

AN INVESTIGATION OF THE ROLE OF THE NUCLEUS ACCUMBENS IN THE HIPPOCAMPAL LEARNING-BEHAVIOUR TRANSLATION

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Abstract

The hippocampus is required for important types of rapid everyday learning, including place learning. However, the pathways via which hippocampal place learning is translated into behaviour remain to be determined. The intermediate hippocampus is critical for the hippocampal learning-behaviour translation and combines neural substrates of accurate place encoding with links to prefrontal and subcortical behavioural control sites, which may contribute to this translation. The nucleus accumbens (NAc) is a main candidate due to strong hippocampo-NAc projections that have been implicated in behaviour based on place memory (Chapter 1).

To examine the role of the NAc, we combined functional inhibition (via microinfusion of the GABA agonist muscimol) with measurements of behavioural performance based on hippocampus-dependent rapid place learning using the watermaze delayed-matching-to-place (DMTP) test. Electrophysiological (Chapter 2) and sensorimotor experiments (locomotor activity, startle response/prepulse inhibition) (Chapter 3) showed that muscimol (125-250ng/0.5 µl/side) reduced NAc neurone firing around the infusion site by about 50% and caused, if at all, only moderate sensorimotor effects. The open field locomotor experiments indicated that NAc functional inhibition impaired between-session open field habituation, a process that has been suggested to require hippocampal contextual learning and memory. Our first watermaze experiment (Chapter 4), testing the impact of NAc muscimol within-subjects, supported that the NAc is required for DMP performance; however, there appeared to be a carry-over effect, with NAc muscimol eroding the typical DMTP search strategy on subsequent test days, possibly reflecting that NAc is required to reinforce the strategy. A second watermaze experiment (Chapter 5), using a between-subjects design, confirmed that NAc muscimol impairs expression of hippocampus-dependent rapid place learning on the DMP test, with a significant impairment of 1-trial place learning performance in the muscimol group on infusion days when compared to performance on washout days as reflected by reduced

search preference for the correct location as compared to the saline group on infusions days, as well as by a significant impairment within the muscimol group, but not the saline group, on infusion days as compared to washout days.

Overall, these findings support that the NAc contributes to translation of rapidly-acquired place memory into behaviour as shown by performance impairment on the DMTP task following functional inhibition of the NAc. Beyond this, further investigation is required to demonstrate the direct hippocampal-NAc interaction.

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Contents

Abstract…… 2

Acknowledgements…… 4

Chapter 1: Introduction…… 7

Chapter 2: Electrophysiological effects: Reduction of firing rate and burst firing following accumbal muscimol infusion…… 23

Introduction…… 23

Material and Methods…… 24

Results…… 30

Discussion…… 37

Chapter 3: Sensorimotor effects: Minor impairment of locomotor activity and prepulse inhibition following accumbal muscimol infusion…… 41

Introduction…… 41

Material and Methods …… 43

Results …… 48

Discussion …… 52

Chapter 4: Watermaze delayed-matching-to-place (DMTP) within-subjects experiment: Preliminary evidence that NAc muscimol impairs expression of 1-trial place learning …… 55

Introduction…… 55

Material and Methods…… 56

Results …… 62

Discussion …… 69

Chapter 5: Watermaze DMTP between-subjects experiment: Muscimol infusion into NAc impairs expression of rapid place learning …… 72

Introduction…… 72

Material and Methods …… 72

Results …… 76

Discussion …… 82

Summary/Discussion…… 86

Conclusions 89
References 90
Appendices 104
Appendix 1: Protocols 104
Appendix 2: Additional work 111
Appendix 3: PIPS reflective statement..... 117

Chapter 1: Introduction

Although the importance of the hippocampus for some types of learning and memory, including declarative and place learning and memory, is well established (Andersen, Morris, Amaral, Bliss, & O'Keefe, 2007; Eichenbaum, Stewart, & Morris, 1990; O'Keefe, 1979), the process via which hippocampus-dependant place learning is translated into behaviour is still largely unknown (Bast, 2011). While a lot of research has shown the anatomy and function of the hippocampus and the nature of its connections to other key brain regions (Andersen et al., 2007), it remains to be shown which of these connections are important for the learning-behaviour translation and the mechanism by which this occurs. There are several candidate brain regions that may be important, including the prefrontal cortex (PFC), amygdala and other subcortical brain structures (Bast, 2011; Bast, Wilson, Witter, & Morris, 2009). One such structure that we will be concentrating on is the nucleus accumbens (NAc) (Bast et al., 2009; Humphries & Prescott, 2010; Pennartz, Groenewegen, & Lopes da Silva, 1994). Muscimol, a GABA-A agonist, causes inactivation in the target region. This is preferable over a more permanent lesioning technique as it allows for within-subject testing due to its temporary effect (Edeline, Hars, Hennevin, & Cotillon, 2002). Another advantage is that functional compensation, which may occur with permanent lesion, is less likely with temporary functional inhibition (Bast & Feldon, 2003; Wang, Bast, Wang, & Zhang, 2015).

Hippocampus – Learning and Performance

The idea that the hippocampus is important in the encoding of place learning via 'place cells' has long been investigated. Research shows that these 'place cells' are located within the hippocampus and selectively fire depending on the location of the animal within its environment (O'Keefe, 1979). Studies have demonstrated that upon exploring a novel environment, rats will form an internal place map with these place cells demonstrating place-specific firing (Frank, Stanley, & Brown, 2004), with these spatial representations then being reinforced during both sleep periods (Lee &

Wilson, 2002; Louie & Wilson, 2001) and during awake periods immediately following place memory formation (Foster & Wilson, 2006). Since the discovery of hippocampal place cells, a variety of other cell types have been discovered in the hippocampus and other closely related structures. These cells relay information about different aspects of the animal's position in space, such as 'head-direction cells', which provide information about the direction the animal is facing relative to environmental cues (Taube, 1998). There are also 'grid cells' within the entorhinal cortex, a key region interacting with the hippocampus in the processing of multimodal information processing, which provide further information about the location of the animal within the environment. These cells are still active in the absence of visual input, as they represent the location with respect to a grid of interconnecting triangles of space (producing an internal 'map') within the entorhinal cortex, implying that visual input is not necessary, but entrainment of the grid itself can be achieved via environmental cues (i.e. movement in the environment leads to movement of the grid) (Hafting, Fyhn, Molden, Moser, & Moser, 2005; E. I. Moser, Kropff, & Moser, 2008; E. I. Moser et al., 2014).

Many studies have been carried out using pharmacological manipulation and lesioning techniques to confirm the role of the hippocampus in the formation, and subsequent translation to behaviour, of place memories. Whole hippocampal lesioning has been shown to impair place learning, as carried out in one study by aspiration of the entire hippocampus producing performance impairments on the standard watermaze task (R. G. Morris, Garrud, Rawlins, & O'Keefe, 1982). Ibotenic acid-induced hippocampal lesioning experiments also demonstrated that rats show an initial impairment of performance on a standard, incrementally-learned watermaze task but over time reach performance levels comparable to controls (R. G. Morris, Schenk, Tweedie, & Jarrard, 1990). The standard watermaze task is a behavioural paradigm used frequently to determine place learning, usually with mice or rats. It consists of four trials, the first of which the rat must find the hidden platform in the water maze without prior knowledge of its location. The second trial therefore is representative of how much learning has occurred, as the rat should be able to use visual cues in the environment to find the platform much quicker. The

third and fourth trials are then used to confirm the win-stay rule for the rat. This was then compared to performance on a delayed matching to place (DMTP) version of the watermaze, which involved changing of the platform location on a daily basis. Performance on this task was shown to be impaired in hippocampal lesioned groups, highlighting the specific hippocampus-dependence of rapid, especially 1-trial, place learning as compared to slower, incremental place learning (also compare Bast et al., 2009). Further work then utilised this DMTP task to determine the role of the hippocampus in rapidly-acquired place memory, with rats showing performance impairment following NMDA-receptor blockade, thereby reducing synaptic plasticity due to the role of NMDA receptors in mediating the induction of long-term potentiation within the hippocampus (Steele & Morris, 1999). Further work suggested that NMDA receptors are involved in the encoding, but not retrieval, of one-trial place memory on a food finding open arena task, whereas AMPA receptors are also involved in the retrieval of this place memory (Bast, da Silva, & Morris, 2005). These findings were also seen in a task involving rats being required to encode and recall paired associates (food flavour and spatial location), with NMDA receptor blockade impairing encoding and AMPA receptor blockade impairing both encoding and retrieval (Day, Langston, & Morris, 2003). Impairment on the DMTP task is also seen during blockade of hippocampal dopamine receptors (Pezze & Bast, 2012) and following dorsal hippocampal kindling (Hannesson et al., 2004). Hippocampal involvement in place memory has also been shown outside of rodent models. For example, human transient global amnesia patients with focal lesions in the CA1 region of the hippocampus have been shown to be impaired on a virtual watermaze task (Bartsch et al., 2010).

Different subdivisions of the hippocampus show different patterns of anatomical connectivity (figure 1). For example, the hippocampus shows evidence of topographical gradients of neural connections along its septotemporal axis, with them getting weaker towards the septal or temporal poles and leading to three hippocampal ‘domains’: a septal domain, a temporal domain, and an intermediate domain (Bast et al., 2009; Fanselow & Dong, 2010; Humphries & Prescott, 2010; Risold & Swanson, 1996). For the translation of rapid-place learning into behaviour,

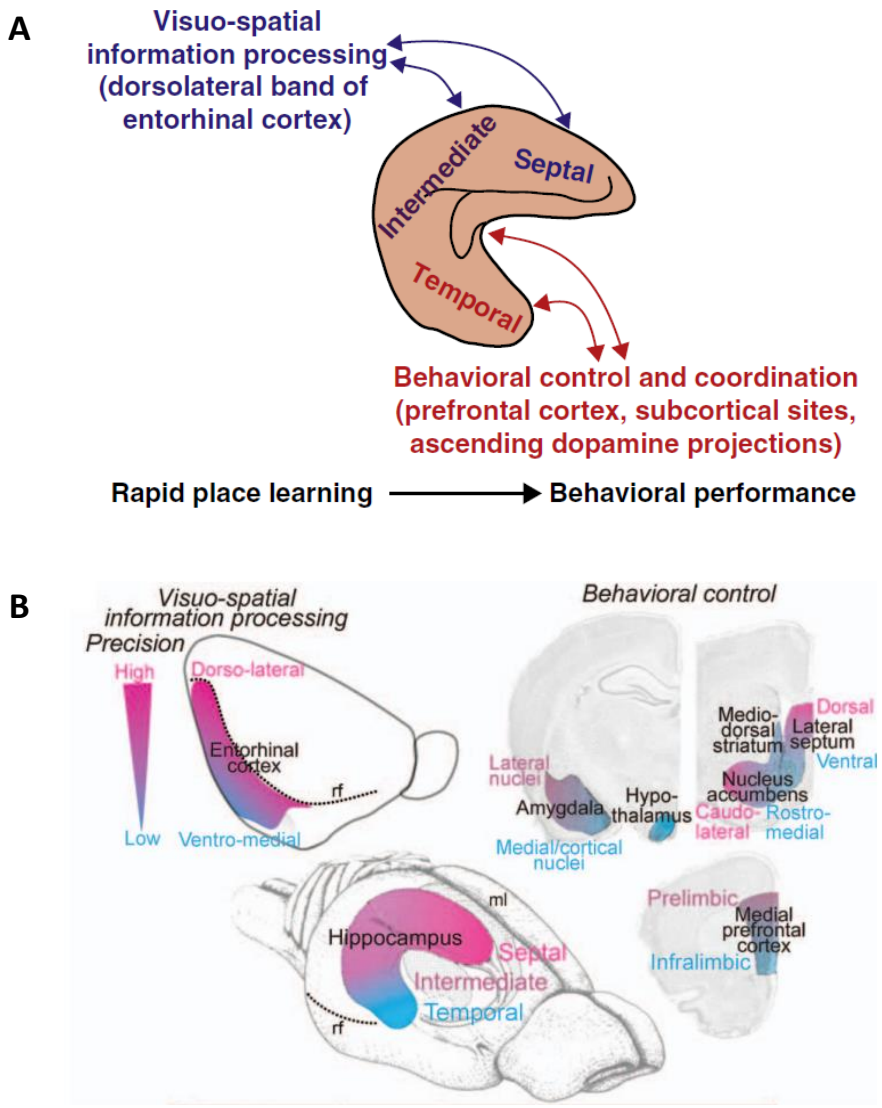


Fig. 1. A) Diagram showing gradient of visuo-spatial information processing shown along the septotemporal axis of the hippocampus, showing strong visuo-spatial input towards the septal pole and more connections to regions of behavioural control towards the temporal pole. Figure from Bast, 2009. **B)** Diagram showing gradients of connectivity within the hippocampus, and key brain regions (nucleus accumbens, medial prefrontal cortex, amygdala, etc.) that could be involved in the translation of rapidly-acquired place memory into behaviour due to their connectivity with the hippocampus. Figure from Bast et al., 2009.

it has been suggested that it is important for there to be direct connections from the hippocampus to brain regions involved in behavioural control, such as the PFC and various subcortical regions (amygdala, NAc - areas which assist in the coordination of motor activity and behavioural aspects such a goal orientation and motivation) (Bast, 2007; Johnson, van der Meer, & Redish, 2007; Petrovich, Canteras, & Swanson, 2001; Poucet et al., 2004; Yu & Frank, 2015). The temporal and intermediate domains of

the hippocampus show strong connectivity to these regions including direct connections from CA1 and subiculum and indirect connectivity via the ventromedial entorhinal cortex, indicating that this region may be more involved in the translation of rapid place-learning to behaviour (Amaral & Lavenex, 2007; Bast, 2007, 2011; Bast et al., 2009). On the other hand, the septal pole of the hippocampus shows more connectivity to the dorsolateral entorhinal cortex, which has a strong visuospatial input from the neocortex (Amaral & Lavenex, 2007; Bast & Feldon, 2003; Bast et al., 2009). This may suggest that the septal domain is more involved with accurate place encoding as opposed to being directly linked to areas of behavioural control.

The distinction between learning and performance as two separate aspects of memory-based behaviour highlights the importance of the functional-anatomical gradient of neural connectivity along the septotemporal axis of the hippocampus (Amaral & Witter, 1989; Strange, Witter, Lein, & Moser, 2014). While learning refers to the encoding of information, performance refers to the translation of this encoded information into behaviour. In the context of the present study, we hypothesise that the encoding is the place learning and primarily occurs within the septal to intermediate region of the hippocampus due to this region's high connectivity with the dorsolateral entorhinal cortex (Fyhn, Molden, Witter, Moser, & Moser, 2004; Kjelstrup et al., 2008; Steffenach, Witter, Moser, & Moser, 2005). On the other hand, the performance in this situation would refer to the translation of place information into behaviour. This translation may depend on the temporal to intermediate region of the hippocampus due to its connectivity with the PFC and subcortical regions (Bast, 2007, 2011; Bast et al., 2009; M. B. Moser & Moser, 1998). In circumstances where place memory is consolidated into the neocortex (for example if the place information is consistent, i.e. where you live), the direct hippocampal- 'behavioural control site' connection would not be required. Therefore, the direct connectivity would only be necessary with rapidly-acquired place information that frequently changes (Bast, 2011). For example, if you were to park your car at work every day, the location that the car is parked would usually change on a day-to-day basis. As there is no need to consolidate each location in the neocortex, the knowledge of where the car is parked each day must be encoded and then translated directly into

behaviour. Alternatively, when the information has already been consolidated in the neocortex, the hippocampus is not necessary for translation of this information into behaviour as this may occur via direct connections between the neocortex and prefrontal/subcortical areas, therefore bypassing the hippocampus altogether.

A recent study (Bast et al., 2009) highlights the importance of the hippocampus in rapid place learning, with watermaze DMTP performance highly sensitive to even partial hippocampal lesions, whereas incremental place learning on the water maze task can be mediated without the hippocampus, i.e. following complete hippocampal lesions. This suggests the presence of two separate pathways for rapidly-acquired place information and consolidated place information. The study consisted of use of two different water maze tasks, one involving changing goal locations daily (DMTP task mentioned previously, representing rapidly-acquired place information) and one involving a consistent goal location (representing incrementally acquired place information). Following complete hippocampal lesion, it was found that rats which were trained on the DMTP test with a new platform location in the water maze each day were more impaired than those trained with a consistent platform location. On the DMTP task, the complete hippocampal lesion group demonstrated a lower percentage of time spent searching within the correct zone and were unable to display accurate place navigation. In contrast, when tested with a constant platform location over many trials, the rats with complete hippocampal lesions were eventually able to display accurate place navigation and locate the platform on each trial, although slower than a control group (sham-operated). As the slow incremental place learning task did not appear to require an intact hippocampus to perform, it confirms the hypothesis that the place information had already been consolidated in another brain region (the neocortex). However, as they were slower than the control group, it is consistent with the idea that hippocampal learning is faster than extra hippocampal place learning.

Bast and colleagues (2009) also demonstrated the importance of the intermediate 'domain' of the hippocampus in the translation of rapidly-acquired place information into behaviour. As outlined above, there are neuroanatomical topographical

gradients running from each pole of the hippocampus, with more accurate place encoding occurring toward the septal pole and connections to behavioural control occurring toward the temporal pole. Therefore, it could be hypothesised that the intermediate region may play a large role in the translation of place information into behaviour (Bast, 2007). Bast and colleagues (2009) demonstrated this by causing localised lesions to either the septal, temporal or intermediate regions of the hippocampus in rats, and testing their performance on the water maze DMTP task. They found that performance was only maintained in groups in which the intermediate hippocampus was spared, with percentage of time spent searching in the correct zone being significantly higher than in groups in which the intermediate hippocampus was lesioned. Using electrophysiological techniques, they were also able to show that when the septal pole is spared, it is still able to accurately encode place information in the absence of intermediate and temporal regions of the hippocampus. However, without other hippocampal regions, it is unable to translate this into behavioural performance and therefore, the group in which only the septal pole was spared were unable to maintain task performance.

Another study (McGarrity, Mason, Fone, Pezze, & Bast, 2017) showed the effects of picrotoxin infusion into the hippocampus (intermediate to temporal regions) on neuronal activity and behaviour. They noted that the picrotoxin caused an increased firing rate within the infusion site, locomotor hyperactivity, attentional deficits on the 5CSRT test (indicating altered prefrontal activity) and memory deficits.

While the importance of the intermediate hippocampus has been well established, the pathways and mechanisms by which it is able to mediate the learning-behaviour translation remain to be clarified. With its connections to multiple brain regions, it is important to determine which of these connections is more implicated in this translation of rapidly-acquired place information to behaviour and the mechanism by which this occurs. The following sections discuss the research that has been carried out with regards to some of these connected regions, including the area chosen as the primary focus of this study: the NAc.

Hippocampal-Prefrontal Projections

The PFC is known to be involved with many complex cognitive and behavioural functions, including aspects of personality expression, decision making, attention and goal-orientated behaviours (Glascher, Hampton, & O'Doherty, 2009; Wunderlich, Rangel, & O'Doherty, 2009). There are strong projections from the hippocampus to the prefrontal cortex (Jay & Witter, 1991). Stimulation of the hippocampus has been shown to produce excitatory potentials in the pyramidal cells of the prelimbic cortex within the mPFC (Degenetais, Thierry, Glowinski, & Gioanni, 2003; Laroche, Jay, & Thierry, 1990), which suggests that the hippocampus can directly modulate prefrontal function. These projections are unidirectional, with almost no reciprocal projections back to the hippocampus as shown by anterograde tracer studies, although there is an indirect projection back via the nucleus reuniens of the thalamus (Hurley, Herbert, Moga, & Saper, 1991; Takagishi & Chiba, 1991). Due to these connections, it has been hypothesised that this hippocampal-prefrontal connection may be involved in the translation of place learning to behaviour (Bast, 2011; Bast et al., 2009).

A study carried out by Bruin and colleagues (de Bruin, Sanchez-Santed, Heinsbroek, Donker, & Postmes, 1994) attempted to determine what role the PFC played in spatial learning by causing either selective bilateral lesions within different prefrontal areas (dorsomedial and ventromedial prefrontal cortices) or whole prefrontal cortical lesions, and then testing performance on the incremental Morris water maze task using a hidden platform. They found that, during 4 days of 4-trial training sessions, there were no significant differences between these groups and sham-operated control animals in their ability to learn the platform location over successive 4-block trials. Following this, the platform was removed on the 5th day and the percentage of time the animal spent in the correct zone was observes. They found that there was no significant difference between groups, with each spending approximately 45% of the time during the trial in the correct quadrant. They also carried out 'reversal' learning trials in which the platform was moved to the opposite

quadrant, and all groups demonstrated the ability to learn the new location of the platform (although the group with whole prefrontal lesions was initially slower in escaping). These results taken together suggest that the PFC plays no major role in performance on the standard incremental place learning watermaze task, as following damage to this structure there was no significant effect on behaviour.

A study investigating up- and down-regulation of neuronal activity within the PFC via pharmacological manipulation (Pezze, McGarrity, Mason, Fone, & Bast, 2014) investigated behavioural changes following increasing or decreasing activity in the PFC via micro infusion of picrotoxin (GABA-A antagonist) or muscimol (GABA-A agonist), respectively. The study showed these neurological changes via electrophysiology, with muscimol causing inhibition, reducing within-burst firing rates, and picrotoxin resulting in disinhibition, increasing within-burst firing rates. This provided evidence that the drugs were having a physiological effect on the PFC, and therefore may suggest that any behavioural changes seen could be attributed to the pharmacological manipulations. Using the five-choice serial reaction time (5CSRT) task, it was shown that both muscimol and picrotoxin infusion resulted in attentional deficits. This suggests that the prefrontal cortex requires an appropriately balanced level of neuronal activity to maintain normal attentional performance, as increasing or decreasing the prefrontal neuronal activity resulted in loss of attentional control. Taken with the knowledge that the PFC has extensive afferent connections from the hippocampus, it could be hypothesised that altered activity within the hippocampus would lead to changing neural activity in the PFC. However, this does not necessarily mean that the PFC is involved in rapid place learning being translated into behaviour. From a methodological perspective, this paper demonstrates many techniques which will be used to modify and measure neuronal activity in the present study on the NAc, with similar pharmacological manipulations and electrophysiological techniques being used.

Recent work has focused specifically on the PFC and its involvement in rapid-place learning. One study (McGarrity et al., in preparation – See (McGarrity et al., 2015)) investigated the role of prefrontal neural activity in performance based on

hippocampus-dependent one-trial place memory by infusing muscimol into the mPFC to cause functional inhibition. Using the DMTP task, they found that there were no significant memory deficits following muscimol infusion as there was an unaffected search preference for the target location. However, disinhibition of the mPFC via picrotoxin infusion produced a change in the swim task behaviour of the rats during the DMTP task, as shown by an increased swim-stop alteration (though disinhibition also did not significantly affect place memory). These findings suggest that the mPFC is not required for behavioural performance based on rapidly-acquired place information, but may instead may play more of a modulatory role in the behaviour as demonstrated by the changes in the search pattern.

A study carried out by Floresco and colleagues (Floresco, Seamans, & Phillips, 1997) looked into the functional relationships between the hippocampus and the prefrontal cortex, and what the effect of reversible lesions of these structures is on various behavioural tasks. Following unilateral lesion of the ventral subiculum (vSub), foraging behaviour is not affected due to the ipsilateral hemisphere being able to compensate for this loss. Therefore, following unilateral lesion of the vSub in one hemisphere and simultaneous lesion of either the NAc or prefrontal cortex in the ipsilateral hemisphere, the functional connection between the two structures (vSub and PFC/NAc) can be determined. It was found that, following lidocaine infusion into vSub/prefrontal cortex, foraging activity was impaired during a two-phase delayed task and unaffected in a single-phase non-delayed task. These results follow the same pattern as bilateral lidocaine infusions into the rat prefrontal cortex (Seamans, Floresco, & Phillips, 1995). This indicates the involvement of the PFC in incrementally learnt place information, but not in the translation of rapidly-acquired place information, as the impairment was only seen following a delay between the initial learning period and the subsequent foraging behaviour.

Following the results of recent prefrontal work, we will now consider if certain subcortical regions such as the ventral striatum may be more involved in the translation of place-information to behaviour, which led to the focus of this study on the NAc and how modulating its activity may produce behavioural changes.

Ventral Striatum/Nucleus Accumbens

The basal ganglia consists of a series of structures including the small subthalamic nucleus, globus pallidus, substantia nigra pars reticula, entopeduncular nucleus and striatum (Bolam, Hanley, Booth, & Bevan, 2000). The striatum is of key concern to us, with the NAc being located within the ventral striatum (along with the olfactory tubercle) (Zaborszky et al., 1985). The NAc is composed of two subdivisions – the core, which is surrounded laterally, medially and ventrally by the shell. These sections are similar in terms of their cell types, with both consisting mainly of medium spiny neurones (MSN) which contain D1- or D2- like dopamine receptors, receive glutamatergic excitatory inputs from multiple brain regions (including hippocampus and PFC), and are interconnected by GABA-A synapses and gap junctions (Wolf, Schroeder, & Finkel, 2001; Zahm, 2000). Due to the abundance of GABA-A receptors on these MSNs, muscimol infusion into the NAc (see Chapters 2 – 5) will act predominantly at these receptors and reduce MSN firing. There is also the possibility that the muscimol will act at receptors on inhibitory interneurons and lead to disinhibition of the MSNs, though the net effect would still be inhibition within the NAc and reduction of output from the region. MSNs are unique in that they display a 'two-state' membrane potential: a hyperpolarised down-state and a depolarised up-state, with action potentials only being possible in the up-state (Wilson & Kawaguchi, 1996). Maintenance of the hyperpolarised down-state is carried out by 'inward rectifying' K^+ channels, and following depolarisation due to excitatory inputs from hippocampus and PFC, these channels are closed, thereby transitioning the membrane potential to the depolarised up-state (Mermelstein, Song, Tkatch, Yan, & Surmeier, 1998; Steephen & Manchanda, 2009). MSNs are considered the primary output neurones of the NAc, while the NAc core in humans also shows evidence of pyramidal-like neurones and some multipolar neurones, and shell showing evidence of groups of fusiform and multipolar neurones (Sazdanovic et al., 2011). The shell has also been shown to have MSNs which are significantly smaller and with a lower dendritic spine density in rats (Meredith, Baldo, Andrezjewski, & Kelley, 2008). Core and shell also appear to differ in the receptors and neuroactive substances that they

contain, with increased quantities of serotonin, dopamine, and substance P expressing neurones in the shell than core (Deutch & Cameron, 1992; Mermelstein et al., 1998), and more calbindin and enkephalin expressing neurones located in core than shell (Prensa, Richard, & Parent, 2003; Rogard, Caboche, Julien, & Besson, 1993). Another key difference between these two regions is their functional role. Overall, the NAc shell is thought to be involved with motivation and emotional aspects of behaviour, as opposed to the NAc core and dorsal striatum, which are associated with more cognitive functions or motor functions such as reaching (Pennartz, Ito, Verschure, Battaglia, & Robbins, 2011). These functional differences are due to the differences in their projections, with core projecting to more premotor brain regions, and shell projecting to prefrontal cortex and subcortical motor regions (Alexander, Crutcher, & DeLong, 1990; Zahm & Brog, 1992). Despite functional differences, the various striatal sub-regions form an ascending spiral of connectivity from the shell to the core and then on to central and dorsal striatal regions through connectivity of midbrain dopamine cells (Haber & Knutson, 2010).

The role of the NAc in reward and motivation has long be studied, particularly when looking at dopaminergic systems. Dopamine has been suggested to be one of the primary reward transmitters in the brain (Wise & Rompre, 1989), with studies showing that anticipation of reward increases dopamine levels in the nucleus accumbens due to dopaminergic projections from the ventral tegmental area (Hernandez & Hoebel, 1988) and that dopamine antagonists can prevent the sensation of reward (Wise, Spindler, deWit, & Gerberg, 1978). Further work looking at the involvement of the NAc has demonstrated that dopamine depletion or antagonism on the NAc does not result in suppressed food intake, i.e. decreased motivation for a natural reward such as food (Baldo, Sadeghian, Basso, & Kelley, 2002; Salamone & Correa, 2012; Salamone, Mahan, & Rogers, 1993). Similarly, measures of appetitive taste reactivity (pleasure from eating food) have been used to show that dopamine depletion in the NAc does not impact the hedonic sensation usually associated with eating (Berridge, 2007). Despite the lack of effect on food intake and hedonic reactivity, the NAc has been shown to be important in various aspects of food-seeking behaviour. For example, dopamine depletion in the NAc has

been shown to reduce the motivation to find food on an effort-based decision making task (Mai, Sommer, & Hauber, 2012; Salamone, Correa, Nunes, Randall, & Pardo, 2012), and dopamine antagonism in the NAc suppresses spontaneous locomotor activity and rearing. These data tend to agree with the incentive salience theory of dopamine function, which suggests that dopamine manipulations affect the "wanting", or motivational aspects, of the stimuli, but not the "liking", or hedonic aspects (Baldo et al., 2002; Berridge & Robinson, 1998).

In terms of hippocampal connectivity to the NAc, tracer studies have shown that projections primarily originate in the subiculum, the major output structure within the hippocampus, and to a lesser extent in the CA1 region, and travel via the fornix fibre bundle to the caudomedial NAc (Groenewegen, Vermeulen-Van der Zee, te Kortschot, & Witter, 1987; Humphries & Prescott, 2010; Kelley & Domesick, 1982; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Other studies have demonstrated the hippocampal connections to NAc via direct stimulation of the subiculum, resulting in concurrent activity in the NAc (Yang & Mogenson, 1984), or by demonstrating synchronous activity between the hippocampus and NAc by showing that hippocampal LFP correlates with the 'up-state' of NAc MSNs, indicating that hippocampal input is important for the transition of MSNs from the 'down-state' to the 'up-state' (Goto & O'Donnell, 2001). Further research then showed that synchronous activity between the hippocampus and NAc can be measured during spatial exploration, as noted by a synchronisation of theta rhythms between the two regions (Gruber, Hussain, & O'Donnell, 2009). The potential for the connectivity between NAC and hippocampus to be involved in spatial navigation and place learning has already been seen to an extent, with numerous studies combining lesion and behavioural experiments to show that disrupting these connections result in behavioural deficits. One such study (Whishaw, Cassel, & Jarrad, 1995) used fimbria-fornix lesions in rats, and they used a variation of the standard watermaze task to demonstrate that rats with fimbria-fornix lesions were able to form place memories. They determined this by training all rats with a visible platform in single location over a period of 20 days, starting with rats being placed onto the platform directly, and ending training with rats swimming to the platform from various start points.

Subsequently, rats carried out probe trials (platform was either hidden or removed entirely), and it was found that rats would swim directly to the correct location, indicating that they had formed a place memory for the platform location and were able to recall where it was. Following tests were then carried out in which the hidden platform was moved to a distinct location on each day for a period of 5 days, in which they found that fimbria-fornix lesioned rats were significantly impaired in their ability to form place memories for the changing hidden platform. This indicates that, while lesioned rats were still able to form place memories when the platform location was consistent, they were unable to form place memories for platform locations that were constantly changing. Similar fornix-fimbria lesion studies have also shown altered exploratory behaviour in an open-field environment, with lesioned animals demonstrating fewer return trips to a designated 'home-base' than controls, again indicating intact place information (where the home-base is) but altered behavioural responses (Gorny, Gorny, Wallace, & Whishaw, 2002). These findings may not be entirely representative of NAc involvement in place memory as fimbria-fornix also carries many other fibres in addition to NAc projections.

Following on from previous discussion, findings from a study in which the functional connection between vSub and NAc was disrupted show that transient disconnection between vSub and NAc produces an impairment in foraging behaviour during a non-delayed foraging task but not during the delayed task (Floresco et al., 1997). This data suggests that the NAc is involved in the translation of rapidly-acquired place memory into behaviour, but has no involvement in incremental learning. This can be seen as in the situation where there was no delay between learning and behaviour, the connection between hippocampus and NAc showed to be necessary for performance, whereas disruption of this connection had no effect on the foraging task when there was a delay between learning and behaviour. Various studies have also looked at the effect of direct NAc lesioning on place memory. One such study used ibotenic acid-induced lesions on performance on a T-maze and watermaze task, showing that both learning and reversal were impaired in the lesioned group on a spatial discrimination task and that acquisition of the platform location on the watermaze task was impaired (Annett, McGregor, & Robbins, 1989). Another study

demonstrated a lack of impairment on a watermaze task following lidocaine-induced lesioning of the NAc, though this may reflect that NAc is only required for rapid place learning which is not tested using a standard watermaze paradigm (Floresco, Seamans, & Phillips, 1996).

Aims of the PhD project

The primary aim of this study is to determine the functional significance of the NAc for behaviour based on hippocampus-dependant rapid place learning. To this end, we will be utilising a combination of pharmacological manipulation (functional inhibition using the GABA agonist muscimol) of the NAc and behavioural testing. Initially, in vivo electrophysiological techniques were used to measure the effect of accumbal muscimol infusion on neuronal activity within the NAc (outlined in Chapter 2). The purpose of the electrophysiological experiments was to ensure that these muscimol doses produced inhibition as we would expect, and therefore act as a positive control for our behavioural experiments. Sensorimotor testing was carried out in order to rule out any gross sensorimotor side effects when using the chosen muscimol doses (Chapter 3). These doses (125ng & 250ng/0.5µl muscimol) were based on previous research (Pezze et al., 2014) in which they produced appropriate levels of inhibition in the medial prefrontal cortex. The sensorimotor tests used were locomotor activity (LMA) testing and prepulse inhibition (PPI) of the startle response. LMA is a measure that looks at exploration and motivation, mechanisms which have both been linked to accumbal activity (Ikemoto & Panksepp, 1999; Salamone, Correa, Mingote, & Weber, 2003). PPI is the percentage reduction of a startle response to a startle-eliciting stimulus (a pulse of sound) when it is preceded by a lesser stimulus (a quieter 'pre-pulse'), and occurs due to activation of sensory gating mechanisms (Swerdlow, Caine, Braff, & Geyer, 1992). Increased accumbal dopamine has been shown to affect both of these measures, inducing hyperactivity and impairing PPI (Bast & Feldon, 2003; Ikemoto, 2002; Swerdlow, Geyer, & Braff, 2001).

Following on from the electrophysiological (Chapter 2) and sensorimotor experiments (Chapter 3), we then conducted two watermaze studies (Chapters 4 and

5) to test the hypothesis that functional inhibition of the NAc by muscimol will impair the expression of hippocampus-dependent rapid, 1-trial place learning as measured on the water maze DMTP task (Bast et al., 2009; Steele & Morris, 1999). This task is a good method of measuring rapidly-acquired place memory using an animal model. As mentioned, the DMTP task differs from the standard incremental place learning task (R. Morris, 1984) in that the platform location changes each day (as opposed to remaining consistent in the standard task). Multiple measures can be taken from this task, including the latency and pathlength of the rat to finding the platform. The measure that we mostly focus on is the search preference, which measures the percentage of time that the rat spends in the correct location's 'zone' when compared to the amount of time it spends in all other possible platform location's 'zones' (see Chapter 4 for full details). Using the DMTP task, we can look at the effects of functional inhibition of the NAc on one-trial place learning, which has been shown to be hippocampus dependent (Bast, 2007; M. B. Moser & Moser, 1998).

Chapter 2: Electrophysiological Effects: Reduction of firing rate and burst firing following accumbal muscimol infusion

Introduction

The primary method via which information is transmitted throughout the nervous system is through neuronal activity. Within neural circuits, complex information can be transmitted through changes in the firing rate of neurones and changes in burst firing of groups of neurones to transmit a 'neural code' (Izhikevich, 2003; Legendy & Salcman, 1985). In this manner, by manipulating neuronal activity it is possible to determine the functional role of specific brain regions. In the case of this thesis, the aim is to determine whether or not functional activity in the NAc is required for behavioural performance based on hippocampal rapid place learning. When carrying out behavioural testing, it is important to understand the underlying biological mechanisms at work to directly link any changes in behaviour to changes in neural activity within the NAc. Therefore, we carried out in vivo electrophysiological recording of multi-unit activity in the NAc of anaesthetised rats to measure neuronal activity and understand the underlying neurobiology. In order to test the hypothesis that the NAc is involved in this learning-behaviour translation, we therefore required a method of altering the neuronal activity specifically within the NAc. There are multiple methods available to achieve this (such as lesioning or optogenetic silencing), but the method that was chosen for this study is pharmacological inhibition via the GABA-agonist muscimol. This method was chosen due to its temporary action as this allows for within-subjects testing due to the transient inactivation of neuronal activity resulting in it returning to baseline and allowing for multiple testing conditions per animal, as well as its advantages over other methods (such as sodium channel blockers) in that it only affects local neurones without affecting fibres of passage (Edeline et al., 2002). We combined muscimol

microinfusion with multi-unit recording by implanting an 8-electrode recording array with attached injector into the NAc shell of anaesthetised rats and recording both pre- and post-infusion periods, comparing the effects of 2 muscimol infusion doses and saline infusion. Using this method, we measured neuronal firing rate over the course of the experiments (recording period lasting approximately 90 minutes per rat) and burst firing using different parameters. These measures were chosen based on previous studies in which drug infusion into distinct brain regions showed significant effects on firing rate and burst firing (McGarrity et al., 2017; Pezze et al., 2014).

Materials and methods

Subjects

We used thirty male Lister hooded rats (Charles River, UK), approximately 10-13 weeks old and weighing between 308g and 498g when the experiment was carried out. Rats were kept in cages of four where possible with food and water provided ad libitum, temperature control (20-23°C), humidity control (40-55%) and a light-dark cycle of 12 hours per phase (lights on between 7am and 7pm), with all experimental work being carried out during the light phase. Prior to the start of any experimental procedures, rats were handled regularly to habituate them to the experimenters. All procedures were conducted in accordance with the requirements of the UK Animals (Scientific procedures) Act 1986. All efforts were made to minimise animal suffering and to reduce the number of animals used.

Implantation of recording array and injector assembly for acute in vivo electrophysiology

For these experiments, rats underwent stereotaxic implantation of an 8-electrode recording array with attached injector. Rats had anaesthesia induced using 4-5% isoflurane in medical air and maintained at 1-3% for the remainder of the

experiment. They were then secured in a stereotaxic frame using ear bars (EMLA cream (5%; AstraZeneca) applied to reduce the risk of pain), with temperature regulation throughout using a heating pad with temperature adjusted using a rectal probe. Depth of anaesthesia was monitored via breathing rate throughout the experiment, with it kept to between 40 and 60 breaths per minute as much as possible. The scalp was then incised (bregma and lambda aligned horizontally), with coordinates for the NAc being taken from bregma as follows: 1.5 mm anterior, 1.0 mm lateral from bregma and 6.7 mm ventral from the skull surface (targeting the right NAc shell). A small section of skull was then removed above the NAc via drill, exposing dura, which was then incised to expose the surface of the brain beneath (which was kept moist with 0.9% saline). The electrode recording assembly, which consisted of a 33-gauge stainless steel injector attached to an 8-electrode recording microwire array (figure 2C) (each electrode being a 50 µm Teflon-coated stainless-steel wire, impedance of ~100 kΩ measured at 1 kHz) and a stainless steel ground wire (NB Labs), was then lowered slowly (over approximately 45 minutes) to the target dorsoventral coordinate (McGarrity et al., 2017; Pezze et al., 2014), with the electrodes arranged parallel to the midline and the injector being positioned between the electrodes and the midline. The row of electrodes was approximately 2 mm long between the 1st and 8th electrode with the tip of the injector being positioned towards the centre of the array (between electrodes 4 and 5) and around 0.5 mm above the tips of these electrodes. The array was then connected to the recording system via a head stage. The injector was connected via Teflon tubing (0.12 mm internal diameter, Bioanalytical Systems) to a 1 µl Hamilton syringe, with the tubing and injector system being filled with saline, 125 ng or 250 ng/0.5 µl muscimol. A small air bubble was included at the end of the tubing towards the syringe, as movement of this bubble was used to determine whether or not the infusion was successful. A 0.25 µl air plug was also included at the injector end of the infusion system before it was inserted into the brain in order to prevent early infusion of the drug during the pre-infusion period due to leakage. Upon reaching the target dorsoventral coordinates, there was at least 30 minutes before recording to allow stabilisation of the system.

Multiunit and Local Field Potential recordings

The recording array was connected to a multichannel preamplifier (Plexon, US) via a unity-gain multichannel head stage. The preamplifier acted to 1000x amplify the analogue signal and apply a band-pass filter in order to separate multiunit activity and local field potential signals (250-8 kHz and 0.7-170 Hz respectively). These signals were then processed by a multichannel acquisition processor system (Plexon, US) which allowed for additional computer-controlled amplification with a final gain of up to 32,000, further multiunit data filtering (500-5 kHz), and digitisation of spikes at 40kHz. Multiunit and Local Field Potential (LFP) data could then be viewed using Real-Time Acquisition Systems Programs for Unit Timing in Neuroscience (RASPUTIN) software (Plexon, US), with this then being used to record our 30 minute pre-infusion period and 60 minute post-infusion period. All recording were made with the ground wire of the array clamped to the ear bars using crocodile clips which were connected to the stereotaxic frame.

Verification of electrode placements

At the end of each experiment, some electrodes (usually 1, 4, and 8) were marked via electrolytic lesioning. This was done by passing a 1 mA current for 10 s through two pairs of the electrodes to deposit ferric ions at the tip of the positive electrode. Successful markings were determined by current successfully flowing (as seen by an increase on the amp gauge on the electrolytic lesioner, indicating a successful circuit has been made and lesioning has occurred). Upon unsuccessful marking, two other electrodes were used to mark (usually the most anterior/posterior that would mark properly). The rat was then euthanised by an overdose of anaesthetic and brains removed and stored in a solution consisting of 4% paraformaldehyde with 4% potassium ferrocyanide. These were stored for at least 2 days before being sliced via vibratome into 80 µm coronal sections and mounted onto slides. Electrode tip positioning was determined using a light microscope and mapped onto coronal sections of stereotaxic rat brain atlas (Paxinos & Watson, 1998). Ferric ion deposits were shown by the Prussian blue reaction.

Microinfusion procedure and experimental design

Following a pre-infusion period of at least 30 minutes, the drug of interest (0.5 µl plus 0.25 µl air plug) was manually infused over the course of approximately 90 seconds (resulting in an infusion speed of approximately 0.5 µl/min to match behavioural studies). This was done by slowly moving the piston of the Hamilton syringe until the 0.25 µl air plug and 0.5 µl drug had been infused into the NAc shell, with confirmation of successful infusion based on movement of the air bubble that was included in the Teflon tubing. Start and end times of the infusion were noted so this period of time could be removed from recording, with accurate end times for pre-infusion periods and start times for post-infusion periods being acquired. Following the infusion, a 60 minute post-infusion period was recorded, with any periods of noise being noted and manually removed from analysis. Experiments were run in batches of 4 rats where possible and rats were randomly allocated to one of the three infusion groups (125 ng group added in last half of experiments, so a larger proportion of the rats were allocated into this infusion group). Final drug group sizes were as follows: saline, n = 8; 125 ng/0.5 µl muscimol, n = 7; 250 ng/0.5 µl muscimol, n = 8. A sample size of 8 per group was targeted due to similar sized groups being used in previous studies to reveal statistically highly significant effects of drug infusion that corresponded to significant behavioural changes (McGarrity et al., 2017; Pezze et al., 2014). 7 additional rats were tested but excluded from analysis: 2 rats due to failed infusions as noted by the air bubble not moving, 3 due to noise interference during recording producing unusable data, 1 due to problems maintaining consistent levels of anaesthesia making the changes in neuronal activity unrepresentative of the action of muscimol, and 1 due to electrode placement being significantly outside of the NAc shell.

Analysis of electrophysiological data

Analysis was carried out using NeuroExplorer version 4 (Nex Technologies, US) software, utilising multi-unit data to look at firing rate and burst parameters. These parameters were measured from each of the 8 electrodes per rat and normalised

within each channel by dividing each time point (analysis done in 5 minute bins) by the average of the 6 baseline time bins. Large variations in activity between channels in a single rat were common due to differences in the areas that each electrode was being implanted into, with some channels showing little to no spiking (potentially being implanted into white matter tracts) and others showing more consistent activity. These were then averaged across the 8 channels to produce 1 set of 18 time bins (6 pre- and 12 post-infusion) per rat which could then be used to analyse drug infusion effects. All data are presented as the mean ± SEM. All data was analysed with GraphPad Prism version 7 using ANOVA with a between-subjects factor of infusion group and a within-subjects factor of 5 minute block across all 6 baseline blocks and all 12 post-infusion blocks. As in our previous studies (McGarrity et al., 2017; Pezze et al., 2014), to support drug-induced changes in neural activity, data was examined for significant interactions between infusion group and 5 minute block, with an accepted level of significance set as $p < 0.05$. When analysing burst firing parameters (periods of high spiking separated by periods of quiescence), we used the interval method of determining bursts with a set maximum inter-spike-interval within burst of 40 ms and a minimum of 40 ms inter-burst-interval and a minimum of 3 spikes per burst, as similar parameters have been previously used to measure burst activity within the NAc (Cooper & White, 2000). We also looked at the surprise method (named as such due to the improbable nature of a burst of neuronal spiking as compared to the average firing rate). The surprise value (S) of a burst is defined as the negative natural logarithm of the probability that a high burst firing rate occurs due to chance (Legendy & Salcman, 1985). For this analysis, bursts with a surprise value of greater than 3 are included (Pezze et al., 2014; Stevenson, Halliday, Marsden, & Mason, 2007). For each 5 minute block, we calculated the number of bursts, percentage of spikes that are within bursts, mean burst duration, mean within-burst firing rate, and mean inter-burst-interval. For the analysis of mean burst duration, mean within-burst firing rate, and mean inter-burst interval, any channel where the number of bursts was 0 in one or more 5 minute blocks were excluded. All channels with a mean baseline firing rate of less than 0.1Hz were excluded (Pezze et al., 2014) in order to be able to detect a decrease in firing rate following infusion and to minimise the risk of poor signal to noise ratio. With the surprise 3 data, all channels

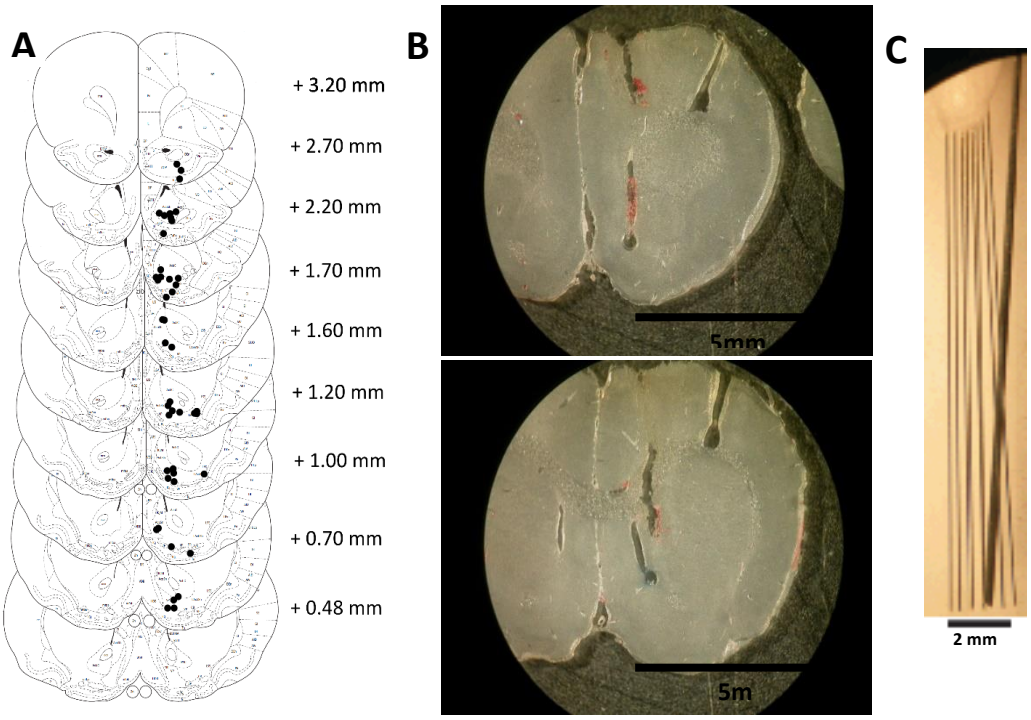


Fig. 2. A) Approximate locations of electrode tips (most anterior and most posterior electrodes represented by dots) as verified by light microscope examination of 80 µm coronal brain sections. Placements mapped onto coronal sections from Paxinos & Watson brain atlas, with numbers on the right indicating distance from bregma. B) Coronal brain sections showing anterior (top, electrode 2) and posterior (bottom, electrode 6) electrode placements under light microscope. Bottom picture shows ferric ion deposit following Prussian blue reaction. C) Layout of electrode-injector array as seen via light microscopy, with 8 electrodes and a stainless steel injector attached.

with a low baseline number of bursts (0 bursts in any of the baseline time bins) were excluded from burst analysis. This usually occurs in bins with very high firing rate, with the burst surprise method showing a low number of bursts with a higher firing rate due to the inverse relationship between the two measures. This is problematic for the burst analysis as increasing firing rates following infusion actually show as a decrease in post-infusion bursting in these high firing channels, which causes the data to become unrepresentative of the changes in burst firing that are actually occurring. Separate analysis for firing rates and both burst parameters was also conducted using only the first 30 minutes post-infusion, to reflect the 30 minutes post-infusion period seen in behavioural testing (Chapter 3). Baseline values (Table 1) were calculated by averaging across the 6 baseline time bins for each channel, then averaging across each channel included in analysis for each rat, and finally averaging across all rats included in analysis for each measure. These were also compared using ANOVA in GraphPad Prism version 7.

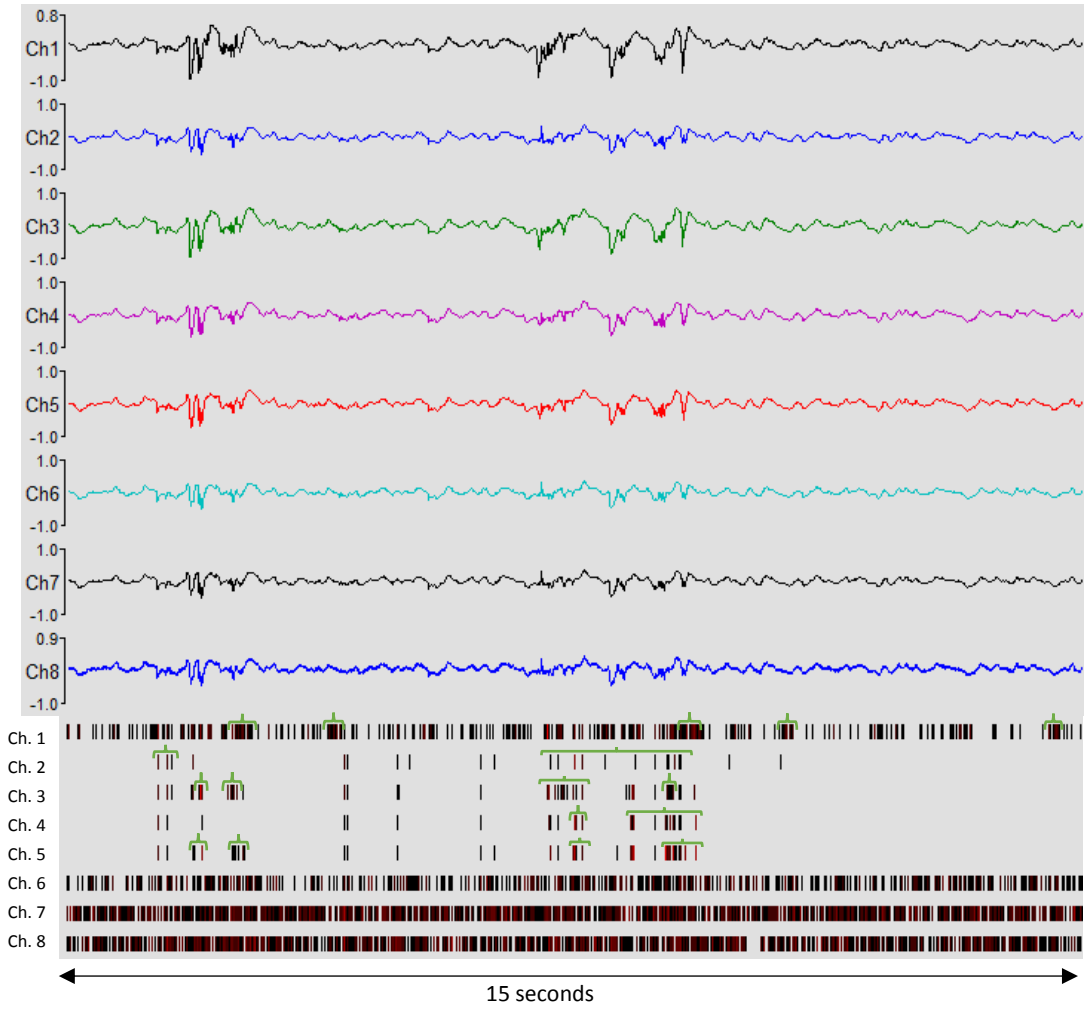


Fig. 3. Example of raw firing (LFP data above, raster plot below) for 8 channels in a single rat over a 15 second period during baseline recording. Y-axis on LFP data in mV, bursts as defined by the surprise 6 parameter marked in green on raster plot. Bursts seen in the raster plot correspond with negative deflections in the LFP.

Results

Verification of electrode placements

Electrode tips were primarily positioned within the NAc shell between 0.7 mm and 3.2 mm anterior to bregma (figure 2A shows most anterior and most posterior electrode placement). Anterior and posterior electrodes were primarily located within the NAc shell, indicating that all electrodes would have been placed appropriately. In some circumstances, the more anterior or posterior tips were slightly outside the shell, indicating that these may record activity from the shell and

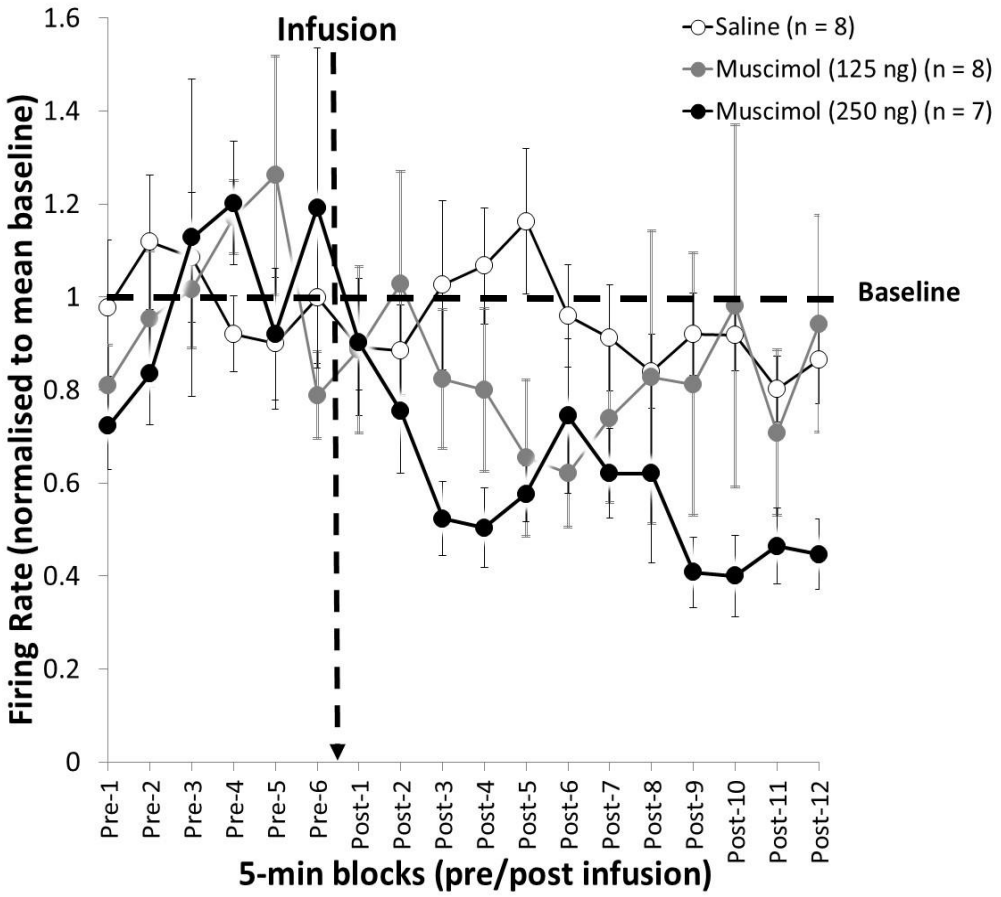


Fig. 4. Effects of monolateral accumbal muscimol infusion on neural activity as measured by firing rate (normalised to mean baseline, between-subjects study (saline and 125 ng muscimol groups n = 8, 250 ng muscimol group n = 7); data presented as mean ± SEM). Data presented and analysed across 5-min time blocks, with pre 1-6 representing the 30 mins preceding the start of the infusion, and post 1-12 representing the 60 mins following the end of infusion. As compared to saline infusion, overall firing rate decreased following 250 ng muscimol infusion across the 60 min post-infusion period, and also decreased following 125 ng muscimol infusion for 30 min following infusion before returning to baseline.

other areas. However, the rest of the electrodes would have been appropriately positioned. In some circumstances, the distance between the electrode tips was less than 2 mm (determined based on the number of sections and thickness of sections), though this could have been due to slight 'bunching' of the electrodes upon implantation, causing them to converge slightly. 1 rat was also excluded based on placement locations outside of the NAc shell. Visual inspection showed damage primarily surrounding the tips of the electrodes where the electrolytic lesions had occurred, with brain sections shown (figure 2B). An example of raw baseline firing (raster plot and LFP data) over a 15 second window during baseline is shown in figure 3.

Baseline Firing Parameters

Baseline values for overall firing rate and nearly all measures of burst analysis (Table 1) were not significantly different between drug groups. The only exception to this is the percentage spikes fired in bursts for the 40 ms interval burst analysis method, with a significant effect of infusion group ($F(2, 20) = 3.783$, $P = 0.0404$).

Overall firing rate

Muscimol dose-dependently reduced overall firing rate as compared to saline infusions (figure 4). The firing rate of all groups in the pre-infusion period remains close to the normalised baseline (± approximately 20%). Following the infusion, the saline group does not show consistent changes from baseline (still ± approximately 20%), indicating a lack of non-specific infusion effects or baseline drift. The largest effect of muscimol infusion can be seen in the high dose 250 ng group, with overall

	Overall firing rate (1/s)	**Bursts (1/min)**	**% spikes fired in bursts**	**Within-burst firing rate**	**Burst duration (ms)**	**Inter-burst-interval (s)**
Saline	35.5±12.9 (n = 8)	297.5±59.8 (n = 8)	61.2±7.9 (n = 8)	144.7±16.1 (n = 8)	11.1±10.5 (n = 8)	4.9±1.9 (n = 8)
Muscimol (125 ng)	15.7±5.4 (n = 8)	239.8±68.8 (n = 8)	46.3±5.5 (n = 8)	136.8±35.7 (n = 7)	5.7±5.5 (n = 7)	5.9±2.6 (n = 7)
Muscimol (250 ng)	17.6±10.3 (n = 7)	145.1±40.6 (n = 7)	35.3±6.0 (n = 7)	170.5±25.9 (n = 7)	1.2±1.1 (n = 7)	11.4±3.1 (n = 7)
Effect of infusion	$F_{2,20}$=1.2, P = 0.3	$F_{2,20}$=1.6, P=0.2	$F_{2,20}$=3.8, P=0.04	$F_{2,19}$<1	$F_{2,19}$<1	$F_{2,19}$=1.9, P=0.2
Saline	35.5±12.9 (n = 8)	87.9±26.0 (n = 8)	35.3±7.5 (n = 8)	219.8±24.2 (n = 8)	0.2±0.04 (n = 8)	9.0±1.6 (n = 8)
Muscimol (125 ng)	15.7±5.4 (n = 8)	51.3±15.5 (n = 8)	32.2±3.3 (n = 8)	157.0±23.0 (n = 8)	0.3±0.08 (n = 8)	18.8±5.2 (n = 8)
Muscimol (250 ng)	17.6±10.3 (n = 7)	56.8±13.2 (n = 7)	34.8±6.2 (n = 7)	150.6±33.6 (n = 7)	0.6±0.2 (n = 7)	17.7±4.2 (n = 7)
Effect of infusion	$F_{2,20}$=1.2, P=0.3	$F_{2,20}$=1.1, P=0.4	$F_{2,20}$<1	$F_{2,20}$=2.1, P=0.2	$F_{2,20}$=2.4, P=0.1	$F_{2,20}$=1.9, P=0.2

Table 1. Pre-infusion baseline values (mean ± SEM) of electrophysiological multi-unit measures in the three prospective infusion groups. Burst parameters shown in the top are based on burst detection using inter-spike-intervals of 40 ms or less to detect bursts, parameters in the bottom are based on burst detection using the Poisson Surprise method with a surprise value of 3. Sample size contributing to each measure is indicated in brackets.

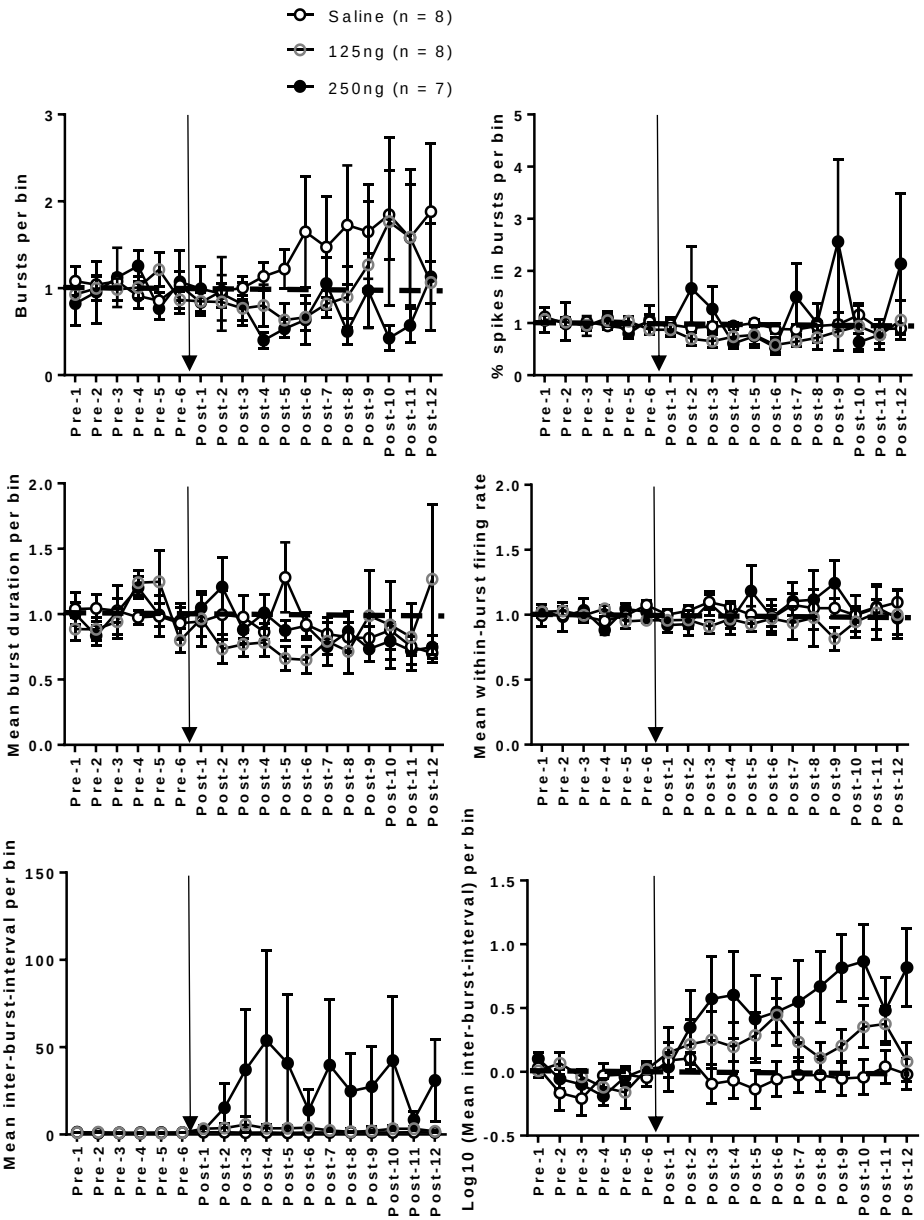


Fig. 5. Effects of monolateral accumbal muscimol infusion on neural activity as measured by 40 ms interval burst firing parameter (all normalised to mean baseline, between-subjects study (saline and 125 ng muscimol groups n = 8, 250 ng muscimol group n = 7); data presented as mean ± SEM). Data presented and analysed across 5-min time blocks, with pre 1-6 representing the 30 mins preceding the start of the infusion, and post 1-12 representing the 60 mins following the end of infusion. Bevelled horizontal line indicates baseline and vertical arrow indicates infusion.

normalised firing rate in the NAc dropping to 0.5-0.4, indicating a 50-60% firing rate reduction by the end of the 60-minute post-infusion recording period. A shorter numerical reduction in overall firing rate can also be seen in the 125 ng group, with the normalised firing rate dropping to around 0.6 at post-infusion block 6, showing an approximate 40% reduction half an hour following infusion. After this point, the firing rate returns to baseline over the final 30 minutes of recording, indicating a

shorter-lasting action of the lower muscimol dose. In spite of this numerical reduction in firing rate after infusion, the interaction and main effect involving the factor infusion failed to reach significance in the overall 2-way ANOVA of the data from all three infusion groups (interaction infusion x 5 min blocks: $F(34,340) = 1.215$, $P = 0.1962$). However, carrying out separate ANOVA comparing the saline and 250 ng group shows a significant effect (2-way ANOVA between saline and 250 ng muscimol group: main effect of infusion group: $F(1, 13) = 16.32$, $P = 0.0014$; interaction infusion x 5 min block: $F(17, 221) = 1.856$, $P = 0.0231$). A separate ANOVA comparing saline and 125 ng muscimol did not reveal a significant main effect or interaction involving the factor infusion (interaction infusion x 5 min block: $F(17, 238) = 0.975$, $P = 0.4878$) or between 125 ng and 250 ng muscimol (interaction infusion x 5 min block: $F(17, 221) = 1.02$, $P = 0.4379$). Separate analysis looking only at the 30 minutes post-infusion period showed a significant interaction ($F(10, 100) = 2.028$, $P = 0.038$), with additional ANOVA showing significant main effect of infusion only between saline and 250 ng groups (infusion: $F(1, 13) = 12.97$, $P = 0.0032$, interaction: $F(5, 65) = 2.158$, $P = 0.0695$). No significant main effect or interaction was detected between saline and 125 ng groups or between 250 ng and 125 ng groups (all $P > 0.06$).

Burst firing – Burst detection based on 40 ms ISI

When looking at burst firing parameters using the 40 ms interval method, a variety of effects can be seen. When bursts are defined based on a 40 ms ISI, muscimol tends to induce several numerical changes as compared to saline that are broadly consistent with a relative suppression of burst firing, although none of these changes approach statistical significance (figure 5). First, muscimol reduces the number of bursts as compared to saline infusion. The number of bursts in each bin increases in the saline group and to an extent in the 125 ng muscimol group, but the 250 ng muscimol group appears to decrease slightly (interaction infusion group X 5 min bin: $F(34, 340) = 0.880$, $P = 0.6642$). Second, percentage of spikes that are within-burst, mean burst duration within-burst and mean within-burst firing rate all appear to remain around baseline (though with some variability), with no significant interactions (all $F < 1.053$, all $P > 0.3933$). Finally, Inter-burst-interval data appears to

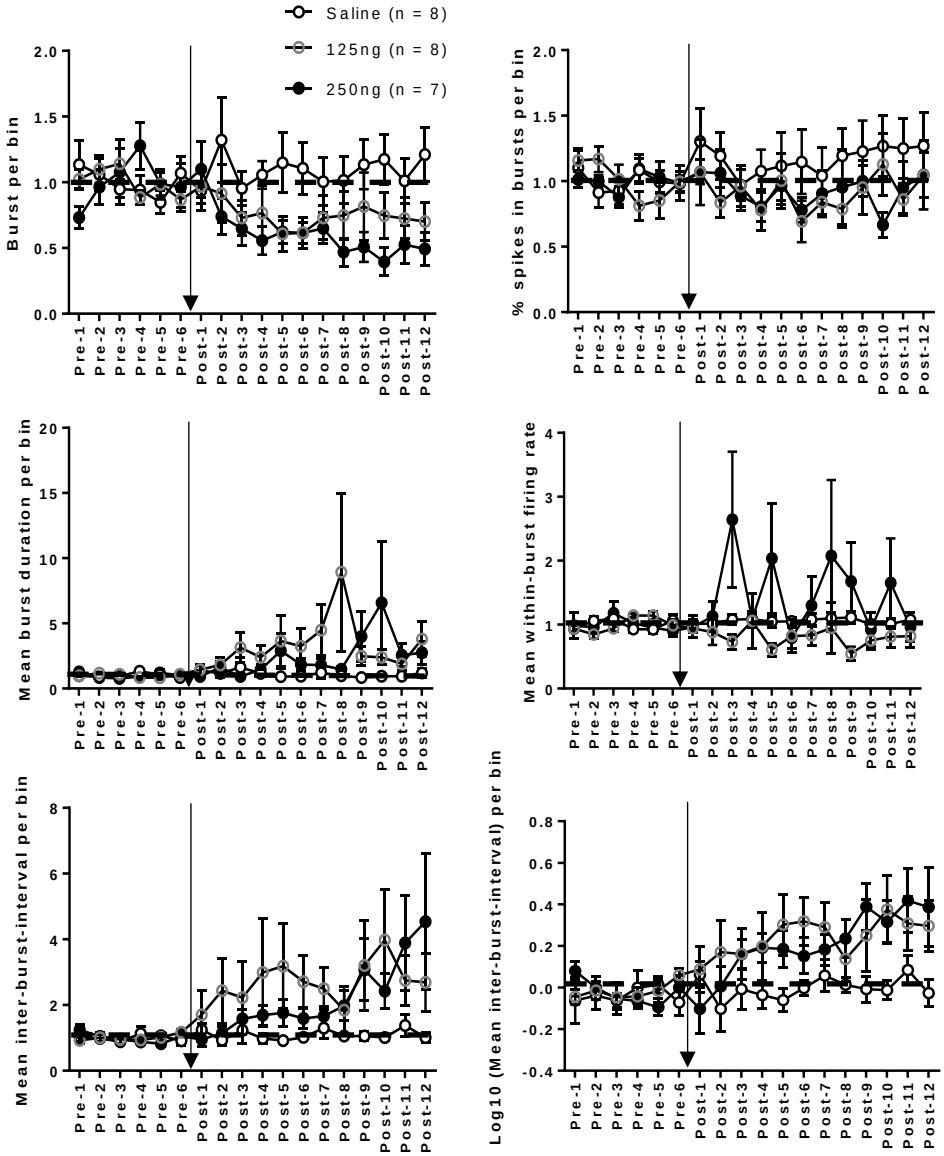


Fig. 6. Effects of monolateral accumbal muscimol infusion on neural activity as measured by surprise 3 burst firing parameter (all normalised to mean baseline, between-subjects study (saline and 125 ng muscimol groups n = 8, 250 ng muscimol group n = 7); data presented as mean ± SEM). Data presented and analysed across 5-min time blocks, with pre 1-6 representing the 30 mins preceding the start of the infusion, and post 1-12 representing the 60 mins following the end of infusion. Bevelled horizontal line indicates baseline and vertical arrow indicates infusion.

show that saline and the low dose of muscimol stay close to baseline levels, but following infusion the variability within the high dose muscimol group increases massively (interaction: $F(34, 323) = 1.098$, $P = 0.3297$). Due to this high variability, a log plot of the data was also produced and analysed, this time showing a significant interaction ($F(34, 323) = 2.084$, $P = 0.0006$). Separate analysis of the first 30 minutes post-infusion alone shows a similar pattern. Almost all analysis shows no significant interaction or main effect (all $F < 2.363$, all $P > 0.1212$). There is, however, a significant

interaction when analysing the log plot data of the mean inter-burst-interval ($F(10, 95) = 3.052$, $P = 0.0021$).

Burst firing – Surprise 3 parameter

When bursts are defined using the Poisson surprise method (with a surprise factor of 3), the analysis of burst parameters reveals significant dose-dependent changes induced by muscimol as compared to saline, which suggest that muscimol suppresses burst firing (figure 6). First, muscimol infusion into the NAc decreased the number of bursts per bin by approximately 50% in the high dose group and approximately 30% in the low dose group, with these changes emerging within the first 10-15 min following infusion and lasting until the end of the recording session in both cases (interaction infusion group X 5 min block: $F(34, 340) = 1.749$, $P = 0.0074$). Separate ANOVAs show a significant infusion X time interaction when comparing the saline and high dose groups (interaction: $F(17, 221) = 2.838$, $P = 0.0002$), but not when comparing saline and the low dose (interaction: $F(17, 238) = 1.334$, $P = 0.1723$). There was also an increase in inter-burst-interval following muscimol infusion, with the low dose steadily increasing over the hour post-infusion period and the high dose increasing slower in the first half an hour, followed by a much more rapid increase in the final half an hour (interaction: $F(34, 340) = 1.693$, $P = 0.0110$). Separate ANOVAs for this measure show significant differences between saline and both groups (interactions: both $F > 2.024$, both $P < 0.0108$).

Despite the number of bursts decreasing and an increase in inter-burst-interval, the percentage of overall spikes that are within bursts remains around baseline for all groups (potentially due to the decrease in firing rate accompanying the decrease in burst firing following muscimol infusion; interaction: $F(34, 340) = 0.756$, $P = 0.8378$). There is also a strong trend towards an increase in the mean burst duration in both muscimol groups (interaction: $F(34, 340) = 1.4$, $P = 0.0732$) with an increase in variability in the final 30 minutes for both groups (as seen by the increase in SEMs, figure 6). This increase in variability can also be seen when looking at the within-burst firing rate, with variability in the 250 ng muscimol group increasing massively during

the post-infusion period. Strangely, the 125 ng group appears to stay around baseline (or slightly decrease if anything), whereas the 250 ng group tends to be increased (interaction: $F(34, 340) = 1.555$, $P = 0.0281$). However, further ANOVAs shows that this significance can only be seen when comparing the effects of the two muscimol groups (interaction: $F(17, 221) = 1.797$, $P = 0.0296$) and not between saline and the two muscimol groups (both $F < 1.321$, both $P > 0.1808$). As with the 40 ms parameter, a log plot of the mean inter-burst-interval data was produced and analysed, with this showing a significant interaction ($F(34, 340) = 1.515$, $P = 0.0364$). Separate analysis of the first 30 minutes post-infusion alone show no significance in any group (all $F < 2.678$, all $P > 0.0932$), except for a significant interaction in the mean within-burst firing rate ($F(10, 100) = 2.228$, $P = 0.0218$).

Discussion

Muscimol infusion into the NAc produces a reduction of neuronal firing rate, with the 250 ng dose producing a reduction of approximately 50-60%, with this effect peaking after 50 minutes and lasting for the entire post-infusion period. The 125 ng muscimol dose also decreased firing rate but to a lesser extent, with firing rate dropping by approximately 40% (peak 30 minute after infusion) and then returning to baseline. Baseline firing rates did not differ significantly between saline and both muscimol doses indicating a lack of variability between rats. This is also the case for burst firing measures with the exception of percentage spikes fired as bursts in the 40 ms interval burst analysis. These data indicate that muscimol will not have 'leaked' into the brain before infusion, as this possibility was countered by inclusion of the 0.25 µl air plug in the injector and the effects of the muscimol can only be seen starting 10-15 minutes post-infusion (arguably the effects can be seen straight away, but due to the approximate 20% variability during baseline this cannot be stated with certainty). Burst analysis using the 40 ms interval parameter does not show any significant effects of muscimol infusion of various burst firing measures when compared to saline. Interestingly, post-infusion bursting numerically increases in the saline group, whereas it tends to decrease in the high dose muscimol group, consistent with

muscimol suppressing burst firing, though these effects fail to even approach significance. Percentage of spikes that are within bursts and inter-burst-interval also seem to increase following the high dose, though variability also increases a lot in the post-infusion period as measured by an increase in SEM. Burst analysis using the surprise 3 parameter seems to be a more appropriate method of measuring bursting in the NAc, with burst firing decreasing in both muscimol groups as compared to the saline group, and inter-burst interval increasing following muscimol infusion. This data seems more representative of the physiological changes seen following visual inspection of the multi-unit data.

These findings show that muscimol infusion does have a neural effect within the NAc, with the reduced firing rate acting as a positive control for our behavioural experiments. Previous studies have also shown the effects of muscimol microinfusion into discrete brain regions such as the medial prefrontal cortex (Pezze et al., 2014), in which 250 ng/0.5 µl muscimol infusion reduced neuronal activity by up to 50%, similar to the results seen in our experiments. Despite the low dose group showing a smaller and shorter-lasting effect on firing rate, this may reflect the potentiation of GABA-A receptors seen when using certain inhalation anaesthetics such as isoflurane (Lecker, Yin, Wang, & Orser, 2013), thereby reducing the neuronal effects of muscimol acting on GABA-A receptors in the NAc. This is supported by our sensorimotor experimental data (see Chapter 3), which shows that effects of both doses of muscimol infusion into awake, non-anaesthetised rats can be measured as far as 24 hours post-infusion. Due to the high density of MSNs in the NAc, it is likely that the recordings from this experiment primarily represent MSN activity. The fact that MSNs express a large quantity of GABA-A receptors (Meredith, Pennartz, & Groenewegen, 1993) further reinforces this speculation, as infusion of the GABA agonist muscimol produced a well characterised decrease in neuronal activity. As mentioned in Chapter 1, a small proportion of cells present in the NAc shell are multipolar and fusiform neurones, and so may represent some of the recorded neuronal activity.

Burst analysis data indicates the amount of variability that can be seen when using different criteria to define bursts to test the effects of muscimol infusion in the NAc. The 40 ms interval parameter was chosen due to similar parameters being used in previous studies looking at burst firing in the NAc (Cooper & White, 2000; Nason, Adhikari, Bozinoski, Gordon, & Role, 2011). Despite this, data from our experiments indicate a lack of changes in various burst measures, indicating that while the overall firing rate was reduced following muscimol infusion, bursting remained unchanged. We also utilised the surprise 3 burst parameter used in previous experiments involving muscimol infusion causing significant changes in burst firing measures (Pezze et al., 2014). The surprise method defines a burst as a short period of significantly higher firing as compared to adjacent periods, which may be a better measure of burst firing within the NAc due to the more variable firing rate seen in this region. Despite this, there are problems with this method, such as in the case of faster firing rates resulting in a significantly lower burst count (due to the inverse relationship of overall firing rate with burst counts using this parameter). This means that channels in which firing rate was fast in the baseline period and decreased post-infusion would be represented as an increase in bursting from pre- to post-infusion, which is not representative of what is happening physiologically. As a result, channels in which burst counts were low during baseline (0 bursts in any bin) were excluded from this analysis. This method also, on occasion, counts spikes with a large inter-spike-interval as being close enough to count as a burst when the firing rate is extremely low (this leads to the variability in mean burst duration seen in both muscimol groups). As very few studies in the literature have examined burst firing in the NAc, an appropriate burst parameter has never been properly verified, leading to some of the problems seen in these experiments. Other burst parameters were also tested based on previous studies (McGarrity et al., 2017; Pezze et al., 2014) such as intervals of 3 and 6 ms, and bursts with a Surprise value of 6 or greater. All of these methods showed to be inadequate, giving burst counts of 0 in the majority of time-bins.

Overall, these data together indicate that muscimol has an in vivo effect on neuronal activity in the NAc, reducing overall firing rate due to its action at GABA-A receptors,

and potentially reducing burst firing at the same time (when looking at the surprise 3 burst parameter).

Chapter 3: Sensorimotor Effects: Minor effects on locomotor activity and prepulse inhibition following accumbal muscimol infusion

Introduction

While the role of the NAc in functions such as motivation and reward is relatively well established (Salamone, 1994) (see Chapter 1), it has also been implicated in a range of sensorimotor processes, particularly when focusing on the effects of altered dopaminergic transmission, including open field locomotor activity and sensorimotor gating as reflected by prepulse inhibition of the startle response. For example, stimulation or inhibition of NAc dopamine transmission increase or decrease, respectively, locomotor activity (Ikemoto, 2002; Svensson & Ahlenius, 1983). Moreover, dopamine infusion into the NAc has been shown to impair sensorimotor gating mechanisms as measured by PPI of the startle response: a mechanism that produces a decrease in startle response to an auditory stimulus when it is preceded by a quieter 'prepulse' stimulus. This phenomenon can be seen in patients with schizophrenia due to abnormal dopaminergic transmission (Swerdlow, Braff, Masten, & Geyer, 1990). Excitotoxic lesioning of the shell or core of the NAc via *N*-Methyl-D-Aspartate (NMDA) administration has also shown mild locomotor (shell) and PPI (core) effect (Jongen-Relo, Kaufmann, & Feldon, 2002). Similarly, lesioning via ibotenic acid infusion has been shown to produce changes in locomotor activity with core lesions resulting in hyperactivity (Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999). Depletion of endogenous dopamine in the NAc via bilateral 6-OHDA injection has also been shown to block the locomotor response usually induced following amphetamine administration (Kelly, Seviour, & Iversen, 1975).

As mentioned in Chapter 1, the NAc is comprised primarily of medium-spiny neurones expressing D_1- and D_2- receptors, and featuring outputs to brain regions such as the ventral tegmental area, ventral pallidum and substantia nigra (Meredith

et al., 2008). These brain regions act as a feedback loop to further alter dopaminergic input to the NAc and other brain regions, as well as projecting to striatal areas involved in motor control. NAc connectivity to the ventral pallidum in particular has been shown to have effects on the pedunculopontine nucleus, an area involved in locomotion, with hippocampal output to the pedunculoptine nucleus (via subpallidal output neurones) being modulated by D_2 receptor-mediated mechanisms (Yang & Mogenson, 1987). This connectivity indicates accumbal influence on sensorimotor processes via up- and down-regulation of dopaminergic transmission. For example, intermittent stressors (such as tail-shocks) produce an increase in extracellular dopamine levels in the NAc, resulting in sensorimotor changes and producing active avoidance responses to prevent further stress (Abercrombie, DeBoer, & Heeringa, 1998; Salamone, 1992).

Sensorimotor effects have been suggested to be specific to inactivation of the accumbal core as opposed to the shell. Upon muscimol infusion into each of the NAc subdivisions, PPI and locomotor activity impairment could only be seen in the core-inhibited group when compared to the control group, whereas shell inhibition did not differ from controls (Pothuizen, Jongen-Relo, & Feldon, 2005). This suggests distinct functional roles for the two subdivisions (Jeanblanc, Hoeltzel, & Louilot, 2002), with the core demonstrating involvement in the modulation of motor behaviour (Parkinson et al., 1999), and the shell being more involved with motivational aspects of behaviour and reward (Di Chiara et al., 2004).

The present experiments aimed to establish suitable muscimol doses to sufficiently inhibit NAc neuronal activity without causing gross sensorimotor side effects that might interfere with DMTP testing. Gross changes in PPI/sensorimotor gating or locomotor activity may impact upon the rats' ability to perform the watermaze task, so it is important to rule out this possibility before carrying out watermaze testing. To this end, we combined NAc muscimol infusion with testing of locomotor activity and of prepulse inhibition (PPI) to the startle response. These experiments also help to clarify the contribution of the NAc to the modulation of PPI and locomotor activity. In light of previous studies suggesting that sensorimotor effects are mainly linked to

functional inhibition of the core, rather than the shell (Pothuizen et al., 2005), we expected that the muscimol infusions should have relatively minor effects on locomotor activity and PPI due to our targeting of the shell as opposed to the core.

Materials and methods

All methods are as in Pezze et al (2014) and McGarrity et al (2017) and adapted as necessary for the present study.

Subjects

We used thirty-nine male lister hooded rats (Charles River, UK), approximately 10-13 weeks old and weighing between 300g and 380g for surgery at the beginning of the experiment (two different replications of twelve rats and one replication of fifteen rats). Rats were kept in cages of four where possible with food and water provided ad libitum, temperature control (20-23°C), humidity control (40-55%) and a light-dark cycle of 12 hours per phase (lights on between 7am and 7pm), with all experimental work being carried out during the light phase. Prior to the start of any experimental procedures, rats were allowed at least 7 days of acclimatisation to the holding room and handled regularly to habituate them to the experimenters. All procedures were conducted in accordance with the requirements of the UK Animals (Scientific procedures) Act 1986. All efforts were made to minimise animal suffering and to reduce the number of animals used.

Implantation of NAc infusion guide cannulae and verification of placements

All rats were implanted with infusion guide cannulae targeting the NAc prior to any experimental work. Rats were initially anaesthetised with isoflurane delivered in oxygen and prepared for surgery in a preparation room. Induction of anaesthesia was carried out with 4-5% isoflurane and maintenance with 1-3% for the remainder of

the surgery and adapted as necessary (depth of anaesthesia assessed via monitoring of breathing rate, kept between 40-60 breaths per minute). Rats were placed into a stereotaxic frame with bregma and lambda aligned horizontally upon skull exposure. In an effort to reduce pain, EMLA cream was applied to the ear bars (5%, AstraZeneca) and Rimadyl was administered perioperatively. Bilateral infusion guide cannulae (plastic pedestals holding two 26 gage metal tubes, 2 mm centre-centre distance) were implanted through holes drilled in the skull, with the tips aimed at the NAc with the following coordinates: 1.5 mm anterior, 1.0 mm lateral from bregma and 6.7 mm ventral from the skull surface. These coordinates were adapted based on a previous study in Wistar rats (Pothuizen et al., 2005), using our in vivo MR Atlas of the Lister hooded rat brain (Prior, McGarrity, Goldschmidt, Bast, Pitiot, in preparation) and confirmed via pilot surgeries. Cannulae were then secured in place with stainless-steel screws and dental acrylic, at which point stylets were inserted into the guides with a 0.5 mm protrusion from the bottom of the guide cannulae, and then sealed with a dust cap. Rats were then given at least 5 days of recovery before any testing took place. Following surgery, rats were checked daily and injected with subcutaneous antibiotic Ceporex (supplier: Schering-Plough Animal Health, active ingredient: cephalexin – 0.01ml/100g) to prevent infection. Regular handling was necessary to habituate the rats to manual restraint in preparation for drug microinfusions. At the end of the behavioural experiments, all rats were transcardially perfused using saline followed by 4% formaldehyde solution before brains were extracted for verification and documentation of cannula placements.

Startle and PPI

Startle and PPI testing was carried out using four startle response systems (San Diego Instruments, US) as in previous studies (McGarrity et al., 2017; Pezze et al., 2014). Each system consisted of a clear Perspex cylinder (dimensions 19.5 cm length, 8.8 cm diameter) on a Perspex base that is linked to an accelerometer, all inside a ventilated sound attenuated chamber (39 x 38 x 58 cm, interior lighting 15W). Sounds could be played into the chamber via a speaker positioned above the Perspex cylinder, with a noise generator controlled by the SR-Lab System (San Diego Instruments, US). Startle

response can be measured following a pulse of sound by measuring whole-body startle response, with the amplitude of the response defined as the average of 100 accelerometer readings (1 ms per reading). For the experiment, rats were placed into the cylinder for a period of acclimatisation lasting 5 minutes, during which time a continuous 62 dB background noise was played. Following this, three test blocks took place. The first consisted of 10 startle pulses alone (40 ms, 120 dB broad-band bursts) to habituate the rats to a stable level of startle responsiveness for the rest of the test session. The second block consisted of 50 trials, with 5 different types of trial presented 10 times each: pulse-alone trials and four trials which played a different intensity prepulse 100 ms before the 120 dB pulse (72, 76, 80, or 84 dB).These trials were presented in a pseudorandom order and with varying inter-trial-intervals (10-20 second duration). Percentage of PPI (%PPI) could then be calculated using the following method: [(mean startle amplitude on pulse-alone trials − mean startle amplitude on prepulse-plus-pulse trials)/ (mean startle amplitude on pulse-alone trial)] × 100%. The third block consisted of 5 more pulse alone trials. A test session lasted for 23 minutes. Habituation could be measured by measuring startle amplitude of the pulse alone trials across the three blocks.

Open-field locomotor activity

Locomotor activity was also measured as in previous studies (McGarrity et al., 2017; Pothuizen et al., 2005). Rats were placed into the centre of 12 clear Perspex boxes (39.5 x 23.5 x 24.5 cm) with metal grid lids (50-70 lux inside each box) to start a session. Boxes were place in photobeam frames, with 2 levels of photobeams in a 4 x 8 configuration (Photobeam Activity System; San Diego Instruments, US). Locomotor activity could then be measured as two consecutive beam breaks in the bottom level of photobeams indicating a count.

Infusion procedure and drugs

On days in which infusion effects were measured, rats were gently restrained and stylets were removed. 33-gauge injectors connected via polyethylene tubing to a

microinfusion pump holding two 5 µl Hamilton syringes were then inserted bilaterally into the guide cannulae, with the injector tips extending 0.5 mm further than the ends of the guides (final DV coordinate for NAc shell -7.2 mm). Infusions of either saline or muscimol solution (125 ng/0.5 µl or 250 ng/0.5 µl, 0.5 µl/side volume for all groups) were then carried out, lasting for 1 minute. Doses were chosen based on previous studies involving prefrontal (Pezze et al., 2014) and hippocampal (McGarrity et al., in preparation – See (McGarrity et al., 2015)) infusions and the positive effect during electrophysiology experiments (see Chapter 2). Injectors were left in the guide for 1 minute to allow for drug absorption, after which they were removed and the stylets put back in. Successful infusion was confirmed by the movement of a small air bubble included in the tubing. Muscimol was diluted in 0.9% saline and frozen until use. Infusions took place in a room next to the testing room.

Visual inspection of rats following infusions

Muscimol infusion into the NAc has been demonstrated to cause behavioural effects, such as increased defensiveness and vigorous eating (Reynolds & Berridge, 2001). Rats were observed for these and any other obvious behavioural changes following infusions, before and after sensorimotor testing. Behavioural changes were noted as qualitative observations.

Experimental Design

We used a between-subjects design similar to that used in previous studies looking at the effect of pharmacological manipulations on locomotor activity and prepulse inhibition (McGarrity et al., 2017; Pezze et al., 2014). Rats were randomly allocated to one of the three infusion groups while ensuring that each cage of four rats included at least one rat allocated to each infusion group, and then all rats had baseline measurements of locomotor activity and PPI (order of LMA/PPI testing alternated between replications). These baseline measures allowed us to see if there were any differences between the groups and reallocate as necessary to ensure groups were matched as far as possible. The second day of testing for both sensorimotor

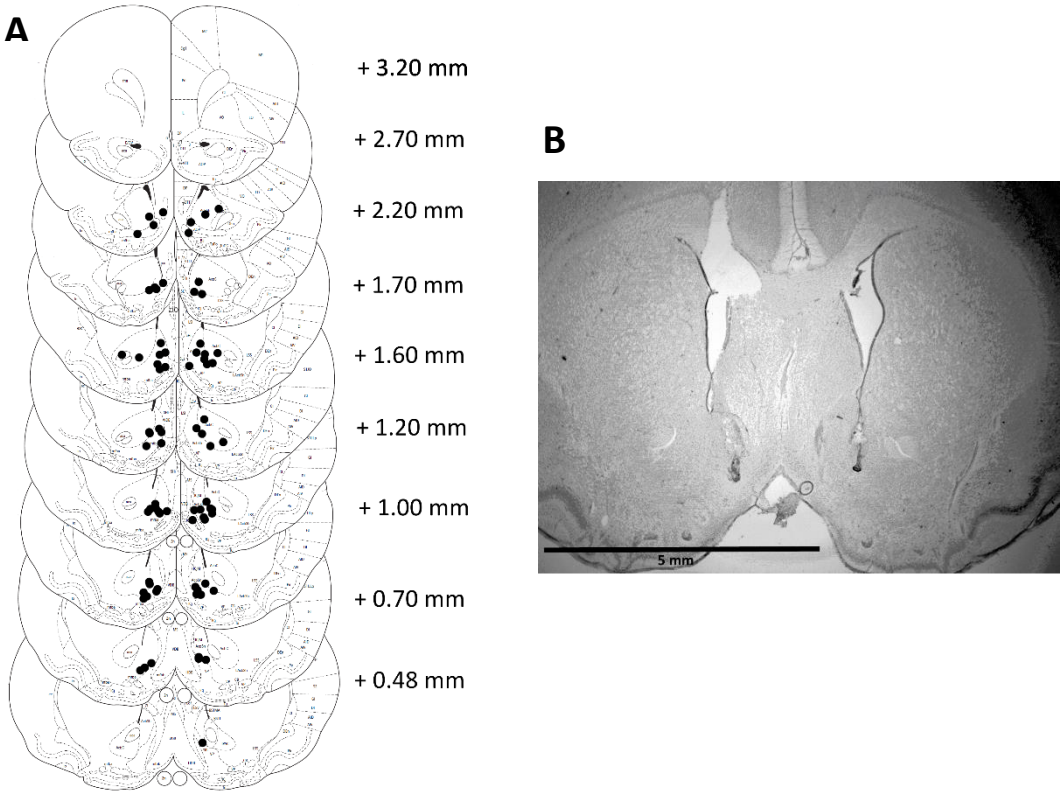


Fig. 7. A) Approximate locations of guide cannulae tips (represented by dots) as verified by light microscope examination of cresyl violet stained coronal brain sections. Placements mapped onto coronal sections from Paxinos & Watson (1998) brain atlas coronal sections, with numbers on the right indicating distance from bregma. B) Representative coronal brain section showing guide cannula tip placements under light microscope.

measures was to test the effects of the infusions. Rats were also retested on the day following infusion to determine if there were any persistent effects of the infusions (with locomotor activity testing being carried out for a 4th day due to locomotor differences between infusion groups found on day 3). A sample size of 13 per infusion group was targeted based on previous studies (McGarrity et al., 2017; Pezze et al., 2014), in which similar sample sizes were used to reveal statistically significant sensorimotor changes.

Locomotor Activity. On day 1, all thirty-nine rats were placed into the locomotor activity test chambers to measure baseline locomotor activity for 60 minutes. Infusion effects were tested on day 2, with an infusion (saline, 125 ng or 250 ng muscimol; n = 13 per group) carried out before being tested for 30 minutes. Days 3

& 4 were used to test re-baseline values for 30 minutes per day to assess long-term changes.

Startle/PPI. All thirty-nine rats used for locomotor testing were also used to test the effect of accumbal muscimol infusion on startle response/PPI. On day 1, all rats had baselines startle/PPI testing with infusion. On day 2, rats again had an infusion carried before testing in the same manner as during locomotor activity testing. On day 3, all rats had re-baseline tests without infusions to assess long-term effects of the infusions. For one rat, data could not be collected correctly on infusion day or rebaseline day due to movement of the cylinder in the PPI box. This rat was excluded from all analysis, resulting in n = 12 in the 125ng group for analysis on infusion and rebaseline days. In addition, baseline day data could not be collected correctly for three rats due to problems with the equipment (one due to movement of the cylinder, two due to the PPI box not working correctly). This resulted (including exclusion of the data from the rat that was not collected correctly on infusion day) in n = 11 for both 125ng and 250 ng groups, and n = 13 for the saline group.

Results

Verification of infusion cannulae placements

Tips were primarily positioned within the NAc shell between 2.7 mm and 0.48 mm anterior to bregma (figure 7a). Some tips were located either on the border of the NAc shell or just outside of the shell, though these were included in all analyses as the spread of the infusion bolus would still produce effects within the shell. Though we target the shell for these experiments, the muscimol spread will inevitably affect surrounding brain regions. Visual inspection showed damage primarily surrounding the tip of the guide cannulae (in the vicinity of the infusion site – figure 7b).

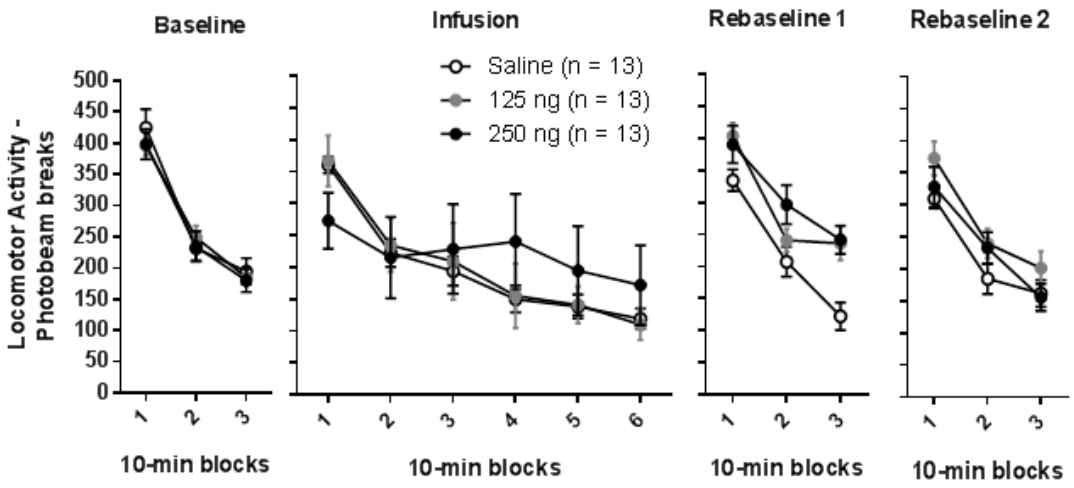


Fig. 8. Effects of accumbal muscimol infusion on locomotor activity (between subjects study, n = 39 (n = 13 per group); data presented as mean ± SEM).

Locomotor Activity

Locomotor activity was measured by consecutive beam breaks and separated into 10-min blocks (figure 8). Baseline activity during Day 1 was not different between the prospective infusion groups (main effect or interaction involving infusion: $F < 1$). During the 60-min testing session on infusion day, locomotion in the 250 ng group tended to be slightly decreased during the first half and slightly increased during the second half (as compared to the other two groups). Despite this numerical pattern of data, there was no significant effect of infusion group ($F(2, 36) = 0.109$, $P = 0.897$) or interaction between test block and infusion group ($F(10, 180) = 1.485$, $P = 0.148$). The effect of 10-min block alone was significant however ($F(5,180) = 15.74$, $P < 0.0001$), indicating overall habituation during the test session. During the first rebaseline testing, 1d after infusion, activity in the muscimol groups was higher than that in the saline group ($F(2, 36) = 6.044$, $P = 0.0055$), independently from the 10-min block (interaction infusion group X 10-min block: $F(4, 72) = 2.277$, $P = 0.0692$), suggesting reduced between-session habituation in the muscimol as compared to the saline groups. During the second rebaseline testing, 2d after infusion, any group differences had disappeared (main effect of group: $F(2, 36) = 1.89$, $P = 0.1657$; interaction group X 10-min bin: $F(4, 72) < 1$). On both days, all groups showed normal

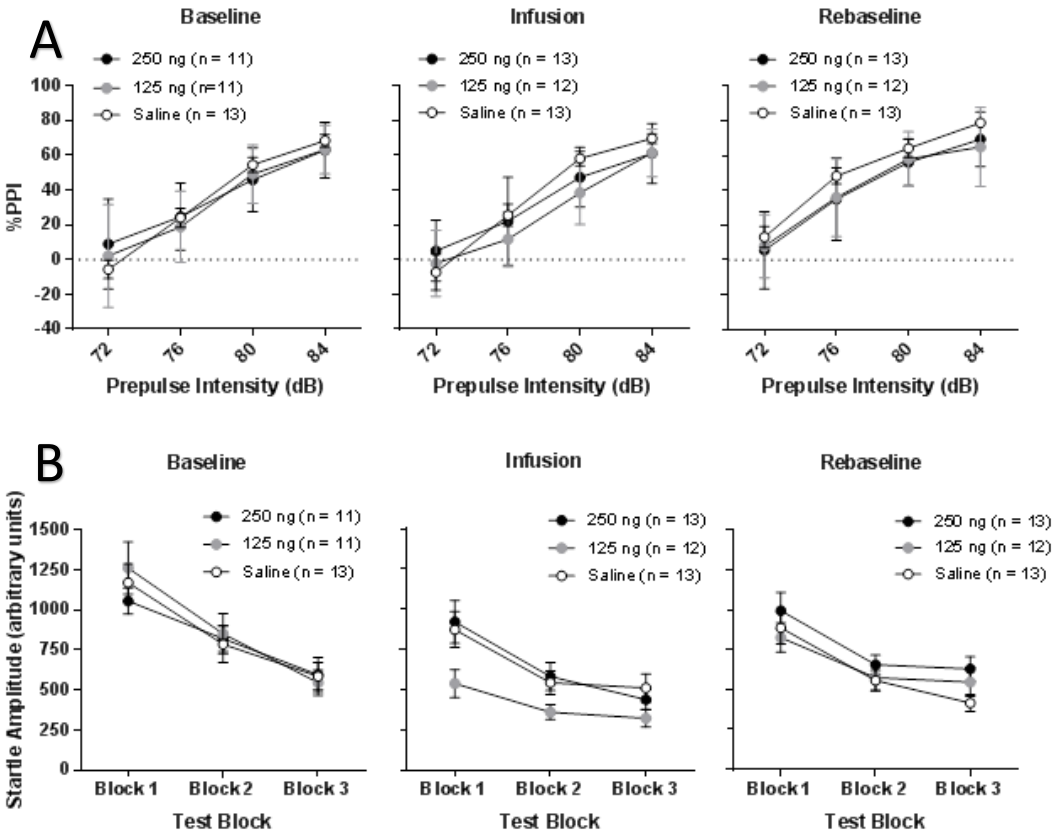


Fig. 9. Effects of accumbal muscimol infusion on PPI (between subjects study, n = 39; data presented as mean ± SEM). A. %PPI when subjected to differing prepulse intensities. B. Startle amplitude across trial blocks (as described in methods).

within-session habituation, as indicated by significant effects of 10-min bins (rebaseline day 1: $F(2, 72) = 87.78$, $P < 0.0001$; rebaseline day 2: $F(2, 72) = 70.22$, $P < 0.0001$). When measured across days, there is a significant effect of type of day ($F(3, 108) = 9.788$, $P < 0.001$), indicating between-session habituation across all groups. Individual ANOVAs also show a significant interaction between type of day and infusion group for 250 ng muscimol vs saline ($F(3, 72) = 2.821$, $P = 0.0449$) and a near significant interaction for 125 ng muscimol vs saline ($F(3, 72) = 2.716$, $P = 0.0510$).

PPI and startle

During baseline, there was no significant main effect or interaction involving prospective infusion group on %PPI or startle response across blocks (all $F < 1$) (figure 9). On the day of infusion, analysis of PPI data revealed a significant interaction between prepulse intensity and infusion group ($F(6, 105) = 2.654$, $P = 0.0195$). This

reflected that the saline group showed numerically slightly higher %PPI than the two muscimol groups at prepulse intensities of 76, 80 and 84 dB, but slightly lower %PPI at 72 dB. As to startle amplitude data, there was clear habituation of the startle response across the three test blocks (effect of block: $F(2, 70) = 49.62$, $P < 0.0001$) (figure 9b). There were strong trends for a main effect of infusion group ($F(2, 35) = 2.913$, $P = 0.0676$) and towards a significant interaction between block and infusion group ($F(4, 70) = 2.346$, $P = 0.063$), reflecting that rats that had received 125 ng muscimol per side into the NAc showed reduced startle amplitude, particularly during the first test block, before startle habituation led to relatively low levels of startle in all groups. One day after infusion, on the rebaseline day 3, all three infusion groups showed similar PPI and startle data (main effects or interaction involving infusion groups: all $F < 1.545$, all $P > 0.2275$).

Visual inspection of rats following infusions

After rats were removed from the startle/PPI (30 min) and locomotor (60 min) test sessions on the infusion days, those infused with muscimol, particularly at the higher dose, often or occasionally showed one of the following:

- 'Floppiness', characterised by reduced muscle tone combined with decreased activity.
- Defensiveness towards either experimenter or other rat, as determined by the stance of the rat ('defensive treading' behaviour - Reynolds & Berridge, 2001)
- Oral tendencies, determined by licking/biting on various objects, such as gloves, towels and boxes. Also includes increased appetitive eating behaviour (as reflected by vigorous eating of food pellets left in the cage, which typically would not be interrupted even if the rat was disturbed by experimenter or other rat).

We recorded how often we observed these behavioural phenotypes (Table 2). With two post infusion periods per rat (1 after locomotor, 1 after startle/PPI testing) and n = 13 rats per group, there was a maximum number of 26 observations of each behavioural phenotype in each group. Oral tendencies was the most common phenotype, observed after nearly half of the 125 ng muscimol infusion and more than two thirds of the 250 ng muscimol infusions. Defensiveness was observed after nearly half of the 250 ng muscimol infusions, but not after infusion of the lower dose. Floppiness was only observed very rarely.

	'Floppiness'	Defensiveness	Oral Tendencies
Saline	0	0	1
Muscimol 125 ng	1	0	11
Muscimol 250 ng	4	11	20

Table 2. Qualitative observations of behaviours following accumbal muscimol and saline infusions. Data show how often the different behaviours were observed for each group (with an n = 13 per group and 2 infusions per rat, the maximum number of observations of each behavioural phenotype was 26 per group).

Discussion

Overall, accumbal muscimol infused did not cause marked changes in locomotor or startle/PPI testing. In both the saline and 125 ng groups, there is a clear habituation over the course of the hour session, with decreasing numbers of photobeam breaks from one block to the next. However, there is numerical evidence for impaired habituation in rats following 250 ng muscimol infusion. These showed slightly reduced activity compared to the other groups at the start of the session and a slight increase in activity during the final 30 minutes, indicating impaired within-session habituation. A significant effect of type of day also indicates appropriate between-session habituation, but individual comparisons indicate an impairment of between-session habituation in both muscimol groups. PPI data indicates only a very mild disruption of PPI, without any impact on startle response on pulse-alone trials,

following muscimol infusion. There was a significant interaction between PPI percentage and prepulse intensity, which reflects the saline group having a higher PPI at the three higher prepulse intensities than other groups, pointing towards a mild impairment of sensorimotor gating. However, the prepulse dependence of the PPI is still intact, with increasing prepulse intensities resulting in greater percentage PPI. There is also a clear habituation of the startle response, with startle amplitude decreasing across test blocks throughout the session ($F(2, 40) = 34.48$, $p < 0.0001$). However, the low muscimol dose, but not the high dose, tended to reduce the startle response on pulse-alone trials. Qualitative observations following the locomotor and PPI test sessions indicated that rats infused with muscimol into the NAc showed increased oral behaviour (feeding on foot pellets scattered within the home cage, chewing on the experimenter coat). Following infusion of 250 ng, defensiveness when touched by the experimenter was also common.

Interestingly, locomotor habituation has been linked to hippocampus-dependent learning of the testing context (Bast & Feldon, 2003; Fanselow, 2000). Therefore, reduced locomotor habituation following NAc muscimol infusion is consistent with the hypothesis that the NAc mediates the impact of hippocampus-dependent learning on behaviour. There is also potential for some muscimol being infused into the accumbal core, which (as mentioned in the introduction) has been suggested to be more involved in the modulation of motor activity. While this habituation effect is still present one day post-infusion, after two days the muscimol effect on locomotor activity has disappeared, indicating the length of time that the muscimol acts. The limited effects of muscimol infusion on PPI are comparable with previous studies (Pothuizen et al., 2005), in which muscimol infusion into the NAc shell did not significantly affect the %PPI when compared to a control group. They also observed a rebound in locomotor activity for 2 days following infusion, though this effect was seen in NAc core manipulations as opposed to shell. Similarly, qualitative observations are consistent with previous reports following muscimol infusions targeting the medial NAc, which showed an increase in defensive and appetitive eating behaviours (Reynolds & Berridge, 2001). As mentioned previously, while we target NAc shell with our infusion, the volume of drug infused per side (0.5 µl) would

instantly affect the region surrounding the cannula tip (0.5 mm in each direction). Beyond this, diffusion would then be expected to increase this distance, therefore reducing the specificity of the infusion. As a result, this would result in muscimol having action in regions surrounding the shell, including NAc core, septum, and ventral pallidum. It is therefore possible that the qualitative observations are due to muscimol action in these brain regions, such as the defensive treading noted being more related to changes in motor function rather than increased defensiveness. Also, decreased activity in the ventral pallidum, which is also recognised as being key in reward and motivation (Salgado & Kaplitt, 2015), may result in behavioural changes noted in this experiment. For example, previous research has shown that the ventral pallidum is important in the regulation of response allocation in food-seeking, with muscimol infusion in the ventral pallidum leading to an increase in food consumption (when the food is readily available and easily accessible) (Farrar et al., 2008). In the context of this thesis, the current experiment indicated that there are minimal effects on motor function, suggesting that impairment seen during watermaze testing is unrelated to motor changes. However, observations should be made during watermaze testing as to whether changes in motivation become apparent, such as rats treading water and not trying to find the escape platform.

This data indicates that functional inhibition of the NAc via microinfusion of the GABA-A agonist muscimol does not cause gross sensorimotor side effects when looking at locomotor activity or startle response/prepulse inhibition. Both doses of muscimol may cause impaired habituation in the locomotor activity chamber, potentially due to hippocampal interactions with the NAc. Also, there appears to be a moderate disruption to prepulse inhibition following muscimol infusion, though this effect appears minor and does not remove prepulse dependence (may just represent a sampling error).

Chapter 4: Watermaze delayed-matching-to-place (DMTP) within-subjects experiment: Preliminary evidence that NAc muscimol impairs expression of 1-trial place learning

Introduction

The present experiment aims to use the watermaze DMTP task to determine if muscimol infusion into the NAc during learning results in impaired 1-trial learning with no delay, thereby denoting the role of the NAc in the translation of rapidly-acquired place information into behaviour. The watermaze task is an accurate method of measuring spatial memory, with the DMTP task being highly hippocampus-dependent (Bast, 2011; Bast et al., 2009). This is important in determining the role of the NAc, as we chose this brain region to look at due to its connectivity with the hippocampus, therefore making a hippocampus-dependent behavioural task the best method. As previously, we combined pharmacological manipulation (infusion of GABA agonist muscimol into the NAc) with watermaze testing, using doses that have been established in sensorimotor (Chapter 3) and electrophysiological (Chapter 2) testing, as these doses have been shown to sufficiently reduce neuronal firing in the NAc without causing gross sensorimotor side effects. The within-subjects design of this experiment allowed us to test each rat with all doses, which increases the number of comparisons that can be made and reduces potential problems due to variability between rats. We also carried out two replications of this study as repeated measures to further increase the number of comparisons that can be made, thereby increasing statistical power. Measures that will be taken include search preference (% of time spent in the correct zone), latency, path length and swim speed.

Materials and methods

All methods are as in Bast et al (2009), Pezze & Bast (2012) and McGarrity et al (2017) and adapted as necessary for the present study.

Subjects

For this study, we used sixteen male Lister hooded rats (Charles River, UK), approximately 10-13 weeks old and weighing between 319g and 384g for surgery at the beginning of the experiment. General husbandry and procedures related to animal welfare were as described in the previous chapter (see Chapter 3 for full details).

Implantation of NAc infusion guide cannulae and verification of placements

As in the previous study (see chapter 3 for full details), rats were implanted with infusion guide cannulae targeting the NAc prior to any experimental procedure, and, at the end of the behavioural experiments, all rats were transcardially perfused using saline followed by 4% formaldehyde solution before brains were extracted for verification and documentation of cannula placements.

Watermaze apparatus

The Morris watermaze (Morris, 1984) consists of a 2m diameter pool (60 cm high) filled with water made opaque by the addition of approximately 200ml of white water-soluble non-toxic children's paint and kept at 25±1°C during testing. Water was filled up to approximately 20 cm below the rim of the pool (temperature and water surface height adjusted on the morning of all experimental days). Surrounding the pool were three-dimensional visual cues that were visible from the water surface, which can be used by the rats to aid in spatial navigation and the formation of place memories (cues include a traffic cone, lampshades, boxes and posters with high

contrast stripes/patterns). 4 points equally distributed around the rim of the pool (every 90%) were designated start positions (North, N; South, S; East, E; and West, W – all arbitrarily defined), and were counterbalanced during testing. Hidden 1-2 cm below the surface of the water was a 12 cm diameter platform that acted as the only method of escape from the pool for the rats, with the idea behind the watermaze design being that the rat could use the surrounding pool cues as allocentric orientation assists and form a place memory for the platform location upon discovering it. We used an 'Atlantis Platform' (Spooner, Thomson, Hall, Morris, & Salter, 1994), so-called due to its ability to be held >30 cm below the surface of the water using a computer-controlled electromagnet, making it inaccessible for the rats (Med Associates Inc., US). Recording of the rats' swimming behaviour was carried out via a ceiling mounted camera located above the centre of the watermaze which could be viewed from an adjacent room via a computer. Video recording and tracking of the rats was done using Ethovision tracking software (Version XT 7, Noldus Technology, Netherlands), allowing us to analyse various measure such as latency to finding the platform, path length, swim speed and search preference. On occasion, tracking of the rat failed due to insufficient contrast between the rat's head and the surrounding water. These trials were manually retracked from the video file recorded with detection settings changed from tracking something 'darker' in contrast to its surroundings to something 'brighter & darker' than its surroundings.

Delayed-matching-to-place (DMTP) task

For this experiment, we used the DMTP version of the watermaze task (Steele & Morris, 1999). On this task, rats receive 4 trials of testing per day. The platform is hidden in 1 of 8 potential locations (depending on the testing block) on either an inner or out ring concentric with the pool (0.8 m & 1.4 m diameter respectively). The rat is then released from one of the four start locations with the goal of finding the platform and escaping. Trials began with the rat being placed against the wall of the pool (facing away from the centre) in one of four symmetrically arranged start locations (designated N,E,W,S) and then gently being released, at which point they are given a maximum of 120 seconds to find the platform. Failure to find the platform

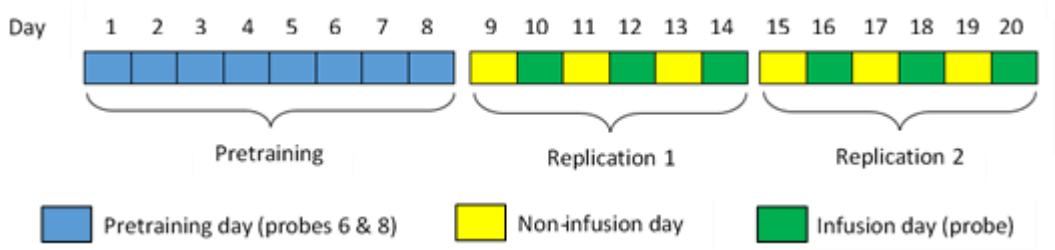


Fig. 10. Timeline of experimental procedure, showing 8 days of pretraining (days 6 & 8 run with trial 2 as a probe and with mock infusions) and two test blocks of 6 days each, with three non-infusion days and three infusion days (trial 2 run as probe) for each block.

would result in the experimenter guiding the rat to the platform location, at which point they would be left on the platform for 30 seconds in order to learn the platform's location. The platform is placed in a new location within the pool on trial 1 each day and remains in that location for the remaining 3 trials. The rat is released from a different start location on each trial to prevent egocentric strategies. For the DMTP version of the task, the platform is moved to a novel location each day, thereby acting as a measure of hippocampus-dependent rapidly-acquired place memory. Overall, a total of 20 different platform locations were used throughout the experiment (1 platform location per day; 8 days of pretraining plus two series of 6 days, during which the effect of NAc drug infusions were tested; figure 10). On infusion days, trial 2 was run as a probe trial (Bast et al., 2009; Jackson et al., 2011), during which the platform was held under the water for a predetermined length of time (60 seconds), after which it would rise to just underneath the surface of the water so the rats could climb onto it. These probe trials were carried out to reinforce spatially focussed searching and allow for us to accurately measure search preference for the zone containing the platform location without the platform being available for escape. Analysis focussed on trial 2 of each day as performance on this trial relies on the place information encoded in trial 1 alone, with trials 3 and 4 being used to reinforce the win-stay rule. A sample size of n = 16 was chosen as previous studies with this sample size produced relatively large effect sizes (Cohen's d of approximately 1 or more; McGarrity et al., 2017).

Measures of 1-trial place memory

Latencies and path lengths

Two of the measures used to determine place learning were latencies and path lengths. Path lengths are a better measure than latencies due to them being independent of swim speed. Latencies (measured in seconds) and path lengths (measured in cm) were both measured for all four trials, with the beginning of the measurement being when they are released into the water and lasting until any part of the rat crossed the threshold of the platform, even if the rat did not then proceed to immediately get onto the platform (for probe trials, these end points were taken when the rat enters the location in which the platform would usually have been located). Good one trial-place learning is indicated by a large reduction in latency/path length between trials 1 and 2.

Search preference

The primary measure used as an indication of place memory was search preference for the correct platform location's zone on trial 2 of infusion days (probe trial sessions). In order to measure this, the correct 'zones' around the possible platform locations (8 possible locations for each test block) were defined as 40 cm diameter areas around each platform location, with all zones being non-overlapping and equally space around the pool (4 on the inner ring, 4 on the outer ring). Ethovision software was then used to measure the amount of time that the rat would spend in each of these zones, and calculate the search preference as the percentage of time that the rat spent in the correct platform location's zone as compared to all of the possible platform location zones. The '% time spent in the correct zone was therefore calculated as follows: (time in 'correct zone' [s]/time in all 8 zones [s]) x 100%, with chance performance therefore being 1 in 8 (12.5%) and anything above this indicating search preference for the correct zone.

Swim speed

Swim speeds were measured up until the rat found the platform location on non-probe trials and for the first 60 seconds on probe trials.

Pretraining on the DMTP task

Before carrying out and test sessions with drug microinfusions into the NAc, rats were given 8 days of pretraining in the watermaze in order to reach asymptotic performance levels (Bast et al., 2009; Jackson et al., 2011; McGarrity et al., 2017). Training was carried out with 8 platform locations, with half of the rats given one sequence of platform locations and the other half given another sequence in order to ensure that performance on any given day was not biased by the platform location. Rats were tested with a new platform location each day. For the first 4 days, inter-trial interval (ITI) between trials 1 and 2 were 10-30 seconds for convenience, and for the final 4 days ITI was 20 minutes in order to simulate infusion day conditions. ITI for trials 2-4 were always 10-30 seconds for convenience. During the ITI, rats were placed in a holding box on the floor of the watermaze room, with rats being manually towel dried by the experimenter upon being removed from the platform following every trial. Pretraining days 6 and 8 were also run with trial 2 as a probe, again to simulate infusion day procedures. These days were also run with mock infusions to acclimatise the rats to this procedure, with half of the rats undergoing mock infusions on day 6 and the other half on day 8 (start positions and platform location counterbalanced between the 2 groups).

Infusion procedure and drugs

The infusion procedure was similar to that used in the sensorimotor experiments (see Chapter 3 for full details). Infusions (saline, 125 ng/0.5 µl muscimol, 250 ng/0.5 µl muscimol) were carried out in a room close to the watermaze room, and were carried out starting 10-minutes following trial 1. Infusions were then carried out as described previously. Following this, rats were returned to their holding box until the remainder

of the 20 minute ITI was complete, at which point they would carry out their 2nd trial. Mock infusions during pretraining were carried out as if they were normal infusions, with the only difference being that the infusion system was not filled with any liquid.

Visual inspection of rats following infusions

Following infusions, rats were observed for various behavioural changes (increased eating, oral fixations, defensiveness, etc.), as in the sensorimotor experiments (see Chapter 3: Sensorimotor Effects). Behavioural changes were noted at the time as qualitative observations.

Testing the effects of muscimol infusion on 1-trial place memory at a 20-min retention delay

The 12 days following pretraining were carried out to determine the effect of muscimol microinfusion between trials 1 and 2 on 1-trial place memory at a 20 minute retention delay. Infusion procedures were as described previously, with a 20 minute ITI between trials 1 and 2 to accommodate for the time taken for the infusion to be carried out and take effect and an ITI of 10-30 seconds for trials 2 to 4. As with pretraining, a novel platform location was used each day, with half of the rats being tested of one of two sequences of platform locations. Again, this is so that two platform locations can be used each day and therefore ensure that performance on any given day was independent of the specific platform location. Every second day was carried out as an infusion day, with rats receiving one of three accumbal microinfusions: saline, 125 ng/0.5 µl muscimol, or 250 ng/0.5 µl muscimol. The order that each rat received these three infusions for each test block of 6 days was counterbalanced using a latin-square design. The series of three different infusions was repeated once more to obtain more accurate estimates of performance measures in each infusion condition from all rats. Within each infusion series, start positions and platform locations were counterbalanced across infusion conditions. On infusion days only, trial 2 was run as a probe trial to allow for measurement of search preference. Preceding infusion days were days of testing without infusion in

order to minimise carry-over effects of drug infusions/probe trials. ITI on these pre-infusion days were always 10-30 seconds for convenience. Following 250 ng muscimol infusion on some trials, rats would prioritise grooming over swimming (see Results – Visual inspection of rats following infusion), meaning that the trial could not be completed. This did not occur on any trial 2, leaving search preference data unaffected, but only occurred on trials 3 and 4. In these cases, the trial was rerun at the end of the test session in order to confirm the win-stay rule for the rat and the latency/pathlength from these trials were used for their respective analyses.

Data analysis

Latencies, path lengths, % time spent in the correct zone and swim speed were analysed. All numerical data are presented as mean ± SEM. Data were analysed using GraphPad Prism version 7, using ANOVA with infusion and trials (and series for search preference) as within-subject factors. Paired one-tailed t-tests were carried out using GraphPad Prism version 7 to compare latencies on trials 1 and 2 to determine one-trial place learning. Search preference measures were all compared to chance (12.5%) using one sample t-tests. The level of significance was set at $P < 0.05$ for all analyses.

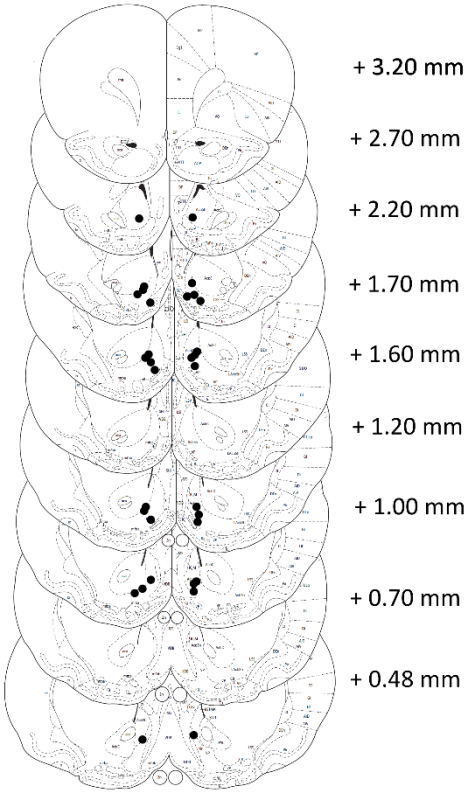


Fig. 11. Approximate locations of guide cannulae tips (represented by dots) as verified by light microscope examination of cresyl violet stained coronal brain sections. Placements mapped onto coronal sections from Paxinos & Watson brain atlas coronal sections, with numbers on the right indicating distance from bregma.

Results

Verification of infusion cannulae placements

Cannula tip locations were mapped onto coronal sections adapted from Paxinos & Watson (1998) 'The Rat Brain' (figure 11). Tips were well positioned within the NAc shell between 0.48 mm and 2.7 mm anterior to bregma. Qualitative observation showed that damage occurred primarily surrounding the tip of the guide cannula where the injection site was located (0.5 mm bellow guide cannulae tips).

Latency and Path Length

While latency and path length data supported learning across trials and 1-trial place learning, especially in the saline condition, during series 1 there was no clear place

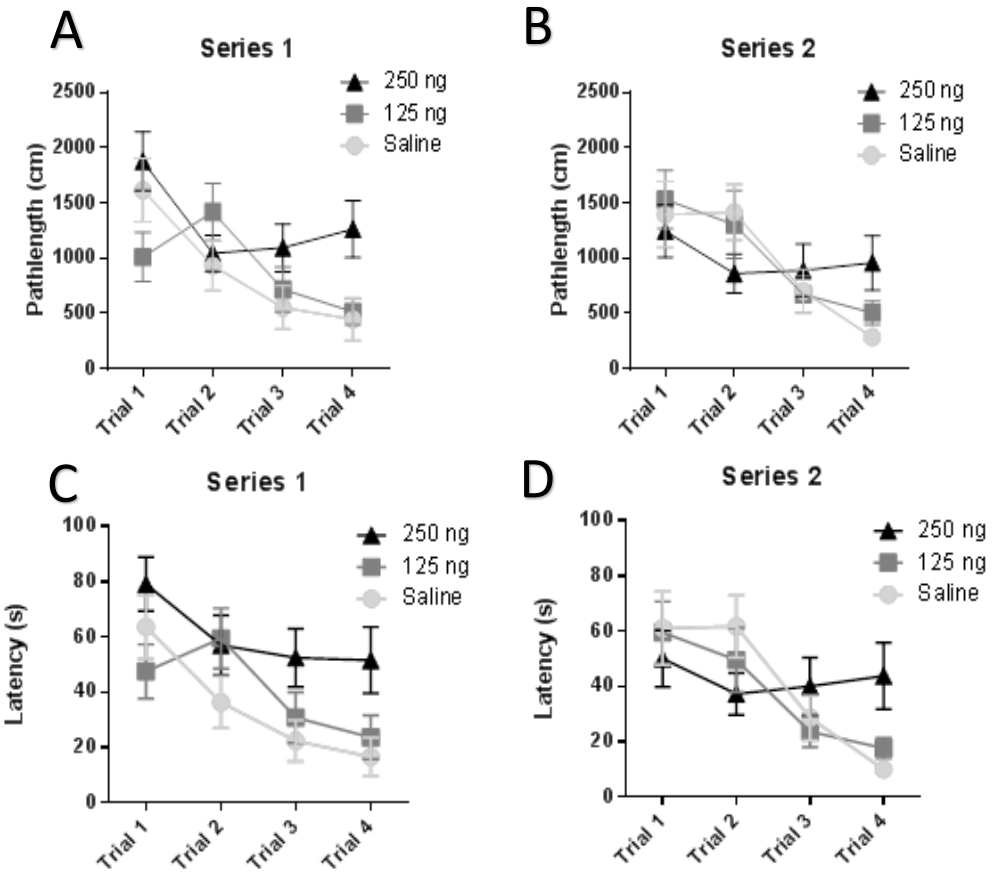


Fig. 12. Effects of accumbal muscimol infusion following trial 1 of the DMTP watermaze task on latency (A & B) and pathlength (C & D) to reach platform location (within subjects study, n = 16 for series 1, n = 15 for series 2; data presented as mean ± SEM). Infusions carried out between trials 1 & 2. A. Series 1 of infusions (effect of trial: $F(3, 45) = 10.22$, $P < 0.0001$, effect of infusion: $F(2, 30) = 5.056$, $P = 0.0128$). B. Series 2 of infusions (effect of trial: $F(3, 42) = 10.14$, $P < 0.0001$, interaction of trial and infusion: $F(6, 84) = 2.474$, $P = 0.0298$). C. Series 1 of infusions (effect of trial: $F(3, 39) = 9.924$, $P < 0.0001$, effect of infusion: $F(2, 26) = 3.452$, $P = 0.0452$). D. Series 2 of infusions (effect of trial: $F(3, 42) = 12.27$, $P < 0.0001$).

learning across trials in any group in series 2. Overall latencies from infusion days (n = 16) during the first series of infusions demonstrated overall learning occurring across the four trials (figure 12C), with a significant effect of trial ($F(3, 45) = 10.22$, $P < 0.0001$). This is due to relatively high latencies during trial 1, followed by lower latencies during subsequent trials. With the saline and 250 ng groups, the largest change occurred between trials 1 and 2, indicating good 1-trial learning, though a significant difference could only be found between trial 1 & 2 for saline infused rats (paired one-tailed t test: $t(15) = 2.046$, $P = 0.0294$), with no significance in either drug group (both $t < 1.454$, both $P > 0.0833$). There was also a significant effect of infusion ($F(2, 30) = 5.056$, $P = 0.0128$) indicating that latencies tended to be increased following muscimol infusions as compared to saline, especially during trial 2 to 4, though the interaction infusion X trial failed to reach significance ($F(6, 90) = 1.168$, $P = 0.3303$). In the second series of infusions (figure 12D), the main effect of the

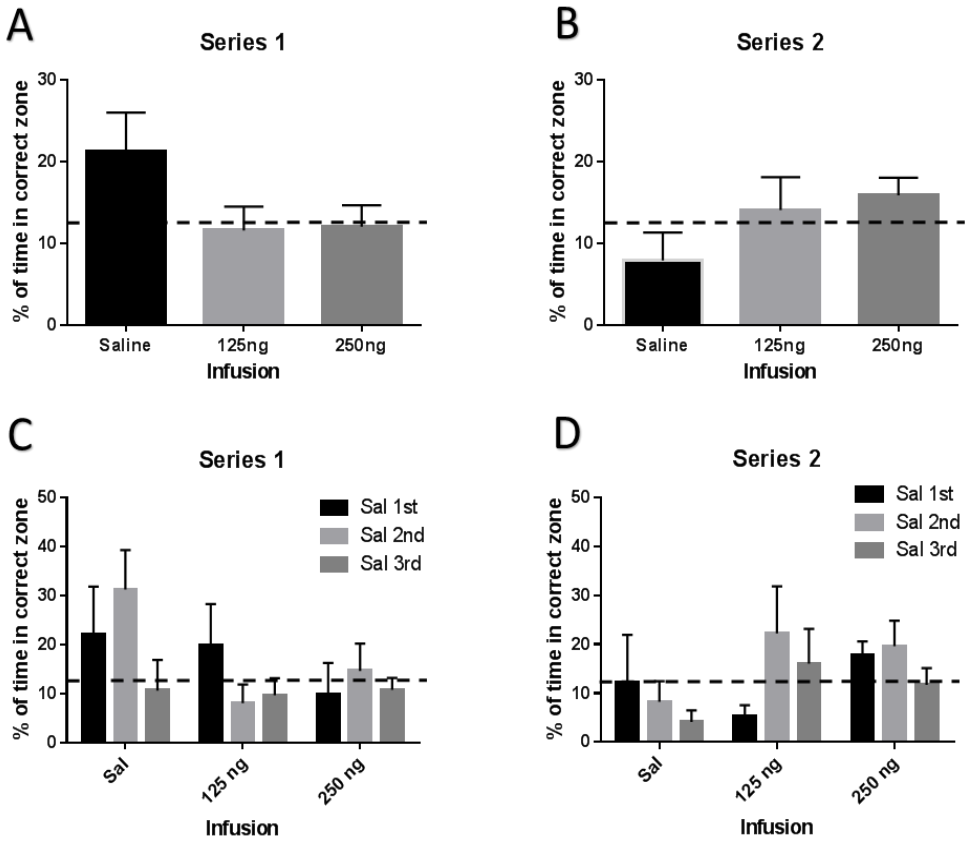


Fig.13. Effects of accumbal muscimol infusion following trial 1 of the DMTP watermaze task on search preference as measured by % time spent in correct zone (within subjects study, n = 16; data presented as mean ± SEM, dotted line = chance). A. Series 1 of infusions, no significant effect. B. Series 2 of infusions, no significant effect. C. Series 1, each infusion group divided into the sequence in which infusions were carried out (effect of sequence: $F(2, 13) = 3.603$, $P = 0.0569$). D. Series 2, each infusion group divided into the sequence in which infusion were carried out (effect of sequence: $F(2, 12) = 0.6834$, $P = 0.5235$).

infusion disappeared (F (2, 28) = 0.23, P = 0.8041), but there was a significant interaction of infusion and trial (F (6, 84) = 2.474, P = 0.0298), potentially due to the 250 ng muscimol condition showing lower latencies during trials 1 & 2, but higher latencies during trials 3 & 4, as compared to the other two conditions. The second series showed poor 1-trial learning overall, with minimal latency reduction between trials 1 and 2 across all groups (paired one-tailed t tests: all $t < 1.527$, $P > 0.0745$), though there was an overall main effect of trial (F (3, 42) = 10.14, $P < 0.0001$).

Path lengths were also measured, as these are independent of swim speeds and therefore a more accurate measure. Again, path lengths were measured from the time that the rat was set into the pool until the head of the rat crossed the threshold of the platform. As swim speeds were similar across infusion conditions, path lengths showed a similar pattern of results (figure 12A and B), with series 1 demonstrating significant effects of trial (F (3, 39) = 9.924, $P < 0.0001$), infusion (F (2, 26) = 3.452, P = 0.0452) and interaction between the two (F (6, 78) = 2.221, P = 0.0497). However, while series 2 still demonstrates efficient learning across the four trials (F (3, 42) = 12.27, $P < 0.0001$) and there was no significant effect of infusion again, there was also no significant interaction (F (6, 84) = 1.800, P = 0.1089).

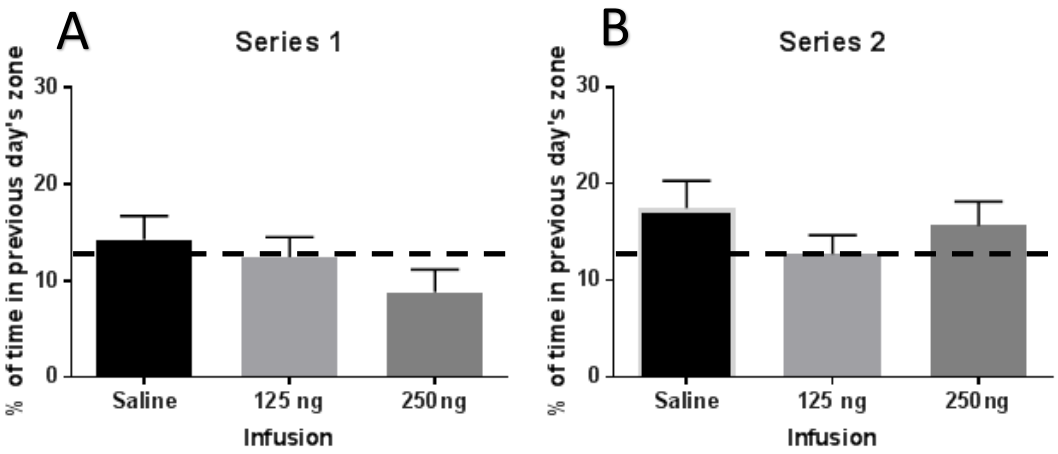


Fig. 14. Effects of accumbal muscimol infusion following trial 1 of the DMTP watermaze task on search preference for the previous day's platform location as measured by % time spent in the previous day's zone (within subjects study, series 1 - n = 16, series 2 - n = 15; data presented as mean ± SEM, dotted line = chance). A. Series 1 of infusions, no significant effect. B. Series 2 of infusions, no significant effect.

Search Preference

Series 1 data supported impairment following NAc muscimol infusion, whereas performance during series 2 was at chance in all infusion conditions (interaction infusion group X series: $F(2, 28) = 4.552$, $P = 0.0194$; figure 13A & B). Although, numerically search preference in the saline condition was nearly twice as high as in the two muscimol conditions during series 1, the main effect of infusion failed to reach significance ($F(2,30) = 1.901$, $P = 0.1670$), with all three infusion groups not showing significant difference from chance (all $t < 1.822$, $P > 0.0884$). During series 2, performance was similarly poor (effect of infusions: $F(2, 28) = 1.778$, $P = 0.1875$), with search preference not differing from chance in any of the three conditions (all $t < 1.537$, $P > 0.1467$).

Overall, this data indicates that performance, in terms of search preference, deteriorated across conditions as the infusion study went on, with none of the three conditions showing significant search preference for the correct location during series 2. This may reflect a carryover effect from the muscimol infusions. Interestingly, within series 1, there was evidence that performance in the saline condition was poor in those rats that received saline infusion on the third infusion day for the saline condition (i.e. had received muscimol infusions on the two preceding infusion days). A two-way ANOVA of the search preference data from series 1, including infusion as within-subjects factor and the sequence of infusions as between-subjects factors (three levels: saline first, saline second and saline third) showed a very strong trend towards a main effect of sequence ($F(2, 13) = 3.603$, $P = 0.0569$; interaction infusion group X sequence: $f(4, 26) = 1.125$, $P = 0.3663$) (figure 13C & D). However, following the second series of infusions, there was no longer an effect of sequence ($F(2, 12) = 0.6834$, $P = 0.5235$; interaction infusion group X series: $F(4, 24) = 1.347$, $P = 0.2818$).

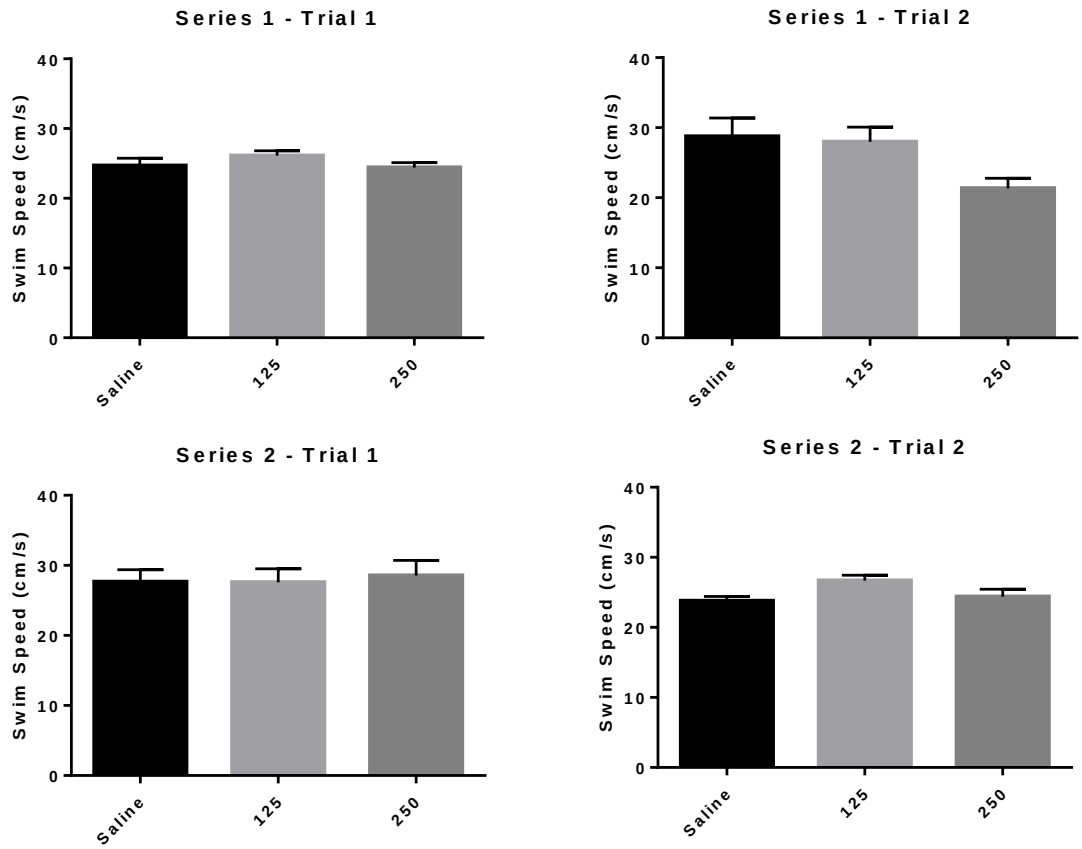


Fig. 15. Effects of accumbal muscimol infusion following trial 1 of the DMTP watermaze task on swim speed (within subjects study, series 1 - n = 16, series 2 – n = 15; data presented as mean ± SEM). Infusions carried out between trials 1 & 2. Trial 1 for series 1 and 2 show no statistical significance, though trial 2 for series 1 ($F(2, 45) = 3.222$, $P = 0.0492$) shows significance and series 2 ($F(2, 42) = 2.932$, $P = 0.0643$) shows a numerical trend towards significance.

Search Preference – Previous Day's Location

Search preference for the previous day's zone was also analysed (figure 14). In both series all groups show search preference around chance level (one sample t-test comparing all groups to chance - 12.5% – all $t < 1.734$, all $P > 0.1048$), with ANOVA showing no significant effect of infusion for series 1 ($F(2,30) = 1.503$, $P = 0.2388$) or series 2 ($F(2,28) = 0.769$, $P = 0.4730$).

Swim Speed

Swim speed data seems to indicate that NAc muscimol infusion may have a moderate effect on swim speed when given at the higher dose, as can be seen by the reduced swim speed for the 250 ng muscimol group in the second trial of series 1 (figure 15).

Series 1 shows a statistically significant reduction of swim speed (effect of infusion: $F(2, 45) = 3.222$, $P = 0.0492$), though Fisher's LSD test only shows significance between the two muscimol groups ($P = 0.0157$). Trial 2 of series 2 data show a slight increase in swim speed for both the 250ng and 125 ng muscimol groups (effect of infusion: $F(2, 42) = 2.932$, $P = 0.0643$), with Fisher's LSD test only showing significance for saline vs. 125 ng muscimol ($P = 0.0199$).

Visual inspection of rats following infusions

During and immediately following watermaze testing, rats were qualitatively observed and often showed behavioural changes, such as increased defensiveness, oral tendencies and instability after climbing on the platform (see Chapter 3: Results for full details).

We recorded how often we observed these behavioural phenotypes (Table 3). With each rat receiving each infusion condition (3 conditions: saline, 125 ng muscimol and 250 ng muscimol) twice and n = 16 rats, there was a maximum number of 32 observations for each behavioural phenotype in each group. Additionally, some rats would also appear to prioritise grooming over swimming and therefore sink, requiring the experimenter to retrieve the rat from the watermaze and end the trial. This category is defined here as 'Grooming Priority', though this phenotype was

	Defensiveness	Oral Tendencies	Instability	Grooming Priority
Saline	0	0	0	0
Muscimol 125 ng	0	8	0	1
Muscimol 250 ng	2	25	8	6

Table 3. Qualitative observations of behaviours following accumbal muscimol and saline infusions. Data show how often the different behaviours were observed for each group (with an n = 16 and 2 infusions per rat, the maximum number of observations of each behavioural phenotype was 32 per group – Grooming Priority numbers indicate phenotype seen on trials 3 and 4).

never noted on trial 2, only ever on trials 3 and 4. Oral tendencies appear to be the most common phenotype (as was seen following infusion during sensorimotor testing), with quarter of the 125 ng muscimol infusion group and more than three quarters of the 250 ng muscimol group showing an increase in biting/eating behaviours. Instability on the platform and grooming priority during swimming were mainly observed in the 250 ng group, and defensiveness was observed rarely.

Discussion

Accumbal muscimol infusion appears to reduce numerically search preference on the DMTP task as compared to saline, though all three groups were not statistically different from chance performance. However, this numerical decrease was only apparent in the first series of infusions and only in the subset of rats that received saline as one of their first two infusions. In contrast, in those rats that received saline as their last infusion (i.e. had received muscimol during first two infusion), performance was also very poor. During the second series of infusions, rats did not show significant rapid place learning performance in any infusion condition, again with no group showing a significant difference from chance performance. This pattern of findings could indicate a carry-over effect of muscimol infusion, with muscimol infusions apparently causing poorer performances on future trials and also resulting in the second series of performances being much poorer, particularly in the saline infused group. The nature of this carryover effect should be considered in a bit more detail, as it is not clear if this is a pharmacological effect due to infusion-induced damage, or psychological pro-active interference in which poor performances on early trials (potentially due to functional inhibition of the NAc) results in the rats adopting inappropriate search strategies on later trials. For example, rats would return to a similar pattern of behaviour as they showed at the start of pretraining in the watermaze. This includes an emphasis on swimming around the edge of the pool by the wall (thigmotaxic swimming), either to find an exit from the water where they are able to make physical contact with visible objects, or due to increased anxiety causing them to want to stay closer to the wall (Devan, McDonald, & White, 1999;

Treit & Fundytus, 1988). Rats would also dive a lot more, potentially in an effort to find a submerged escape path, which has also previously been suggested to be attributed to strategy impairment (Enomoto et al., 2008). This would potentially explain the performance difference between the different sequences of infusions and the overall poor performances in the second series (particularly the decrease from saline series 1 to saline series 2). There is also the possibility that these carryover effects could be due to long-lasting drug effects, with muscimol still reducing accumbal activity on the following days. This could be due to increased long term potentiation (LTP) in the synapses between hippocampus and accumbens following repeated infusions, thereby allowing behavioural effects to be seen long after muscimol infusion (LeGates et al., 2018). However, sensorimotor testing (see Chapter 3) indicates that long lasting drug effects are not necessarily the case, as the rebaseline effects seen during LMA testing could only be seen for 1 day following infusion, and had returned to baseline by the second day. As our watermaze design allows for this extra day between infusions, it seems like the carryover muscimol effects shouldn't affect subsequent infusions. Reduced swim speed following infusion in the first series may not be due to decreased locomotor activity, as our previous work has indicated that muscimol infusion does not affect basal activity, although there was a numerical trend for reduced locomotor activity early on. Instead, it could be attributed to changes in search strategies following inhibition of the NAc, with slightly slower swim speeds due to a more focused search. However, this is not supported by search preference data, with the higher muscimol group showing search preference at chance level, indicating that the rat was not conducting a focused search in a particular area of the pool.

A potential side-effect of the higher muscimol dose was noted during several test sessions. On certain trials, swim speed would be very slow initially, followed by complete cessation of swimming. Rats were then removed from the pool by an experimenter. Observations were that the rat would prioritise grooming instead of swimming in many of the cases and would sink as a result. This indicates, along with previous behavioural work, that it is potentially not due to reduced locomotor activity but due to changes in other processes. These could include changes in

motivation, with rats being less motivated to continue swimming and deciding to remove the water from their face instead, or an inability to efficiently prioritise activity, with them deeming grooming as being more important at the time than swimming. Other observations made about these rats include that they were relatively unstable if they made it onto the platform due to persistent grooming, they demonstrated more oral tendencies (biting edge of the platform, biting towel, persistent eating upon being returned to their cage), as well as more general defensive behaviour, with rats acting more defensively when approached by other rats or the experimenters.

Our initial findings are comparable to similar work in which NAc – hippocampal disconnection resulted in impaired foraging behaviour during a non-delayed task, indicating reduced translation of rapidly-acquired place information into behaviour (Floresco et al., 1997). Despite this trend, after each infusion group was split further to take sequence of infusions into account, it is clear (particularly in the saline group) that there is a lot of variation between rats which received saline first, second and third. Specifically, rats which received saline third (and had therefore already received 125 ng and 250 ng doses on previous days) showed much poorer search preference and latency (data not shown).

Functional inhibition of the NAc via microinfusion of GABA-A agonist muscimol shows a trend towards impairment on the watermaze delayed-matching-to-place task, potentially due to accumbal involvement in the translation of rapidly-acquired place information into behaviour. However, it appears as though consistent muscimol infusions result in carryover effects which cause this trend to disappear. Therefore, a second watermaze series was planned to reduce these confounding effects by removing the high dose group, running the experiment as a between-subjects study to reduce muscimol effects on the control group, and increase the number of washout days between infusions in order to allow appropriate search strategies to be readopted.

Chapter 5: Watermaze DMTP between-subjects experiment: Muscimol infusion into NAc impairs expression of rapid place learning

Introduction

In the previous study, there was evidence for a carryover effect of muscimol resulting in poor performances on later test sessions due to the rats adopting inappropriate search strategies. In order to counter this, we carried out the present study as a between-subjects study, thereby separating the saline group from the muscimol group entirely in an effort to reduce potential muscimol effects on the controls. Another change that was made was to increase the number of days between infusions in order to ensure performance measures were back up to baseline before carrying out the next infusion. Therefore, every other probe session was carried out as a 'washout' day (i.e. a probe trial for trial 2 but without an infusion taking place). This was carried out as saline performances on infusion days were still poor following previous muscimol infusions, indicating that rats did not have enough time to readopt appropriate search strategies without any infusions taking place. Finally, we decided to remove the higher dose of muscimol (250 ng) for this study as this dose demonstrated the most non-specific side effects, such as prioritising grooming over swimming resulting in incomplete trials. As in the previous within-subjects study, measures that will be taken include search preference (% of time spent in the correct zone), latency, path length and swim speed (see Chapter 4).

Materials and methods

All methods are as in Bast et al (2009) and adapted as necessary for the present study.

Subjects

For this study, we used twenty four male Lister hooded rats (Charles River, UK), approximately 10-13 weeks old and weighing between 326g and 390g for surgery at the beginning of the experiment. Housing, husbandry and ethical considerations are as described in previous chapters (see Chapter 3 for full details).

Implantation of NAc infusion guide cannulae and verification of placements

As in the previous studies (see chapter 3 for full details), rats were implanted with infusion guide cannulae targeting the NAc prior to any experimental procedure, and, at the end of the behavioural experiments, all rats were transcardially perfused using saline followed by 4% formaldehyde solution before brains were extracted for verification and documentation of cannula placements.

Watermaze apparatus

Watermaze and associated equipment and set-up were as described in Chapter 4.

Delayed-matching-to-place (DMTP) task & measures of 1-trial place memory

This study used the same DMTP watermaze experimental design (Bast et al., 2009; Jackson et al., 2011; Steele & Morris, 1999) as the within-subjects study, with the exception of the number of days of testing. Following 8 days of pretraining, 16 days of testing were carried out, with probe-trial days being preceded by non-probe days, and with probe days alternating between infusion and washout. This gives a total of 8 non-probe days, 4 infusion days and 4 washout days (figure 16). Measures of 1-trial place memory (latency, path length, search preference, and swim speed) are also as in the previous study. See Chapter 4 for full details on the DMTP task design and measures taken during testing.

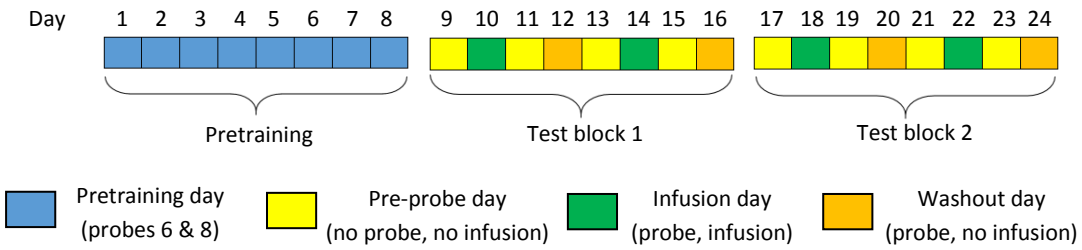


Fig. 16. Timeline of experimental procedure, showing 8 days of pretraining (days 6 & 8 run with trial 2 as a probe and with mock infusions) and two test blocks of 8 days each, 2 infusion days and 2 washout days in each block (trial 2 run as a probe on these days), and days with no infusion or probe trial separating these.

Pretraining on the DMTP task

As mentioned, 8 days of pretraining were carried out before any pharmacological manipulations/testing occurred. See Chapter 4 for pretraining design.

Infusion procedure and drugs

Infusion procedure is as described in Chapter 4. However for this study, only saline (0.9%, 0.5 µl/side) and 125 ng/0.5 µl muscimol were used, with the 250 ng/0.5 µl muscimol group being excluded from the between-subjects study due to non-specific effects observed during the within-subjects study (details of these observations can also be seen in Chapter 4). Final numbers were n = 11 for the saline group and n = 8 for the muscimol group. 3 rats were excluded from analysis due to misplaced guide cannulae noted during examination of brain histology, and 2 rats were excluded from analysis due to illness late in the study leading to them being euthanized and missing the final days of testing. These 2 rats could therefore not be included in analysis due to problems with counterbalancing of platform locations. A sample size of n = 12 was targeted for each group, as previous studies with this sample size produced relatively large effect sizes (Cohen's d of approximately 1 or more) (Heath et al., 2015).

Visual inspection of rats following infusions

Qualitative observations of the rats were noted during and immediately following infusions, as in previous chapters (see Chapter 3 & 4 for details of behavioural characteristics). Due to the exclusion of the high muscimol dose, 'Grooming Priority' was not seen and therefore not included in this chapter.

Testing the effects of muscimol infusion on 1-trial place memory at a 20-min retention delay

As mentioned, DMTP testing was carried out immediately following the 8 days of pretraining to determine if muscimol infusion in the NAc 10 minutes after trial 1 affect 1-trial place memory at a 20 minute retention delay. Testing procedures were similar to that seen in the within-subjects DMTP study (see Chapter 4). For the present study, we preceded all probe days by a day of standard DMTP training without probe trial and without infusion to minimise carry-over effects of probe trials and drug infusions. On the 1st, 3rd, 5th and 7th probe trial days, rats received one of two bilateral accumbal infusions 10 min after encoding trial (i.e. infusion would be completed 11 min after the encoding trial/9 min before trial 2): saline only or 125ng/0.5 µl muscimol. Rats were randomly allocated to one of these groups post-surgery, and then performance of the two groups compared following pretraining to ensure group performances did not differ. Every other probe trial day was run as a non-infusion washout day. Start positions and platform locations were counterbalanced between infusion groups.

Data analysis

Latencies (during pretraining), path lengths, % time spent in the correct zone and swim speed were analysed. All numerical data are presented as mean ± SEM. Data were analysed with IBM SPSS Statistics version 22, using three-way ANOVA for latency and pathlength data with trials and type of day as within-subjects factors and infusion group as a between subjects factor or two-way ANOVA for search preference

data with type of day as a within-subject factor and infusion group as a between-subjects factor. Further analyses (i.e. non-repeated measures two-way ANOVA) and figures were carried out using GraphPad Prism version 7. Paired one-tailed t-tests were carried out using GraphPad Prism version 7 to compare latencies on trials 1 and 2 to determine one-trial place learning. Search preference measures were all compared to chance (12.5%) using one sample t-tests. The level of significance was set at $P < 0.05$ for all analyses.

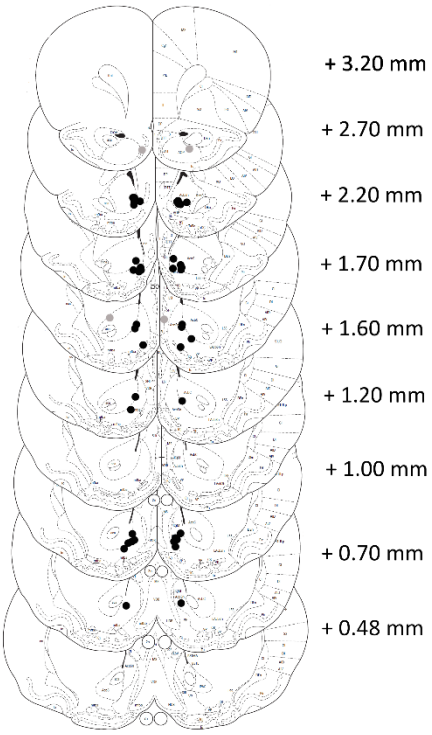


Fig. 17. Approximate locations of guide cannulae tips (represented by dots) as verified by light microscope examination of cresyl violet stained coronal brain sections. Placements mapped onto coronal sections from Paxinos & Watson brain atlas, with numbers on the right indicating distance from bregma. Grey dots indicate tips that were not in the correct location.

Results

Verification of infusion cannulae placements

Cannulae tip locations were mapped onto coronal sections adapted from Paxinos & Watson (1998) 'The Rat Brain' (figure 17). Tips were primarily positioned within the NAc shell between 0.7mm and 2.7mm anterior to bregma. Some tips were positioned borderline between NAc shell and core, indicating that these infusions may reflect effects of inhibition in both of these regions. Although we target NAc shell, due to the spread of the muscimol being infused NAc shell will be affected along with some surrounding brain regions. 3 rats were also excluded based on placement locations outside of the NAc shell. Visual inspection showed damage primarily surrounding the tip location within the infusion site (0.5mm below guide cannulae tips).

Pretraining performance

Comparison of search preferences on mock infusion days during pretraining (data not shown) shows that there was no difference between performance in the saline infused group and the muscimol infused group ($t(22) = 0.494$, $P = 0.6266$).

Latency and Path Length

Latency and path length data for the 4 infusion days combined shows learning across the four trials for both the saline group (n = 11) and 125 ng muscimol group (n = 8; see figure 18). Comparisons for between trials 1 and 2 for latencies show a significant decrease in both infusion groups for both types of day (all $t > 2.609$, all $P < 0.0131$). This can be seen by the large reduction in both measures between trials 1 & 2, indicating strong 1-trial place learning, and an overall reduction in both measures across the four trials with a significant effect of trial in each case (latency - infusion: $F(3, 51) = 64$, $P < 0.0001$; pathlength - infusion: $F(3, 51) = 52.45$, $P < 0.0001$). When comparing the effects of infusion days vs. washout days, there are significant effects of type of day for both measures (latency: $F(1, 17) = 6.626$, $P = 0.02$; path length: $F(1, 17) = 6.769$, $P = 0.019$), though no interaction type of day X infusion group (all $P > 0.1$). When comparing the effects of the infusions, 125 ng muscimol infusion appears to increase both latency and path lengths as compared to saline infused rats on infusion days. ANOVA of the latency data on infusion day shows a significant main effect of infusion ($F(1, 17) = 7.83$, $P = 0.0124$; interaction infusion X trial: $F(3, 51) = 3.517$, $P = 0.0215$), as does ANOVA of the pathlength data ($F(1, 17) = 8.318$, $P = 0.0103$), though this does not show a significant interaction ($F(3, 51) = 1.18$, $P = 0.3264$). This reflects similar results seen in the first series of our within subjects watermaze DMTP experiment (see Chapter 4) and indicates that inhibition of the NAc leads to impaired performance. Analysis of washout data alone does not show significant effects of infusion or interactions for either latency or pathlength (all $F < 1$, all $P > 0.3$).

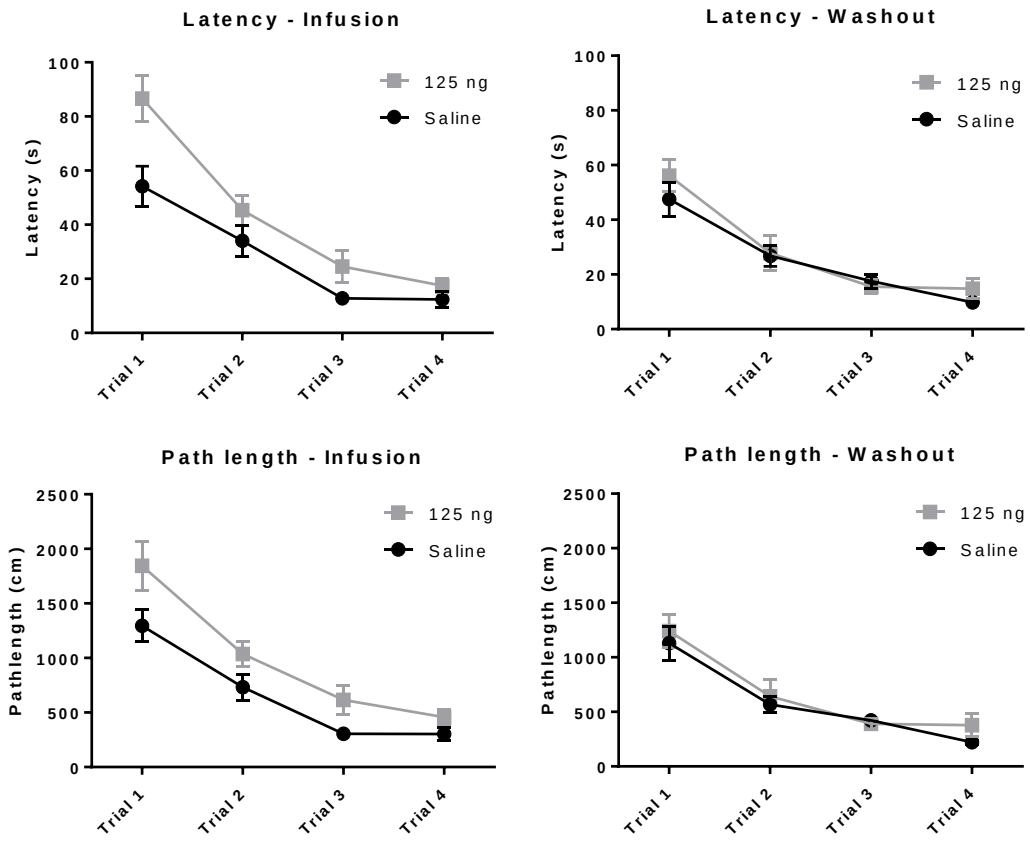


Fig. 18. Effects of accumbal muscimol infusion following trial 1 of the DMTP watermaze task on latency and pathlength to reach platform location on infusion days and washout days (between subjects study, saline n = 11 muscimol 125 ng n = 8; data presented as mean ± SEM). Infusions carried out between trials 1& 2. Latency and pathlength decrease across the four trials in both groups on all days, with the muscimol infused group being mildly impaired in comparison to saline on infusion days for both measures (latency interaction infusion X type of day: F (1, 17) =2.985, P = 0.102; pathlength interaction infusion X type of day: F (1, 17) = 2.710, P = 0.118).

Search Preference – Correct platform location

Search preference data for this experiment shows that performance is significantly impaired in the 125 ng muscimol group on infusion days as compared to performance on washout days (probe session days without an infusion, carried out 2 days following each infusion day), whereas this is not the case for the saline group (interaction infusion group X type of day: F (1, 17) = 8.422, P = 0.01; figure 19). The data also indicates that the muscimol group is impaired compared to saline on infusion days alone. Analysis of infusion day alone shows that the difference between saline and muscimol is significant (effect of infusion: F (1, 17) = 4.756, P = 0.0435). ANOVA comparing infusion groups on washout days alone showed no significant

(effect of infusion: F (1, 17) < 1). For this between subjects experiment, comparison between infusion days and washout days is more appropriate as it has a higher statistical power to detect difference because it is within-subjects, i.e. rats in the 125 ng muscimol group having their performance compared between days, rather than saline rats and 125 ng rats being compared with each other. We therefore also analysed the difference between infusion day muscimol group and washout day muscimol group, which showed a significant effect of type of day (F (1, 14) = 21.08, P = 0.0004). This comparison between infusion day saline group and washout day saline group showed no effect of type of day (F (1, 20) = 0.196, P = 0.6626). Comparisons to chance show that muscimol on infusion day was not different to chance (t (3) = 1.377, P = 0.2622), whereas all other groups were significantly above chance level (all t > 4.411, P < 0.0216).

Search Preference – Previous day's platform location

We also compared search preference of each group for the previous day's location. While there was no interaction between type of day and infusion group (F (1, 17) = 1.663, P = 0.215), there was a main effect of infusion group (F (1, 17) = 5.850, P = 0.027), indicating that saline infused rats were more likely to go to the previous day's location than muscimol rats (see figure 19). Search preference for the previous day's zone was significantly above chance for the saline group on both infusion and washout day (both t > 3.827, P < 0.0314) and not different to chance for the muscimol group in either type of day (both t < 0.656, P > 0.5587).

Swim Speed

Analysis of swim speed during probes on infusion and washout days (figure 19) shows no significance of infusion effect (F (1, 17) = 8.608, P = 0.782), though does show an effect of type of day (main effect: F (1, 17) = 10.183, P = 0.005; interaction infusion X type of day: F (1, 17) = 14.328, P = 0.001) indicating that rats generally swam slower on washout days as compared to infusion days, particularly in the

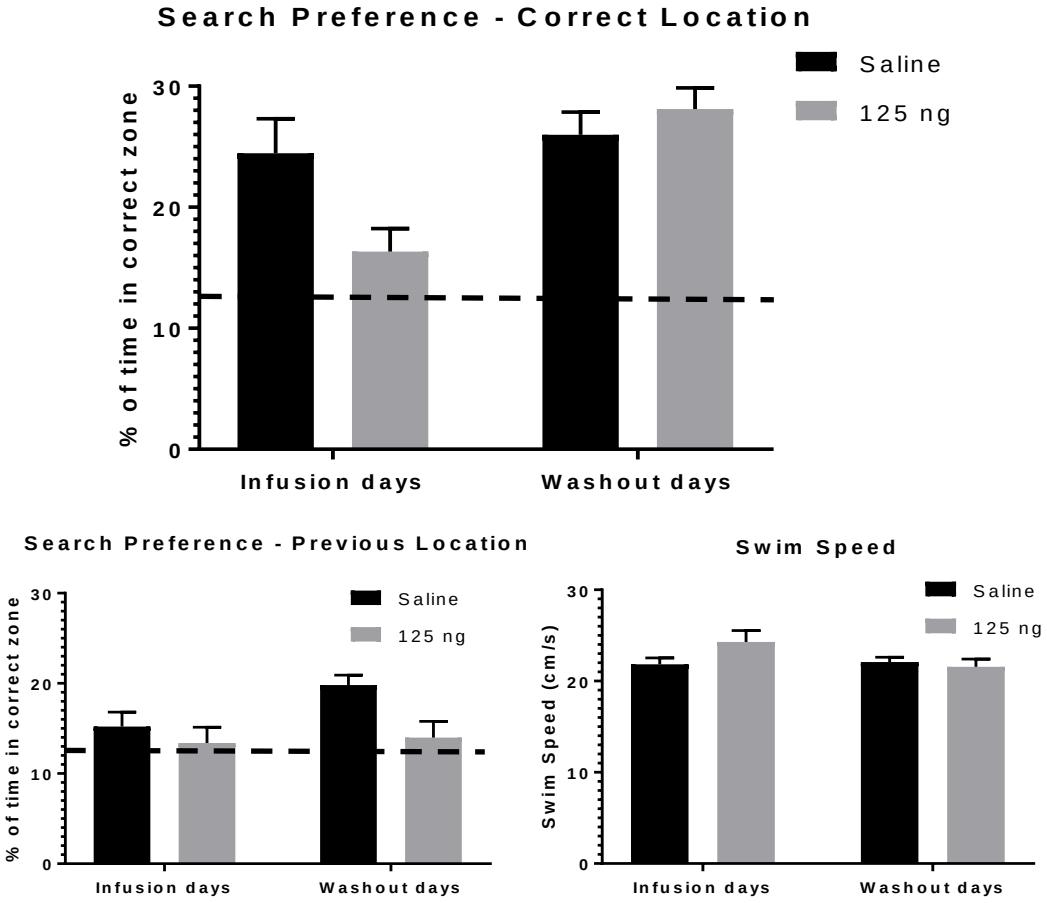


Fig. 19. Effects of accumbal muscimol infusion following trial 1 of the DMTP watermaze task on search preference for the correct zone location and previous day's zone location as measured by % time spent in correct zone & swim speed (between subjects study, saline group n = 11, 125 ng muscimol group n = 8; data presented as mean ± SEM, bevelled line indicates chance performance: 12.5%). Infusions carried out between trials 1& 2. Search preference for the correct zone is significantly impaired in the muscimol group on washout days as compared to performance on infusion days (interaction infusion x type of day: F (1, 17) = 8.422, P = 0.01). Search preference for the previous day's zone is lower in the muscimol group than the saline group (effect of infusion: (F (1, 17) = 5.850, P = 0.027). Swim speed is higher on infusion days than washout days (interaction infusion X type of day: (F (1, 17) = 14.328, P = 0.001).

muscimol group. Fisher's LSD comparisons show that there is a significant difference between infusion groups on infusion days (P = 0.0421) but not on washout days (P = 0.6571), and that there is a significant difference between muscimol group infusion and washout (P = 0.0003) but not between saline group infusion and washout (P = 0.6529).

Visual inspection of rats following infusions

As in the within-subjects study, rats were observed following infusion for the following behavioural characteristics: increased defensiveness, as characterised by a change in stance; increased oral tendencies, which includes chewing on towels, gloves, and general increases in appetitive behaviours; and instability on the watermaze platform.

During and following testing, rats were observed and notes were made by the experimenters of which of these behavioural phenotypes were being displayed. As there were 4 infusions per rat, the maximum number of possible observations for each phenotype was 44 for the saline group (n = 11) and 32 for the muscimol group (n = 8). Table 4 shows that saline rats did not show any of these phenotypes, with it mostly being noted that they would groom themselves upon being returned to their cage following testing. The most common phenotype was the oral tendencies in the muscimol group, being displayed 12 out of a possible 36 times. Defensiveness was observed rarely, on two occasions, in the muscimol group, and no rats appeared unstable when climbing onto/sitting on the platform.

	Defensiveness	Oral Tendencies	Instability
Saline	0	0	0
Muscimol 125 ng	2	12	0

Table 4. Qualitative observations of behaviours following accumbal muscimol and saline infusions. Data show how often the different behaviours were observed for each group (with an n = 11 for saline n = 8 for muscimol and 4 infusions per rat, the maximum number of observations of each behavioural phenotype was 44 for the saline group and 32 for the muscimol group).

Discussion

Functional inhibition of the NAc via microinfusion of the GABA agonist muscimol resulted in impaired 1-trial place learning on the watermaze DMTP task, indicating the involvement of this brain region in the translation of rapidly acquired place memory into behaviour. Latency and path length data indicated an impairment in the 125 ng muscimol group as compared to the saline group on infusion days, with both measures showing a significant effect of infusion. This is interesting as effects could be seen in trial 1, even though the infusion occurred after this. It is important to note that these effects cannot reflect artifacts caused by the use of different combinations of start positions and platform locations because the platform locations were counterbalanced across the two groups and the same daily start positions were used for both groups. Also, our pre-training results suggest that there were no pre-existing group differences in trial 1 performance. Therefore, the most likely explanation may be that this is a chance finding, reflecting the high variability of the pathlength/latency measure, which is one of the reasons why our analysis of watermaze DMTP data focuses on the statistically more reliable search preference measure (da Silva, Bast, & Morris, 2013). Washout day data for these two measures then show no difference between infusion groups, supporting the fact that the muscimol infusion produced the impairment on infusion days. The main findings of this study show that search preference on trial 2 (probe trials) is impaired in muscimol infused rats on infusion days (average search preference approximately 16%) in comparison to this group's performance on washout days (average search preference approximately 28%), with no difference in performance between saline group infusion day and washout day (24.5% and 26% search preference respectively). Due to the between-subjects nature of this study, this comparison is more appropriate than the comparison between the muscimol infused group and the saline infused group due to the increased statistical power of the within-subjects comparison. However, the between-subjects comparison of groups on infusion days alone revealed significantly impaired performance in the muscimol as compare to the saline group. Qualitative observations of the rats following infusion show that

behavioural phenotypes related to sensorimotor deficits are rare in muscimol infused rats, with oral tendencies being the most common observation seen in this group (less than 50% of muscimol rats demonstrated this behaviour) and defensiveness being extremely rare. No rats in this study showed signs of instability on the platform, suggesting that motor control and balance were unaffected by the muscimol infusion, and there were no incidences of 'grooming priority' as in the previous experiment (see Chapter 4), suggesting that there are less non-specific effects in the 125 ng infused group as compared to the higher dose 250 ng infused group. Taken together, these results suggest that the muscimol dose used in this experiment was sufficient to cause performance impairment on the DMTP task without causing any gross sensorimotor side effects. Swim speed data shows that rats infused with muscimol into the NAc swam faster than those infused with saline, but only on infusion days. This may reflect that functional inhibition of the NAc directly increases swim speed or, more likely, reflect that rats infused with muscimol have impaired place memory, as reflected by reduced search preference, and, therefore, slow down less in the correct location.

The levels of performance seen in this experiment are comparable with those seen in a previous watermaze study (McGarrity et al., 2017), which reported appropriate baseline search preference at around 25-30% and impaired search preference (in this study due to picrotoxin infusion into the hippocampus) showing search preference around chance level (12.5%). Muscimol infusion into the NAc during this experiment showed performance drop to almost 16% (not significantly different from chance), which is a good indicator of impairment and may indicate that the NAc is involved. This data also supports previous work showing impaired non-delayed foraging behaviour (potentially mediated by translation of rapidly-acquired place memory into behaviour) following loss of the functional connection between ventral subiculum and NAc (Floresco et al., 1997), as functional loss of NAc activity in our experiment showed impaired rapid place learning translation comparable to the impairment following disconnection of the NAc from these hippocampal inputs. Other work looking at hippocampal output showed the connectivity between proximal subiculum and NAc, and showed that silencing of the proximal subiculum

did not impair performance on the DMTP watermaze task (Cembrowski et al., 2018), indicating that the functional connection between the hippocampus and NAc is not required for the translation of rapidly-acquired place memory into behaviour. However, search preference was only measured with respect to one of the four quadrants of the maze, indicating less specificity when it comes to the rat searching for the correct platform location. Also, proximal subiculum is not the only output of the hippocampus to the NAc, with research showing connections from the CA1 region of the hippocampus (Kelley & Domesick, 1982), and circuits involving other brain regions also providing a connection, such as circuitry in the midbrain involving the ventral tegmental area (Kahn & Shohamy, 2013) or prefrontal cortex (Lisman & Grace, 2005).

Due to the involvement of the NAc in motivation and reward, it is possible that the results seen in this study could be attributed to impaired motivation for finding the platform following muscimol infusion, resulting in the reduced search preference that occurred. Research has demonstrated that hippocampal activity induces LTP in hippocampal-NAc synapses, and as a result also induces goal-directed behaviour for a reward (LeGates et al., 2018). However, observations of the swim behaviour of the rats indicate that there is no loss of motivation following muscimol infusion, as seen by the consistent swimming to find the escape platform, and the re-adoption of early-pretraining search strategies in order to find an exit from the water. As there is no obvious 'reward' in this circumstance (the escape platform is not visible to the rats), these behaviours can therefore be seen as being more aligned with punishment avoidance than reward-seeking, indicating that changes in motivational processes in the NAc may not affect this task. Despite this, further study looking specifically at the role of motivation in this task is necessary to rule this out as a possibility.

Taken together, these data indicate the involvement of the NAc in the translation of rapidly acquired place memory into behaviour, as functional inhibition of the NAc via GABA agonist muscimol microinfusion into the NAc produced impairment of a DMTP watermaze task. Combined with evidence from previous studies, it is likely that the

NAc is involved but not sufficient for this translation, with hippocampal output to other brain regions likely to be involved.

Summary/Discussion

The purpose of this thesis was to address the role the NAc played in the translation of rapidly acquired place information into behaviour. The hippocampus has long been shown to be integral for place memory formation (Frank et al., 2004; R. G. Morris et al., 1982; R. G. Morris et al., 1990; O'Keefe, 1979). More recent work suggests that different subdivisions along its septotemporal axis are largely responsible for different aspects: a septal region that is more involved in the encoding of place memory due to its strong visuospatial input; a temporal region that is more involved with the translation of place memory to behaviour due to connectivity to regions of behavioural control; and an intermediate region that combines neural substrates for both of these and is therefore necessary for the translation of place memory that does not require consolidation into behaviour (Bast, 2011; Bast et al., 2009). We therefore chose to focus on the NAc for this thesis due to its high connectivity with the intermediate hippocampus (Bast et al., 2009; Witter & Amaral, 2004) and previous studies implicating hippocampal-accumbal interactions in the performance on spatial learning tasks (Floresco et al., 1997).

In order to determine the involvement of the NAc in the translation of place learning into behaviour, we used pharmacological manipulation in the form of microinfusion of the GABA-A agonist muscimol into the NAc of Lister Hooded rats to produce functional inhibition. Initially, we carried out in vivo electrophysiological measurements (Chapter 2), confirming that muscimol infusion in the NAc inhibits local neural activity, as reflected by a reduction in overall multi-unit firing rate and evidence for an inhibition of burst firing. Moreover, our sensorimotor experiments (Chapter 3) revealed that functional inhibition of the NAc causes only marginal changes on spontaneous locomotor activity and – if at all – only a very small reduction of PPI. This lack of sensorimotor effects may reflect that our infusions targeted the medial NAc, with previous studies showing that locomotor and PPI changes are mainly associated with muscimol infusions into the lateral NAc (Pothuizen et al., 2015). Interestingly, the open field locomotor experiments showed

that functional inhibition of the NAc impaired between-day habituation to the open field. Given that locomotor habituation has been linked to the hippocampus (Fanselow, 2000), this finding is consistent with the hypothesis that NAc activity is important to mediate the impact of hippocampal memory on behaviour. We then combined muscimol infusion with behavioural testing on the DMTP version of the watermaze task, which is a good test of this learning-behaviour translation due to the constantly changing goal location and has been demonstrated to be highly hippocampus-dependent (Bast et al., 2009; R. G. Morris et al., 1990; Steele & Morris, 1999). This task is a good animal model for the human car park problem, in which changing parking spots on a daily basis result in the need for a mechanism in which place memory can be translated directly into behaviour, without requiring consolidation into the neocortex. We carried out two DMTP studies, the first as a within-subjects study (Chapter 4) and the second as a between-subjects study (Chapter 5) due to potential carryover effects of muscimol during the within-subjects study.

In the first DMTP experiment (within-subjects, three infusion groups, infusion occurs every other day during the testing period), accumbal muscimol infusion numerically reduced search preference as compared to saline during the first infusion series (first three infusion days), though none of the groups performed significantly above chance level. Following this, performances in all groups during the second series were very poor, with the saline group performing much closer to chance than in the previous series. This effect was apparent even after the second infusion day during series 1, with saline performance impairment being immediately apparent. We therefore hypothesised that the decline in performance was due to a muscimol carry-over effect, with accumbal muscimol infusion potentially interfering with the reinforcement of the 1-trial place learning strategy, leading to the rats dropping this strategy on subsequent sessions. Following on from this, we carried out our second DMTP experiment with multiple key changes to experimental design. The main changes were the use of a between-subjects design, the exclusion of the 250 ng muscimol group, and carrying out alternate probe days as 'washout' days in order to increase the number of days between infusions (1 day in the within-subjects study, 3

days in the between-subjects study). This study showed that accumbal muscimol infusion reduced 1-trial place learning performance, as reflected by reduced search preference on infusion days as compared to washout days, indicating the NAc is involved in the translation of rapidly-encoded place information (learning of the platform location) into behaviour (returning to the platform on each subsequent trial).

Previous work has suggested that the NAc is not required in the translation of place information into behaviour when place information remains consistent, as shown by unimpaired performance on the watermaze task when the platform location was learnt prior to NAc lesioning (Sutherland & Rodriguez, 1989). Lidocaine-induced lesions of the NAc shell also showed little effect on performance on a standard incrementally-learned watermaze task (Floresco et al., 1996). Bast et al (2009) also demonstrated that this incremental learning can occur without the hippocampus, but also that the hippocampus is necessary for constantly changing place information. The impairment seen during our experiments are similar to the effects seen following transient disconnection between the vSub and the NAc on a radial-arm maze task when foraging behaviour is assessed with either a delay or without a delay following training (Floresco et al., 1997). Findings from this experiment further suggest that the NAc is not involved in incremental place learning due to the lack of impairment in the delayed task, but that it is involved in rapidly-acquired place learning due to the impairment being seen during the non-delayed task. It is therefore suggested that place information that is constantly changing via hippocampal place encoding mechanisms (or place information that requires immediate retrieval) may be sent via the NAc for rapid translation into behaviour. In order to determine if hippocampus-NAc interactions are required for 1-trial place learning performance on the DMTP task, future studies are required combining the task with functional disconnection approaches to disconnect the hippocampus from NAc.

Conclusions

Accumbal muscimol infusion reduces neuronal firing rate and burst firing, resulting in performance impairments on the watermaze DMTP task. It has been suggested that place memory formed in the hippocampus is either consolidated into the neocortex (and retrieved as needed) or else consolidation is not required and it is translated directly into behaviour by prefrontal or subcortical connectivity of the hippocampus (Bast, 2011; Bast et al., 2009). Following previous work indicating that prefrontal cortex may not be involved in this (unpublished results), the research presented in this thesis suggests that this hippocampal learning-behaviour translation involves hippocampal projections to NAc and then onto areas of behavioural control. This work adds to the idea that the NAc does not exclusively play roles involving reward and motivation, but also plays an important role in memory translation, including place memory and other declarative memory types requiring the hippocampus (Setlow, 1997). The involvement of other brain regions, such as the amygdala and ventral tegmental area, is yet to be determined. Also, the direct hippocampus-NAc interaction remains to be demonstrated, meaning that further research is needed to form a better understanding of all mechanisms at play.

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Appendix 1: Protocols

Cannula stereotaxic implantation (recovery surgery)

Isoflurane anaesthesia induction

(5% in 1 litre O2). When rat is down, shave head + inject rimadyl (SC) + Antibiotic (SC)

Wipe shaved head with chlorhexedine/water.

Transfer to frame – assistant monitor breathing every ~5 minutes during surgery

O2 at 1ltr, Iso at 3%:

- Rotate rat head to fit into tooth-bar – tilt back and from rotate 90 degrees to vertical
- Take breathing count – breaths per 15 seconds X4
- Have breathing rate between 40-60 breaths. Adjust isofluorane as needed
- Eye Lubricant
- Ear bars - EMLA cream
- **Check toe-pinch reflex – no reflex should be visible**
- Insert bars – so that bone surrounds and head is level
- Make sure ears bars distance is equal
- Check by loosening nose-cone that head stays in place with bars.

Disinfect:

Alcohol wipe scalp

Wash hands and dress in sterile gown/gloves with assistance)

Buster-op cover on rat (cut out triangle “collar” for rat).

Incision:

- Cut skin layers – expose skull
- clip back at four corners ***the under-layer*** of skin (don’t clip outer/furry skin) (Use Cotton buds; mop blood and push back skin over skull “ridge”).

Take Coordinates from bregma:

- Fasten drill to frame – use tip to find bregma and read frame coordinates – AP/ML

- *assistant calculate final coordinates
- Drill cannula holes + screw holes around these – don't puncture meninges
- Remove drill
- Bend injection needle into hook – gently pierce dura
- Insert screws, leaving about 0.5 mm between screw head and skull.
- Attach cannula holder to frame - lower to measure DV coordinates* either from skull or dura (depending on target region)
- Lower cannula guides to required level.

Fix guides in place with acrylic pedestal

- Keeping cannula holder attached, mix dental acrylic powder with liquid. Medium viscosity, drip mixture between screws and guide, to attach and build a pedestal. Work around 3/4 of reachable area around holder. Do not get acrylic on holder (otherwise Scrape off with forceps). Mop away any acrylic spilled to edge of wound.
- Touch acrylic with forceps to check if hard. Then carefully unscrew cannula holder and remove from frame, ensuring cannula guide doesn't move out of place.
- Apply acrylic to remaining areas, ensuring no gaps are left between screws, skull and guide. Leave enough thread visible to screw caps on guide. Smooth over any bumps in pedestal with low viscosity acrylic.
- When dry, remove clips from skin.
- Stitch wound closed with sterile suture thread. At least 2 stitches in front of pedestal and two behind. Leave a slight gap around guide (to still see dried acrylic) rather than stitching too tight, to aid wound healing.
- IP injection of NaCl Solution to rehydrate rat. Remove rat from stereotactic frame and attach any stylets, caps etc.
- Put rat on top of recovery pad in recovery cage on heat mat, no wire grid food holder, with fresh mash food and water. Observe until righting reflex regained.
- Monitor daily, recording weight and administering antibiotics as prescribed.

Version July 2018, R Hock

Electrophysiological recording experiments

1. Computer and Plexon server on (computer pwd: scn)
2. Double click server item on desktop → "Program loaded"
3. Sort client – Settings – Analogue channels –Open sorting parameters, save as new – Update server
   (Open PicoScope 6 & Ref2 from desktop)
4. Open gas bottle (medical oxygen) and switch on scavenging system (anaesthesia vent)
5. Prepare surgical instruments: scalpel, forceps, clamps, dura cutter, cotton buds
6. Prepare syringes: saline (2 needles: clean for pulling up new saline and normal one for use)
7. Check injector on electrode array
8. Check glass syringe (1 ul Hamilton, SYR7226 from SLS)
9. Get rat (write down weight and tracking number)
10. Prepare rat: Induction of anaesthesia (4% isofluorane in 600 ml/min medical oxygen), transfer to frame (600 ml/min medical oxygen; 4-3% isofluorane to begin, reduce gradually, steps of 0.25%, to stable breathing rate of ca. 60/min ca. 2%),
11. Insert rectal probe; heat blanket on – !Earth on! On heat blanket control unit
12. Connect electrode array to head stage
13. Remove bone and dura over region of interest, keep moist with saline (try to keep bregma)
14. Fill injector with drug, add air bubble at end of tubing and connect to 1 ul syringe (piston out)
15. Move piston to 0.5ul (check for proper function); pull back 0.25ul for air plug
16. Mark bubble!
17. Insert injector-array assembly into brain surface
18. Connect ground wire to ear bar with big clip and little clip. Connect a croc wire between surgery clamp and croc clip for ground wire.
19. Turn off lamp and drill (at plug socket)
20. Switch pre-amplifier on (on Plexon server), then switch blue box on
21. Switch on oscilloscope
22. (Switch Neurolog Control on for loudspeaker)

23. Slowly move down with assembly to target coordinates (stop close to target coordinates with good signal)
24. Wait for at least 30 min
25. Record 30 min baseline activity
26. Infusion, can be marked in data file using Alt+1 (start) and Alt+2 (end).
27. Record post-infusion activity
28. At completion of experiment: stop recording, switch blue box off, switch pre-amplifier off, switch Plexon server off, close Sort client.
29. Overdose of anaesthesia
30. Marking of electrode locations: Remove head stage; connect + (blue) wire of lesion generator to electrode to be marked and – (white) wire to any other electrode. Pass 0.2 mA for 10 s.
31. Measure bubble movement from infusion
32. Store brain in cyanous ferranide PFA
33. **ALL GASES OFF AND SCAVENGER OFF**

Possible problems:

- Noise in recording
    - Lamp still on → make sure the plug sockets are off (lamp; drill; lesion marker)
    - Ground wire not attached properly
    - Heat blanket earth not on
    - Microscope touching blue box
    - If recording seems very noisy try touching microscope, frame, table and isoflurane vaporiser.
- Anaesthesia levels to high or low
    - Adapt anaesthesia levels in 0.25% steps → avoids missing appropriate level
    - If at 4% and still toe pinch reflex → disconnect scavenging tube
    - If very low and breathing rate low → check if blanket is on! If it is → increase scavenging level
    - If breathing looks very laboured → check if ear bars are not too far back → check that mask does not press on nose

12 Feb 2018

Watermaze DMP task

Before day of experiment

- On PC1, open Ethovision XT 7 .
- If possible use template and rename
- Check arena settings are still ok.
- If not see water maze build from scratch

On day of experiment

Preparation

Water maze room (F35A)

- Switch the lights on by the switch next to the dimmer switch. Ensure the dimmer switch is all the way up. If only one side lights up, turn plug (wall opposite side) on. Illumination level just above the water surface in the centre of the pool: 200 lx.
- Turn on Atlantis Platform controller
- Fill the water maze so that the water level is 1-3 cm above the platform and at 25º C ± 1º C.
- Use approximately 200ml of liquid latex/water-based paint to make the water white and opaque on the first day. Add about 100ml latex/water-based paint if water is not opaque enough after a couple of days over which the water is partly exchanged (every 4-6 days).

Computer room (F35B)

- Switch on:
    - PC1 (Username: ad\'username' + password)
    - PC1 Monitor
    - PSU3
    - Med Associates Inc. SG-501 box
    - Noldus Information Technology USB-IO box
- On PC1, open Ethovision XT 7 .
    - In Ethovision, open the appropriate experiment template file.
    - Save as experiment of the day (Axxxx_day _date_manipulation)

- Copy paste trial list form excel file (to prepare in advance) to Ethovision file
- Move platform to correct location

Experiment

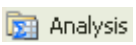


- In Ethovision, click on 

then 

on the left side.
- Reset the background  and start the trial 
- **Probe trial: push platform down**!!!
- Release the animal from the appropriate starting position at the edge of the water maze, with the animal facing the wall,
- Simultaneously press “IN-1” on the USB-IO TTL tester in order to start tracking.
- Once the rat has found the platform, note the latency.
- The trial can be stopped in Ethovision. The rat must be left on the platform for 30 s. (Should the rat not find the platform within 120 s, guide it to the platform and have it complete the 30 s on the platform).
- Stop recording in pinnacle (for 15-30 s delay record 1 video for each rat; for 20 min delay record first trials separately and then trial 2-4 on one video)

Data extraction

- Training days: only need latencies. Use manual values.
- Probe trials:
  - Search preference
    - (Make sure the arena settings are correct)
    - Click on 

    - In 

select 

.
    - In 

select 

then select
      - Velocity
      - In Zone (select all zones around platforms)
    - Select Results then Analysis Output
    - Click Calculate (top left) to get your values
    - select 

.
  - Pathlengths
    - You need to extract pathlength for every trial individually

- Select System Independent Variable → Trial name → select trial x
- Select Nesting → Time → Select time interval form 0 to end of trial time
- Place both Filter box and Nesting box on arrow between Start and Result1 box
- Go to Analysis Profiles, make sure Distance moved is selected!
- Select Results → Analysis Output
- Click Calculate (top left) to get your values
- select Export → Analysis Output.

Trial retracking

- Open new file from template, save with title of trial to be retracked.
- Open acquisition to load settings.
- Go to: Experiment settings – video source – from video file.
- Choose appropriate video file in the acquisition window.
- Copy trial list info into trials page.
- Set up arena settings as appropriate.
- Amend detection settings as appropriate: usually set to 'Subject is Brighter and Darker than background'.
- Reset background and press play, using the TTL tester to start trial upon rat being released into the pool.

Appendix 2: Additional work

BNA 2017 abstract

Poster number: P-W100

Theme: Learning & memory

A role for the nucleus accumbens in the hippocampal learning-behaviour translation?

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The role of the hippocampus in important types of rapid everyday learning, such as place learning, is well established. However, the mechanisms via which rapidly-acquired place memory may be translated into behaviour are yet to be determined. The intermediate hippocampus, which has been shown to be critical for the hippocampal learning-behaviour translation, combines neural substrates of accurate place encoding with links to prefrontal and subcortical behavioural control sites. This supports that these sites, including the nucleus accumbens (NAc), may be critical for this translation (Bast et al., 2009, PLoS Biol; Bast, 2011, Curr Opin Neurobiol). The NAc is a main candidate due to strong hippocampo-NAc projections that have been implicated in behaviour based on place memory (van der Meer et al., 2014, In: Space, Time and Memory in the Hippocampal Formation, ed. Derdikman & Knierim).

To examine the role of the NAc in the hippocampal learning-behaviour translation, we combined functional inhibition of the NAc (via microinfusion of the GABA agonist muscimol) with measurements of behavioural performance based on hippocampus-dependent rapid place learning using the watermaze delayed-matching-to-place test (DMTP) (Bast et al., 2009). In preparation for these studies, we conducted in vivo electrophysiological and sensorimotor experiments (locomotor activity, LMA and startle response/prepulse inhibition, PPI) to establish suitable muscimol doses to reduce NAc neuronal activity without causing gross sensorimotor side effects. Muscimol infusions into the NAc (125-250 ng/0.5

µl/side) reduced neuronal firing in the infusion site vicinity by 40-50% and, if at all, only moderately reduced LMA and PPI; the latter is consistent with findings that these sensorimotor functions mainly depend on the NAc core (Pothuizen et al., 2005, Neuropsychopharmacology), whereas our infusions targeted the NAc shell, which is the main recipient of hippocampo-NAc projections (Humphreys & Preston, 2010, Prog Neurobiol). Preliminary findings from our studies combining functional inhibition of the NAc with DMTP testing support that the NAc is required for performance based on hippocampus-dependent rapid place learning. Additional experiments to confirm and extend these findings are on the way.

P116-F.02.h Animal studies: Pharmacology of learning and memory - part I

Abstract: 4073

F106 - A role for the nucleus accumbens in the hippocampal learning-behaviour translation

Adam Seaton[1], Rebecca Hock[1], Miriam Gwilt[1], Stuart Williams[1], Charles Greenspon[2], Robert Mason[2], Tobias Bast[1]

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The hippocampus is required for important types of rapid everyday learning, including place learning. However, the pathways via which hippocampal place learning is translated into behaviour remain to be determined. The intermediate hippocampus is critical for the hippocampal learning-behaviour translation and combines neural substrates of accurate place encoding with links to prefrontal and subcortical behavioural control sites, which may contribute to this translation (Bast et al.,2009,PLoSBiol; Bast,2011,CurrOpinNeurobiol). The nucleus accumbens (NAc) is a main candidate due to strong hippocampo-NAc projections that have been implicated in behaviour based on place memory (Humphries & Prescott, 2010, Prog Neurobiol).

To examine the role of the NAc, we combined functional inhibition (via microinfusion of the GABA agonist muscimol) with measurements of behavioural performance based on hippocampus-dependent rapid place learning using the watermaze delayed-matching-to-place (DMP) test (Bast et al., 2009). Electrophysiological and sensorimotor experiments (locomotor activity, startle response/prepulse inhibition) showed that muscimol (125-250ng/0.5 µl/side) reduced NAc neuron firing around the infusion site by about 50% and caused, if at all, only moderate sensorimotor effects. Watermaze Experiment 1, testing the impact of NAc muscimol within-subjects, supported that the NAc is required for DMP performance; however, there

appeared to be a carry-over effect, with NAc muscimol eroding the typical DMP search strategy on subsequent test days, possibly reflecting that NAc is required to reinforce the strategy. Watermaze Experiment 2, using a between-subjects design, confirmed that NAc muscimol impairs expression of hippocampus-dependent rapid place learning on the DMP test.

Overall, these findings support that the NAc contributes to the hippocampal learning-behaviour translation.

Abstract: 4072

F105 - Too little and too much: impact of functional inhibition and disinhibition of the medial prefrontal cortex on cognitive flexibility assessed using an operant strategy-shifting task

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Attentional performance requires balanced medial prefrontal cortex (mPFC) activity, with functional inhibition or disinhibition (by micro-infusion of GABA agonist muscimol or antagonist picrotoxin, respectively) causing impairments (Pezze et al., 2014, J Neurosci). Cognitive flexibility has also been suggested to require balanced mPFC activity (Brady & Floresco, 2016, JoVE). To confirm this, we examined how mPFC muscimol and picrotoxin affect cognitive flexibility in Lister-hooded rats, using an operant strategy-shifting task involving shifts from egocentric-spatial to cue light-based responses or vice versa (Brady&Floresco,2016).

Remarkably, Lister-hooded rats (irrespective of infusion) required three times as many trials compared to previous studies in other strains to shift from egocentric to cue light-based responses (Brady & Floresco, 2016). These slow shifts were unaffected by the prefrontal manipulations. However, disinhibition, but not inhibition, impaired egocentric-response expression and increased trial omissions, showing that regional neural disinhibition can disrupt functions not normally requiring the disinhibited region (Bast et al., 2017, BrJPharmacol). Additional behavioural studies showed that although Lister-hooded rats struggled to shift from egocentric to cue-based responses, they readily acquired cue-based responses when

they had not first been trained the egocentric strategy. Moreover, they then readily shifted from cue-based to egocentric strategy, and could perform egocentric-response reversals. However, there was always a higher shift-cost for egocentric-to-cue than for cue-to-egocentric shifting.

Overall, although Lister hooded rats readily acquired both strategies, they overcame the egocentric strategy very slowly, and this slow strategy change was not mPFC dependent. Contrastingly, rats readily performed cue-to-egocentric-strategy shifts; we are currently examining the impact of mPFC functional inhibition and disinhibition on such flexible shifts.

Appendix 3: PIPS reflective statement

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 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.

PIPS Reflective Statement

For my PIP placement, I spent 3 months working at RenaSci Ltd., a contract research organisation (CRO) based in Nottingham. During my time there, I was working as part of the rat microdialysis team, whose role is to carry out intracerebral microdialysis in combination with pharmacological manipulation in order to assess the action of centrally-acting compounds in order to further drug development for clients. During the initial part of my stay I was based in their main offices in the centre of Nottingham in order to understand the role of a CRO and how they conduct their business, including how they develop their own techniques, how they are primarily funded and what steps they have to undertake to work with a client. To this end, I worked closely with a member of the microdialysis team during the preparation for a client study, learning how they get in contact with the clients and what paperwork needs to be completed for a study to take place. As the purpose of the research is to test the effects of a novel centrally-acting drug, the drug is provided by the clients with instructions provided on dosing and method of delivery. This means that the initial stages of a project are spent determining the experimental design (dosing procedure, sample size, etc.), as well as ordering animals and preparing rooms in preparation for the experiment. I was then involved in the experimental procedures, initially watching other members of the team performing the surgery required for the microdialysis procedure and ensuring full recovery of the rats following this (ensuring rats regain righting reflex, are given food and water, etc.).

Surgery involved stereotaxic implantation of a microdialysis probe into the brain region of interest in a similar way to the stereotaxic cannula implantation techniques carried out during my own research. Briefly, this involved induction of anaesthesia followed by incision of the scalp, drilling holes through the skull, implanting probes and securing these with dental cement (including the addition of an 'anchor'). The experimental design of the experiment was set out such that each surgery day was followed by an experimental day. Therefore, on each experimental day I was involved in conducting the microdialysis procedure, including set-up of the bowls in which the rats were tested, preparation of the rats following surgery for sample collection from the microdialysis probes (attached to pumps with the rats freely moving in the bowls attached to a 'crane' device via the 'anchor' implanted during surgery), and collecting of the samples throughout the session. These samples were then transported in dry ice back to the main offices where they were stored at -80° until the levels of neurotransmitters/neuromodulators using High Performance Liquid Chromatography (HPLC) separation, which I was briefly shown how to carry out. Following this study, the microdialysis team carried out an 'in-house' study, which allows the company to carry out research and develop techniques for them to be able to advertise these to prospective clients. As this was not a client study, I was more directly involved with this and was able to carry out a couple of the surgeries for the experiment and be generally more involved in the running of the experiment, including the initial set-up/paperwork and daily care for the rats (weighing, feeding, etc.).

Overall, my time at RenaSci was very useful as I developed skills such as experimental design, surgical techniques, and new experimental procedures. I also gained an understanding of the role that a CRO plays and how the business itself is run. Due to my passion for carrying out experimental procedures, including surgical procedures and testing, my time at RenaSci has helped me determine that I was like to be involved in a career in industry as opposed to staying in academia.