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Ventral hippocampal GABAergic inhibition and neurobehavioural function

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Abstract

The hippocampus has been implicated in specific memory functions, including declarative and place memory, but is also involved in a wide range of other cognitive and behavioural functions. In addition to these functions, the hippocampus can modulate behaviours dependent on other brain regions, including the medial prefrontal cortex (mPFC), to which the ventral to intermediate hippocampus has strong projections. The ventral hippocampus, in particular, has been linked to behavioural functions outside this narrow domain of specific memory functions, including anxiety and sensorimotor functions. Hippocampal dysfunction, including reduced GABAergic inhibition, so-called neural disinhibition, and hyperactivity in the hippocampus, has been implicated in several neuropsychiatric disorders, including schizophrenia. This thesis investigated the role of the ventral hippocampus and of local GABAergic inhibition, including of α5-containing GABA-A receptors (α5-GABAARs), which are preferentially expressed in the hippocampus, in selected innate and learnt behaviours.

In Chapter 1, we reviewed the current evidence for a role of the hippocampus in reversal learning and for the behavioural significance of hippocampal α5-GABAARs, with a focus on selected sensorimotor functions, including locomotor activity, prepulse inhibition (PPI) and startle.

In Chapter 2, we examined the role of the ventral hippocampus and of local GABAergic inhibition in distinct stages of reversal learning in rats. Reversal learning is a type of cognitive flexibility which involves switching from one response to another when the reward contingencies of the responses are reversed, and is disrupted in several neuropsychiatric disorders, including schizophrenia. In a previous study, we found reversal learning to depend on mPFC activity. Here, we examined the idea that ventral hippocampal disinhibition may manifest as impairment on tasks that depend on prefrontal activity. Additionally, we investigated how reduced activation of the ventral hippocampus would affect reversal learning, given that there is some evidence suggesting that the hippocampus may be required for aspects of reversal

learning. We used bidirectional manipulations of ventral hippocampal activity, including disinhibition by infusion of the GABA-A receptor antagonist, picrotoxin, and inhibition by infusion of the GABA-A receptor agonist, muscimol, and examined the impact of these manipulations on two stages of a food-reinforced two-lever reversal task. More specifically, we examined early serial reversal learning, where rats were unfamiliar with the task demands, and late serial reversal learning, where rats were familiar with the task demands. We found that ventral hippocampal disinhibition tended to improve early serial reversals, which may have partly reflected a disruption in the expression of the previous response; in contrast, ventral hippocampal inhibition did not affect early reversal performance. For late serial reversals, ventral hippocampal inhibition tended to impair performance, whereas ventral hippocampal disinhibition did not affect performance. This suggests that the ventral hippocampus may normally contribute to learning how to reverse and is then required to express such learning. However, if the ventral hippocampus is not available during early reversals, while rats learn to reverse, other brain regions can compensate, and rats show intact reversal performance.

In Chapter 3, we investigated behavioural functions of α5-GABAARs in the ventral hippocampus, which have been reported to be reduced in patients with schizophrenia. We focused on the contribution of ventral hippocampal α5-GABAARs to behaviours that have previously been shown to be affected by ventral hippocampal disinhibition, namely locomotor activity, PPI and startle. Changes in these behaviours in rodents have been widely used to study neural mechanisms associated with schizophrenia. More specifically, we examined the effect of negative allosteric modulation of ventral hippocampal α5-GABAARs, by infusions of L-655,708, on locomotor activity, PPI and startle. We did not find an effect of ventral hippocampal infusion of L-655,708 on any of these behaviours. Then, we examined if, although L-655,708 did not increase baseline locomotor activity, it may instead sensitise the dopamine system to stimulation and thus augment a locomotor response to amphetamine. Indeed, we found that L-655,708 tended to augment amphetamine-induced hyperlocomotion.

Our findings furthered our understanding of the role of the ventral hippocampus and of local GABAergic inhibition in behavioural functions. The ventral hippocampus tended to be required for late serial reversal learning, but not early serial reversal learning, whilst ventral hippocampal disinhibition tended to improve early serial reversal learning but had no effect on late serial reversal learning. This suggests that the reversal learning deficits in schizophrenia may not be a consequence of reduced hippocampal GABAergic inhibition and hyperactivity and instead are likely to be the consequence of dysfunction in other brain areas. Additionally, negative modulation of ventral hippocampal α5-GABAARs tended to augment the locomotor response to dopamine stimulation, but did not affect baseline locomotor activity, PPI or startle. This suggests that reduced expression of hippocampal α5-GABAARs, which has been shown in schizophrenia, may contribute to the heightened dopamine system responsivity characterising this disorder.

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List of abbreviations

5-CSRT: 5-choice-serial-reaction-time

α5-GABAARs: alpha-5 containing GABA-A receptors

ANOVA: Analysis of Variance

ARRIVE: Animal Research: Reporting of In Vivo Experiments

AWERB: Animal Welfare and Ethical Review Board

CA: Cornu Ammonis

CANTAB: Cambridge Neuropsychological Test Automated Battery

DMP: delayed-matching-to-place

DMSO: dimethyl sulfoxide

DREADD(s): designer receptors exclusively activated by designer drugs

GABA: gamma-aminobutyric acid

GAD: glutamic acid decarboxylase

GAT: GABA transporter

IED: intra-extra dimensional

i.p.: intraperitoneal

LFP: local field potential

LSD: Least Significant Difference

LTP: long term potentiation

MAM: methylazoxymethanol acetate

mPFC: medial prefrontal cortex

MUS: muscimol

NAc: nucleus accumbens

NAM: negative allosteric modulator

NMDA: N-methyl-d-aspartate

NVHL: neonatal ventral hippocampal lesion

OFC: orbitofrontal cortex

P: probability

PAM: positive allosteric modulator

PFC: prefrontal cortex

PPI: prepulse inhibition

PTX: picrotoxin

R: reversal

RTC: responses to criterion

s.c.: subcutaneous

SAL: saline

SD: spatial discrimination

SEM: standard error of the mean

SLM: stratum lacunosum-moleculare

SPECT: single-photon emission computed tomography

SST+: somatostatin-expressing

TLE: temporal lobe epilepsy

VGAT: vesicular GABA transporter

VP: ventral palladium

VS: ventral striatum

VTA: ventral tegmental area

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Chapter 1: Introduction

The hippocampus is one of the most studied parts of the human brain and has been of interest for centuries (Engelhardt, 2016; Knierim, 2015; S. J. Martin & Clark, 2007). A structure within the medial temporal lobe of the brain, the hippocampus plays a significant role in certain forms of memory, including aspects of declarative and place memory, but the hippocampus, particularly its ventral part, has also been linked to behavioural functions outside that narrow domain of specific memory functions, including emotional and sensorimotor functions (Bast, 2011; Bast et al., 2017; Bast & Feldon, 2003; Knierim, 2015; McGarrity et al., 2017). Cognition and behaviour depend on a balance of excitatory and inhibitory neurotransmission. Hippocampal inhibitory gamma-aminobutyric acid (GABA) neurotransmission has been linked to mechanisms which are important for cognitive and behavioural functions, such as the regulation of burst firing and the generation of neural oscillations (Bast et al., 2017; Buzśaki & Wang, 2012; Isaacson & Scanziani, 2011; Lovett-Barron et al., 2012; Mann & Paulsen, 2007; McGarrity et al., 2017; Royer et al., 2012). The hippocampus is dysfunctional in many brain disorders, including schizophrenia (Heckers & Konradi, 2010, 2015), Alzheimer's disease (Barnes et al., 2009; Small et al., 2011) and depression (Campbell & Macqueen, 2004; Fogaça & Duman, 2019). One of the key processes that is known to be dysfunctional in such disorders is GABAergic inhibition (Ambrad Giovannetti & Fuhrmann, 2019; Bast et al., 2017; Benes & Berretta, 2001; Dienel & Lewis, 2019; Heckers & Konradi, 2015; Lisman et al., 2008; Luscher et al., 2011; Rissman et al., 2007). Specifically, increased hippocampal neural disinhibition, i.e. reduced inhibitory GABA transmission, has emerged as a key characteristic of schizophrenia pathophysiology and has been linked to both cognitive impairments and psychosis (Heckers & Konradi, 2015; Lisman et al., 2008; Marín, 2012). Indeed, a causal link has been found between hippocampal disinhibition and some of the characteristic cognitive impairments of schizophrenia, including deficits in attention and memory in rats (McGarrity et al., 2017; Taylor et al., 2025).

Here, we will discuss the contributions of hippocampal GABAergic inhibition to neurobehavioural function, particularly the contribution of reduced GABAergic

inhibition, so-called neural disinhibition, to behavioural impairment. The connectivity between the ventral hippocampus and the prefrontal cortex (PFC; McGarrity et al., 2017; Sigurdsson & Duvarci, 2016; Simons & Spiers, 2003; Thierry et al., 2000) will also be considered, particularly the implications of this for behavioural functions. A specific subtype of GABA-A receptor, the α5 subunit-containing receptor (α5-GABAARs), will be investigated, as these receptors are preferentially expressed in the hippocampus (McKernan & Whiting, 1996). The role α5-GABAARs for behavioural function will be explored using negative allosteric modulation of α5-GABAARs. We were interested in the extent to which these receptors contribute to behaviours that are known to be affected by hippocampal disinhibition (McGarrity et al., 2017).

This thesis aims to contribute to the understanding of the role of the ventral hippocampus in selected behavioural functions outside the narrow domain of memory functions typically associated with the hippocampus. Specifically, we investigated the significance of the ventral hippocampus and of local GABAergic inhibition in reversal learning, an important aspect of behavioural flexibility, and of ventral hippocampal α5-GABAARs for selected sensorimotor processes: locomotor activity, the acoustic startle response and prepulse inhibition (PPI) of the acoustic startle response.

1 The hippocampus and GABAergic inhibition

1.1 Structure and connectivity of the hippocampus

The hippocampus is a structure in the medial temporal lobe of the brain, comprised of the dentate gyrus, the subiculum, and the hippocampus proper: the Cornu Ammonis (CA) regions, CA1, CA2 and CA3. The CA1–3 regions have a laminar organisation made up of the stratum lacunosum-moleculare (SLM; most superficial layer), the stratum radiatum, the stratum pyramidale and the stratum oriens (the deepest layer). Information is passed around these subregions (dentate gyrus, subiculum, CA1–3) of the hippocampus via the trisynaptic pathway. Stellate cells from

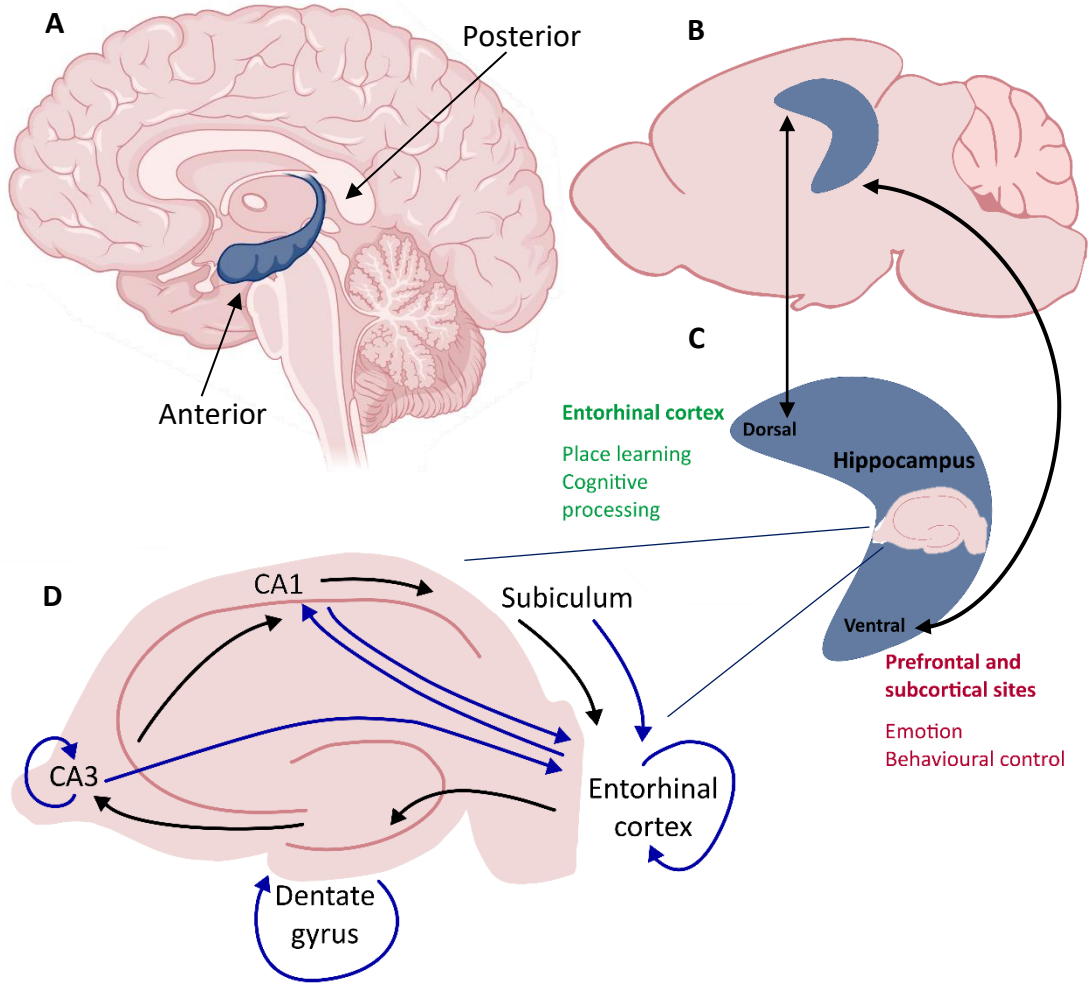
the entorhinal cortex, which acts as a relay between the neocortex and the hippocampus (Lavenex & Amaral, 2000), project to the granule cells in the dentate gyrus through the perforant pathway. The mossy fibres of the dentate gyrus then connect to the CA3 pyramidal neurons, CA3 neurons project to CA1 neurons via the Schaffer collateral pathway and CA1 projects to the subiculum (Amaral & Witter, 1989; Basu & Siegelbaum, 2015). There are also direct connections between the entorhinal cortex and the CA1 and CA3 regions and the subiculum (Amaral & Witter, 1989). The trisynaptic circuit and these additional connections are shown in Figure 1-1.

Importantly, the hippocampus is differentiated anatomically and functionally along its longitudinal axis, which is referred to as posterior-anterior in humans and either septotemporal or dorsoventral in rodents. Many connections to and from the hippocampus and other structures show a dorsoventral (or posterior-anterior) topographical gradient, and, consistent with this anatomical dorsoventral differentiation, many behavioural functions of the hippocampus have been found to rely differentially on the dorsal and ventral hippocampus (Amaral & Witter, 1989; Bast, 2007, 2011; de Hoz & Martin, 2014; Moser & Moser, 1998b; Poppenk et al., 2013). This longitudinal axis of the hippocampus and some key anatomical connections and functions are shown in Figure 1-1.

1.2 Dorsal and ventral hippocampus

In this thesis, we will use the terminology ‘dorsal’ (septal/posterior) and ‘ventral’ (temporal/anterior). The two regions of the hippocampus have different afferent and efferent connectivity with cortical and subcortical areas. Regarding hippocampal afferents, one key dorsoventral difference is that the dorsolateral portion of the entorhinal cortex, which receives highly processed multimodal (including visuo-spatial) information from the sensory association cortices, mainly projects to the dorsal hippocampus, whereas the ventromedial portion of the entorhinal cortex, the inputs of which include projections from the prelimbic and the infralimbic cortices,

Figure 1-1: Neuroanatomy and connections of the hippocampus.



Note. The position of the hippocampus in the human brain (A) and in the rat brain (B). Some key connections of the dorsal and ventral hippocampus and associated functions, adapted from Bast (2007 and 2011; C). The trisynaptic circuit of the hippocampus in black, with additional connections shown in blue, adapted from Amaral and Witter (1989; D). Arrows between B and C show the positioning of the dorsoventral axis in the rat brain. Lines between C and D indicate how the transverse slice of the hippocampus in D relates to the dorsoventral axis in C. Figures produced using Biorender.com and Affinity 3.0.2.3912.

projects mostly to the ventral hippocampus (B. F. Jones & Witter, 2007; Strange et al., 2014; Witter, 1993). With respect to hippocampal projections, some key dorsoventral differences include that it is mainly the dorsal hippocampus that projects to the entorhinal cortex, the retrosplenial and anterior cingulate cortices, the mammillary bodies and the medial septum, whereas it is mainly the ventral

hippocampus that projects to the infralimbic and prelimbic cortices, the amygdala, the olfactory bulb, the hypothalamus and the lateral septum (Fanselow & Dong, 2010; Knierim, 2015; Strange et al., 2014; Swanson & Cowan, 1977). Consistent with the dorsoventral anatomical differentiation, there is also a dorsoventral functional differentiation in the hippocampus (Bannerman et al., 2004; Bast, 2007, 2011; Moser et al., 1995; Strange et al., 2014). In line with the dorsal hippocampus receiving the bulk of visuo-spatial inputs to the hippocampus, the dorsal hippocampus has been more closely associated than the ventral hippocampus in fine-grained visuo-spatial processing and place representations (Moser et al., 1995; Pothuizen et al., 2004). In contrast, the ventral hippocampus has been more closely associated than dorsal hippocampus with functions associated with prefrontal and subcortical sites, including emotional processing and sensorimotor functions (Bannerman et al., 2004; Bast, 2007, 2011; Gray & McNaughton, 2003). This thesis will focus on the ventral hippocampus, especially GABAergic inhibition within this region, building on previous work investigating the functional significance of ventral hippocampal GABAergic inhibition (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022).

1.3 GABAergic inhibition

Of the neurons in the hippocampus, only around 10% are GABAergic inhibitory interneurons, with the majority being glutamatergic pyramidal cells (Freund & Buzsáki, 1998; Pelkey et al., 2017). Despite the small proportion of GABAergic interneurons, these interneurons are important to regulate excitatory glutamatergic neuron activity (Buzśaki & Wang, 2012; Isaacson & Scanziani, 2011; Mann & Paulsen, 2007) and have been shown to regulate hippocampal burst firing (Lovett-Barron et al., 2012; McGarrity et al., 2017; Royer et al., 2012).

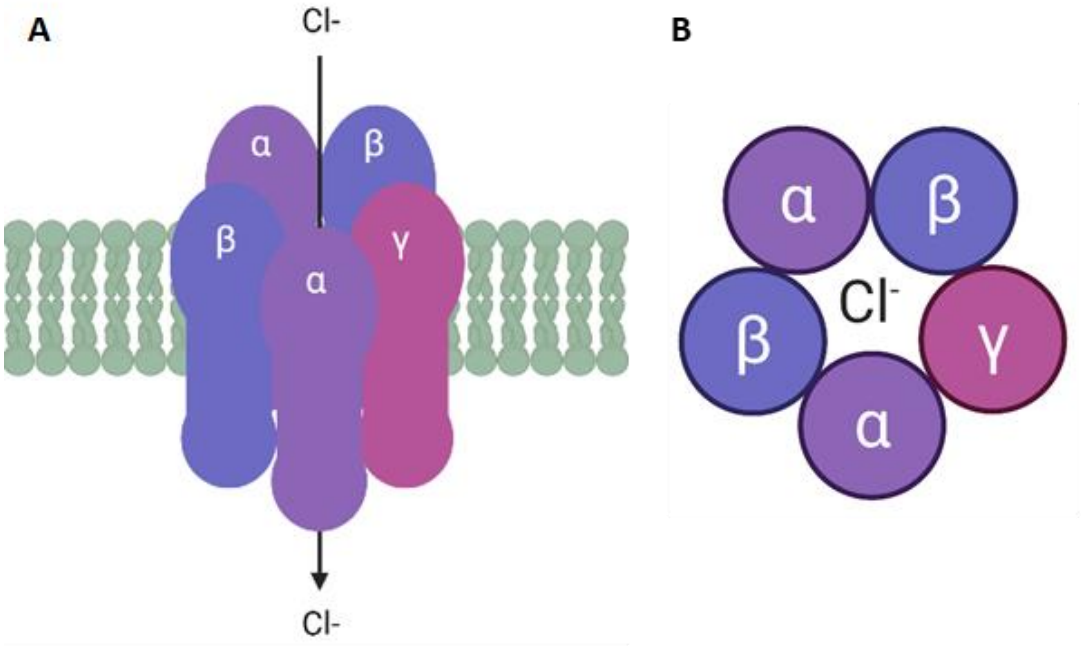
GABA is synthesised from glutamate by the enzyme glutamic acid decarboxylase (GAD), of which there are two isoforms: GAD65 and GAD67. GAD65 is more prevalent at nerve endings, and synthesises GABA for vesicular release, whereas GAD67

produces GABA for cytoplasmic stores (Conti et al., 2011; Soghomonian & Martin, 1998). After synaptic release of GABA, it can be taken up by either GABA transporter 1 (GAT-1, neuronal) or 3 (GAT-3, glial) and recycled into glutamate via the tricarboxylic acid cycle, then GABA can be synthesised again from the glutamate (Jin et al., 2011; Schousboe et al., 2013; Walls et al., 2015; Zafar & Jabeen, 2018). Vesicular GABA transporter (VGAT) transports GABA into synaptic vesicles and is localised at synaptic terminals (Chaudhry et al., 1998).

Normal functioning of the brain has been suggested to require a balance of excitatory and inhibitory neurotransmission, and disturbances to this balance have been implicated in brain disorders and cognitive and behavioural impairments (Bast et al., 2017; Sears & Hewett, 2021). There are two types of GABA-mediated inhibition, phasic and tonic (Farrant & Nusser, 2005). Phasic inhibition refers to the activation of synaptic GABA-A receptors by the release of GABA into the synapse, whereas tonic inhibition is a sustained activation by GABA in the extracellular space, typically at extrasynaptic and perisynaptic GABA-A receptors. Phasic inhibition results in fast, transient GABAergic conductance and tonic inhibition results in a persistent GABAergic conductance which decreases membrane resistance (V. Lee & Maguire, 2014). The two forms of inhibition have distinct roles in controlling excitatory transmission and are controlled by different subtypes of GABA-A receptor (Farrant & Nusser, 2005; Prenosil et al., 2006). Tonic GABAergic inhibition has a key role in controlling the excitability in the hippocampus (Farrant & Nusser, 2005; V. Lee & Maguire, 2014) and α5-GABAARs in particular regulate tonic inhibition (discussed in section 5 and Chapter 3).

There are two main classes of GABA receptor, GABA-A and GABA-B. GABA-A receptors are ionotropic chloride ion channels, whereas GABA-B receptors are metabotropic G-protein coupled receptors (Bormann, 2000; Ghit et al., 2021). Although interest in GABA-B receptors has increased more recently and their functional and clinical relevance is emerging (Heaney & Kinney, 2016; Hyland & Cryan, 2010), these will not be discussed further, as our research focuses on GABA-A receptor-mediated processes. Within the class of GABA-A receptors, receptors can

Figure 1-2: Structure of a typical GABA-A receptor.



Note. GABA-A receptors are ionotropic chloride ion channels. They are pentameric receptors, most commonly comprised of two α, two β and one γ subunits. Front view (A) and top view (B) of a typical GABA-A receptor. Abbreviations: Cl-: chloride ion. Figures produced using Biorender.com

be further classified according to their subunit composition. GABA-A receptors are pentameric receptors and are typically comprised of α, β, γ subunits, most commonly in a 2:2:1 stoichiometry (Figure 1-2; Maramai et al., 2020). There have been 19 genes identified that encode the GABA-A receptor subunits: α1–α6, β1–β3, γ1–γ3, δ, ε, θ, π and ρ1–ρ3 (Olsen & Sieghart, 2008). The subunit composition of GABA-A receptors determines both the brain-wide and the subcellular localisation of the receptors, as well as the intrinsic properties of receptors. As a result, the various subtypes of GABA-A receptors are differentially expressed across the brain and have distinct functions (Engin et al., 2018; Maramai et al., 2020). For example, regarding location, α1–3 receptors are more commonly located at post-synaptic sites, α4–5 at extrasynaptic and perisynaptic sites, α6 receptors mostly expressed in the cerebellum and the ρ subunits are typically located in the retina (Engin et al., 2018; Fritschy & Panzanelli, 2014; Ghit et al., 2021). With regards to associated behavioural functions, α2 and 3

subunit-containing receptors have been found to mediate anxiolytic effects, whereas α5 subunit-containing receptors have been particularly implicated in aspects of learning and memory (Rudolph & Möhler, 2006). The α5 subunit is of particular interest with respect to hippocampal GABAergic inhibition because this subunit is highly expressed in the hippocampus and is discussed in depth in section 5 and Chapter 3.

2 Hippocampal neural disinhibition

Reduced GABAergic inhibition, so-called neural disinhibition, including within the hippocampus, has been implicated in neuropsychiatric disorders (Bast et al., 2017; Marín, 2012), such as schizophrenia (Heckers & Konradi, 2015; Jahangir et al., 2021; Ruzicka et al., 2015), major depressive disorder (Fogaça & Duman, 2019), Alzheimer's disease and age-related cognitive decline (Ambrad Giovannetti & Fuhrmann, 2019; Bi et al., 2020; Thomé et al., 2016), and autism-related disorders (Ramamoorthi & Lin, 2011; Tang et al., 2021). Disinhibition disturbs balanced neurotransmission and results in an increase in excitatory transmission. This can cause dysfunction not only in the hippocampus itself, but also in regions that the hippocampus projects to, for instance, the medial prefrontal cortex (mPFC; McGarrity et al., 2017). Cognitive impairments are a key feature of neuropsychiatric disorders (Millan et al., 2012) and have been linked to neural disinhibition in the hippocampus and the PFC (Bast et al., 2017). The implications of hippocampal neural disinhibition for clinically relevant behavioural functions will be discussed, including those affected in schizophrenia.

2.1 Functions associated with the hippocampus and prefrontal cortex, and the implications for schizophrenia

Schizophrenia is a neuropsychiatric disorder characterised by three classes of symptoms: positive, negative, and cognitive. Positive symptoms refer to hallucinations and delusions, negative symptoms include social withdrawal and lack

of motivation, and cognitive symptoms can include working memory and executive function deficits (Lewis & Gonzalez-Burgos, 2006; Lisman et al., 2008). There have been multiple theories on the cause of schizophrenia, including the idea that there is excess dopamine activity (Brisch et al., 2014; Howes and Kapur, 2009; Toda and Abi-Dargham, 2007). It has been proposed that this dopamine dysfunction is a consequence of upstream pathways, rather than being the cause itself (Lau et al., 2013; Perez et al., 2022; Stahl, 2018). The hippocampus can regulate dopamine activity and so hyperactivity of the hippocampus may cause this excess dopamine activity (Grace, 2016; Lisman et al., 2008; Lodge & Grace, 2006, 2007, 2011). Indeed, hippocampal disinhibition has been implicated in schizophrenia (Heckers & Konradi, 2015).

Hyperactivity of the hippocampus has been observed in both human studies and animal models. Imaging studies have consistently reported hippocampal hyperactivity in schizophrenia (McHugo et al., 2019; Schobel et al., 2009, 2013; Talati et al., 2014; Tregellas et al., 2014) and this increased hippocampal activity observed in patients with schizophrenia has been significantly associated with a reduction in grey matter in the PFC (Molina et al., 2005), another region of the brain known to be affected in schizophrenia. Additionally, a reduction in GABA markers has been observed in schizophrenia, including GAD67 and GAT-1 mRNA (Curley et al., 2011; de Jonge et al., 2017; Dienel & Lewis, 2019; Gonzalez-Burgos et al., 2010; Volk et al., 2001) and GABA transmission has been reported to be impaired in patients with schizophrenia (Frankle et al., 2015), suggesting GABA dysfunction may contribute to the pathophysiology of schizophrenia.

As discussed, hyperactivity of the hippocampus is likely a result of GABAergic disinhibition, resulting in reduced inhibition of excitatory glutamatergic neurons (Heckers & Konradi, 2015). Recent studies using single-photon emission computed tomography (SPECT) measures of regional cerebral blood flow in rats also support this possibility, with the finding that hippocampal disinhibition, by infusion of the GABA-A receptor antagonist, picrotoxin, caused marked metabolic hippocampal hyperactivity, resembling findings in patients with schizophrenia (Williams, 2021).

Other previous work in rats has examined the impact of hippocampal neural disinhibition on clinically relevant cognitive functions, including attention and memory, and found deficits in such functions with hippocampal disinhibition (Bast, Zhang, & Feldon, 2001b; Gwilt et al., 2020; McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022).

Ventral hippocampal disinhibition increased burst firing of neurons in the vicinity of the infusion site (Gwilt et al., 2020; McGarrity et al., 2017). This is consistent with the increase in hippocampal resting activity revealed by blood-flow based brain imaging in schizophrenia (Heckers & Konradi, 2015; McHugo et al., 2019; Molina et al., 2005; Talati et al., 2014; Tregellas et al., 2014). Hippocampal disinhibition also increased locomotor activity and decreased startle reactivity and, in Wistar but not Lister hooded rats, reduced PPI (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017). Locomotor hyperactivity reflects increased dopamine, impaired PPI is an indication of sensorimotor gating deficits and reduced startle may reflect impairments in attention and sensorimotor gating; hence such measures are widely used as a psychosis-related indices (Bast & Feldon, 2003; Braff, 1992; Braff et al., 2001; Buuse et al., 2005; N. R. Swerdlow et al., 2006; Weiss & Feldon, 2001). Additionally, ventral hippocampal disinhibition has been found to disrupt performance on the delayed-matching-to-place (DMP) watermaze task of rapid place learning, which is highly dependent on the hippocampus (Bast et al., 2009; McGarrity et al., 2017; Pezze & Bast, 2012; Steele & Morris, 1999). This finding is in line with impairments in people with schizophrenia in an analogue of the DMP watermaze (Fajnerová et al., 2014).

In addition, hippocampal disinhibition impaired attentional performance on the 5-choice-serial-reaction-time test (5-CSRT), which is highly dependent on the mPFC and, specifically, balanced neural activity within this region (McGarrity et al., 2017; Pezze et al., 2014). This is relevant to schizophrenia as attention is impaired in patients with schizophrenia (reviewed in Cornblatt and Keilp (1994)). It was suggested that this may be due to strong hippocampal-prefrontal connectivity: disinhibition of the hippocampus activated projections of the hippocampus to the PFC, thereby disrupting balanced prefrontal activity and attention (McGarrity et al.,

2017). Indeed, hippocampal-prefrontal connections have an important role in cognitive, behavioural and emotional functions (reviewed in Sigurdsson & Duvarci (2016)) and in schizophrenia, there are disruptions to these hippocampal-prefrontal connections (reviewed in Godsil et al. (2013)).

The implications of this hippocampal-prefrontal connectivity for other cognitive and behavioural processes warrants further investigation. This is something we aim to address in this thesis, where we will examine the idea that hippocampal disinhibition may manifest as impairments on tasks that depend on balanced prefrontal activity, namely reversal learning (Renström et al., 2025, discussed in section 4 and Chapter 2). Additionally, there seems to be a causal link between hippocampal hyperactivity and the excessive dopamine seen in schizophrenia (Grace, 2016; Lisman et al., 2008; Lodge & Grace, 2007, 2011; Perez & Lodge, 2013). We will begin to investigate the specific contribution of ventral hippocampal α5-GABAARs to this control of dopamine in Chapter 3, based on previous findings that hippocampal α5-GABAARs can modulate ventral tegmental area (VTA) dopamine activity (Perez et al., 2022; see section 5).

3 The role of the hippocampus in innate versus learnt behaviour

This thesis will examine ventral hippocampal activity in neurobehavioural function. We will consider both innate (natural) and learnt (acquired) behaviours. Innate behaviours refer to those that an animal, or person, is born with, whereas learnt behaviours refer to those gained through experience or practice. For this thesis, locomotor activity, PPI and startle (Chapter 3) are innate behaviours, whereas reversal learning is a learnt behaviour (Chapter 2).

3.1 Innate behaviours

The acoustic startle response and PPI of the startle response, which have been suggested to reflect sensorimotor gating, are innate reflexes, i.e. they are automatic behaviours that do not require prior experience or learning. Startle reactivity is a fast response to sudden, intense stimuli and the acoustic startle response is mostly studied alongside PPI, where the primary interest is startle modulation by the prepulse (Koch, 1999). PPI occurs when a weak stimulus (the prepulse) precedes a strong startling stimulus and reduces the startle response. PPI has been suggested to be a mechanism to protect information processing by gating out interfering information (N. R. Swerdlow et al., 2001, 2008). While the hippocampus is not required for startle and PPI, hippocampal activity changes can affect these behaviours. Indeed, ventral hippocampal disinhibition (with picrotoxin) decreased startle reactivity, and in Wistar, but not Lister hooded rats, disrupted PPI (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017), whilst ventral hippocampal inactivation (with tetrodotoxin), inhibition (with muscimol) and stimulation (with N-methyl-d-aspartate; NMDA) have been shown to disrupt PPI (Bast, Zhang, Heidbreder, et al., 2001; Zhang et al., 2002b). Locomotor activity is, to an extent, a natural behaviour as animals are born with basic locomotion and a drive to explore new environments. However, some aspects of locomotion, as measured by an open-field test, are shaped by experience, such as habituation to the open-field apparatus. The open-field test provides a measure of how much an animal moves around within the apparatus, for example, through infrared beam breaks. Changes in spontaneous locomotor activity can provide insight into neurobiological processes, for instance, locomotor hyperactivity can indicate an increase in dopamine activity: increased dopamine transmission in the ventral striatum (VS), which receives dopaminergic input from the VTA, has been found to increase locomotor activity (Beninger, 1983; Ikemoto & Panksepp, 1999). Basic locomotion does not require the hippocampus, i.e. animals without a hippocampus still show locomotor activity. However, hippocampal activity changes affect locomotor activity, with ventral hippocampal disinhibition and stimulation producing hyperactivity (Bast, Zhang, & Feldon, 2001b; Bast, Zhang,

Heidbreder, et al., 2001; McGarrity et al., 2017). The hippocampus can also modulate other innate behaviours, including fear, anxiety and stress (Bannerman et al., 2004; Bast, Zhang, & Feldon, 2001c, 2001a; Engin & Treit, 2007; Williams et al., 2022; Zhang et al., 2014) but these will not be discussed further. Given the role of the ventral hippocampus, and particularly of ventral hippocampal inhibition, in modulating locomotor activity, PPI and startle, we were interested in the extent to which ventral hippocampal α5-GABAARs contribute to these behaviours (discussed in Chapter 3).

3.2 Learnt behaviours

The hippocampus is involved in learning and memory, particularly consolidation of short-term memories into long-term memories (Alvarez & Squire, 1994; S. J. Martin & Clark, 2007; Squire et al., 2015; Squire & Zola-Morgan, 1991). It is associated with declarative memory, including episodic memory, spatial learning, contextual learning and decision making (Eichenbaum, 2004; O'Keefe & Nadel, 1978; Riedel et al., 1999; Squire et al., 2004). Additionally, the ventral hippocampus projects to the mPFC and other cortical and subcortical sites and can, thereby, play a role in behaviours dependent on these regions (Bast, 2011; Bast et al., 2017; McGarrity et al., 2017). In this thesis, we will discuss the contribution of the ventral hippocampus to cognitive flexibility, and more specifically, reversal learning.

3.2.1 Cognitive flexibility

Cognitive flexibility is the ability to adapt behaviour in response to a changing environment and is usually assessed by changing reward contingencies in response to changing stimuli (Izquierdo et al., 2017). Studying cognitive flexibility allows us to investigate its neural basis and the underlying processes required to be flexible, and as such, provides valuable insight into several neuropsychiatric disorders which are characterised by inflexibility, including schizophrenia (Leeson et al., 2009; G. K. Murray et al., 2008). To study cognitive flexibility, reversal learning and set-shifting

tasks are typically used. These tasks have the advantage that they can be used across species (Izquierdo et al., 2017). In this thesis, we will focus on reversal learning.

4 Reversal learning

Reversal learning is an important aspect of cognitive flexibility, which is disrupted in a range of neuropsychiatric conditions, including schizophrenia (Izquierdo et al., 2017). Reversal learning requires the reversal of a response-reward association. This involves an initial discrimination, where a stimulus feature results in a reward. Then, a reversal of the response-reward association occurs, such that the previously correct feature is now incorrect whilst the previously incorrect feature is now correct. The subject is required to 'reverse' their response, switching from the old rule to the newly correct rule. An example of this, based on the reversal task used within this thesis, is that for the initial discrimination a subject (rat) would be introduced to two levers (left and right) and a press on one lever (the left) would provide a reward. Then, for the reversal, the reward contingencies would be reversed, such that the left lever is no longer rewarded (old rule) and instead the right lever is now rewarded (new rule). Reversal learning can be investigated using different paradigms and has been investigated across species, and so such experiments have translational value (Izquierdo et al., 2017). Here, we will discuss the differences in reversal learning tasks.

4.1 Reversal learning tasks

One of the main differences in reversal learning tasks is the task used, and this naturally differs across species. Classical experiments in humans involve training people to discriminate between visual stimuli or spatial locations, with one associated with a reward. Once this has been learnt, the stimulus-outcome associations are reversed (Fellows, 2003). Additionally, one aspect of the Cambridge Neuropsychological Test Automated Battery (CANTAB; a battery of cognitive tests), the intra-extra dimensional (IED) test, is used in humans. This measures simple and

compound reversal tasks of visual stimuli (Leeson et al., 2009; Robbins et al., 1994). Participants are required to choose between two stimuli which have two dimensions (e.g. shapes and lines) and receive feedback on their choice, again learning through trial and error. Participants use the feedback to decipher the correct rule, which can be within a dimension (e.g. shape is relevant) or extra-dimensional (line becomes relevant), and the rule switches (Robbins et al., 1994, 1998). Reversal learning tasks in non-human primates can included modified versions of the Wisconsin General Testing Apparatus, including object reversal learning (Izquierdo et al., 2004; Jang et al., 2015; B. Jones & Mishkin, 1972).

Tasks used in rodents tend to vary more, and generally include bowl-digging tasks, lever tasks and mazes. Here, a difference between tasks is the dimension that is reversed. Some reversal tasks, like bowl-digging tasks, use olfactory or tactile stimulus dimensions (Dhawan et al., 2019; McAlonan & Brown, 2003; Tait & Brown, 2008; Wang et al., 2019), while many reversal tasks depend on a spatial dimension: this could be levers or cue lights in a lever task (Brady & Floresco, 2015; Kosaki & Watanabe, 2012; Renström et al., 2025), or position within a maze, such as the T-maze (Bortz et al., 2019), Y-maze (Kimble & Kimble, 1965; Silveira & Kimble, 1968), or radial arm maze (McDonald et al., 2002, 2007). Touch-screen tasks can also be used in rodents, which are designed to be similar to the CANTAB test in humans (Brigman & Rothblat, 2008; Bussey et al., 1997). Tasks can also involve either a positive reward for a successful response, or an aversive outcome for the incorrect response (Cernotova et al., 2021), though our primary focus is tasks using a reward, as in our reversal learning experiments (Chapter 2).

4.1.1 Deterministic versus probabilistic tasks

Another difference in reversal learning tasks is whether they are deterministic or probabilistic. In a deterministic task, each choice has a certain outcome e.g. either it is rewarded, or it is not rewarded. In a probabilistic task, the outcome of a choice is uncertain, for example, it may be rewarded 80% of the time, but not the other 20%. Deterministic tasks have the advantage that they are easier to learn, whereas

probabilistic tasks have the advantage that they are more ecologically valid. Deterministic tasks are commonly employed in rodent research (Bortz et al., 2019; Boulougouris et al., 2007; Brady & Floresco, 2015; Cernotova et al., 2021; Kosaki & Watanabe, 2012; Renström et al., 2025), whereas many human and non-human primate reversal tasks are probabilistic (Costa et al., 2015; Vilà-Balló et al., 2017), as the rate of learning on deterministic tasks is typically considered too quick in primates (Izquierdo et al., 2017). There are, of course, exceptions, with some non-human primate investigations using deterministic tasks (Jang et al., 2015), and some rodent investigations using probabilistic tasks (Mishchanchuk et al., 2024). Deterministic and probabilistic reversal tasks may also rely on different brain regions. Whilst the orbitofrontal cortex (OFC) has been strongly implicated in reversal tasks (discussed in section 4.2.1), it has been reported that probabilistic tasks rely more on an extended network of the OFC, anterior cingulate cortex and subcortical structures, including the striatum (Habiby Alaoui et al., 2021). The mPFC has also been reported to have different roles in deterministic and probabilistic reversal learning, with mPFC inhibition impairing early deterministic reversal learning (Renström et al., 2025), whilst mPFC inhibition improved performance on a probabilistic reversal task (Dalton et al., 2016). This may be because the mPFC contributes to reversal learning through suppressing perseverative errors. As such, mPFC inhibition impairs deterministic reversal learning, but can in some instances improve probabilistic reversal learning, for example through decreasing the rats' tendency to change their response selection after the occasional non-rewarded, but correct, responses (Renström et al., 2025). Additionally, it has been suggested that the basolateral amygdala is required more so for probabilistic tasks than for deterministic tasks, which may be because the amygdala provides a representation of the difference between expected rewards (based on previously received rewards) and received rewards (Izquierdo et al., 2017). Reversal tasks can also be classical or operant, and these involve involuntary and voluntary behaviours, respectively. Most of the research into reversal learning uses operant tasks, but some work has focused on classical conditioning tasks (Burke et al., 2009), including earlier research which suggested a role for the hippocampus in

reversal learning (discussed further in section 4.2.2 and Chapter 2; Berger & Orr, 1983; Weikart & Berger, 1986).

4.1.2 Different stages of reversal learning

A further difference in reversal tasks is the stage of reversal learning being studied. Either the acquisition of, or early, reversal learning is studied, where the focus is on the initial discrimination and then one or two reversals (Berger & Orr, 1983; Cernotova et al., 2021; Chudasama & Robbins, 2003; Enomoto et al., 2011; Floresco et al., 2008; Morellini et al., 2010), or late serial reversal learning, where rats have undergone the initial discrimination and several reversals until relatively stable reversal performance is shown and serials reversals are investigated (Boulougouris et al., 2007; Hervig et al., 2020; Izquierdo et al., 2004; Jackson et al., 2019; Jang et al., 2015; Manning, 1972; E. A. Murray et al., 1998; Ridley et al., 1985; Rygula et al., 2010; Schoenbaum et al., 2002; Silveira & Kimble, 1968). It has been suggested that during reversal learning, there are two learning processes involved. Subjects must learn and reverse stimulus-reward associations and subjects must learn that reversals can occur, which has been referred to as a 'learning set' (Costa et al., 2015; Jang et al., 2015). During the acquisition of reversal learning/early reversals, subjects have had limited experience with reversals and so take longer to reverse. They form a belief or expectation that reversals can occur. This can then facilitate later serial reversal learning, as at this point subjects have learnt to expect reversals to occur. While early reversals may depend more on cognitive control, to overcome prepotent behavioural responses, later serial reversals may depend more so on mechanisms supporting the knowledge that reversals can occur (Izquierdo et al., 2017; Jang et al., 2015). Notably, early and serial reversal learning have been found to be affected differently with prefrontal lesions, with OFC lesions in rats impairing initial reversal learning on reversal one but improving performance at later stages (Boulougouris et al., 2007; Schoenbaum et al., 2002) and mPFC inhibition impairing early reversal learning, but mPFC disinhibition impairing late serial reversal learning (Renström et al., 2025).

Reversal stages can be further distinguished by the when the manipulation, e.g. a lesion or inactivation, is introduced. In this thesis, we have divided these stages into: pre-acquisition manipulation for early reversal learning (the manipulation, e.g. a hippocampal lesion, is made *before* the task is learnt and the initial discrimination and one or two reversals are investigated), pre-acquisition manipulation for early serial reversal learning (the initial discrimination and several reversals are investigated) and post-acquisition manipulation for late serial reversal learning (the manipulation is made *after* the task has been learnt and the reversals after the manipulation are investigated). In our experiments in Chapter 2, we investigated two of these stages: pre-acquisition early serial reversal learning and post-acquisition late serial reversal learning.

4.2 Neural basis of reversal learning

4.2.1 OFC and mPFC

Extensive literature has implicated the PFC, especially the OFC, in reversal learning across species: in humans (Cools et al., 2002; Fellows, 2003; Ghahremani et al., 2010; Nagahama et al., 2001; Remijnse et al., 2005), in non-human primates (Dias et al., 1996, 1997; Izquierdo et al., 2004; B. Jones & Mishkin, 1972; Rygula et al., 2010) and in rodents (Bissonette et al., 2015; Bohn et al., 2003; Boulougouris et al., 2007; Chudasama & Robbins, 2003; Enomoto et al., 2011; Hervig et al., 2020; McAlonan & Brown, 2003; Ragozzino, 2007; Schoenbaum et al., 2002). In contrast, many studies have reported that the mPFC is not required for reversal learning (Boulougouris et al., 2007; Brigman & Rothblat, 2008; Enomoto et al., 2011; Floresco et al., 2008; McDonald et al., 2007; Ragozzino et al., 1999). However, other studies have indicated that the mPFC was required under certain conditions, including when there is greater attentional demand, such as when more complex stimuli are used, or when multiple stimuli need to be monitored simultaneously (Bussey et al., 1997; Kosaki & Watanabe, 2012; Renström et al., 2025). We have recently found that the mPFC is required for the early serial reversal learning in a two-lever task in rats (pre-

acquisition manipulation, early serial reversal; Renström et al., 2025). Inhibition of the mPFC (by infusion of the GABA-A receptor agonist, muscimol) increased responses to criterion and perseverative errors and it was suggested that this may reflect the mPFC overcoming pre-potent responses. Interestingly, mPFC disinhibition (by infusion of the GABA-A receptor antagonist, picrotoxin) improved early serial reversal, though this was mainly due to impaired expression of the previous response. For late serial reversal learning performance (post-acquisition manipulation, late serial), we found mPFC disinhibition, but not inhibition, impaired performance, suggesting that the mPFC is not required for late serial reversal learning, but that disinhibition of the mPFC may disrupt activity in mPFC projection sites, such as the OFC (Renström et al., 2025).

4.2.2 Hippocampus

Our reason for investigating the hippocampus was twofold: firstly, although some evidence suggests that the hippocampus may play a role in reversal learning, specific hippocampal contributions, especially to early versus late serial reversal, remain to be clarified. As such, we investigated how ventral hippocampal inhibition affects reversal learning. Secondly, ventral hippocampus projects to structures involved in reversal learning, namely the mPFC and OFC, and so we were interested in how ventral hippocampal disinhibition affects reversal learning as changes in performance with disinhibition may reflect changes in activity in hippocampal projections to other regions.

Numerous studies have implicated the hippocampus in reversal learning; the findings of the following studies are summarised in Table 1-1. Earlier studies that implicated the hippocampus in reversal learning included classical conditioning tasks (Berger & Orr, 1983; Weikart & Berger, 1986), pre-acquisition early (Whishaw & Tomie, 1997) and pre-acquisition early serial reversal learning (Kimble & Kimble, 1965; Mahut, 1971; E. A. Murray et al., 1998; Silveira & Kimble, 1968). More recent research has also indicated a role for the hippocampus in pre-acquisition early (Bortz et al., 2019; Cernotova et al., 2021; Morellini et al., 2010), pre-acquisition early serial (Jang et al.,

2015), post-acquisition late serial reversal learning (Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024), and more widely in cognitive flexibility (Bortz et al., 2022; Torres-Berrío et al., 2019). Though it must be noted that some of this research involves maze tasks such as T- and Y-mazes (Bortz et al., 2019; Kimble & Kimble, 1965; Silveira & Kimble, 1968), rotating arena tasks (Cernotova et al., 2021) and watermazes (Whishaw & Tomie, 1997). The hippocampus is required for such spatial tasks (Clark et al., 2007; S. J. Martin & Clark, 2007; Morris et al., 1982; O'Keefe & Nadel, 1978) so it is difficult to discern whether the contribution of the hippocampus is for the reversal learning aspect or the navigation aspect of the task. Nevertheless, there is research indicating a role of the hippocampus in reversal learning tasks that do not require the hippocampus (e.g. lever tasks) in rodents and non-human primates.

Most of the research for pre-acquisition early, and early serial, reversal learning has indicated that hippocampal lesions or inactivation leads to a deficit in reversal learning, but not in the initial acquisition of the discrimination (Cernotova et al., 2021; Jang et al., 2015; Kimble & Kimble, 1965; Mahut, 1971; Silveira & Kimble, 1968), though some studies have reported deficits on the initial discrimination (E. A. Murray et al., 1998; Whishaw & Tomie, 1997). However, in the neonatal ventral hippocampal lesion (NVHL) rat model, no impairments in the initial spatial discrimination or early reversal learning were observed in a two-lever task (Placek et al., 2013). In contrast, activation of the ventral subiculum (with Designer Receptors Exclusively Activated by Designer Drugs; DREADDs) improved T-maze reversal learning (Bortz et al., 2019). An improvement in reversal learning performance, both in a watermaze and an odour task, was also observed in a mouse model with enhanced GABAergic inhibition in the dentate gyrus (Morellini et al., 2010).

There has been less research into post-acquisition manipulation late serial reversal learning. A deficit in reversal learning has been reported with ventral hippocampal lesions on a three-lever task in which rats learnt the task before lesion surgery and performance was compared pre- and post-surgery (Kosaki & Watanabe, 2012). More recent work has found the ventral hippocampus to be required for serial probabilistic

reversal learning on a two-lever task in mice, but only with bilateral ventral CA1 lesions; unilateral ventral CA1 lesions did not affect behaviour (Mishchanchuk et al., 2024). Additionally, dopamine activity in nucleus accumbens (NAc) was found to track the inferred state of the task, and unilateral ventral CA1 lesions resulted in a loss of these NAc dopamine activity representations of task state. The authors suggested that this reflected that the ventral hippocampus provided a representation of task contexts (Mishchanchuk et al., 2024). The findings of a link between ventral hippocampus and NAc dopamine activity are in line with previous findings in which the ventral hippocampal – NAc pathway was found to mediate deficits in reversal learning (Donegan et al., 2019). Here, increased trials to criterion were observed in the methylazoxymethanol acetate (MAM) rat model (which has increased hippocampal activity amongst other traits), and this deficit was attenuated by chemogenetic inhibition of the ventral hippocampal – NAc pathway.

In addition to the research with rodents and non-human primates, a study in humans has also cited a role for the hippocampus in reversal learning (Vilà-Balló et al., 2017). This study used patients with temporal lobe epilepsy (TLE) and unilateral hippocampal sclerosis. On the one hand this is similar to a pre-acquisition manipulation as patients had hippocampal sclerosis before completing the task, but on the other hand, patients were told there would be reversals so perhaps this is more similar to post-acquisition manipulations, in that patients expected reversals to occur. Patients with TLE, who acted as a hippocampal lesion model, were less able to anticipate reversals than healthy controls, had a lower accuracy rate and gained less rewards than controls in a probabilistic reversal learning task. It was also found that hippocampal volume correlated with anticipatory switching, with those with a smaller volume less able to predict reversals. The authors suggested that the hippocampus was providing a representation of the task (Vilà-Balló et al., 2017), in line with findings in mice (Mishchanchuk et al., 2024). This idea is also supported by Knudsen & Wallis (2021) who proposed that the primate anterior hippocampus (corresponding to rodent ventral hippocampus) can provide a representation of the relative value of options, so called value place fields. It may be that the hippocampus

provides a representation of the task structure and/or the value of the options in reversal learning tasks.

Additionally, the ventral hippocampus projects to structures involved in reversal learning, such as the mPFC and OFC, and ventral hippocampal disinhibition has been shown to affect prefrontal dependent processes, such as attention (Bast et al., 2017; McGarrity et al., 2017). It is plausible that by manipulating ventral hippocampal activity, such as through disinhibition, reversal learning would be affected in a similar manner, through disrupting activity in hippocampal-prefrontal connections. Equally, disinhibition may improve performance (Bortz et al., 2019).

There has been limited research linking the hippocampus and the prefrontal cortices for reversal learning. In a ventral hippocampal-OFC disconnection model, reversal learning in the Morris watermaze was impaired (Thonnard et al., 2021), and in rats with both mPFC and fimbria-fornix lesions, reversal learning in a T-maze was impaired more so than with either lesion independently (Malá et al., 2015), though both these studies used a maze tasks, which require the hippocampus and, as such, this may explain the involvement of the hippocampus, rather than a specific role of the hippocampus for reversal learning (Clark et al., 2007; S. J. Martin & Clark, 2007; Morris et al., 1982; O'Keefe & Nadel, 1978). It has also been reported that the hippocampus and the OFC synchronise theta activity in reward-based learning, and disruption to hippocampal activity impairs such learning (Knudsen & Wallis, 2020), and that an intact ventral subiculum is required for the OFC neural representation of a nose poke odour reversal task (Wikenheiser et al., 2017).

Table 1-1: Reversal studies focusing on hippocampal manipulations.

Study	Species and strain	Manipulation	Stage of reversal	Reversal task	Main findings
Whishaw & Tomie (1997)	Long-Evans rats	Bilateral lesions of either CA1–4 and dentate gyrus or of the fimbria-fornix	Pre-acquisition early	Watermaze	• Increased latencies during initial spatial learning, but preference for correct quadrant in both groups • Increased time spent in previous (now incorrect) quadrant during reversal in both groups
Morellini et al. (2010)	C57BL/6J mice	TNR-deficient (TNR–/–): enhanced GABAergic inhibition in dentate gyrus	Pre-acquisition early	• Watermaze • Olfactory (2 odours)	• Improved reversal learning: shorter distance and reduced latency to find new platform during reversal

					• Improved reversal learning: preference for new (reversed) odour; increased percentage of correct approaches, learnt in fewer trials
Placek et al. (2013)	Sprague-Dawley rats	Neonatal ventral hippocampal lesion	Pre-acquisition early	Two-lever reversal task	• No impairment in initial discrimination • No impairment in reversal task • Increased latencies to respond
Bortz et al. (2019)	Sprague-Dawley rats	DREADD activation of ventral subiculum	Pre-acquisition early	T-maze	• Decreased trials to criterion • Decreased entries into previously baited arm • Decreased win-shift errors

Cernotova et al. (2021)	Long-Evans rats	Inhibition of ventral hippocampus with muscimol (1 μg/μl)	Pre-acquisition early	Place avoidance on rotating arena (mild shocks)	• Increased number of entrances into and time spent in forbidden sector, and more shocks, during reversal 2 • Increased number of entrances into forbidden sector during last quarter (5 min) of reversal 1 and during quarters 2–4 of reversal 2
Kimble & Kimble (1965)	Sprague-Dawley rats	Bilateral hippocampal lesions	Pre-acquisition early serial	Y-maze	• No effect on initial discrimination • Increased trials to criterion • Increased errors • Decreased number of reversals

Silveira & Kimble (1968)	Sprague-Dawley rats	Bilateral hippocampal lesions	Pre-acquisition early serial	Y-maze	• No effect on initial discrimination • Increased trials to criterion • Decreased number of reversals
Mahut (1971)	Rhesus macaque monkeys	Bilateral hippocampal lesions	Pre-acquisition early serial	Modified Wisconsin apparatus: Spatial reversal	• No effect on initial discrimination • Increased errors during reversals
Murray et al. (1998)	Rhesus macaque monkeys	Bilateral hippocampal lesions	Pre-acquisition early serial	Wisconsin General Test Apparatus: • Object reversal • Place reversal	• Increased errors to criterion on initial discrimination (object) • Increased errors to criterion on reversals 1–7 (object)

					• No effect on discrimination or reversals (place)
Jang et al. (2015)	Rhesus macaque monkeys	Bilateral hippocampal lesions (grouped into a cluster with OFC, rhinal cortex and unilateral OFC-amygdala lesions)	Pre-acquisition early serial	Wisconsin General Testing Apparatus: Object reversal	• Deficit compared to controls, took longer to switch rules • Less win-stay behaviour • Lower belief that reversals occur but developed a stronger belief as they gained experience across reversals • Required more evidence that the object signalling reward had changed but this decreased across reversals
Vilà-Balló et al. (2017)	Humans	Patients with refractory temporal lobe epilepsy and	Pre-acquisition manipulation, but	Probabilistic reversal: two identical stimuli on	• Less accurate • Gained less money (reward)

		unilateral hippocampal sclerosis	knowledge of reversals; early serial	left and right of computer screen	• Could not anticipate potential reversals • No improvement in accuracy across the task
Kosaki & Watanabe (2012)	Wistar rats	Bilateral hippocampal lesions	Post-acquisition late serial	Three-lever reversal task	• Increased trials to criterion • Increased errors to criterion • Increased perseverative and non-perseverative errors
Mishchanchuk et al. (2014)	C57BL/6 mice	Uni- and bilateral lesions of ventral hippocampal CA1	Post-acquisition late serial	Probabilistic two-lever task	• Bilateral: decrease in accuracy of state inference predictions • Bilateral: decreased proportion of choices consistent with a state inference strategy • Unilateral: no effect on behaviour

					• Ventral CA1 maintained a generalised representation of abstract context during task performance
Berger & Orr (1983)	New Zealand white rabbits	Bilateral hippocampal lesions	Classical Conditioning	CS+: tone with airpuff CS-: tone without airpuff	• No effect on initial discrimination • Increased trials to criterion during reversal
Weikart & Berger (1986)	New Zealand white rabbits	Bilateral hippocampal lesions	Classical Conditioning	CS+: tone with airpuff CS-: tone without airpuff	• No effect on initial discrimination • Increased trials to criterion during reversal

Note. Abbreviations: CA: Cornu Ammonis; DREADD: Designer receptors exclusively activated by designer drugs; OFC: orbitofrontal cortex.

It could be proposed that there is a monotonic linear relationship between hippocampal activity and reversal learning performance, with reduced hippocampal function (due to lesioning or inhibition) disrupting reversal learning, and enhanced activity of the ventral subiculum facilitating reversal learning performance. Early serial reversal learning and late serial reversal learning may also be affected differently by hippocampal manipulations. Ventral hippocampal manipulations *before* spatial learning in a watermaze have been reported to not affect subsequent learning of a task, whilst ventral hippocampal manipulations *after* rats have learnt the task do affect subsequent learning (de Hoz & Martin, 2014; M.-B. Moser & Moser, 1998). This suggests that if the hippocampus is intact for learning, it will then be required for performance, but it if it is disrupted during learning, it will not be required for subsequent performance.

4.3 Questions regarding the role of the hippocampus in reversal learning stages that we address in this thesis

We were interested in the role of bidirectional hippocampal activity in pre-acquisition early serial reversal learning and post-acquisition late serial reversal learning (Chapter 2). Although there is some evidence for the hippocampus in reversal learning, much of this has been for pre-acquisition manipulations, many studies have used tasks that depend on the hippocampus, and few studies have focused specifically on the ventral hippocampus. Overall, there have been mixed findings as to the exact contributions of the hippocampus. As such, further investigation is required into how the ventral hippocampus is involved at these distinct stages of reversal learning.

5 Functional significance of hippocampal α5-GABAARs

GABA-A receptors containing the α5 subunit (referred to as α5-GABAARs) are preferentially expressed in the hippocampus, accounting for around 20–25% of hippocampal GABA-A receptors, while representing less than 5% of the GABA-A receptors in the brain (McKernan & Whiting, 1996; Sur et al., 1999). They are expressed more highly in the ventral hippocampus than the dorsal hippocampus, particularly in CA1 (Sarantis et al., 2008; Sotiriou et al., 2005). Typically, α5-GABAARs are located at extrasynaptic sites at the base of pyramidal cell dendritic spines (Brünig et al., 2002; Serwanski et al., 2006). α5-GABAARs have been mainly implicated in mediating tonic inhibition (Caraiscos et al., 2004) and have been shown to control pyramidal neuron activity through regulating action potential firing (Bonin et al., 2007). However, more recent evidence clearly supports that they also contribute to phasic inhibition at GABA-A receptor synapses (Schulz et al., 2018), and α5-GABAARs have been observed using electron microscopy to be localised at synapses in the dendrites of hippocampal pyramidal cells, as well as extrasynaptically (Serwanski et al., 2006). Within CA1 specifically, α5-GABAARs are localised to the distal apical dendrites of pyramidal neurons, which are located in the SLM. Here they mediate both tonic and slow, phasic inhibition. Somatostatin-expressing (SST+) interneurons that target the distal apical dendrites of pyramidal neurons in the SLM preferentially activate α5-GABAARs and this α5-GABAAR synaptic inhibition negatively modulates NMDA receptor-dependent burst firing and synaptic plasticity in CA1 pyramidal neurons (Schulz et al., 2018). Interestingly, different types of interneurons have been found to differentially regulate ventral hippocampal pyramidal neurons depending on their projection target, with SST+ interneurons preferentially regulating the activity of NAc-projecting cells (Lodge et al., 2023). Therefore, the neurons that preferentially target α5-GABAARs also preferentially target projections to the NAc.

Hippocampal α5-GABAAR expression is decreased in antipsychotic-free patients with schizophrenia (Marques et al., 2020), and in vitro studies have implicated α5-GABAARs in controlling hippocampal neural activity (Atack et al., 2006; Bugay et al., 2020; Engin et al., 2018; Papatheodoropoulos & Koniaris, 2011; Schulz et al., 2018).

As discussed, α5-GABAARs have been implicated in controlling hippocampal burst firing in vitro (Schulz et al., 2018) which suggests that α5-GABAARs in the hippocampus may contribute to the control of hippocampal burst firing that has previously been revealed with a non-specific GABA-A receptor blockade (McGarrity et al., 2017). Additionally, intra-hippocampal modulation of α5-GABAARs was reported to regulate VTA dopaminergic neuron activity (Perez et al., 2022), and SST+ interneurons selectively activating α5-GABAARs preferentially regulate the activity of NAc-projecting cells. This has implications for schizophrenia as hippocampal hyperactivity has been linked to aberrant dopamine activity in the NAc (Lodge & Grace, 2007; Perez & Lodge, 2013).

The prevalence of α5-GABAARs in the hippocampus, their location at synaptic and extrasynaptic sites, and their involvement in neuropsychiatric disorders like schizophrenia, has prompted research into their role in hippocampus-dependent learning and memory (Atack et al., 2006; Collinson et al., 2006; Davenport et al., 2021; Dawson et al., 2006; Donegan et al., 2019; Engin et al., 2015; L. J. Martin, Bonin, et al., 2009) and α5-GABAARs have been proposed as targets to treat disorders associated with hippocampal dysfunction (Maramai et al., 2020). The two primary mouse models that have been used to study the role of α5-GABAARs in cognitive processes are the α5-subunit knockout (Gabra5$^{-/-}$) and the α5(H105R) point mutation models (Jacob, 2019). In addition to these transgenic models, specific positive and negative allosteric modulators (PAMs/NAMs) of α5-GABAARs have been investigated, though much of the research focuses on systemic administration of these modulators, rather than site-specific administration.

5.1 Negative modulation of α5-GABAARs

5.1.1 Innate behaviour

The α5(H105R) transgenic mouse model is one of the models that has been used to study the role of α5-GABAARs. This model uses gene targeting to induce a point

mutation in position 105 of the α5 subunit gene, reducing the number of hippocampal α5-GABAARs (Rudolph et al., 1999). It is a partial knockdown of α5-GABAARs which should make any compensatory changes less likely. This transgenic mouse model was used to investigate the role of hippocampal α5-GABAARs for PPI, startle and locomotor activity. PPI was found to be attenuated in mice with the H105R mutation, and an increase in locomotor activity was also observed, but no effect was seen for startle (Hauser et al., 2005). PPI deficits are a key feature of schizophrenia (Braff, 1992; Grillon et al., 1992; Parwani et al., 2000) and locomotor hyperactivity can indicate increased dopamine activity and so has been used as a psychosis-related behavioural index (Bast & Feldon, 2003; Buuse et al., 2005). However, systemic negative allosteric modulation of α5-GABAARs with L-655,708 did not affect PPI or locomotor activity (Bugay et al., 2020; Carreno et al., 2017; Fischell et al., 2015; Magnin et al., 2019), and in a transgenic mouse model where only α5-GABAARs were sensitive to diazepam (α1–3 desensitised; DZα5), positive modulation of α5-GABAARs with systemic diazepam had no effect on locomotor activity (Behlke et al., 2016).

5.1.2 Learnt behaviour

Due to the localisation of α5-GABAARs in the hippocampus, it has been suggested that these receptors may have a role in hippocampus-dependent learning and memory. Research has highlighted the importance of α5-GABAARs for hippocampal-dependent functions.

An improvement in spatial learning on the Morris watermaze task was observed in two transgenic mouse models: Gabra5$^{-/-}$ mice and a CA1-specific α5-GABAAR knockout mouse model (Engin et al., 2020). Systemic injection of the α5-GABAAR NAM, L-655,708, has also been investigated in the Morris watermaze task and an improvement has been reported (Atack et al., 2006), but no improvement has also been observed (L. J. Martin, Bonin, et al., 2009). Additionally, systemic injection of two other α5-GABAAR NAMs, RO4938581 and Basmisanil, was found to attenuate a diazepam-induced GABAergic deficit on the Morris watermaze (Ballard et al., 2009; Hipp et al., 2021). Furthermore, an improvement in performance on the DMP

watermaze task, which is particularly sensitive to hippocampal dysfunction, has been reported in the Gabra5$^{-/-}$ transgenic mouse model and in mice and rats with a systemic injection of an inverse agonist of α5-GABAARs, α5IA (Collinson et al., 2002; Dawson et al., 2006).

5.1.3 Hippocampus-specific negative allosteric modulation of α5-GABAARs

There has been limited research that has specifically targeted hippocampal α5-GABAARs, with much of the research focusing on genetic models or systemic administration of NAMs. One study that did specifically target α5-GABAARs in the ventral hippocampus found L-655,708 decreased immobility and increased climbing in a forced swim test in rats, though this effect was reported 48 h after L-655,708 administration (Bugay et al., 2020). Additionally, 20 min after infusion of L-655,708 or TB 21007 (both α5-GABAAR NAMs) into the ventral hippocampus, VTA dopamine activity was increased in saline treated (control) rats, whereas the same manipulation with L-655,708 (but not TB 21007) attenuated aberrant dopamine activity in the MAM rat model 20 min after infusion (Perez et al., 2022). The MAM model displays brain and behavioural changes reminiscent of schizophrenia, including ventral hippocampal hyperactivity, reduced GABAergic neuron function and increased dopaminergic activity (Lodge et al., 2009; Lodge & Grace, 2007; Moore et al., 2006). It is unclear why L-655,708 increased dopamine neuron activity in saline treated (control) rats, whilst reducing activity in MAM treated rats (Perez et al., 2022), although these findings suggest that the impact of negative modulation of ventral hippocampal α5-GABAARs on VTA dopamine neuron activity is dependent on the baseline activation state of VTA dopamine neurons.

5.2 Positive modulation of α5-GABAARs

Positive modulation of α5-GABAARs has also been reported to reverse aberrant dopamine activity in MAM rats (Gill et al., 2011; Perez et al., 2022). It has been proposed that PAMs, by increasing α5-GABAARs mediated inhibition, may reduce the hippocampal hyperactivity reported in schizophrenia, and therefore may be effective

in treating symptoms of these disorders (Maramai et al., 2020; Möhler & Rudolph, 2017; Perez et al., 2022).

Treatment of MAM rats with an α5-GABAAR PAM (SH-053-20F-R-CH3), both intravenously and by infusion into the ventral hippocampus, resulted in a reduction in midbrain dopaminergic activity (Gill et al., 2011). This negative modulation of midbrain dopaminergic neuron activity by hippocampal α5-GABAARs is supported by another study which used two different α5 PAMs (GL-II-73 and MP-III-022). Ventral hippocampal infusion of MP-III-022 or GL-II-73 was observed to attenuate dopamine activity in MAM rats, but for systemic injection, only MP-III-022, and not GL-II-73, attenuated this excessive dopamine activity (Perez et al., 2022).

PAMs have also been investigated for the treatment of disorders affecting cognition, including schizophrenia and age-related cognitive decline. It has been reported that two different α5-GABAAR PAMs (Compound 6 and 44) improved performance on the radial arm maze in aged rats (Koh et al., 2013). Additionally, intracerebroventricular infusion of Compound 44 led to a partial improvement in spatial memory in aged rats in the water maze task. It was suggested that these compounds may ameliorate the hippocampal hyperactivity that has been associated with ageing (Koh et al., 2013).

5.3 Questions regarding hippocampal α5-GABAARs that we begin to address in this thesis

Research on α5-GABAARs has indicated that targeting α5-GABAARs may be an effective way to treat disorders such as schizophrenia. Both NAMs and PAMs of α5-GABAARs have been observed to improve memory in rodent models and to attenuate excessive dopamine activity. It has been suggested that although these modulators have essentially opposite effects, they may both be acting to restore the balance between excitatory and inhibitory neurotransmission (Prévot & Sibille, 2020). NAMs can act to decrease inhibition of glutamatergic activity, while PAMs decrease noise (Perez et al., 2022). However, there is limited research that has looked at the effects

of site-specifically pharmacologically targeting hippocampal α5-GABAARs on behavioural functions associated with the hippocampus. We aimed to begin to address this in this thesis. We investigated the effect of negative allosteric modulation of α5-GABAARs, using infusions of a α5-GABAAR NAM (L-655,708) into the ventral hippocampus of rats, on behaviours that are disrupted by hippocampal disinhibition, namely locomotor activity, PPI and startle (McGarrity et al., 2017). Key papers that are relevant to our aims are summarised in Table 1-2.

Table 1-2: Studies investigating α5-GABAARs relevant for our thesis aims.

Study	Species and strain	Manipulation	Behaviour or Activity Measure	Main Findings
Hauser et al. (2005)	129X1/SvJ mice	α5(H105R): partial reduction of α5-GABAARs in CA1–3 of hippocampus	• Locomotor activity • PPI • Startle	• Increase in locomotor activity • Reduced PPI • No effect on startle
Behlke et al. (2016)	129X1/SvJ mice	Dzα5: positive modulation of only α5-GABAARs with diazepam	• Locomotor activity	• No effect on locomotor activity
Fischell et al. (2015)	Sprague Dawley rats	Systemic injection of L-655,708 (0.7 mg/kg, i.p.)	• Locomotor activity	• No effect on locomotor activity
Carreno et al. (2017)	Sprague Dawley rats	Systemic injection of L-655,708 (3.0 mg/kg, i.p.)	• PPI	• No effect on PPI
Magnin et al. (2019)	C57BL/6 mice	Systemic injection of L-655,708 (0.5 mg/kg, i.p.)	• Locomotor activity	• No effect on locomotor activity

Bugay et al. (2020)	Sprague Dawley rats	Systemic injection of L-655,708 (3.0 mg/kg, i.p.) Bilateral ventral hippocampal infusion of L-655,708 (30 or 90 ng/1 µl)	• Locomotor activity • Forced swim test	• No effect on locomotor activity (systemic) • Decreased immobility and increased climbing 48 h after infusion (infusion, 90 ng)
Perez et al. (2022)	Sprague Dawley rats and MAM rats	Unilateral ventral hippocampal infusion of L-655,708 (0.75 µl of 100 ng/ µl)	• Dopamine neuron activity in the VTA 20 min after infusion	• Increased dopamine neuron activity in control rats • Reduction in dopamine neuron activity in MAM rats

Note. Abbreviations: i.p.: intraperitoneal; MAM: methylazoxymethanol acetate model; PPI: prepulse inhibition; VTA: ventral tegmental area.

6 Aims of Thesis

This thesis aims to contribute to the understanding of the behavioural significance of the ventral hippocampus, and especially local GABAergic inhibition, for innate and learnt behaviours. First, we investigated the impact of ventral hippocampal GABAergic inhibition and disinhibition on early serial reversal learning and late serial reversal learning (Aim 1). Second, we began to assess the significance of ventral hippocampal α5-GABAARs for behaviour, focusing on locomotor activity, PPI and startle (Aim 2).

Aim 1: Investigate the impact of bidirectional ventral hippocampal activity changes on early serial and late serial reversal learning (Chapter 2).

In experiments reported in Chapter 2, we examined the impact of bidirectional ventral hippocampal activity manipulations on early serial and late serial reversal learning performance using a food-reinforced two-lever discrimination task in rats. We aimed to investigate the impact of ventral hippocampal inhibition on these two stages of reversal learning to examine if the ventral hippocampus is required for distinct aspects of reversal learning. We also further examined the idea that hippocampal disinhibition may manifest as impairments of cognitive processes depending on balanced prefrontal activity (McGarrity et al., 2017) by investigating the impact of ventral hippocampal disinhibition on reversal learning performance. Performance on a two-lever reversal learning task (Brady & Floresco, 2015) has recently been found to be markedly disrupted by bidirectional changes of mPFC activity, with mPFC inhibition impairing early serial reversal learning and mPFC disinhibition impairing late serial reversal learning (Renström et al., 2025). Here, we examined the idea that, because of strong hippocampal-prefrontal connectivity, hippocampal disinhibition manifests partly in the form of impairments in functions depending on prefrontal integrity (McGarrity et al., 2017). These experiments have some clinical relevance, because impairments in reversal learning are a feature of schizophrenia (Leeson et al., 2009) and hippocampal dysfunction, especially

hippocampal overactivity and disinhibition, has been heavily implicated in schizophrenia (Heckers & Konradi, 2010, 2015).

Aim 2: Investigate the significance of ventral hippocampal α5-GABAARs for locomotor activity, PPI and startle (Chapter 3)

In Chapter 3, we reported our studies that began to investigate the significance of ventral hippocampal α5-GABAARs for behavioural functions disrupted by hippocampal disinhibition (McGarrity et al., 2017). We aimed to investigate the role of ventral hippocampal α5-GABAARs for locomotor activity, PPI and startle, using an α5-GABAAR NAM, L-655,708 (Perez et al., 2022). These experiments have some clinical relevance because α5-GABAARs have been shown to be deceased in schizophrenia (Marques et al., 2020), and this may be linked to the hyperactivity (Heckers & Konradi, 2015) and increased dopamine responsivity (Lodge & Grace, 2006, 2007, 2011) also observed in schizophrenia.

Chapter 2: Ventral hippocampal inhibition tends to disrupt late serial reversal learning, whereas disinhibition tends to improve early serial reversal learning

Declaration: Jacob Juty, Jacco Renström, Patricia Radu, Charlotte Taylor, Luke O'Hara and Joanna Loayza contributed to surgeries and/or data collection. Silvia Maggi ran the Bayesian analysis code.

1 Introduction

The hippocampus has a significant role in certain types of memory, including rapid event and place learning, but also has strong connectivity with prefrontal and subcortical sites and so may modulate a wide range of other cognitive and behavioural functions (Bast, 2011; Bast et al., 2017). Changes in hippocampal function, including both increased and decreased activation, have been implicated in a range of neuropsychiatric disorders (Small et al., 2011). Specifically, hippocampal neural disinhibition, i.e. reduced inhibitory GABA transmission, is implicated in the pathophysiology of neuropsychiatric disorders, such as schizophrenia (Heckers & Konradi, 2015). The hippocampus has strong projections to the PFC and, therefore, it has been suggested that hippocampal activity changes may affect cognitive and behavioural functions depending on the PFC (Bast, 2011; Eichenbaum, 2017; McGarrity et al., 2017; Sigurdsson & Duvarci, 2016). An important aspect of cognition is cognitive flexibility. Cognitive flexibility is the ability to change behaviour in response to a changing environment and is of great clinical relevance because it is disrupted in many neuropsychiatric disorders (Izquierdo et al., 2017). The PFC has been widely implicated in cognitive flexibility (discussed in section 1.1), but knowledge of role of the hippocampus, particularly the ventral hippocampus, is more limited (section 1.2).

1.1 Reversal learning and the prefrontal cortex

Reversal learning is a form of cognitive flexibility and involves switching from one response to another when the reward contingencies of the responses are reversed. Reversal learning is disrupted in several neuropsychiatric disorders (Izquierdo et al., 2017), including schizophrenia (discussed in section 1.2.1). Several brain regions have been investigated for their role in reversal learning and cognitive flexibility. Studies in rodents (Bissonette et al., 2015; Bohn et al., 2003; Boulougouris et al., 2007; Chudasama & Robbins, 2003; Hervig et al., 2020; McAlonan & Brown, 2003; Ragozzino, 2007; Schoenbaum et al., 2002), and non-human primates (Izquierdo et al., 2004; Rygula et al., 2010) support that the OFC is required for reversal learning, and imaging studies suggest that the OFC is also important for reversal learning in humans (Ghahremani et al., 2010; Hampshire et al., 2012). In contrast, many studies investigating the mPFC have reported that this region is not required for reversal learning (Brigman & Rothblat, 2008; Enomoto et al., 2011; Floresco et al., 2008; McDonald et al., 2007; Ragozzino et al., 1999). However, Bussey et al. (1997) found that mPFC lesions did affect reversal learning (increased errors), but only when the stimuli were difficult to discriminate, and Kosaki & Watanabe (2012) found a deficit in serial reversal learning with mPFC lesions (increased errors and perseveration). We have recently found that the mPFC is required for early serial reversal learning in a two-lever task in rats (pre-acquisition manipulation early serial reversal learning). Inhibition of the mPFC increased responses to criterion and perseverative errors, and we suggested that this may reflect the mPFC overcoming pre-potent responses (Renström et al., 2025). Interestingly, mPFC disinhibition improved early serial reversals, though we suggested this may be due to impaired expression of the previous response. Disinhibition of the mPFC impaired late serial reversal learning performance (post-acquisition manipulation late serial reversal learning; Renström et al., 2025). This suggests that the mPFC is not required for late serial reversal learning, but that disinhibition of the mPFC may disrupt activity in mPFC projection sites, including the OFC. Considering the evidence regarding the role of the mPFC in reversal learning together, the mPFC may be required when the task demand is

higher. This could include situations when stimuli are difficult to discriminate (Bussey et al., 1997), when there are additional levers (Kosaki & Watanabe, 2012) and early reversal learning, which may require more mPFC-dependent attention and cognitive control (to overcome prepotent responses and to learn that reward contingencies can reverse; also see section 4.1.2), than late reversals (Renström et al., 2025).

There has been some research linking the prefrontal cortices and the hippocampus in reversal learning. It has been reported that lesions to the mPFC and to the fimbria-fornix in rats impaired performance on a T-maze reversal learning task, more so than either lesion did independently (pre-acquisition early reversal; Malá et al., 2015). Regarding the OFC, reversal learning performance on the Morris water maze reversal task was impaired in a ventral hippocampal-OFC disconnection model in mice (pre-acquisition early serial reversal; Thonnard et al., 2021). However, as discussed in Chapter 1, such maze tasks have a hippocampus-dependent spatial component, and so this may explain the impact of the hippocampal manipulations, rather than some specific role of hippocampus in reversal processes. Additionally, the OFC neural representation of a nose poke odour reversal learning task in rats was found to require an intact ventral subiculum (pre-acquisition early reversal; Wikenheiser et al., 2017). It has also been reported that the hippocampus and the OFC synchronise theta activity in reward-based learning, and disruption to hippocampal activity impairs such learning (Knudsen & Wallis, 2020). Furthermore, OFC and CA1 activity have been shown to coordinate during reversal learning, though this was CA1 in the dorsal hippocampus, and was a spatial plus maze task (pre-acquisition early serial reversal; Riceberg et al., 2022).

1.2 Reversal learning and the hippocampus

There is some evidence for the role of the hippocampus in reversal learning. Numerous earlier studies in animals have found reversal learning to be impaired with hippocampal lesions (Berger & Orr, 1983; Kimble & Kimble, 1965; Mahut, 1971; E. A. Murray et al., 1998; Silveira & Kimble, 1968; Whishaw & Tomie, 1997). It should be

noted that the study by Berger & Orr (1983) focused on classical conditioning, while the present study and much of the other research focuses on operant conditioning. More recently, evidence for the role of the hippocampus in reversal learning has been found in rodents (Bortz et al., 2019; Cernotova et al., 2021; Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024; Morellini et al., 2010), non-human primates (Jang et al., 2015) and humans (Vilà-Balló et al., 2017).

As discussed in Chapter 1, it is important to note the timing of the hippocampal manipulations in relation to different stages of the reversal task in previous research. In much of the literature hippocampal manipulations (lesions, inactivation) were performed prior to learning a reversal; pre-acquisition manipulations (Berger & Orr, 1983; Bortz et al., 2019; Cernotova et al., 2021; Jang et al., 2015; Kimble & Kimble, 1965; Mahut, 1971; Morellini et al., 2010; E. A. Murray et al., 1998; Placek et al., 2013; Silveira & Kimble, 1968; Whishaw & Tomie, 1997). The design of these experiments is similar to the first experiment we will describe in this chapter, where we investigated early serial reversal learning. It is unclear what the role of the hippocampus is in early reversal learning, as an impairment (Berger & Orr, 1983; Cernotova et al., 2021; Jang et al., 2015; Kimble & Kimble, 1965; Mahut, 1971; E. A. Murray et al., 1998; Silveira & Kimble, 1968; Whishaw & Tomie, 1997), an improvement (Bortz et al., 2019; Morellini et al., 2010) and no effect (Placek et al., 2013) have been reported with hippocampal manipulations. The second experiment described in this chapter focuses on late serial reversal, where the ventral hippocampal manipulation was given after the rats had learnt to reverse. There is less research into this stage of reversal, but it has been suggested that the hippocampus is required for this stage of learning (Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024). Vilà-Balló et al. (2017) investigated reversal learning in humans, both in healthy controls and in patients with TLE and unilateral sclerotic hippocampus, and so on the one hand this is similar to pre-acquisition manipulations. However, participants were informed that reversals were going to occur so on the other hand this study could be likened to the post-acquisition manipulation studies, as in these

studies animals have learnt reversals can occur, whilst in pre-acquisition manipulation studies they have not learnt reversals can occur.

The hippocampus has been proposed to create a multi-dimensional representation, or a map, of the environment (Bird & Burgess, 2008; Eichenbaum, 2004; Eichenbaum & Cohen, 1988; Konkel & Cohen, 2009; O'Keefe & Nadel, 1978; Stachenfeld et al., 2017). More recently, Vilà-Balló and colleagues (2017) proposed that the hippocampus creates a representation of task structure and uses this to execute appropriate strategies. They found patients with TLE and a unilateral sclerotic hippocampus (who served as a hippocampal lesion model) had reduced performance in a probabilistic reversal learning task. These deficits in reversal learning following hippocampal sclerosis were suggested to be due to the role of the hippocampus in providing a representation of the task structure (Vilà-Balló et al., 2017). This idea is supported by Knudsen & Wallis (2021) who proposed that the primate anterior hippocampus (corresponding to rodent ventral hippocampus) can provide a representation of the relative value of options, so called value place fields. For example, in the case of a reversal learning experiment, this could be a representation of the task's cues and their reward/lack of reward e.g. a left and right lever, and a sugar pellet reward/no reward. This is supported by recent findings in mice that found the ventral hippocampus provides a representation of task context in a probabilistic lever task (Mishchanchuk et al., 2024).

As suggested in Chapter 1, it could be proposed that there is a monotonic linear relationship between hippocampal activity and reversal learning performance, with reduced hippocampal function (due to lesioning or inhibition) disrupting reversal learning, and enhanced activity of the ventral subiculum facilitating reversal learning performance. However, disinhibition may also impair reversal learning. Firstly, hippocampal disinhibition has been shown to impair prefrontal-dependent sustained attention in rats, likely through alterations in the activity of hippocampal-PFC projections (McGarrity et al., 2017), indicating that manipulating hippocampal activity can affect prefrontal behaviours, and we have recently found the mPFC to be required for early serial reversal learning (Renström et al., 2025). Secondly,

disinhibition may disrupt hippocampal representations which have been suggested to be important for reversal learning, as hippocampal disinhibition can impair hippocampal function in a similar manner to hippocampal inhibition (McGarrity et al., 2017; Taylor et al., 2025).

1.2.1 Clinical significance: reversal learning in schizophrenia

Cognitive flexibility, and more specifically reversal learning, is affected in neuropsychiatric disorders like schizophrenia and psychosis (Leeson et al., 2009; G. K. Murray et al., 2008; Reddy et al., 2016; Waltz & Gold, 2007). These deficits have been proposed to be a consequence of dysfunction in the prefrontal cortices (Fellows, 2003; Floresco et al., 2008; Hampshire et al., 2012; Hervig et al., 2020; Waltz & Gold, 2007). However, it is plausible that hippocampal dysfunction may also contribute to these reversal learning deficits. First, as discussed in the preceding section, the hippocampus has also been implicated in reversal learning. Second, extensive research has indicated the involvement of the hippocampus in schizophrenia (reviewed in Heckers & Konradi (2010)), with hippocampal hyperactivity (McHugo et al., 2019; Schobel et al., 2009, 2013; Talati et al., 2014; Tregellas et al., 2014), and impairments in GABAergic inhibition (de Jonge et al., 2017; Dienel & Lewis, 2019; Gonzalez-Burgos et al., 2010), including hippocampal disinhibition (Heckers & Konradi, 2015; Lisman et al., 2008; Marín, 2012), being linked to schizophrenia.

1.3 Aims and Hypotheses

The hippocampus may have a role in reversal learning through providing a representation of the task (Knudsen & Wallis, 2021; Mishchanchuk et al., 2024; Vilà-Balló et al., 2017). The ventral hippocampus specifically may be involved, as neurons in the anterior hippocampus in monkeys (which corresponds to the ventral hippocampus in rats) have been shown to encode value-place fields (Knudsen & Wallis, 2021), and the ventral CA1 in mice has been found to provide a representation

of task contexts during reversal learning (Mishchanchuk et al., 2024). Additionally, the ventral hippocampus has projections to the mPFC and is able to disrupt mPFC-dependent behaviours (McGarrity et al., 2017) and we have recently found the mPFC to be required for early serial reversal learning (Renström et al., 2025).

Based on this notion that the hippocampus creates a flexible representation of tasks such as reversal learning, as well as previous findings that suggest the hippocampus is required for reversal learning (Cernotova et al., 2021; Jang et al., 2015; Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024; Vilà-Balló et al., 2017), we hypothesised that ventral hippocampal inhibition may disrupt reversal learning. Based on the available evidence, it is difficult to predict the effect of ventral hippocampal disinhibition. On the one hand, disinhibition may disrupt reversal learning by disrupting prefrontal processing, due to aberrant activity in hippocampal-prefrontal projections (Bast et al., 2017; McGarrity et al., 2017), or by disrupting hippocampal task representations, as hippocampal disinhibition has previously been found to disrupt tasks dependent on hippocampal function (McGarrity et al., 2017; Taylor et al., 2025). On the other hand, as previous work has suggested (Bortz et al., 2019), ventral hippocampal disinhibition may improve early reversal learning.

This chapter reports two key pharmacological investigations examining the impact of bidirectional activity changes of the ventral hippocampus on two distinct stages of a two-lever reversal task in rats (Brady & Floresco, 2015; Renström et al., 2025; Renstrom, 2023). We examined the effects of ventral hippocampal inhibition, with the GABA-A receptor agonist, muscimol, and ventral hippocampal disinhibition, with the GABA-A receptor antagonist, picrotoxin, to discern a potential contribution of inhibitory control within the hippocampus, as in our previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). In Experiment 1, we examined the impact of ventral hippocampal manipulations on the initial spatial discrimination (left or right lever) task and the first few reversals (pre-acquisition manipulation early serial reversal learning). In Experiment 2, we examined late serial reversal performance once rats were proficient in reversal learning and had reached relatively

stable reversal performance levels (post-acquisition manipulation late serial reversal learning).

2 Methods

2.1 Rats

In total, 64 young adult male Lister hooded rats (Envigo, UK) were used, weighing around 250–350 g (8–11 weeks old) when they underwent surgery. We ran two experiments (Experiment 1 and 2) in three separate cohorts: Experiment 1, two cohorts of n=24 rats and Experiment 2, one cohort of n=16 rats. Sample size justification, exclusions and final sample sizes can be found in Section 2.4. Rats were housed in groups of four, in individually ventilated, two-level, 'Double Decker' cages (462 mm X 403 mm X 404 mm; Tecniplast, UK). Environmental conditions were controlled: temperature (21.0 ± 1.5 °C); humidity (50 ± 8%); light, alternating in 12-h day/night cycles (lights on at 7.00 am). All procedures were carried out during the light phase. Rats initially had ad libitum access to water and food (Teklad Global 18% Protein Rodent Diet 2018C; Envigo) until seven days following completion of surgery to implant guide cannulae into the ventral hippocampus. Access to food was then mildly restricted (12–24 g per rat per day) to motivate responding on the food-reinforced reversal task. Food restriction started four days before pretraining and lasted until completion of the testing. Rats' weights were monitored daily to maintain about 90% (85–95%) of their free-feeding weights, as projected by a pre-established weight growth curve. All procedures were conducted in accordance with the United Kingdom (UK) Animals (Scientific Procedures) Act 1986, approved by the University of Nottingham's Animal Welfare and Ethical Review Board (AWERB) and run under the authority of Home Office project license PP1257468. For the reporting of our studies, we followed ARRIVE guidelines (du Sert et al., 2020).

2.2 Ventral hippocampal cannula implantation

Experiment 2 was completed before Experiment 1, but the experiments are labelled as 1 and 2 as early serial reversal learning (Experiment 1) precedes late serial reversal learning (Experiment 2). Guide cannulae were implanted bilaterally above the ventral

hippocampus targeting the same coordinates and using similar procedures as in previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). In Experiment 1, the coordinates were adjusted so that the dorsal-ventral coordinate was taken from skull, rather than from dura. Stainless steel stylets (33 gauge, Plastics One, Bilaney, UK), with a dust cap, were placed into the guide cannulae for the surgery and protruded 0.5 mm beyond the tips of the guide cannulae. The protrusion of the stylets was the same as the infusion cannula. The stylets with the dust cap were implanted with the tips aimed at the infusion coordinates (see below).

Surgery and testing of the second cohort of rats in Experiment 1 was carried out at a new facility, and there were some adjustments made to the surgical procedures. Rats were anaesthetised using isoflurane delivered in oxygen (induction: 5%; maintenance: 1–3%; Experiment 1, cohort 1, and Experiment 2: flow rate: 1 l/min, Experiment 1, cohort 2: flow rate: 2 l/min) and prepared for surgery by shaving and disinfecting the scalp with 2% chlorhexidine (Experiment 1, cohort 1, and Experiment 2: Clinell, UK; Experiment 1, cohort 2: ChloraPrep, Becton Dickinson, UK). For Experiment 1, cohort 2, bupivacaine was administered subcutaneously (s.c.) into the incision site (MercuryPharma, UK; 2 mg/kg) to further reduce the risk of the rats experiencing discomfort during, and after, the surgical procedures. Their heads were secured in a stereotaxic frame with blunt ear bars; EMLA cream (5%, lidocaine/prilocaine, Aspen) was applied to the ear bars to minimise pain, and a lubricant gel was used (Lubrithal, Dechra, Uldum) to prevent the eyes from drying out during surgery. Rats received perioperative analgesia (s.c.; Metacam; Boehringer Ingelheim, UK; Experiment 1, cohort 1, and Experiment 2: 1 mg/kg; Experiment 1, cohort 2: 2 mg/kg) and 2–6 ml of saline (s.c.; Vetivex, Dechra, UK) to prevent dehydration. The skull was exposed and bregma and lambda were aligned horizontally. Bilateral infusion guide cannulae (stainless steel, 26-gauge, Plastics One, Bilaney Consultants, UK) were implanted through small holes drilled in the skull. The target coordinates for the infusion sites into the ventral hippocampus were 5.2 mm posterior to bregma, ± 4.6mm lateral from the midline and 6.5 mm ventral from dura (In Experiment 1, the dorsal-ventral coordinate was taken from skull instead of dura

and was adjusted to 7.0 mm ventral from the skull; the skull is approximately 0.5 mm thick, so these coordinates targeted a location 6.5 mm ventral from dura, but taking coordinates from skull is less subjective than from dura, so should be more accurate and consistent between surgeons). Cannulae were secured to the skull using cement and four stainless steel screws. In Experiment 1, cohort 1, and Experiment 2, dental cement (Simplex Rapid, Kemdent, UK) and light curing cement (HS Maxima Natural Elegance, Henry Schein, UK), which was used mostly around the trepanations, were used. In Experiment 1, cohort 2, bone cement containing gentamicin (Eurofix Gun G Vet, Synimed, France) was used. The scalp incision was sutured around the pedestal to reduce exposure of the skull and open wound margins. For Experiment 1, cohort 2, following suturing, the skin was glued to the pedestal with a tissue adhesive (GLUture, Abbott Laboratories, USA), so that no skull or open wound remained exposed. For Experiment 1, cohort 1, and Experiment 2, an antibiotic was administered, for infection prophylaxis, preoperatively and subsequently every day for the duration of the study (s.c.; Synulox; 140 mg amoxicillin, 35 mg clavulanic acid/ml; Pfizer; 0.02 ml/100 g). For Experiment 1, cohort 2, antibiotics were only administered perioperatively on the day of surgery and again 48 h later (each rat received two doses). However, the cement contained gentamicin. After surgery, the rats were allowed at least seven days of recovery before food restriction or reversal testing commenced. During the recovery period, rats were checked daily and habituated to the manual restraint necessary for the subsequent drug infusions.

2.3 Ventral hippocampal infusions

We used infusion procedures as described in our previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). Rats were manually restrained during infusions. Stylets were removed and replaced by infusion injectors (protruding 0.5 mm from the tip of guide cannulae, stainless steel, 33 gauge; Plastics One, Bilaney, UK), which were connected with flexible polyethylene tubing to two 5 µl SGE micro syringes mounted on a microinfusion pump (sp200IZ, World Precision Instruments,

UK). A volume of 0.5 µl/side of 0.9% sterile saline (Vetivex, Dechra, UK), the GABA-A receptor agonist, muscimol ($C_4H_6N_2O_2$, Sigma-Aldrich, UK) in saline (250 ng/0.5 µl/side), or the GABA-A receptor antagonist, picrotoxin ($C_{30}H_{34}O_{13}$, Sigma-Aldrich, UK) in saline (100 ng/0.5 µl/side), was infused bilaterally over 1 min. These doses were based on previous studies, in which they had specific behavioural effects following hippocampal infusions, but with limited gross behavioural effects (Taylor et al., 2025). Movement of an air bubble, trapped in the tubing, was used to monitor the infusion flow. Injectors were left in the cannulae for another minute before being removed and replaced by the stylets to allow for absorption of the infusion bolus. Rats were then put in the operant boxes and 10 min later the testing session began. The timings for the start of operant testing following infusions were based on previous electrophysiological measurements, where the peak effect of picrotoxin on neuronal firing in the ventral hippocampus was recorded between 10–35 min following infusion (McGarrity et al., 2017). All rats receiving infusions were carefully monitored during and after each infusion for any behavioural effects that would be evident from visual inspection, for example wet dog shakes and agonistic behaviour (Taylor et al., 2025; Williams et al., 2022). Not all rats displayed such behavioural effects. See Table 2-1 and Table 2-2 for the full list of behavioural effects observed in these experiments. The number of infusions per rat was limited to six, as in our previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022) to reduce the risk that reactive gliosis caused by repeated infusions may reduce effectiveness of the infused drug (Bast, Zhang, Heidbreder, et al., 2001; Cunningham et al., 2008).

2.4 Operant reversal task

We examined reversal learning on a food-reinforced two-lever discrimination task. Our testing procedures were the same as in our recent study investigating the impact of bidirectional activation changes in mPFC on reversal learning (Renström et al., 2025) and adapted from our earlier reversal studies (Gonçalves et al., 2023; Hock, 2020), which used protocols from Brady & Floresco (2015). Rats were pretrained to

press levers to obtain a food reward (sucrose pellet), before acquiring a 'spatial discrimination' (SD), where either the left or the right lever was associated with the reward. This reward contingency was then reversed, and rats had to learn to switch levers to obtain the food reward (see bottom part of Figure 2-1). For Experiment 1, rats were pretrained and then received ventral hippocampal infusions before SD and reversals 1–5 to investigate the impact of inhibition and disinhibition of the ventral hippocampus on the initial discrimination and early serial reversal learning. For Experiment 2, the rats were pretrained and then completed SD and four reversal sessions, to achieve stable performance levels. Ventral hippocampal infusions were then given before reversals 5–10 to investigate the impact of inhibition and disinhibition of the ventral hippocampus on late serial reversal learning.

2.4.1 Apparatus

Rats were tested in standard operant conditioning boxes (30.5 cm x 24.1 cm x 21.0 cm; MedAssociates, St. Albans, VT, USA) equipped with one house light (40 lux), two stimulus lights (40 lux each), a food pellet dispenser and two retractable levers either side of the dispenser, as shown at the bottom of Figure 2-1. Extraction fans in each box masked any external noise. Lever control, pellet delivery, and data collection were operated via a connected computer (Dell; OS: Windows XP) running MED-PC software. The code used was taken from Renström et al. (2025), which was previously adapted from Hock, (2020). Throughout all sessions, one of the two cue lights was chosen pseudo-randomly to be on, and which cue light was on was irrelevant to the correct lever choice (i.e., the cue lights were essentially acting as distractors); this was similar to previous reversal studies (Brady & Floresco, 2015; Floresco et al., 2008; Hock, 2020; Renström et al., 2025).

2.4.2 Habituation and pretraining

Rats were always tested in the same operant box and were habituated to the boxes four days before pretraining began. For the habituation, rats were placed in their designated boxes each day (of the four days) for 10 min. Rats were then pretrained

for 10–11 days before SD and reversal sessions. Rats completed one session per day for pretraining, SD and reversals.

Stage 1 of pretraining consisted of single lever press training in which only one lever, either left or right, was presented, with each press of the lever delivering a sucrose pellet (5TUL 45mg; Testdiet, UK). The testing order for the levers was counterbalanced, with half of the rats starting with the left lever, and half with the right. The house light was lit continuously throughout these trials. Rats were required to make a minimum of 50 rewarded responses on the lever presented to progress to pretraining on the opposite lever. Again, the lever was extended and there was a criterion of 50 lever presses. Stage 1 pretraining sessions lasted for 30 min (number of lever presses was not limited within this time). Once rats had made at least 50 lever presses within one session, for both levers, they progressed to stage 2.

Stage 2 of pretraining involved training on retractable levers. Rats were placed in the operant boxes with the house light off. After 20 s, both stimulus lights illuminated, one of the levers extended randomly, and the house light turned on. The rats had 10 s to make a response. If they pressed the lever, this caused the lever to retract, and they were rewarded with a sucrose pellet. Failure to make a response within the 10 s also led to lever retraction but was recorded as an omission for the trial. There were 90 trials, and all rats were tested on this stage for a minimum of five consecutive days. Rats were expected to make fewer than five omissions by the fifth day.

Stage 3 of pretraining was the side preference test and was conducted immediately after the last retractable lever session (rats remained in the operant boxes after finishing the retractable lever training). Side preference trials were divided into seven main trials with several sub-trials. On each main trial, both levers were extended into the box, with no cue light illuminated and either a left or right response would result in a sucrose pellet reward. Then, following a 20 s interval, the sub-trial began, where the opposite lever to the main trial was rewarded, whilst response on the same lever was unrewarded. Only when the correct lever was pressed in the sub-trial would the next main trial begin. For instance, if a rat pressed the left lever on the main trial, it

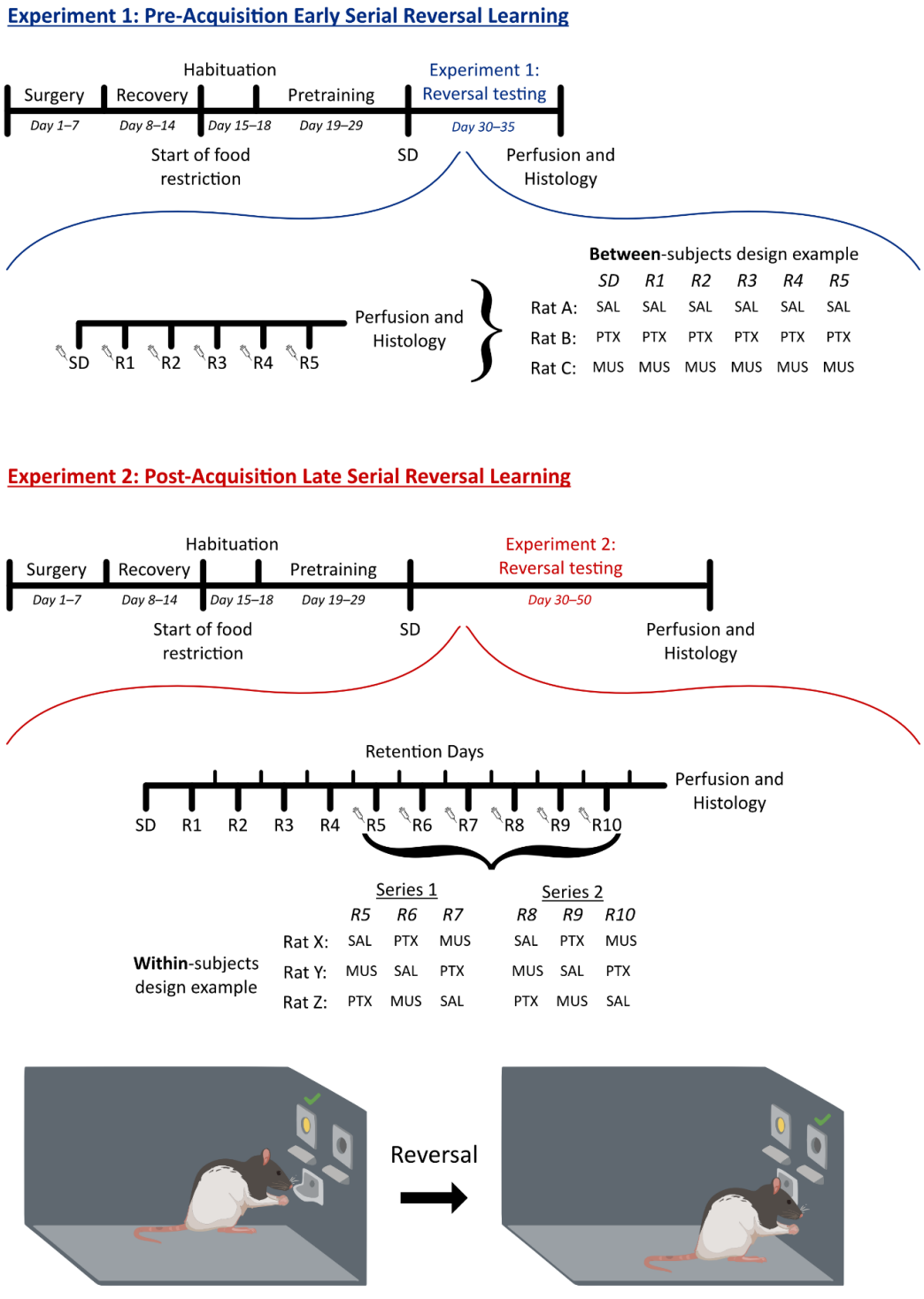
would need to press the right lever on the sub-trial to progress to the next main trial. Overall, rats were required to make 14 rewarded responses (seven pairs of main lever responses and sub-trial responses on the opposite lever). When seven pairs of responses were made, the test ended, and the rats' side preference was established based on the ratio of main trial responses (minimum ratio of 4:3) i.e., if a rat had four left responses and three right responses, the side preference would be left.

2.4.3 Experiment 1: Impact of ventral hippocampal muscimol and picrotoxin on spatial discrimination and early serial reversal learning

Experiment 1 investigated the role of ventral hippocampal activity for the initial SD learning and the subsequent early serial reversal learning. Rats received ventral hippocampal infusions before the SD session and before each of up to five subsequent reversal sessions (Reversals 1–5; R1–5). These sessions were on consecutive days and rats received the same infusion (saline, muscimol or picrotoxin) each day, i.e. the impact of the different infusions was compared between-subjects (see Figure 2-1).

Following pretraining, the first session was the SD task where rats were required to press the lever opposite to their side preference (as determined during pretraining, see above). Trials began every 20 s, with a 10 s response window and an inter-trial interval of 20 s. On each trial, both levers were presented, but only the lever opposite to the rats' side bias was rewarded. For example, if left was the side preference, for SD pressing the right lever was rewarded and pressing the left lever was not rewarded. This session was terminated once the rat had reached the criterion of 10 consecutive correct responses, or after a maximum of 200 trials, though a minimum of 30 trials was required (Brady & Floresco, 2015). If the success criterion was not reached within 200 trials, rats were retested the next day with the same protocol (in the example given, the right lever would be the correct choice again). For example, if a rat failed to reach criterion on the first day of SD and then took 90 trials to reach criterion on the following day, the responses to criterion for this rat would be 290.

Figure 2-1: Experimental timeline and two-lever task for Experiments 1 and 2.



Note. Experiment 1 (between-subjects, early serial reversal, n=40) combined ventral hippocampal activity manipulations with testing in reversal-naïve rats between SD–R5, whilst Experiment 2 (within-subjects, late serial reversal; n=12) introduced these manipulations once the reversal task had been learnt (across two series of reversals: R5–7 and R8–10). For Experiment 1, statistical analysis was restricted to SD and R1–3, as these were the sessions completed by all rats, with fewer rats successfully completing all five reversals as rats could take multiple days to reach criterion for one SD or reversal session. Syringes indicate ventral hippocampal infusions. Retention days were included between reversal stages for Experiment 2 to allow rats to achieve similar baseline levels and to serve as 'wash out' days between infusions. Examples of between- and within-subject infusion schedules for three example subjects are included adjacent to timelines. The bottom of the figure shows the operant lever task adapted from Brady & Floresco (2015). Operant boxes contained two retractable levers either side of a food hopper with cue light above each lever, as well as a house light. Green tick above levers indicates correct response. Abbreviations: MUS: muscimol (250 ng/0.5 µl/side); PTX: picrotoxin (100 ng/0.5 µl/side); SAL: saline (0.5 µl/side); SD: spatial discrimination; R: reversal. Figure was produced using BioRender.com and Affinity by Canva 3.0.2.3912.

All rats reached the criterion of 10 consecutive responses in one or two sessions. One rat in each of the two cohorts of rats used in Experiment 1 completed the SD session in less than 30 trials. For these rats, this initial < 30 trial session was used for all analyses (responses to criterion etc.), but the rats continued the session until they had experienced 30 trials, so they had a similar exposure to the procedure as other rats (see Brady and Floresco (2015)).

The day after rats completed the SD stage, reversal sessions began. Again, trials began every 20 s, with a 10 s response window and an inter-trial interval of 20 s. Each reversal session started with 20 reminder trials, during which rats were rewarded for pressing the same lever as on the previous day; for the example, if the right lever was rewarded during the SD session, the right lever would also be rewarded during the reminder trials of the first reversal session. Reminder trials were included to allow us to determine whether differences in performance during the reversal were due changes in flexibility, e.g. enhanced flexibility, or differences in retrieval of the previous rule, e.g. impaired retrieval which may facilitate learning of a new rule

during the reversal (Brady & Floresco, 2015). The reversal occurred at trial 21, where the correct and incorrect lever were reversed so that the rewarded side was now the opposite (in the example given, this would now be the left lever). Rats were required to reach a criterion of 10 consecutive correct responses, and the session ended once this was achieved. If this was not achieved within the maximum of 200 reversal trials, the rat continued to be reinforced on the same lever the following day, and these trials were included in the responses to criterion performance measure. As described for SD, if a rat failed to reach criterion on the reversal day and then took 90 trials to reach criterion on the following day, the responses to criterion for this rat would be 290. Once rats had completed one reversal stage, they moved to the next reversal stage. Each reversal stage started with 20 reminder trials, where the same lever was reinforced as during the preceding reversal stage, followed by reversal trials, where the opposite lever was reinforced. Rats were allowed a maximum of three sessions (across three days) for each reversal stage (see Brady and Floresco (2015)); none of the rats exceeded this, with most rats passing within one session. Because we limited the number of ventral hippocampal infusions to six per rat, in each rat, the impact of infusions could be tested on the initial SD and up to five reversal stages, if the rat completed each reversal stage within one session. Some rats completed fewer reversal stages, either because they required more than one session for at least one of the reversal stages, or because they had to be culled before the end of testing due to illness or behavioural effects.

2.4.3.1 Sample size

Experiment 1 compared the impact of muscimol, picrotoxin and saline infusion during early serial reversal learning (SD and R1–5) using a between-subjects design. Our target sample size was n=16 per group to give a power of >80% for pairwise comparisons to detect differences between infusion groups that correspond to an effect size of Cohen's d of around 1, using pairwise comparisons (two-tailed independent t-tests, $p < 0.05$; G*Power, Faul et al., 2007). Rats were allocated to one of three infusion groups: muscimol, picrotoxin or saline, using a randomised block design, with at least one rat in each cage of four being randomly allocated to each

infusion group. Testing order was counterbalanced using a Latin square design. Furthermore, Experiment 1 was run as a sequential design (Neumann et al., 2017) using two cohorts of 24 rats (cohort 1 and 2). In Experiment 1, cohort 1, six rats were excluded from analyses because they did not complete three reversals: four rats (three in the picrotoxin group and one in the saline group) were culled early due to illness, one rat in the muscimol group was culled due to behavioural effects that did not improve within 1 h, and one rat in the saline group did not complete a test session due to a mechanical error with the operant testing box. In Experiment 1, cohort 2, two rats were excluded from analyses: one rat in the muscimol group died shortly after surgery due to an unexpected complication and one rat in the saline group was culled early during pretraining due to illness. This gave a final sample size of n=40 rats (muscimol: n=14; picrotoxin: n=13; saline: n=13). Statistical analysis was restricted to SD and R1–3, as these were the sessions completed by all rats, with fewer rats successfully completing all five reversals.

2.4.4 Experiment 2: Impact of ventral hippocampal muscimol and picrotoxin on late serial reversal learning

Experiment 2 investigated the role of ventral hippocampal activity in late (well-established) serial reversal learning. Rats initially completed SD and four reversal stages (R1–4) and there were no infusions for these stages. The SD and these reversals allowed the rats to learn the reversal task, with all rats reaching similar performance on the task by the fourth reversal day. This was based on previous work that found a significant reversal cost at R1 and R2, which then improved at R3 and plateaued from around R5 (Renström et al., 2025; Renstrom, 2023). Then the impact of the three different ventral hippocampal drug infusions (muscimol, picrotoxin, saline) on late serial reversal learning was compared within-subjects across six reversal sessions (R5–10). Each rat received two series of the three different ventral hippocampal infusions, with infusion series 1 consisting of infusions applied 10 min before R5–7 and infusion series 2 consisting of infusions applied 10 min before R8–10. Testing order of the three infusion conditions within each series was counterbalanced using a Latin square design (Figure 2-1).

Following pretraining, the rats completed the SD stage, as in Experiment 1. In Experiment 2, all rats reached the criterion of 10 consecutive correct responses within less than 200 trials. The day after completing the SD stage, reversal sessions began. As in Experiment 1, reversal sessions started with 20 reminder trials, before reversal trials started. Reversal sessions ended after rats had achieved the criterion of 10 consecutive correct responses, or after a maximum of 200 reversal trials. However, in this experiment, if a rat did not achieve the criterion of 10 consecutive correct trials within the maximum 200 trials, the rat was excluded from the analyses. Importantly, in this experiment, there were retention days interleaved between the reversal sessions. These involved the same task as for SD, and the rats had to respond to the same side as they did in the reversal trials the previous day (if rats had a right to left reversal, they would be reinforced on the left lever on the retention day). Sessions on the retention days were terminated when the rat had achieved the criterion of 10 consecutive correct responses or after a maximum of 200 trials. On all retention days, all rats achieved the criterion in less than 200 trials. The retention days were important for the within-subjects testing of infusion effects from R5 onwards. Rats were always tested without drug infusions on retention days, so that the retention days served as 'wash out' days between different drug infusions and allowed all rats to achieve the criterion of 10 consecutive correct responses, i.e. similar performance levels, before the impact of the next drug infusion was tested in another reversal session. Rats completed four reversals and four retention days, without any infusions, before ventral hippocampal infusions were administered for six reversals (5–10), with retention days interleaved between reversals.

2.4.4.1 Sample size

Experiment 2 compared the impact of ventral hippocampal muscimol, picrotoxin, and saline infusion on late serial reversal performance using a within-subjects design. Testing order was counterbalanced using a Latin square design and this was blocked by series. Our target sample size was n=12. This sample size would give a power of >80% to detect differences between infusion conditions that correspond to an effect size of Cohen's d of around 1, using pairwise comparisons (two-tailed paired t-tests,

$p < 0.05$; G*Power, Faul et al., 2007). 16 rats completed pretraining and the first four reversals, 12 rats completed all reversals, and 11 rats completed all testing (including the final retention day after the final reversal). One rat was excluded because it failed one of the reversal sessions (in the muscimol condition in series 1) and three rats were excluded from analyses because they were culled early: one due to behavioural effects in the picrotoxin condition, one due to illness and one due to an injector snapping inside the cannula preventing further infusions. Therefore, the analysis of infusion effects across both infusion series was based on data from n=12 rats, whereas the analysis of infusion effects on retention days was based on n=11 rats (as one rat had to be culled due to illness on the morning of the final retention day before testing). For analysis of some of the performance measures, such as during the reminder trials, the n was further reduced. For example, during the reminder trials one rat omitted for all 20 trials and, therefore, this rat was removed from the percentage correct and latency analyses. See individual performance measure analyses for full details.

2.4.5 Blinding

The lead experimenter was blinded to the drug conditions for the duration of the Experiments 1 and 2 and the statistical analyses, though the efficacy of the blinding was limited given that the muscimol and picrotoxin infusions have known behavioural effects (see Table 2-1 and Table 2-2 for more details).

2.5 Verification of cannula placements

Cannula placements were verified as in our previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). On completion of the experiments, rats were anesthetised with a lethal dose of sodium pentobarbital (1–2 ml intraperitoneal (i.p).; sodium pentobarbital, 200 mg/ml, Dolethal, Vetoquinol, UK) and transcardially perfused with 0.9% saline followed by 4% formaldehyde solution (Sigma-Aldrich, UK) made up in 0.9% saline. Brains were removed from the skull, stored in 4%

formaldehyde made up in 0.9% saline and cut into 80 µm coronal sections on a vibratome (Leica VT 1000 S, UK). Sections containing the relevant brain regions were mounted on microscope slides and placement of the injectors was determined using a light microscope and mapped onto coronal sections of a rat brain stereotaxic atlas (Paxinos & Watson, 1998). For each experiment, the sections of some brains were cresyl-violet stained for presentation purposes.

2.6 Performance measures

As in our previous studies (Maggi et al., 2024; Renström et al., 2025), we used classical, whole-session performance measures as well as trial-by-trial probabilities, determined by a Bayesian strategy analysis to assess rats' performance (Maggi et al., 2024).

2.6.1 Classical performance measures

Performance was measured during SD and reversals, reminder trials and retention days. The main measure of task performance was responses to criterion (RTC) i.e., the number of trials a rat required to achieve our success criterion of 10 consecutive correct responses, excluding omission trials. We excluded omissions from RTC to separate the impact of the infusions on errors of commission (incorrect lever presses) from errors of omission (no lever press). RTC is not applicable for reminder trials, as there is no success criterion on these trials, and instead percentage correct was used, which calculates the percentage of correct reminder trial responses divided by the total number of reminder trial responses, excluding omissions. Response latencies were separated by correct or incorrect responses and analysed separately for reminder trials and SD/reversal trials. There we no reminder trials for SD.

As in our recent study (Renström et al., 2025), we differentiated errors by type (perseverative/regressive), to examine if rats had difficulties to cease responding to the previous rule or to learn the new rule. Different approaches can be used to differentiate perseverative and regressive errors, and we used three of these

approaches: 1. “Block” method (Brady and Floresco, 2015): errors were counted as perseverative errors until a threshold of less than 10 errors in a block of 16 responses was reached. The blocks would be as follows: block one, trials 1–16; block two, trials 17–32; block 3, trials 33–48, and so on. Following the first block with less than 10 errors, all subsequent errors were counted as regressive; 2. “Sliding window” method: this is similar to the “Block” method, but rather than having the blocks of 16 trials as discrete blocks, instead the block is moved per trial i.e. block one consists of trials 1–16, block two of trials 2–17 and block three is trials 3–18 etc.; 3. “Simple” method: this refers to counting errors as perseverative until the first correct response, then all errors after this first correct response are classed as regressive, as in Dhawan et al. (2019). For our experiments the direction of findings were the same across the three methods. Reported analyses of perseverative and regressive errors are using the “Block” method as in Brady and Floresco (2015) and Renström et al. (2025).

2.6.2 Trial-by-trial Bayesian strategy analysis

Complementing the use of the classical performance measures outlined above, data were analysed using a trial-by-trial Bayesian strategy analysis protocol (Maggi et al., 2024). We were interested in the strategies that were used to learn the rule. The Bayesian analysis protocol estimates the probability that a particular strategy has been used on a trial. Each strategy (e.g. win-stay) was estimated independently from each other based on a set of input parameters. These parameters included the lever choice (left or right lever) and the outcomes (rewarded or unrewarded) of the current and preceding responses. For example, a “win-stay” strategy is defined as a correct/rewarded response (‘win’), followed by re-selection of the same lever (‘stay’) on the following trial. Estimating strategy probabilities, therefore, requires information about the choice and outcome on trial t and the choice on trial t+1. The equation below refers to the computation, using Bayes theorem, used to calculate the *posterior probability*, $P(\text{strategy}_i(t) \mid \text{choices}(1{:}t))$, which is an estimate of the probability of strategy_i being applied by the subject at trial t. The *likelihood*, $P(\text{choices}(1{:}t) \mid \text{strategy}_i(t))$ is the consistency of the choices in the strategy we are

assessing, and the *prior*, P(strategy$_i$(t)), is the initial estimate of the probability that the subject applies the current strategy at a hypothetical trial '0', and thus plays a role in the initial estimation of early probabilities, defined in the range of [0,1]. In the current model the prior is set to 0.5. (i.e., chance).

$$P(strategy_i(t)\,|\,\text{choices (1: t)}) \propto \text{P(choices(1: t)}\,|\,strategy_i(t)) \times P(strategy_i(t))$$

The Bayesian analysis protocol estimates the probability of a rat applying a particular strategy on any given trial based on evidence collected up to this trial. This is done by keeping a running total of past success or failures to execute the strategy in question, whereby positive evidence (i.e., for a given strategy, such as win-stay) and negative evidence (i.e., for an opposing strategy, such as win-shift) are related to α and β values, respectively, with positive or negative evidence incrementally increasing its respective value. If no evidence is available, probabilities remain the same. Evidence used to update the Bayesian model is weighted based on recency using a decay function (γ). More specifically, γ reduces α and β on every trial, and this decay is exponentially weighted by how far in the past evidence was accumulated. In the current model a γ = 0.9 was used. The relationship between the α and β values produces an iteratively changing beta distribution, whereby the peak is a product of the relationship between the positive and negative evidence for any given strategy at t, based on all trials up to t (Maggi et al., 2024). If no evidence is present, this value will decrease with time due to the decay function. This model allows for a continuously updating estimation of strategies employed by the rats at a trial-by-trial resolution.

To establish a robust estimation of strategy probabilities in Experiment 1, we used the SD stage to accumulate evidence for each strategy before statistically comparing the impact of ventral hippocampal manipulations on the probabilities for R1–3. In Experiment 2, evidence for each strategy was accumulated across the SD stage and R1–4, before comparing the impact of ventral hippocampal manipulations on probabilities for R5–10.

Strategies were investigated “around reversal” and “around learning”. Around reversal refers to the trials preceding and following the rule reversal i.e., the reminder trials and the reversal trials immediately after the rule reversal. Around learning refers to responses preceding the 10 consecutive correct responses that we defined as criterion of successful learning, and that ended the testing session. These time points were of interest, as response strategies around the rule reversal reveal how the rats responded to the rule change, whereas the strategies preceding learning reveal how rats learnt the new rule. The number of responses included in our Bayesian strategy analysis was limited by the number of responses that were completed by most rats across all sessions. For the analysis of strategies around reversal, we included six reminder trial responses and the 20 responses post rule reversal, i.e. 26 responses total. For analysis of the strategies around learning, this was limited to the 11 responses preceding learning the rule. The last 11 responses of every session were identical (one incorrect response before the success criterion of 10 correct responses), which is why we only included those preceding this learning point.

In our experiments, we aimed to investigate what strategies rats were using to learn the task. First, we examined strategies that reflect if rats follow the task rule. For around reversal, this was “go-previous” which indicates the trial-by-trial probability that the rat applies the strategy that was correct prior to rule change (i.e., go-left or go-right). Typically, rats strongly apply this strategy on the reminder trials, where this is the correct strategy, then the probability of the strategy decreases after reversal, as the rat begins to switch responses to the newly rewarded lever. Therefore, this strategy captures perseveration around rule change at a trial-by-trial resolution, as rats struggle to move away from the previously rewarded, but now incorrect response. For around learning, we used the complementary strategy, “go-new”, which indicates the correct choice strategy after the reversal and reveals how quickly the rats learned this new rule. Go-previous and go-new strategies are complementary, i.e. their probabilities add up to P=1, whereby as one increases the other one decreases, and vice versa.

Second, we investigated other task-appropriate strategies. This included “win-stay” and “lose-shift”, which describe either ‘stay’ behaviour on the same rule (i.e., same lever) following a win-trial (rewarded trial), and ‘shift’ behaviour to the opposite rule following a lose-trial (unrewarded trial).

Third, we investigated task-inappropriate strategies which included the “go-cued” strategy and the complementary strategies “sticky” and “alternate”. Go-cued is when a rat’s response choice depends on the cue light. In our task, the cue light was not indicative of reward as our rule was always left or right lever, meaning this strategy was not appropriate for task success. We wanted to investigate whether rats used this cue to learn the task. Sticky is when the rat stays on the same lever, regardless of reward, and its complementary strategy, alternate, is when a rat continuously switches between the left and right lever, regardless of reward. Again, we were interested in whether these were strategies that the rats used.

When interpreting outcomes of the Bayesian strategy analysis it is important to consider that the strategies are not mutually exclusive, for example, any win-stay trial may also alter the probability of other exploratory strategies. As such, changes in the probabilities of strategies that are not dominantly applied (i.e., whose probability is below chance, $P < 0.5$) need to be interpreted in context of other, dominant, strategies ($P > 0.5$). In these experiments, only one of two complementary strategies (e.g., win-stay and win-shift or sticky and alternate) was examined, as the opposite strategy always mirrors the investigated strategy, i.e. the probability of one of these two strategies is implied by the probability of the other, therefore we only analysed the probabilities of the dominant strategy ($P > 0.5$) out of these pairs of strategies.

2.7 Statistical analysis

Data were analysed using ANOVAs, as in our recent reversal study (Renström et al., 2025). For Experiment 1, infusion group (muscimol, picrotoxin or saline) was used as the between-subjects factor and task stage (SD, R1–3) as the within-subjects factor.

For Experiment 2, infusion condition (muscimol, picrotoxin or saline) and infusion series (series 1 and 2) were used as within-subject factors. Analysis of Bayesian strategy profiles additionally included response as a within-subjects factor. Where the assumption of sphericity (Mauchly's test) was violated, Greenhouse-Geisser, when $\varepsilon < 0.75$, or Huynh-Feldt, when $\varepsilon > 0.75$, correction was applied to the degrees of freedom. Significant interactions were further analysed using simple main effects analyses and significant three-way interactions were followed by separate two-way ANOVAs for each reversal stage or for each series. Significant main effects were analysed by post-hoc pairwise comparisons using Fisher's LSD test. Because JASP does not offer the Fisher's LSD test, the p values for the Fisher's LSD were computed by taking the Bonferroni corrected p values from JASP and dividing these by the number of multiple comparisons; for the present experiments, the number of comparisons was three as we had three infusion groups or conditions (saline, muscimol and picrotoxin). Because Bonferroni-corrected p values are 'capped' at p=1, if the number of comparisons is three, JASP returns Bonferroni-corrected p values of p=1 whenever the uncorrected p value is $\geq 1/3$. Therefore, whenever a Bonferroni-corrected p value of p=1 was reported by JASP, we could not compute an accurate uncorrected p value, and instead we report $p \geq 0.3$ in these circumstances.

Additionally, a one-tailed Fisher's Exact Test was used to compare the number of rats that showed gross behavioural side effects, such as wet dog shakes (which were observed after some infusions, see sections 3.3 and 3.5) between infusion groups or conditions. After each infusion, we recorded if a rat showed a behavioural effect at least once and the unit of analysis was the number of rats showing a certain behaviour at any time point, rather than the number of observations of a behaviour. As we expected ventral hippocampal picrotoxin and muscimol to increase these behavioural effects compared to saline, based on previous work (Taylor et al., 2025; Williams et al., 2022), we used one-tailed Fisher's Exact Tests.

Data are presented as mean ± SEM and p values of < 0.05 were considered to indicate statistical significance. Data analysis was performed using JASP (JASP Team (2024). JASP Version 0.18.3), analysis of gross behavioural effects was completed with, and

graphs were produced using, GraphPad Prism (version 10.2.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com). Bayesian strategy analysis and perseverative and regressive errors were computed using The MathWorks Inc. (2022). MATLAB version: 9.13.0 (R2020b and R2022b, respectively), Natick, Massachusetts: The MathWorks Inc. https://www.mathworks.com. Affinity by Canva version 3.0.2.3912 and BioRender (BioRender.com) were also used to produce figures.

3 Results

3.1 Experiment 1 and 2: Infusion sites in the ventral hippocampus

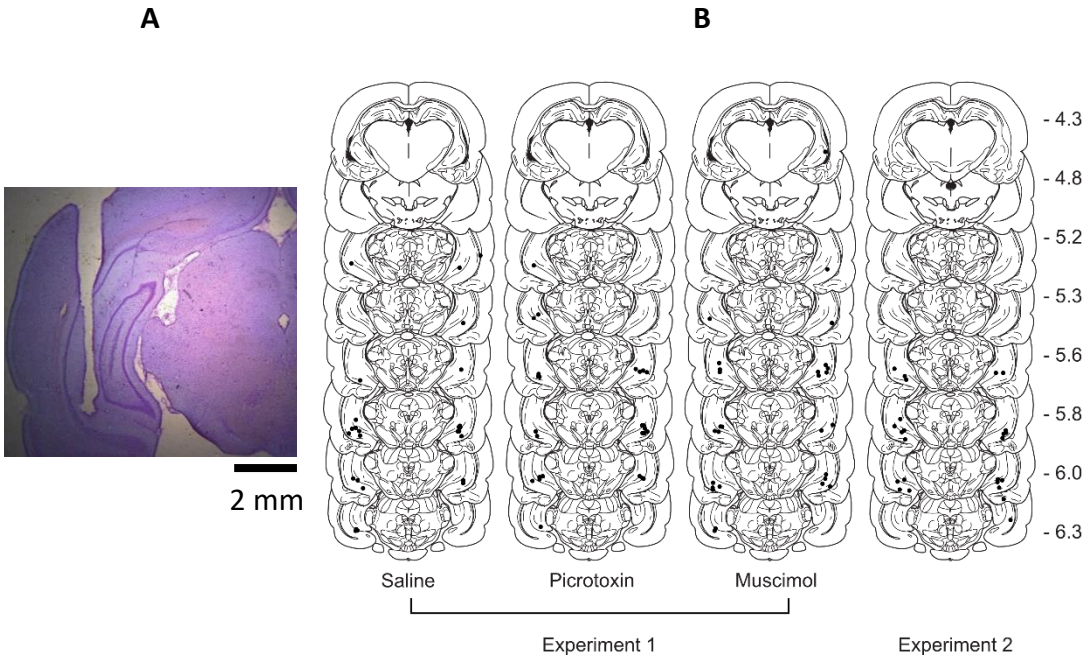
Infusion cannula tips were primarily placed within the ventral to intermediate hippocampus (Figure 2-2), in coronal brain sections corresponding to between 4.3–6.3 mm posterior to bregma in the rat brain atlas by (Paxinos & Watson, 1998). In Experiment 1, one cannula was implanted too lateral (seen on the right side of the -5.2 section in the saline group). This infusion would likely not have targeted the ventral hippocampus due to the densely packed fibre bundles surrounding the hippocampus (see McGarrity et al. (2017)). However, this rat received a saline infusion and so could still serve as a control given the rat experienced the same infusion procedure. In Experiment 2, one cannula was located quite deep, encroaching on the entorhinal cortex (seen on the right side of the -6.0 section). However, due to drug spread up the cannula tract, the infusion would still have affected the ventral hippocampus. Statistical analyses revealed essentially the same findings, regardless of whether data from these rats were included. We only present the outcomes from the analyses including these rats.

3.2 Experiment 1: Disinhibition of the ventral hippocampus tended to improve performance across spatial discrimination and early serial reversal learning, whilst inhibition did not affect performance

3.2.1 Ventral hippocampal disinhibition tended to decrease RTC across sessions, whereas inhibition had no effect on RTC

All groups showed a clear initial reversal cost, with rats requiring more responses to reach criterion at R1 than at the SD stage, and this cost gradually diminished across reversals, as rats learned to reverse; overall, the RTC values in the saline group at different stages were similar to our recent study involving prefrontal manipulations (Renström et al., 2025; Renstrom, 2023).

Figure 2-2: Experiment 1 and 2: Infusions sites in the ventral hippocampus.



Note. Illustrative coronal brain section showing an infusion site in the ventral hippocampus, cresyl-violet stained for presentation purposes (A). Approximate locations of infusion cannula tips (black dots) mapped onto coronal sections adapted from the Paxinos and Watson (1998) rat brain atlas in Experiments 1 and 2 (B; Experiment 1: Saline, n=13, Picrotoxin, n=13, Muscimol, n=14; Experiment 2, n=12). Numbers on the right indicate posterior distance from bregma in millimetres.

A two-way repeated measures ANOVA revealed a main effect of stage ($F_{(3,111)}$ = 43.488, $p < 0.001$), reflecting that RTC increased from SD to R1 and then decreased from R1 through to R3, when RTC were similar to the SD stage again. Post hoc pairwise comparisons revealed differences in RTC between all sessions (largest p = 0.004), except between SD and R3. Disinhibition of the ventral hippocampus tended to improve performance on SD and R1–3, whereas inhibition of the ventral hippocampus had no effect (Figure 2-3A). There was a trend towards an effect of infusion group ($F_{(2,37)}$ = 2.728, p = 0.078), and pairwise comparisons to examine this trend further revealed a difference between the picrotoxin and saline groups (p = 0.040), a trend towards a difference between picrotoxin and muscimol (p = 0.064)

and no difference between muscimol and saline ($p \geq 0.3$). There was no infusion group X stage interaction ($F < 1$).

3.2.2 Neither ventral hippocampal disinhibition nor inhibition affected perseverative and regressive errors

Ventral hippocampal infusions of picrotoxin and muscimol did not significantly affect perseverative or regressive errors (main effect or interaction involving infusion group, all $F < 2.0$, $p > 0.15$), although perseverative errors were numerically decreased across all reversals in the picrotoxin group compared to the other two groups. There was only a main effect of reversal stage for both error types ($F > 8.0$, $p < 0.001$), which reflected a decrease in errors across the three reversals (Figure 2-3 B and C).

3.2.3 Ventral hippocampal inhibition, but not disinhibition, tended to increase omissions

A two-way repeated measures ANOVA revealed a trend for a main effect of infusion group on the number of omissions ($F_{(2,37)} = 2.800$, $p = 0.074$; Figure 2-3D). Pairwise comparisons to examine this trend indicated an increase in omissions in the muscimol group compared to the saline group ($p = 0.025$), but not the picrotoxin group ($p \geq 0.3$), and there was no difference between the picrotoxin and saline groups ($p = 0.135$). There was no main effect of task stage or an infusion group X stage interaction (both $F < 1$). When outliers were excluded (using boxplots, n=25), the trend towards a main effect of infusion remained ($F_{(2,22)} = 2.765$, $p = 0.085$; data not shown). There was a main effect of stage ($F_{(1.422,31.273)} = 9.135$, $p = 0.002$) and simple main effects revealed an increase in omissions in R1 versus SD, R2 and R3 (highest $p = 0.003$). There was no infusion group X stage interaction ($F_{(2.843,31.273)} = 2.092$, $p = 0.124$).

3.2.4 Ventral hippocampal disinhibition increased, and inhibition tended to increase, incorrect, but not correct, response latencies

Correct response latencies were not affected by infusion group ($F_{(2,37)} = 2.340$, $p = 0.110$), or stage ($F < 1$), and there was no infusion group X stage interaction ($F < 1$; Figure 2-3E). Ventral hippocampal disinhibition increased incorrect response

latencies across the SD and three reversal stages (main effect of infusion group: $F_{(2,37)}$ = 4.621, p = 0.016; Figure 2-3F) compared to saline (p = 0.005) and ventral hippocampal inhibition tended to increase incorrect response latencies compared to saline (p = 0.062), and there was no difference between ventral hippocampal picrotoxin and muscimol (p = 0.264). There was a trend towards a main effect of stage ($F_{(3,111)}$ = 2.360, p = 0.075), reflecting a decrease in incorrect response latencies from SD to R3. There was no interaction of infusion group X stage (F < 1).

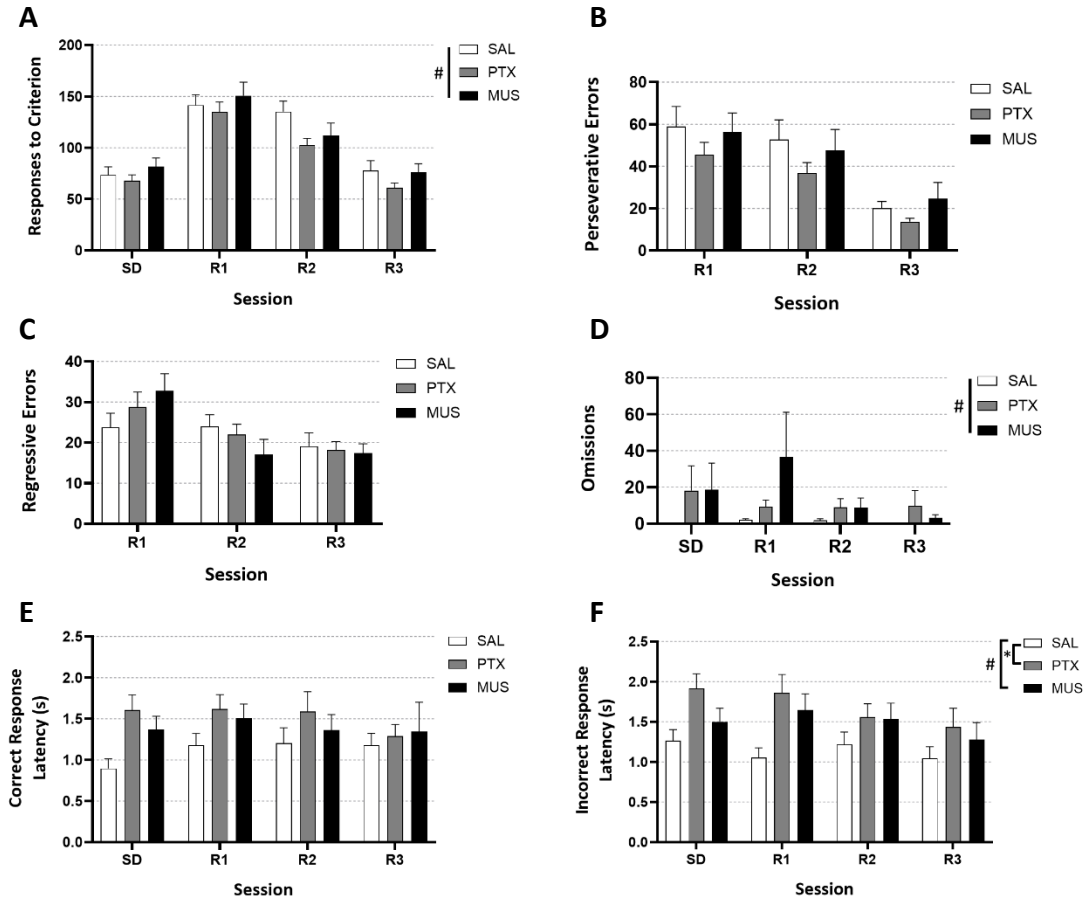
3.2.5 Ventral hippocampal inhibition and disinhibition impaired performance during reminder trials

Ventral hippocampal inhibition, but not disinhibition, decreased the percentage of correct responses during reminder trials before reversals (Figure 2-4A). A two-way repeated measures ANOVA found a main effect of infusion group ($F_{(2,37)}$ = 4.003, p = 0.027), without an infusion group X stage interaction (F < 1). Post hoc pairwise comparisons revealed that ventral hippocampal muscimol decreased the percentage of correct responses during reminder trials compared to saline infusion (p = 0.007), but not picrotoxin (p = 0.208) and there was no difference between picrotoxin and saline (p = 0.138). There was also a trend towards a main effect of stage ($F_{(2,74)}$ = 3.030, p = 0.054) and pairwise comparisons of this trend revealed a decrease in percentage correct from R1 to R2 (p = 0.012).

Ventral hippocampal disinhibition increased the number of omissions during the reminder trials (Figure 2-4B). A two-way repeated measures ANOVA revealed a main effect of infusion group on omissions ($F_{(2,37)}$ = 3.391, p = 0.044) and post hoc pairwise comparisons revealed that picrotoxin increased omissions compared to saline (p = 0.018) and tended to increase omissions compared to muscimol (p = 0.058), whilst muscimol and saline did not differ from each other (p ≥ 0.3). There was no effect of stage ($F_{(1.662,61.494)}$ = 1.431, p = 0.246) or an infusion group X stage interaction ($F_{(3.324,61.494)}$ = 1.208, p = 0.316).

Ventral hippocampal disinhibition increased response correct latencies (Figure 2-4C). There was a main effect of infusion group ($F_{(2,37)}$ = 7.759, p = 0.002) and post hoc

Figure 2-3: Experiment 1: Disinhibition of the ventral hippocampus tended to improve performance across spatial discrimination and early reversal learning, whilst inhibition did not affect performance.



Note. Data are shown as mean (± SEM), between-subjects design. Performance measures during SD and R1–3: Responses to criterion (A); Perseverative errors (B); Regressive errors (C); Omissions (D); Correct latency (E) and Incorrect latency (F). Abbreviations: MUS: muscimol (250 ng/0.5 µl/side; n=14); PTX: picrotoxin (100 ng/0.5 µl/side; n=13); SAL: saline (0.5 µl/side; n=13); SD: spatial discrimination; R: reversal. Only effects involving infusion group are indicated. # indicates trend, p = 0.051–0.095; * indicates p < 0.05 (Fisher's LSD test following a significant main effect of infusion with a two-way repeated measures ANOVA).

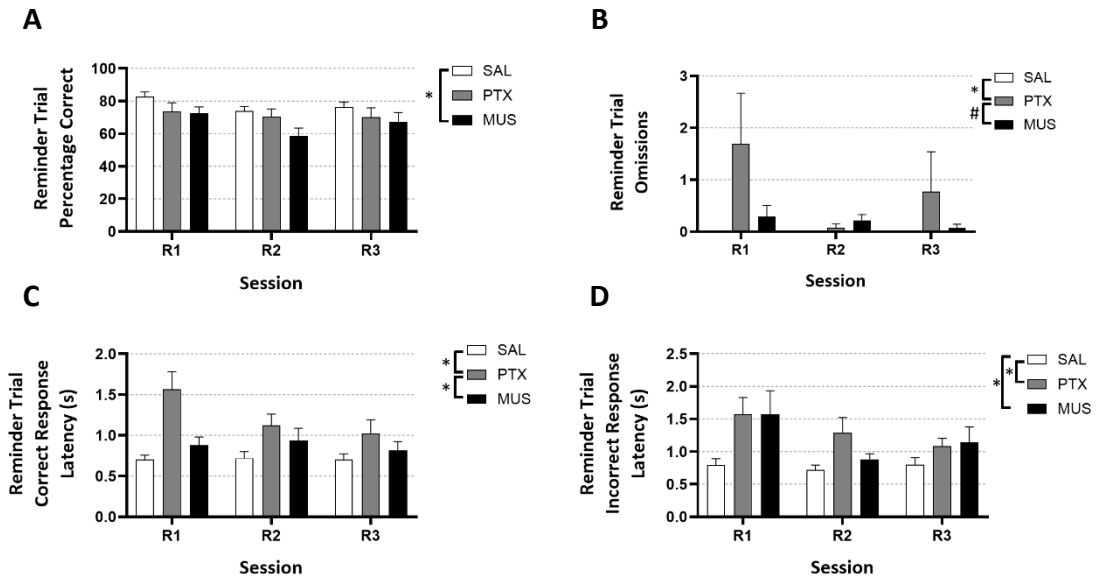
pairwise comparisons revealed that picrotoxin increased correct response latencies compared to saline and muscimol ($p < 0.011$), but muscimol and saline did not differ from each other ($p = 0.222$). There was a trend towards an effect of stage ($F_{(2,74)} = 2.730$, $p = 0.072$) and a trend towards an infusion group X stage interaction ($F_{(4,74)} = 2.424$, $p = 0.056$). These reflected a decrease in latencies from R1 to R3, and that

picrotoxin particularly increased latencies during R1. Ventral hippocampal inhibition and disinhibition increased incorrect response latencies (Figure 2-4D; picrotoxin group reduced to n=11 so total n=38). There was a main effect of infusion group ($F_{(2,37)} = 4.160$, $p = 0.024$), without an infusion group X stage interaction ($F_{(4,74)} = 1.172$, $p = 0.331$). Post hoc pairwise comparisons revealed that picrotoxin and muscimol increased incorrect latencies compared to saline ($p < 0.033$) but not differ from each other ($p \geq 0.3$). There was also a main effect of stage ($F_{(2,74)} = 3.234$, $p = 0.045$) and pairwise comparisons revealed this reflected a decrease in latencies from R1 to R3 (R1 versus R2 and R1 versus R3, $p < 0.046$; R2 versus R3, $p \geq 0.3$).

3.2.6 Ventral hippocampal inhibition, but not disinhibition, reduced the use of the previous rule during reversal 2

To identify fine behavioural changes around rule reversal across the three reversal stages, we used a Bayesian strategy analysis (Maggi et al., 2024). First, we examined the probabilities of the go-previous strategy, i.e. of pressing the lever that was correct *before* reversal, to measure the speed of rule abandonment after reversal (Figure 2-5A). The saline group exhibited a high probability of go-previous behaviour prior to rule change, indicative of good reminder trial performance. However, the probability of the use of this strategy remained high after reversal indicating that rats were not switching to the opposite, newly rewarded lever. A three-way repeated measures ANOVA revealed an infusion group X stage X response interaction ($F_{(104,1924)} = 1.513$, $p = < 0.001$). Based on this three-way interaction, we ran separate two-way (infusion group X task stage) ANOVAs at the three reversal stages. At R2, there was a main effect of infusion group ($F_{(2,37)} = 8.237$, $p = 0.001$), which was independent of response (infusion group X response interaction: $F < 1$). Pairwise comparisons revealed there was a decrease in go-previous in the muscimol group compared to the saline and picrotoxin groups ($p = 0.001$), which did not differ from each other ($p \geq 0.3$). There was also a main effect of response ($F_{(2.671,98.831)} = 4.384$, $p = 0.008$). At R1 and R3, there was no main effect of infusion group or response, or an infusion group X response interaction (all $F < 2.1$, all $p > 0.12$).

Figure 2-4: Experiment 1: Ventral hippocampal inhibition and disinhibition impaired performance during reminder trials.



Note. Data are shown as mean (± SEM), between-subjects design. Performance measures during the reminder trials preceding R1–3: Percentage correct (A); Omissions (B); Correct latency (C) and Incorrect latency (D). Abbreviations: MUS: muscimol (250 ng/0.5 µl/side; n=14); PTX: picrotoxin (100 ng/0.5 µl/side; n=13); SAL: saline (0.5 µl/side; n=13); R: reversal. Only effects involving infusion group are indicated. # indicates trend, p = 0.051–0.095; * indicates p < 0.05 (Fisher's LSD test following a significant main effect of infusion with a two-way repeated measures ANOVA).

3.2.7 Ventral hippocampal inhibition disrupted exploitative behaviour around reversal but improved exploratory behaviour post-reversal, whilst disinhibition had no effect

Next, we wanted to examine the implementation of task-appropriate strategies including win-stay and lose-shift. Regarding the former, in the saline group there was high, stable, implementation of the win-stay strategy prior to reversal which was followed by a gradual decline post-reversal (Figure 2-5B). This decline of win-stay after reversal suggests that despite winning trials animals go back to the previously (pre-reversal) rewarded choices (i.e. using a win-shift strategy), indicating perseveration on the previously successful stimulus-action association, a typical behaviour just after reversal. Win-stay behaviour was reduced in the muscimol group

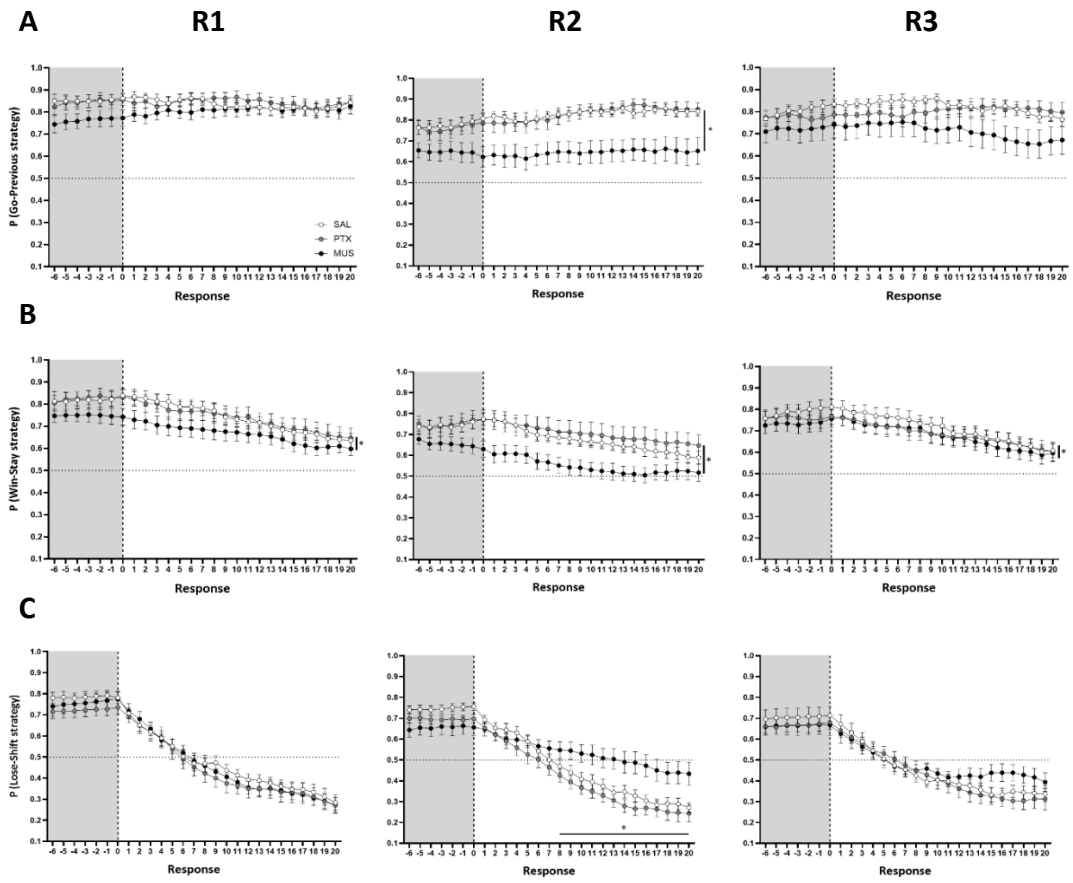
across reversals: there was a main effect of infusion group ($F_{(2,37)} = 3.912$, $p = 0.029$), and post hoc pairwise comparisons revealed a decrease in win-stay behaviour in the muscimol group compared to the saline ($p = 0.022$) and picrotoxin groups ($p = 0.021$), which did not differ from each other ($p \geq 0.3$). There was also a main effect of stage ($F_{(2,74)} = 3.599$, $p = 0.032$) and pairwise comparisons revealed a decrease in win-stay behaviour at R2 compared to R1 ($p = 0.010$) and trend towards a decrease at R2 versus R3 ($p = 0.087$). There was a main effect of response ($F_{(2.485,91.962)} = 91.095$, $p = < 0.001$). There was no infusion group X stage X response ($F < 1$), infusion group X stage ($F < 1$), infusion group X response ($F_{(4.971,91.962)} = 1.004$, $p = 0.420$), or stage X response interactions ($F_{(52,1924)} = 1.233$, $p = 0.125$).

For the lose-shift strategy, in the saline group there was strong lose-shift behaviour prior to reversal, indicating that, where necessary, responses were shifted to search out the rewarding lever. Immediately after the rule reversal, however, there was a steep decline, indicating evidence for the opposing, lose-stay, strategy (Figure 2-5C). This coincides with perseveration which is typical following a sudden change in reward contingency. There was an infusion group X stage X response interaction ($F_{(104,1924)} = 1.767$, $p = < 0.001$). Based on this three-way interaction, we ran separate two-way (infusion group X task stage) ANOVAs at the three reversal stages. At R2, there was an infusion group X response interaction ($F_{(3.574,66.112)} = 5.214$, $p = 0.002$) and simple main effects analysis revealed a significant main effect of drug at trials 8–20 (smallest $F = 3.297$, largest $p = 0.048$), with increased use of lose-shift in the muscimol group for these trials. At R1 and R3, there was no main effect of infusion group or an interaction between infusion group X response ($F < 1.4$, $p > 0.26$), but there was a main effect of response ($F > 67.0$, $p < 0.001$).

3.2.8 Ventral hippocampal inhibition increased the use of cue-based strategy and decreased the use of sticky strategy at reversal 2, whilst disinhibition had no effect

In addition to the task-appropriate strategies, we investigated the effect of ventral hippocampal manipulations on task-inappropriate strategies, including go-cued and sticky/alternate. The rule in our task was always based on the lever (i.e., rats had to

Figure 2-5: Experiment 1: Ventral hippocampal inhibition reduced use of the previous rule and increased exploratory behaviour at reversal 2 but disrupted exploitative behaviour across reversals 1–3, whilst disinhibition had no effect on these strategies.



Note. Data are shown as mean (± SEM), between-subjects design. Bayesian trial-by-trial strategy profiles of task-appropriate strategies around R1–3. Go-previous (A), win-stay (B), and lose-shift (C). Grey-shaded region indicates reminder trial performance. Vertical dashed line indicates rule reversal and horizontal dashed line indicates chance. Abbreviations: MUS: muscimol (250 ng/0.5 µl/side; n=14); PTX: picrotoxin (100 ng/0.5 µl/side; n=13); SAL: saline (0.5 µl/side; n=13); P: probability; R: reversal. Only effects involving infusion group are indicated. * indicates $p < 0.05$ (Simple main effects analysis following a significant interaction or Fisher's LSD test following a significant main effect of infusion with repeated measures ANOVA).

press the left or right lever) and, therefore, following the cue light was a task-inappropriate strategy. During R2, muscimol increased go-cued behaviour, which was supported by an infusion group X stage interaction ($F_{(4,74)} = 3.097$, $p = 0.021$; Figure 2-6A). Simple main effects revealed a difference at R2 ($p = 0.010$), and for the

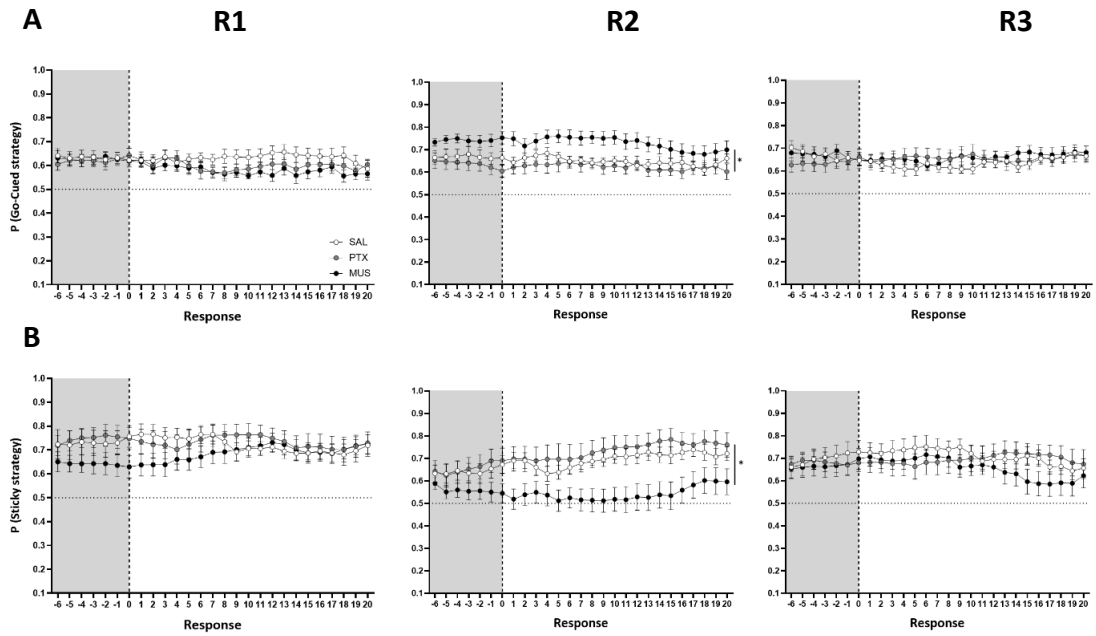
muscimol group ($p < 0.001$). There was also a stage X response interaction ($F_{(52,1924)} = 1.741$, $p = < 0.001$). Simple main effects revealed a difference at R1 and R2 ($p < 0.007$) and this was across nearly all trials. There was also a main effect of stage ($F_{(2,74)} = 7.000$, $p = 0.002$). There was no infusion group X stage X response interaction ($F_{(104,1924)} = 1.113$, $p = 0.211$), no infusion group X response interaction ($F < 1$), no main effect of infusion group ($F_{(2,37)} = 1.346$, $p = 0.273$), and no main effect of response ($F_{(4.342,160.661)} = 1.792$, $p = 0.127$).

There was also a decrease in use of the sticky strategy at R2 in the muscimol group; this is where the same lever is consistently pressed regardless of reward contingency (Figure 2-6B). There was an infusion group X stage X response interaction ($F_{(104,1924)} = 1.323$, $p = 0.018$). Based on this three-way interaction, we ran separate two-way (infusion group X task stage) ANOVAs at the three reversal stages. At R2, there was a main effect of infusion group ($F_{(2,37)} = 5.907$, $p = 0.006$), which was independent of response (infusion group X response interaction: $F_{(4.871,90.113)} = 1.004$, $p = 0.419$). Pairwise comparisons revealed a decrease in sticky with the muscimol group compared to the saline and picrotoxin groups ($p < 0.013$), which did not differ from each other ($p \geq 0.3$). At R1 and R3, there was no main effect of infusion group or response, or an infusion group X response interaction (all $F < 1.4$, all $p > 0.25$).

3.2.9 Ventral hippocampal inhibition and disinhibition had no effect on task-appropriate strategies around learning, whilst inhibition and disinhibition tended to decrease use of the sticky strategy around learning at reversal 2

We also used the Bayesian strategy analysis to identify fine behavioural changes around learning (Maggi et al., 2024). We examined go-new (this is the complementary strategy to the go-previous strategy used for reversal and was the probability of using the correct rule), win-stay, lose-shift, go-cued and sticky/alternate. Ventral hippocampal inhibition and disinhibition had no effect on any of the task-appropriate strategies (go-new, win-stay or lose-shift; Figure 2-7A–C) or on go-cued around learning (data not shown). There were no main effects of infusion group or interactions with infusion group (all $F < 1.9$, all $p > 0.10$).

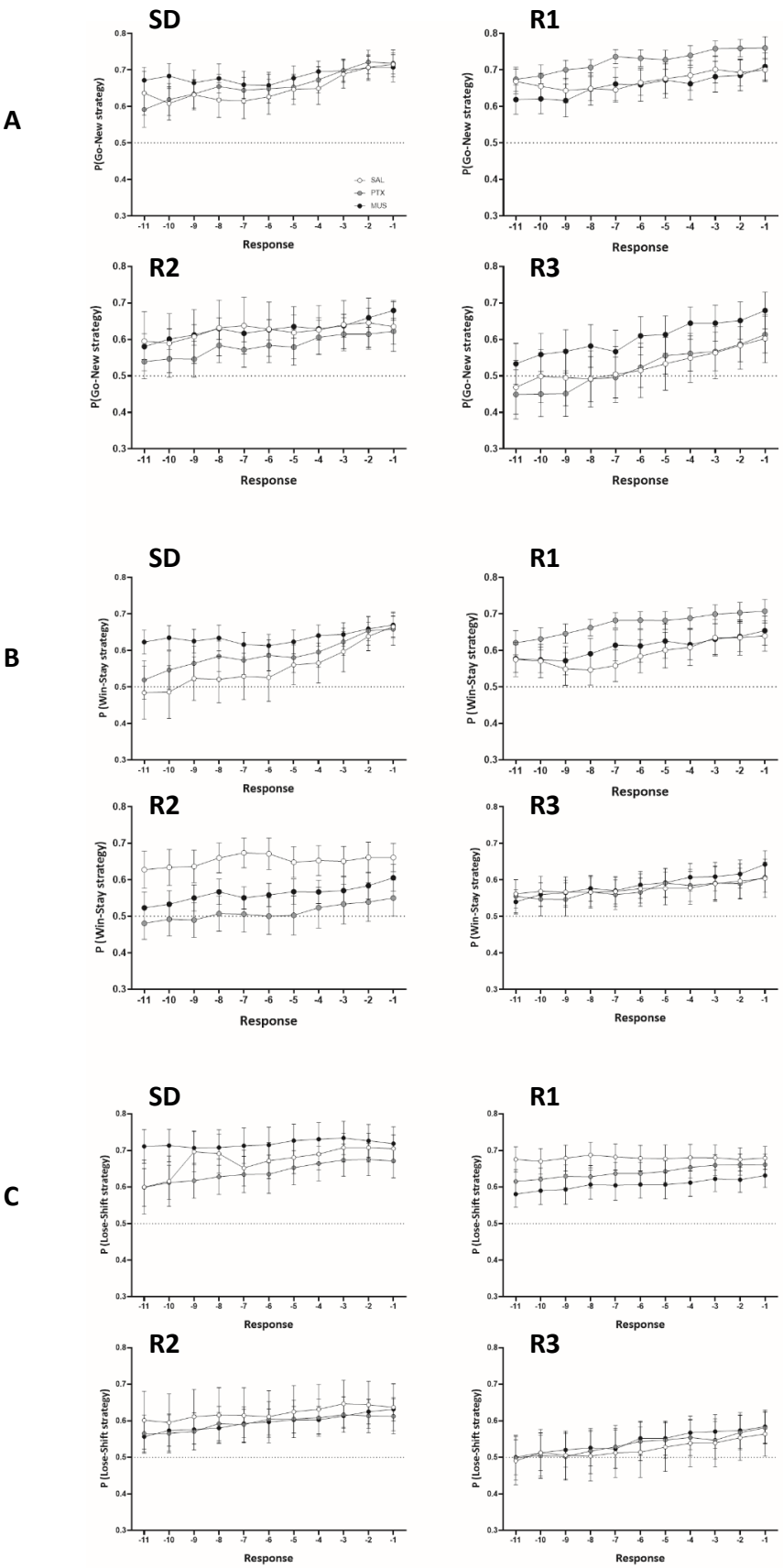
Figure 2-6: Experiment 1: Ventral hippocampal inhibition increased the use of a cue-based strategy and decreased the use of the sticky strategy at reversal 2, whilst disinhibition had no effect.



Note. Data are shown as mean (± SEM), between-subjects design. Bayesian trial-by-trial strategy profiles of task-inappropriate strategies around R1–3. Go-cued (A) and sticky (B). Grey-shaded region indicates reminder trial performance. Vertical dashed line indicates rule reversal and horizontal dashed line indicates chance.Abbreviations: MUS: muscimol (250 ng/0.5 µl/side; n=14); PTX: picrotoxin (100 ng/0.5 µl/side; n=13); SAL: saline (0.5 µl/side; n=13); P: probability; R: reversal. Only effects involving infusion group are indicated. * indicates $p < 0.05$ (Simple main effects analysis following a significant interaction or Fisher's LSD test following a significant main effect of infusion with repeated measures ANOVA).

For the sticky strategy (data not shown), there was a trend towards an infusion group X stage interaction ($F_{(6,111)} = 2.004$, $p = 0.071$), reflecting a difference in infusion groups at R2, with picrotoxin and muscimol groups showing lower sticky probabilities than the saline group. There was also main effect of response ($F_{(2.687,99.416)} = 5.932$, $p = 0.001$). There was no infusion group X stage X response interaction ($F < 1$), no infusion group X response ($F < 1$), no stage X response interaction ($F_{(6.881,254.604)} = 1.568$, $p = 0.147$) and no main effect of infusion group or stage (both $F < 1$).

Figure 2-7: Experiment 1: Ventral hippocampal inhibition and disinhibition had no effect on task-appropriate strategies around learning.



Note. Data are shown as mean (± SEM), between-subjects design. Bayesian trial-by-trial strategy profiles of task-appropriate strategies around learning for SD and R1–3. Go-new (A) win-stay (B) and lose-shift (C). Horizontal dashed line indicates chance. Abbreviations: MUS: muscimol (250 ng/0.5 µl/side; n=14); PTX: picrotoxin (100 ng/0.5 µl/side; n=13); SAL: saline (0.5 µl/side; n=13); P: probability; SD: spatial discrimination; R: reversal.

3.3 Experiment 1: Behavioural effects of ventral hippocampal infusions that were evident based on visual inspection

Some rats displayed gross behavioural effects after ventral hippocampal picrotoxin and muscimol infusions that were evident based on visual inspection. Behavioural effects are reported for all rats that received infusions, regardless of whether they were included in the data analysis (n=46). The full list of behavioural effects can be found in Table 2-1. 'Wet dog shakes' refers to when a rat shook its head and torso, much like when a wet dog shakes. 'Agonistic behaviour' refers to when the rats would face each other standing on their hind legs, showing their teeth and occasionally hissing at one another. 'Oral tendencies' refers to when the rats put their tail in their mouth and/or chewing or licking the testing box (particularly the bars the rat was stood on in the operant box). Wet dog shakes, wild running and jumpiness were typically seen shortly after infusion (5–30 min), oral tendencies and reversing tended to be 10–60 min after infusion, and agonistic and aggressive and fighting behaviour was observed several hours after infusion in the home cages. No adverse effects were observed with saline infusions.

Analyses with a one-tailed Fisher's Exact test revealed there was a significant increase in wet dog shakes with picrotoxin compared to saline ($p < 0.001$). There was also a significant increase in jumpiness ($p = 0.006$), aggression and fighting ($p = 0.013$) and oral tendencies ($p = 0.028$) with muscimol versus saline. There was a trend towards an increase in agonistic behaviour with muscimol compared to saline ($p = 0.058$). For the other behavioural effects, no statistically significant differences were found between picrotoxin/muscimol and saline.

Table 2-1: Gross behavioural effects after ventral hippocampal infusions of picrotoxin and muscimol in Experiment 1.

Observed behaviour	**Number of rats displaying behavioural effect**			
	PTX, Cohort 1	PTX, Cohort 2	MUS, Cohort 1	MUS, Cohort 2
Wet dog shakes	8	7		1
Wild running	3			
Jumpiness		1	2	5
'Agonistic behaviour' – on hindlegs, hissing	1	1	2	2
Oral tendencies			4	1
Aggression and fighting	2		4	2
Reversing (into the corner of the box)			2	1

Note. Rats were given picrotoxin (PTX), muscimol (MUS) and saline for six consecutive days in a between-subjects design (n=46). Definitions of the behaviours can be found in section 3.3. No behavioural effects were seen with saline. Rats often displayed behaviours on multiple days, but each rat is only included once.

3.4 Experiment 2: Ventral hippocampal inhibition, but not disinhibition, tended to impair late serial reversal learning

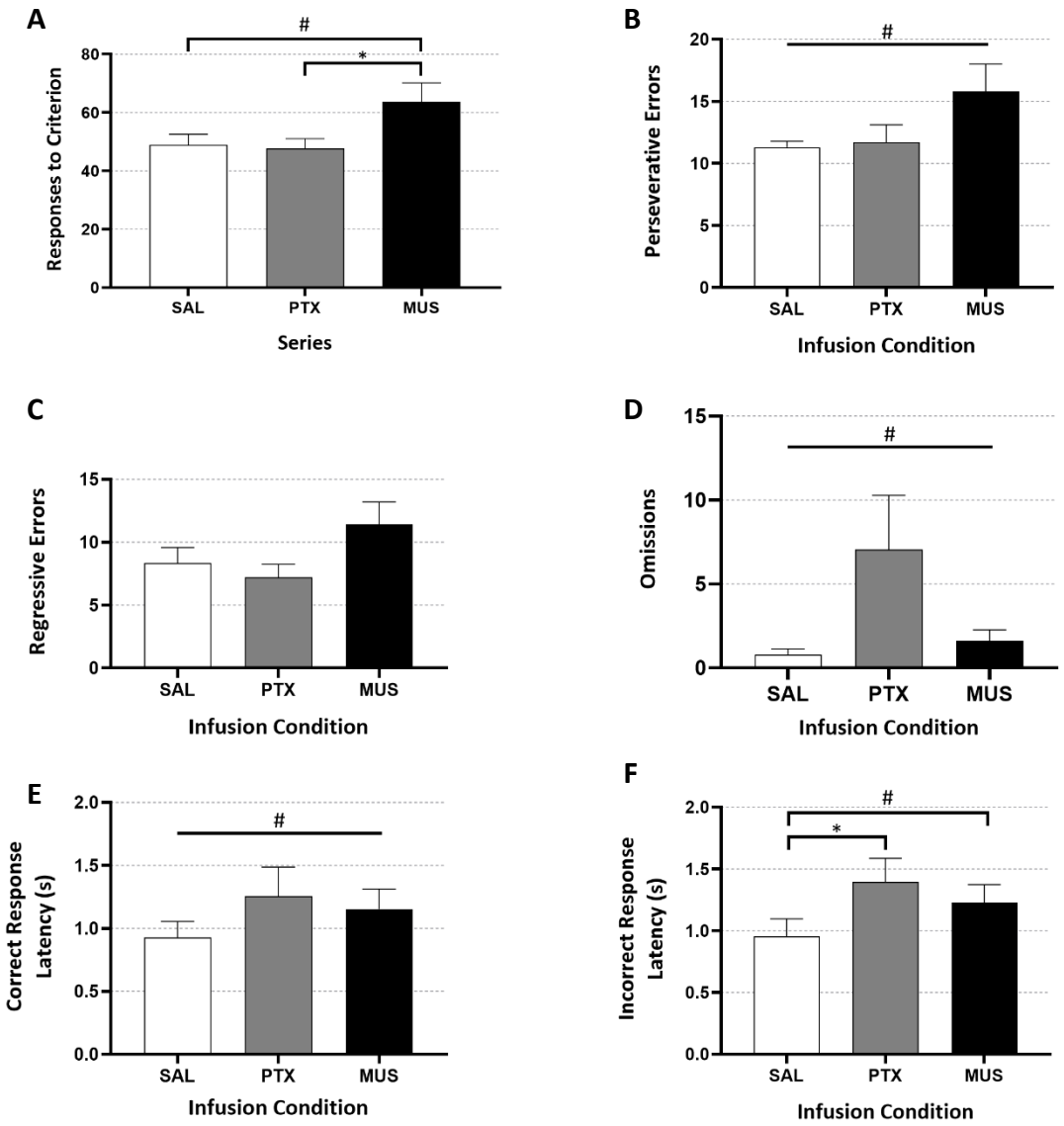
3.4.1 Ventral hippocampal inhibition tended to increase RTC, whereas disinhibition had no effect

Ventral hippocampal inhibition by muscimol impaired late serial reversal learning across R5–10, as reflected by increased RTC, whilst disinhibition by picrotoxin did not affect RTC (Figure 2-8A). A two-way repeated measures ANOVA of RTC revealed a significant main effect of infusion condition ($F_{(2,22)}$ = 3.766, p = 0.039), which was independent of series (infusion condition X series interaction, F < 1), and there was no significant effect of series ($F_{(1,11)}$ = 1.415, p = 0.259). Post hoc pairwise comparisons revealed that ventral hippocampal muscimol increased RTC compared to picrotoxin (p = 0.024) and tended to increase RTC compared saline (p = 0.090), whilst picrotoxin and saline did not differ from each other (p ≥ 0.3).

3.4.2 Ventral hippocampal inhibition tended to increase perseverative but not regressive errors, whereas disinhibition had no effect

Ventral hippocampal inhibition, but not disinhibition, tended to increase perseverative errors (Figure 2-8B). There was a trend towards a main effect of infusion condition ($F_{(2,22)}$ = 2.768, p = 0.085). Pairwise comparisons of this trend revealed an increase in perseverative errors with muscimol compared to saline (p = 0.047) and trend towards an increase compared to picrotoxin (p = 0.066), whilst picrotoxin and saline did not differ from each other (p ≥ 0.3). There was no infusion condition X series interaction (F < 1) and no main effect of series (F < 1). Neither ventral hippocampal inhibition, nor disinhibition, affected regressive errors (Figure 2-8C). There was no main effect of infusion condition ($F_{(1.343,14.777)}$ = 2.570, p = 0.123) and no infusion condition X series interaction (F < 1). There was a trend towards a main effect of series ($F_{(1,11)}$ = 3.545, p = 0.086; data not shown).

Figure 2-8: Experiment 2: Ventral hippocampal inhibition, but not disinhibition, tended to impair late serial reversal learning.



Note. Data are shown as mean (± SEM), within-subjects design (n=12). Performance measures during the reversals: Responses to criterion (A); Perseverative errors (B); Regressive errors (C); Omissions (D); Correct latency (E) and Incorrect latency (F). Abbreviations: MUS: muscimol (250 ng/0.5 µl/side); PTX: picrotoxin (100 ng/0.5 µl/side); SAL: saline (0.5 µl/side). Only effects involving infusion group are indicated. # indicates trend, p = 0.051–0.095; * indicates $p < 0.05$ (Fisher's LSD test following a significant main effect of infusion with a two-way repeated measures ANOVA).

3.4.3 Ventral hippocampal disinhibition, but not inhibition, tended to increase omissions

Ventral hippocampal disinhibition tended to increase the number of omissions (Figure 2-8D). A two-way repeated measures ANOVA revealed a trend towards a main effect of infusion condition ($F_{(1.048,11.525)} = 3.772$, $p = 0.076$). Pairwise comparisons to examine this trend revealed an increase with picrotoxin compared to saline ($p = 0.019$) and muscimol ($p = 0.039$), which did not differ from each other ($p \geq 0.3$). There was no infusion condition X series interaction and no main effect of series (both $F < 1$). The results of the analyses were unchanged when outliers were excluded (using boxplots, n=8).

3.4.4 Ventral hippocampal disinhibition increased incorrect response latencies and tended to increase correct response latencies, whilst inhibition tended to increase correct and incorrect latencies

Both ventral hippocampal inhibition and disinhibition numerically increased latencies of correct and incorrect responses during late serial reversal learning (Figure 2-8E and F). For correct-response latencies, a two-way repeated measures ANOVA revealed a trend towards a main effect of infusion condition ($F_{(1.320,14.517)} = 3.409$, $p = 0.076$). Pairwise comparisons to examine this trend revealed an increase in correct response latencies with picrotoxin versus saline ($p = 0.018$) and a tendency towards an increase with muscimol versus saline ($p = 0.093$), whilst picrotoxin and muscimol did not differ from each other ($p \geq 0.3$). There was no infusion condition X series interaction ($F < 1$) and no effect of series ($F_{(1,11)} = 2.919$, $p = 0.116$).

For incorrect response latencies, a two-way repeated measures ANOVA revealed a main effect of infusion condition ($F_{(2,22)} = 4.998$, $p = 0.016$), and this was independent of series (infusion condition X series interaction, $F_{(2,22)} = 1.368$, $p = 0.275$). Pairwise comparisons revealed that ventral hippocampal picrotoxin increased incorrect response latencies compared to saline ($p = 0.005$), and a tendency towards an increase with muscimol versus saline ($p = 0.064$), whilst picrotoxin and muscimol did

not differ from each other ($p \geq 0.3$). There was no significant effect of series ($F_{(1,11)} = 1.451$, $p = 0.254$).

3.4.5 Ventral hippocampal disinhibition, but not inhibition, impaired performance during reminder trials

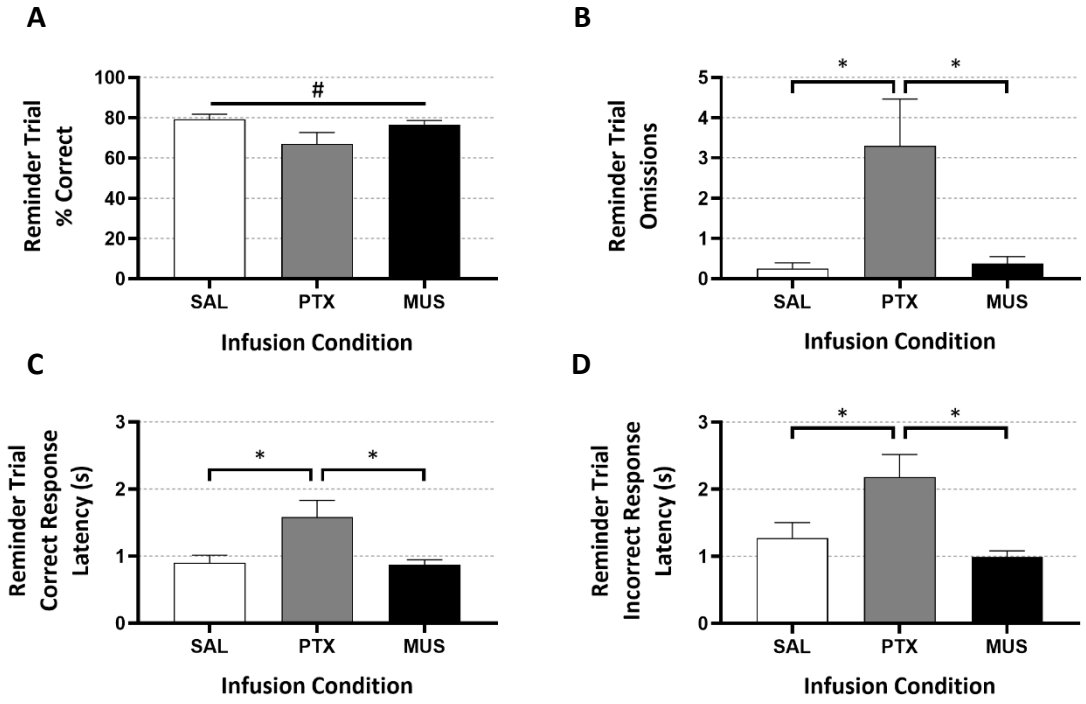
Ventral hippocampal disinhibition tended to decrease percentage correct during reminder trials (Figure 2-9A; n=11). There was a trend towards a main effect of infusion condition ($F_{(2,20)} = 3.041$, $p = 0.070$). Pairwise comparisons to examine this trend revealed an increase with picrotoxin compared to saline ($p = 0.029$), and tendency towards an increase compared to muscimol ($p = 0.082$), whilst muscimol and saline did not differ from each other ($p \geq 0.3$). Percentage correct increased from series 1 to series 2, which was supported by a main effect of series ($F_{(1,10)} = 5.258$, $p = 0.045$; data not shown). There was no infusion condition X series interaction ($F < 1$).

Ventral hippocampal disinhibition increased omissions during reminder trials (Figure 2-9B). There was a main effect of infusion condition ($F_{(1.067,11.741)} = 6.253$, $p = 0.027$) and pairwise comparisons revealed an increase with picrotoxin compared to saline and muscimol (both $p = 0.005$), which did not differ from each other ($p = 0.300$). Omissions tended to decrease from series 1 to series 2 ($F_{(1,11)} = 4.000$, $p = 0.071$; data not shown). There was no infusion condition X series interaction ($F_{(1.037,11.410)} = 2.144$, $p = 0.170$).

Ventral hippocampal disinhibition increased correct response latencies (Figure 2-9C; n=10). There was a main effect of infusion condition ($F_{(1.248,11.236)} = 7.701$, $p = 0.014$) and post hoc pairwise comparisons revealed that picrotoxin increased latencies compared to saline ($p = 0.004$) and muscimol ($p = 0.003$), which did not differ ($p \geq 0.3$). There was also a main effect of series ($F_{(1,9)} = 18.887$, $p = 0.002$) which reflected a decrease in latencies from series 1 to series 2. There was a trend towards an infusion condition X series interaction ($F_{(1.168,10.508)} = 3.703$, $p = 0.078$), which revealed picrotoxin mainly increased latencies in series 1 (data not shown).

Ventral hippocampal disinhibition also increased incorrect response latencies in both series (Figure 2-9D, n=11). There was a main effect of infusion condition ($F_{(1.543,15.434)}$ = 7.473, p = 0.008) and pairwise comparisons revealed that picrotoxin increased latencies compared to saline (p = 0.011) and muscimol (p = 0.001), which did not differ from each other (p ≥ 0.3). There was no infusion condition X series interaction (F < 1) and no main effect of series ($F_{(1,10)}$ = 3.036, p = 0.112).

Figure 2-9: Experiment 2: Ventral hippocampal disinhibition, but not inhibition, impaired performance during reminder trials.



Note. Data are shown as mean (± SEM), within-subjects design. Performance measures during the reminder trials preceding reversals: Percentage correct (A, n=11); Omissions (B, n=12); Correct latency (C, n=10) and Incorrect latency (D, n=11). Abbreviations: MUS: muscimol (250 ng/0.5 µl/side); PTX: picrotoxin (100 ng/0.5 µl/side); SAL: saline (0.5 µl/side). Only effects involving infusion group are indicated; # indicates trend, p = 0.051–0.095; * indicates p < 0.05 (Fisher's LSD test following a significant main effect of infusion with a two-way repeated measures ANOVA).

3.4.6 Ventral hippocampal inhibition tended to increase use of the previous rule, whereas disinhibition tended to decrease use of previous rule around reversal

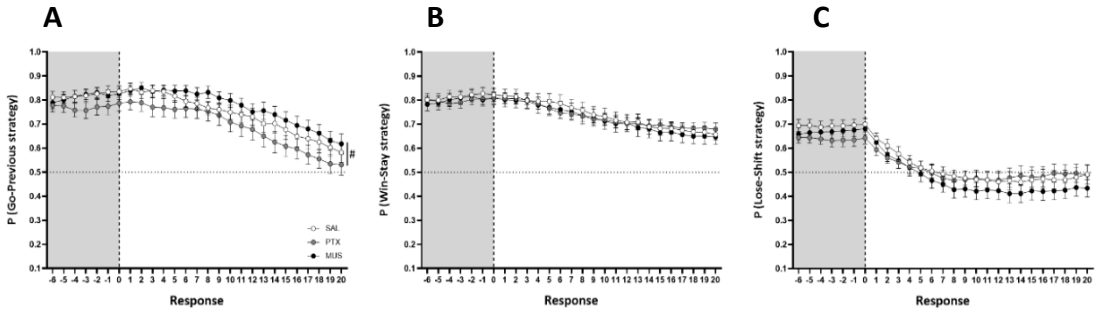
Again, Bayesian strategy analysis was employed to investigate the strategies used by the rats during the reversal sessions on a trial-by-trial basis (Maggi et al., 2024). Due to high omissions during the reminder trials, the analyses were limited to six responses before the rule reversal and twenty responses after the reversal (n=10 rats).

Ventral hippocampal inhibition and disinhibition tended to affect the use of the go-previous strategy (Figure 2-10A). There was a trend towards a main effect of infusion condition ($F_{(2,18)} = 3.216$, $p = 0.064$) and this was independent of series and response (infusion condition X series, infusion condition X response and infusion condition X series X response interactions all $F < 1$). This reflected that ventral hippocampal picrotoxin tended to decrease, and muscimol tended to increase, go-previous probabilities compared to saline. Pairwise comparisons revealed picrotoxin increased go-previous probabilities compared to muscimol ($p = 0.024$), whilst neither picrotoxin nor muscimol differed from saline ($p > 0.100$). There was a series X response interaction ($F_{(26,234)} = 3.284$, $p = < 0.001$; data not shown) and simple main effects analyses revealed an increase in use of the go-previous strategy from series 1 to series 2 for trials -6–3 (highest $p = 0.049$). There was also a main effect of trial ($F_{(26,234)} = 58.340$, $p = < 0.001$) but no main effect of series ($F < 1$).

3.4.7 Neither ventral hippocampal inhibition nor disinhibition affected use of task-appropriate or task-inappropriate strategies around reversal

Ventral hippocampal inhibition and disinhibition had no effect on the task-appropriate (win-stay or lose-shift; Figure 2-10B and C) or task-inappropriate strategies (go-cued and sticky/alternate; data not shown) around reversal. No main effects of infusion condition or interactions with infusion condition were observed (all $F < 2.0$, $p > 0.17$).

Figure 2-10: Experiment 2: Ventral hippocampal inhibition tended to increase use of the previous rule, whereas disinhibition tended to decrease use of previous rule around reversal, while neither affected exploitation nor exploration.



Note. Data are shown as mean (± SEM), within-subjects design (n=10). Bayesian trial-by-trial strategy profiles of task-appropriate strategies around reversal across series. Go-previous (A), win-stay (B), and lose-shift (C). Grey-shaded region indicates reminder trial performance. Vertical dashed line indicates rule reversal and horizontal dashed line indicates chance. Abbreviations: MUS: muscimol (250 ng/0.5 µl/side); PTX: picrotoxin (100 ng/0.5 µl/side); SAL: saline (0.5 µl/side); P: probability. Only effects involving infusion group are indicated. # indicates trend, p = 0.051–0.095 (Fisher's LSD test following a significant main effect of infusion with a three-way repeated measures ANOVA).

3.4.8 Ventral hippocampal inhibition and disinhibition had no effect on the use of the correct rule around learning

We also used the Bayesian strategy analysis to identify fine behavioural changes around learning. We used the same strategies: go-new (the complementary strategy to go-previous, the probability of using the correct rule), win-stay, lose-shift, go-cued and sticky/alternate. Analyses included all rats included in the main analyses (n=12).

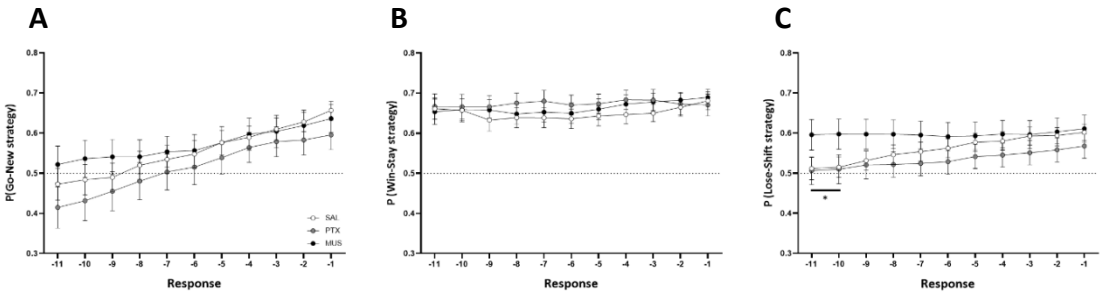
Ventral hippocampal inhibition and disinhibition did not affect go-new around learning (Figure 2-11A). A three-way repeated measures ANOVA revealed no main effect of infusion condition and no interactions with infusion condition (all $F < 1.3$, all $p > 0.20$). There was a main effect of response ($F_{(2.132,23.451)} = 48.119$, $p < 0.001$).

3.4.9 Ventral hippocampal inhibition increased exploration around learning, but did not affect exploitation, whilst disinhibition had no effect on either strategy

Ventral hippocampal inhibition and disinhibition did not affect exploitative (win-stay) behaviour (Figure 2-11B). A three-way repeated measures ANOVA revealed no main effect of infusion condition and no interactions with infusion condition (all $F < 1$, all $p > 0.45$). There was a series X response interaction ($F_{(2.543,27.968)} = 3.249$, $p = 0.043$; data not shown) reflecting increased win-stay from series 1 to series 2.

Ventral hippocampal inhibition increased exploration (lose-shift) around learning (Figure 2-11C). A three-way repeated measures ANOVA revealed an infusion condition X response interaction ($F_{(20,220)} = 5.360$, $p < 0.001$). Simple main effects revealed a main effect of infusion condition at trials -11 and -10 ($F > 3.5$, $p < 0.05$), with increased use of lose-shift in the muscimol condition for these trials. There was no infusion condition X series X response or infusion condition X series interaction or a main effect of infusion condition (all $F < 1.3$, all $p > 0.29$).

Figure 2-11: Experiment 2: Ventral hippocampal inhibition increased exploration around initial learning trials, but not exploitation or go-new, whilst disinhibition had no effect.



Note. Data are shown as mean (± SEM), within-subjects design (n=12). Bayesian trial-by-trial strategy profiles of task-appropriate strategies around learning across series. Go-new (A), win-stay (B), and lose-shift (C). Dashed line indicates chance. Abbreviations: MUS: muscimol (250 ng/0.5 µl/side); PTX: picrotoxin (100 ng/0.5 µl/side); SAL: saline (0.5 µl/side); P: probability. Only effects involving infusion group are indicated, * indicates $p < 0.05$ (Simple main effects analysis following a significant interaction with a three-way repeated measures ANOVA).

3.4.10 Ventral hippocampal inhibition and disinhibition had no effect on the go-cued or sticky strategies around learning

In addition to the task-appropriate strategies, we investigated the effect of ventral hippocampal manipulations on task-inappropriate strategies, including go-cued and sticky/alternate (data not shown). Neither ventral hippocampal inhibition nor disinhibition affected go-cued or sticky behaviour (all main effects or interactions involving infusion condition: $F < 2.2$, $p > 0.14$).

3.5 Experiment 2: Behavioural effects of ventral hippocampal infusions that were evident based on visual inspection

As in Experiment 1, some rats displayed gross behavioural effects after ventral hippocampal picrotoxin or muscimol infusion that were evident based on visual inspections. Please refer to section 3.3 for full descriptions of the behaviours and when these typically occurred (hyperactivity was typically 5–30 min after infusion and lethargic and withdrawn behaviour were after testing in the home cage, 60+ min after infusion). Behavioural effects are reported for all rats that received infusions, regardless of whether they were included in the data analysis (n=16). The full list of behavioural effects and the number of rats that displayed these behaviours in Experiment 2 can be found in Table 2-2.

We compared the number of rats showing these behavioural effects between infusion conditions using a one-tailed Fisher's Exact Test. If a rat had an effect in both series, it was only included in the analyses once. There was a significant increase in rats showing wet dog shakes with picrotoxin compared to saline ($p < 0.001$). There was also a trend towards an increase in agonistic behaviour with muscimol compared to saline ($p = 0.051$). For the other behavioural effects, no statistically significant differences were found between picrotoxin/muscimol and saline.

Table 2-2: Gross behavioural effects after ventral hippocampal infusions of picrotoxin and muscimol in Experiment 2.

Observed behaviour	Number of rats displaying behavioural effect			
	PTX, Infusion Series 1	**PTX, Infusion Series 2**	**MUS, Infusion Series 1**	**MUS, Infusion Series 2**
Wet dog shakes	5	7***	1	
'Agonistic behaviour' – on hindlegs, hissing			4	3***
Oral tendencies			3	1*
Aggression and fighting			3	2*
Lethargic	1			1
Withdrawn	3			
Hyperactivity	3			

Note. Rats were given picrotoxin (PTX), muscimol (MUS) and saline in two series in a within-subjects design (n=16). Each rat received two infusions of each drug, one in each series. Behavioural definitions can be found in section 3.3. No behavioural effects were observed with saline. * Indicates the same rat having this effect in both series (multiple * indicates multiple rats).

3.6 Experiment 2: The effect of ventral hippocampal disinhibition on reminder trial performance, but not on reversals, was not due to the effect of picrotoxin wearing off

3.6.1 No evidence for the effect of picrotoxin diminishing across reversal trial blocks

In Experiment 2 (which we ran before Experiment 1), ventral hippocampal disinhibition affected responses during reminder, but not reversal, trials. We reasoned this may have been due to the effect of picrotoxin wearing off. Previous research has found the largest effect of picrotoxin to be between 10–35 min post-infusion, with the peak of the effect being between 20–25 min (McGarrity et al., 2017). For our experimental design, the experiment began 10 min post infusion, the reminder trials lasted on average for 7 min and the reversals took on average 25–30 min. We therefore investigated the effects of the infusions on the performance measures in blocks of 10 trials so see if there was an effect of picrotoxin infusions early in the reversal session that diminished with time.

For the first 80 trials, after which most rats had completed the task, the performance measures did not differ much across trial blocks (data not shown). We analysed within the first four trials blocks (four blocks of ten trials) whether there was a difference in infusion conditions across trial blocks using a three-way repeated measures ANOVA, with infusion condition, series and trial block as within-subjects factors. We only analysed the first four blocks as the mean RTC was 44–67 across conditions, so there were very few rats to include in the analysis for block five onwards. The n number for these exploratory analyses was considerably reduced by "missing data", such as a rat having no correct or incorrect lever presses within that block (n=6–8). There were no main effects or interactions involving infusion condition (all $F < 1.7$, all $p > 0.19$). These data indicate that the effect of picrotoxin was not diminishing across trials.

3.6.2 No evidence for the effect of picrotoxin diminishing across reminder trial blocks

We also investigated the reminder trial performance measures in trial blocks (four blocks of five trials) using a three-way repeated measures ANOVA, with infusion condition, series and trial block as within-subjects factors (data not shown). Again, the n number was considerably reduced by "missing data," such as a rat having too many omissions or only incorrect lever presses (n=6–12). There was a main effect of infusion condition for omissions ($F_{(1.067,11.741)} = 6.253$, $p = 0.027$), which reflected an increase in omissions with picrotoxin. There was also a main effect of infusion condition for correct response latencies ($F_{(2,10)} = 4.960$, $p = 0.032$), again reflecting an increase with picrotoxin. These findings are line with the reminder trial analyses. There were no interactions involving infusion condition (all $F < 2.2$, all $p > 0.11$). These data indicate that the effect of picrotoxin was not diminishing across trials.

3.7 Experiment 2: Neither ventral hippocampal inhibition nor disinhibition affected performance on retention days

Neither ventral hippocampal inhibition nor disinhibition affected any of the performance measures on retention days, i.e. the day after the infusion (data not shown, n=11). There was no significant main effect of infusion condition, or interactions with infusion condition, for RTC, omissions, correct latency or incorrect latency (all $F < 3.0$, all $p > 0.095$). These findings indicate that, after the success criterion is reached on the reversal day, the rule remains salient the following day. Additionally, these findings confirm that rats started each infusion day from a similar baseline and that there were not any long-lasting effects of the infusions that would interfere with our within-subjects design.

4 Discussion

In order to study the role of the ventral hippocampus in distinct stages of reversal learning, we investigated bidirectional ventral hippocampal activity manipulations for early serial and late serial reversal learning. We found that ventral hippocampal disinhibition tended to improve early serial reversal learning, decreasing RTC, but did not affect behavioural strategies during responses around reversal or preceding learning of the new rule. Ventral hippocampal inhibition did not affect whole-session classical performance measures during the early serial reversal but changed rats' behavioural strategies during responses around reversal, particularly at R2. Inhibition decreased go-previous and increased lose-shift around R2, and decreased win-stay across reversals. Neither disinhibition nor inhibition affected behavioural strategies around learning. In contrast, ventral hippocampal inhibition tended to disrupt late serial reversal learning, increasing RTC, but had minimal effect on behavioural strategies during responses around reversal and learning. Inhibition tended to increase go-previous around reversal and increased lose-shift around learning. Ventral hippocampal disinhibition did not affect late serial reversal learning (classical measures) and had minimal effect on behavioural strategies around reversal or learning, tending to decrease go-previous around reversal. Both ventral hippocampal inhibition and disinhibition affected expression of the previous rule on reminder trials, with the effect of inhibition being limited to the early reversal stages examined in Experiment 1. This reduced expression of the previous rule during reminder trials may contribute to the tendency towards an improvement during early serial reversal learning with disinhibition.

4.1 Ventral hippocampal inhibition tended to disrupt late serial but not early serial reversal learning

Ventral hippocampal inhibition tended to impair late serial reversal learning. This suggests that the post-acquisition manipulation of late serial reversal learning tends to require ventral hippocampal activity. Although this effect did not reach statistical

significance, this impairment in late serial reversal learning with ventral hippocampal inhibition is consistent with previous findings. Bilateral hippocampal lesions were reported to impair serial reversal learning performance on a three-lever reversal task in rats (Kosaki & Watanabe, 2012), and bilateral CA1 lesions were found to impair performance on a two-lever probabilistic serial reversal task in mice (Mishchanchuk et al., 2024). As has been suggested, it may be that the ventral hippocampus provides a representation of the task (Mishchanchuk et al., 2024; Vilà-Balló et al., 2017) and the relative value of cues (Knudsen & Wallis, 2021), that is particularly important for late serial reversal and inhibition disrupts this representation, impairing performance. Ventral hippocampal inhibition tended to increase use of the previous rule around reversal, suggesting the rats in this condition tend to stick with the old rule for longer, supported by a tendency towards an in perseverative errors. Interestingly, exploration (lose-shift) preceding learning was somewhat improved with inhibition, though this may reflect that the learning point in this condition is later than in the other two conditions i.e. rats in the muscimol condition had been doing the task for longer (mean RTC: 65) when they reached the trials around learning than rats in the saline (mean RTC: 49) and picrotoxin (mean RTC: 48) conditions.

In contrast to late serial reversal learning, ventral hippocampal inhibition had no effect on the classical measures of early serial reversal learning, suggesting the ventral hippocampus is not required for SD and early reversals. This is in-line with previous findings in the NVHL model, in which no impairments in discrimination learning or reversals were seen on a two-lever task (Placek et al., 2013). However, these findings contrast with other previous research which found hippocampal lesions impaired early reversal learning in animal models (Berger & Orr, 1983; Jang et al., 2015; Kimble & Kimble, 1965; Mahut, 1971; E. A. Murray et al., 1998; Silveira & Kimble, 1968; Whishaw & Tomie, 1997). Although, these tasks used hippocampal lesions that were not specific to the ventral portion of the hippocampus, and used different tasks, including mazes which have a hippocampal spatial component (discussed in Chapter 1).

Whilst ventral hippocampal inhibition did not affect classical performance measures during early reversals, inhibition did affect behavioural strategies around reversal. Inhibition with muscimol reduced the use of the previous rule (go-previous) which was in line with the impaired reminder trial performance (decreased percentage correct and increased response latencies) also observed with muscimol. However, reduced go-previous post-reversal is advantageous, and additionally, muscimol increased exploration (lose-shift) around R2. Although these suggest there should be improved performance, inhibition also increased use of the cue-based strategy at R2, which is an inappropriate strategy, and inhibition decreased exploitation (win-stay) across reversals, which may mitigate any benefit from the decreased go-previous (post-reversal) and increased lose-shift. Exploitative behaviour has been suggested to be more important for maintaining a learnt behaviour (Maggi et al., 2024), so perhaps this reduction in win-stay across reversals explains why no improvement in classical measures was seen (such as RTC), despite the improvement in other task-appropriate strategies.

We hypothesised that the hippocampus may be required for early (Jang et al., 2015; Kimble & Kimble, 1965; E. A. Murray et al., 1998) and late serial reversal learning (Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024) and that it could create a representation of the task (Mishchanchuk et al., 2024; Vilà-Balló et al., 2017) that encoded the relative value of the cues (Knudsen & Wallis, 2021). Ventral hippocampal inhibition did not disrupt early serial reversal learning, suggesting that the ventral hippocampus is not required here, but did disrupt late serial reversal learning. This may be because during early reversals, the hippocampus was disrupted during the acquisition of the reversal, and so rats learnt the task without intact hippocampal function. As such, other brain regions, such as the OFC and mPFC, could have compensated for the absence of intact hippocampal function. During late reversals, rats learnt to reverse with an intact hippocampus and, therefore, subsequent performance was dependent on hippocampal function. This is in line with studies of spatial memory performance in the Morris watermaze (de Hoz & Martin, 2014; Moser & Moser, 1998b) and studies of contextual fear conditioning (J. Q. Lee et al.,

2017) where hippocampal inhibition or lesions before learning did not affect subsequent performance, but manipulations after learning disrupted performance. Regarding the role of the ventral hippocampus creating a representation of the task, it may create such a representation but if so, this is particularly required for efficient performance during late serial reversals and is not required for early serial reversal learning, and does not require balanced ventral hippocampal activity (which would be disrupted by ventral hippocampal disinhibition; discussed in section 4.2 below).

4.2 Ventral hippocampal disinhibition tended to improve SD and early reversals but not late serial reversal learning

Ventral hippocampal disinhibition tended to improve early serial reversal learning across SD and R1–3, with a trend towards a decrease in RTC. Although this effect did not reach statistical significance, it is consistent with the finding that increased activation of the medial septum-ventral subiculum pathway improved performance on a T-maze reversal task (Bortz et al., 2019). In our experiments, it is likely that the spread of the infusion volume would have covered all subregions in the ventral hippocampus, including the subiculum (Williams et al., 2022).

Ventral hippocampal disinhibition has been suggested to disrupt mPFC-dependent functions, such as sustained attention (McGarrity et al., 2017). However, ventral hippocampal disinhibition did not impair early serial reversal learning, which we have recently found to require the mPFC (Renström et al., 2025). This may reflect that sustained attention requires balanced levels of mPFC activity, being disrupted by both mPFC inhibition and disinhibition, whereas early reversals are only disrupted by mPFC inhibition, but not disinhibition. Indeed, the improvement we observed in the present study with ventral hippocampal disinhibition was similar to the improvement seen with mPFC disinhibition for early reversals (Renström et al., 2025). It may be that the ventral hippocampal disinhibition in our experiment resulted in changes in mPFC activation akin to disinhibition. The improvement with mPFC disinhibition was specific to R2 and likely due to an impairment in the expression of the previous

response (Renström et al., 2025). On the one hand, an impairment in the expression of the previous rule may also contribute to the tendency for ventral hippocampal disinhibition to improve early serial reversal learning in the present study. Ventral hippocampal disinhibition increased correct and incorrect response latencies during reminder trials and tended to increase omissions at early reversal stages. However, unlike for mPFC disinhibition, an improvement was also seen with ventral hippocampal disinhibition for SD, which has no reminder trials. Therefore, it is likely that other mechanisms contribute to this improvement in reversal performance. Ventral hippocampal disinhibition had no effect on behavioural strategies around reversal or around learning, suggesting disinhibition did not improve learning through increased use of task-appropriate behavioural strategies. Ventral hippocampal disinhibition also had no effect on perseverative or regressive errors, suggesting the improvement was not through reduced perseveration on the old rule, nor through increased engagement in behaviour facilitating learning of the new rule.

Ventral hippocampal disinhibition did not affect performance during late serial reversals. We expected that disinhibition of the ventral hippocampus would have a similar effect to mPFC disinhibition, which disrupted late serial reversal learning (Renström et al., 2025), due to the connectivity between these regions (Bast, 2011; Ishikawa & Nakamura, 2006). However, reversal learning is not typically a mPFC dependent process (Brigman & Rothblat, 2008; Floresco et al., 2008; McDonald et al., 2007; Ragozzino et al., 1999), and in our recent work we found that the mPFC was not required for late serial reversal learning (Renström et al., 2025). Additionally, we have proposed that the ventral hippocampus may provide a representation of the task, particularly for late reversals, as has previously been suggested (Knudsen & Wallis, 2021; Mishchanchuk et al., 2024; Vilà-Balló et al., 2017). However, as disinhibition did not affect task performance during late reversals, it seems such a representation may require the ventral hippocampus but does not require balanced ventral hippocampal activity.

4.3 Ventral hippocampal inhibition and disinhibition disrupted performance on reminder trials

Ventral hippocampal inhibition and disinhibition affected reminder trial performance differently across the two reversal stages. During early serial reversals, both inhibition and disinhibition impaired reminder trial performance, whilst for late serial reversal learning, only disinhibition impaired performance on reminder trials.

We have suggested that this disruption to reminder trial performance may explain the lack of impairment on early reversals with inhibition, and the tendency towards an improvement with disinhibition: impaired expression of the previous response makes it easier for rats to reverse to the new rule. Equally, it may be that these two processes (reversals and reminder trials) rely on different learning processes. During the reminder trials, the rats are required to remember yesterday's rule, whereas for the reversals they must realise that the rule has been switched and change their response accordingly, though in the late serial reversal learning experiment the rats did have previous experience of reversals prior to the ventral hippocampal manipulations.

4.4 Clinical implications

Schizophrenia is characterised by deficits in reversal learning (Leeson et al., 2009; G. K. Murray et al., 2008; Reddy et al., 2016; Waltz & Gold, 2007), as well as hippocampal hyperactivity (Heckers & Konradi, 2010, 2015; McHugo et al., 2024; Talati et al., 2014; Tregellas et al., 2014). We proposed that there may be a link between this hippocampal hyperactivity and deficits in reversal learning. However, we did not find that ventral hippocampal disinhibition impaired performance on early or late serial reversals and instead found a tendency towards an improvement with disinhibition. Therefore, our findings do not support that hippocampal hyperactivity leads to the reversal learning deficits seen in schizophrenia. However, at later stages of schizophrenia, hippocampal hypofunction due to hippocampal atrophy may

dominate (Achim & Lepage, 2005). Leeson and colleagues investigated reversal learning in schizophrenia across six years and reported deficits in reversal learning that persisted over three years (Leeson et al., 2009), while Reddy and colleagues reported reduced performance in patients with schizophrenia in a probabilistic reversal learning task at baseline and after four weeks (Reddy et al., 2016). We found that ventral hippocampal inhibition impaired late, but not early, serial reversal learning. Our findings may support that hippocampal hypofunction later in schizophrenia could contribute to this serial reversal impairment.

4.5 Conclusions

Ventral hippocampal inhibition tended to disrupt late serial but not early serial reversal learning. We propose that this may be because during early reversals, the hippocampus was disrupted during initial learning of the reversal, and therefore rats learnt the task without intact hippocampal function, whereas during late reversals, rats learnt to reverse with an intact hippocampus and, therefore, subsequent late reversal performance was dependent on hippocampal function. This is in line with studies of spatial memory performance and contextual fear conditioning (de Hoz & Martin, 2014; J. Q. Lee et al., 2017; Moser & Moser, 1998b). We also suggest that the ventral hippocampus may provide a representation of the task, in line with previous work (Knudsen & Wallis, 2021; Mishchanchuk et al., 2024; Vilà-Balló et al., 2017), that is more important for late than early serial reversals, but that does not depend on balanced ventral hippocampal activity.

Ventral hippocampal disinhibition tended to improve performance across SD and early reversals, in line with previous work (Bortz et al., 2019), but did not affect late serial reversal learning. On the one hand, this may be due to impaired expression of the previous response, but on the other hand, disinhibition improved performance during SD, which did not have reminder trials. The improvement we observed with ventral hippocampal disinhibition was similar to the improvement seen with mPFC disinhibition for early reversals (Renström et al., 2025), though here no improvement

was observed for SD. Interestingly, for reversal learning, ventral hippocampal inhibition is more disruptive than ventral hippocampal disinhibition, which contrasts with the impact of ventral hippocampal disinhibition on other cognitive functions, including rapid place learning and attention (McGarrity et al., 2017), and novel object recognition (Taylor et al., 2025). It should be noted that our findings did not reach conventional levels of statistical significance. As such, they should be interpreted with caution, and they require replication in future work.

Chapter 3: Ventral hippocampal infusion of the α5-GABAAR negative allosteric modulator L-655,708 does not affect baseline locomotor activity, prepulse inhibition or startle, but tends to enhance amphetamine-induced hyperlocomotion in rats

Declaration: Jacob Juty, Charlotte Taylor, Patricia Radu, Jacco Renström, Joanna Loayza and Ayesha Mohamed Sherief contributed to surgeries and/or data collection.

1 Introduction

Previous research in rats has indicated that hippocampal GABA-A receptor mediated inhibition is required to sustain cognitive and behavioural functions of the hippocampus and its projection sites (Bast et al., 2017; McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). GABA-A receptors are pentameric receptors that are most commonly found in a 2:2:1 stoichiometry made up of α, β and γ subunits (Maramai et al., 2020). GABA-A receptor subtypes are differentially expressed across different brain regions, and some subtypes are preferentially expressed in specific brain regions. GABA-A receptors that contain the α5 subunit (α5-GABAARs) are preferentially expressed in the hippocampus, accounting for around 20–25% of hippocampal GABA-A receptors but less than 5% of the GABA-A receptors in the brain (McKernan & Whiting, 1996; Sur et al., 1999). In vitro studies have implicated α5-GABAARs in controlling hippocampal activity, specifically controlling network excitability through control of oscillatory activity and establishing a high threshold for induction of long-term potentiation (LTP; Engin et al., 2018; Papatheodoropoulos & Koniaris, 2011; Schulz et al., 2018). α5-GABAARs are predominantly found in the CA1–3 subfields and in the dentate gyrus (Serwanski et al., 2006; Sur et al., 1999), with limited expression in the subiculum (Curia et al., 2009; Prenosil et al., 2006). They are expressed more highly in the ventral hippocampus than the dorsal hippocampus, particularly in CA1 (Sarantis et al., 2008; Sotiriou et al., 2005). Typically, α5-GABAA5 are located at extrasynaptic sites at the base of pyramidal cell dendritic spines (Brünig et al., 2002; Serwanski et al., 2006) where they are anchored by the protein radixin

(Hausrat et al., 2015; Loebrich et al., 2006). Here, they mediate tonic inhibition (Caraiscos et al., 2004). However, α5-GABAARs are also found at synaptic sites, where they contribute to phasic inhibition (Schulz et al., 2018). α5-GABAARs have been found to redistribute to synapses in an activity-dependent manner (Davenport et al., 2021). Within CA1, α5-GABAARs are localised to the distal apical dendrites of pyramidal neurons in the SLM. Here, they are preferentially targeted by SST+ interneurons and mediate slow phasic inhibition (Schulz et al., 2018). SST+ interneurons were reported to preferentially target pyramidal neurons which project to the NAc (Lodge et al., 2023). Due to their specific localisation in the hippocampus, and their implication in both phasic and tonic transmission, as they are found at synaptic and extrasynaptic sites, α5-GABAARs have received substantial interest for their role in hippocampus-dependent learning and memory (Atack et al., 2006; Collinson et al., 2002; Davenport et al., 2021; Dawson et al., 2006; Donegan et al., 2019; Engin et al., 2015; Hausrat et al., 2015; L. J. Martin, Oh, et al., 2009). Modulators of α5-GABAARs have also received interest as candidate drugs to treat neuropsychiatric disorders, including schizophrenia and depression (Bugay et al., 2020; Carreno et al., 2017; Fischell et al., 2015; Gill et al., 2011; Gill & Grace, 2014; Rudolph & Möhler, 2014). It has been suggested that hippocampal hyperactivity may drive the dopamine system dysfunction in schizophrenia and that positive modulation of α5-GABAARs may reduce this hippocampal hyperactivity (Gill et al., 2011; Lodge & Grace, 2007, 2011). Additionally, the hippocampus has been implicated in depression (MacMaster & Kusumakar, 2004; Stockmeier et al., 2004; Videbech & Ravnkilde, 2015), and negative modulators of α5-GABAARs have been shown to have antidepressant effects (Bugay et al., 2020; Carreno et al., 2017; Fischell et al., 2015).

1.1 Hippocampal GABA-A receptors and sensorimotor processes

Previous studies have shown that the behavioural effects of ventral hippocampal disinhibition by the non-specific GABA-A receptor antagonist, picrotoxin, include a

range of sensorimotor effects: enhanced locomotor activity, decreased startle reactivity and disruption of PPI, though disruption of PPI is strain dependent (Bast, Zhang, & Feldon, 2001b; Bast & Feldon, 2003; McGarrity et al., 2017). Locomotor activity is a behavioural measure of movement. Locomotor hyperactivity can indicate an increase in dopamine activity: increased dopamine transmission in the VS, which receives dopaminergic input from the VTA, has been found to increase locomotor activity (Beninger, 1983; Ikemoto & Panksepp, 1999). As such, locomotor hyperactivity has been used as a psychosis-related behavioural index in rodents (Buuse et al., 2005). PPI is widely used as a measure of sensorimotor gating, which has been suggested to be a mechanism to protect information processing by gating out interfering information (N. R. Swerdlow et al., 2001, 2008) and is disrupted in schizophrenia (Braff et al., 2001; Geyer et al., 2001; N. R. Swerdlow et al., 2006). PPI occurs when a weak stimulus (the prepulse) precedes a strong startling stimulus and reduces the startle response. The hippocampus has been implicated in the modulation of PPI (Bast & Feldon, 2003; Caine et al., 1992). However, the hippocampal modulation of PPI in rats may be strain dependent. Ventral hippocampal disinhibition by the non-specific GABA-A receptor antagonist, picrotoxin, inhibition by the non-specific GABA-A receptor agonist, muscimol, and inactivation with the sodium channel blocker, tetrodotoxin, disrupted PPI in Wistar rats (Bast, Zhang, & Feldon, 2001b; Zhang et al., 2002b). However, ventral hippocampal disinhibition with picrotoxin did not disrupt PPI in Lister hooded rats (McGarrity et al., 2017).

Startle reactivity is a fast response to sudden, intense stimuli (Koch, 1999). Here, we refer to acoustic startle, a reaction to a loud noise. The acoustic startle response is mostly studied alongside PPI, where the primary interest is startle modulation by the prepulse. However, apart from ‘prepulses’, a wide range of other factors modulate startle reactivity, including attention and emotional states (Bast & Feldon, 2003; Koch, 1999; Lang, 1995). Enhanced startle reactivity is a diagnostic criterion for anxiety disorders in humans and used as an animal model of anxiety (Rodgers, 1997; Walker & Davis, 1997). Moreover, numerical startle reductions have been reported

in people with schizophrenia (Bast & Feldon, 2003; Braff, 1992; Ludewig et al., 2002) and significant startle reductions in the prodromal stage of schizophrenia, with the latter being related to symptom severity (Quednow et al., 2008). Ventral hippocampal disinhibition with picrotoxin decreased startle reactivity in Wistar and Lister hooded rats (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017). As we will outline in the following sections, there is limited evidence for the specific role of α5-GABAARs in the ventral hippocampus in regulating locomotor activity, PPI and startle.

1.2 Hippocampal α5-GABAARs and locomotor activity

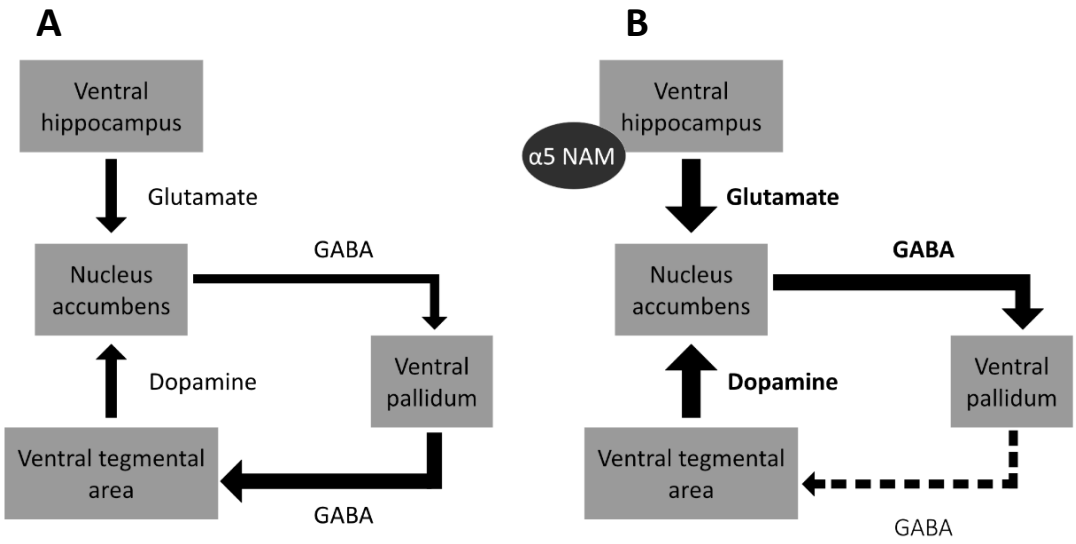
The evidence regarding the specific role of hippocampal α5-GABAARs is limited. On the one hand, there is some evidence for the role of hippocampal α5-GABAAR activity in locomotor activity. Mice with a mutation (α5(H105R)) that reduces the expression of α5-GABAARs in CA1–3 of the hippocampus had higher locomotor activity than wildtype controls, suggesting that hippocampal α5-GABAARs negatively modulate locomotor activity (Hauser et al., 2005). To our knowledge, there has not been further research into the specific contribution of hippocampal α5-GABAARs for locomotor activity. Interestingly, ventral hippocampal infusion of the α5-GABAAR NAM, L-655,708, was reported to increase VTA dopamine neuron population activity in anaesthetised rats (Perez et al., 2022). This suggests that negative modulation of ventral hippocampal α5-GABAARs may increase locomotor activity in awake rats, given that increased dopamine transmission in the VS, which receives dopaminergic input from the VTA, increases locomotor activity (Beninger, 1983; Ikemoto & Panksepp, 1999). Additionally, as discussed, ventral hippocampal disinhibition of GABA-A receptors by picrotoxin increases locomotor activity (Bast et al., 2001; McGarrity et al., 2017), indicating that modulating the activity of hippocampal GABA-A receptors can drive the dopamine system. We propose that ventral hippocampal infusion of an α5-GABAAR NAM, L-655,708, may increase locomotor activity, but this could be to a lesser extent than picrotoxin, as only the α5-GABAARs are targeted with this NAM, whereas picrotoxin targets all GABA-A receptors. Additionally, picrotoxin

is an antagonist, which blocks GABAergic inhibition, whilst L-655,708 is a negative allosteric modulator, which leads to a partial loss of GABAergic inhibition. The ventral hippocampus can modulate dopamine transmission in the VS via a direct glutamatergic projection from the CA1 or subiculum region of the ventral hippocampus to the NAc of the VS (Lindenbach et al., 2022; Taepavarapruk et al., 2008) or via a polysynaptic pathway: the ventral CA1/subiculum – NAc – ventral palladium (VP) – VTA – VS; shown in Figure 3-1A (Floresco et al., 2001, 2003; Groenewegen et al., 1987). There is limited expression of α5-GABAARs in the subiculum (Curia et al., 2009; Prenosil et al., 2006). However, in CA1, α5-GABAARs are localised to the distal apical dendrites of pyramidal neurons in the SLM, where they preferentially mediate slow phasic inhibition by SST+ interneurons (Schulz et al., 2018) that were reported to preferentially target NAc-projecting pyramidal neurons (Lodge et al., 2023). Ventral hippocampal α5-GABAARs have been shown to modulate dopamine activity in the VTA: ventral hippocampal infusion of L-655,708 increased dopamine neuron activity in the VTA and it was suggested this was through the polysynaptic pathway (Perez et al., 2022). Therefore, we hypothesise that locomotor activity may be increased with ventral hippocampal infusion of L-655,708, in line with findings in mice that hippocampal α5-GABAARs negatively modulate locomotor activity (Hauser et al., 2005). Ventral hippocampal L-655,708 may increase locomotor activity by increasing activity in the dopamine pathway from the VTA to the VS; shown in Figure 3-1B (Beninger, 1983; Ikemoto & Panksepp, 1999; Perez et al., 2022), or by increasing glutamatergic activity in the direct projection from the ventral CA1/subiculum to the NAc, which has been shown to increase NAc dopamine and locomotion (Lindenbach et al., 2022; Taepavarapruk et al., 2008). Interestingly, SST+ interneurons target α5-GABAARs in CA1 and SST+ neurons preferentially activate NAc-projecting pyramidal cells (Lodge et al., 2023), suggesting α5-GABAARs specifically may be important in the ventral hippocampal – NAc projections.

On the other hand, several other studies suggest that α5-GABAARs do not modulate locomotor activity, but this was not specifically for hippocampal α5-GABAARs. In mice with three point mutations that desensitise the α1, 2 and 3 receptors to diazepam

(leaving only α5 receptors sensitive to diazepam; DZα5), it was found that positive modulation with diazepam at α5-GABAARs had no effect on locomotion, suggesting that α5-GABAARs, including in the hippocampus, do not mediate the negative modulation of locomotor activity by diazepam (Behlke et al., 2016). In line with this, studies using systemic administration of L-655,708 in mice and rats have found no effect on locomotor activity (Bugay et al., 2020; Fischell et al., 2015; Magnin et al., 2019), though it is not clear to what extent the systemic injections would have reduced the function of hippocampal α5-GABAARs.

Figure 3-1: The pathway in which ventral hippocampal activity can increase dopamine activity and the effect of an α5-GABAAR NAM on this pathway.



Note. The pathway in which the ventral hippocampus can increase dopamine activity under normal conditions (A) or with an α5-GABAAR NAM (B). The α5 NAM decreases inhibition in the ventral hippocampus, increasing the glutamatergic output from the hippocampus to the nucleus accumbens. This increases the inhibitory drive from the nucleus accumbens to the ventral pallidum, which decreases the inhibitory drive from the ventral pallidum to the ventral tegmental area. This thereby increases dopamine neuron activity in the ventral tegmental area, increasing the activity in the projections from the ventral tegmental area back to the nucleus accumbens. Adapted from Perez et al. (2022). Abbreviations: GABA: gamma-aminobutyric acid; NAM: negative allosteric modulator. Figure produced using Affinity by Canva 3.0.2.3912.

Overall, it is not clear from previous studies how hippocampal α5-GABAAR modulation contributes to locomotor activity. Specific reduction of hippocampal α5-GABAARs in mice increased locomotor activity (Hauser et al., 2005), whereas systemic modulation of α5-GABAARs did not affect locomotion (Behlke et al., 2016; Bugay et al., 2020; Fischell et al., 2015; Magnin et al., 2019). To our knowledge, there has been no research that has investigated the effect of pharmacologically targeting specifically the ventral hippocampal α5-GABAARs on locomotor activity. We aimed to address this in these experiments by investigating the impact of ventral hippocampal infusion of L-655,708 on locomotor activity.

It is possible that ventral hippocampal α5-GABAAR modulation may not affect baseline dopamine release and baseline locomotor activity but may instead sensitise the dopamine system's response to stimulation, and thereby, augment the locomotor response to amphetamine as has been suggested for other manipulations of hippocampal activity (Lodge & Grace, 2007; Shah & Lodge, 2013; Wilkinson et al., 1993). The hippocampus is known to modulate midbrain dopamine neuron activity, with increased hippocampal activity increasing dopamine neuron activity (Floresco et al., 2001; Legault et al., 2000; Legault & Wise, 1999; Lodge & Grace, 2007). However, it has been found that changes to hippocampal activity, such as by lesioning, do not affect baseline locomotor activity, but when combined with amphetamine administration, are able to augment the response to amphetamine by further increasing locomotion and dopamine activity (Wilkinson et al., 1993). Additionally, it has been reported that increased ventral hippocampal activity, due to disruption of perineuronal nets in the ventral hippocampus (Shah & Lodge, 2013), or in the MAM rat model (Lodge & Grace, 2007), augmented the locomotor response to amphetamine, but had no effect on baseline locomotor activity. It has been proposed that increased ventral hippocampal activity leads to an increased number of spontaneously active VTA dopamine neurons which then increases dopamine release in response to stimulation of the dopamine system (such as, with amphetamine), and with this increase in dopamine release there is associated behavioural effects, including increased locomotion (Lodge & Grace, 2011).

1.3 Hippocampal α5-GABAARs, PPI and startle

As discussed, the ventral hippocampus, and local GABA-A receptors, have been shown to be involved in PPI and startle (Bast, Zhang, & Feldon, 2001b; Bast & Feldon, 2003; Caine et al., 1992; Zhang et al., 2002b). To our knowledge the effect of pharmacologically targeting ventral hippocampal α5-GABAARs on PPI and startle has not been examined. There has been limited research into the involvement of α5-GABAARs in startle and PPI. A study using α5(H105R) mice with reduced expression of α5-GABAARs in CA1–3 of the hippocampus found PPI to be attenuated in α5(H105R) mice compared to wildtype mice, while startle was unaffected in the mutant mice (Hauser et al., 2005). Systemic administration of L-655,708 in Sprague Dawley rats had no effect on PPI, and the effect on startle was not reported (Carreno et al., 2017). However, systemically targeting α5-GABAARs allows all α5-GABAARs to be targeted, whereas site specific reduction of α5-GABAARs only affects the α5-GABAARs in CA1–3 of the hippocampus. It is difficult to extrapolate from these studies what effect pharmacological manipulation of ventral hippocampal α5-GABAARs would have. On the one hand, the hippocampus has, and more specifically α5-GABAARs in the hippocampus have, been shown to modulate PPI across species (Bast, Zhang, & Feldon, 2001b; Hauser et al., 2005; Kumari et al., 2005). On the other hand, the contributions of the hippocampus to PPI have been shown to have strain differences (McGarrity et al., 2017), and it may be that it is not specifically the α5-GABAARs in the hippocampus that modulate PPI. In our experiments, we therefore investigated the extent to which α5-GABAARs contribute to PPI and startle by investigating the impact of ventral hippocampal infusion of L-655,708 on these behaviours.

1.4 Aims and Hypotheses

In the present study, we investigated the impact of negative modulation of ventral hippocampal α5-GABAARs on locomotor activity, PPI and startle in Lister hooded rats. To this end, we combined ventral hippocampal infusion of the α5-GABAAR NAM, L-

655,708 (Perez et al., 2022), with behavioural tests of these processes, using procedures similar to our previous studies examining the impact of ventral hippocampal picrotoxin on these behaviours (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017). We hypothesised that there would be an increase in locomotor activity with ventral hippocampal L-655,708 infusion, considering the finding that ventral hippocampal L-655,708 infusion increased VTA dopamine activity (Perez et al., 2022), which could increase dopamine in the NAc and, thereby, locomotion (Ikemoto & Panksepp, 1999). Based on the findings of our initial experiments examining the effect of α5-GABAAR negative allosteric modulation on baseline locomotor activity, we also investigated the impact of α5-GABAAR negative allosteric modulation on amphetamine-induced hyperlocomotion. Regarding PPI and startle, it is not clear from the literature to what extent ventral hippocampal α5-GABAARs contribute to the role of the ventral hippocampus in these sensorimotor gating processes. L-655,708 may reduce startle reactivity, as ventral hippocampal picrotoxin reduces startle reactivity, but PPI may remain unaffected, as ventral hippocampal picrotoxin does not affect PPI in Lister hooded rats.

2 Methods

2.1 Rats

This study used a total of 48 male young adult Lister hooded rats (Envigo, UK), weighing 260–350 g (about 8–11 weeks old) at surgery. We ran three experiments (Experiment 1–3) in three separate cohorts of n=16 rats. Sample size justification, exclusions and final sample sizes can be found in Section 2.8. Rats were housed in groups of four, in individually ventilated, two-level, 'Double Decker' cages (462 mm X 403 mm X 404 mm; Tecniplast, UK). Environmental conditions were controlled: temperature (21.0 ± 1.5 °C); humidity (50 ± 8%); light, alternating in 12-h day/night cycles (lights on at 7.00 am). All procedures were carried out during the light phase. Rats had ad libitum water and food (Teklad Global 18% Protein Rodent Diet 2018C; Envigo). Rats had at least seven days of acclimatisation and handling before surgery. All procedures were conducted in accordance with the United Kingdom (UK) Animals (Scientific Procedures) Act 1986, approved by the University of Nottingham's AWERB and run under the authority of Home Office project license PP1257468. For the reporting of our studies, we followed ARRIVE guidelines (du Sert et al., 2020).

2.2 Ventral hippocampal cannula implantation

For Experiments 1 and 2, guide cannulae were implanted bilaterally above the ventral hippocampus using the same coordinates and similar procedures as in previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). For Experiment 3, the coordinates were adjusted so that the dorsal-ventral coordinate was taken from skull, rather than from dura. Stainless steel stylets (33 gauge, Plastics One, Bilaney), with a dust cap, were placed into the guide cannulae for the surgery and protruded either 0.5 mm (Experiments 1 and 2), or 1.0 mm (Experiment 3) beyond the tips of the guide cannulae (the guide cannulae were 0.5 mm shorter for Experiment 3; all tips were aimed at the same location within the ventral hippocampus). The protrusion of the stylets was the same as the infusion cannula.

The stylets with the dust cap were implanted with the tips aimed at the infusion coordinates (see below).

Experiment 3 was carried out at a new facility, and there were some adjustments made in the surgical procedures for Experiment 3 compared to the other two experiments. Rats were anaesthetised using isoflurane delivered in oxygen (induction: 5%; maintenance: 1–3%; Experiment 1 and 2: flow rate: 1 l/min, Experiment 3: flow rate: 2 l/min) and prepared for surgery by shaving and disinfecting the scalp with 2% chlorhexidine (Experiment 1 and 2: Clinell, UK; Experiment 3: ChloraPrep, Becton Dickinson, UK). For Experiment 3, bupivacaine was administered into the incision site (s.c.; MercuryPharma, UK; 2 mg/kg), to further reduce the risk of the rats experiencing discomfort during, and after, the surgical procedures. The rats' heads were secured in a stereotaxic frame with blunt ear bars; EMLA cream (5%, lidocaine/prilocaine, Aspen) was applied to the ear bars to minimise the risk of pain, and a lubricant gel was used (Lubrithal, Dechra, Uldum) to prevent the eyes from drying out during surgery. Rats received perioperative analgesia (s.c.; Metacam; Boehringer Ingelheim, UK; Experiment 1 and 2: 1 mg/kg, Experiment 3: 2 mg/kg) and 2–6 ml of saline (s.c.; Vetivex, Dechra, UK) to prevent dehydration. The skull was exposed and bregma and lambda were aligned horizontally. Bilateral infusion guide cannulae (stainless steel, 26-gauge, Plastics One, Bilaney Consultants, UK) were implanted through small holes drilled in the skull. The coordinates for infusions into the ventral hippocampus were 5.2 mm posterior to bregma, ± 4.6mm lateral from the midline and 6.5 mm ventral from dura for Experiment 1 and 2. For Experiment 3, the dorsal-ventral coordinate was taken from skull instead of dura and was adjusted to 7.0 mm ventral from the skull. The skull is approximately 0.5 mm thick, so these coordinates targeted the same location, but taking coordinates from skull is less subjective than from dura, so should be more accurate and consistent between surgeons. Cannulae were secured to the skull using cement and four stainless steel screws. In Experiments 1 and 2, dental cement (Simplex Rapid, Kemdent, UK) and light curing cement (HS Maxima Natural Elegance, Henry Schein, UK), which was used mostly around the trepanations, were used. In Experiment 3, bone cement containing

gentamicin (Eurofix Gun G Vet, Synimed, France) was used. The scalp incision was sutured around the pedestal to reduce exposure of the skull and open wound margins. For Experiment 3, following suturing, the skin was glued to the pedestal with a tissue adhesive (GLUture, Abbott Laboratories, USA) so that no skull or open wound remained exposed. For Experiments 1 and 2, an antibiotic was administered, for infection prophylaxis, preoperatively and subsequently every day for the duration of the study (s.c.; Synulox; 140 mg amoxicillin, 35 mg clavulanic acid/ml; Pfizer; 0.02 ml/100 g). For Experiment 3, no antibiotics were administered, but the cement contained gentamicin. For Experiment 3, rats received post-operative Metacam in jelly for three days. Rats were housed singly to eat the jelly for approximately 15–30 min, and each rat received one cube (2 ml) per day (1 mg/kg Metacam made up in strawberry jelly; Hartley's, UK). After surgery, the rats were allowed at least five days of recovery before any testing commenced. During the recovery period, rats were checked daily and habituated to the manual restraint necessary for the subsequent drug infusions (Experiments 1–3) and systemic (i.p.) injections (Experiment 3).

2.3 Ventral hippocampal infusions

Rats were manually restrained during infusions. Stylets were removed and replaced by infusion injectors protruding 0.5 mm (Experiments 1 and 2) or 1.0 mm (Experiment 3) from the tip of guide cannulae (both targeted 7.0 mm ventral from skull; stainless steel, 33 gauge; Plastics One, Bilaney), which were connected with flexible polyethylene tubing to two 5 µl SGE micro syringes mounted on a microinfusion pump (sp200IZ, World Precision Instruments, UK). A volume of either 0.5 µl/side (Experiment 1) or 1 µl/side (Experiments 2 and 3) of vehicle (50% dimethyl sulfoxide (DMSO), Tocris, UK and 50% saline, Vetivex, Dechra, UK), or a dose of L-655,708 ($C_{18}H_{19}N_3O_4$, Tocris, UK) made up in vehicle was infused bilaterally over 1 min (Experiment 1), or 2 min (Experiments 2 and 3). The doses of L-655,708 were 50 or 100 ng/0.5 µl per side (Experiment 1), 100, 200, 400 or 600 ng/1 µl per side (Experiment 2) and 600 ng/1 µl per side (Experiment 3). Initial doses were based on

previous studies (Bugay et al., 2020; Perez et al., 2022); see section 2.4 for more details. Movement of an air bubble, trapped in the tubing, was used to monitor the infusion flow. Injectors were left in the cannulae for another minute before being removed and replaced by the stylets to allow for absorption of the infusion bolus. Rats were then placed in the locomotor boxes (section 2.6), or PPI/startle boxes (section 2.7), and the testing session began. Two rats were infused at the same time, which took 5–10 min, and then placed into the test boxes. All rats receiving infusions were carefully monitored during and after each infusion for any behavioural signs potentially related to seizure development, including wet dog shakes (Chapter 2; Taylor et al., 2025; Williams et al., 2022). Not all rats showed behavioural effects. See Table 3-2 for the full list of behavioural effects observed in these studies.

2.4 Doses of L-655,708

For clarity, all doses and volumes can be found in Table 3-1. In Experiment 1, the doses of L-655,708 used were based on previous studies, which found ventral hippocampal infusion of L-655,708 in Sprague-Dawley rats to decrease immobility and increase climbing in the forced swim test 48 h after bilateral infusion (90 ng/1 µl/side; Bugay et al., 2020), and to increase dopamine activity in the VTA 20 min after unilateral infusion (0.75 µl of 100 ng/µl, i.e. 75 ng in 0.75 ul; Perez et al., 2022). The doses we used were 50 ng/0.5 µl and 100 ng/0.5 µl, and we chose to infuse 0.5 µl/side, as this volume limits the spread of the drug outside of the ventral hippocampus, and is the volume we have used in previous experiments with ventral hippocampal infusions (Chapter 2; Gwilt et al., 2020; McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022).

Because Experiment 1 did not reveal any effect of L-655,708 on locomotor activity (or any other gross behavioural effects evident from visual inspection), we decided to increase the volume of the infusion from 0.5 µl/side to 1 µl/side for Experiment 2. This was based on the consideration that a larger volume may be required to achieve better drug spread, which may have been limited due to the viscosity of the drug

solution, which was observed during infusions in Experiment 1. Previous studies infusing L-655,708 into the ventral hippocampus had used 1 µl (Bugay et al., 2020) and 0.75 µl (Perez et al., 2022). For the first part of Experiment 2 (Experiment 2a), we bilaterally infused 100 ng or 200 ng /1 µl/side. Because this did not cause a marked change in locomotor activity or any other gross behavioural effects that were evident based on visual inspection of the rats, we increased the doses further to 400 and 600 ng/1 µl/side for the second part of Experiment 2 (Experiment 2b). This was a large increase from previous studies, but strain differences in doses are not uncommon (Deiana et al., 2007; López-Rubalcava & Lucki, 2000). For Experiment 3, we used the highest dose of 600 ng/1 µl/side based on there being some gross behavioural effects at this dose (a positive control for the drug acting), but not serious behavioural effects (see section 3.6).

Table 3-1: Doses of L-655,708 and amphetamine used in Experiments 1–3.

Experiment and behavioural test	**Volume infused per side**	**Amount of drug infused into ventral hippocampus <u>per side</u>**	**Amount of amphetamine given by systemic injection**
1: LMA and PPI/startle	0.5 µl	0, 50 or 100 ng	N/A
2a: LMA	1 µl	0, 100 or 200 ng	N/A
2b: LMA	1 µl	0, 400 or 600 ng	N/A
3: LMA	1 µl	0 or 600 ng	0 or 0.5 mg/kg

Note. Vehicle was 50% DMSO, 50% saline. Abbreviations: DMSO: Dimethyl sulfoxide, LMA: Locomotor activity, N/A: not applicable; PPI: Prepulse inhibition.

2.5 Systemic injection of amphetamine

In Experiment 3, the effect of ventral hippocampal L-655,708 infusion on amphetamine-induced hyperlocomotion was examined. As such, 30 min post infusion, rats received either a systemic injection of amphetamine (i.p.; Dextroamphetamine sulphate, Biosynth, UK made up in 0.9% saline, Vetivex, Dechra, UK) at a dose of 0.5 mg/kg (Gill et al., 2011; Lodge & Grace, 2007; Perez & Lodge, 2013; Redrobe et al., 2012; Wilkinson et al., 1993), or systemic injection of 0.9% saline.

2.6 Locomotor activity testing

Locomotor activity was measured as in McGarrity et al., (2017), and as described in detail in Pezze et al., (2014). Briefly, locomotor activity was measured using 12 clear Perspex boxes (39.5 cm long x 23.5 cm wide x 24.5 cm deep) with metal grid lids placed in a dimly lit box (25–40 lux, note this is lower than in McGarrity et al., (2017) and in Pezze et al., (2014)). The boxes were placed in frames containing two levels of a 4 x 8 photobeam configuration (Photobeam Activity System; San Diego Instruments, USA). Two consecutive breaks of adjacent beams within the lower level of photobeams generated a locomotor count. To start a session, rats were placed into the centre of the boxes and rats were always tested in the same box. Total locomotor counts were calculated for each 10-min block of testing.

2.7 PPI and startle testing

PPI and startle were measured as in McGarrity et al., (2017), and as described in detail in Pezze et al., (2014). Four startle response boxes (San Diego Instrument) were used to measure PPI and startle. Each box contained a clear Perspex cylinder (8.8 cm diameter, 19.5 cm long) with a solid Perspex base, which was linked to an accelerometer. The box was a well-lit (15 W) and ventilated sound-attenuated

chamber (39 x 38 x 58 cm). Background noise and acoustic stimuli were produced by a speaker located centrally above the cylinder, linked to a noise generator which was controlled by the SR-Lab system (San Diego Instrument). Whole-body startle responses were recorded for each rat by the accelerometer connected to Reflex Testing software (San Diego Instruments). The amplitude of the whole-body startle response to a pulse was defined as the average of 100 one ms accelerometer readings collected from pulse onset. Rats were always tested in the same box.

Each test session began with the rat being put into the cylinder. At the start of the session, there was a five-min acclimatisation period with a 62 dB background noise level. This background 62 dB level was continued throughout the session. After the acclimatisation period, there were three test blocks. In the first block, ten startle pulses (40 ms, 120 dB) were presented alone. The second block consisted of 50 trials to measure PPI. There were five different trial types: pulse alone trials (120 dB) and four types of prepulse plus pulse trials in which a weak 20 ms prepulse (72, 76, 80, or 84 dB) preceded the startle pulse by 100 ms. Each trial type was presented ten times, in pseudorandom order and with a variable intertrial interval of 10–20 s duration (average 15 s) to ensure they were unpredictable. The third block consisted of five startle pulses (120 dB). A complete test session lasted 23 min. The percentage of PPI was calculated for each of the four prepulses using:

$$\frac{(\text{mean startle amplitude on pulse alone trials}) - (\text{mean startle amplitude on prepulse plus pulse trials})}{(\text{mean startle amplitude on pulse alone trials})} \times 100$$

Startle amplitude was measured on pulse alone trials across the three blocks to determine startle habituation.

2.8 Experimental design

All experiments were run using a within-subjects design with a target sample size of n=12. This sample size would give a power of >80% to detect differences between

infusion conditions that correspond to an effect size of Cohen's d of around 1, using pairwise comparisons (two-tailed paired t-tests, $p < 0.05$; G*Power, Faul et al., 2007).

In Experiment 1 and 2, each rat received a total of six infusions of vehicle or a dose of L-655,708. In Experiment 3, each rat received four infusions. The number of infusions per rat was limited to six to reduce the risk that reactive gliosis, caused by repeated infusions, may reduce effectiveness of the infused drug (Bast, Zhang, Heidbreder, et al., 2001; Cunningham et al., 2008). In all experiments, we included baseline and re-baseline days before and after drug infusions. These days served as 'wash out' days between infusions and their purpose was to reduce the risk of carry over effects between the infusion conditions of our within-subjects design. Testing order of different infusion and systemic injection conditions was counterbalanced using a Latin square design. The lead experimenter was blinded to the drug conditions for the duration of the experiment and the statistical analysis. The design of Experiments 1–3 is described below and depicted in Figure 3-2.

2.8.1 Experiment 1

In Experiment 1, locomotor activity was measured during the first part of the experiment (Experiment 1a). On day 1 of Experiment 1, locomotor activity was measured for 30 min without infusion (baseline). On day 2, locomotor activity was measured for 30 min without infusion (pre-infusion baseline), then rats were infused with either vehicle or a dose of L-655,708 (this took approximately 5–10 min) and placed back in the box for 60 min of testing. On day 3, locomotor activity was measured again without infusion for 30 min (re-baseline), as on day 1. This was repeated three times, so each rat was subject to each infusion condition (vehicle, 50 ng and 100 ng L-655,708), giving a total of four baseline/re-baseline days and three infusion days. The rats from this study were then used for PPI and startle testing the following week.

In the second part of Experiment 1 (Experiment 1b), PPI and startle were measured, in the week following locomotor activity testing. The same rats were used for locomotor activity and PPI testing, except one rat that was culled early during week

1 of testing. In Experiment 1b, on day 1, PPI was measured without infusion (baseline). On day 2, PPI was measured 5–10 min after infusion with either vehicle or a dose of L-655,708. On day 3, PPI was measured again without infusion (re-baseline), as on day 1. This was repeated three times, so each rat was subject to each infusion condition (vehicle, 50 ng and 100 ng L-655,708), giving a total of four baseline/re-baseline days and three infusion days.

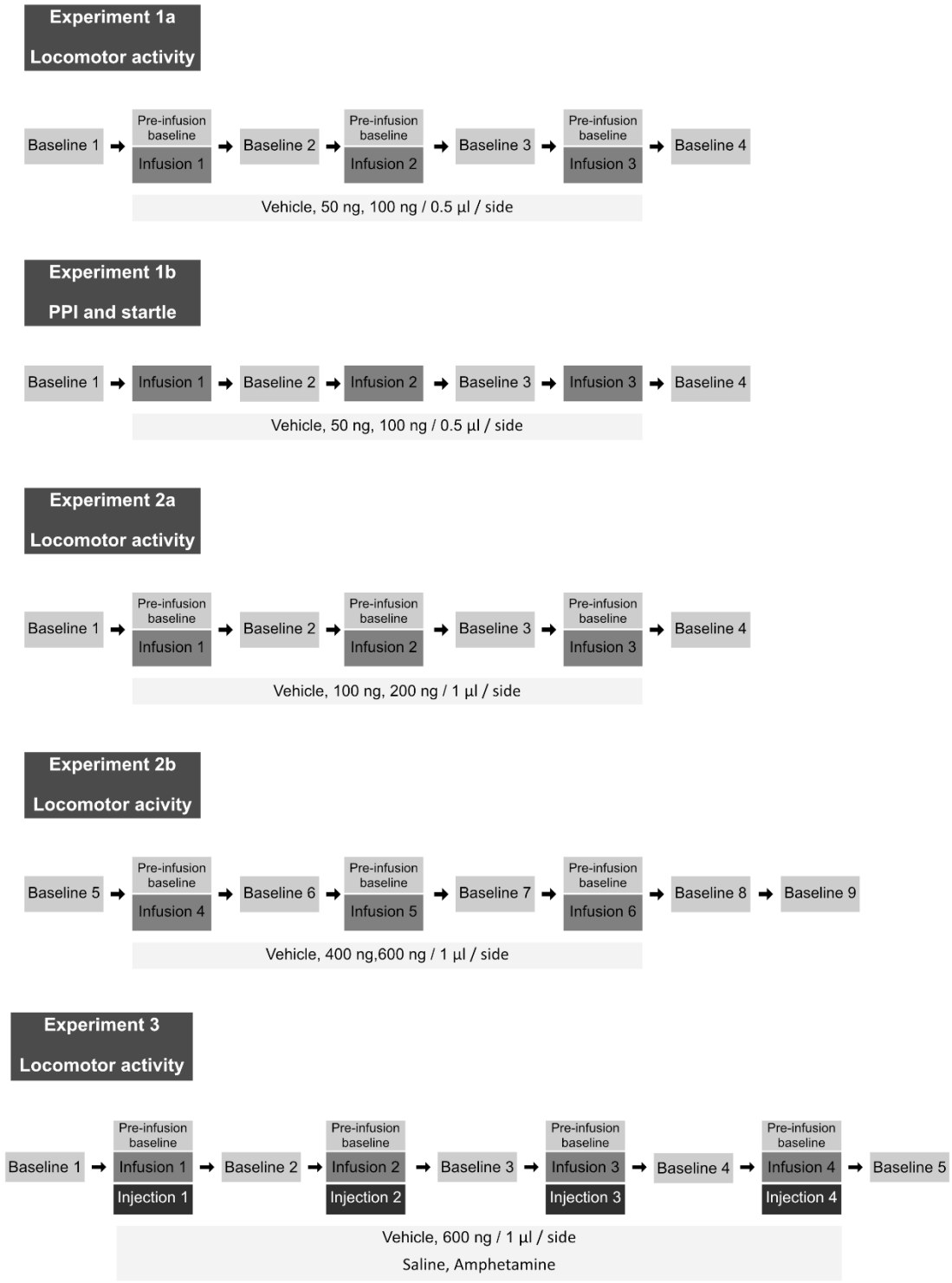
2.8.2 Experiment 2

In Experiment 2, the design was the same (baseline day, infusion day, baseline day) as for the locomotor activity testing in Experiment 1. During part 1 (Experiment 2a), rats were infused with either vehicle, 100 ng or 200 ng of L-655,708 on days 2, 4 and 6. During part 2 (Experiment 2b), rats were infused with either vehicle, 400 ng or 600 ng of L-655708, on infusion days. Experiment 2a consisted of four baseline/re-baseline days and three infusion days. Experiment 2b consisted of five baseline/re-baseline days and three infusion days, as two re-baseline days were measured after the final infusion, to allow us to also investigate locomotor activity 48 h after infusion (see Section 3.4).

2.8.3 Experiment 3

In Experiment 3, the design was similar (baseline day, infusion day, baseline day) as for Experiment 1 and 2. However, in Experiment 3, the infusion days also involved a systemic injection (i.p.) of amphetamine (0.5 mg/kg) or saline. Only the highest dose of L-655,708 was used (600 ng) and rats received either L-655,708 or vehicle, and amphetamine or saline, resulting in four different ventral hippocampal infusion/systemic injection conditions: L-655,708 ventral hippocampal infusion and amphetamine systemic injection, L-655,708 infusion and saline injection, vehicle infusion and amphetamine injection and vehicle infusion and saline injection. There were five baseline days and four infusion/injection days. An additional re-baseline day was not included after the final infusion/injection as no effect was present 48 h later in Experiment 2 (see section 3.4).

Figure 3-2: Study Design for Experiments 1–3.



Note. In Experiment 1a, locomotor activity was measured. There was a 30-min baseline day to habituate the rats to the locomotor activity boxes. Infusions were preceded by 30-min pre-infusion baseline recordings, and post-infusion testing (50 or 100 ng/0.5 µl/side L-655,708 or vehicle) was for 60 min. 30-min re-baseline days were included between infusion days.
In Experiment 1b, PPI and startle were measured. A baseline day was included to habituate the rats to the testing boxes, followed by three infusion days (50 or 100 ng/0.5 µl/side L-655,708 or vehicle), with baseline days interleaved between infusions. All PPI sessions lasted 23 min.
In Experiment 2, the procedure was the same as in 1a, except an increased infusion volume and increased doses of L-655,708 were used: 100 and 200 ng/1 µl/side (2a); 400 and 600 ng/1 µl/side (2b), and Baselines 5 and 9 were included so activity 48 h after infusion could be measured.
In Experiment 3, the procedure was the similar to in 1a. There was an initial baseline day, and re-baseline days between infusion days. The infusion day differed: there was a 30 min pre-infusion baseline, then an infusion was given (600 ng/1 µl/side L-655,708 or vehicle), and rats were tested for 30 min. Then a systemic injection (i.p.; 0.5 mg/kg amphetamine or saline) was given, and the rats were tested for a further 60 min.
Rats were infused with either a dose of L-655,708 or vehicle (50:50 DMSO, saline), and for Experiment 3, injected with either amphetamine or saline, in a within-subjects design, see the Methods for details. Abbreviations: DMSO: Dimethyl sulfoxide; i.p.: intraperitoneal; PPI: Prepulse inhibition. Figure produced using Affinity by Canva 3.0.2.3912.

In some experiments, some rats had to be excluded from the analysis of the whole experiment or some stages of the experiment. In Experiment 1, one rat was excluded from Experiment 1a (locomotor activity testing) because it received the incorrect infusion on one of the testing days (but the rat was included in Experiment 1b). A second rat was excluded from Experiment 1 because it had to be culled early, due to an injector snapping inside the cannula, before data collection for Experiment 1a or 1b could be completed. In Experiment 2b, two rats only had an approximately 23-min pre-infusion baseline on one of the infusion days, instead of a 30-min pre-baseline. The results of the ANOVA were unaffected when these two rats were excluded from analyses and so they were included in the analyses. In Experiment 3, one rat was culled after surgery due to illness and could not complete the experiment. Therefore, the final sample sizes for the three experiments were as follows: n=14 rats for Experiment 1a (locomotor activity), n=15 rats for Experiment 1b (PPI/startle), n=16

rats for Experiment 2a and 2b (locomotor activity) and n=15 rats for Experiment 3 (locomotor activity).

2.9 Verification of cannula placements

Cannula placements were verified as in our previous studies (McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022). On completion of the experiments, rats were anaesthetised with a lethal dose of sodium pentobarbital (1–2 ml i.p.; sodium pentobarbital, 200 mg/ml, Dolethal, Vetoquinol, UK) and transcardially perfused with 0.9% saline followed by 4% formaldehyde solution (Sigma-Aldrich, UK) in 0.9% saline. Brains were removed from the skull, stored in 4% formaldehyde in 0.9% saline and cut into 80 µm coronal sections on a vibratome (Leica VT 1000 S, UK). Sections containing the relevant brain regions were mounted on microscope slides and placement of the infusion cannulae was determined using a light microscope and mapped onto coronal sections of a rat brain stereotaxic atlas (Paxinos & Watson, 1998). For each experiment, the sections of some brains were cresyl-violet stained for presentation purposes.

2.10 Statistical analysis

Data were analysed by multi-factorial repeated measures ANOVA, using infusion condition (dose of L-655,708 or vehicle), systemic injection condition (amphetamine or vehicle), time block (10-min bin; locomotor activity), prepulse intensity (72, 76, 80, 84 dB; PPI) and startle test block (trials 1–10, 11–60 or 61–65; startle) as within-subjects factors. Where the assumption of sphericity (Mauchly's test) was violated, Greenhouse-Geisser, when $\varepsilon < 0.75$ or Huynh-Feldt, when $\varepsilon > 0.75$, correction was applied to the degrees of freedom. Significant main effects revealed by ANOVAs were followed by post-hoc pairwise comparisons using Fisher's LSD test. Any significant interactions observed were further examined by simple-main effects analyses. Additionally, a one-tailed Fisher's Exact Test was used to compare the number of rats

showing wet dog shakes (which were observed after some infusions, see section 3.6) between conditions. Data are presented as mean ± SEM and p values of < 0.05 were considered to indicate statistical significance. Data analysis was performed using JASP (JASP Team (2024). JASP Version 0.18.3) and graphs were produced using GraphPad Prism (version 10.2.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com) and Affinity by Canva version 3.0.2.3912.

3 Results

3.1 Verification of cannula placements in the ventral hippocampus

Infusion cannula tips were primarily placed within the ventral to intermediate hippocampus, in coronal brain sections corresponding to between 4.3–6.3 mm posterior to bregma in the rat brain atlas by Paxinos & Watson (1998; Figure 3-3).

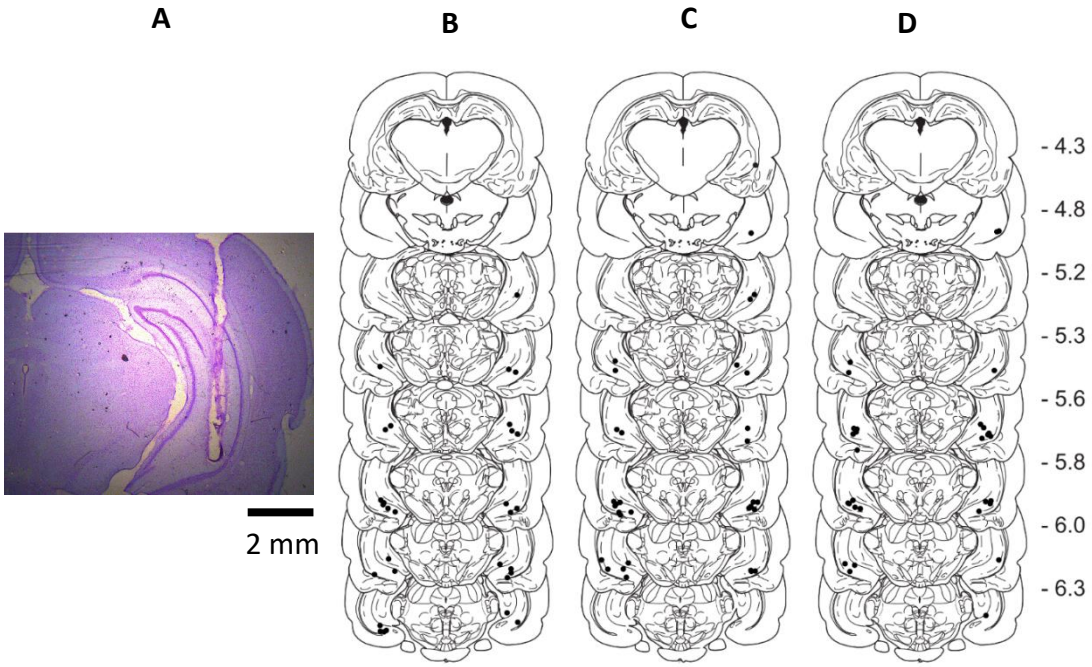
In Experiment 1 (Figure 3-3B), four rats had cannula placements within the subiculum of the ventral hippocampus. There is limited expression of α5-GABAARs within the subiculum (Curia et al., 2009; Prenosil et al., 2006). However, even if individual infusion cannula placements are within distinct hippocampal subregions, such as subiculum, the drug will spread beyond one subregion of the ventral hippocampus. The spread of the infusion volume of 0.5 µl (as used Experiment 1) would occupy a sphere with a radius of 0.5 mm, if an isotropic spread of the infusion volume is assumed. It is likely it would spread further, given that spread could be facilitated dorsally by the cannula tracks and diffusion would further add to drug spread (Jacobs et al., 2013; Williams et al., 2022). Therefore, even with an infusion tip placed in the subiculum, the drug would have spread to the other subregions of the ventral hippocampus, where α5-GABAARs are more highly expressed. The analyses were conducted with and without these rats, but the findings were unchanged when these rats were excluded. Only the analyses with these rats included are reported in the Results section.

In Experiment 2 (Figure 3-3C), one cannula was implanted slightly too lateral (seen on the right side of the -4.3 section). Given the proximity of this placement to the ventral tip of CA3, and the likely spread of the infusion volume (see above), it is likely that the infusion would have targeted the ventral hippocampus. Analyses were run with and without this rat and the results of the analyses did not change so the rat was included.

In Experiment 3 (Figure 3-3D), one cannula was slightly too deep (seen on the left side of the -5.6 section). However, the drug infusion spreads back up the cannula

tract, so the ventral hippocampus would still have been affected by the infusion. Analyses were run with and without this rat and the results of the analyses did not change so the rat was included.

Figure 3-3: Infusions sites in the ventral hippocampus in Experiments 1, 2 and 3.



Note. Example coronal brain section showing infusion sites in the ventral hippocampus, cresyl-violet stained for presentation purposes (A). Approximate locations of infusion cannula tips (black dots) mapped onto coronal sections adapted from the Paxinos and Watson (1998) rat brain atlas in Experiments 1 (B, n=14–15), 2 (C, n=16) and 3 (D, n=15). Numbers on the right indicate posterior distance from bregma in millimetres.

3.2 Experiment 1 and 2: Ventral hippocampal infusion of L-655,708 had no effect on locomotor activity, prepulse inhibition or startle

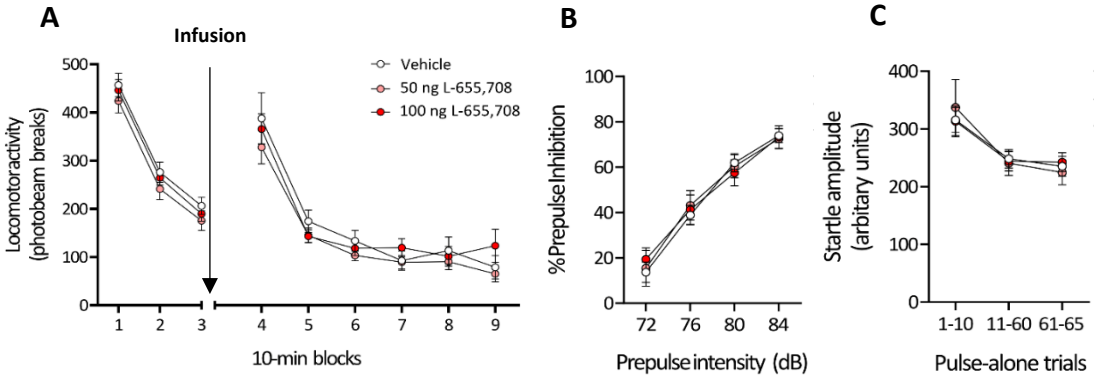
In Experiment 1a, there was no effect of ventral hippocampal L-655,708 (50 or 100 ng/0.5 µl/side) on locomotor activity (Figure 3-4A). All prospective infusion conditions showed similar locomotor activity during the 30-min pre-infusion baseline (main effect of infusion condition: $F_{(2,26)} = 1.817$, $p = 0.183$, infusion condition X time

block interaction: F < 1). There was no significant effect of L-655,708 infusion condition on locomotor activity at either dose of L-655,708 (main effect of infusion condition: $F_{(1.511,19.637)}$ = 1.637, p = 0.221) or an infusion condition X time block interaction: (F < 1). Only a main effect of time was observed ($F_{(2.857,37.136)}$ = 83.613, p < 0.001), reflecting decreased activity across time as the rats habituated to the boxes.

In Experiment 1b, there was no effect of ventral hippocampal L-655,708 (50 or 100 ng/0.5 µl/side) on PPI (Figure 3-4B). There was no main effect of infusion condition (F < 1) or an infusion condition X prepulse intensity interaction (F < 1). There was only a main effect of prepulse intensity ($F_{(1.551,21.700)}$ = 94.709, p < 0.001), reflecting increased PPI with increased prepulse intensity.

L-655,708 did not affect startle reactivity on pulse alone trials during any of the test blocks (Figure 3-4C). There was no main effect of infusion condition (F < 1) and no infusion condition X startle test block interaction (F < 1). There was only a main effect of startle test block ($F_{(1.223,17.119)}$ = 18.265, p < 0.001), reflecting startle habituation.

Figure 3-4: Experiment 1: Ventral hippocampal infusion of L-655,708 (50 or 100 ng/0.5 µl/side) did not affect locomotor activity, prepulse inhibition or startle.



Note. Data are shown as mean (± SEM). Infusion of L-655,708 at two doses (50 and 100 ng/0.5 µl/side) had no effect on locomotor activity (A, n=14 rats), prepulse inhibition (B, n=15 rats), or startle (C, n=15 rats). Vehicle: 50:50 DMSO, saline.

In Experiment 2a, there was no effect of ventral hippocampal L-655,708 (100 or 200 ng/1 µl/side) on locomotor activity (Figure 3-5A). In Experiment 2a, all prospective infusion conditions showed similar locomotor activity during the 30-min pre-baseline (main effect of infusion condition: $F_{(2,30)} = 1.744$, $p = 0.192$, infusion condition X time block interaction: $F_{(4,60)} = 1.38$, $p = 0.252$). For the post-infusion analysis, there was no significant effect of L-655,708 infusion on locomotor activity at either dose (main effect of infusion condition: $F_{(2,30)} = 2.42$, $p = 0.106$), and there was no significant interaction (infusion condition X time block interaction: $F_{(3.554,53.315)} = 1.292$, $p = 0.286$). Only a main effect of time was observed ($F_{(2.527,37.902)} = 65.444$, $p < 0.001$), reflecting decreased activity across time.

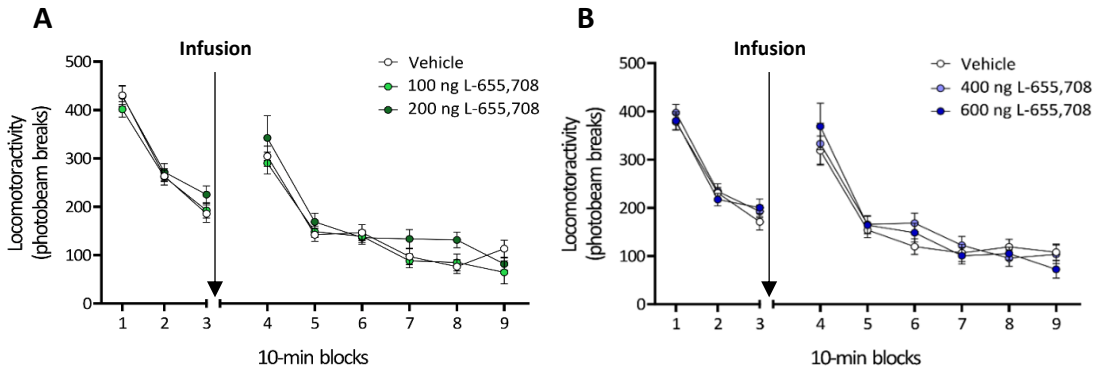
In Experiment 2b, ventral hippocampal L-655,708 (400 or 600 ng/1 µl/side) did not affect locomotor activity (Figure 3-5B). All prospective infusion conditions showed similar locomotor activity during the 30-min pre-baseline (main effect of infusion condition: $F_{(2,30)} = 1.240$, $p = 0.304$, infusion condition X time block interaction: $F < 1$). For the post-infusion analysis, there was no significant main effect of L-655,708 infusion at either dose, or an interaction (main effect of infusion condition: $F < 1$; infusion condition X time block interaction: $F_{(2.888,43.322)} = 1.039$, $p = 0.383$). Only a main effect of time was observed ($F_{(1.593,23.899)} = 48.205$, $p < 0.001$), reflecting decreased activity across time.

3.3 Experiment 1 and 2: No effect of ventral hippocampal infusion of L-655,708 on locomotor activity 24 h after infusion

We investigated whether there was an effect 24 h after ventral hippocampal infusion of L-655,708, as we had this data from our baseline days. For Experiment 1, ventral hippocampal infusion of L-655,708 (50 or 100 ng/0.5 µl/side) did not affect locomotor activity 24 h after infusion (main effect of infusion condition and infusion condition X time interaction: $F < 1$, $p > 0.55$; data not shown). For Experiment 2, again ventral hippocampal infusion of L-655,708 (100 ng, 200 ng, 400 ng and 600 ng/1 µl/side) did not affect locomotor activity 24 h after infusion (Experiment 2a or b, all main effects

of infusion condition or interactions with infusion condition, $F < 1.3$, $p > 0.29$; data not shown).

Figure 3-5: Experiment 2: Ventral hippocampal infusion of L-655,708 (100, 200, 400 and 600 ng/1 µl/side) did not affect locomotor activity.



Note. Data are shown as mean (± SEM). Infusion of L-655,708 at four doses (100, 200, 400 and 600 ng/1 µl/side) had no effect on locomotor activity. Experiment 2a (A; n = 16 rats); Experiment 2b (B; n = 16 rats). Vehicle: 50:50 DMSO, saline.

3.4 Experiment 2: No effect of ventral hippocampal infusion of L-655,708 on locomotor activity 48 h after infusion

A previous study reported decreased immobility and increased climbing on the forced swim test 48 h after ventral hippocampal infusion of L-655,708 (90 ng/1 µl; Bugay et al., 2020). In Experiment 2, we included extra baseline days so the effect of L-655,708 after 48 h could be measured. Ventral hippocampal infusion of L-655,708 (100 ng, 200 ng, 400 ng and 600 ng/1 µl/side) did not affect locomotor activity 48 h after infusion during Experiment 2 (a or b, all main effects of infusion condition or interactions with infusion condition, $F < 1.7$, $p > 0.16$; data not shown).

3.5 Ventral hippocampal infusion of L-655,708 tended to enhance amphetamine-induced locomotor hyperactivity

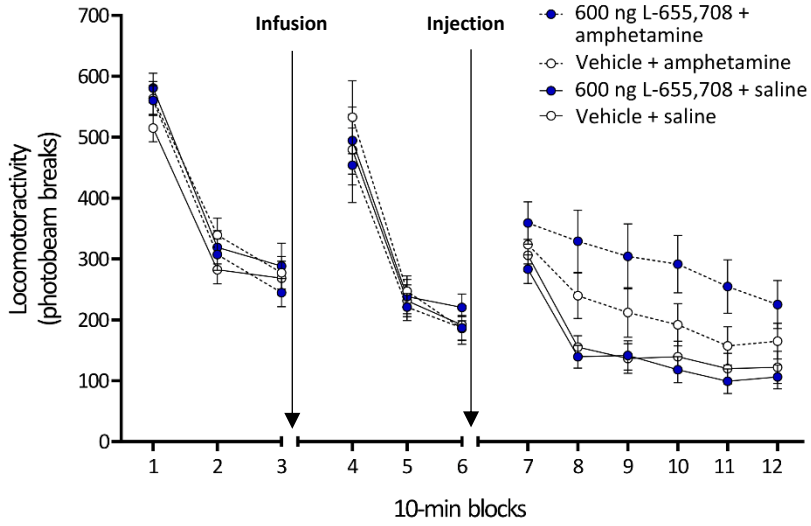
In Experiment 3, L-655,708 (600 ng/1 µl/side) tended to enhance amphetamine-induced hyperlocomotion (Figure 3-6). All prospective ventral hippocampal infusion conditions/systemic injection conditions showed similar locomotor activity during the 30-min pre-infusion/injection baseline (main effect of prospective infusion condition: $F < 1$, main effect of prospective injection condition: $F < 1$, prospective infusion condition X prospective injection condition X time block interaction: $F < 1$). However, there was a trend for a prospective infusion condition X prospective injection condition interaction ($F_{(1,14)} = 3.666$, $p = 0.076$). This reflected a trend (simple main effects: $F = 4.012$, $p = 0.065$) for rats in the L-655,708 infusion + saline injection condition to have slightly higher locomotor activity during the pre-infusion/injection baseline than the rats in the vehicle infusion + saline injection condition.

Following ventral hippocampal infusion of L-655,708 or vehicle, all conditions showed similar locomotor activity (all main effects and interactions involving infusion condition and prospective injection condition: $F < 1$, $p > 0.36$). Only a main effect of time was observed ($F_{(1.211,16.957)} = 79.620$, $p < 0.001$), reflecting decreased activity across time.

Following systemic injection of amphetamine or saline in rats that had received ventral hippocampal infusion of L-655,708 or vehicle, there was a main effect of systemic injection condition ($F_{(1,14)} = 16.067$, $p = 0.001$), reflecting that amphetamine increased locomotion, a main effect of time ($F_{(4.217,59.041)} = 28.481$, $p < 0.001$), indicating decreased activity across time and no effect of ventral hippocampal infusion condition ($F_{(1,14)} = 1.924$, $p = 0.187$) or a ventral hippocampal infusion condition X time interaction ($F < 1$). There was a trend towards a systemic injection condition X time interaction ($F_{(2.751,48.878)} = 2.610$, $p = 0.070$), reflecting that the amphetamine-induced hyperactivity was somewhat weaker during the first 10-min block after systemic injection compared to the other 10-min blocks. Interestingly, there was also a strong trend towards a ventral hippocampal infusion condition X

systemic injection condition interaction ($F_{(1,14)}$ = 4.445, p = 0.054). This reflected that ventral hippocampal infusion of L-655,708 tended to enhance amphetamine-induced hyperlocomotion, in line with our hypothesis. Simple main effects analysis of this trend revealed a trend towards a difference between the L-655,708 and vehicle infusion conditions in the amphetamine injection condition ($F_{(1,14)}$ = 3.383, p = 0.091) but not in the saline injection condition ($F_{(1,14)}$ = 1.474, p = 0.245).

Figure 3-6: Experiment 3: Ventral hippocampal infusion of L-655,708 (600 ng/1 µl/side) tended to enhance amphetamine-induced hyperlocomotion.



Note. Data are shown as mean (± SEM); n = 15 rats. Ventral hippocampal infusion of L-655,708 (600 ng/1 µl/side) tended to enhance amphetamine-induced hyperlocomotion. Vehicle: 50:50 DMSO, saline.

3.6 Behavioural effects of ventral hippocampal infusions that were evident based on visual inspection

Some rats displayed behavioural effects after ventral hippocampal infusion that were evident based on visual inspections (Table 3-2). Behavioural effects are reported for all rats that received infusions, regardless of whether they were included in the data analysis (Experiment 1: n=16; Experiment 2: n=16; Experiment 3: n=15). 'Small head shakes' refer to small rotational movements (in both directions) of just the head of

the rat. ‘Wet dog shakes’ refer to when a rat shook its head and torso, much like when a wet dog shakes (the same as the wet dog shakes described in Chapter 2). ‘Jumpiness’ refers to when a rat would jump when an experimenter went to pick the rat up. ‘Wild running’ refers to the rat running around the locomotor activity box and trying to run up the sides of the box. The ‘small head shakes’ were observed at the time of infusion, whereas the other behavioural effects were mainly observed in the locomotor activity boxes post-infusion.

These gross behavioural effects observed were observed with the DMSO vehicle alone and with L-655,708. In previous studies, no behavioural effects have been observed with saline infusions (Taylor et al., 2025; Williams et al., 2022), suggesting the observed behavioural effects with vehicle infusions were due to the DMSO. No gross behavioural effects evident based on visual inspection were recorded due to the systemic amphetamine and saline injections in Experiment 3. More behavioural effects were observed when the dose of L-655,708 was increased from Experiments 1 and 2a to 2b and 3. We analysed the difference in the number wet dog shakes between the 600 ng condition and the vehicle condition in Experiments 2 and 3. As we expected that there would be an increase in wet dog shakes with the highest dose (600 ng; similar to our findings with picrotoxin), we used a one-tailed Fisher’s Exact Test. A trend level ($p = 0.086$) increase in the number of rats showing wet dog shakes was observed with 600 ng versus vehicle in Experiment 2. Interestingly, in Experiment 3 there was a similar number of rats showing wet dog shakes in the 600 ng and vehicle conditions. It is unclear why there was such an increase in gross behavioural effects in the vehicle condition in this experiment. Overall, while L-655,708 may have induced some gross behavioural effects, this was much less pronounced compared to what we observed following ventral hippocampal infusion of picrotoxin (Chapter 2; Taylor et al., 2025; Williams et al., 2022). In addition, ventral hippocampal DMSO may also have caused some weak behavioural effects.

Table 3-2: Gross behavioural effects after ventral hippocampal infusion of doses of L-655,708 or vehicle (50:50 DMSO, saline) in Experiments 1–3.

		Number of rats for which the behaviour was observed.			
		Small head shakes	Wet dog shakes	Jumpiness	Wild running
Experiment 1a: LMA	Vehicle	4			
	50 ng / 0.5 µl	3			
	100 ng / 0.5 µl	6			
Experiment 1b: PPI/startle	Vehicle	2			
	50 ng / 0.5 µl	1			
	100 ng / 0.5 µl	1			
Experiment 2a: LMA	Vehicle	1			
	100 ng / 1 µl	5			
	200 ng / 1 µl	3			
Experiment 2b: LMA	Vehicle	1	1		
	400 ng / 1 µl	1*	2*†	1†	
	600 ng / 1 µl	1*	5*†		2†
Experiment 3: LMA	Vehicle	11	12		
	600 ng / 1 µl	7	11	1‡	1‡

Note. Behaviours observed following ventral hippocampal infusions of a dose of L-655,708 or vehicle during LMA and PPI/startle testing in Experiment 1 (n=16 rats), 2 (n=16 rats) and 3 (n=15 rats).

Behavioural definitions can be found in section 3.6. *, † and ‡ indicate multiple behaviours from the same rat, such as showing small head shakes and wet dog shakes. No behavioural effects were observed with amphetamine or saline injections. Vehicle: 50:50 DMSO, saline. Abbreviations: DMSO: Dimethyl sulfoxide; LMA: Locomotor activity: PPI: Prepulse inhibition.

4 Discussion

Ventral hippocampal infusion of the α5-GABAAR NAM, L-655,708, did not affect baseline locomotor activity, PPI or startle. The effect of L-655,708 on locomotor activity was investigated using six doses, ranging from 50 ng/0.5 µl/side to 600 ng/1 µl/side, and no effect was observed on locomotor activity at any dose. PPI and startle were investigated using two doses of L-655,708 (50 ng or 100 ng/0.5 µl/side), and neither measure was affected by L-655,708 infusion. Although ventral hippocampal L-655,708 (600 ng/1 µl/side) did not affect spontaneous locomotor activity, it tended to increase amphetamine-induced locomotor hyperactivity.

4.1 Ventral hippocampal L-655,708 did not affect baseline locomotor activity

In Experiment 1, ventral hippocampal L-655,708, at doses of 50 and 100 ng/0.5 µl/side, did not affect locomotor activity. We hypothesised that a larger volume may be required to achieve better drug spread, which may have been limited due to the viscosity of the drug solution. In Experiment 2, the infusion volume was increased to 1 µl, as previous studies had used higher volumes (1 µl and 0.75 µl; Bugay et al., 2020; Perez et al., 2022). In Experiment 2, four doses were investigated (100, 200, 400 and 600 ng/1 µl/side) for their effect on locomotor activity, and again no effect of L-655,708 was observed on baseline locomotor activity.

There has been no previous research, to our knowledge, that has site specifically targeted the ventral hippocampal α5-GABAARs and investigated the effect on locomotor activity. Other studies addressing the role of α5-GABAARs in locomotor activity, using genetic methods to manipulate hippocampal α5-GABAARs and systemic administration of α5-GABAAR modulators, were inconclusive. On the one hand, we thought that decreasing the activity at ventral hippocampal α5-GABAARs with L-655,708 may have increased locomotor activity. Firstly, it has been found that a mutation that decreased the number of α5-GABAARs in the hippocampus led to increased locomotor activity (Hauser et al., 2005). Secondly, we hypothesised that L-

655,708 ventral hippocampal negative allosteric modulation may have acted on the dopamine system to increase locomotor activity. It has been previously reported that disinhibition of GABA-A receptors in the ventral hippocampus results in increased hippocampal activity (McGarrity et al., 2017) and increased ventral hippocampal activity can increase dopamine activity (Lodge & Grace, 2007). This can be through the direct glutamatergic projection of the ventral hippocampus to the NAc (Lindenbach et al., 2022; Taepavarapruk et al., 2008), or through a polysynaptic pathway: the ventral hippocampus has glutamatergic projections to the NAc, the NAc in turn has inhibitory projections to the VP. The VP then has inhibitory projections to the VTA, which has projections back to the NAc of the VS; see Figure 3-1 (Floresco et al., 2001, 2003; Lodge & Grace, 2006). Increased dopamine activity in the VS has been found to increase locomotor activity (Beninger, 1983; Ikemoto & Panksepp, 1999). Interestingly, SST+ interneurons selectively activate α5-GABAARs (Schulz et al., 2018), and SST+ interneurons preferentially target pyramidal neurons that project to the NAc (Lodge et al., 2023), suggesting α5-GABAARs may contribute particularly to the control of these ventral hippocampal – NAc projections. Through ventral hippocampal L-655,708 infusion increasing dopamine activity, either by the direct ventral hippocampal – NAc pathway, or through the polysynaptic pathway involving VTA dopamine neurons (see Figure 3-1B), it was plausible that an increase in locomotor activity would have be seen. Ventral hippocampal infusion of L-655,708 increased dopamine neuron activity in the VTA, and it was suggested this was through the polysynaptic pathway (Perez et al., 2022). However, in our first two experiments no increase in locomotor activity was observed with L-655,708 infusion.

In contrast, systemic administration of L-655,708 was reported to have no effect on locomotor activity 5–30 min post-injection (Bugay et al., 2020; Fischell et al., 2015; Magnin et al., 2019), which is supported by our findings with ventral hippocampal L-655,708 infusion. Additionally, in an α receptor knockout model, rats with three point mutations that desensitise the α1, α2 and α3 receptors to diazepam (leaving only α5 receptors sensitive to diazepam; DZα5), positive modulation of α5-GABAARs with systemic administration of did not affect locomotor activity (Behlke et al., 2016),

suggesting that α5-GABAARs may not be involved in the modulation of locomotor activity. It is also possible we did not observe an increase in locomotor activity with ventral hippocampal L-655,708 infusion because the dose was not high enough. However, we increased the dose considerably compared to previous studies that have used hippocampal L-655,708 infusion (Bugay et al., 2020; Perez et al., 2022), and we also observed a higher incidence of behavioural effects in the higher dose conditions (Table 3-2), suggesting L-655,708 was having some effect in the brain, though we note that side effects may also be due to the DMSO in the vehicle (discussed further in section 4.4).

Interestingly, a decrease in immobility and an increase in climbing were observed in the forced swim test 48 h after ventral hippocampal infusion of L-655,708 (Bugay et al., 2020). To our knowledge, this is the only study to report the effect of site specific ventral hippocampal α5-GABAAR pharmacological manipulations on behaviour. However, the effect of ventral hippocampal L-655,708 on dopamine neuron activity was observed 20 min post-infusion (Perez et al., 2022), so we expected to see an effect of L-655,708 on locomotor activity soon after infusion, as we expected L-655,708 would increase locomotion through its action on the dopamine system. In Experiment 2, we analysed the effect of the L-655,708 infusion 48 h later, but no differences were observed between infusion conditions.

It has been suggested that increased activity in the VTA alone due to hippocampal hyperactivity may not increase NAc dopamine release but may instead increase susceptibility to stimulation of the dopamine system (Lodge & Grace, 2008). For example, the MAM rat model shows increased ventral hippocampal activity, but basal locomotor is unaffected. MAM rats do, however, show an increased response to amphetamine, with a significantly increased locomotor response compared to control rats receiving amphetamine (Flagstad et al., 2004; Lodge & Grace, 2007; Moore et al., 2006), suggesting that increased hippocampal activity sensitises the dopaminergic response and augments the locomotor response to amphetamine. Indeed, when we investigated the effect of ventral hippocampal L-655,708 infusion

on amphetamine-induced hyperlocomotion, this hyperlocomotion tended to be augmented by ventral hippocampal infusion of L-655,708.

4.2 Ventral hippocampal infusions of L-655,708 tended to enhance amphetamine-induced hyperlocomotion

Based on this idea that α5-GABAAR modulation may not affect baseline dopamine release and subsequent locomotor activity but may instead sensitise the dopamine system's response to stimulation (Lodge & Grace, 2007, 2008), we predicted that infusion of the ventral hippocampal α5-GABAAR NAM, L-655,708, may augment a locomotor response to amphetamine. We found that ventral hippocampal infusion of L-655,708 tended to augment the increase in locomotion with amphetamine. Although the effect did not reach statistical significance, our findings are in line with research showing that modulating hippocampal activity enhances the locomotor response to amphetamine, while not affecting baseline locomotion. Hippocampal lesions have been reported to augment the locomotor response to amphetamine, but not to affect baseline locomotor activity (Wilkinson et al., 1993), increased ventral hippocampal activity in MAM rats augmented amphetamine-induced hyperlocomotion (Flagstad et al., 2004; Lodge & Grace, 2007; Moore et al., 2006), and removal of ventral hippocampal perineuronal nets, which increased ventral hippocampal activity, enhanced amphetamine-induced hyperlocomotion, but did not increase baseline locomotion (Shah & Lodge, 2013). Furthermore, our findings are consistent with the idea that the ventral hippocampus modulates midbrain dopamine activity (Floresco et al., 2001, 2003; Wilkinson et al., 1993) and that L-655,708 specifically increases dopamine neuron activity in the VTA (Perez et al., 2022), as we suggest that the tendency towards an increase in amphetamine-induced hyperlocomotion with L-655,708 is through the action of ventral hippocampal activity on midbrain dopaminergic neuron activity, although this would need to be confirmed in future work. α5-GABAARs specifically may contribute to this modulation of midbrain dopaminergic activity through activation of SST+ interneurons, which

selectively activate α5-GABAARs in CA1 (Schulz et al., 2018), and preferentially target pyramidal neurons that project to the NAc (Lodge et al., 2023). In contrast to our findings, negative α5-GABAAR modulation was found to attenuate the increase in locomotor activity with amphetamine, though this was through systemic administration of an α5-GABAAR NAM. Here, they also found systemic administration of an α5-GABAAR PAM to attenuate the increase in locomotor activity with amphetamine (Redrobe et al., 2012). This was not in line with previous work that found positive modulation of α5-GABAARs did not alter the response to amphetamine, but that positive modulation attenuated the increased response to amphetamine in MAM rats (Gill et al., 2011). Hippocampal activity can regulate midbrain dopamine activity, and our findings suggest that ventral hippocampal α5-GABAARs have a role in this modulation of dopamine activity.

4.3 Ventral hippocampal infusions of L-655,708 did not affect PPI or startle

Ventral hippocampal infusions of L-655,708 at doses of 50 and 100 ng/0.5 µl/side, did not affect PPI or startle. This is consistent with previous findings that systemic administration of L-655,708 did not affect PPI in rats (Carreno et al., 2017), and that ventral hippocampal disinhibition with the non-specific GABAAR antagonist, picrotoxin, in Lister hooded rats had no effect on PPI (McGarrity et al., 2017), although ventral hippocampal picrotoxin did disrupt PPI in Wistar rats (Bast, Zhang, & Feldon, 2001b). In contrast to our findings, an attenuation in PPI with a reduction in hippocampal α5-GABAARs in a knockdown mouse model (α5(H105R)) has been reported (Hauser et al., 2005). However, it should be noted that the α5(H105R) model targets the whole dorsal-ventral axis of the hippocampus, while our manipulation focused on the ventral hippocampus. The dorsal hippocampus also has a role in PPI (Zhang et al., 2002b), so this may explain the difference in findings. Additionally, although the α5(H105R) model is a partial knockdown, which should reduce compensatory changes, these may still occur and there may be upregulation of other

α subunits and shifts in the excitatory/inhibitory balance; such alterations are less likely to occur with an acute ventral hippocampal infusion of L-655,708.

We expected that ventral hippocampal L-655,808 infusion may reduce startle reactivity, as a decrease in startle reactivity was seen with picrotoxin in Lister hooded rats (McGarrity et al., 2017). However, our findings did not reveal any effect of ventral hippocampal L-655,708 on startle reactivity, which may reflect that α5-GABAARs, the target of L-655,708, account for only 20–25% of hippocampal GABA-A receptors (McKernan & Whiting, 1996; Sur et al., 1999). In line with our findings, no effect on startle was observed in the α5(H105R) model (Hauser et al., 2005). It should also be noted that we used a much lower dose for our PPI and startle experiments (50–100 ng/0.5 µl/side) than the dose of L-655,708 that tended to augment the amphetamine-induced increase in locomotor activity (600 ng/1 µl/side). We cannot rule out that a higher dose of L-655,708 would have had an effect on PPI and startle.

4.4 Gross behavioural effects of ventral hippocampal infusions of L-655,708

In Experiment 2b, we found that rats were more likely to show wet dog shakes following ventral hippocampal infusion of the higher doses of L-655,708, compared to the vehicle condition. There was a trend towards increased wet dog shakes with the highest dose of L-655,708 (600 ng/1 µl/side) compared to vehicle infusions in Experiment 2b, but this was not replicated in Experiment 3. Wet dog shakes, which were observed in some rats when given 400 or 600 ng/1 µl/side L-655,708 (and one rat in the vehicle condition in Experiment 2; this rat had wet dog shakes in all conditions), are observed in many rats following ventral hippocampal picrotoxin infusions (Chapter 2; Taylor et al., 2025; Williams et al., 2022). The tendency towards an increase in wet dog shakes observed with the higher doses of L-655,708 supports that these doses had some effect in the brain, although it is noted that some behavioural effects were observed in the vehicle condition, especially in Experiment 3. It is unclear why there was a higher prevalence of behavioural effects in the vehicle condition in this experiment compared to the previous experiments. In previous

work, no behavioural effects were observed in the saline vehicle condition in a similar locomotory activity experiment (McGarrity et al., 2017; Taylor et al., 2025), suggesting the effects observed in these experiments were due to the DMSO. Future work could include a saline only control group.

4.5 Clinical implications

Imaging studies have shown hippocampal hyperactivity in schizophrenia (McHugo et al., 2019; Schobel et al., 2009, 2013; Talati et al., 2014; Tregellas et al., 2014), with specific impairments in GABA transmission (Frankle et al., 2015), while post-mortem studies have revealed reductions in GABAergic markers within the hippocampus in schizophrenia (de Jonge et al., 2017; Dienel & Lewis, 2019; Gonzalez-Burgos et al., 2010). More specifically, antipsychotic free patients with schizophrenia have reduced expression of hippocampal α5-GABAARs, and this may contribute to this increase in hippocampal excitability, with the reduction in α5-GABAARs decreasing tonic inhibition (Marques et al., 2020). Patients with schizophrenia also have increased dopamine system responsivity (Howes et al., 2012; Laruelle et al., 1999), reflected by increased behavioural and neurochemical responses to dopamine-releasing drugs, such as amphetamine (Weidenauer et al., 2020). Our findings suggest that these features of schizophrenia may be linked, with reduced hippocampal α5-GABAARs contributing to this heightened dopamine system responsivity. Hippocampal hyperactivity has been linked to the dopamine dysfunction in schizophrenia (Grace, 2016; Heckers & Konradi, 2015; Lisman et al., 2008; Lodge & Grace, 2007, 2011), but it may be that a reduction in α5-GABAAR activity specifically leads to this increased dopamine system responsivity. Indeed, overexpression of α5-GABAARs within the ventral hippocampus was found to normalise hippocampal pyramidal cell activity and VTA dopamine neuron activity in the MAM rat model, which led to an attenuation in extradimensional set-shifting, but not reversal learning, deficits (Donegan et al., 2019).

4.6 Conclusions and future studies

We found that ventral hippocampal α5-GABAARs play no major role in controlling locomotor activity, PPI or the acoustic startle response. Negative allosteric modulation of α5-GABAARs, however, tended to augment amphetamine-induced locomotor hyperactivity, supporting that ventral hippocampal α5-GABAARs negatively modulate responsivity of dopamine neurons projecting from the VTA to the VS, consistent with electrophysiological studies (Perez et al., 2022). Though these results should be interpreted with caution, and should be replicated in future work, given the effect did not reach statistical significance. Our findings may have implications for schizophrenia, which is characterised by hippocampal hyperactivity and dopamine dysfunction (Heckers & Konradi, 2015; Howes et al., 2012). In future work, it would be of interest to examine if ventral hippocampal α5-GABAARs contribute specifically to memory and other cognitive functions disrupted by ventral hippocampal GABA-A receptor activity changes (inhibition and/or disinhibition), including rapid place learning (McGarrity et al., 2017), novel object recognition (Taylor et al., 2025) and reversal learning (Chapter 2). Given the lack of effect on baseline sensorimotor processes, infusion of L-655,708 for such cognitive and behavioural tasks should not interfere with the rats' ability to perform these tasks.

Chapter 4: General Discussion

1 Project aims

This thesis investigated the role of the ventral hippocampus and of local GABAergic inhibition, including of α5-GABAARs, in neurobehavioural function in rats. Hippocampal neural disinhibition i.e. reduced GABAergic inhibition, has been linked to schizophrenia pathophysiology and cognitive and behavioural impairments (Heckers & Konradi, 2015; Marín, 2012). Here, we examined the contribution of ventral hippocampal activity to selected innate and learnt behaviours. We set out to address two aims. Firstly, we aimed to investigate the role of ventral hippocampal activity in early and late serial reversal learning. To this end, we examined the impact of bidirectional changes of ventral hippocampal activity, caused by ventral hippocampal inhibition and disinhibition (by local infusion of the GABA-A receptor agonist, muscimol, and antagonist, picrotoxin) on reversal learning on a food-reinforced two-lever discrimination task in rats. Secondly, we aimed to investigate the significance of ventral hippocampal α5-GABAARs for locomotor activity, PPI and startle reactivity. To this end, we examined the impact of negative allosteric modulation of ventral hippocampal α5-GABAARs (by local infusion of L-655,708) on these behaviours in rats.

2 Overview of the main findings

First, we considered how ventral hippocampal GABA-A receptor activity influences reversal learning, following on from previous work which investigated contributions of ventral hippocampal activity to other cognitive and behaviour functions, including locomotor activity, PPI, startle, attention, rapid place learning and novel object recognition (McGarrity et al., 2017; Taylor et al., 2025), and from our recent work considering the role of mPFC activity for reversal learning (Renström et al., 2025). We then focused on negative modulation of a specific type of GABA-A receptors, α5-GABAARs, which are preferentially expressed in the hippocampus (McKernan &

Whiting, 1996). Ventral hippocampal disinhibition with picrotoxin and negative modulation of ventral hippocampal α5-GABAARs with L-655,708 both decrease GABAergic inhibition, thereby increasing excitability (Atack et al., 2006; Bugay et al., 2020; McGarrity et al., 2017; Papatheodoropoulos & Koniaris, 2011; Schulz et al., 2018; Williams, 2021). Here, we began to investigate the significance of ventral hippocampal α5-GABAARs for behaviours previously found to be affected by hippocampal disinhibition, namely locomotor activity, PPI and startle (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017).

2.1 Ventral hippocampal inhibition tended to disrupt late serial reversal learning, whereas disinhibition tended to improve early serial reversal learning

In Chapter 1, we reviewed the current evidence for a role of the hippocampus in reversal learning. We highlighted that previous studies examined different stages of reversal learning and separated the literature into studies that manipulated the hippocampus before the acquisition of reversal learning, or after, and whether they investigated early or late serial learning; these were termed pre-acquisition manipulation early, pre-acquisition manipulation early serial and post-acquisition manipulation late serial reversal learning studies. These differences are important, as early and late stages of reversal learning require distinct psychological mechanisms, and the timing of the manipulation can also change how the task is learnt. There was some evidence for the requirement of the hippocampus in the pre-acquisition manipulation early reversal studies (Berger & Orr, 1983; Jang et al., 2015; Kimble & Kimble, 1965; E. A. Murray et al., 1998; Silveira & Kimble, 1968; Weikart & Berger, 1986), as well as in post-acquisition manipulation late reversal tasks (Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024).

In Chapter 2, we examined the impact of ventral hippocampal inhibition and disinhibition on two stages of a two-lever reversal task in rats: pre-acquisition early serial reversal and post-acquisition late serial reversal. We found that ventral hippocampal disinhibition tended to improve early serial reversal performance,

whilst ventral hippocampal inhibition tended to disrupt late serial reversal performance. These findings suggest that ventral hippocampal activity may play a role in reversal learning, though the effects did not reach statistical significance. Therefore, our results should be interpreted with caution and would benefit from confirmation by future studies.

We propose that in our early serial reversal study, rats in the ventral hippocampal inhibition group had normal performance (not different from the control group) because the hippocampus was disrupted during initial learning of the reversal, and therefore rats learnt the task without intact hippocampal function, which may have allowed for extra-hippocampal brain regions to compensate for the absence of intact hippocampal function. In contrast, rats in the ventral hippocampal inhibition condition in the late serial reversal study tended to have impaired performance, which may reflect that they learnt to reverse with an intact hippocampus during early reversals, when the ventral hippocampus was not manipulated, and, therefore, subsequent late reversal performance was dependent on hippocampal function. This suggests that the ventral hippocampus may normally contribute to learning how to reverse and is then required for expressing such learning. However, if ventral hippocampus is not available while rats learn to reverse, other brain regions can compensate, and rats are still able to reverse. This is in line with studies of spatial memory performance in the Morris watermaze, where ventral hippocampal inhibition or lesions before learning did not affect subsequent performance, but manipulations after learning disrupted performance (de Hoz & Martin, 2014; Moser & Moser, 1998b), and with contextual fear conditioning studies, which found hippocampal lesions after training caused retrograde amnesia for context discrimination, but lesions before training did not induce anterograde amnesia (J. Q. Lee et al., 2017). The contribution of the ventral hippocampus to reversal learning may be that the ventral hippocampus creates a representation of the task, as has been proposed for ventral CA1 in mice (Mishchanchuk et al., 2024), the anterior hippocampus in non-human primates (Knudsen & Wallis, 2021), and the hippocampus in humans (Vilà-Balló et al., 2017), that contributes to efficient

performance during late serial reversals. Additionally, it should be noted that whilst the inhibition of the ventral hippocampus tended to disrupt late serial reversal learning, ventral hippocampal disinhibition did not affect late serial reversal learning. This suggests that, although the ventral hippocampus tends to be required late reversal learning (if the ventral hippocampus is intact during early reversal learning), balanced ventral hippocampal activity (which would be disrupted by ventral hippocampal disinhibition) is not required. This contrasts with previous findings that both lesions and disinhibition of hippocampal activity disrupted rapid place learning performance in the DMP watermaze, suggesting that such performance requires balanced levels of ventral hippocampal activity (Bast et al., 2009; McGarrity et al., 2017).

Interestingly, ventral hippocampal disinhibition tended to improve performance during early serial reversal learning, in line with previous work (Bortz et al., 2019). As indicated above, ventral hippocampal disinhibition did not disrupt late serial reversal learning, suggesting that balanced hippocampal activity is not required for late serial reversal learning. One of our hypotheses was that ventral hippocampal disinhibition may disrupt both stages of reversal learning through downstream actions on projection sites, including the mPFC, which we have recently found to be required for early serial reversal learning (Renström et al., 2025). Ventral hippocampal disinhibition disrupted expression of the previous response in a similar manner to mPFC disinhibition, so this may reflect that ventral hippocampal disinhibition changed mPFC activation. For mPFC disinhibition, we suggested that this disruption to the previous rule may have contributed to the facilitation of early reversal learning, which was observed at R2 (Renström et al., 2025). This may also contribute to the facilitation by ventral hippocampal disinhibition in the present study, however, unlike for mPFC disinhibition, ventral hippocampal disinhibition also improved SD, and facilitation of reversal learning was not limited to R2. Other mechanisms, in addition to mPFC activation and disruption of the previous response, may therefore contribute to the trend towards an improvement in performance across SD and reversals. It may be that activation of the midbrain dopamine system plays a role, as

has been suggested for operant learning (Robbins & Everitt, 2006). Indeed, dopamine antagonism was found to prevent the enhancement of reversal learning performance observed with ventral subiculum activation (Bortz et al., 2019).

2.2 Ventral hippocampal infusion of the α5-GABAAR negative allosteric modulator L-655,708 did not affect baseline locomotor activity, prepulse inhibition or startle, but tended to enhance amphetamine-induced hyperlocomotion in rats

In Chapter 1, we discussed current evidence for a contribution of hippocampal α5-GABAARs to the regulation of locomotor activity, PPI and startle. Evidence for the role of α5-GABAARs specifically in the hippocampus was limited to transgenic mouse models (Hauser et al., 2005), with much of the literature focused on systemic targeting of α5-GABAARs (Bugay et al., 2020; Carreno et al., 2017; Fischell et al., 2015; Magnin et al., 2019).

In Chapter 3, we examined the impact of negative allosteric modulation of ventral hippocampal α5-GABAARs on locomotor activity, PPI and startle. Previous research reported an increase in VTA dopamine neuron activity with ventral hippocampal infusion of L-655,708 (Perez et al., 2022). Therefore, we hypothesised that ventral hippocampal α5-GABAAR negative modulation may increase locomotor activity through increasing dopamine neuron activity in the VTA – VS pathway. However, using local infusions of the α5-GABAAR NAM, L-655,708, at doses ranging from 50 ng/0.5 µl/side to 600 ng/1 µl/side, we found that there was no change in baseline locomotor activity. We then examined the idea that ventral hippocampal α5-GABAAR negative modulation may not affect baseline locomotor activity but may instead sensitise the dopamine system's response to stimulation and, thereby, augment the locomotor response to amphetamine as previously suggested for other manipulations of hippocampal activity (Lodge & Grace, 2007, 2008; Shah & Lodge, 2013; Wilkinson et al., 1993). In line with this hypothesis, we found ventral hippocampal infusion of L-655,708 tended to augment amphetamine-induced hyperactivity. However, this result did not reach statistical significance and would

benefit from confirmation by future work. Regarding PPI and startle, we found no effect of ventral hippocampal infusion of L-655,708 on either behaviour at 50 or 100 ng/0.5 µl/side. However, we cannot rule out that a higher dose of L-655,708 would have affected these behaviours.

3 Relevance of our findings to schizophrenia

Our findings regarding the role of the ventral hippocampus in reversal learning, and of ventral hippocampal α5-GABAARs in sensorimotor functions, are relevant for the understanding of behavioural impairments in schizophrenia. Schizophrenia is characterised by hippocampal hyperactivity, which may be a consequence of neural disinhibition, and there is substantial evidence for disrupted hippocampal GABAergic inhibition, as well as some evidence for reduced expression of α5-GABAARs, in schizophrenia (discussed in Chapter 1).

Deficits in reversal learning (Leeson et al., 2009; G. K. Murray et al., 2008; Reddy et al., 2016; Waltz & Gold, 2007) and hippocampal hyperactivity (Heckers & Konradi, 2010, 2015; McHugo et al., 2019; Talati et al., 2014; Tregellas et al., 2014) are characteristic of schizophrenia. However, our findings do not support that the latter contributes to the former. We did not find ventral hippocampal disinhibition to impair early or late serial reversal learning and instead found a tendency towards an improvement in early serial reversal performance with ventral hippocampal disinhibition. Our findings do not support that the reversal learning deficits in schizophrenia are a consequence of hippocampal hyperactivity and instead suggest that such deficits are more likely to be the consequence of dysfunction in other brain areas implicated in reversal learning, such as the OFC or mPFC (discussed in Renström et al. (2025) and Renstrom (2023)).

Reduced expression of hippocampal α5-GABAARs has been reported in antipsychotic free patients with schizophrenia (Marques et al., 2020), and this reduction may contribute to the increase in hippocampal hyperactivity observed in schizophrenia

(Heckers & Konradi, 2010, 2015; McHugo et al., 2019; Talati et al., 2014; Tregellas et al., 2014). Patients with schizophrenia also have increased dopamine system responsivity (Howes et al., 2012; Laruelle et al., 1999). Our findings that negative modulation of ventral hippocampal α5-GABAARs tended to increase locomotor hyperactivity in response to amphetamine injection suggest that a reduction in hippocampal α5-GABAARs contributes to this heightened dopamine system responsivity.

4 General limitations

Some of the effects in this thesis did not reach conventional statistical significance and as such would benefit from replication in a larger sample in future work. The trends we observed towards a facilitation in early reversal learning with ventral hippocampal disinhibition and a disruption in late serial reversal learning with ventral hippocampal inhibition are in line with previous findings (Bortz et al., 2019; Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024). Additionally, the trend effect of L-655,708 to enhance amphetamine-induced locomotion is in line with what we predicted based on other hippocampal activity manipulations (Lodge & Grace, 2008; Shah & Lodge, 2013; Wilkinson et al., 1993).

All experiments in this thesis were carried out in young adult male rats. We used male rats because our experiments built on previous work in male rats, using the same stereotaxic coordinates as our previous studies involving ventral hippocampal manipulations (Bast, Zhang, & Feldon, 2001b; McGarrity et al., 2017; Taylor et al., 2025; Williams et al., 2022), and the same behavioural tests that have been developed using male rats (Gonçalves et al., 2023; Hock, 2020; McGarrity et al., 2017; Pezze et al., 2014; Renström et al., 2025; Taylor et al., 2025). Our experiments are also in line with other related studies of reversal learning (Bortz et al., 2019; Cernotova et al., 2021; Kosaki & Watanabe, 2012; Mishchanchuk et al., 2024; Morellini et al., 2010) and sensorimotor functions (Carreno et al., 2017; Fischell et al., 2015) that used male rats. For use of female rats, different stereotaxic coordinates

may be required, doses for ventral hippocampal drug infusions may need to be adapted to ensure similar physiological effects and behavioural tests may need to be adapted to ensure performance is at an appropriate level, so that both increases and decreases in performance could be detected. Additionally, we expect that fundamental brain mechanisms as examined in this thesis apply to both male and female rats, although for the reasons listed, such mechanisms are, at present, easier to study in male rats. However, we acknowledge that this is a limitation in our study design and that future work would benefit from inclusion of female rats.

Many neuroscience studies in rodents only use one sex (mostly males, but some studies include only females, for example, see Gigg et al. (2020)), and studies that do include both sexes often do not analyse sex differences (Beery & Zucker, 2011; Karp & Reavey, 2019). In studies that have investigated sex differences, differences have been reported in rats and non-human primates for reversal learning, with females performing worse than males (Gargiulo et al., 2022; Laclair et al., 2019). This does not necessarily imply that the basic hippocampal mechanisms found to be relevant to reversal learning in this thesis do not apply to females. However, it may suggest that any impairments in reversal learning may be more difficult to detect in females, because the lower baseline performance may lead to a floor effect. Additionally, we have recently found sex differences in locomotor activity and startle, with female rats showing increased locomotor activity and male rats showing an increased startle response (Taylor et al., 2025). These findings are in line with research in mice, where female mice showed increased locomotor activity and male mice showed increased startle reactivity (Hauser et al., 2005). Again, this does not necessarily mean that the hippocampal mechanisms revealed in the current thesis do not apply to females. In future work, it would be of interest to investigate whether our findings of the involvement of ventral hippocampus in reversal learning and of ventral hippocampal α5-GABAARs in amphetamine-induced hyperlocomotion can be replicated in female rats.

Another potential limitation of the work discussed in this thesis is the light cycles under which rats were housed and tested. All handling and behavioural procedures

used for the studies in this thesis were conducted during the light cycle (12-h cycle; lights on at 07.00), but rats are nocturnal. Whilst this is in line with much of the previous work on reversal learning (Cernotova et al., 2021; Chudasama & Robbins, 2003; Renström et al., 2025), locomotor activity (Fischell et al., 2015; McGarrity et al., 2017; Pezze et al., 2014; Taylor et al., 2025; Wilkinson et al., 1993) and PPI and startle (Fischell et al., 2015; McGarrity et al., 2017; Pezze et al., 2014), and is experimentally practical, it is important to acknowledge this could have affected the influence of our ventral hippocampal manipulations on reversal learning performance and sensorimotor functions. Indeed, research has indicated that the time-of-day rodents are tested can have effects on their behaviour and physiology, such as increased heart rate, which is indicative of stress, as well as changes to circadian rhythms (Castelhano-Carlos & Baumans, 2009; Hawkins & Golledge, 2018). Stress has been reported to cause a reversal impairment in rats (Lapiz-Bluhm et al., 2008), to increase the locomotor response to amphetamine (Herman et al., 1984), and to reduce PPI and startle (Santos-Carrasco et al., 2025), though in these studies the stressor was not light. Therefore, the impact of light-induced stress may be important, but we believe this was controlled in our experiments via several aspects. Reversal and locomotor activity testing were always conducted in dark boxes with light levels controlled and limited to low levels (reversal boxes: 40 lux; locomotor activity boxes: 25–40 lux); PPI and startle were tested in brighter light due to the nature of the experiment. Additionally, rats have been shown to adapt to changes in light-dark cycles within four to five days (Van Den Buuse, 1999), and rats in our studies always had a minimum of seven days acclimation period prior to cannula implantation surgeries and a further minimum of five days before behavioural testing. This should have given rats sufficient time to adapt prior to behavioural testing, likely reducing stress at the time of testing. As such, we believe the influence of light-induced stress is controlled within our experiments, but as we did not specifically measure signs of stress, we cannot rule out that this could have influenced our ventral hippocampal manipulations or behavioural measures. Furthermore, we measured differences between or within groups/conditions and all rats were tested in the same light levels, so any effects of light-induced stress would have been across

groups/conditions. An additional consideration is that, with rats being tested in the light phase, this could have affected our hippocampal manipulations, as sleep-wake cycle alteration has been shown to affect hippocampal activity (Weerasinghe-Mudiyanselage & Moon, 2024) and diurnal differences have been reported in synaptic plasticity in the hippocampus (Schröder et al., 2023). Again, we measured differences between or within groups and all rats were tested within the same light-dark cycles so any effects of sleep-wake cycle alteration would have been consistent across groups/conditions. We expect that these group/condition differences would persist if rats were tested in the dark phase, but this is something that would need to be investigated. Indeed, rats with OFC lesions housed under a reversed light-cycle were impaired on a two-lever reversal task (Boulougouris et al., 2007), in line with findings with OFC lesions under a standard light-cycle (Chudasama & Robbins, 2003; McAlonan & Brown, 2003; Schoenbaum et al., 2002).

A further limitation of our experiments is that the gross behavioural effects, such as wet dog shakes, we observed with ventral hippocampal infusions, particularly of picrotoxin and muscimol, limit the efficacy of our blinding. However, the behavioural measures for our experiments are automated and so any potential bias would not have affected recording of these performance measures. Additionally, it could be argued that the behavioural effects we observed may contribute to the specific changes in reversal learning and locomotor activity that we observed following ventral hippocampal manipulations. However, the behaviours observed during our reversal learning experiments with ventral hippocampal picrotoxin were most pronounced prior to testing and less noticeable during testing (although we did not formally examine the time course of these effects), and, most importantly, we did not see impairments in reversal performance; instead ventral hippocampal picrotoxin tended to improve SD and reversal performance. Therefore, it is unlikely the behavioural effects, including wet dog shakes, contributed to the changes in task performance. Ventral hippocampal muscimol specifically affected late, but not early, serial reversal learning. This specific effect cannot be accounted for by non-specific impairments caused by gross behavioural effects of ventral hippocampal muscimol.

Regarding our L-655,708 studies, behavioural effects were observed in the vehicle condition as well as the L-655,708 conditions, and such minor non-specific effects could not have accounted for the specific effect on amphetamine-induced hyperactivity, without an effect on baseline locomotor activity.

5 Future studies

In future work, it would be interesting to complement our ventral hippocampal infusion studies with chemogenetic methods. We have recently found that chemogenetic mPFC disinhibition using DREADDs impaired late serial reversal learning in rats (Renström et al., 2025). First, we confirmed that inhibition of mPFC GABAergic neurons via activation with DREADDs indeed caused disinhibition. DREADD activation resulted in increased mPFC burst firing and local field potential (LFP) power (Renström et al., 2025; Renstrom, 2023), similar to what we observed following pharmacological mPFC disinhibition with picrotoxin (Pezze et al., 2014). We then investigated the effects of mPFC DREADD activation on our late serial reversal learning paradigm and found an increase in RTC, in line with what we observed with mPFC disinhibition with picrotoxin (Renström et al., 2025). It would be interesting to investigate the effect of chemogenetic ventral hippocampal disinhibition by DREADD-induced inhibition of GABAergic neurons on reversal learning, particularly early serial reversal learning where we found ventral hippocampal disinhibition by picrotoxin tended to improve performance, in line with other work using DREADD activation of the ventral subiculum in reversal learning (Bortz et al., 2019).

It would also be relevant to investigate the role of the dorsal hippocampus in reversal learning. Unlike the ventral hippocampus, the dorsal hippocampus does not feature direct prefrontal projections (Bast, 2011). There has been limited research into the dorsal hippocampus for reversal learning, although it was reported that dorsal CA1 activity coordinates with OFC activity during reversal learning in a plus maze task (Riceberg et al., 2022) and that inactivation of dorsal hippocampal α5-GABAARs improved reversal learning on a nose poke operant task (Davenport et al., 2021).

Additionally, examining the role of the dorsal hippocampal α5-GABAARs for amphetamine-induced locomotor hyperactivity would be valuable. In line with the functional differentiation of the hippocampus (discussed in Chapter 1), previous work has suggested that dorsal hippocampus is less relevant for modulation of locomotor activity and of midbrain dopamine systems (Bast, 2007; Bast & Feldon, 2003; Zhang et al., 2002a). Additionally, α5-GABAARs are less strongly expressed in the dorsal than the ventral hippocampus (Sarantis et al., 2008; Sotiriou et al., 2005). Therefore, we would expect negative allosteric modulation of dorsal hippocampal α5-GABAARs to have less impact than of ventral hippocampal α5-GABAARs on amphetamine-induced locomotor hyperactivity, but this remains to be confirmed in future work.

It would also be important to clarify the local neuronal effects of ventral hippocampal infusion of L-655,708 using in vivo electrophysiological investigations. Ventral hippocampal infusions of the non-specific GABA-A receptor antagonist, picrotoxin, increased hippocampal neuron activity and enhanced burst firing (McGarrity et al., 2017). Infusion of L-655,708 may lead to similar neuronal disinhibition in the ventral hippocampus, akin to this disinhibition caused by picrotoxin (McGarrity et al., 2017), albeit with potentially weaker effects. Studies in hippocampal slices have found L-655,708 to reduce inhibition and increase excitability, with increased bursting, prolonged sharp-wave ripples and enhanced LTP (Atack et al., 2006; Bugay et al., 2020; Papatheodoropoulos & Koniaris, 2011), and α5-GABAARs have been implicated in controlling hippocampal burst firing in vitro (with the α5-GABAAR NAM, RO4938581; Schulz et al., 2018). This suggests that blockade of α5-GABAARs in hippocampus may contribute to the enhancement of hippocampal burst firing observed with picrotoxin (McGarrity et al., 2017). The impact of ventral hippocampal infusion of L-655,708 has been investigated in vivo for its effect on VTA dopamine neuron activity (Perez et al., 2022), but to our knowledge the local effect within the ventral hippocampus has not been explored in vivo.

An important future direction would be to investigate the role of the ventral hippocampal α5-GABAARs in memory and other cognitive functions disrupted by ventral hippocampal inhibition and/or disinhibition, including rapid place learning

(McGarrity et al., 2017), novel object recognition (Taylor et al., 2025) and reversal learning (Chapter 2). Whilst hippocampal disinhibition has been found to impair rapid place learning on the DMP watermaze in rats, negative modulation or knockout of hippocampal α5-GABAARs has been reported to improve performance on the DMP watermaze in rodents (Collinson et al., 2002; Dawson et al., 2006). Regarding novel object recognition, we have recently found ventral hippocampal disinhibition to impair novel object recognition over a 1-min retention period (Taylor et al., 2025). In contrast, negative allosteric modulation of α5-GABAARs, with the inverse agonist, PWZ-029, has been found to improve object recognition after a 24-h delay in rats (Milić et al., 2013), and the α5-GABAAR antagonist, S44819, improved object recognition after a 24-h delay in rats (Etherington et al., 2017) and in mice (Gacsályi et al., 2017). Furthermore, the α5-GABAAR inverse agonist, α5IA, improved object recognition memory in control mice, and alleviated object recognition memory deficits in a Downs syndrome mouse model (Ts65Dn), over a 10-min retention interval (Vidal et al., 2017). There has been limited research into the role of α5-GABAARs for reversal learning, but it has been reported that ventral hippocampal α5-GABAARs do not modulate reversal learning. Overexpression of α5-GABAARs in the ventral hippocampus in rats did not affect reversal learning in control rats (Donegan et al., 2019). However, it may be that a reduction in, or reduced activity of, ventral hippocampal α5-GABAARs could affect reversal learning, even though overexpression does not improve behaviour. Interestingly, photoblocking dorsal hippocampal α5-GABAARs with PAG1C was found to improve reversal learning performance in mice (Davenport et al., 2021). It would be of interest to investigate the specific role of ventral hippocampal α5-GABAARs for rapid place learning, novel object recognition and reversal learning in future work.

6 Conclusions

This thesis has contributed to the characterisation of the role of the ventral hippocampus, and of local GABAergic inhibition, in innate and learnt behaviours. Firstly, we found ventral hippocampal disinhibition tended to improve early serial reversal learning, whereas ventral hippocampal inhibition tended to impair late serial reversal learning. Secondly, we found that negative allosteric modulation of ventral hippocampal α5-GABAARs did not affect baseline locomotor activity, PPI and startle, but tended to augment amphetamine-induced hyperlocomotion. Additionally, implications of our findings for clinical populations characterised by hippocampal dysfunction, namely schizophrenia, have been discussed.

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Appendices

1. Publications and Posters

Breaking barriers towards credible research: Outcomes of a workshop at the BNA Festival of Neuroscience 2025

Josefina Weinerova, Roni Tibon, Tobias Bast, **Rachel Grasmeder Allen**

Brain and Neuroscience Advances (2025); 10.1177/23982128251363046

Understanding the specific barriers and possible solutions to credible research for early career researchers is key to enabling the next generation of scientists to implement these practices. Here we describe the outcomes of a workshop that tackled these issues, held at the BNA Festival of Neuroscience 2025. Workshop attendees highlighted various challenges to credible research that are highly relevant for early career researchers. In particular, time constraints were highlighted as a universal barrier across multiple credible research practices, and attitudes of more senior researchers/supervisors were perceived as an important challenge. Participants also highlighted solutions to help implement credible research that can be pursued by individual researchers, but, importantly, also emphasised the importance of "structural" solutions, requiring initiative by stakeholders, including academic institutions, journals and funders. We hope the summary of the workshop will foster active debate on the topic within the neuroscience community.

Too little and too much: balanced hippocampal, but not medial prefrontal, neural activity is required for intact novel object recognition in rats

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Journal of Neuroscience (2025); 10.1523/JNEUROSCI.1141-25.2025

Impaired GABAergic inhibition, so-called neural disinhibition, in the prefrontal cortex and hippocampus has been linked to cognitive deficits. The novel object recognition (NOR) task has been used widely to study cognitive deficits in rodents. However, the contribution of prefrontal cortex and hippocampal GABAergic inhibition to NOR task performance has not been established. Here, we investigated NOR task performance in male Lister Hooded rats following regional neural disinhibition or functional inhibition, using intra-cerebral microinfusion of the GABA-A receptor antagonist picrotoxin or agonist muscimol, respectively. Our infusion targets were the medial prefrontal cortex (mPFC), dorsal hippocampus and ventral hippocampus. Using a within-subjects design, we compared NOR task performance (1-min retention delay) following bilateral regional saline, picrotoxin or muscimol infusions made before the acquisition phase. In mPFC, neither functional inhibition nor neural disinhibition affected object recognition memory. However, in both dorsal and ventral hippocampus, neural disinhibition impaired NOR relative to saline control, mainly by reducing novel object exploration time. In addition, functional inhibition of dorsal hippocampus impaired NOR, whereas ventral hippocampal functional inhibition tended to reduce novel object exploration at the highest dose used (alongside substantial non-specific behavioural effects). Overall, our data suggest that hippocampal, but not prefrontal, GABAergic inhibition contributes to NOR at a 1-min retention delay. Moreover, such NOR performance likely requires balanced neural activity in the dorsal hippocampus, with both too little and too much dorsal hippocampal activity impairing NOR memory. Our findings support that the NOR task can be used to investigate hippocampal GABAergic dysfunction in rodent models.

Too little and too much: medial prefrontal functional inhibition impairs early, whereas neural disinhibition impairs serial reversal performance in rats

Jacco G. Renström, Charlotte J.L. Taylor, **Rachel Grasmeder Allen**, Luke O'Hara, Joanna Loayza, Jacob Juty, Paula M. Moran, Moritz von Heimendahl, Serena Deiana, Johann Du Hoffman, Carl W. Stevenson, Silvia Maggi, Tobias Bast

BioRxiv (2025); 10.1101/2025.02.10.637529

Schizophrenia is associated with reduced activation ('hypofrontality') and neural disinhibition (reduced GABAergic inhibition) in the dorsolateral prefrontal cortex (dlPFC), as well as reversal learning deficits. Whilst reversal learning has been strongly linked to the orbitofrontal cortex, its dependence on the primate dlPFC – and its rodent analogue, the medial PFC (mPFC) – is less clear. Nevertheless, we hypothesized that the mPFC may be required for reversal learning if the reversal is demanding. Furthermore, even if the mPFC is not required, mPFC disinhibition may impair reversals, because it may disrupt processing in mPFC projection sites.

To test these hypotheses, we combined bidirectional manipulations of mPFC GABAergic inhibition, using intracerebral drug microinfusion and chemogenetic/DREADD methods, with reversal testing on a food-reinforced two-lever discrimination task in rats. First, we induced mPFC functional inhibition and disinhibition, by microinfusion of the GABA-A receptor agonist muscimol or antagonist picrotoxin, respectively, and examined the impact on early reversals (reversals 1-3) and well-established serial reversals (reversal 5 onwards).

Using classical performance measures and Bayesian trial-by-trial strategy analysis, we found that mPFC muscimol impaired early, but not serial, reversals, increasing perseveration and impairing exploratory (lose-shift) behavior at reversal 2. In contrast, mPFC picrotoxin impaired serial reversals, reducing exploratory (lose-shift) and exploitative (win-stay) behavior. Second, to inhibit mPFC GABAergic neurons, we expressed the inhibitory DREADD hM4Di in these neurons; chemogenetic mPFC disinhibition by activation of hM4Di also impaired serial reversal learning, primarily

disrupting exploitation. Our findings suggest that mPFC hypoactivation and disinhibition disrupt distinct aspects of reversal learning by different mechanisms.

Neuro-behavioral impact of Tourette-related striatal disinhibition in rats

Joanna Loayza, Charlotte Taylor, Jacco Renstrom, **Rachel Grasmeder Allen**, Stephen Jackson, Tobias Bast

BioRxiv (2025); 10.1101/2025.02.02.636049

Tourette syndrome has been linked to reduced GABAergic inhibition, so called neural disinhibition, in the dorsal striatum. Dorsal-striatal neural disinhibition in animal models, caused by local microinfusion of GABA-A-receptor antagonists, produces striatal local field potential (LFP) spike-wave discharges and tic-like movements resembling motor tics. Here, we characterized further the neuro-behavioral impact of striatal disinhibition, by unilateral picrotoxin infusion into the anterior dorsal striatum, in adult male Lister hooded rats. In vivo electrophysiology under anesthesia revealed enhanced neuronal burst firing in the striatum, alongside spike-wave LFP discharges. In freely moving rats, striatal picrotoxin reliably induced tic-like movements, which mainly involved lifting of the contralateral forelimb and concomitant rotational movements of head and torso, as well as occasional rotations of the whole body around its long axis. Prepulse inhibition (PPI) of the acoustic startle response was not affected, but startle reactivity tended to be reduced. Both locomotor and non-ambulatory movements, measured in photo-beam cages, were increased. Our findings suggest that, apart from generating tic-like movements, dorsal striatal disinhibition may contribute to hyperactivity, which is often comorbid with Tourette syndrome. Enhanced striatal burst firing may be important for these behavioral effects. Our findings do not support that striatal disinhibition contributes to PPI disruption, which has been associated with Tourette syndrome and suggested to contribute to tic generation.

Ventral hippocampal functional inhibition disrupts repeated reversal learning, whereas disinhibition disrupts expression of the previous response

Rachel Grasmeder Allen, Jacob Juty, Jacco Renstrom, Charlotte Taylor, Joanna Loayza, Luke O'Hara, Patricia Radu, Silvia Maggi, Tobias Bast

Brain and Neuroscience Advances (2025); 10.1177/23982128251339668

Reversal learning, a form of cognitive flexibility disrupted in many neuropsychiatric disorders, involves switching from one response to another when the reward contingencies of the responses are reversed. The hippocampus, especially ventral hippocampus (VH), projects to fronto-striatal circuits regulating reversal learning. In previous animal studies, hippocampal lesions impaired, whereas chemogenetic activation of VH facilitated, the acquisition of reversal learning. However, there is limited evidence on how VH activity affects distinct stages of reversal learning, including reversal acquisition and during established reversal learning.

We examined how VH functional inhibition or disinhibition, via infusion of a GABAA receptor agonist (muscimol) and antagonist (picrotoxin), affects repeated reversal learning and reversal acquisition, in young adult male Lister hooded rats performing a two-lever discrimination task. To study repeated reversals, rats were trained to acquire a spatial discrimination (press left or right lever) followed by four reversals, to achieve stable performance levels. Then, the impact of VH muscimol, picrotoxin and saline infusions on repeated reversals was compared within-subjects (n=13). To study reversal acquisition, we examined the impact of VH muscimol, picrotoxin or saline on spatial discrimination and three reversals, using a between-subjects sequential design (currently n=5–7 per group, study ongoing). Alongside classical performance measures, we used Bayesian trial-by-trial analysis of behavioural strategies. Data were analysed by ANOVAs, using infusion as within-subjects factor (repeated reversal), or infusion as between-subjects and task stage as within-subjects factor (reversal acquisition).

VH inhibition, but not disinhibition, impaired repeated reversal learning, as reflected by increased responses to criterion and increased regressive errors. This suggests that repeated reversal learning requires VH activity, but not balanced levels of VH activity. In contrast, VH disinhibition impaired expression of the previous response during reminder trials preceding reversal trials, increasing response latencies and omissions. This was supported by strategy analysis, which revealed VH inhibition tended to increase, and VH disinhibition tended to decrease, the use of the previous rule around reversal. VH manipulations did not affect win-stay or lose-shift strategies around reversal, suggesting that VH activity changes do not affect exploration or exploitation, and contrasting with our findings for the medial prefrontal cortex. Our reversal acquisition study is ongoing: VH disinhibition tended to improve reversal acquisition, but this may reflect impaired expression of the previous response during reminder trials. Our findings suggest the VH is required for repeated reversals, whereas VH disinhibition disrupts expression of the old rule during reversals, which may facilitate reversal learning acquisition.

Impact of ventral hippocampal chondroitinase-ABC infusion on perineuronal nets and diffuse extracellular matrix in male Lister hooded rats.

Jacob Juty, **Rachel Grasmeder Allen**, Jacco Renström, Jennifer Fletcher, Charlotte Taylor, John Gigg, Michael Harte, Tobias Bas

FENS Forum 2024

Perineuronal nets (PNNs) in the hippocampus primarily surround inhibitory interneurons. PNNs have been suggested to regulate synaptic plasticity and support GABAergic inhibition, and PNN disruption has been implicated in brain disorders, including schizophrenia. Yet, the cognitive and behavioural consequences of hippocampal PNN loss largely remain to be studied. The enzyme chondroitinase-ABC (chABC) degrades chondroitin-sulfate proteoglycans (CSPGs), a main component of PNNs and the diffuse extracellular matrix (ECM), and has been used to study PNN function in animal models. Here, we characterised hippocampal PNN expression in male Lister hooded rats and the impact of ventral hippocampal chABC infusion. Control rats were infused with penicillinase, an enzyme with no endogenous substrate. Sensorimotor testing indicated no major impact of ventral hippocampal chABC infusion on locomotor activity, startle reactivity, or prepulse inhibition (PPI), although group sizes were small. Post-mortem staining of CSPGs by WFA lectin histochemistry revealed that PNNs were mainly expressed in CA2/CA3 of dorsal hippocampus and CA1 of ventral hippocampus. ChABC-treated rats showed profound ablation of WFA-stained PNNs and of diffuse ECM CSPGs in the ventral to intermediate hippocampus. Immunohistochemistry revealed extensive chondroitin-4-sulfate (C4S) "stubs" (products of CSPG degradation by chABC), in the ventral to intermediate hippocampus of chABC-treated rats 1 day post-infusion. 10 days post-infusion, PNN staining had recovered to some extent, whereas the ECM CSPG staining remained attenuated and C4S-stub staining remained marked. Future work will assess the impact of ventral hippocampal PNN removal with chABC on selected cognitive and behavioural functions linked to the hippocampus and regional inhibitory function.

Impaired reversal learning in the Dlg2+/− rat model of genetic risk for psychiatric disorder: Important questions regarding the neuro-behavioral mechanisms of reversal learning

Tobias Bast, **Rachel Grasmeder Allen**, Silvia Maggi, Jacco Renstrom

Genes, Brain and Behaviour (2023); 10.1111/gbb.12870

In this issue, Griesius et al report that heterozygous Dlg2+/- rats showed a reversal learning impairment on a specific bowl-digging task, whereas other reversal tasks were unaffected. The study suggests that Dlg2 gene disruption, which has been linked to neuropsychiatric disorders, including schizophrenia, may cause relatively specific impairments in reversal learning, an important aspect of cognitive flexibility. The study draws attention to two important issues regarding the neuro-behavioral mechanisms of reversal learning, namely that hippocampal dysfunction, which is prominent in Dlg2+/- rats, may contribute to reversal learning impairments and that, depending on the task and previous experience, brain and behavioral mechanisms of reversal learning may differ.

Contribution of medial prefrontal cortex D1 receptors to early and repeated reversal learning in rats

Luke O'Hara, Jacco Renstrom, **Rachel Grasmeder Allen**, Charlotte Taylor, Joanna Loayza, Tobias Bast, Silvia Maggi

Brain and Neuroscience Advances (2023); 10.1177/23982128231180246

Cognitive flexibility allows individuals to adjust their behaviour in response to changes in their environment. A form of cognitive flexibility is reversal learning: the ability to switch between responses when the reward contingency of the responses is reversed. Impaired in Schizophrenia and other neuropsychiatric disorders, reversal learning depends on frontal cortex function. The prefrontal cortex receives midbrain dopamine projections, which convey information about reward feedback signals. Moreover, dopamine D1 receptors in the medial prefrontal cortex (mPFC) have been implicated in learning and attention. However, whether mPFC D1 receptors play a role in the acquisition of reversal learning or in flexibly switching between response rules after repeated exposure is not fully understood.

To address this, we examined the impact of mPFC infusion of the D1 receptor agonist SKF 81297 or the antagonist SCH 23390 on reversal performance on a two-lever task in male Lister hooded rats. In one cohort, we tested the impact on early reversals, when rats were naïve and showed significant between-session performance improvements. In another cohort, we tested the impact on later repeated reversals, when rats had 'learnt to reverse' and their performance was relatively stable across reversals.

In both experiments, mPFC SCH, but not SKF, increased response omissions and latencies compared to saline infusions. Furthermore, mPFC SCH impaired the expression of the previous rule and decreased perseverative errors during early reversals, but not later reversals. Moreover, a Bayesian trial-by-trial analysis revealed that mPFC SKF increased perseveration, whereas SCH decreased perseveration, during later reversals. These findings suggest that mPFC D1 receptors modulate the

expression/exploitation of recently learnt rules and inhibit exploratory behaviour. Specifically, D1 antagonism reduces expression of a previously learned rule and promotes exploration, whereas D1 agonism promotes exploitation at the expense of exploration. Additional Bayesian trial-by-trial analyses are on the way to examine the emergence of exploratory versus exploitative behaviour across reversals.

Poster Presentations

Ventral hippocampal functional inhibition disrupts repeated reversal learning, whereas disinhibition disrupts expression of the previous response

- BNA International Festival of Neuroscience (2025), Liverpool, UK
- SfN (2024), Chicago, USA
- FENS Forum (2024), Vienna, Austria
- Psychology Postgraduate Conference (2024), Nottingham, UK
- Neuroscience at Nottingham Symposium (2024), Nottingham, UK
- BNA International Festival of Neuroscience (2023)
- BBSRC DTP Spring Conference (2023), Nottingham, UK

Oral Presentations

- School of Psychology Seminar Series (2025), 30-min talk, Nottingham, UK
- BBSRC DTP Spring Conference (2025), 3 Minute Thesis, Nottingham, UK
- BNA Member's Meeting (2024), 15-min talk, online
- BBSRC DTP Spring Conference (2024), 15-min talk, Nottingham, UK
- Behavioural Neuroscience (Learning & Memory) Group seminar (2023), 30-min talk, Nottingham, UK
- Psychology Postgraduate conference (2023), 15-min talk, Nottingham, UK
- BBSRC DTP Spring Conference (2022), 2-min talk, Nottingham, UK

Awards

- School of Psychology REF quality enhancement fund (£3,000)
- BBSRC In Vivo Strategic Skills Award 2023 (£9,945)

- Postgraduate Research Student Award for Participation and Contribution to Public Engagement Activities (School of Psychology, University of Nottingham, 2023 and 2024)
- Oral Presentation Prize at the Psychology PGR Conference (School of Psychology, University of Nottingham, 2023)
- Poster Prize at the BBSRC DTP Spring Conference (BBSRC DTP, University of Nottingham, 2023)
- Presentation Prize at the BBSRC DTP Spring Conference (BBSRC DTP, University of Nottingham, 2022)

Other work

Five policy briefs written for Series 1 of Notts Psych Pod (https://www.nottingham.ac.uk/psychology/outreach/notts-psych-pod.aspx):

- Access to care for neurodiversity - ADHD and Tourette Syndrome
- Neurodiversity in the workplace
- Autism and Driving
- Autism and Policing
- Autism and Suicide Prevention

2. Reflective Statement on experience during PIPS project

Note to examiners: This statement is included as an appendix to the thesis in order that the thesis accurately captures the PhD training experienced by the candidate as a BBSRC Doctoral Training Partnership student.

The Professional Internship for PhD Students (PIPS) is a compulsory 3-month placement which must be undertaken by DTP students. It is usually centred on a specific project and must not be related to the PhD project. This reflective statement is designed to capture the skills development which has taken place during the student's placement and the impact on their career plans it has had.

For my PIPS, I completed an internship at Costello Medical, a medical communications company, and I was based in their Cambridge Office. Whilst at Costello Medical, I worked in the Publications division and the Pro Bono division. It was a great opportunity to work cross-divisionally and experience the different ways projects are managed and completed. Alongside my divisional work, I also completed a lot of training. This included training on proofreading, using generative AI, client communication and using the various Microsoft Office programmes. Working in a fast-paced environment, I strengthened my time management and organisational skills. I planned each week's work, whilst remaining flexible to changes in deadlines or unexpected tasks. I also developed strong communication skills through working with my project teams and with the clients.

For my Publications work, I worked with pharmaceutical company and produced two posters and three abstracts for upcoming congresses related to a recent clinical trial. For these projects, I developed the posters and abstracts from initial outlines to complete posters and abstracts that were submitted to two congresses. For this client, I also helped implement author comments on a manuscript, assisted with going through manuscript proofs and produced a slide deck for a kick-off call about another manuscript. I gained a lot of experience in client communication, regularly being involved in client calls and emailing the client and external authors. I developed my skills in writing, proofreading and client communication, as well as learning the

processes at Costello Medical. Whilst working in the Publications team, I also did many quality checks of other work, including abstracts, posters and manuscripts. This was a great way to learn about work being done across the company, and to develop my skills in quality checking documents. During my internship, I attended a "Publications Team Away Day". This was a fantastic opportunity to meet the wider UK Publications team. During this event, we had workshops on designing figures and omnichannel customer service.

For my Pro Bono work, I worked with two charities. Both projects were a targeted literature review, but I joined each project at a different stage which allowed me to experience different phases of the project. For one project, I attended the kick-off call with the client, developed the targeted literature review protocol and completed extractions of literature. For the other project, I produced the kick-off call slide deck and attended the kick-off call, developed the targeted literature review protocol and began the abstract sifting phase. For these projects, I learnt a different way of doing literature reviews which was very insightful.

I received excellent feedback from both my colleagues and from our clients. My colleagues noted that I adapted well to life at Costello Medical and were impressed at how quickly I picked up the processes and how I integrated within the team. They said I produced high quality work and that I always kept to deadlines. I received feedback that I improved in confidence in client calls during my internship, and that I was clear and professional. My team were impressed at how I handled juggling numerous deliverables and that I took responsibility for each of these.

My PIPS was an invaluable experience. I developed numerous skills, worked on different types of projects and gained an insight into working in the medical communications industry. I thoroughly enjoyed my experience and have secured a position as a Medical Writer at Costello Medical for after my PhD.