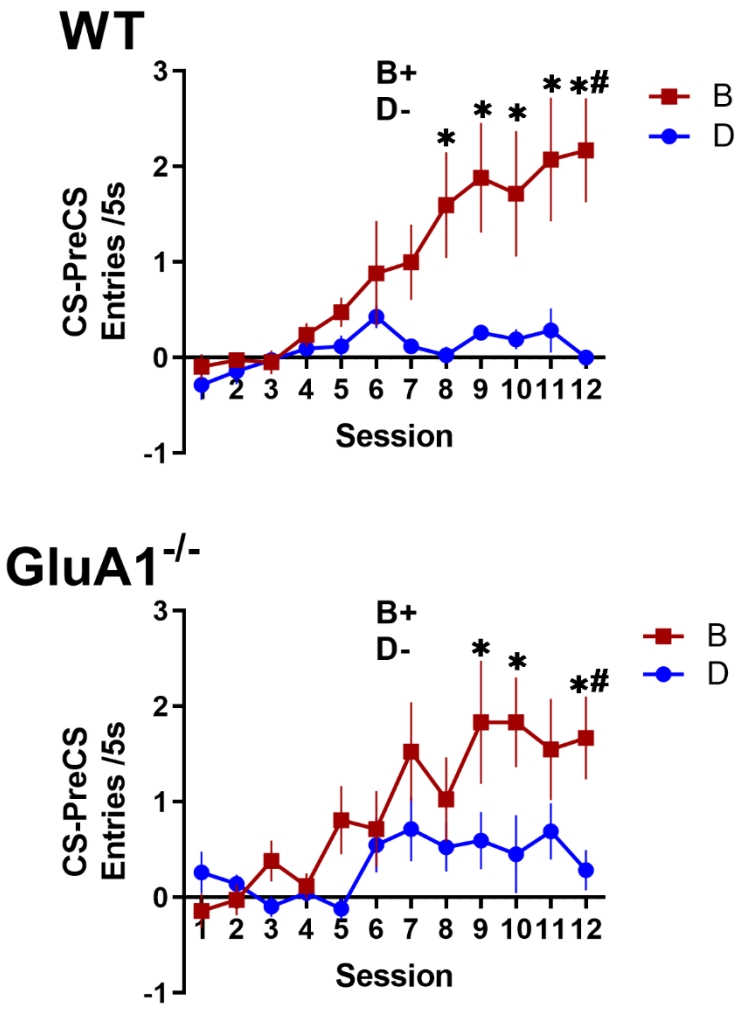

Figure 5.6 Normal conditioning in GluA1$^{-/-}$ mice. WT and GluA1$^{-/-}$ mice displayed normal conditioning to auditory CS+ and CS- cues over training. Both genotypes learnt to discriminate between rewarded and unrewarded cues and increased anticipatory responding selectively to B+ from session 7 of conditioning. All data are presented as mean ± SEM. * $p < 0.05$ between cues, # $p < 0.05$ cue*session interaction vs. session 1.

A cue*session interaction was observed, however, confirming acquisition of the conditioned discrimination across training ($F_{(11,132)} = 8.555$, $p < 0.001$).

To confirm the lack of genotype effects we did also investigate each genotype separately. A main effect of cue and cue*session interaction was seen in WT mice (cue: $F_{(1,6)} = 10.074$, $p = 0.019$; cue*session: $F_{(11,66)} = 6.282$, $p < 0.001$). Importantly, we also observed the same pattern in GluA1$^{-/-}$ mice (cue: $F_{(1,6)} = 9.322$, $p = 0.022$; cue*session: $F_{(11,66)} = 3.601$, $p < 0.001$). *Post-hoc* comparisons were investigated separately for each genotype, but data were not Sidak- corrected in this experiment; with only 7 animals per group this was deemed unfairly

restrictive and would potentially eradicate any significant differences. WT mice displayed significant discrimination between B and D from session 8 onwards (all $p < 0.05$). In GluA1$^{-/-}$ mice, differences were seen at sessions 9, 10 and 12 (all $p < 0.05$). Simple contrasts comparing discrimination at session 12 compared to session 1 revealed significantly elevated responding to cue B in both groups (WT: $F_{(1,6)} = 9.017$, $p = 0.024$; GluA1$^{-/-}$: $F_{(1,6)} = 15.582$, $p = 0.008$). Surprisingly, if we had stopped conditioning after 8 sessions, neither WTs nor GluA1$^{-/-}$ mice would have shown convincing discrimination.

Discussion

Similar to the intact performance described by Sanderson *et al.* (2017) on a conditioned discrimination paradigm, we found no evidence of impairments in conditioning in GluA1$^{-/-}$ mice in this experiment. While visually, it appeared that GluA1$^{-/-}$ mice were slightly slower to acquire a discrimination between the two stimuli, statistically there were no differences. In previous studies we ended conditioning after 8 sessions and had intended to do the same here. However, surprisingly, neither group expressed convincing discrimination here after 8 sessions. It may be that some pre-exposure to stimuli during preconditioning in earlier experiments actually facilitated learning about these cues during conditioning. It could be that in SPC, some pre-exposure to these auditory stimuli during preconditioning allowed animals to form unique memory representations of them (e.g., perceptual learning) that helped to expedite future learning when those tones gained physiological relevance (Macphail, Good, and Honey 1995).

Because of this lack of convincing discrimination in either group at session 8, we trained animals over a further four sessions. Importantly, after this extended training, it was clear that there were no overt differences between genotypes on conditioned discrimination.

This result of intact discrimination in GluA1$^{-/-}$ mice in certain circumstances is in line with what we observed in the simple-complex condition in SPC (Figure 5.5). This may indicate that increased complexity of pre-exposure stimuli contributes to the deficits in conditioning seen in the complex-complex condition (Figure 5.2). However, there was still some suggestion that learning was marginally slower in GluA1$^{-/-}$ mice here, despite no significant genotype effects. It may be that increased task complexity can affect learning, but only a combination of increased complexity across both SPC training stages (pre-exposure and conditioning) was sufficient to impair SPC during the probe test.

Discussion

Enhanced associative learning in GluA1$^{-/-}$ mice

Previous work on GluA1$^{-/-}$ mice has indicated that associative learning in spatial memory tasks is intact in these animals, at least in situations less reliant on working memory (Reisel *et al.,* 2002; Schmitt *et al.,* 2003). These mice can be trained to learn the spatial location of food rewards on appetitively-motivated Y-maze or radial maze paradigms. However, it was important to try and understand the nature of associations learned in GluA1$^{-/-}$ mice, and whether they are similar to those of a WT counterpart. This is an interesting question, given our findings so far indicating the translational relevance of this model to aberrant salience mechanisms. Interestingly, under simple conditions, we actually showed enhanced sensory preconditioning in GluA1$^{-/-}$ mice, supporting our prediction that these mice may form associations more quickly, or more robustly, at least under some conditions.

Intact sensory preconditioning was seen in simple conditions, in the absence of a clear learning effect at test in WT mice. However, it should be noted that the magnitude of

learning during conditioning was also less in WT mice than in other experiments where only one conditioning cue was presented (Figure 5.4). It is possible that the lack of effect at test is due to weaker conditioning in this earlier stage, compared to other cohorts tested under different conditions. The sensory preconditioning effect is highly dependent on learning in prior stages, and training at these stages is also highly sensitive to context and testing parameters. This represents a potential limitation of the task, and findings in this chapter; that sensory preconditioning was only absent in WT mice when conditioning was less robust, and this may be driving seemingly enhanced performance in GluA1$^{-/-}$ mice. This is not to say conditioning was entirely absent; there was still a significant increase in conditioning between the first and last training sessions, and conditioning on the last session was well above the chance level of 0 nose-pokes. This study was also well powered, with 16 animals per cohort, and run over a number of replications, further validating these findings. Overall, while alternative accounts cannot be ruled out, this result certainly alludes to an enhanced propensity to form associations in GluA1$^{-/-}$ animals, and supports the use of this model as a tool for studying psychosis and its aetiology preclinically.

That being said, GluA1$^{-/-}$ performance was only enhanced under these specific conditions, and when the task complexity was increased across both preconditioning and conditioning stages, they expressed reduced SPC. While this could be argued to reflect an impairment in the basic mechanisms of associative learning, this seems unlikely given SPC was intact in all other conditions tested (and enhanced in simple-simple). This may instead be the result of altered associative learning leading to the formation of inappropriate associations in the absence of GluA1. We have argued previously that these mice may express an increased propensity to form associations, and that in the simple scenario this appears to have been beneficial to learning the SPC task. But if animals are forming associations more readily, in

the complex-complex condition, this may have led to inappropriate associations forming between separate stimuli, despite them being presented temporally in isolation.

It is possible that stimuli persisting in A1 long after they have offset leads to stronger or more robust associations forming between stimuli. In this scenario, it is then possible that after decaying back to the inactive state there is increased retrieval-generated priming of cue elements into A2 through their association with the context, and subsequently an increased window for mediated A2-A2 conditioning to occur (Aitken and Dickinson 2005). There is some precedent for associative learning across stimuli even in their absence (Holland and Sherwood 2008), which could explain how inappropriate associations across cues could form long after they were presented. Subtle differences in task parameters may have a profound impact on learning, making it difficult to say from these results alone what exactly is being learnt in GluA1$^{-/-}$ mice across training stages. Temporal contiguity of stimulus presentations could affect whether excitatory or inhibitory associations are forming, but further work would be required to test these possibilities.

Enhanced or increased mediated conditioning may explain how maladaptive associative learning could occur during preconditioning to impair later SPC performance. However, given that SPC was intact under complex-simple and simple-complex conditions, there must be maladaptive association formation across both stages of training. It is possible, then, that this is happening to some extent across both stages of training but only the combined effect of this over both stages affects associative learning to the point that SPC is impaired.

Deficits in SPC under complex-complex conditions could still represent increased associative learning, but under conditions where an increased propensity to form

associations is no longer beneficial. While SPC was left intact when one task stage was more complex, in the complex-complex scenario GluA1$^{-/-}$ mice were seemingly unable to acquire SPC, and instead seemed to generalise across cues A and C at test. While we can't say with certainty that this is due to an increased propensity for associative learning, this appears to be the most parsimonious explanation for the enhanced SPC in simple conditions, shifting to normal or even impaired SPC in more complex conditions seen in this chapter. It would be valuable to test this hypothesis further, by manipulating task parameters in such a way that complex stimuli are presented but in a way that may facilitate appropriate association formation in GluA1$^{-/-}$ mice – such as presenting preconditioning stimuli in isolation across separate sessions.

Alternative explanations of associative learning deficits

It is possible that at least some of these findings could alternatively be explained by impaired associative learning, although it is difficult to see how this could explain the simple-simple result. However, there is a report of impaired associative learning in models of reduced GluA1 in the literature. A paper investigating blockade of synaptic GluA1 delivery found impairments in an inhibitory avoidance (IA) task (Mitsushima *et al.,* 2011). In this paradigm, animals were exposed to light and dark sides of an arena, but crossing in to the dark side was paired with electric footshock. Control animals, but not rats lacking GluA1, were subsequently much slower to re-enter the dark chamber in a test session 30 minutes later. This paper followed findings by Whitlock *et al.* (2006) of upregulated GluA1 expression in the period immediately following IA training, which declined after 2 hours. Mitshushima *et al.* showed that rats lacking GluA1 were much faster to re-enter the dark side of the box, where they had previously experienced footshock, than uninfected

controls. Furthermore, the authors demonstrated a correlation between the latency to enter, and the extent to which synaptic delivery had been blocked. By virally blocking synaptic GluA1 delivery in rats, and impairing IA performance, they argued there was an inability to form associations in the absence of GluA1.

This seemingly contradicts some of the findings on intact associative learning not just in this thesis, but also in earlier reports where GluA1$^{-/-}$ mice have demonstrated intact reference memory in the water maze, appetitive Y-maze, and radial maze (Reisel *et al.,* 2002; Schmitt *et al.,* 2003; Zamanillo *et al.,* 1999). There is a substantial body of evidence arguing for unimpaired associative learning in GluA1$^{-/-}$ mice and expression of a memory trace of a rewarded location/stimulus. These results, alongside the enhanced SPC result seen in Experiment 5.3 in particular seems to disprove this argument by Mitsushima *et al*.

Furthermore, there were a number of flaws in the methodological design of the IA study that make it difficult to interpret this result as an impairment in associative learning. Mitsushima *et al.* (2011) tested rats for learning just 30 minutes after training. It cannot be ruled out that expression of IA may be intact in these rats, but that GluA1-independent learning was not expressed. We have argued that GluA1 deletion impairs self-generated priming and increases the likelihood of stimulus elements entering or persisting in A1 leading to locomotor hyperactivity in a novel environment. If the same is true in this model of reduced GluA1 synaptic delivery, animals may not have sufficiently habituated to the testing environment, which could have detrimentally affected animals' ability to control their locomotor responding.

Critically, during IA training, rats were removed just 10s after exposure to the dark side of the chamber and subsequent footshock. Given the lack of prior habituation to the arena,

and the close temporal proximity between light and dark side exposures, and footshock, animals may have formed associations across all of these competing cues, potentially similar to behavioural abnormalities observed here under complex-complex conditions (Figure 5.2). It is possible that elements representing both sides of the arena (light and dark) were present in A1 at the same time as the footshock was delivered. This could make it more difficult for rats lacking synaptic GluA1 to recognise the light side as safe, but the dark side as aversive.

These results could thus instead be an impairment in credit assignment, something which the results of this chapter support. It is likely that due to the close temporal proximity of light and dark side exposures, and footshock, a memory trace of both chambers of the arena were in short-term memory at the time of footshock. Animals may have assigned negative valence to both sides of the arena as a result. It would be interesting to test performance on IA in GluA1$^{-/-}$ mice, or in the rats used by Mitsushima *et al.,* but with separate exposure to the light side, from those to the dark side and subsequent footshock, such that there is less potential for overlap of competing cues in short-term memory. Enhanced mediated conditioning could also explain the results seen in SPC here. It is possible that there is overlap of representations of auditory stimuli in short-term memory, potentially due to impaired self-generated priming in GluA1$^{-/-}$ mice, or through retrieval-generated priming of stimulus elements back into short-term memory after decaying to I.

A key aim of this chapter was to demonstrate alterations in associative learning that may lend support to the use of this model, as one with high construct validity in relation to clinical psychosis. The findings of enhanced associative learning under simple conditions, but impaired SPC under complex conditions suggest that the formation of associations is

affected in these mice, potentially in a way that is translationally relevant to the clinical condition.

Chapter 6: General Discussion

This thesis has investigated the contribution of synaptic plasticity to salience, attention, habituation and associative learning, and the effects of impaired plasticity on these components of behaviour in preclinical models. Through the study of two models of impaired synaptic plasticity, and a third model – of hyperdopaminergia – commonly used in the psychosis literature, we have demonstrated intriguing model-specific deficits that provide insight into how the behavioural phenotype associated with these models may arise, and what this may mean for clinical psychosis.

Furthermore, in the case of GluA1 knockout, we have gone on to demonstrate how these impairments in habituation and attention may consequentially affect how animals learn about their surroundings in a task of associative learning. This is particularly important for considering the construct validity of models commonly used in drug discovery research, an important aspect of this research project. The aberrant salience framework (Kapur 2003) is widely recognised as a mechanism through which psychotic symptoms may arise in the context of schizophrenia, although this may also be relevant to other disorders where psychosis can develop. However, it has been difficult to say with certainty whether a similar framework could exist in preclinical models that are commonly used in drug discovery research. By demonstrating deficits in habituation and attention, and subsequently inappropriate association formation in SPC, we have shown parallels between this model and the clinical condition that indicate high construct validity for GluA1$^{-/-}$ mice as an experimental tool to study psychosis.

In this final chapter, we will discuss in more detail the phenotype observed with each model used throughout this thesis, in terms of behaviour reported here but also in the wider

literature. We will consider the psychological and neural mechanisms that may lead to these observed behavioural phenotypes, and the utility of each model as a tool for drug discovery research.

Amphetamine as a model of hyperarousal, but intact short-term memory

In our studies, amphetamine induced hyperactivity in a novel environment, but also when administered in the highly familiar homecage. Despite this, expression of short-term memory was seemingly intact when tested in a spatial novelty preference test in the Y-maze; animals appropriately responded to the novel arm of the Y-maze, choosing to spend more of the test phase exploring this arm than a previously visited location. Interestingly, in a separate test of short-term habituation, NCR, amphetamine-treated mice were impaired. However, rather than reflecting a genuine impairment in short-term memory following amphetamine treatment, this may highlight potential limitations of the NCR assay (e.g., the absence of a choice measure in this task). It is vital that these limitations are considered when choosing any behavioural paradigm to test for deficits; a point we will return to later in the discussion.

Overall, these findings are supported by other reports in the literature. Hyperactivity has been confirmed elsewhere, in novel environments (Schiørring 1971), but also in familiar locations (Khroyan *et al.,* 2012; Zhuang *et al.,* 2001), though prior exposure to these environments is typically less extensive than the 8 days of acclimation to a homecage used here, and often at different doses of amphetamine or in rats. Preserved short-term memory in the Y-maze has also been confirmed recently in a separate report from the

Bannerman group (Wolff *et al.,* 2018), as well as in private communications with David Sanderson and colleagues, further reinforcing the argument that this behaviour is unaffected by enhanced dopamine transmission.

These findings point to a model of hyperarousal in response to an animals' environment or changing internal state with amphetamine, but no evidence of impaired short-term memory. This fits with our current understanding of how antipsychotic treatments work to alleviate positive symptoms of schizophrenia. By reducing the salience of distressing delusions or hallucinations, patients learn to disregard them and eventually deconstruct them to the point that they fade from the patients awareness (Kapur 2003). However, these drugs don't selectively target aberrantly salient events, and seemingly don't fix the underlying problem. By dampening salience at a more general level to all stimuli, not just those that are aberrantly salient, neuroleptics have been criticized for inducing a state of dysphoria in patients (King, Burke, and Lucas 1995). Furthermore, after stopping antipsychotic treatment, symptoms typically return in schizophrenic patients. The nonspecific effect of neuroleptic drugs, combined with the fact that they fail to rescue cognitive or negative symptoms of schizophrenia have led many to believe they are working downstream of the cause of schizophrenia (Howes and Kapur 2009).

The results of Chapter 3 seem to support this. Preclinically a psychotomimetic compound increased locomotor activity independently of context or familiarity of the environment, but seemingly failed to induce deficits in short-term memory that may be expected to arise if priming was impaired.

However, we observed profound impairments in attentional processing in the 5-CSRTT. There are contrasting reports in the wider literature around this behaviour, with some

reporting impairments similar to those found in this thesis (Baarendse and Vanderschuren 2012; Fletcher *et al.,* 2011; Papaleo *et al.,* 2012), and others suggesting performance is relatively normal (Cole and Robbins 1987; Harrison *et al.,* 1997). These differences may reflect variation in doses used, or comparisons of rat versus mouse models, but could also be due to any differences in task parameters between studies during the lengthy training schedule required for 5-CSRTT. One clear finding across all studies of amphetamine, as well as our own in Chapter 4, is that of increased impulsivity. Where deficits in focused attention are seen, it is only in terms of choice accuracy, omissions always remain similar to control levels. Impaired choice accuracy in some circumstances may reflect a deficit in inhibitory control with high doses of amphetamine, affecting not only premature responding, but also accuracy if animals are unable to inhibit an impulsive response at any port both before and after cue onset.

In summary, our studies of amphetamine argue for a generally arousing effect of amphetamine that may mimic some of the psychotomimetic effects seen with amphetamine in humans, but the drug remains a relatively poor model for the wider features of schizophrenia typically seen in patients. Drugs targeting aberrantly high dopamine have failed to do more than reduce positive symptoms in schizophrenia patients, and even then, they come with substantial side effects. In the same way, preclinical models of increased dopamine transmission may be a model of some aspects of schizophrenia but they fail to effectively replicate the wider disease framework. It is important to emphasise that this does not necessarily mean amphetamine, and other drugs inducing hyperdopaminergia, are a poor choice of model for preclinical studies aiming to provide translational relevance to human disease states, but these limitations should be considered when choosing a disease model to work with.

Aberrant salience in models of impaired synaptic plasticity

Both GluA1 knockout and MK-801 induced deficits in short-term habituation, but expression of long-term habituation was mostly preserved, and GluA1$^{-/-}$ mice were still able to form associations across stimuli or events. Investigation of these models across Chapters 2 and 3 found increasing locomotor hyperactivity in a novel environment indicating sensitisation to their surroundings. However, in a well-habituated homecage environment no such hyperlocomotion was observed. While MK-801 treatment showed some suggestion of subtle increases in homecage activity these were not significant. This could be a result of mild hyperarousal in these animals following compound administration or could reflect heightened emotional responses to the internal state of the animals after drug treatment. It could also indicate a mild deficit in long-term habituation, the exact nature of these abnormalities are unclear from these current results.

In the NCR task, performance was similar between the two manipulations, with both expressing stimulus-specific sensitisation to context A over repeated presentations that declined when placed in context B on the third trial. In the spatial novelty preference Y-maze, both manipulations were impaired in their expression of short-term habituation. Interestingly however, the distribution of responses across individual animals was very different between the two groups. While GluA1$^{-/-}$ mice typically expressed a 50% preference for novel and familiar locations, resulting in chance discrimination with little variance, MK-801 induced very divergent preferences either purely for the novel arm, or the familiar, or somewhere in between – the net result of this being average performance at chance levels. We ruled out a number of simple explanations for this pattern of

behaviour in MK-801; such as specific arms that were assigned either novel or familiar, arms assigned as novel for previous mice that may have left an odour trail, location of the experimenter in the testing room. Crucially, the results of the long-term habituation study indicate that impairments were unlikely due to perceptual deficits. Currently, it is uncertain why this behaviour arose, but it appeared fairly consistent after testing 19 animals after MK-801 treatment.

It was interesting to see that impairments in short-term spatial novelty preference did not translate to impaired performance in the long-term variant, and therefore are unlikely due to visual discrimination deficits. MK-801 had no effect on novelty discrimination when animals were extensively habituated to the two sample arms prior to receiving the drug. A similar effect was seen in GluA1$^{-/-}$ performance in the Y-maze, although here long-term habituation was not just shown to be intact, but enhanced, compared to controls (Sanderson *et al.,* 2009). Given the results of the simple-simple SPC experiment (Figure 5.3), it could be that GluA1$^{-/-}$ mice formed more robust associations across stimuli in the familiar arm locations during sample phases. Using the NCR assay we also demonstrated intact (though not enhanced under these circumstances) long-term habituation in GluA1$^{-/-}$ mice, confirming that this behaviour was preserved, but also emphasising the utility of this behavioural paradigm for investigating measures of habituation and the timescales affecting this behaviour.

These findings on measures of habituation indicate that both models demonstrate stimulus-specific sensitisation to novel environmental stimuli, indicative of aberrant salience at a psychological level in these manipulations. This raises interesting questions around how specifically these animals attend to stimuli, and whether this is restricted to

bottom-up control of attending to sensory inputs, or whether top-down mechanisms that modulate behavioural responding to salient stimuli are also affected. MK-801 was shown to significantly disrupt performance on the 5-CSRTT of attentional processing, supporting other literature looking at this NMDAR antagonist (Barlow *et al.,* 2018; Smith *et al.,* 2011). MK-801-treated mice were more impulsive, impaired on choice accuracy, missed more trials, and slower to respond, suggesting that overall they were unable to sustain attention and were generally out of synchrony with the task structure. Whereas amphetamine-treated and GluA1$^{-/-}$ mice were generally still engaging with the task to complete trials (despite being considerably more impulsive), MK-801 appeared to disrupt animals' ability to appropriately move through the task structure without getting distracted by other elements of the box. This could explain impairments on most measures of performance investigated here, as well as increased latencies to respond or to then collect reward. It is possible that this reflects some mild impairment of long-term habituation to the environment in this situation, or an effect of MK-801 on an animals' ability to complete a pattern of goal-directed model-based behaviour.

In GluA1$^{-/-}$ mice, accuracy was only impaired when task conditions became unpredictable, or distractors were introduced, although they were generally much more impulsive. This could indicate a disconnect between GluA1 knockout and MK-801, with largely preserved top-down attentional control in GluA1$^{-/-}$ mice, and disrupted top-down control with MK-801. However, animals were trained over 2-3 months and so a constitutive knockout model may have learnt to compensate for deficits across training such that performance during later stages was largely unaffected. Further work is required to better understand differences observed between these manipulations. Of particular interest, would be follow-up behavioural studies in animals where expression of GluA1-containing AMPARs at

hippocampal synapses was temporarily blocked, as has recently been achieved through antibody infusion (Farhy-Tselnicker *et al.,* 2017). Alternatively, it may be relevant to investigate attentional processes in chronic NMDAR knockout models such as the *Grin1*$^{\Delta DGCA1}$ model (Bannerman *et al.,* 2012, 2014; Taylor *et al.,* 2014).

Dopamine and associative learning

For many years, schizophrenia has been linked with a hyperdopaminergic state, and the aberrant salience framework outlined by Shitij Kapur (2003). This details a mechanism through which elevated dopaminergic activity, and increased attending to stimuli, could lead to an increased propensity for association formation, and potentially psychotic delusions. But there is considerable evidence that dopaminergic abnormalities may occur downstream of other events that induce this state of aberrant salience (Howes and Kapur 2009).

However, how exactly neural dysfunction in schizophrenia leads to maladaptive associative learning is still somewhat unclear. In our preclinical investigations of GluA1$^{-/-}$ mice, we have shown that glutamatergic hypofunction leads to deficits in short-term habituation which in some situations can lead to stimulus-specific sensitisation to novel stimuli, and in Chapter 5 went on to show how this can generate an increased propensity to form associations. Associations formed were shown to be beneficial under very simple conditions, but detrimental to performance when the task was more complex, though in both situations this learning was abnormal compared to controls. Separately, recent outputs from the Bannerman group have also shown these mice to be hyperdopaminergic, with elevated dopamine release in the nucleus accumbens across multiple exposures to unexpected auditory stimuli (Panayi *et al.,* 2021).

These findings point to a potentially important role of midbrain dopaminergic activity in forming associations between environmental cues, and the potential role of aberrant signalling in this pathway in forming maladaptive associations, relevant to clinical psychosis. How exactly this may occur, however, is still unclear. A recent paper investigating the effects of VTA dopaminergic signalling on tests of SPC has helped to shed some light on the function of this pathway (Sharpe *et al.,* 2020). In this paper, rats were first pre-exposed to a visual cue A, followed by an auditory tone, X (A→X), in the absence of reward. Animals then received a second session of preconditioning, where A was presented as a compound cue, paired with two different cues (C or D), followed again by X (AC→X, AD→X), preventing learning about the relationship with C or D and X through blocking (Kamin 1968). Importantly, in half of rats, a virus containing photosensitive channel rhodopsin 2 (ChR2) was injected into the VTA, and in the other group, an inactive control protein was injected, eYFP. During the second session of preconditioning, when X was presented after an AC compound stimulus, 1s of blue light (473nM) was delivered alongside X onset, activating ChR2 to stimulate dopaminergic VTA neurons.

This light delivery had no effect, in either group, during preconditioning or conditioning, where animals then learnt to associate cue X with delivery of two sucrose pellets. Prior to probe testing, Sharpe *et al.* tested animals on the value assigned to individual cues in two sessions of a conditioned reinforcement paradigm. In the first session, animals were presented with two levers; pressing of lever 1 led to presentation of cue D, whilst lever 2 was paired with cue C. No preference was seen for either lever in the eYFP controls, but also not in the ChR2 group where VTA dopaminergic transients had been evoked after presentations of C. In the second session, the levers were paired with cue D again, or with the conditioned cue X, and both groups showed a robust preference for the lever paired

with X. These findings indicate that cue X has acquired value through pairing with reward, but the AC→X cue pairing, coinciding with optogenetic stimulation, has no effect on an animals perceived value of cue C.

Finally, in a probe test, rats were tested for SPC, by delivering interspersed presentations of cue C or cue D, across a session. Both cues had been blocked during preconditioning, by pairing with the preconditioned cue A. In the eYFP group, there was no preference for either cue. However, in the ChR2 group, a preference for cue C was seen, indicating that by stimulating dopamine neurons in the VTA, cue C was unblocked during preconditioning, and associative learning was facilitated. This occurred in the absence of cue C acquiring value, as might be expected by prediction error accounts, and argues for a role of midbrain dopamine transmission in the formation of model-based associations.

A follow-up experiment in the same paper followed a similar SPC approach to the complex-complex condition in Chapter 5, with a day of preconditioning to cue-pairs A→B and C→D, followed by conditioning to B as a CS+, and D as a CS-. However, during preconditioning, 1s of blue light was delivered alongside the onset of cue B. No difference was seen between ChR2 and eYFP groups in responding to cues during preconditioning, or in rates of acquisition of conditioning. There was no difference between groups in a conditioned reinforcement test comparing preferences for levers associated with cue A or cue C, or in a second session comparing cues B and D (where both groups displayed a preference for the lever linked with B). During a probe test measuring SPC eYFP controls showed intact SPC, responding more to cue A, preconditioned with B, compared to C. However, interestingly, the SPC effect disappeared in the ChR2 group. The only difference between groups was that animals infused with virus containing ChR2 were sensitive to the effects of

blue light delivery during cue B presentation in preconditioning, artificially evoking dopamine transients coinciding with cue B. This was sufficient to eliminate the SPC behaviour, and appears to be an effect of overlearning during the preconditioning phase – in a separate cohort of rats, training animals to 48 cue pre-exposures, rather than the usual 12, also eradicated SPC. These experiments argue for a mechanism of valueless associative learning after artificial induction of VTA dopaminergic transients.

In a final experiment investigating the effect of optogenetic stimulation on associative learning, rats were trained on a configural learning paradigm, where two cues were either rewarded or unrewarded. On separate trials, when cues were preceded by a second cue, the configural structure was reversed, meaning the opposite cue was now rewarded. ChR2 animals, sensitive to VTA stimulation by blue light delivery, learnt this conditioning rule faster, and more robustly than eYFP controls. Importantly, in a probe test, stimulation was only presented on 50% of trials, but no differences in performance were seen in trials where stimulation was applied, vs trials where it was not. This result suggests that VTA dopaminergic activity is important for acquisition of associative learning, but not for its subsequent expression.

These findings linking dopamine transmission with the formation of associations may be highly relevant for understanding onset of psychotic symptoms in the clinic, where exaggerated dopaminergic prediction error signals have been shown to occur during learning (Corlett, Murray, *et al.,* 2007). These findings can also potentially be applied to our understanding of deficits in GluA1$^{-/-}$ mice. In this thesis, and elsewhere (Sanderson *et al.,* 2009), these animals have been shown to display an increased propensity to form associations, and notably there is also evidence of elevated dopamine (Panayi *et al.,* 2021).

Whereas WT mice showed an attenuation of NAc dopamine activity across repeated presentations of the same stimulus, dopamine activity remained high in GluA1$^{-/-}$ mice. These findings, in the context of findings reported by Sharpe *et al.* suggest a mechanistic link between increased dopamine transmission and maladaptive associative learning in GluA1$^{-/-}$ mice. This suggests a potential pathway to the increased propensity to form associations that we observed in Chapter 5.

Associative learning and habit formation

We have seen interesting comparisons between GluA1$^{-/-}$ mice and clinical schizophrenia, where hyperdopaminergia seemingly leads to aberrant salience and subsequent formation of inappropriate or maladaptive associations. In the aberrant salience framework, the final outcome of this pathway is the onset of delusions as a means to explain why physiologically irrelevant or neutral events take on significance (Kapur 2003). Delusions represent false beliefs that are resistant to re-evaluation despite overwhelming evidence arguing against them. While it is very difficult to say with certainty whether a mouse model is capable of delusional false beliefs, we will consider the evidence for a similar mechanism in GluA1$^{-/-}$ mice here.

The formation of instrumental associations can lead to the development of habitual behaviours, model-free repetition of behaviour, that is not re-evaluated despite evidence against its benefit (Corlett *et al.,* 2009, 2010). In comparison, model-based behaviour represents goal-directed learning about the world where individuals are able to change their perspective based on results. Habitual behaviour is not inherently a bad thing, or an indication of underlying psychiatric conditions; when faced with highly stable conditions where events are predictable, it could be advantageous to conserve attentional resources

for unexpected events. But an unwillingness to update ones perspective and persist with repetitive behaviours can be debilitating to an individual, and their onset and tenacity have been linked with the tenacity of delusions in schizophrenia.

It is possible, given the increased propensity for GluA1$^{-/-}$ mice to form associations between environmental events, that habit formation is also affected in this model. In an early study of GluA1$^{-/-}$ mice, it was found that they were insensitive to the effects of reward devaluation in a paradigm where they had to run along a track for reward (Johnson *et al.,* 2005). In this task each end of a linear track was associated with a unique reward (liquid sucrose or pellets). After training to run along this track for reward, animals were tested for outcome preferences after reward devaluation. WT mice were slower to cross the track to reach the devalued goal area, compared to the non-devalued. However, no differences were observed in running speeds for GluA1$^{-/-}$ mice. In a follow-up experiment from the same paper, they also showed GluA1$^{-/-}$ nose-poke responding in an operant chamber to be insensitive to outcome devaluation. Despite this, both WT and GluA1$^{-/-}$ mice consumed less of the devalued reward during a choice test, indicating that there was no change in the motivational valence of the reward in GluA1$^{-/-}$.

One explanation of this result is a lack of inhibitory control, a theory that would fit with observations in the 5-CSRTT in Chapter 4 of this thesis. The authors argued against this possibility in a follow-up study (Johnson *et al.,* 2007). They showed that testing animals in a non-deprived state reduced their willingness to perform an instrumental behaviour previously associated with reward, during an extinction test session. Instead, they argued, GluA1$^{-/-}$ mice may be unable to form an association between the reward-predicting cue, and sensory-specific properties of the reward outcome.

However, an alternative account is that GluA1$^{-/-}$ mice more readily fall into habitual, model-free behaviours and so are less able to re-evaluate their actions at test. Recent work from the Bannerman/Walton group has investigated the propensity of GluA1$^{-/-}$ mice to form habits in an instrumental response paradigm (Fernando *et al*., manuscript in preparation). Here, assessment of responding after outcome devaluation took place (in extinction) after just two 30-minute sessions of responding to a lever for rewards. They were then trained for two more days, then received a second test of devaluation. They then received a third round of this process, with two more days of training of instrumental responding for reward, followed by a final test of devaluation.

In this experiment, after two days of instrumental response training, there was no evidence of habitual responding in either group, and outcome devaluation was intact. Both WTs and GluA1$^{-/-}$ displayed reduced rates of lever pressing in the first extinction test session, compared to the previous day of training. However, in the second and third test sessions, rates of lever pressing increased in GluA1$^{-/-}$ mice, whereas they stayed stable in WTs. Over 6 days of training, WT mice continued to exhibit reduced responding to the conditioned lever following outcome devaluation. While GluA1$^{-/-}$ mice initially showed similar, goal-directed, behaviour, they transitioned between the first and second test sessions to being more model-free, or habitual.

The results of this study indicate that GluA1$^{-/-}$ mice may more readily develop habitual behaviours, a finding that could be explained by our analysis of SPC performance where we found that GluA1$^{-/-}$ mice expressed an increased propensity for association formation. The study described above by Sharpe *et al.* further indicates how “overlearning” (in this case via overtraining during preconditioning, or activation of VTA dopaminergic neurons)

could lead to maladaptive associations. While this was expressed as impaired SPC in the Sharpe *et al*. study, and in our own work using the same paradigm, in the experiments performed by Fernando *et al.* this appears to have led to the development of habitual behaviours.

Furthermore, it is possible that an increased propensity for model-free, habitual behaviours, could explain the results of earlier studies investigating outcome devaluation in GluA1$^{-/-}$ mice (Johnson *et al.,* 2005, 2007). In the experiments described in these studies, there was fairly extensive training to the task conditions (9+ training sessions per experiment) prior to tests of outcome devaluation. It is possible that GluA1$^{-/-}$ mice became habitual prior to the test, and the lack of outcome-specific devaluation was a result of habitual behaviours.

GluA1 and synaptic homeostasis

The results of this thesis argue overwhelmingly for an impaired self-generated priming account to explain deficits, at least in GluA1$^{-/-}$ mice, but this may also translate to other models of glutamatergic dysfunction, like MK-801, where similar behavioural impairments were observed. The existence of stimulus-specific sensitisation in these animals suggests that behavioural deficits are the result of salience misattribution and inappropriate behavioural responding to novelty – rather than an inability to discriminate or respond to novelty.

Earlier accounts of the role of hippocampal glutamatergic transmission are unable to accommodate the behavioural findings presented in this thesis. Early work argued for a causal role for hippocampal glutamatergic function in hippocampus-dependent associative learning and memory, supported by impaired water maze performance in hippocampal

lesioned animals (Morris *et al.,* 1990), or in the presence of NMDAR antagonists (Morris *et al.,* 1986; Tsien *et al.,* 1996). There is now substantial evidence that this is not the case (Bannerman *et al.,* 1995, 2012; Sanderson *et al.,* 2009), including the findings of this thesis. We have shown that GluA1$^{-/-}$ (Figure 2.10) and MK-801-treated mice (Figure 3.7) are able to learn about their environments and express appropriate long-term habituation, attending more to novel contexts or locations than familiar ones. Furthermore, in the short-term, deficits are not just driven by a failure to recognise novelty. Instead, these animals displayed an inappropriate increase in attending to stimuli over prolonged or repeated exposure. These findings provide convincing evidence for a memory trace of a stimulus, suggesting there may be a different explanation to how behavioural impairments arise.

Impaired self-generated priming can accommodate all of the behavioural abnormalities described throughout this thesis. Certainly in the case of GluA1, a blockade from A1 to A2 attentional states could explain stimulus-specific sensitisation, impaired short-term but enhanced long-term habituation, and maladaptive associative learning. Furthermore, a priming account for GluA1 could explain the results observed by Whitlock *et al.* (2006), where they saw long-term changes in expression of behaviour after inhibitory avoidance training, despite only reporting elevated GluA1 levels for 1-2 hours post-training. Synaptic recruitment of GluA1-containing AMPARs in the hippocampus may be important for self-generated priming, without affecting long-term memory or retrieval-generated priming.

This role for GluA1 could also explain changes in its expression throughout the sleep/wake cycle; increasing during periods of wakefulness and returning to baseline during sleep (Vyazovskiy *et al.,* 2017). In the General Introduction, we described how sleep homeostasis

helps to regulate the balance of synaptic plasticity; synaptic proteins accumulate throughout periods of wakefulness, increasing sleep burden or pressure. Sleep acts to restore the homeostatic balance by scaling down synaptic plasticity potentially through a LTD like mechanism during slow-wave sleep (Tononi and Cirelli 2006, 2014). Through this mechanism, sleep may play a role in restoring attentional capacity, dishabituating an individual to recent events and readying the brain for new learning (i.e., elements pass from the A2 state back to the I state in the context of the SOP model). If GluA1 represents a molecular candidate for self-generated priming, its accumulation at synapses throughout the day, coinciding with increased sleep burden, may reflect the priming of numerous memory traces over prolonged periods of wakefulness. In doing so, habituation may be a means to conserve metabolic resources, by disengaging from expected events that have previously been learned about. If habituation is impaired in GluA1$^{-/-}$ mice, and attending to stimuli remains aberrantly high, sleep pressure may accumulate faster. This has recently been shown to be the case in GluA1$^{-/-}$ mice (Ang *et al.,* 2018). These results could explain the increased propensity for habitual, model-free behaviours with less scope for retrospective re-evaluation.

Downstream consequences of disrupted synaptic plasticity

Throughout this thesis we have demonstrated a behavioural phenotype of aberrant salience and stimulus-specific sensitisation in two models of disrupted synaptic plasticity; MK-801 and GluA1 knockout. It would be important, going forwards, to expand on these results by investigating what is happening at a physiological level that may drive behavioural abnormalities. This was an original aim of the current project but was

abandoned due to time constraints. The 5-choice testing kit was designed with the potential for future recording studies in mind, and with consideration of the need for a steady-state task investigating attentional processes that could potentially be used to generate physiological recording data over multiple sessions.

Fibre photometry has been used over the last ten years to measure changes in neural activity or neurotransmitter release in real-time, during behavioural testing (Cui *et al.,* 2013; Gunaydin *et al.,* 2014). Early studies using this technique used genetically encoded calcium indicators to measure intracellular fluctuations in Ca^{2+} concentration as a proxy of neural activity. More recently, proteins sensitive to neurotransmitters have been developed, including the dopamine sensor dLight (Cosme, Palissery, and Lerner 2018; Patriarchi *et al.,* 2018). In the Walton group, this sensor has successfully been used in tandem with fibre photometry recordings to measure dopamine release in the nucleus accumbens, and dorsomedial striatum during behavioural testing (Blanco-Pozo, Akam, and Walton 2021).

Further work investigating the relationship between GluA1, MK-801, and salience/priming would benefit from physiological investigation of dopamine responses during tests of habituation and attention. One of the advantages of the newer generation of genetically encoded sensors such as dLight, is they do not require a Cre-lox transgenic mouse line; dLight can be injected into any mouse model of interest to measure synaptic dopamine release. This has opened up new possibilities for measuring physiological changes in genetic mouse models of interest, without the need for generating new transgenic lines. Using this methodology, it would be possible to compare changes in dopamine transmission in GluA1 and MK-801-treated mice, or in mice treated with an antibody

blocking GluA1-dependent AMPAR function (Farhy-Tselnicker *et al.,* 2017) during behavioural testing. In studies investigating orienting responses to stimuli, GluA1$^{-/-}$ mice exhibited behavioural sensitisation, and a lack of habituation of the NAc dopamine response over a session (Panayi *et al.,* 2021; Sanderson, Sprengel, *et al.,* 2011). Sensitisation was not seen behaviourally in the orienting to lights task with MK-801, though this may be more due to task parameters than a genuine effect (Boerner 2018). When tested in NCR, we did observe stimulus-specific sensitisation. Further work is warranted both to better understand behavioural similarities or differences between models, but also to probe the physiological nature of behavioural deficits.

Further work is also warranted in the investigation of alterations in dopamine release at other VTA projection sites in response to novel/familiar stimuli, or during tasks of attention. It could potentially be very interesting to investigate changes in dopamine release in areas such as prefrontal cortex, important for guiding attention during the 5-CSRTT (Tan *et al.,* 2018). Furthermore, it would be important to consider the effect of dopamine release in the hippocampus, which may reveal insights into a potential positive feedback loop in models of glutamatergic dysfunction (Lisman *et al.,* 2008).

Alternative mechanisms of habituation and associative learning

Throughout this thesis, efforts to explain abnormalities in habituation and formation of associations have largely been viewed through the lens of Wagner's model of SOP (Wagner 1981). Previous reports investigating GluA1$^{-/-}$ mice have posited that this model may offer a psychological mechanism that can account for observed behavioural outcomes (Barkus

et al., 2014; Barkus, Feyder, *et al.,* 2012; Sanderson and Bannerman 2012). This thesis has expanded on the predictions made in these earlier reports, but some consideration should be given to alternative theories that could be used to explain these processes under normal and impaired conditions.

One of the strengths of Wagner's model is that it attempts to integrate mechanisms of habituation with effects on associative learning, whereas many other learning theorists have given little regard to how habituation affects stimulus processing (Hall and Rodríguez 2017). A recent review of the habituation literature found only three proposals that could account for habituation processes (Thompson 2009). The dual-process theory put forward by Groves and Thompson (1970) has been relatively successful in accounting for habituation following repeated stimulus presentation, and sensitisation as an opposing process modulating an incremental increase in responding. However, this model does little to explain changes in attending to stimuli over longer timeframes (in contrast to mechanisms of self- vs. retrieval-generated priming described by Wagner (1976)).

While the Groves & Thompson model is useful for explaining how responding diminishes over repeated stimulus presentations, it makes little effort to reconcile these changes with underlying psychological mechanisms. A second theory of habituation indicates that the stimulus becomes less effective as it comes to match an internal representation of the stimulus encoded following prior experience (Sokolov 1963). However, beyond some consideration in the context of the orienting response to novel/familiar stimuli, little consideration has been given to Sokolov's model in the context of associative learning. Wagner's model of SOP has received by far the most attention by learning theorists, for its ability to reconcile processes of habituation, with subsequent formation of associations.

A number of models of associative learning theory have been developed over the last century. These were summarised briefly in the Introduction to this thesis, but it would be prudent to consider the strengths and limitations of some of these theories, given the efforts made to understand glutamatergic hypofunction-induced behavioural deficits in terms of SOP.

The Mackintosh model built on the foundations developed by Rescorla and Wagner (1972). The key addition was that the amount of attention paid to a stimulus would be higher if that stimulus was a good predictor of the US (changing the value of α in the Rescorla-Wagner equation)(Mackintosh 1975). However, this model has been contradicted by others. When rats are trained to learn a tone-shock association, if the shock intensity is then increased learning is delayed about the new US compared to animals who were pre-trained with a different CS identity. According to Mackintosh's model the associative strength of the tone CS should be increased but the opposite was observed, a phenomenon termed Hall-Pearce negative transfer (Hall and Pearce 1979).

Pearce and Hall went on to develop their own ideas of associative learning; the Pearce-Hall model (Pearce and Hall 1980). In this model, attention is again represented by α, but varies depending on the surprisingness of the US on the previous trial. When the US is surprising, attention is high, as in Mackintosh's model, but is reduced when the US is unsurprising (i.e., when the CS-US relationship is well understood, or in latent inhibition).

However, neither the Mackintosh nor the Pearce-Hall models are able to explain associations formed between two CSs, such as those described in the sensory preconditioning experiments of Chapter 5. SOP is the only model capable of explaining how associations may develop between two neutral stimuli during preconditioning, as well as

how associative strength of a CS may increase without the occurrence of that CS as in the conditioning stage. Similarly, other studies looking at changes in associative strength between two CSs, in the absence of either CS occurring, cannot readily be explained by other learning theory models (Holland and Sherwood 2008).

Similarly, through its integration of processes of both habituation and conditioning, SOP is better able to explain the decline in responding seen over days to familiar arms of the Y-maze, as shown in GluA1$^{-/-}$ and WT mice (Sanderson *et al.,* 2009; Sanderson and Bannerman 2011), but also following MK-801 treatment (Figure 3.7).

However, there are limitations to the utility of SOP. Whilst it has proven to be effective at determining how bottom-up attentional processing of incoming sensory inputs changes across repeated or prolonged exposure, it was less applicable to mechanisms of top-down attention. The 5-CSRTT experiments of Chapter 4 and deficits seen across each manipulation cannot be easily reconciled with an SOP explanation. Furthermore, SOP has come under criticism for being so flexible that it cannot easily be disproved. Whilst this shouldn't necessarily be considered a criticism of the model, this fact combined with the lack of biological substrate for the mechanism make its occurrence difficult to prove categorically. This model remains the most likely explanation for the results obtained in this thesis, but further work is clearly needed to elucidate the mechanisms controlling these processes.

Use of appropriate paradigms in behavioural profiling

A core feature of this project has been the behavioural phenotyping of models commonly used in the psychosis field, in order to draw comparisons between models and assess their relevance to the clinical condition. We have emphasised throughout this thesis, the importance of careful consideration of behavioural assay required to probe the psychological mechanism of interest. But there are a number of other important aspects of study design that are worth iterating here that should be considered when initiating a study. Appropriate planning of study design is critical, not just for the quality of scientific data collected, but also from an animal welfare perspective, to reduce the number of animals required as much as possible, and refine techniques to do the least harm where animals must be used.

Firstly, it is important to consider sample size – where a minimum number of animals may be required to observe group differences, but no more animals should be used than absolutely necessary. Throughout this thesis, sample sizes have been used that are comparable to previous studies showing deficits in behaviours tested here (e.g., Gilmour *et al*., 2009; Wolff *et al*., 2018). However, where results have been particularly surprising, or approaching significance where they may benefit from increased power, additional cohorts were used in replications of the experiment (such as in the striking Y-maze novelty preference pattern exhibited by MK-801-treated mice, Figure 3.6).

The use of one or both sexes can also affect power; variability in patterns of activity seen in male vs. female mice may increase the variance in data where both sexes are used.

GluA1$^{-/-}$ mice were bred and maintained by the Bannerman research group. In order to reduce the number of animals used overall, both male and female mice were used in experiments with this strain. C57Bl/6j mice used in this thesis were ordered from breeders, either within the University of Oxford, or from external suppliers. Because other researchers would use mice from the same colonies, only male mice were used when ordering from elsewhere.

Doses of amphetamine and MK-801 were chosen based on reports in previous literature of doses that can induce locomotor hyperactivity and deficits in spatial recognition (Bygrave *et al.,* 2016; Wolff *et al.,* 2018). Where experiments required regular handling of the animals (e.g., spatial novelty preference, novel context recognition), experimenters ran the animals blind to pharmacological treatment group. This wasn't feasible to be controlled for in every experiment ran, but assays that largely relied on automated recording (e.g, open-field, homecage activity, 5-CSRTT) should be less susceptible to experimenter biases as there is less experimenter-subject interaction.

Finally, it is vital to consider the relevance of different behavioural approaches, and what we can learn from each task either individually or in the context of the wider behavioural profile observed across multiple tasks in each model. As an example, locomotor activity has been used for decades as a proxy of underlying neural dysfunction. Pharmacology that induces hyperlocomotor activity, and subsequent efforts to restore normal responding through administration of novel compounds has been used as a method of drug discovery research for years in the schizophrenia field, but recent papers have called the utility of this approach into question (Gilmour *et al.,* 2015; Pratt *et al.,* 2012). There are countless

drugs that could reduce locomotor responding but would not be a suitable candidate as a potential antipsychotic (Fell *et al.,* 2008; Moghaddam and Adams 1998; Patil *et al.,* 2007).

That does not necessarily mean that the novel open-field assay has no utility as a test to assess an animals' behavioural phenotype. In Chapters 2 and 3 of this thesis we initially looked at locomotor responses, in novel or familiar environments, in our models of interest and revealed some interesting differences between models. Furthermore, through investigation of the early stages of a novel open-field test at high temporal resolution we were able to see some interesting patterns of how hyperlocomotor responding develops, which in the context of other behavioural paradigms tested throughout this thesis lends further support to the argument that GluA1$^{-/-}$ and MK-801-treated mice exhibit deficits in short-term habituation that can lead to stimulus-specific sensitisation to novel stimuli.

The strength of the data generated in Chapters 2 and 3 lies not in any single compelling result highlighting a specific behavioural impairment, but in the profile of an animal model developed across multiple assays investigating different components of short- or long-term habituation and learning more generally. No single behavioural test can provide a comprehensive phenotype of an animal, to the extent that we can understand the relationship between circuit-level dysfunction induced by a manipulation and behavioural outcomes. This can only be achieved through a broad profile of behaviour probing different components of relevant memory circuits (and ideally through physiological recording of neural activity during behaviourally relevant tests). In the case of this thesis, we have carefully considered the choice of behavioural paradigms that would provide insight into how aberrant salience may develop in GluA1$^{-/-}$ mice. But this is also apparent when considering the choice of behavioural assay for measuring short-term habituation. An

interesting, and at face value contradictory, result came from our studies of amphetamine showing intact short-term memory in the Y-maze, but seemingly impaired habituation in the NCR assay. However, these differences can be reconciled by considering the outcomes being investigated in each paradigm, alongside their potential limitations.

The spatial novelty preference Y-maze has the advantage of presenting animals simultaneously with both novel and familiar locations, where the primary outcome was time spent in these arms, and thus generating a choice measure of performance. A preference for novelty, measured as increased time spent in the novel vs. familiar arm, was seen as analogous to novelty discrimination and appropriate expression of habituation to familiar stimuli.

In the NCR assay, animals were also presented with novel or recently familiar stimuli, but this time in series, across three discrete trials. Because of this, preferences for novel over familiar cues could not be assessed directly and were instead measured in a between-subjects comparison of exploratory activity within each environment, to avoid response competition – an important consideration given impairments seen in other assays in GluA1$^{-/-}$ mice where stimuli are presented in parallel. Importantly, here the primary outcome was locomotor activity within each context. This was not a problem in control animals, where we observed declining locomotor responding across repeated exposures to the same context. However, following amphetamine treatment locomotor activity remained high, independent of context (and importantly was also elevated in a highly familiar homecage environment). Apparent deficits in performance, following amphetamine treatment, may instead have been an inability to inhibit locomotor hyperactivity, and the context-independent hyperactivity may represent a ceiling effect of how much animals can move

in a 30s window. Whilst this represents a potential limitation of the NCR task, it remains a valuable measure of habituation if we keep in mind exactly what is being recorded – by avoiding response competition this task revealed sensitisation in GluA1$^{-/-}$ mice, for example. From the results of this task alone, we may deduce that habituation is impaired by amphetamine. But given the result of the Y-maze, we know that these animals can habituate, and our assessment of activity in the homecage also emphasises that these animals become hyperactive independent of novelty.

These results have emphasised the importance of considering what exactly is being measured as an outcome in a behavioural paradigm when designing a study, and how this may represent a limitation when assessing a manipulation of interest. Interestingly, in the case of MK-801 and GluA1$^{-/-}$ mice, the utility of the Y-maze and NCR task for measuring short-term habituation were essentially reversed, compared to their use when assessing amphetamine. By presenting stimuli in parallel in the Y-maze, glutamatergic models were impaired but we couldn't deduce a reason for these deficits. It was only through investigation of these deficits in NCR that we were able to conclude that these manipulations induce not just impaired short-term habituation, but sensitisation to novel stimuli over repeated or prolonged exposure.

Psychosis across disease domains

The focus of this thesis has been on how impaired synaptic plasticity could generate aberrant salience, largely in the context of schizophrenia and preclinical models used in this field of research. However, as discussed in the General Introduction, psychosis is prevalent in many diseases, and coincides with impairments in synaptic plasticity that hints at a possible overlap in its aetiology. Across AD, depression and schizophrenia, patients

present with a range of cognitive, positive and negative symptoms. Psychosis has been reported across all three disease domains, although there is heterogeneity in the nature of psychotic symptoms both across disorders and individually within patients of each disease. To some extent, there are also reports of episodic memory deficits and low mood across these diseases.

Importantly, impaired LTP-like plasticity has been implicated in all of these disorders. We have already reviewed the evidence for this at length in the context of schizophrenia. But in AD patients, and AD-relevant rodent models, plasticity has also been shown to be reduced (Battaglia *et al.,* 2007), and soluble Aβ oligomers have been shown to disrupt hippocampal LTP (Walsh *et al.,* 2002). In research of depression, exposing rats to unavoidable footshocks led to reduced hippocampal LTP (Foy *et al.,* 1987; Shors *et al.,* 1989). There are also suggestions that the benefits of currently available, or promising new therapeutics for depression are driven largely by their effects in promoting neural plasticity (Branchi 2011; Castrén 2005).

While the focus of this thesis has been on psychosis in the context of schizophrenia, it is clear that symptomatology across these disorders is very similar in a number of ways. Through further research of the causes of psychotic symptoms and the contribution of circuit-level dysregulation in rodent models, we may gain a deeper understanding of the aetiology of psychosis that could be of benefit not just in schizophrenia, but in other disease domains where psychotic symptoms feature.

There is no single clear biological marker of a psychotic phenotype, and it is clear there is still a long way to go for us to fully understand how and why these delusions develop. But here we have outlined a comprehensive behavioural analysis of a rodent model with a

relevant circuit-level manipulation, that could be applied to investigation of other relevant circuits. By investigating behaviour across multiple levels of cognition we have shown how impairments in basic habituation and attention go on to have significant consequences on how an individual forms associations and learns about their environment. This approach could be very useful in furthering our understanding of how cognitive and positive symptoms may arise as a result of dysfunction or pathology found in other disorders, such as AD where there are suggestions that low level behaviours such as habituation and locomotor activity could be similarly affected (Blackmore *et al.,* 2017). It would be very interesting to investigate these deficits more closely, and see how this could go on to affect associative learning.

Concluding remarks

One of the strengths of this thesis is in its insight into the contribution of impaired synaptic plasticity to behaviour across multiple domains; ranging from basic habituation and attention, through to how this goes on to affect associative learning and potentially induces symptoms of psychosis. Taken in the context of other reports of the effect of impaired associative learning on habit formation and the aetiology of delusions, this work is potentially highly relevant to psychosis in schizophrenia, though there may also be mechanistic overlap with psychotic symptoms that present in other diseases such as depression and AD.

Additionally, this work has demonstrated dissociable behavioural phenotypes across three rodent models of relevance to psychosis research. Through careful and extensive profiling of behaviour, we have seen clear differences in both the behavioural deficits that present in each model, as well as how these may develop over time in response to features of an

animals' environment. In the case of amphetamine, as a model of hyperdopaminergia, deficits were largely limited to hyperarousal, whilst short-term memory was preserved. On the other hand, NMDAR antagonism via MK-801, or reduced AMPAR trafficking through genetic ablation of the *Gria1* gene, led to pronounced deficits on short-term habituation. This impairment in habituation was identified as the most likely factor for the hyperactivity seen in response to novelty. Crucially, associative learning and retrieval-generated priming appeared to be intact in both of these models, at least in the relatively simple behavioural measures investigated over longer timescales in this thesis (but see also Bannerman *et al.*, 2012). Deficits were instead limited to the short-term where we also demonstrated stimulus-specific sensitisation in response to repeated exposures of novel stimuli. The differentiable phenotypes observed based on specific circuit-level manipulations could reflect some of the heterogeneity seen in clinical schizophrenia populations.

These preclinical findings suggest high construct validity of the glutamatergic models investigated as a research tool for clinical psychosis, particularly in the case of GluA1$^{-/-}$ mice where we went on to demonstrate the consequences of stimulus-specific sensitisation on associative learning. However, equally as important as the findings on specific models, is to highlight the importance of the approach taken; to gain a deeper understanding of the behavioural phenotype of a preclinical model. It is important to consider carefully both models and methods used in preclinical analyses, if we are to gain insight into the aetiology of highly complex disorders. Psychiatric and neurodegenerative disease represents an area of profound unmet medical need at present, and previous clinical failures may be, to some extent, a result of poor choice of preclinical model or assay to test for efficacy. It is only through a better understanding of the tools available to us as researchers that we can hope to gain new insights into potential therapeutic treatments.

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