

**THE ROLE OF HIPPOCAMPAL NMDA RECEPTORS IN ENCODING
AND CONSOLIDATION OF SPATIAL INFORMATION ON THE SPATIAL
VERSION OF THE WATER TASK**

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**The role of hippocampal NMDA receptors in encoding and consolidation of
spatial information on the spatial version of the water task**

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Abstract

The NMDA receptor is a proposed molecular mechanism responsible for the structural changes that occur in neurons during learning and memory formation. I investigate the role that NMDA receptors have in hippocampal spatial learning and memory. Three projects were done, in which hippocampal NMDA receptors were pharmacologically blocked in groups of rats. They were pre-trained on a spatial version of the Morris water task with mass reversal training occurring in same or different training environments as pre-training. I measured expression of Arc protein throughout the main hippocampal subfields, CA1, CA3, and dentate gyrus, after training. I observed that NMDA receptor blockade allowed spatial learning but not consolidation when using previously acquired environmental information, and impaired learning when this information was novel. Arc protein expression in the dentate gyrus followed this pattern of NMDA receptor dependent spatial behavior. These results implicate dentate NMDA receptors in the acquisition of novel environmental information.

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List of abbreviations

- AMPA receptor - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APV - 2-amino-5-phosphonovalerate
Arc – Activity related cytoskeletal protein
CA1 – Cornu ammonis 1
CA3 – Cornu ammonis 3
Cav1.2 – Voltage gated calcium channel 1.2
CamkII – Calmodulin dependent protein kinase II
CREB - cAMP response element-binding protein
CPP - 3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid
LTP – Long term potentiation
NMDA receptor - N-methyl-D-aspartate receptor

Chapter 1

General Introduction

The mammalian central nervous system appears to contain multiple memory subsystems for acquiring information about an animal's environment and storing that information in a way that can be retrieved for future use. One such memory system centers on the hippocampal formation, a medial temporal lobe structure (Scoville & Millner, 1957; O'Keefe & Nadel, 1978). Through its extensive connections with cortical and other limbic structures, the hippocampus is thought to form associative representations between stimuli in the environment and use these representations to influence a variety of autonomic and voluntary movements (Gruber & McDonald, 2012; McDonald & Hong, 2013). The internal circuitry of the hippocampus and parahippocampal structures, combined with compelling behavioural/neurophysiological correlates characteristic of hippocampal principal neurons, has led to the idea that it encodes information about space and context as well as the temporal ordering of events. The discovery of place cells in the hippocampus, cells whose activity is related to where the organism is positioned in its environment, has reinforced this idea (O'Keefe & Dostrovsky, 1971). This combination of spatial, contextual and temporal information provides evidence for the hippocampus' primary hypothesized role: episodic memory. The behavioural effects of damage to the hippocampus in humans and rats provide further evidence for this claim (Morris, Garrud, Rawlins, & O'Keefe, 1982; Scoville & Millner, 1957; Sutherland, Whishaw, & Kolb, 1983).

The neurobiology and physiology underlying hippocampal learning and memory is an exciting and ever growing field of study. One widely studied neural property proposed to allow for the formation of memories is synaptic plasticity. Synaptic plasticity

is the strengthening or weakening of synaptic connections between neurons and is the result of what is called long term potentiation (LTP) and long term depression (LTD) respectively. LTP is an amplification of the excitatory post synaptic potential as a result of simultaneous high frequency inputs (Bliss & Lømo, 1973; Levy & Steward, 1979). Typically, LTP is induced artificially through electrical stimulation by an experimenter, however, some of the bio-markers for LTP have been found in freely learning animals providing evidence that LTP also occurs naturally (Whitlock, Heynen, Shuler & Bear, 2006)

NMDA receptors and LTP

N-methyl-D-aspartate receptors (NMDA receptor) are a class of postsynaptic glutamate receptors located in many regions of the brain, and are highly expressed in the hippocampus (Dingledine, 1983). Although not necessary for normal synaptic transmission of signals between neurons, NMDA receptors have been thought to play an important role in mediating synaptic plasticity (Collingridge, Kehl & McLennan, 1983; Harris, Ganong & Cotman, 1984). By manipulating extracellular ion concentrations that are critically involved in NMDA receptor function like calcium or magnesium, LTP can be inhibited (Dunwiddie & Lynch, 1979; Herron, Lester, Coan, & Collingridge, 1985). LTP can also be inhibited with NMDA receptor antagonists like o-2-amino-5-phosphonovalerate (APV) that block the receptor (Abraham & Kairiss, 1988; Herron, Lester, Coan, & Collingridge, 1986). Genetic knockouts for the NMDA receptor in mice have been associated with reduced synaptic plasticity and LTP in the hippocampus (Sakimura et al, 1995). A significant body of evidence over the past decades has shown a strong relationship between the expression of LTP and the NMDA receptor.

One brain area in which LTP and NMDA receptors have been studied extensively is the hippocampus because of its links with memory functions. Research has consistently shown that LTP and the NMDA receptor fit many of the requirements necessary for a proposed neural mechanism for associative memory (Hebb, 1949). LTP is induced rapidly after stimulation and because most NMDA receptors are present in the postsynaptic membrane, they are activated immediately following neurotransmitter release. Like long term memory, LTP also persists over time. Once stimulated, LTP can last anywhere from 3 days (Bliss & Gardner-Medwin, 1973) to several months (Abraham, Logan, Greenwood & Dragunow, 2002), although it is subject to decay. LTP and NMDA receptors also have associative and cooperative properties (McNaughton, Douglas & Goddard, 1978). When neurons that are coactive cooperate in depolarizing a target neuron, the connections of those neurons will be strengthened, i.e., associated, while other connections will not. The NMDA receptor possesses similar associative properties. Glutamate release from the active presynaptic neuron must bind to the receptor while the postsynaptic neuron is currently depolarized, causing a release of its magnesium plug allowing for calcium influx (Herron, Lester, Coan, & Collingridge, 1985). This has led to the receptor often being labelled as a “coincidence detector”. This necessary coactivity property is what makes LTP and the postsynaptic NMDA receptor prime candidates for synaptic mechanisms of associative memory (Bashir et al., 1993).

The biochemical connection between NMDA receptors and LTP is thought to be mediated by calcium regulated second messenger systems located in the dendritic space of the postsynaptic neuron. Calcium is introduced to the intracellular space via NMDA receptors and the second messenger systems involved can cause immediate changes in the

proteins present or cause transcriptional changes within the nucleus. When calcium enters the dendrite, it binds to the proteins Calmodulin and Protein Kinase C (PKC). These proteins phosphorylate several other proteins in a series of cascades thought to mediate LTP. One of the most widely studied of these proteins is calcium/Calmodulin-dependent protein kinase CaMKII which is phosphorylated by Calmodulin to alter its structure (Leonard et al., 1999). If enough calcium, and hence Calmodulin, is present, CaMKII will alter its structure in such a way that it will auto-phosphorylate itself and persist in its activated state. The activated CaMKII plays a critical role in the regulation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptor) in the postsynaptic membrane (Barria et al., 1997). AMPA receptors depolarize the cell under normal neural activity and if more are present then neurotransmitter release from the presynaptic neuron will result in more stimulation of the post-synapse, in other words, a strengthening of the connection. LTP cannot be induced in CaMKII knockout mice (Silva, Stevens, Tonegawa & Wang, 1992). By manipulating intracellular levels of PKC, LTP can be induced or inhibited (Wang & Feng, 1992). It is important to note that many other molecules are involved in the potentiation of synapses. Another mechanism behind synaptic plasticity is Immediate Early Genes (IEG). These are genes that are transcribed and translated into functional proteins immediately following high levels of neural activity. Because the transcription of genes takes a much longer time than it takes to simply induce LTP, some IEGs are thought to regulate proteins involved in the maintenance and consolidation of LTP. The molecular biology behind LTP induction and consolidation is complex and extensive.

NMDA receptors and spatial learning

In a breakthrough study linking NMDA receptor function, LTP, and hippocampal based memory, Morris, Anderson, Lynch, and Baudry (1986) bilaterally infused APV into the ventricles of rats and observed a blocking of LTP in the hippocampus. When subjected to a hippocampal-dependent spatial water task that requires the rats to locate and learn the position of a hidden platform under a pool of milky water, they failed to accurately find the hidden escape platform location. These results are similar to those of rats with hippocampal lesions (Sutherland, Whishaw, & Kolb, 1983). NMDA receptor inactivation has been associated with impairments in other learning tasks as well. Intraventricular administration of APV has been shown to impair performance on operant conditioning (Tonkiss, Morris & Rawlins, 1988). The use of other NMDA receptor antagonists such as 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) and Dizocilpine (MK-801) also produce impairments in rats on other types of hippocampus-dependent tasks like the 8 arm radial maze (Ward, Mason & Abraham, 1990). NMDA receptor antagonists like APV and MK 801 have not been found to influence the retrieval of already formed memories. If rats acquire spatial information and are given NMDA receptor antagonists 24 hours later before being tested for retention of that learned information there is no effect (Shapiro & Caramanos, 1990). In other words, these drugs do not affect already formed memories or retrieval processes. The results of this early research using NMDA receptor antagonists combined with electrophysiological and behavioral techniques has generated the popular theory that these receptors are critical for

inducing synaptic plasticity, and therefore are critical for the formation of learned associations and memories. (Collingridge & Bliss, 1987).

However, separate lines of research suggest that this story is not as straightforward as it might seem. NMDA receptor antagonists may have multiple effects on behavior because when administered intraperitoneally or intraventricularly, which are common methods (Morris, Anderson, Lynch & Baudry, 1986; Morris, 1989; Ward, Mason & Abraham, 1990), NMDA receptors are blocked throughout the entire central nervous system. NMDA receptors are located in the cerebral cortex, hippocampus, amygdala, striatum, thalamus, and brainstem (Monaghan & Cotman, 1985). Not all of the brain regions that express NMDA receptors are primarily involved in memory functions and so blocking receptor function in these areas may produce confounding behavioral effects. NMDA receptor antagonists have been shown to induce a wide variety of electrophysiological and behavioral impairments outside associative learning involving sensory (Salt, 1986), motor coordination, (Cain et al., 1996), and anxiolytic responses (Stephens et al., 1986). Some claim that NMDA receptor antagonists only affect the rats' ability to learn new information by impairing their sensorimotor skills. In the spatial water task, motor impairments like poor swimming performance and speed, deflecting, jumping off, or falling off the platform, can severely limit the rats' ability to learn the location of the platform (Cain et al., 1996). In fact, many of the studies examining the role of NMDA receptors in spatial learning have also observed motor problems in their rats when subjected to antagonists (Morris, Steele, Bell, Martin, 2013; Shapiro & Caramanos, 1990; Robinson, Crooks, Shinkman & Gallagher 1989). These factors bring into question the validity of much of the past research on NMDA receptor based memory.

These impairments present a potential confound that begs the question: When applied universally, are the behavioral effects that these drugs have on learning and memory tasks the result of their effects on memory systems or sensorimotor, attentional, and motivational systems? Several behavioral methodologies have been proposed to avoid these potential confounds. Non-spatial visual discrimination tasks can be used to dissociate the effects that NMDA receptor antagonists might have on sensori-motor processes from their effects on learning. Typically, rats are impaired on the spatial task and successful at the visual discrimination task. Although this task can be learned normally without a hippocampus, the visual discrimination task still requires learning and rats with APV in their brains can learn this task successfully, indicating that NMDA receptors may not be a universal plasticity mechanism in the brain (Morris, 1989).

Pre-training

More surprisingly, pre-training laboratory rats on the spatial water task has been shown to eliminate the learning deficits associated with NMDA receptor antagonists (Bannerman et al., 1995; Saucier & Cain 1995). Briefly, if a rat is procedurally trained to learn the spatial water task, prior to drug administration and standard training, the rat is capable of learning new spatial information independent of NMDA receptor function and can perform perfectly compared to controls. It is argued that by making the rat procedurally skilled in a task, the potential effects that the antagonist has on the sensory or motor functions of the subject is diminished. If the sensori-motor impairments disappear then whatever effects the antagonist has on learning can be independently observed. Pre-training can take the form of standard training involving finding a hidden platform (McDonald et al., 2005), training in an entirely different context (Bannerman et

al., 1995) or navigating the pool in a non-spatial way with either curtains drawn or a non-fixed platform position (Hoh et al., 1999, Cain et al., 1996). Hoh et al. (1999) pre-trained rats in a non-spatial version of the water task while they were exposed to a NMDA receptor antagonist, and then successfully trained rats to find a fixed hidden platform with NMDA receptors blocked. These results showed that not only are NMDA receptors unnecessary for the rats to learn place information, but they are potentially unnecessary for learning the behavioral strategies required to navigate the task during pre-training. Studies utilizing pre-training have produced results very different from the earlier research and suggest that the proposed role of NMDA receptors in the acquisition of information may be incorrect.

The functional significance of the NMDA receptor is not as clear in other aspects of associative learning either. NMDA receptor antagonists prevent rats from retaining contextual information and associative fear memories 24 hours after exposure when administered to the appropriate memory system, the dorsal hippocampus and amygdala respectively (Matus-Amat et al., 2007). However, expression of fear memories does not seem to be affected when tested immediately after exposure (Kim, DeCola, Landeira, & Fanselow, 1991; Kim, Fanselow, DeCola, & Landeira, 1992). Tonkiss and Rawlins (1991) used a T maze to analyze reference memory and working memory deficits. Rats had to correctly choose a baited arm from an un-baited one using memory from previous trials. If they were familiarized with the task during pre-training, the NMDA receptor antagonist APV did not influence the rats ability to make correct arm choices. However, if a 20 second time delay was added, rats made more errors and failed to learn the task. Steele & Morris (1999) added time delays to the training trial intervals in the spatial water

task. Pre-trained rats could rapidly learn new spatial positions if the inter-trial intervals were kept short. When the intervals were increased the rats' performance worsened and eventually the subjects failed to learn altogether.

NMDA receptors and memory consolidation

Much of the spatial training that rats undergo while being administered NMDA receptor antagonist occurs over multiple days, however, if training occurs rapidly within a short time period then the rats may be able to acquire the spatial information (McDonald et al., 2005). Research using time delays suggests that the NMDA receptors role in memory may be in the acquisition of associative information, but in the consolidation of that information, a process that occurs later in time. The distinction between rapidly acquired short term memory and long term memory that is acquired over the course of days may explain the inability of rats to learn in several NMDA receptor antagonist studies, even those utilizing pre-training. Indeed, the majority of research testing the role of NMDA receptors and spatial memory are done over several days of drug administration (Morris, 1989; Inglis, Martin & Morris, 2013; Robinson, Crooks, Shinkman & Gallagher 1989)

Some of the most critical support for the consolidation idea came from Kentros et al., (1998). They examined the effects that a NMDA receptor antagonist would have on the formation and stability of place fields in the hippocampus. What they found was that the drug did not prevent the formation of new place field representations in the hippocampus when the rat was located in a new environment, and that this new representation lasted for approximately 1.5 hours. The antagonist did however prevent the long term stability of the representation as it disappeared the following day. This study

provided electrophysiological support for behavioral results showing that the acquisition of new spatial information is possible independent of NMDA receptor function.

Further support came from Santini, Muller, and Quirk (2001) and McDonald et al., (2005). Santini, Muller, and Quirk (2001) found that when rats were given CPP peripherally they could acquire extinction memories on a conditioned fear task but could not remember the extinction 24 hours later. Similarly, when given CPP in the rest period directly after acquiring extinction memory, the rats also could not remember what they had learned at a later time. This means that the availability of NMDA receptors after training had an impact on memory. In 2005, McDonald et al., pre-trained rats to find a hidden location in the spatial water task, then later rapidly trained to a new location while given intraperitoneal or intrahippocampal injections of CPP. The rats successfully learned the new location during the training period showing acquisition without NMDA receptor function. When tested 24 hours, the rats did not remember what they had learned. They preferentially swam towards and spent most of their time in the location of the original platform location, showing a complete forgetting of the new, rapidly acquired location. This means that their ability to learn was intact while their ability to consolidate the information into long term memory was impaired. The McDonald et al., (2005) study will be the foundation for much of the proposed research below.

Other molecular mechanisms

If NMDA receptors are necessary for the consolidation of spatial information in the hippocampus and not acquisition, then what might a possible mechanism for acquisition be? Or conversely, is there a non-LTP form of plasticity that supports hippocampal learning? Instead of throwing out LTP all together, I propose the simpler

explanation, that a NMDA receptor independent form of LTP induction is possible and that it can support certain forms of spatial learning. LTP can be induced in hippocampal neurons without NMDA receptor function if the frequency of stimulation is higher than what is normally used for induction. Usually, a 100 Hz stimulation is used but a 200 Hz stimulation can induce LTP in hippocampal principle cells when NMDA receptors are blocked (Grover & Teyler, 1990). NMDA receptor independent forms of LTP have been induced in many synapses of the hippocampus such as those at mossy fibres (Harris & Cotman, 1986), the perforant path (Bramham, Milgram & Srebro, 1991), and Schaffer collaterals (Grover, 1998). This means that some other non-NMDA mediated synaptic mechanism is potentially responsible for acquisition of spatial information in tasks like the water maze.

Some forms of LTP may not be NMDA receptor dependent but something that LTP is dependent on is intracellular calcium. There exist other mechanisms by which calcium can enter the intracellular space following synaptic activity that are non-NMDA receptor based. Voltage gated calcium channels located on the postsynaptic membrane can provide this calcium to the intracellular space. These channels are located throughout the central and peripheral nervous systems with the $\text{Ca}_v1.2$ being the most abundant in the brain. Work done with $\text{Ca}_v1.2$ channels has shown that they may play an important role in the induction of LTP. LTP can be induced with a 200 Hz stimulation in CA1 pyramidal neurons when NMDA receptors are blocked but not when $\text{Ca}_v1.2$ channels are blocked using nifedipine, a calcium channel antagonist (Grover & Teyler, 1990). Similarly, genetic knockout mice missing the $\text{Ca}_v1.2$ receptor can exhibit normal NMDA receptor dependent LTP in CA1 neurons, but when an NMDA receptor antagonist is administered

LTP cannot be induced (Moosmang, et al., 2005). Behaviorally, these mice expressed impaired performance on a variety of hippocampal dependent spatial tasks. Other behavioral research has produced mixed results. Bauer, Schafe and LeDoux (2002) found that blocking NMDA receptors impaired learning contextual fear learning and that blocking calcium channels impaired retention of this information. The complex relationship that $\text{Ca}_v1.2$ s and NMDA receptors have in the production of synaptic plasticity may explain the contradictory behavioral results (Freir & Herron 2003) found in the research literature. Intracellular signaling cascades involved in LTP induction such as ERK/MAPK and CREB phosphorylation have been found to be affected by manipulation of postsynaptic calcium channels (Dolmetsch et al., 2001). Postsynaptic voltage gated calcium channels are a prime candidate mechanism for NMDA receptor independent LTP as they can support spatial learning and trigger its necessary molecular signals. Therefore, the $\text{Ca}_v1.2$ channel should be examined as a potential mechanism behind spatial learning in the hippocampus in the pre-training paradigm.

Another potential mechanism behind the NMDA receptors memory properties are immediate early genes (IEG). Activity-regulated cytoskeleton-associated protein (Arc) is an IEG that is upregulated by NMDA receptor activation (Lyford et al., 1995) and is limited to cells expressing NMDA receptors. Because IEG activation and subsequent protein synthesis takes at least 15 minutes to induce, their expression is associated with the later stages of LTP. Performance on the spatial water task has been shown to induce Arc and zif268 in the hippocampus (Guzowski, Setlow, Wagner, & McGaugh, 2001) and blocking NMDA receptors with various antagonists can eliminate this expression (Wisden et al., 1990). Conversely, when rats are treated with Arc antisense

oligonucleotides, special DNA and RNA strands that disrupt protein translation, they are able to acquire an inhibitory avoidance association but unable to consolidate this association into memories, a pattern of results very similar to McDonald et al., (2005) and Santini, Muller, and Quirk, (2001) (McIntyre et al., 2005). Analyzing the expression of IEG activity as a direct result from behavioral training will provide molecular evidence for the involvement of NMDA receptors in memory consolidation.

Purpose of this thesis and research questions

The purpose of this thesis is to provide an answer to the general question “what role does the NMDA receptor have in learning in memory?” I attempted to answer this question with a series of more specific questions regarding its involvement in hippocampal spatial memory. This was accomplished by the use of animal learning behavioral procedures, neuropharmacology, and molecular imaging techniques. Based on previous research, I hypothesized that the hippocampal NMDA receptor will have a critical role in the consolidation of spatial memory.

Project 1: Does full hippocampal NMDA receptor blockade impair spatial memory acquisition or consolidation?

In the McDonald (2005) study, the NMDA receptor antagonist CPP was only administered to the dorsal hippocampus, leaving the ventral portion unaffected. It is possible that ventral hippocampal NMDA-based plasticity could support place learning which could account for the lack of effect of the NMDA manipulation in this study. Further, there are functional and anatomical differences between the dorsal and ventral regions of the hippocampus (Ferbinteanu, Ray, & McDonald, 2003; McDonald, Jones,

Richards & Hong, 2006). Lesions of the dorsal segment have been associated with poor performance on the spatial water task whereas lesions of the ventral segment were not (Bannerman et al., 1999). However, more recent evidence has pointed to different spatial roles of the dorsal and ventral segments across training (Ruediger, Spirig, Donato & Caroni, 2012). From an experimental design perspective, intracranial injections of NMDA directly into the entire hippocampus is also important because this procedure leaves NMDA receptors in other brain regions unaffected allowing the isolation of the mnemonic effects of this manipulation from any other potential behavioral effects. Experiment 1 was a replication of the McDonald et al, (2005) study using entire hippocampal blockade. I hypothesized that full hippocampal blockade will result in identical results as the McDonald et al., (2005) study, showing that rats should be able to learn new spatial information in the water maze yet fail to consolidate this information, and soon forget it.

Project 2: Does full hippocampal NMDA receptor blockade impair novel contextual learning?

It has been argued that in studies where the same training context is used for both pre-training and standard training (McDonald et al., 2005; Hoh et al., 1999), most of the learning that is crucial for completing the task occurs during the pre-training phase and that later training in the task can be completed without engaging plasticity mechanisms in the hippocampus. This means that even though the platform is in a different position during regular training, the rat can locate and “learn” the new position without needing hippocampal plasticity mediated by NMDA receptors and may either rely on previously acquired hippocampal memories or cortical plasticity (Inglis, Martin & Morris, 2013).

This presents a potential confound because if learning the new platform position doesn't actually require hippocampal plasticity then administering NMDA receptor antagonists will be irrelevant and the rat will "learn" the new position regardless. However, even when two completely different contexts are used (Bannerman et al., 1995), replications have failed to reproduce identical results (Inglis, Martin & Morris, 2013). To determine whether NMDA receptors only encode novel spatial information, and not further learning dependent on previously acquired spatial information, a second version of experiment 1 will be done but using two different contexts for pre-training and rapid training. If the rats can still rapidly acquire spatial information in an entirely different context, then the idea that NMDA receptors are involved in the acquisition of novel spatial information can be ruled out. Experiment 2 will be identical to experiment 1 except that pre-training and NMDA receptor blocked training will occur in two different spatial contexts.

Project 3: Is contextual learning induced Arc expression in the hippocampus NMDA receptor dependent?

Immediate early gene activity is a molecular product of learning and memory related behaviors. Spatial memory tasks such as the water maze have been shown to induce expression of Arc in the hippocampus. Both water maze performance and hippocampal Arc expression can be affected by NMDA receptor antagonism. Project 3 explored this relationship further. Rats were subject to a water maze pre-training procedure, and then trained again in either the same room, a new room, or a new room with an NMDA receptor blocker. The expression of Arc was analyzed in the hippocampus after this experience. I hypothesize that Arc expression will follow the behavioral results of experiments 1 and 2. Namely, that whichever learning scenario, if

any, is found to be NMDA receptor dependent, this will be the scenario that results in the highest Arc expression and that NMDA receptor blockade will inhibit this expression.

Chapter 2

The effects of full hippocampal NMDA receptor blockade on spatial learning and consolidation in the Morris water task

The NMDA receptor is a post-synaptic glutamate receptor expressed both on excitatory and inhibitory synapses in principle cells throughout the central nervous system (Moreau & Kullman, 2013). One of the primary roles in which this receptor is studied is as a molecular mechanism that supports synaptic plasticity and learning new information. The NMDA receptor is indeed an attractive candidate for the neurobiology of learning and memory due to its various properties and associations with other learning and memory phenomena (Hunt & Castillo, 2012). It is distributed throughout the central nervous system, in the spinal cord, brainstem, neocortex, and cerebellum, but concentrated most heavily in brain regions associated with learning and memory, such as the striatum, amygdala, and hippocampal formation (Monaghan & Cotman, 1985). The NMDA receptor is often referred to as a coincidence detector, only being fully opened when both its mg+ plug and channel pore have been opened by simultaneous pre- and post-synaptic activity. Similarly, this type of electrophysiological activity that activates NMDA receptors also produces long term potentiation (LTP) (Nicoll, 2003).

LTP is the strengthening of synaptic connections between neurons and is the most prominent model of physiological memory formation. Blocking NMDA receptors has been shown to prevent the maintenance of LTP in hippocampal neurons. (Abraham & Mason, 1988; Lu et al., 1991) The ability to link two separate but related stimuli together make both the NMDA receptor and LTP prime candidates for Hebbian associative learning (Cotman & Monaghan, 1988). In the opposite direction, long term depression

(LTD) is the weakening of synaptic connections, usually due to repetitive, low frequency stimulations. Both LTP and LTD are typically NMDA receptor dependent processes (Luscher & Malenka 2012). Within the hippocampus, NMDA receptor dependent LTP can be induced at all synaptic connections of the tri-synaptic loop; Schaffer collaterals projections to CA1 (Bashir & Alford, 1991), Mossy fibre projections to CA3 (Kwon & Castillo, 2008), and entorhinal projections neurons that make up the perforant path that project to the granule cells of the dentate gyrus (Xie, Berger, & Barrientos, 1992).

The NMDA receptor, although permeable to both Na^+ and K^+ , is primarily a Ca^+ channel. Ca^+ is a second messenger molecule critical to several intracellular processes thought to underlie the biological formation of memory such as CaMKII, CREB, IEG activation, and AMPA receptor cycling (Leonard et al., 1999; Alberini, 2009). Along with the several biomolecular processes associated with learning and memory, NMDA receptor function can also be studied at the behavioral level. An animal will either be bred as a genetic knockout, missing a key component of the receptor complex in some specified brain region such as CA1 (McHugh et al., 1996) or dentate gyrus (Niewoehner et al., 2007), or will be given an NMDA receptor antagonist and then trained on some learning and memory task. In their original study, Morris, Anderson, Lynch & Baudry (1986) found that intracerebroventricular injection of APV blocked both LTP in the perforant path → dentate gyrus circuits and spatial learning in the Morris water task. Similar studies in his lab and by others have found comparable results (Morris, 1989; Davis, Butcher & Morris 1992; Abraham & Mason, 1988). When injected directly into the amygdala, NMDA receptor blockade prevents the learning of associative fear memories (Matus-Amat et al., 2007; Lee & Kim 1998). And when injected directly into

the striatum, impairs instrumental learning in conditioning experiments (Yin, Knowlton & Balleine 2005; Smith-Row, Sadeghian & Kelley, 1999). The use of genetic mouse models has produced similar results (Cravens, Vargas-Pinto, Christian & Nakazawa, 2006; Sakamura et al., 1995). Studies of these kinds have reinforced the theory that NMDA receptors have a critical role in LTP and memory formation.

However, there exists an extensive contrary literature detailing opposite or different effects: that NMDA receptors are either not necessary for learning at all, (Cain et al. 1996), necessary for learning depending on the parameters of the task (Steele & Morris, 1999, Bannerman et al., 2008), or necessary for different aspects of the learning and memory process (i.e., cellular consolidation, memory decay, resolve memory ambiguity) (Bannerman et al., 2012; McDonald 2005; Roesler et al., 2005; Shinohara & Hata, 2014). First evidence of this kind was found by Bannerman et al., (1995) and Saucier & Cain, (1996), showing that when animals were pre-trained to be procedurally proficient at the water task, that learning new information on the task could be done independent of NMDA receptor function. It was argued that blocking NMDA receptors in naïve rats masked any learning effect by impairing sensori-motor processes. NMDA receptor blockade has been shown to impair visual processing (Salt, 1986), anxiety responses (Stephens et al., 1986) and motor function (Cain et al., 1996).

Motor impairments in the water task can be observed to increase in a dose dependent way in response to NMDA receptor antagonist drugs, regardless of injection site, making the dissociation between learning and motor impairments very difficult (Ahlander, Misane, Schott & Ogren, 1999; Inglis, Martin & Morris, 2013). Not only this, but several aspects of successful water task performance must be learned during training,

such as swimming, strategy, and platform size and stability, not just spatial location. Pre-training ensures that these other aspects, many of which are not related to hippocampal spatial navigation, do not occlude the learning and memory behaviors of further testing. The pre-training effect has also been observed in other tasks like inhibitory avoidance (Roesler et al., 1998) and context fear conditioning (Taylor et al., 2010; although see Lee & Kim, 1998). Extensive reviews of these two streams of literature can be found in Bannerman, Rawlins & Good (2006), Nakazawa, McHugh, Wilson & Tonegawa (2004), and Morris (2013).

There also exist alternative molecular mechanisms that when NMDA receptors are inactive, can support learning and its proposed physiological and molecular correlates like LTP (Bauer, Schafe & LeDoux, 2002; Anwyl, 2006), MAPK/CREB signaling cascade and immediate early gene activation (Moosmang et al., 2005). Voltage Gated Calcium Channels (VGCC) can allow for high levels of calcium influx into synapses in amounts capable of inducing LTP (Freir & Herron, 2003). VGCCs are also capable of supporting learning and memory processes in the presence of NMDA receptor antagonists (Borroni, Fichtenhotlz, Woodside, & Teyler 2000; Woodside, Borroni, Hammonds &Teyler, 2004).

These two bodies of work, those detailing NMDA receptor dependent learning and memory, and those detailing NMDA receptor independent learning and memory leave the question of the mnemonic role of NMDA receptors open. Previous work in our lab has shown that when an animal has been procedurally pre-trained in the Morris water task, that these animals can learn new information in this task when an NMDA receptor antagonist is injected into the dorsal hippocampus, yet fail to consolidate that information when tested at a later time (McDonald et al., 2005).

This current work is an extension of that previous work. The McDonald (2005) work observed the effects of infusing the NMDA receptor antagonist CPP into the dorsal hippocampus on a rapid learning version of the Morris water task. In order to isolate the type of learning required by the animal during the task as purely spatial, rats were given spatial water task pre-training. Pre-training ensures that the only thing that is required for the rat to learn during the drug influenced spatial training is a novel spatial location, because all other aspects of the task are learned during this pre-training period. In order to avoid any potential sensorimotor impairments or anxiolytic responses, bilateral hippocampal cannulations were used to limit the receptor blockade to only the hippocampal formation. NMDA receptors are located in several places throughout the central nervous system, administering an antagonist intra-peritoneally or intra-cerebroventricularly means that the drug will be widely distributed throughout the central nervous system, which adds a potential confound. NMDA receptor antagonism in other brain regions is not only unnecessary but may induce unwanted behavioral effects that might occlude or interfere with learning and memory processes.

In McDonald (2005) only the dorsal segment of the hippocampus was blocked with a receptor antagonist. Although the dorsal portion of the hippocampus is usually recognized as being the portion necessary for spatial navigation and memory (Moser, Moser & Anderson, 1993; Moser & Moser 1998; Ferbinteanu & McDonald, 2001), the ventral portion may still support memory functions or navigation in spatial tasks (Kim & Levin, 1996; Rudy & Matus-Amat, 2005; Ferbinteanu, Ray & McDonald, 2003). Apart from the conditions of global blockade (Morris, Steele, Bell & Martin, 2013), or hippocampal receptor knockouts that are thought to span the entire septo-temporal axis

(Bannerman et al, 2012) very few pharmacological studies have been done using full hippocampal NMDA receptor blockade in the Morris water task (Steele & Morris, 1999; Inglis, Martin & Morris 2013). To determine if the reason why rats with NMDA receptor blockade limited to the dorsal hippocampus could learn new information in the McDonald (2005) study was because of an intact ventral hippocampus NMDA receptor function, rats with full hippocampal NMDA receptor blockade manipulations were trained in the rapid acquisition version of the Morris water task. This study also sought to shed light on comparable studies of its kind, observing what alterations of learning and memory process occur following pharmacological blockade of the NMDA receptor spanning the entire hippocampus.

Rats were given 4 days of spatial pre-training, followed by NMDA receptor antagonist drug infusion 2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and mass spatial training to a new platform location. This was followed by a series of probe tests, one immediately after training to test the hypothesis that NMDA receptors are necessary for the acquisition of novel spatial information, and two others done 8 hours and 24 hours later to examine the effect of NMDA receptor blockade on short term memory consolidation. Based on previous results (McDonald et al, 2005), we hypothesized that rats should be able to acquire new information without the use of NMDA receptors, but that this information would not be consolidated and would disappear after a short period of time.

Methods

Subjects, Acclimation, and Handling

Subjects were male Long Evans rats aged 90 days upon arrival to the facility (n=16). The weight range at the start of the experiment was between 300 – 350g. They were housed in pairs and were kept on a 12h light/12h dark cycle with lights turning on at 7:30 and turning off at 19:30. The rats had *ad libitum* access to both water and food. Rats were allowed 7 days of acclimatization in their home cages to reduce stress induced from travel. After this period all rats were handled for 5 minutes a day for 5 days to familiarize them with the experimenters and being manipulated.

Training room/pool/ apparatus

The training apparatus was a large white circular fibreglass pool 46 cm in height and 127 cm in diameter. The pool was placed roughly in the centre of the room. The pool was filled with water low enough that the rats could not escape by climbing onto the walls but high enough that the rats could see the extra maze cues on the laboratory walls. Water was made non-transparent with non-toxic white paint. The pool was emptied, cleaned, and refilled with fresh water daily. The escape platform was located approximately 2 cm below the surface of the water. It was a white plastic circle 13 cm in diameter and made up approximately 1% of the total surface area of the pool. It had several small holes drilled into the surface to allow the rats to grip for balance. The platform was held down with a small weight to ensure that it did not move out of place between trials. Posters of simple colored geometric shapes (ex. Black square, red triangle) were placed on the walls

of the laboratory room to serve as visual cues along with the computer, experimenter, a large black shelf, and a visible door frame.

Surgery

Permanent guide cannulae were implanted bilaterally into both the dorsal and ventral hippocampi of all rats. Rats received subcutaneous injections of buprenorphine (Temgesic®, Schering-Plough, Hertfordshire, UK) at 0.03 mg/kg prior to surgery to avoid pain wind up and offer post-surgical analgesia. Rats were anesthetized using 4% isoflurane gas (Benson Medical Industries, Inc., ON, Canada) in oxygen with a flow of 1.5 l/min. Surgical anesthetic plane was maintained using 1–2% isoflurane throughout the surgery. The rats were positioned in a standard stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). An incision was made in the scalp, the skin retracted, and seven 0.7 mm holes were drilled into the skull. Three pilot holes were drilled for anchor screws (Small Parts, USA), and 4 holes for guide cannulae. Two 23-gauge stainless steel guide cannulae were lowered bilaterally into the dorsal (A/P -3.5, M/L: ±2.0, D/V:-3.2) and ventral (A/P:-5.8, M/L:±5.2, D/V:-6.0) hippocampus and were held in position using dental acrylic. The guide cannulae were plugged using 30-gauge wire obturators, which stayed inside until infusion. Following surgery, rats were injected with Metacam®, 5 mg/ml, 0.5 mg/kg (Buehringer Integelheim, Burlington, ON, Canada) and monitored for 24 h, then returned to their home cages.

Data collection and statistical analysis

Data was collected with a movement tracking software (Noldus Ethovision 3.1) and a ceiling mounted camera. Statistical analysis was performed using IBM SPSS

Statistic Version 22. Acquisition and probe test data was analyzed with two way repeated measures ANOVA. When a significant interaction occurred, planned post hoc pair wise comparisons were done between the pre- and mass trained quadrants on the trial 1 and immediate probes in experiment 1 as I expected differences to occur. Planned post hoc pairwise comparisons were done for the consolidation probes in experiment 1 comparing % time spent in the pre- vs mass trained quadrants. For the trial 1 probe in experiment 2, post hoc pairwise comparisons were done within groups with Bonferroni correction. For cell count data in experiment 3, post hoc pairwise comparisons were done between groups with Bonferroni correction.

Training/testing

The training procedure consisted of a three phase version of the Morris water task. All training and testing occurred in the same room and occurred between 0700 and 1200, except for phase 3 of experiment 1. Two cohorts of rats were run in this experiment, differing only in the amount of training and time between phases.

PHASE 1

Rats were brought into the testing room in individual cages on a wheeled cart and placed into the north-east corner of the room. Animals were run in squads of 4, one right after the other. For this phase, all rats were trained to find a hidden platform located in the south-west quadrant of the pool. Each rat was given 8 trials a day for 4 days, for a total of 32 trials. The starting position of each trial was randomly assigned to arbitrarily equidistant points labelled NE, SE, SW, and NW. (ex. NE, SW, NW, SE, SW, NE, NW, SW). The sequence of start points varied each day. The rat was placed in the pool at one

of the start positions facing the pool wall. Rats swam until they reached the hidden platform or until 60 seconds had elapsed. If after 60 seconds the rat had not found the platform it would be led to the platform by hand. After every trial the rat would be left on the platform for 10 seconds and then removed and placed back in its transport cage while the next rat was being trained. No drugs were administered during this phase of training. Each training session took approximately 30-40 minutes with an average inter-trial interval of 5 minutes.

PHASE 2

24 hours after completing phase 1 rats began phase 2 (day 5) of training. The platform was moved to the north-east quadrant, opposite to that of phase 1 pre-training. For cohort 1, training consisted of 16 trials within a two-hour period, all on day 5. For cohort 2, training was 20 trials. Similar to phase 1, rats were placed in the pool at one of the cardinal positions in random order, were allowed 60 seconds to find the platform, and remained on it for 10 seconds. The north-east starting point was eliminated during phase 2 because it was closest to the platform. Prior to training, rats were brought into a novel room and assigned to either the treatment group or a control group. The assignment to treatment groups was designed in such a way that there was no difference in the pre-training acquisition between pre-treatment groups. The treatment group (cohort 1:n=6, cohort 2:n=7) received bilateral dorsal and ventral intrahippocampal infusion CPP (0.32 ng/ μ l). The control group (cohort1:n=7, cohort 2:n=7) received artificial cerebral spinal fluid. Obturators were removed and infusions were done at a rate of 0.25 μ l/minute for 4 minutes, for a total of 1 μ l per infusion site. This dose is the same used in the McDoanld

et al (2005) study and is a dose of CPP that has been shown to impair spatial water maze performance in hippocampal injection (Riekkinen & Riekkinen, 1997).

The infusion cannulae were left inside the permanent guide cannula for an extra minute to allow for diffusion of the drug and to prevent any drug being pulled out with the infusion cannula when removed. After this 5 minute procedure, cleaned, new obturators were placed into the permanent guide cannula and rats were returned to their home cage. Training began 30 minutes after infusion.

For cohort 1, the platform was removed after the 16 massed training trials and a 30 second probe test was given. The interval between the last trial and the probe test was 5 minutes. Cohort 2 did not receive a probe test during this time. All training occurred within a two-hour period, a time frame that CPP has been shown to block prime-burst potentiation in the hippocampus (Kentros et al., 1998). The purpose of phase 2 is to determine whether rats can learn a new platform location while their NMDA receptors are blocked across the dorsal and ventral aspects of the hippocampus.

PHASE 3

8 hours after completing the phase 2 probe test, cohort 1 received a second probe test. This was done to determine if what was learned during mass training would be remembered. The rat was placed in the pool in the exact same way, in the same start location, as the prior probe test. After 30 seconds the rat was removed from the pool. Cohort 2 received a probe test 24 hours after completing phase 2. This difference in the two cohorts was used to examine potential differences in periods of consolidation.

Perfusion and Euthanization

The day after phase 3 the rats were euthanized with an intraperitoneal injection of Sodium Pentobarbitol (300mg/kg) and then transcardially perfused with 4% Paraformaldehyde solution and 5% phosphate buffered ACSF. The tissue was left in the 4% paraformaldehyde solution for 24 hours for cryoprotection, then placed into a 30% sucrose + 0.2% sodium azide solution for 5 days. Brain tissue was sliced on a freezing microtome and sections of the hippocampus were stained with a cresyl violet stain. Proper cannulation placement was analyzed and all subjects with cannulation points outside of the hippocampal formation were excluded from statistical analysis.

Results

Cohort 1

Pre-training

The measures of learning and memory used during acquisition were latency to find the platform and path length to the platform. These graphs represent the average of 4 trials across 4 days of training. The average latency of the first trial block was 35.8 seconds for controls and 42.1 seconds for pre-CPP rats. By the end of pre-training, the latency for controls on the last trial block was 4.7 seconds and for pre-CPP rats 4.1 seconds (fig. 1.1). Similarly, the average path length of the first trial block was 11.6 metres for controls and 17.1 metres for pre-CPP rats. By the end of pre-training, the path length for controls on the last trial block was 1.4 metres and for pre-CPP rats 1.3 metres (fig. 1.2). Two way repeated measures ANOVA showed that there was a significant effect of trial ($F_{7,77} = 63.307$, $p < 0.001$) on latency but no effect of group ($F_{1,11} = 0.016$, $P >$

0.05) and no interaction ($F_{7,77} = 1.571$, $P > 0.05$). There is a similar effect of trial on path length ($F_{7,77} = 29.344$, $P < 0.001$) with no effect of group ($F_{1,11} = 1.09$, $P > 0.05$) and no interaction ($F_{7,77} = 1.615$, $P > 0.05$). Over the 4-day pre-training period, all rats from both groups learned the platform position in the pool.

Mass training

Mass training was analyzed in two trial average blocks. Because the platform was in the opposite quadrant of the pool, the latencies and path lengths start high and decrease throughout training. The average latency of the first trial block was 25.6 seconds for ACSF infused controls and 42.4 seconds for CPP infused rats. Despite this difference at the start of mass training, by the end of mass training, the latency for ACSF infused controls on the last trial block was 6.3 seconds and for CPP infused rats 6.9 seconds (fig. 1.3). The average path length of the first trial block was 7.4 metres for ACSF infused controls and 14.9 metres for CPP infused rats. However, by the end of mass training this difference reduced to 2.0 metres ACSF infused controls and 2.3 metres for CPP infused rats (fig. 1.4).

Two way repeated measures ANOVA revealed a significant effect of trial on latency ($F_{7,77} = 22.829$, $P < 0.001$) but not of group ($F_{1,11} = 4.008$, $P > 0.05$) and no interaction ($F_{7,77} = 1.984$, $P > 0.05$). For Path length, there was a significant effect of trial ($F_{7,77} = 24.062$, $P < 0.001$) as well as group ($F_{1,11} = 8.34$, $P = 0.015$) and an interaction ($F_{7,77} = 4.294$, $P < 0.001$). With or without hippocampal NMDA receptor function, rats successfully learned a new platform position over a 2-hour training period.

Probes

To determine if the rats had successfully acquired a spatial memory during pre-training, as well as test the effects of CPP on the expression of already formed memories, the first trial of mass training was analyzed as a probe trial (fig. 1.5). Comparisons were made between the percentage of time spent in the target quadrant where rats were trained during pre-training and an average of the percentage of time spent in the other three quadrants. Because the platform was present during this trial, not all animals had equal latencies on trial one of mass training and so not all animals spent an equal amount of time searching within the pool. The percent of time spent in each quadrant given the total time each animal spent in the pool was used. ACSF infused controls spent an average 39.6 % search time in the target quadrant and an average of 20.1% in all other quadrants. CPP infused rats spent an average 37.8% search time in the target quadrant and an average of 20.7% in all other quadrants. Two-way repeated ANOVA revealed that there was a significant effect of quadrant ($F_{1,11} = 55.660, P < 0.001$) but not of group ($F_{1,11} = 0.247, P > 0.05$) and no interaction ($F_{1,11} = 0.227, P > 0.05$). These data show that rats had developed a spatial memory during pre-training and that CPP did not interfere with the expression of this memory.

After 16 trials of mass training to the new quadrant location, the platform was removed and rats were put through a 30 second probe test to determine if a new spatial preference had been learned (fig. 1.6). Comparisons were made between the percentage of time spent in the new target quadrant where rats were trained during mass training and an average of the percentage of time spent in the other three quadrants. ACSF infused controls spent an average of 42% in the new target quadrant and an average of 19.3% in

the all other quadrants. CPP infused rats spent an average of 36.1% in the new target quadrant and an average of 21.2% in all others. Two way repeated measures ANOVA revealed a significant effect of quadrant ($F_{1,11} = 52.568$, $P < 0.001$) but not of group ($F_{1,11} = 2.289$, $P > 0.05$) with no interaction ($F_{1,11} = 2.280$, $P > 0.05$).

Probe data was also analyzed comparing percentage of time spent in a small region surrounding the platform covering 2% of the total surface area of the pool, to contrast with the 25% surface area of the quadrant. This type of analysis provides information about the spatial specificity of what was learned during mass training, as the region of interest is limited to the area immediately surrounding the platform location. ACSF infused controls spent an average of 10.2% in the new target location and an average of 1.5% in the pre-trained location. CPP infused rats spent an average of 7.9% in the new target quadrant and an average of 2.2% in all others. (fig. 1.7). Two way repeated measures ANOVA revealed a significant effect of quadrant ($F_{2,22} = 51.402$, $P < 0.001$) but not of group ($F_{1,12} = 0.355$, $P > 0.05$) with no interaction ($F_{2,22} = 2.090$, $P > 0.05$).

Along side with this, first quadrant entered was also measured as a rough measure of heading direction. First quadrant entered is the first quadrant that rats swim to when placed into the pool during the probe. Rats were placed into the SW quadrant and so had two options, they could either enter the pretrained or the mass trained quadrant first. 6 out of 7 rats in the control group went to the mass trained quadrant first and 5 out of the 6 CPP rats went to the mass trained quadrant first (fig. 1.8). This indicates that the majority of rats in each group went to the mass trained quadrant immediately after being placed into the pool. Together, these results indicate that both groups had learned a spatial preference over mass training, they both went immediately to the quadrant where they

were trained and both displayed a high degree of spatial preference for the location of the platform.

Previous work has shown that hippocampal NMDA receptors may have a critical role in the consolidation of newly acquired memories (McDonald et al., 2005; Roesler et al., 2005). For cohort 1, a probe test was administered 8 hours after the end of mass training to determine if what was learned during mass training would remain or be forgotten due to a lack of consolidation.

The percentage of time spent in the two trained target quadrants, pre-training and mass training, as well as the average percentage of time spent in the other two non-trained quadrants, were compared within and between groups (fig.1.9). Two way repeated measures ANOVA, showed no effect of quadrant ($F_{2,22} = 2.043$, $P > 0.05$), no effect of group ($F_{1,11} = 11.373$, $P > 0.05$), but a significant group x quadrant interaction ($F_{2,22} = 7.256$, $P < 0.05$). Post hoc pairwise comparisons revealed a significant difference within the CPP group, rats spending more time in the pre-trained quadrant (avg = 37.35%) than in the mass trained quadrant (avg = 17.18%) ($P = 0.03$) No difference was found within the control group between any of the quadrants.

Probe data for the 8 hour consolidation probe was also analyzed comparing percentage of time spent in the 2% area surrounding the pre-trained and mass trained platform locations. ACSF infused controls spent an average of 4.8% in the mass trained location and an average of 1.3% in the pre-trained location. However, CPP infused rats spent an average of 2.3% in the pre-trained location and an average of 5.4% in the mass trained location. (Fig. 1.10). Two way repeated measures ANOVA revealed no significant effect of quadrant ($F_{2,24} = 1.029$, $P < 0.001$) and not of group ($F_{1,12} = 0.001$, $P > 0.05$).

However, there was a significant interaction ($F_{2,22} = 10.936$, $P < 0.001$). Post hoc pairwise comparisons revealed a significant difference within the CPP group, rats spending more time in the pre-trained location than in the mass trained location ($P = 0.012$). Control rats spent significantly more time in the mass trained location than in the pre-trained location ($P = 0.006$).

First quadrant entered was also measured for the 8 hour probe. as a rough measure of heading direction. 4 out of 7 rats in the control group went to the mass trained quadrant first and the other 3 went to the pre-trained quadrant first, indicating that this group displayed no preference in total for either of the quadrants immediately. All of the 6 CPP rats went to the pre-trained quadrant first (Fig. 1.11). Together, these results indicate that control rats had maintained some spatial preference for the mass trained platform location, but overall displayed no preference for either quadrant. CPP rat however displayed a strong preference for the pretrained quadrant as well as a preference for the pre-trained platform location. This all happened 8 hours after mass training, indicating that whatever was learned during mass training was not consolidated in the CPP rats.

Cohort 2

Pre-training

During pre-training the average latency of the first trial block was 44.0 seconds for controls and 44.4 seconds for pre-CPP rats. By the end of pre-training, the latency for controls on the last trial block was 7.4 seconds and for pre-CPP rats 6.8 seconds (fig. 1.12). Similarly, the average path length of the first trial block was 12.0 metres for controls and 14.2 metres for pre-CPP rats. By the end of pre-training, the path length for

controls on the last trial block was 2.1 metres and for pre-CPP rats 1.9 metres (fig. 1.13). Two way repeated measures ANOVA showed that there was a significant effect of trial ($F_{7,84} = 26.965$, $p < 0.001$) on latency but no effect of group ($F_{1,12} = 0.925$, $P > 0.05$) and no interaction ($F_{7,84} = 0.537$, $P > 0.05$). There is a similar effect of trial on path length ($F_{7,84} = 28.479$, $P < 0.001$) with no effect of group ($F_{1,12} = 0.051$, $P > 0.05$) and no interaction ($F_{7,84} = 1.151$, $P > 0.05$). Over the 4-day pre-training period, all rats from both groups learned the platform position in the pool.

Mass training

The average latency of the first trial block for mass training was 39.3 seconds for ACSF infused controls and 32.7 seconds for CPP infused rats. By the end of mass training, the latency for ACSF infused controls on the last trial block was 7.2 seconds and for CPP infused rats 9.8 seconds (fig. 1.13). The average path length of the first trial block was 11.8 metres for ACSF infused controls and 9.8 metres for CPP infused rats. However, by the end of mass training this difference reduced to 2.3 metres ACSF infused controls and 3.0 metres for CPP infused rats (fig. 1.15). Two way repeated measures ANOVA revealed a significant effect of trial on latency ($F_{9,108} = 17.898$, $P < 0.001$) but not of group ($F_{1,12} = 4.106$, $P > 0.05$) and no interaction ($F_{9,108} = 1.412$, $P > 0.05$). For Path length, there was a significant effect of trial ($F_{9,108} = 16.457$, $P < 0.001$) but no effect of group ($F_{1,12} = 4.387$, $P > 0.05$) and no interaction ($F_{9,108} = 1.519$, $P > 0.05$). With or without hippocampal NMDA receptor function, rats successfully learned a new platform position over a 2 hour training period.

Probes

For cohort 2, the probe test for memory consolidation was done 24 hours after completion of mass training. The percentage of time spent in the two trained target quadrants, pre-training and mass training, as well as the average percentage of time spent in the other two non-trained quadrants, were compared within and between groups (fig. 1.16). Two way repeated measures ANOVA, showed a significant effect of quadrant ($F_{2,24} = 4.408$, $P < 0.05$), a significant effect of group ($F_{1,12} = 7.681$, $P < 0.05$), and no group x quadrant interaction ($F_{2,24} = 1.366$, $P > 0.05$). Post hoc pairwise comparisons revealed a significant difference within the CPP group, rats spending more time in the pre-trained quadrant (avg = 33.1%) than in the mass trained quadrant (avg = 18.1%) ($P = 0.05$) No differences were found within any of the control group percentages or between groups. These probe results are similar to those found in cohort 1 and indicate that despite rats learning a new spatial position during mass training, CPP rats reversed their preference while controls did not maintain a preference over 24 hours. This implicates NMDA receptors in the consolidation of spatial memory.

Discussion

In this experiment, rats with bilateral dorsal and ventral cannulations (fig. 1.17A-D) were pre-trained on the spatial version of the Morris water task. After 4 days of pre-training, they were given either the NMDA receptor antagonist CPP or ACSF via intra-hippocampal infusion and mass trained to a new platform location in the task. Three probe tests were done across 2 cohorts. The first was immediately after mass training to assess new learning, and the second was done 8 hours later, and the third 24 hours later, to assess consolidation. There were no significant differences between the two groups during the pre-training phase. For the measures of both latency and swim path length, all

animals started relatively high, and by the end of training significantly reduced their swim times and path lengths.

After intra-hippocampal drug infusion, rats were mass trained to a reversal platform position in the same pool and room. The first trial of this mass training revealed a significant preference for the quadrant where the platform was located during pre-training. This spatial preference shows that the pre-training was sufficient to induce a spatial memory in the animals. It also shows that NMDA receptor blockade does not interfere with previously acquired spatial memories. During the first trial of mass training, both groups latency to find the platform and swim path length drastically increase from their final pre-training measures. This is because the platform is located in a novel position in the pool. Over the course of training, swim path length and latency significantly decrease for both groups. The probe test done immediately after mass training revealed a significant spatial preference for the quadrant that the new platform was located in compared to all others. All groups performed equally well on this probe test.

However, when tested again on a second probe test 8 hours later or 24 hours later, the spatial preference in CPP administered rats reversed back to the pre-training location. CPP rats spent significantly more time in the quadrant where the pre-training platform was located, despite having learned a novel location as indicated by the acquisition curve and probe results 8-24 hours earlier. The control animals in contrast, still spent more time in the mass trained quadrant during the 8 or 24 hour retention probes, but this preference was not statistically significant.

These results provide three insights into the role of NMDA receptors in learning and memory. Firstly, NMDA receptors are not necessary for the expression of previously acquired memories, and the blockade of NMDA receptors does not extinguish or alter previously acquired memories. This effect is in line with most prior research showing NMDA receptor independent memory expression (Matus-Amat et al., 2007; Kim et al., 1991; Shapiro & Caramanos, 1990). NMDA receptor blockade will interfere with types of neural activity associated with plasticity, such as LTP and LTD, yet leave basal neural activity unaltered. Second, NMDA receptors are not necessary for the rapid acquisition of novel information. Despite being under pharmacological NMDA receptor blockade, rats learned the new platform position just as well as controls did in the rapid acquisition water task. This effect has been explored in different ways in the past (Bannerman et al., 1995, Inglis, Martin & Morris, 2013; Otnæss, Brun, Moser & Moser, 1999) and complements the previous work done in our lab (McDonald et al., 2005; Holahan et al., 2005).

Third, NMDA receptors have a role in the consolidation of recently acquired memory. Santini, Muller & Quirk (2001) observed that during extinction of conditioned fear and bar pressing responses, rats could successfully extinguish these memories under the effects of CPP, but could not recall extinction when tested 24 hours later. In a dry-land version of the water task, Kesner & Dakis (1995) injected Phenylciclidine directly into the dentate gyrus of rats. Using multiple training trials per day, over multiple training days, they observed that rats could learn over many trials within a day. However, performance would always return back to its initial level the next day, and learning over the course of several days was impaired. Regarding hippocampal spatial processes, the

formation of place cells has been found to form independent of NMDA receptor function, while its long-term maintenance is impaired without them (Kentros et al., 1998). These results and the results of Experiment 1 supports the idea that rapid memory acquisition in the hippocampus may be an NMDA receptor independent process while its consolidation or maintenance may be NMDA receptor dependent.

This may explain the effect of NMDA receptor antagonism on the ability of rats to learn tasks which require multiple training days such as the Morris water task (Morris, 1989; Davis, Butcher & Morris 1992; Abraham & Mason, 1988). Most of the past research using this task involves several training days and it is possible that the learning impairments reported are actually impairments in memory consolidation. If rats cannot consolidate what they have learned after each training session, then they would never be able to learn over several days. Similarly, if individual learning trials are sufficiently spaced out over time, a lack of consolidation may also appear to be a lack of learning. For example, in a delay matching-to-place (DMP) version of the water task, Steele & Morris (1999) gave intracerebroventricular infusions of APV to rats. Utilizing a pre-training procedure, when rats were given the drug for the DMP training, they were impaired when ITI's were 20 minutes or 2 hours, but not when they were 15 seconds. In other words, when training trials are spaced close together the drug had no effect on learning and only when they were spaced relatively far apart was learning impaired. In our experiment the ITI averaged 2-4 minutes during mass training.

However, pre-training can allow for rats to learn new spatial information in the presence of NMDA receptor blockers, even over the course of several days (Bannerman et al., 1995, Saucier & Cain, 1995, Inglis Martin & Morris, 2013), as well as rapidly in

our experiment. Many possible explanations exist for this effect. During the pre-training phase, rats learn to be procedurally proficient at the water task. This phase of training can be said to be NMDA receptor dependent because receptor antagonists impair novel task acquisition (Morris, 1989; Davis, Butcher & Morris 1992; Abraham & Mason, 1988). It is possible that all NMDA receptor dependent learning processes occur during the pre-training phase, and that mass reversal training does not require new learning that is NMDA receptor dependent.

What exactly is learned during the pre-training phase may also influence its ability to rescue drug induced learning impairments later on. A “spatial strategy” account of pre-training is that what is learned is how to use environmental cues as a means of navigation (Bannerman, Rawlins & Good, 2006). If rats learn a spatial strategy during pre-training, then they can simply employ this previously acquired strategy during later training without drug-induced impairment, since NMDA receptors are not necessary for memory expression. Different types of pre-training have been used before to examine this hypothesis. Hoh (1999) trained rats to learn novel spatial search strategies under NMDA receptor blockade, showing that even if rats could utilize previously learned strategies during later training, the strategies themselves could be learned even without NMDA receptor function. Bannerman et al., (1995) used non-spatial pre-training in one of their cohorts. A black curtain surrounded the pool during the pre-training phase. APV impaired these rats in subsequent spatial water task learning whereas rats pre-trained in the spatial task, or given ASCF, were not. However, the use of a curtain also prevents the formation of a coherent spatial representation of the environment as all the features of the room are occluded. Instead of a spatial strategy being learned, it is possible that hippocampal

NMDA receptors are necessary for rapidly forming a spatial representation of the testing room, and that this is what is learned during pre-training. This might allow for new platform locations to be learned independent of NMDA receptor function as long as it occurs in the same spatial context. The processes and biochemical mechanisms behind learning a new spatial location in the same context is still up for debate. Past experiments have observed no learning impairment in novel contexts (Bannerman et al., 1995) and learning impairments in the same context (Morris, Davis & Butcher, 1990). Inglis, Martin & Morris (2013) propose the possibility that spatial learning can be supported by NMDA receptor dependent plasticity that occurs in the cortex when hippocampal plasticity is not involved. In this account, all learning is molecularly the same, but that different stages of training depend on different brain regions, namely, the hippocampus for novel spatial memory (pre-training), and the cortex for utilizing this information in very similar learning situations (mass training). Given the number of experiments relying on intracerebroventricular or intraperitoneal injection methods, this explanation seems unlikely as cortical NMDA receptors would also be blocked along with hippocampal ones (McDonald et al., 2005, Hoh et a 1999, Bannerman et al., 1995).

One aspect of this idea is intriguing though and that is the potential role of NMDA receptor in rapidly acquiring a global spatial representation when first exposed to a new environment but once formed NMDA receptor function is not required to use that representation to learn to navigate to any location within that environment. These explanations may provide an account for why the rats in our experiment where able to rapidly learn a new spatial location in the same spatial environment without the use of NMDA receptors: The mass reversal training occurs in the same environment as pre-

training, utilizes the same type of spatial search strategy, and occurs rapidly with a very low ITI. Despite this, the relationship of NMDA receptors to novel versus same environment learning and to that of consolidation is still unclear at this time. The hypothesis of an NMDA receptor dependent formation of a spatial representation of a novel environment will be explored in the next experiment.

Chapter 3

The effects of full hippocampal NMDA receptor blockade on spatial learning across environments in the Morris water task

In the first experiment, rats were capable of learning new spatial information in a water task with their NMDA receptors blocked across the dorsal and ventral hippocampus. Although the new platform location was learned as evidenced by the acquisition learning curve and spatial preference in the probe test results, this spatial preference was quickly lost. When tested for this preference again 8 hours or 24 hours later, the rats whose NMDA receptors were blocked during mass training showed a spatial preference to a previously learned position, while the control animals did not. These results implicate NMDA receptors in the consolidation of newly acquired memories, even though the acquisition of that memory may occur independent of NMDA receptor function.

One possible explanation for the occurrence of NMDA receptor independent learning during rapid acquisition of a new platform location in the same room may be that most of the learning that is actually NMDA receptor dependent has already occurred during prior training. A lot of information needs to be learned for successful navigation of the Morris water task (location, direction, cue orientation, functional swimming, platform stability, task strategy, reinforcement contingencies, etc.) and this information is all learned during a pre-training phase. The purpose of pre-training is to create a situation in which the only thing that is required for an animal to learn during mass training is a new spatial location in the same pool, in the same room. NMDA receptors can be blocked during the mass training phase of the experiment to assess their involvement in spatial

learning. However, if all of the NMDA receptor dependent learning occurs during pre-training, then new location training may simply involve using the already learned information to complete the task, with no actual hippocampal NMDA receptor dependent plasticity required.

Many explanations as to what aspects of an initial learning experience are NMDA receptor dependent have been proposed for the hippocampus. Things like behavioral search strategies (Hoh et al, 1999), contextual setup (Taylor et al., 2010) or a region specific change in NMDA receptor plasticity depending on task parameters (Inglis, Martin & Morris, 2013). NMDA receptor antagonists have been shown to interfere with neural plasticity associated with learning, but not basal level activity associated with memory recall and behavioral expression (Bast, Silva & Morris, 2005). Rapid acquisition of a new location in an environment in which rats had already learned a spatial location may be such that it is functionally different from novel spatial learning, and so may involve a different set of neural circuits or molecular mechanisms.

If a behavioral search strategy account is taken, then rats learn how to find the platform during pre-training, and this same strategy can be used during drug administered reversal training without impairment. Saucier & Cain (1995) provided non-spatial pre-training to rats that later received a NMDA receptors antagonist during spatial training and performed as well as controls. Hoh et al., (1999) successfully pre-trained rats during receptor blockade as well as during later training. The ability of rats to learn search strategies without NMDA receptor use, as well as perform on spatial versions of the water task despite non-spatial pre-training counters the behavioral strategy account.

One proposed hypothesis is that all spatial learning is NMDA receptor dependent but where in the brain this plasticity occurs changes throughout training. Initial learning of novel spatial information will take place in the hippocampus, and subsequent learning involving the use of this previously learned information will rely on the neocortex. This would explain why when NMDA receptor antagonists are injected directly into the hippocampus, they produce learning impairments in novel tasks, but not when animals have previously learned the task. (Inglis, Martin & Morris, 2013) This has been called the “pre-training effect”. However, this hippocampal-to-cortical switch hypothesis does not account for when global receptor inactivation produces similar results, such as in Bannerman et al., (1995) or McDonald et al., (2005). During i.c.v. or i.p. injection, cortical NMDA receptors would be blocked as well as hippocampal ones.

A third account is that of spatial representation of novel environments. This is the idea that what is learned during pre-training is the layout of the training environment, i.e., useful information about the environment that can be used for navigation, such as proximal and distal cues, pool shape, and room geometry. In a series of experiments using fear conditioning to context, Taylor et al., (2010) showed that learning about environmental cues could be made NMDA receptor independent if it was similar enough to previously learned ones. The more dissimilar a novel training environment is from previously experienced ones, the more it required NMDA receptors to be learned. However, pre-training has been shown to allow new spatial learning in novel environments such as in Bannerman et al., (1995) as well as its replication Inglis, Martin & Morris (2013) making conclusions about NMDA receptor dependent learning less straight forward.

The spatial environment account will be explored in this experiment. Rats were pre-trained over 4 days in room A in a spatial version water task. After procedural proficiency and learning of the initial platform position, mass training under pharmacological NMDA receptor blockade limited to the dorsal and ventral hippocampus was performed in room B. Probe tests were completed immediately after and 24 hours after mass training to assess memory formation. Much of the procedures were identical to that of experiment 1, the only difference being a change in training rooms across training phases.

If the reason why rats were able to rapidly learn the new platform location in the same room in experiment 1 was because of previously acquired, NMDA receptor dependent spatial information during pre-training, then providing novel environmental information during blockade should result in learning impairments.

Methods

In the multiple room version of the rapid acquisition water task procedure, animals were pre-trained in room A: a separate room on the other end of the facility, with distinct extra maze cues and relationships between constant inter-room cues. These included the door, computer, and experimenter. This training consisted of 4 days of training, 8 trials a day, to the same platform location each day, similar to experiment 1. For the second phase of training, animals were mass trained to a new platform location in room B over 2 hours. Room B was the same room used in experiment 1. The animals were also brought to the room in a different style of transport cage between pre-training and mass training phase. The drug administration process was identical to experiment 1,

either CPP or ASCF was infused bilaterally into the both the dorsal and ventral hippocampus 30 minutes prior to mass training in room B.

Two cohorts of rats were trained on this task. For the cohort 1 (n=13), mass training consisted of 20 trials in Room B. In cohort 2 (n=11), mass training was only 16 trials. Trial one of mass training was analyzed in a similar way as experiment 1 to assess if spatial pre-training in room A had any effects on novel spatial navigation. Previous work from our lab has shown that some aspects of spatial navigation can transfer between training environments (Clark et al., 2015). A 30 second probe test was run 24 hours after mass training to determine if what was learned during mass training was consolidated into long term memory. In experiment 1, control rats did not maintain a spatial preference for very long, as evidenced by a probe test run 8 hours after mass training. This may be due to the two different memories for trained platform locations competing with each other, not allowing for a preference to be maintained. In a novel environment, only 1 platform location is learned and so training may reliably form a stable spatial memory. The second cohort (n=11) received a probe test immediately after mass training to assess whether mass training induced a spatial preference.

Surgical procedure, data and statistical analysis, and histology was identical to that of experiment 1.

Results

Cohort 1

Pre-training

For all training procedures, Latency to find the platform and path length were used as measures of learning and memory. The average latency of the first trial block was 41.6

seconds for controls and 48.1 seconds for pre-CPP rats. By the end of pre-training, the latency for controls on the last trial block was 6.7 seconds and for pre-CPP rats 7.2 seconds (fig. 2.1). Similarly, the average path length of the first trial block was 10.7 metres for controls and 13.9 metres for pre-CPP rats. By the end of pre-training, the path length for controls on the last trial block was 1.8 metres and for pre-CPP rats 1.9 metres (fig. 2.2). Two way repeated measures ANOVA showed that there was a significant effect of trial ($F_{7,77} = 47.696$, $p < 0.001$) on latency but no effect of group ($F_{1,11} = 0.423$, $P > 0.05$) and no interaction ($F_{7,77} = 0.587$, $P > 0.05$). There is a similar effect of trial on path length ($F_{7,77} = 43.133$, $P < 0.001$) with no effect of group ($F_{1,11} = 1.260$, $P > 0.05$) and no interaction ($F_{7,77} = 1.046$, $P > 0.05$). This means that all rats from each group successfully learned the platform position over the 4 days of training and that no group differences were present.

Mass training

Mass training was analyzed in two-trial average blocks. The average latency of the first trial block was 28.5 seconds for ACSF infused controls and 24.3 seconds for CPP infused rats. By the end of mass training, the latency for ACSF infused controls on the last trial block was 6.0 seconds and for CPP infused rats, latency remained high at 20.4 seconds (fig. 2.3). The average path length of the first trial block was 7.9 metres for ACSF infused controls and 8.2 metres for CPP infused rats. However, by the end of mass training, ACSF infused controls swam on average 1.5 metres whereas CPP infused rats swam on average 6.2 metres (fig. 2.4).

Two way repeated measures ANOVA revealed a significant effect of trial on latency ($F_{9,99} = 4.192$, $P < 0.001$) and of group ($F_{1,11} = 11.5$, $P < 0.05$) but no interaction

($F_{9,99} = 1.842$, $P > 0.05$). For Path length, there was a significant effect of trial ($F_{9,99} = 4.084$, $P < 0.001$) as well as group ($F_{1,11} = 12.499$, $P = 0.005$) but no interaction ($F_{9,99} = 1.850$, $P > 0.05$). This means that control rats successfully learned over the course of mass training whereas rats given CPP did not.

Probes

To examine if spatial pre-training in Room A effects spatial training in Room B, trial 1 of mass training was analyzed as a probe. The percent of time spent in each quadrant given the total time each animal spent in the pool was measured (fig. 2.5). Two-way repeated ANOVA revealed that there was a significant effect of quadrant ($F_{3,33} = 9.606$, $P < 0.001$) but not of group ($F_{1,11} = 0.035$, $P > 0.05$) and no interaction ($F_{3,33} = 0.701$, $P > 0.05$). Within groups post hoc pairwise comparisons (LSD) revealed significant differences between percentage swim time spent in some of the quadrants. The only quadrant in either group that was significantly different from the others was quadrant 2. The amount of time spent in quadrant 2 significantly differed from all other quadrants ($P < 0.05$) for the CPP rats. In the controls, amount of time spent in quadrant 2 different from quadrant 1 ($p < 0.05$) but not quadrant 3 or 4. This probe indicated that despite NMDA receptor blockade, rats expressed a spatial preference when they first entered the pool in room B. An possible explanation of this effect will be provided in the discussion,

For the 24 hour probe, the percentage time swam in the quadrant where the platform was located during mass training was compared to an average of the other three quadrants (fig. 2.6). ANOVA revealed an effect of quadrant ($F_{1,11} = 8.565$, $P < 0.05$) but not of group ($F_{1,11} = 0.414$, $P > 0.05$). and no interaction ($F_{1,11} = 0.074$, $P > 0.05$). These

results show that mass training in room B did not produce a spatial preference that lasts past 24 hours.

Cohort 2

Pre-training

The average latency of the first trial block was 47.4 seconds for controls and 41.9 seconds for pre-CPP rats. By the end of pre-training, the latency for controls on the last trial block was 7.5 seconds and for pre-CPP rats 6.3 seconds (fig. 2.7). Similarly, the average path length of the first trial block was 14.0 metres for controls and 10.8 metres for pre-CPP rats. By the end of pre-training, the path length for controls on the last trial block was 2.0 metres and for pre-CPP rats 1.6 metres (fig. 2.8). Two way repeated measures ANOVA showed that there was a significant effect of trial ($F_{7,63} = 39.969$, $p < 0.001$) on latency but no effect of group ($F_{1,9} = 0.704$, $P > 0.05$) and no interaction ($F_{7,63} = 0.704$, $P > 0.05$). There is a similar effect of trial on path length ($F_{7,63} = 40.523$, $P < 0.001$) with no effect of group ($F_{1,9} = 0.843$, $P > 0.05$) and no interaction ($F_{7,63} = 1.070$, $P > 0.05$). Similar to cohort 1, both groups of rats successfully learned the platform location over the course of pre-training.

Mass training

For mass training, the average latency of the first trial block was 23.9 seconds for ACSF infused controls and 27.8 seconds for CPP infused rats. By the end of mass training, the latency for ACSF infused controls on the last trial block was 3.9 seconds and for CPP infused rats, latency remained relatively high at 25.3 seconds (fig. 2.9) The average path length of the first trial block was 6.5 metres for ACSF infused controls and

8.5 metres for CPP infused rats. However, by the end of mass training, ACSF infused controls swam on average 1.4 metres whereas CPP infused rats swam on average 8.4 metres (fig. 2.10).

Two way repeated measures ANOVA revealed a significant effect of trial on latency ($F_{7,63} = 3.599$, $P < 0.05$) and of group ($F_{1,9} = 19.872$, $P < 0.05$) but no interaction ($F_{7,63} = 1.856$, $P > 0.05$). For Path length, there was a significant effect of trial ($F_{7,63} = 2.381$, $P < 0.05$) as well as group ($F_{1,9} = 19.220$, $P < 0.05$) but no interaction ($F_{7,63} = 1.400$, $P > 0.05$). This means that rats given CPP did not learn the platform location over the course of training compared to the control rats.

Probes

For the probe test run immediately after mass training, no significant effects occurred on any of the factors. However, post hoc within group pairwise comparisons (LSD) revealed a significant effect for the control group ($p < 0.05$), indicating that they spent more time in the target quadrant, whereas the CPP group (fig. 2.11) did not. Taken together with the mass training results, this pattern of effects suggests that NMDA receptor blockade impairs rats' ability to learn a spatial location when the available environmental information is novel.

Discussion

When rats were given CPP directly into their dorsal and ventral hippocampus (fig. 2.12A-D), they were incapable of acquiring novel spatial information in room B, despite spatial pre-training in room A. Both latency to find the platform and path length were significantly higher in the NMDA receptor blockade group than in the ACSF control

group. Probe tests revealed that controls acquired a spatial preference for the pool quadrant where the platform was located, CPP rats did not. This contrasts with experiment 1 in which rats were capable of temporarily acquiring new spatial information under hippocampal NMDA receptor blockade in the same room as pre-training. The only factor that differed from these two experiments was the spatial information available to the rats during mass training.

The results of experiment 2 indicate that hippocampal NMDA receptors may be necessary for the acquisition of spatial information during water task training (the arrangement and type of extra-maze cues, room and maze geometry). Presumably, this environmental information would be learned during pre-training in room A. Once this has been learned however, hippocampal NMDA receptors would not be necessary for using this same information in learning new things, such as learning to navigate to a new platform location. When brought to a novel room however, some of the environmental information that was learned during pre-training would not be available to the animal. The task is fundamentally the same, just with a different set of extra-maze cues and overall room shape. In this version of the task rats without hippocampal NMDA receptor function could not perform at the level of controls, indicating their importance in novel spatial learning.

When brought to training room B, both CPP and ASCF controls displayed a spatial preference for one of the pool quadrants during trial 1 of mass training, despite having never been in room B before. This means that some information learned during pre-training in room A carried over to room B. This also provides further evidence that NMDA receptor blockade does not interfere with previously acquired memories. It has

been shown that some components of spatial navigation, namely heading direction, can be transferred between spatially distinct environments (Clark et al., 2015, Dudchenko & Zinyuk, 2005). Head direction cell orientation is often controlled by environmental boundaries and maze shape (Clark, Harris & Taube 2012; Hamilton, Akers, Weisend & Sutherland, 2007). Indeed, in this experiment the overall shape of the testing rooms are similar, but opposite in orientation and different in dimension, (room A: width: 10 ft., length: 20 ft.; Room B: width: 10 ft., length: 15 ft.) as well as using an identical water task pool. Based on Clark et al., (2015), which used the exact same rooms and pools as in this experiment, although with different extra-maze cues, it is very possible that head direction information from room A influenced trial 1 of mass training in room B. Despite this initial effect, it was not maintained throughout training, and rats treated with CPP failed to learn the new platform location.

Changing rooms, thereby changing the environmental information available to be used during learning and memory tasks with NMDA receptor blockade in the hippocampus has been done before, and our work directly conflicts with some of this work, and compliments others. In 1995, Bannerman et al., spatially pre-trained rats in the water task in one room, and then spatially trained these same rats in a novel room with chronic infusion pumps and APV. However, in their experiment, training took place over 8 days, with 1 trial per day. Similarly, in a replication study done by Inglis, Martin & Morris (2013), dorsal hippocampal infusions of APV resulted in no impairment in novel environment water task training, yet impaired LTP. Our work here shows the opposite, that pre-training cannot save learning impairments when performed in a novel

environment. The experiments above utilized training protocols over several days whereas ours was a rapid single session acquisition.

Taylor et al., (2010) has shown that in a context fear conditioning paradigm, rats previously trained to fear one environment can be trained to fear a novel environment without the use of hippocampal NMDA receptors. This effect was only seen when the two training environments were very similar. If they were made to be very distinct, prior training did not make subsequent learning NMDA receptor independent, implicating their role in learning environmental cues. Interestingly, it was also found that NMDA receptor independent learning occurred in the hippocampus, and that the expression of Arc protein depended on the similarity of the two training environments. In similar experiments, Lee & Kim (1998) have shown that context fear pre-training does not make subsequent novel fear training NMDA receptor independent. They also showed that learning tone-shock, or light –shock pairings was also shown to be NMDA receptor dependent.

Given the variability in results from different experiments, the role of hippocampal NMDA receptors in spatial learning is uncertain. The data from our experiment is in line with the traditional view of hippocampal NMDA receptors. Namely, that they have a critical role in the acquisition of spatial information. However, the aspect of spatial learning found to be dependent on hippocampal NMDA receptors was learning about the features of the external environment to guide spatial navigation. They may still have a role in memory consolidation though. Evidence from experiment 1 also indicates NMDA receptors role in the consolidation of spatial memories as well. These findings do not discount the behavioral strategy or cortical plasticity accounts of the pre-training effect, since those accounts were not explored in this experiment. It is clear that reversal

learning in using the previously acquired information and novel learning are different phenomena, involving different task requirements because of the differences in available information. Indeed, the effects of environmental information on reversal learning, inhibition, and extinction have been studied for decades in associative learning paradigms (Bouton, 1993; McDonald, Ko & Hong, 2002). It may be the case that because same room reversal and novel room acquisition depend on different types of information, they will potentially depend on different neural mechanisms, either molecular, or regional. If NMDA receptors are necessary for the consolidation of reversal learning information but the acquisition of novel information, then it is possible that the ultimate function of hippocampal NMDA receptors, as well as what hippocampal plasticity represents mnemonically, is dependent on the type of behavior to be learned and the environmental information available to the animal.

In the next experiment, we examined the effect of NMDA receptor blockade and spatial learning on the expression of Arc protein in the hippocampus.

Chapter 4

The effect of NMDA receptor dependent learning behaviors on Arc expression in the hippocampus

In experiments 1 and 2, the effects of hippocampal specific NMDA receptor blockade on spatial learning was examined. In this final experiment, I set out to determine if there are observable molecular, neurobiological changes in the hippocampus that are associated with the behavioral effects seen in those experiments. The type of impairment seen in experiment 1, in which NMDA receptors were blocked during rapid acquisition of a new spatial position in the same environment, was the consolidation of long term memory formation and not encoding or short-term retention. Accordingly, I chose to examine the immediate early gene (IEG) Activity related cytoskeletal protein (Arc) as it has been implicated in long-term memory processes (Czerniawski et al., 2011).

First described by Lyford et al., (1995), Arc is an IEG, a gene whose mRNA is rapidly increased by high levels of neural activity, with many IEGs being activated by LTP in particular. Arc mRNA is delivered to dendrites of heavily active synapses, implicating its use in synaptic changes associated with plasticity and learning. Expression of Arc in the hippocampus has been reported to be enhanced following novel spatial learning in both the dorsal and ventral segments (Guzowski, Setlow, Wagner & McHugh, 2001; Czerniawski et al., 2011). The NMDA receptor has been heavily implicated in the activation of Arc, with the same type of LTP that is NMDA receptor dependent inducing ARC expression (Lyford et al., 1995). This was later reinforced by Steward & Worley (2001), showing that NMDA receptors are necessary for Arc trafficking and expression in dendrites. Administering Arc antisense oligodeoxynucleotides interferes with both

learning and memory behaviors as well as the molecular processes associated with them (Guzowski et al., 2000; Ploski et al., 2008). Similar effects have been observed with Arc genetic knockout animals (Plath et al., 2006).

IEGs are thought to mediate the molecular consolidation of memory because of their time course of action and the types of subsequent molecular mechanisms they induce. It is proposed that there are two general processes involved in the molecular formation of memory. The first utilizes transient increases in local calcium resulting in the short term heightened reactivity of synapses and shorter forms of LTP. The second involves protein synthesis via genetic activation, namely an increase in immediate early gene protein synthesis resulting in synaptic membrane AMPA receptor insertion. Because of the time involved in gene transcription and translation, and molecular trafficking, it is believed that these latter processes are critical for memory consolidation, and not its initial formation. (Steward & Worley, 2002)

Stable LTP requires structural changes of the synapse relying on F-actin and protein synthesis. When Arc antisense is applied to tissue to disrupt LTP, this disruption can be reversed by F-actin stabilizing drugs. Reciprocally, F-actin is necessary for the localization of Arc at synapses (Haung, Chotiner & Steward, 2007). Arc has also been shown to be critically related to AMPA receptor trafficking, a process by which new AMPA receptors are brought to and inserted in the post synaptic membrane. This process is thought to be one of the primary mechanisms by which the heightened calcium influx seen in LTP is maintained. (Chowdhury et al., 2006)

When applied to hippocampal tissue, Arc antisense will allow for the induction of LTP, yet its long-term maintenance will disappear (Guzowski, Setlow, Wagner &

McHugh, 2001). Knockout mice similarly express heightened early phase LTP and reduced late phase LTP (Plath et al., 2006). In a fear conditioning paradigm, Czerniawski et al., (2011) showed that an NMDA receptor antagonist could prevent arc expression in both the dorsal and ventral hippocampus and that arc antisense would impair memory consolidation but spare initial learning, linking NMDA receptors, Arc, and memory consolidation. Taken together, these electrophysiological, molecular, and behavioral data, all suggest a role for IEGs, and NMDA receptor dependent Arc in particular, in the long-term consolidation of memory.

This link between NMDA receptors and Arc protein with regards to molecular mechanisms of plasticity and learning behaviors is further explored in this experiment. These two molecules' involvement in the consolidation of activity induced synaptic changes is exactly the type of process we think is involved in experiment 1, in which the consolidation of newly acquired memories was impaired by NMDA receptor antagonism. In the present experiment, rats were trained in two versions of the water task: one involving rapid mass training in the same spatial environment as pre-training similar to experiment 1, and the other rapid mass training in a novel spatial environment separate from pre-training, similar to experiment 2. The NMDA receptor antagonist CPP was injected into the dorsal and ventral hippocampus of rats trained to swim to a hidden platform in a novel context. After training, levels of Arc protein expression throughout the hippocampus were measured and compare between groups. In our previous experiment, animals could not learn novel spatial information without NMDA receptors but could learn a new platform location using previously learned environmental cues. Because of these results, we hypothesized that Arc expression would be much higher after

novel spatial learning compared to reversal learning in the same spatial environment, and that NMDA receptor blockade would simultaneously impair learning behaviors and reduce Arc protein expression.

Methods

Behavioral training and testing was very similar to that of experiments 1 and 2. Pre-training was done in context A for all groups except cage controls. There were four groups in this experiment, differing only in how their mass training was done in reference to pre-training: Same room, New room, New room + CPP, and cage controls ($n=5$). For the Same room group ($n=5$), mass reversal training consisted of 16 trials and was performed identically to experiment 1. For the New room group ($n=5$) and New room + CPP group ($n=5$) mass training consisted of 16 trials and was identical to experiment 2. Drug infusion for the New room + CPP rats was done 30 minutes before mass training and was performed identical to the CPP group in experiment 2. The rats were run one at a time during mass training to avoid variation in timing of procedures. After completing mass training, rats were brought back to their home cage until perfusion. Perfusion occurred approximately 70-80 minutes after trial 8 of mass training. This is a time period in which IEG protein expression after learning is active (Lonergan, Gafford, Jerome & Helmstetter, 2010). Euthanization and perfusion procedures were identical to experiments 1 and 2.

Tissue collection and immunohistochemistry

After extraction, the brains were placed in 4% PFA solution for 24 hours, at which point they were then placed in a 30 % sucrose + sodium Azide solution for a 3 day period

of cryoprotection. The brains were then sliced on a freezing microtome in 12 series, to include the entire hippocampal formation. Tissue was stained using a 3 day immunohistochemical protocol that fluorescently stained Arc protein.

On day 1, tissue was pre-washed in 1% PBS solution, 3 x 10 minutes. Tissue was then placed in 2 ml of a 0.3 % triton and PBS solution with a 1:1000 ratio of primary antibody. For Arc protein staining, Arc (c-7) sc-17839 mouse monoclonal IgG (Santa Cruz Biotechnologies) was used. The tissue well was placed on a rotating belly dancer and left to incubate for 24 hours. On day 2, the tissue went through a second wash in 1% PBS solution, 3 x 7 minutes. After the wash, the tissue was placed in a 1:500 solution of PBS and Alexa fluor 488 donkey antimouse IgG (H+L) (Thermo Fisher Scientific). The tissue well was then covered in tin foil to prevent light from affecting the staining process. The well was placed back on the belly dancer and the room light turned off and left for a second 24 hour incubation period. On day 3, the tissue was put through another 1% PBS wash, 3 x 7 minutes and then wet mounted onto 1 % gelatin and 0.2 % chromatin coated slides. The slides were covered and placed in a refrigerator at 3° Celsius for 24 hours.

Microscopy and cell counts

Cell counts were done on a Zeiss AxioImager M1 microscope (Zeiss, Jena, Germany) using the program Stereo Investigator® (MicroBrightField Inc., 2013, Version 10). A constant light intensity exposure was set at 50%. Light was projected through a FITC filter. A 20X magnification was used when counting cells. All labelled granule cells were individually counted in the granule cell layer of the dentate gyrus. All labelled pyramidal cells were individually counted in the pyramidal cell layers of areas CA1 and

CA3. Cell counts in each hippocampal slice for each animal were summed and multiplied by 12 (12 series sections were sliced) to get an approximation of total cell number in each region. Tissue photographs were taken and colored with ImageJ® (ImageJ, R. W., & US National Institutes of Health. (2012). Bethesda, Maryland, USA.)

Results

Water task

The measures of learning and memory used during acquisition were latency to find the platform and path length to the platform. These graphs represent groups of 4 trial averages across 4 days of training for the 3 groups that did water task training. The average latency of the first trial block was 48.0 seconds for Same room group, 41.9 seconds for the new room group, and 30.2 seconds for the New+CPP group. By the end of pre-training, the latency for the Same room group on the last trial block was 7.3 seconds, 6.3 seconds for New room group, and 5.2 seconds and for New+CPP group (fig. 3.1). Similarly, the average path length of the first trial block was 13.7 metre for Same room group, 10.8 metres for New room group and 9.0 metres for New+CPP group. By the end of pre-training, the path length for controls on the last trial block was 1.8 metres for Same room group, 1.6 metres for the New room group, and 1.6 metres for the New+CPP group (fig 3.2). This indicates that Latency and path length follow identical patterns and that all groups reduced these measures over training. Two way repeated measures ANOVA showed that there was a significant effect of trial on latency ($F_{7,84} = 47.373$, $p < 0.001$), no effect of group ($F_{2,12} = 3.636$, $P > 0.05$) but there was an interaction ($F_{7,14} = 2.312$, $P < 0.05$). There was a similar effect of trial on path length ($F_{7,77} = 48.977$, $P < 0.001$) with no effect of group ($F_{2,12} = 2.529$, $P > 0.05$) and no interaction ($F_{7,14} = 1.782$, P

> 0.05). Over the 4-day pre-training period, all rats from all three groups learned the platform position in the pool.

Mass training was analyzed in two trial average blocks. The average latency of the first trial block was 34.8 seconds for the Same room group, 25.9 seconds for the New room group, and 34.6 seconds for the New+CPP group. By the end of mass training the average latency for the last trial block reduced to 7.0 seconds for the Same room group, 4.7 seconds for the New room group, but stayed high at 32.0 seconds for the New+CPP group (fig. 3.3). Similarly, the average path length of the first trial block was 10.4 metres for the Same room group, 7.7 metres for the New room group, and 10.4 metres for the New+CPP group. By the end of mass training this reduced to 2.3 metres for the Same group, 1.6 metres for the New group, but remained high at 10.8 metres for the New+CPP group (fig. 3.4). This indicates that for both measures of learning, latency and path length decreased over training for all groups except for New+CPP.

Two way repeated measures ANOVA revealed a significant effect of trial on latency ($F_{7,84} = 4.861$, $P < 0.001$) as well as group ($F_{2,12} = 7.967$, $P < 0.05$) and no interaction ($F_{7,14} = 1.139$, $P > 0.05$). For Path length, there was a significant effect of trial ($F_{7,84} = 4.160$, $P < 0.001$) as well as group ($F_{2,12} = 7.192$, $P < 0.05$) but no interaction ($F_{7,14} = 1.389$, $P < 0.05$). Collectively, these results indicate that both Same room and New room groups of rats successfully learned the platform locations over the course of mass training while the New+CPP group did not.

Immunohistochemistry

Cell counting was done for all rats in areas CA1, CA3, and dentate gyrus and repeated measures ANOVAs were completed on the data. All three areas were compared between all four behavior groups (fig. 3.5). There were significant effects of area ($F_{2,32} = 28.818$, $P < 0.001$), of group ($F_{1,16} = 3.733$, $P < 0.001$) as well as an interaction ($F_{6,32} = 10.653$, $P < 0.001$). Post hoc pairwise comparisons were made between all the groups and areas. Significant differences were found between cell counts in the dentate gyrus of the new room group. They expressed more Arc positive cells than cage controls ($p < 0.001$), the same room group ($p < 0.001$), and the new+CPP group as well ($p < 0.05$). The New+CPP group also expressed more Arc positive granule cells than cage controls ($p < 0.05$) and but did not differ from the Same room group. For area CA1, the Same room group expressed more Arc positive cells than did the New room rats ($p < 0.05$). no differences were found in area CA3 between any of the groups. This indicates that New room training induces more dentate Arc expression than Same room and control groups, and that CPP diminishes this effect. Images of Arc expression in the dentate gyrus across groups are seen in fig 3.6 and cannulation coordinates are seen in fig 3.7.

Discussion

In this experiment, rats were trained in a version of the Morris water task that involved multiple training rooms. The effect of the NMDA receptor antagonist CPP on Arc protein expression in hippocampal sub-regions in response to learning experiences was examined. When pre-trained in the water task in room A, rats could easily learn a new platform position in room A or in room B. However, when CPP was administered to both the dorsal and ventral hippocampus prior to the second training phase, rats could not

learn a platform position in room B, despite pre-training. These results are identical to those found in experiment 2. They further support the idea that hippocampal NMDA receptors are critical for the acquisition of novel environmental information used in spatial navigation.

The expression of the protein for the immediate early gene Arc differed between group and region, depending on the training conditions. In the dentate gyrus, expression was high for the New group compared to the equally low cage controls and Same room group. When CPP was given, this corresponded with both a reduction in dentate Arc expression and an impairment in learning compared to the New room group. The same room rats expressed a significant increase in Arc in area CA1 compared to New room rats. No differences were observed in area CA3 between any of the groups.

Despite a lack of a defined pattern of behavior related activity in areas CA1 and CA3, the pattern of Arc expression in the dentate gyrus fits with the behaviors exhibited by the rats in their respective learning conditions, both in this experiment and experiments 1 and 2. When rats had to learn a new spatial platform location in the same environment as pre-training, dentate arc expression was not significantly different from cage controls. Learning in the new room however produced a large increase in dentate Arc expression. This would indicate that if Arc is necessarily tied to learning and plasticity, that the same room reversal training in the water task is not the same as novel room training, behaviorally or molecularly.

The results from experiments 1 and 2 also show that depending on the task requirements, learning can be made NMDA receptor dependent. Learning novel environmental information is an NMDA receptor dependent process while utilizing

previously learned environmental information for new navigational purposes is not. The results from this experiment show that Arc expression follows a similar pattern. In a task that was not found to be NMDA receptor dependent (experiment 1), dentate Arc expression was no different from controls. In a task found to be NMDA receptor dependent (experiment 2), Arc expression was high in the dentate gyrus, and reduced in the same condition that produced behavioral impairments (hippocampal NMDA receptor blockade).

This is a specific pattern of results that support the idea that Arc expression is both a plasticity based IEG activated by novel learning behaviors, and that this molecular and behavioral expression is NMDA receptor dependent (Czerniawski et al., 2011; Steward and Worley 2001). From these 3 experiments, our results associate both molecules with learning novel environmental information in the hippocampus, particularly in the dentate gyrus.

Another IEG proposed to be related to learning based plasticity and potentially NMDA receptor dependent is the regulator IEG Zif268 (Richardson et al., 1992; Cole, Saffen, Baraban & Worley, 1989). In a multiple training room water task experiment very similar to this one, rats were trained to multiple platform locations over multiple days in room A, and then trained in room B just once. Zif268 expression was recorded across hippocampal sub-regions after training. After extensive spatial training in one room, new room learning induced Zif268 expression in the dentate gyrus and CA1 much higher compared to same room trained rats.

Guzowski, Wagner, Setlow & McGaugh (2001) have shown that same room water task reversal training induces Arc mRNA in the hippocampus at higher rates than

controls. When spatial water task training was done in a room separate from pre-training, a sub-region analysis showed that Arc mRNA expression increases in the dentate and area CA3 relative to controls, however, this sub-region analysis was not done in same room reversal and so does not speak to reversal learning. Dentate Arc expression has also been observed in object place learning. When learning to match objects to places in a maze, moving the position of the objects while maintaining the extra maze cues induced Arc mRNA in the dentate gyrus of rats (Soule et al., 2009). These conditions are similar to our water task experiments but different in the type of changes that induce dentate Arc expression. These contrasts and others will be discussed in the general discussion section.

Chapter 5

General discussion

In these experiments, we assessed the role that hippocampal NMDA receptors have in spatial memory and the molecular processes associated with it. NMDA-blockade across the dorsal and ventral hippocampus was accomplished using the receptor antagonist CPP and direct cannulations. In each experiment, all animals were pre-trained to become procedurally efficient at the Morris water task. After this, a mass training procedure was done in one of two conditions: in the same training room as pre-training, or in a new training room, different from pre-training. In this way, we could observe how NMDA receptors mediate spatial learning dependent on previously acquired or novel environmental information.

Without the use of NMDA receptor function in the hippocampus, rats were capable of expressing what they had learned previously, and they could also learn new spatial information in the same environment as pre-training. However, they forgot this information as soon as 8 hours or 24 hours later. When brought to a completely novel training environment, rats could not learn new spatial information at all without the use of hippocampal NMDA receptors. These results lead to the conclusion that NMDA receptors mediate the acquisition of novel environmental information to be used in spatial navigation.

This type of NMDA receptor dependent behavior was also found to induce specific patterns of Arc expression in the hippocampus, namely the dentate gyrus. In the task in which NMDA receptors were not found to be necessary for learning (same room), expression was low. In the task in which NMDA receptors were found to be necessary for

learning (new room), expression was high. CPP blocked both learning and reduced Arc expression in animals trained in the new room. This pattern of results points to a link between these three phenomena: NMDA receptor function, Arc expression, and novel spatial learning. The relationship between these will be explored below.

Novel contributions and support for hypotheses

The results from the three experiments above support a series of hypotheses regarding the biological nature of learning and memory behaviors thought to be dependent on the hippocampus and related circuits. First, that depending on what has to be learned and the information available to do so, learning can be NMDA receptor independent. Second, NMDA receptors are not involved in the retrieval of previously learned information. Third, NMDA receptors have a critical role in the consolidation of spatial memory. Fourth, NMDA receptors in the hippocampus also have a critical role in the acquisition of novel environmental information. Fifth, NMDA receptor dependent learning also activates the immediate early gene Arc in the dentate gyrus of the hippocampus. Finally, some aspects of the information acquired during spatial navigation training in the MWT can be applied outside of where it was originally learned. However, much of the scientific research done in the past on these issues does not give support to some of these claims. Here I will discuss the discrepancies between the results of my present experiments and previous work done by others, as well as what novel contributions this work provides.

NMDA receptor blockade in the dorsal versus full hippocampus

The inspiration and design of my experiments was from McDonald et al, (2005). In that series of experiments only the dorsal hippocampus was blocked for the intracranial experiments and no effect of NMDA receptor blockade on mass training of a new spatial position was reported. Experiment 1, reported in the current thesis, was completed to determine if the pattern of results observed in the McDonald et al., (2005) study was due to a functional ventral hippocampus. The ventral portion of the hippocampus has been shown to support certain aspects of spatial learning (Ferbinteanu, Ray & McDonald, 2003). Very few water task studies have been done with full hippocampal NMDA receptor blockade (Steele & Morris, 1999; Inglis, Martin & Morris 2013). Both the experiments cited above utilized pre-training procedures followed further training under receptor blockade, and both experiments resulted in successful spatial learning under specific circumstances, despite full blockade. These experiments will be explained in more detail below.

As a stated hypothesis in experiment 1, the full hippocampal NMDA blockade discounts the possibility that the reason why animals were capable of learning novel spatial locations in the McDonald et al., (2005) study was due to a functional ventral hippocampus. Comparably, some rats in that experiment that were given I.P. injections also learned the novel platform location. Due to the injection method, I.P. necessarily results in global antagonism, and so would presumably cover both dorsal and ventral hippocampus. This leads to a further conclusion that the dorsal and ventral hippocampal NMDA receptors do not play a critical role in this task. With regards to novel environmental learning as assessed in experiment 2 of this thesis, only full hippocampal

blockade was done, and so the respective contributions of the dorsal and ventral segments cannot be elaborated on.

NMDA receptors and memory retrieval

The lack of NMDA receptor involvement in memory retrieval has long been accepted after several experiments outlining rats' ability to use previously acquired information during NMDA receptor blockade (Shapiro & Caramanos, 1990; Bast, Silva, Morris, 2005). Biologically this would make sense, if NMDA receptors are necessary for LTP induction and learning based plasticity, then once the circuit connections representing the memory have been formed and consolidated, plasticity mechanisms should not be involved in simple circuit activation during retrieval. However, recent evidence has shown the contrary; that NMDA receptor blockade can prevent the expression of previously established fear memories (Lopez, Gamache, Schneider & Nader, 2015). Our results in experiment 1 further support the claim that NMDA receptors are not involved in memory retrieval and expression. When analyzed as a probe, trial 1 of mass training revealed that rats from both groups displayed a spatial preference for the pre-training platform location, indicating that it was both successfully learned and retrieved. Trial 1 of mass training in experiment 2 (room B) also supports NMDA receptor independent retrieval, as well as providing some interesting insights into spatial processing.

In experiment 2, rats were brought into room B after being pre-trained in room A. Trial 1 of mass training was analyzed as a probe to see if any spatial preferences could be observed. This was done to see if any information learned from training in room A could be used during training in room B. Past research from our lab has shown that this may be

the case (Clark et al, 2015), and so we hypothesized that rats would show some kind of directed spatial behavior during trial 1, although the specific expression of which was undetermined.

Both ASCF controls and CPP infused groups spent most of their swim time in quadrant 2. This indicates that despite NMDA receptor blockade, both groups behaved identically after spatial training in room A. However, rats placed in a novel water task for the first time almost never display spatial preferences but instead display a type of thigmotaxis characterized by hovering near the pool wall and circling quickly around the perimeter of the pool. The fact that these rats went through pre-training in room A, and then displayed a spatial preference in room B, despite having never been in room B before raises the question of what exactly is learned in the water task that can be transferred between rooms. There are environmental cues that exist in both rooms and both pools are identical in shape, color and size. The geometrical structure of the room has similar features such as wall shape and length. The door and experimenter are also present in both rooms. Indeed, the spatial preference was displayed by both groups for the quadrant which was closest to the door, which was also the quadrant where the platform was located in the pre-training in room A. (fig 2.5).

Our results from experiment 2 indicate that some spatial information learned in one environment can be used in other environments. The specific nature of transferable information between environments or what common environmental features may be used in navigation remains unclear, but its expression is NMDA receptor independent.

NMDA receptors and spatial learning

Starting with Bannerman et al. (1995) and Saucier & Cain (1995), the idea of NMDA receptor independent learning began to be studied. Novel water task training in the presence of NMDA receptor antagonist drugs resulted in learning impairments, but by changing certain parameters of the task, these impairments could be eliminated. Since then, many studies have examined this phenomenon, through NMDA receptor independent extinction (Santini, Muller & Quirk, 2001), place cell formation (Kentros et al., 1998), LTP (Grover & Teyler, 1990), and molecular cascades associated with learning and plasticity (Moosmang, et al., 2005). How exactly learning processes can be made NMDA receptor independent is not certain. Some have shown that behavioral strategies are learned once, and then can be applied across multiple learning scenarios independent of NMDA receptor plasticity (Hoh et al., 1999). Others have argued that where in the brain learning takes place changes over training (Inglis, Martin & Morris, 2013). Some of these issues were discussed in ch2.

Almost all studies examining NMDA receptor independent spatial learning have done so using pre-training, in some form or another. A review of the literature on experiments using NMDA receptor blockade, pre-training, and spatial learning reveals two overall findings. The first is spatial learning can be made NMDA receptor independent when done with the same information available to prior learning, and is also done rapidly. This is made most clear by Steele & Morris (1999) where rats were pre-trained in the water task on a version that involved a new platform location every day. After several days, rats continued training in the same room but with hippocampal NMDA receptor blockade. The manipulated variable was inter-trial interval, and it was

found that when it was kept short at 15 seconds rats learned successfully. However, when it was lengthened to 20 minutes, they failed to learn. This means that rapid learning was possible while learning over longer periods of time was not. Because the platform changes every day, the effect of receptor blockade on long term training cannot be assessed.

Work done by Saucier & Cain (1995) and Hoh et al., (1999) have shown that non-spatial pre-training (NSP) can lead to rapid NMDA receptor independent spatial learning. The argument put forth by these researchers is that NSP allows for behavioral search strategies to be developed prior to spatial training and that this is what leads to NMDA receptor independence later on. However, behavioral search strategies themselves were also found to be learned independent of NMDA receptors. This contrasts with earlier work done (Morris, Davis & Butcher, 1990) in which NSP led to impairments in same room spatial training that was done over the course of several days. NSP is performed with a curtain around the pool and so may potentially conflict with our explanation of previously learned environmental information hypothesis. However, the complete environmental repertoire that contributes to spatial navigation behaviors goes beyond wall cues like posters.

The water task environment can be divided into two separate spaces: the intra-maze (local) environment which contains everything inside the pool walls, and the extra-maze (global) environment which contains everything outside the pool walls but inside the experiment room. Some evidence shows that aspects of spatial navigation can even transfer between multiple contexts and so would constitute potentially larger, environment independent navigation strategies as well, such as heading direction (Taube

& Burton, 1995, Clark et al., 2015). Given the continued use of the maze and global location of the maze throughout training, NSP does not necessarily mean that no usable environmental information is learned. With this in mind, in the studies using NSP, rats successfully learn the spatial water task when trained rapidly (Saucier & Cain, 1995; Hoh et al., 1999).

Our work reinforces these conclusions. It shows that in the absence of NMDA receptors in the hippocampus, rapid learning is possible when the information used to learn a new escape location is identical to what has been previously learned. After both an 8 hour and a 24 hour retention period, everything that was learned during mass training was forgotten. Our specific pattern of results indicates that whatever was learned was not consolidated. A lack of consolidation may explain why rats in these types of tasks can learn rapidly but not with increased ITIs or over several days. NMDA receptor involvement in memory consolidation has been shown on multiple occasions (McDonald et al, 2005; Santini, Muller & Quirk (2001).

The second finding is that novel spatial learning can be made NMDA receptor independent when done over several days. This comes primarily from two experiments. Bannerman et al., (1995) was able to get rats to learn the spatial water task in a room different from that of pre-training, and did so over multiple training days. A similar effect was found in that experiments replication (Inglis, Martin & Morris, 2013). In the Bannerman et al., (1995) study, a NSP experiment was also done, and when done in room 1, it did not allow for spatial learning in room 2 across multiple days. NSP is a very specific type of pre-training that eliminates extra-maze cues. Given that there is no environmental information outside of the pool, it is likely that the rats given NSP pre-

training treated room 2 in the Bannerman study in the same way that the rats in the Cain and Hoh studies treated their respective training rooms. Stated differently, without previous environmental information to compare to new information, all information is new, and so new training done in a different or same room will not matter. A similar intra-maze environment and lack of extra-maze specific cues means that there is no such thing as same room or different room (dependent on extra-maze cues) following NSP, and that all subsequent spatial training will follow the same principles. This would also explain the multiple day learning impairments observed in Morris, Davis & Butcher (1990).

However, multiple day NMDA receptor independent learning does not apply to naïve rats in the water task. This may be because novel water task training does not just involve spatial learning but also developing successful motor patterns for swimming, trying out search strategies, as well as reducing fear. The water task is an aversive learning task and given NMDA receptors involvement in motor function and anxiety, some have argued that learning deficits cannot be dissociated from other types of effects (Ahlander, Misane, Schott & Ogren, 1998, Keith & Rudy, 1990). Because of the ubiquitous nature of the NMDA receptor, it may have a role in many neural and behavioral processes outside of spatial navigation and learning. This is why pre-training is used, it allows for only certain aspects of the task to be isolated and examined.

In all of the work involving water task training that relies on previously learned environmental information, NMDA receptor independent learning is successful when done rapidly but not when done with increased ITIs or over several days. However, when training is done with novel environmental information and the potentially confounding

influence of behavioral strategies, anxiety, and sensori-motor impairments are removed, learning may be NMDA receptor independent when done over several days, but not rapidly. To our knowledge, no one has examined the role of NMDA receptors in rapid novel spatial learning after pre-training. Our work provides support these conclusions by showing that when transferred to a new room with novel environmental cues rats without NMDA receptor function cannot rapidly learn.

The shift in NMDA receptor dependence may have to do with the requirements of the task. Reversal learning in the water task consists of fundamentally different task parameters and learning requirements than novel learning. When put through the water task for the first time, rats must learn many things ranging from behavioral search strategies, spatial locations, and interacting with the maze, the experimenter, and the environment. These include, but are not limited to, swimming away from the pool wall, the size and stability of the hidden platform, the arrangement of extra-maze cues, etc. One of the main reasons why pre-training procedures are used is to limit the type of learning that must occur in subsequent training procedures for successful maze navigation. When put through reversal learning in the same spatial context after a pre-training procedure, the only thing required for the rat to learn is a new platform position. The dimensions and details of the maze and room, as well as a successful search strategy, have already been learned. Also, rats have to extinguish the previously learned spatial information, so that reversal doesn't just involve new learning, but extinction as well. The rat must do this using information and strategies it is already familiar with.

Further training with a completely new set of information such as in experiment 2 is also different from both naïve training and same room reversal. Several components of

training are already familiar to the rat such as swimming and an expectation of the rules of further training. Yet some things are completely unfamiliar like where the animal is and what exists in its environment. In this way, three different stages of water task training, naïve, new room, same room reversal, all have increasing amounts of familiarity to them, respectively.

This means that all three stages of training may involve different search strategies, or the same strategies but based on different types of available information. Given the different task requirements during each stage, it is not unlikely that the role plasticity has will vary across stages. Our own results, as well as a review of the relevant literature, reveal that NMDA receptors in the hippocampus will be heavily involved in several processes during naïve water task training, resulting in an inability to dissociate between learning, anxiety, and sensori-motor processes just from behavioral data alone. After extensive pre-training and task-familiarization, NMDA receptors consolidate changes to existing spatial information. And that when processing completely novel environmental information, are necessary for its rapid learning.

Hippocampal remapping

A potential electrophysiological correlate to these behavioral patterns may be remapping. Remapping occurs when changes to the external information that guides internal place cell activity produces changes to that cell activity. (Bostock, Muller & Kubie, 1991) Slight changes such as single, distinct environmental cues result in rate remapping, in which the population of active cells and their representative spatial location remain the same but their firing rate changes. Large changes to environmental information or even a complete switch in environments results in global remapping, in

which the entire population of active place cells changes its spatial representations and firing rates (Leutgeb et al, 2005).

Neither the initial formation of place fields nor the maintenance of already present place fields is an NMDA receptor dependent process. However, their long-term stability (Kentros et al, 1998) and changes in their representative properties is impaired by pharmacological and genetic manipulation (McHugh et al., 2007; Ekstrom, Metzel, McNaughton & Barnes, 2001) and so rate and global remapping to a novel context would likely involve them as well. Rate remapping in CA3 was impaired between environments in an NMDA receptor knockout mouse (McHugh et al., 2007). LTP also appears to play a critical role in global remapping as well (Dragoi, Hariss & Buzsaki, 2003).

In our experiments, it could be argued that same room reversal might produce rate remapping because of the small change in platform position. While new room training could produce global remapping because of the training room change. Given the link between NMDA receptors, LTP, and remapping, the inability to learn due to blocked NMDA receptors in our experiment 2, could be due to an inability to remap place fields in the hippocampus. However, in our experiments, this remains completely hypothetical. Given the overlap of global and rate remapping across different types of spatial manipulations (training rooms, color, size, shape), what exact features of the environment lead to remapping, and whether these changes are behind pattern separation and navigational behaviors remains to be determined (Jeffery, Gilbert, Burton & Strudwick, 2003; Speirs et al., 2013; Leutgeb et al., 2005; Kentros et al, 1998).

Immediate early genes and the dentate gyrus

The use of IEGs in neuroscience research is commonly used to map behaviorally relevant neural activity. The IEG Arc is thought to be dependent on LTP related activity and so is used to map neurobiological mechanisms of learning and memory. Learning and memory behaviors are known to reliably induce Arc mRNA and protein expression in behaviorally relevant brain regions. Activation of the NMDA receptor has been identified as a critical mechanism that leads to Arc expression. Blocking NMDA receptors with antagonist drugs reduces behavior induced Arc mRNA (Czerniawski et al., 2011). Furthermore, NMDA receptor hypofunction leads to reduced Arc expression (Balu & Coyle, 2014). The links between learning behaviors, NMDA receptors, LTP, and Arc activity is well supported by research (Guzowski et al., 2001; Shepard & Bear, 2011).

My results from experiment 3 show that in one version of a water task that did not require NMDA receptors to learn, Arc protein expression was not significantly different from cage controls. In a version of the task that did require NMDA receptors to learn, Arc protein expression was high compared to cage controls and the NMDA receptor independent task. When an NMDA receptor antagonist was introduced to the hippocampus, it impaired learning as well as reduced Arc protein expression. The level of expression in the CPP group was significantly below the New room group but also significantly higher than the cage control group. No observable patterns emerged in areas CA1 or CA3, however, the pattern of expression described above was observed in the dentate gyrus.

Two important conclusions can be reached from these results. One is that NMDA receptor dependent learning of novel environmental information activates Arc in the

dentate gyrus. The other is that NMDA receptor blockade reduces Arc expression in the dentate gyrus. Because we have did not determine the binding efficacy of CPP administered, it is impossible to know if the heightened Arc expression seen in the CPP+New group relative to cage controls was because of NMDA receptor independent processes or due to still active NMDA receptors. Given the specific pattern of results our experiments produced, as well as the contributions of other work on this topic, we can also make hypotheses regarding the function of dentate gyrus plasticity as it pertains to spatial learning.

Because of the relatively small size of the dentate gyrus, region specific manipulations are limited to specialized techniques. Dentate specific lesions of granule cells can be accomplished with intra-dentate Colchicine injections (Sutherland & Rudy, 1988). This type of lesion has been shown to impair both reference memory across training days and working memory within training days in spatial water tasks (Xavier, Oliveira-filho & Santos, 1999; McNaughton, Barnes, Meltzer & Sutherland, 1989). Adrenalectomy also produces granule cells loss in the dentate gyrus and results in similar impairments in reference and working memory spatial water tasks. (Spanswick, Keith, Epp & Sutherland, 2007). However, spatial learning impairments are not always seen with this type of manipulation (McCormick, McNamara, Mukhopadhyay & Kelsey, 1997).

In a study of object-context associations, adrenalectomized rats with dentate damage were placed in small boxes with objects placed in them. When objects were moved, or new objects were placed inside, ADX rats displayed the same degree of novelty preference as control rats. However, in a scenario where the objects and the

context were both changed, ADX rats displayed no novelty preference, whereas control rats did (Spanswick & Sutherland, 2016). This is not a general spatial learning impairment like the studies mentioned above but a more specific impairment related to objects in a novel spatial environment.

Other forms of dentate manipulation, like NMDA receptor genetic manipulations, produce more nuanced spatial impairments similar to Spanswick & Sutherland (2010). Colchicine and ADX lesions have several other consequences such as hormone changes, damage to neighboring tissue, as well as eliminating entire hippocampal circuits in which the dentate is a key component. Selective genetic knockouts have the advantage of maintaining dentate morphology while eliminating plasticity mechanisms. Selective genetic knockout of NMDA receptor NR1 subunit exclusively in the dentate gyrus of mice produces working memory impairments while maintaining reference memory in an 8 arm radial maze (Niewoehner et al., 2007). In a mouse model of fragile X syndrome, NMDA receptor hypofunction occurs in the dentate gyrus. This sub-region specific receptor loss results in impaired context discrimination (Eadie et al., 2012). Similarly, McHugh et al., (2007) produced a dentate specific knockout mouse that could learn context fear conditioning. However, the mice could not learn context discrimination, supporting the dentates proposed role in pattern separation (Kesner, 2013, Luetgeb, Luetgeb, Moser & Moser, 2007).

In a BAX-KO mouse, overexpression of newly generated granule cells disrupts dentate function. These mice perform as well as controls in learning the spatial version of the water task over several days, however, when the extra maze cues were rotated, controls went to the location relative to the cues and the BAX-KO mice did not. No

impairments were observed in a similarly designed radial maze experiment over several days. The researchers indicate that the dentate gyrus has a role in aligning internal spatial maps to external landmarks, and when not functional, other methods of navigation take over, such as dead reckoning (Lee, Kim sun & Jung 2009). Other genetic studies have provided even more insight into the function of dentate NMDA receptors. Chen et al., (2009) produced a mutant NMDA receptor mouse with GluN1^R subunits being expressed in roughly 10% of total NMDA receptors in the dentate gyrus. Receptors that contain the GluN1^R mutant allele do not act as coincidence detectors and so provides a method for examining subtle changes to specific populations of NMDA receptor functions. The behavioral phenotype of these mice was that of impaired water task performance over multiple days, as well as impaired same room reversal learning over multiple days.

The results from lesion and knockout experiments points to three functions of the dentate gyrus: 1) its role in the hippocampal circuits mediating spatial learning; 2) a role in both working memory with a sparing of reference memory (Niewoehner et al., 2007; Lee, Kim Sun & Jung, 2009); 3) supporting discriminations between multiple learned environments (McHugh, 2007; Eadie et al., 2010). The results from the Chen et al., (2009) experiments suggest differently, as these rats failed to learn anything, on any spatial task. However, as mentioned by the authors themselves, this was not an NMDA receptor knockout, or dentate gyrus lesion, but an altering of the functional capacity of certain NMDA receptors.

Interestingly, genetic experiments show a very specific type of behavioral learning deficit after NMDA receptor manipulation in the dentate gyrus, namely, the inability to discriminate environmental information and learn rapidly where as in our experiment,

rapid novel environmental learning resulted in NMDA receptor dependent activity in the dentate gyrus. The previous work produces behavioral effects after the molecular manipulation, our work shows similar molecular effects after similar behavioral manipulations. Although more work would necessarily have to be done to prove it, this bidirectional effect demonstrates a link between NMDA receptor plasticity in the dentate gyrus and the rapid learning of novel environmental information.

Limitations and considerations of my work

One of the primary problems with pharmacological blockade is the difficulty in directly quantifying drug-receptor binding efficacy. Many techniques have been used such as electrophysiological recordings of LTP (Inglis, Martin & Morris, 2013) given that NMDA receptor dependent LTP has been well characterized and has a unique and identifiable form. Another technique that has been explored is autoradiography (Steele & Morris, 1999).

However, in our work we did no direct measure so there is no way of knowing for sure how much CPP was released or how far its effect spread in the nervous tissue. Even though 1 μ l was injected through each cannula, they sometimes clog, and equipment setup and performance does not always go as planned. The only measure we have is of Arc protein expression and the behavioral results. In the first two experiments, all rats were treated as equally as possible and the only difference between groups being whether CPP was present in the ACSF hippocampal infusions. The behavioral effects demonstrate the presence and effect of CPP, although not its anatomical spread.

A similar issue is that NMDA receptor antagonists have been shown to reduce the excitability and complex spike firing of hippocampal neurons in addition to its effects on LTP induction (Abraham & Kairiss, 1988). This leaves open the possibility that the behavioral results of my experiments were not due to blocked LTP but instead of reduced hippocampal excitability. For example, it may be the case that NMDA receptors are not necessary for memory consolidation as shown in experiment 1, but instead that the overall activity of the hippocampus was reduced, leading to less activation of consolidation mechanisms. If hippocampal excitability was reduced artificially in some other way, similar results may be expected. As with the drug-binding problem, this research did not address this potential confound and so remains speculative.

Another drug issue is that CPP may still be present in the brains of the rats during the 8 hour retention probe in experiment 1. CPP has been shown to fully maintain its antagonist effects for up to 6-8 hours after administration (Abraham & Mason 1988). The first trial of mass training reveals a spatial preference that supports the idea that NMDA receptors do not interfere with the expression of previously formed memories. The probe done immediately after mass training also reveals no differences between the two groups, even though NMDA receptor antagonism is still present in the drug group. Because NMDA receptor antagonism does not interfere with memory expression, any difference observed in the 8 hour retention probe would not be due to non-mnemonic effects of the drug. If NMDA receptors have no role in the learning and memory process, then CPP administered rats should perform identical to controls even during the 8 hour retention probe, despite still having CPP, and hence blocked NMDA receptors, in their hippocampi.

CPP rats showed a clear reversal in spatial preference during this probe, indicating that what was learned during mass training was forgotten or not consolidated.

One neural process important in spatial navigation is the orientation of head direction cells outside of the hippocampal formation, namely the thalamus and post subiculum (Taube, 1995; Taube, Muller & Ranck, 1990). In experiment 1, mass training involved locating a novel platform location in the pool. This type of training also demands that the rats head in a different direction in the room. Heading direction is an important part of navigation, and some research indicates that it plays a primary role in water maze performance (Hamilton et al, 2007). It is possible that because only hippocampal blockade occurred, that the head direction system, with intact NMDA receptor dependent plasticity, may account for the rats ability to learn the novel location. Previous work using mice with a genetic knockout of NR1 subunit in Ca1 indicate that heading direction processes remain intact (McGugh et al., 1996). Many other studies using NMDA receptor antagonists have done I.P. or I.C.V. injections with varying directional requirements in the same training environment (McDonald et al., 2005; Steele & Morris, 1999). This means that the neurophysiology behind spatial behavior in the head direction system may not depend on NMDA receptors, although direct, brain-region specific receptor blockade has not yet been done. Procedures such as spatial disorientation (Clark et al, 2015) prior to training may reveal whether a functional head direction system can account for the lack of learning impairments in my experiments.

Other potential confounds regard consolidation. This first concerns the time course of cellular consolidation and the time we waited between mass training and probes. In experiments 1 and 2, we used a 24 hour retention probe, and in experiment 1

we used an additional 8 hour retention probe. Applying arc antisense 2 hours after the induction of LTP in hippocampal tissue eliminates it, however, applying Arc antisense at 4 hours post induction has no effect (Messauodi et al., 2007), indicating that Arc has a very time specific role in LTP consolidation. PKC inhibitors will disrupt LTP if administered 10-60 minutes after induction but not at 240 minutes (Loverger et al., 1987). Similar patterns of early post training disruption, but not disruption at later times, can be seen with learning behaviors as well. In a fear conditioning experiment, protein synthesis disruption (cAMP) impairs memory retention at 4 hour but not 24 hours after training (Guzowski & McGaugh ,1997).

These results and others indicate that the consolidation of memory is dependent on protein synthesis activated by IEGs, and that the most critical time of activity is in the immediate hours following training. From this it can be assumed that a 24 hour retention probe in our water task experiments is a sufficient time for memory consolidation. Consistent with this claim, the spatial preferences we observed in the experiment 1 8 hour probe were no different from the 24 hour probe. The pattern of behavior was identical across the two probe tests.

The second issue relating to consolidation is differentiating consolidation from extinction. Rats given CPP during mass training in the same room showed a spatial preference for the pre-trained platform location during the 24-hour probe. It could be argued that this happens because NMDA receptor blockade prevented extinction learning from occurring towards the original platform. In a platform reversal training session, presumably two types of learning or going on: new spatial learning and extinction learning for the old platform position. However, it is not consistent that rats would be able

to learn a new spatial location during training while also not being able to learn extinction for an old spatial location, at the same time. This would necessitate novel learning and extinction learning to be molecularly different. Not only this but the mechanisms underlying cellular consolidation of extinction memories are proposed to be very similar to the cellular consolidation of other memories (Quirk, Gregory & Mueller, 2008, Santini, Quirk & Muller, 2001).

A third consolidation problem is that the results of the 8 and 24 hour probes in experiment 1 may be due to state dependent learning during mass training. State dependent learning suggests that when learning is done in a certain psychological state (in this case under the influence of a drug), memory retrieval is most efficient when done in a similar state. State dependent learning has been shown to occur with a number of different psychoactive substances (Overton, 1966, Overton, 1972). The fact that CPP rats in experiment 1 behaved differently 24 hours after mass training compared to immediately after suggests that what was learned was state dependent. Indeed, state dependent learning effects were not controlled for in these experiments. CPP was not administered before any of the consolidation probes, and this leaves open the possibility that if it were, the CPP rats may have behaved similar to controls. However, prior work investigating NMDA receptor involvement in consolidation suggests that it is not state dependent (Santini, Muller & Quirk, 2001; Kentros et al., 1998).

Another potential problem is the pattern of expression of Arc in the hippocampus in experiment 3. Although an explanation for the results is given above, design limitations should also be explored. Many studies examining the effects of spatial learning on IEG expression have found it in all three sub-regions of the hippocampus proper: Dentate

gyrus, CA1, and CA3. (Guzowski, Setlow, Wagner & McGaugh, 2001; Guzowski, McNaughton, Barnes & Worley, 1999). However, in our experiment, same room reversal training did not induce Arc expression any different from cage controls. New room training did induce higher Arc expression but was limited to the dentate gyrus.

An alternative explanation of this effect could be the time course of protein action. The time scales used to detect Arc protein were borrowed from previous research (Lonergan, Gafford, Jerome & Helmstetter, 2010). In our experiment, rats were euthanized and perfused approximately 70-80 minutes after trial 8 of mass training. It is possible, although has not been explored to our knowledge, that IEG protein expression varies throughout the hippocampus after water task training in a time dependent manner. Unlike fear conditioning, water task training takes more time to complete successfully. If animals were euthanized 70 minutes after trial 1 versus after trial 16, then sub-region expression differences may be seen. Consistent with this idea is evidence showing that rats change their search strategies throughout the course of training (Graziano, Petrosini & Bartoletti, 2003). Different strategies may induce activity in different hippocampal sub-regions depending on what the animal is learning.

For example, early on in training the rat may be learning environmental information to set up a context representation, which may be dependent on the dentate gyrus (Kesner, 2013), and further on in training, when the exact location of the platform is being learned, place cell formation in area CA1 may be more dominant. Activation of area CA1 has been shown in water maze tasks using measuring IEGS (Guzowski, McNaughton, Barnes & Worley, 1999). However, given the robust time course of Arc protein action (30-90 minutes), sub-region activation dependent on training time would

likely be observed regardless of which trial the rat was perfused in our experimental design.

Contributions of the present work

In these experiments, we examined what role hippocampal NMDA receptors have in spatial learning and memory and the expression of memory related IEGs. Several conclusions can be drawn from this work including the role of these receptors in hippocampus for memory consolidation of spatial information and the lack NMDA receptor involvement in memory retrieval. However, the novel contributions of this work centre on three main findings. The first is that hippocampal blockade across both dorsal/ventral aspects results in similar behavioral patterns in the spatial water task as a dorsal-limited blockade. This suggests that the lack of an impairment of NMDA dorsal hippocampal blockade on rapid acquisition of a new spatial location, reported in McDonald et al., 2005 was not due to intact ventral hippocampal NMDA function. Second, NMDA receptors appear to be necessary for encoding and consolidation of new environmental information that will be utilized for spatial navigational behaviours but not new spatial navigational behaviours in a previously trained context. Finally, NMDA receptor dependent Arc activation occurs in the dentate gyrus after rapid spatial learning in a new environment. These three novel contributions to the understanding of the neurobiology of learning and memory will hopefully lead to more discoveries in the future.

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Figures

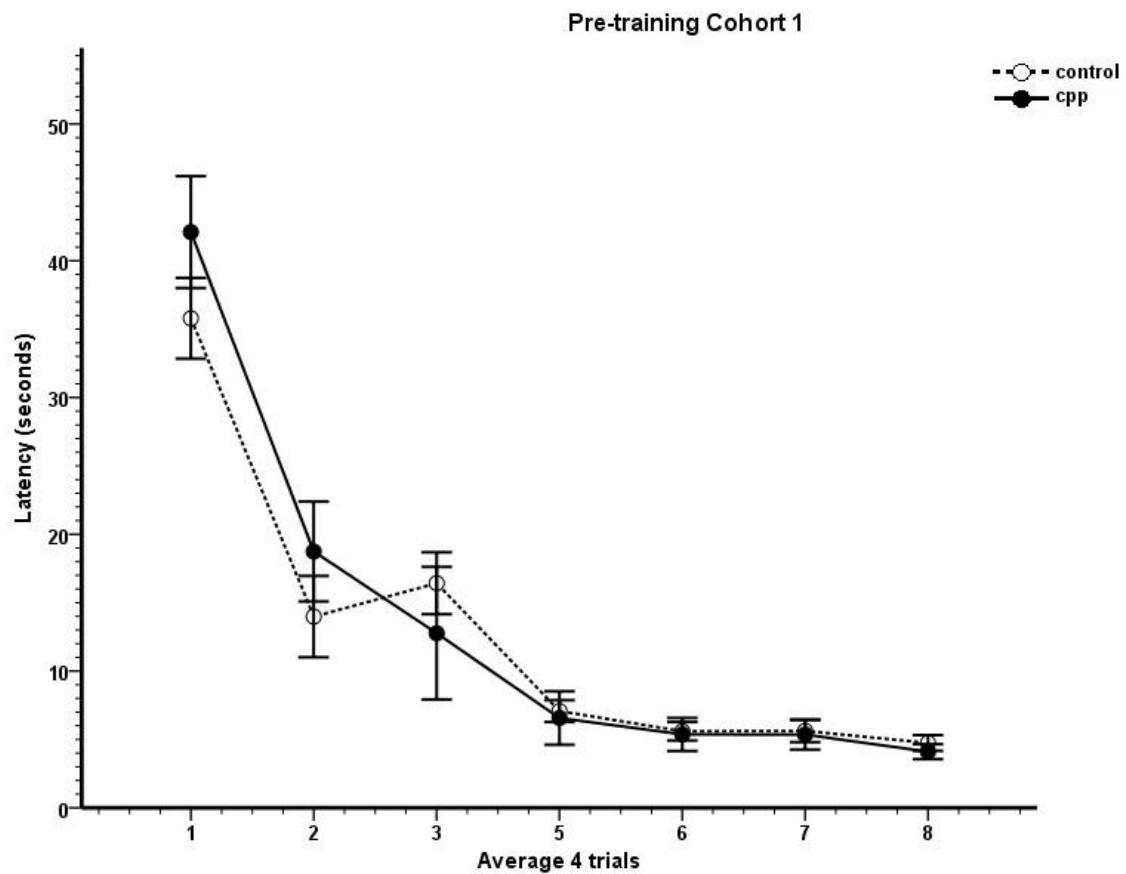


Figure 1.1: Pre-training performance of rats in cohort 1, experiment 1 on the morris water maze- Latency. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

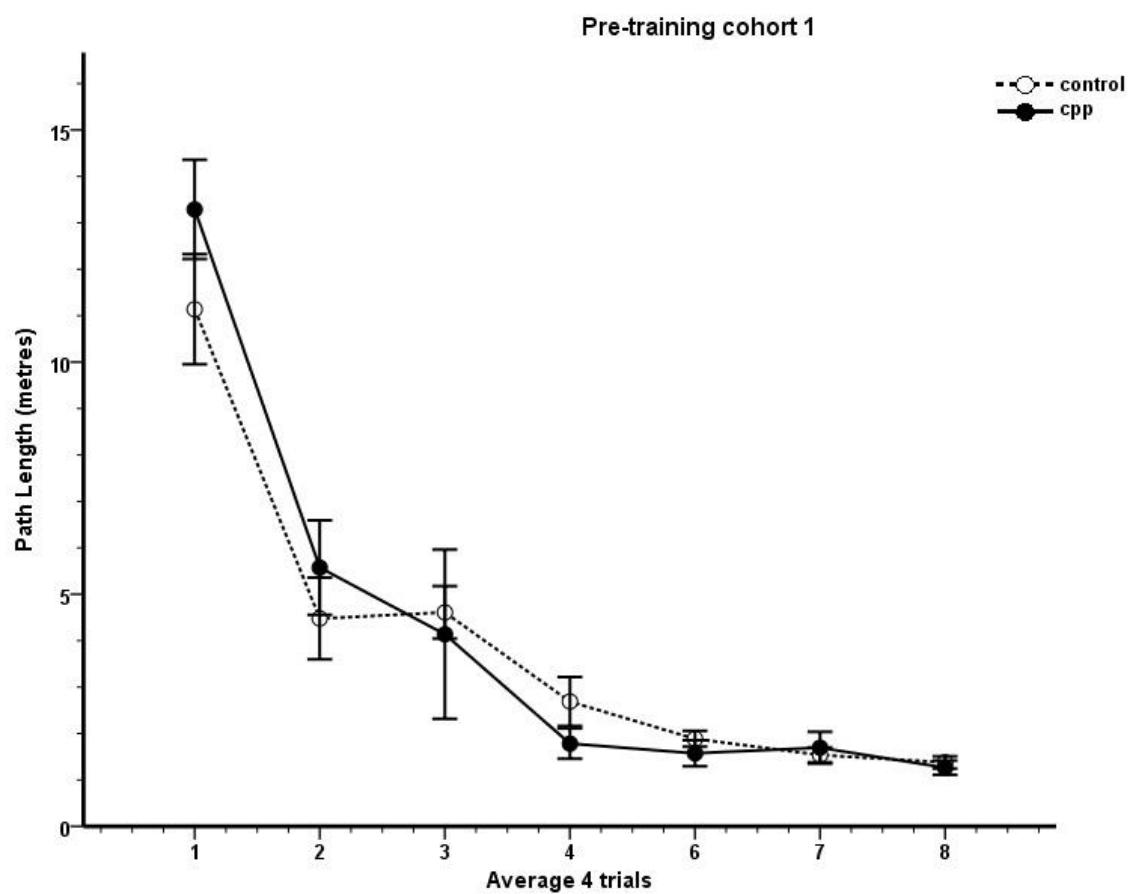


Figure 1.2: Pre-training performance of rats in cohort 1, experiment 1 on the morris water maze- Path length. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

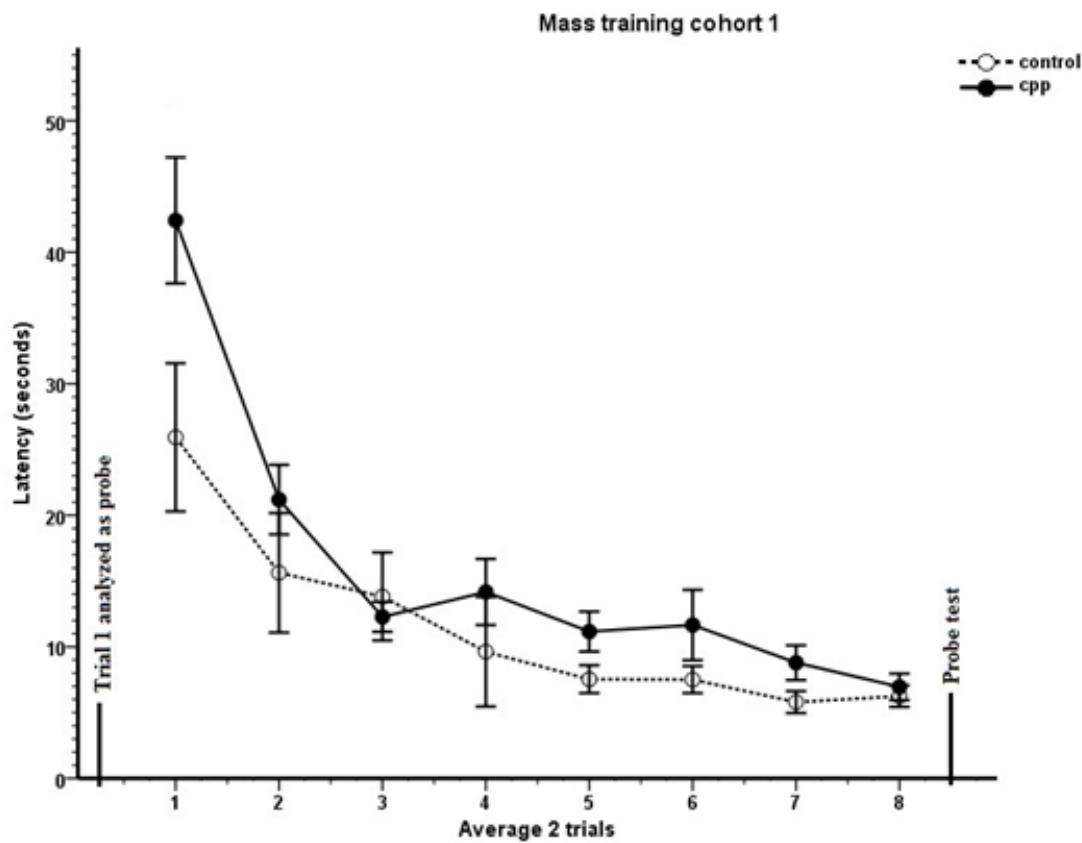


Figure 1.3: Mass training performance of rats in cohort 1, experiment 1 on the morris water maze- Latency. Rats were trained over 2 hours, 16 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE.

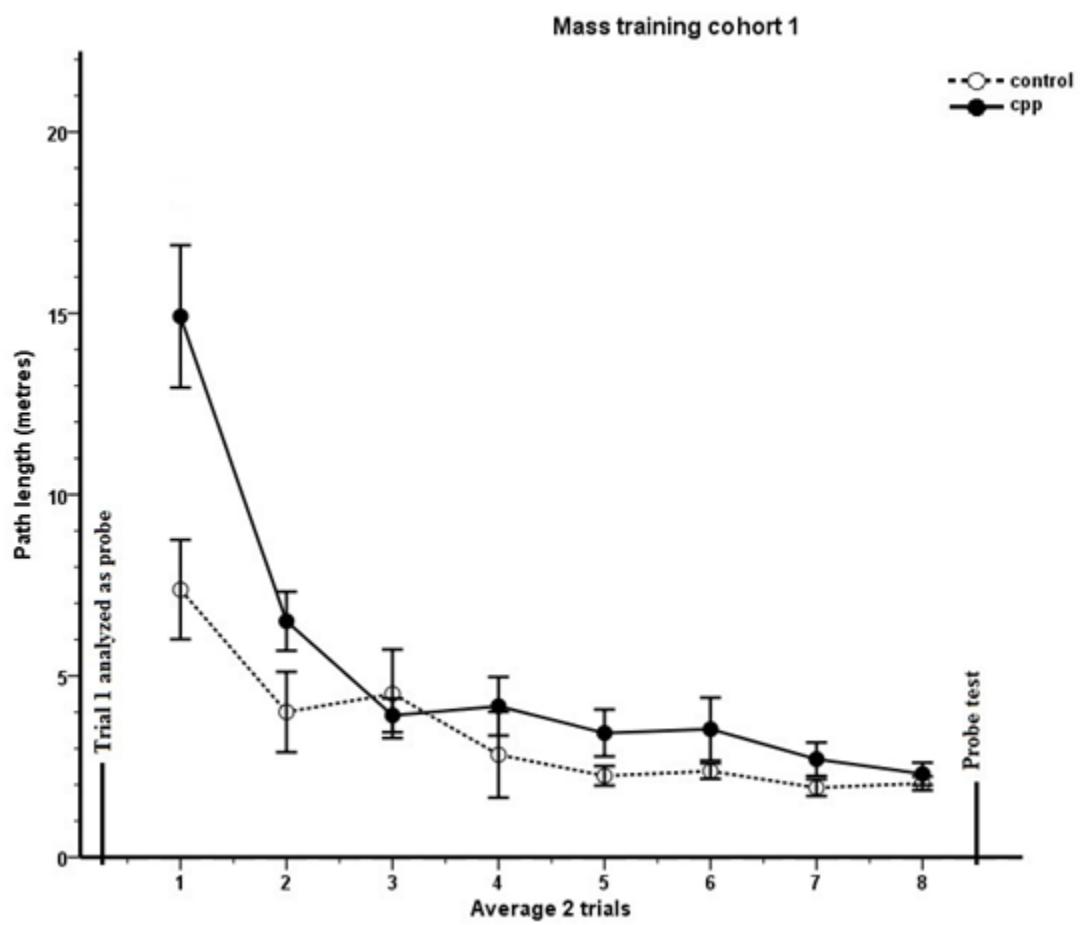


Figure 1.4: Mass training performance of rats in cohort 1, experiment 1 on the morris water maze- Path length. Rats were trained over 2 hours, 16 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

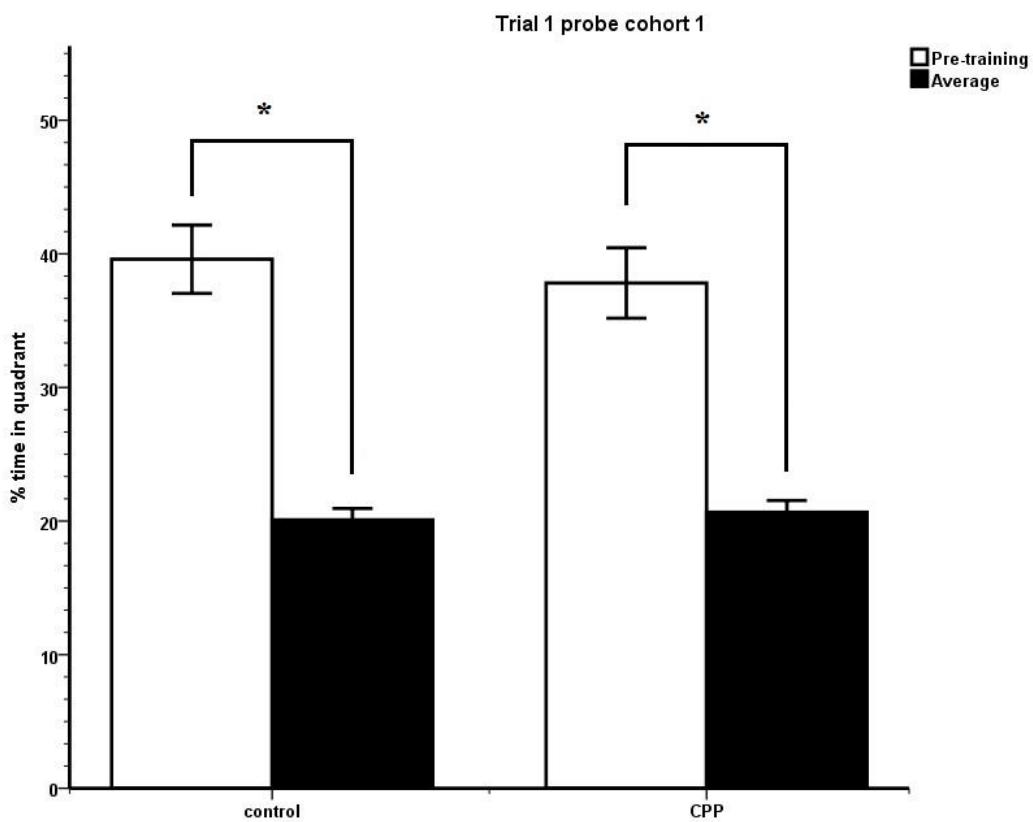


Figure 1.5: Trial 1 of mass training analyzed as a 30 second probe to test pre-training spatial memory in cohort 1, experiment 1. Comparison between time spent in pre-trained quadrant vs an average of the other three shows that both groups show a spatial preference to the pretrained quadrant. * $p < 0.05$, Error bars: ± 1 SE

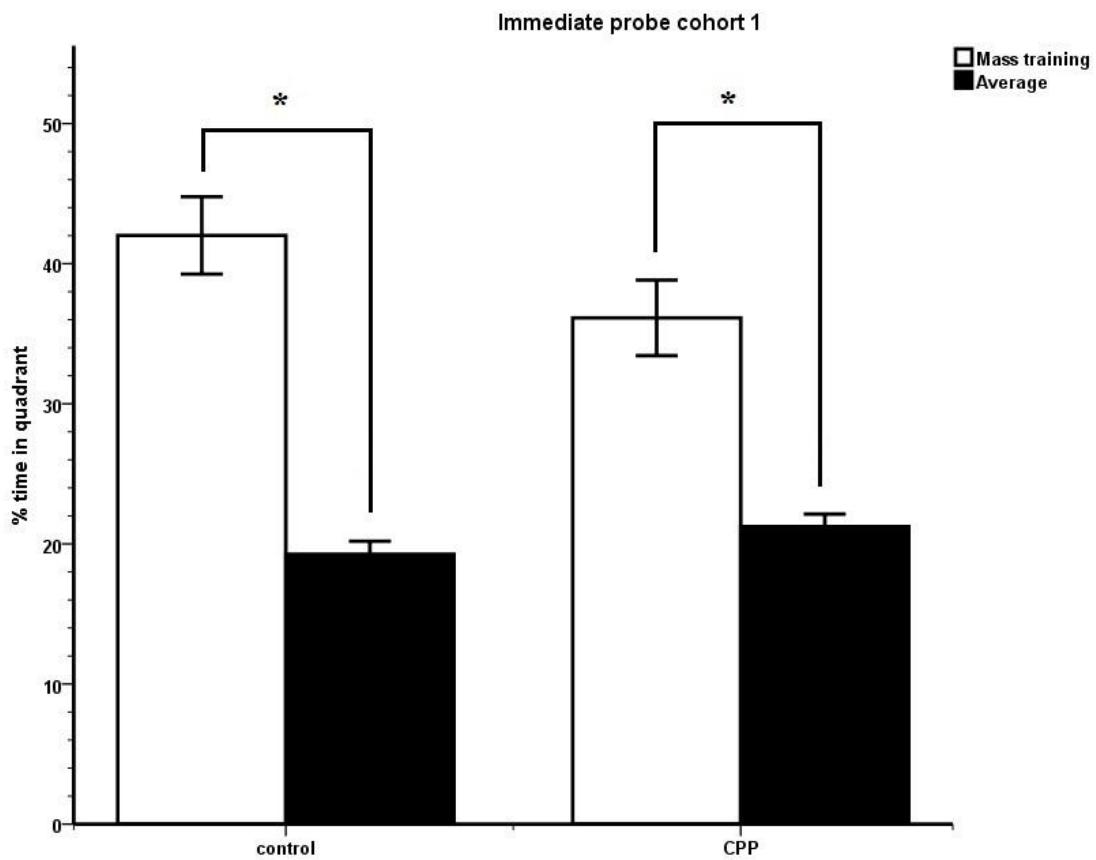


Figure 1.6: 30 second probe test done immediately after massed reversal training comparing time spent in mass trained quadrant to average of the other three in experiment 1, cohort 1. Both groups show preference for the mass trained quadrant immediately after training * $p < 0.05$, Error bars: ± 1 SE

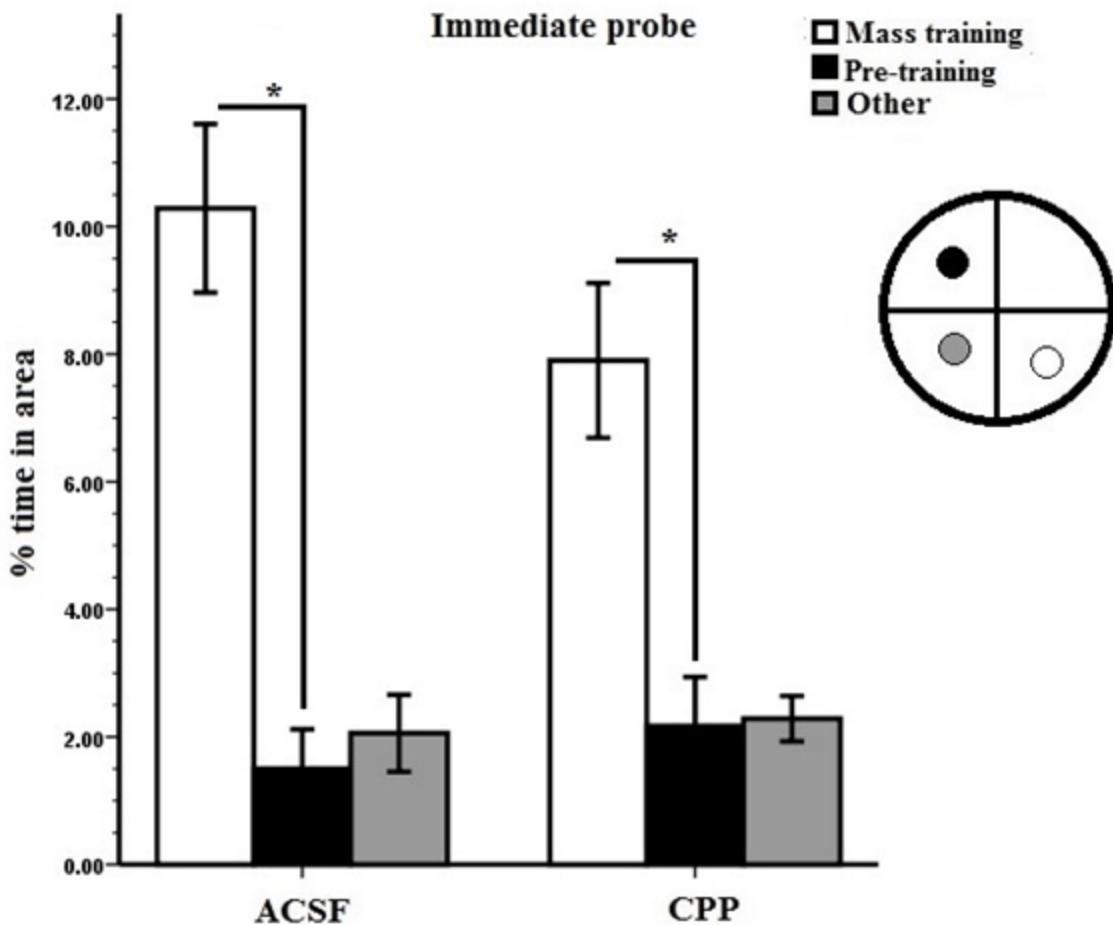


Fig 1.7: 30 second probe test done immediately after massed reversal training comparing time spent in an area immediately surrounding the mass training platform location (2% of total pool surface area) to the pre-training platform location in experiment 1, cohort 1. Both groups show preference for the mass trained location immediately after training * $p < 0.05$, Error bars: ± 1 SE

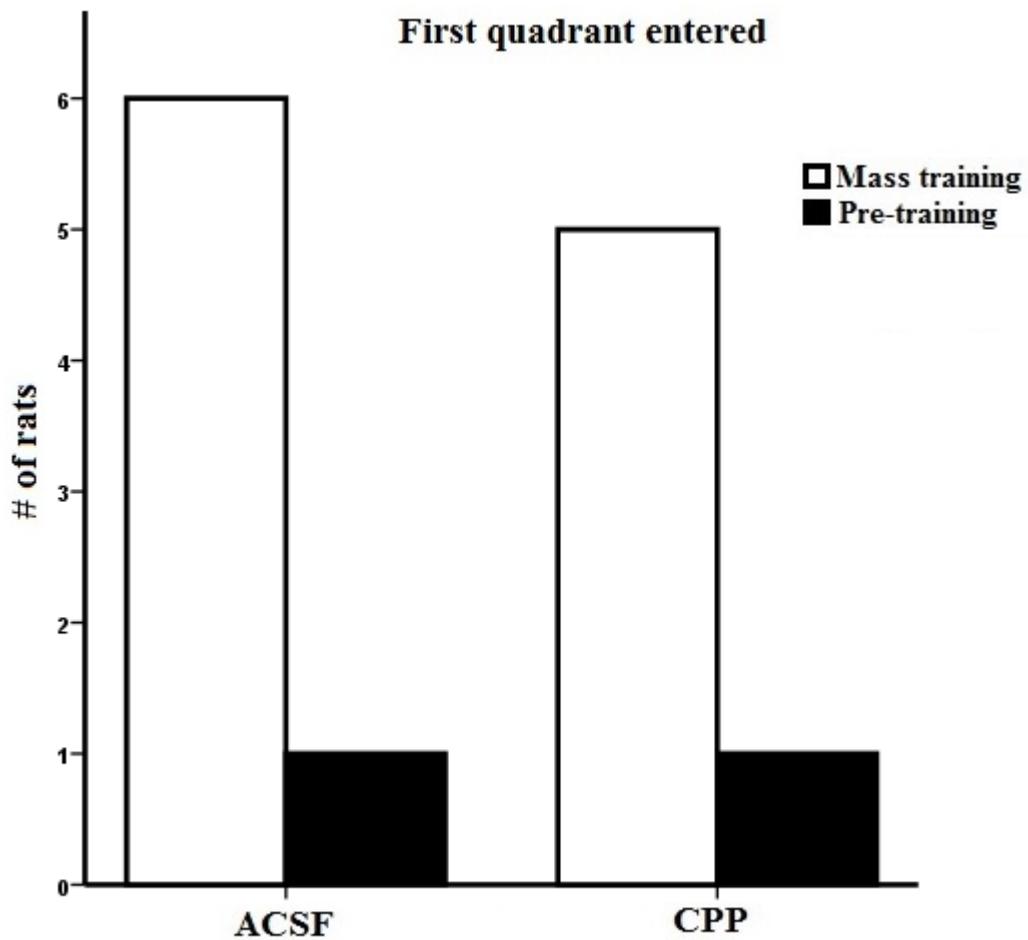


Fig. 1.8: The number of rats that swim to either the mass trained quadrant or pre-trained quadrant first in the 30 second probe done immediately after mass training for each group in experiment 1, cohort 1. Most of the rats from each group swim first to the mass trained quadrant.

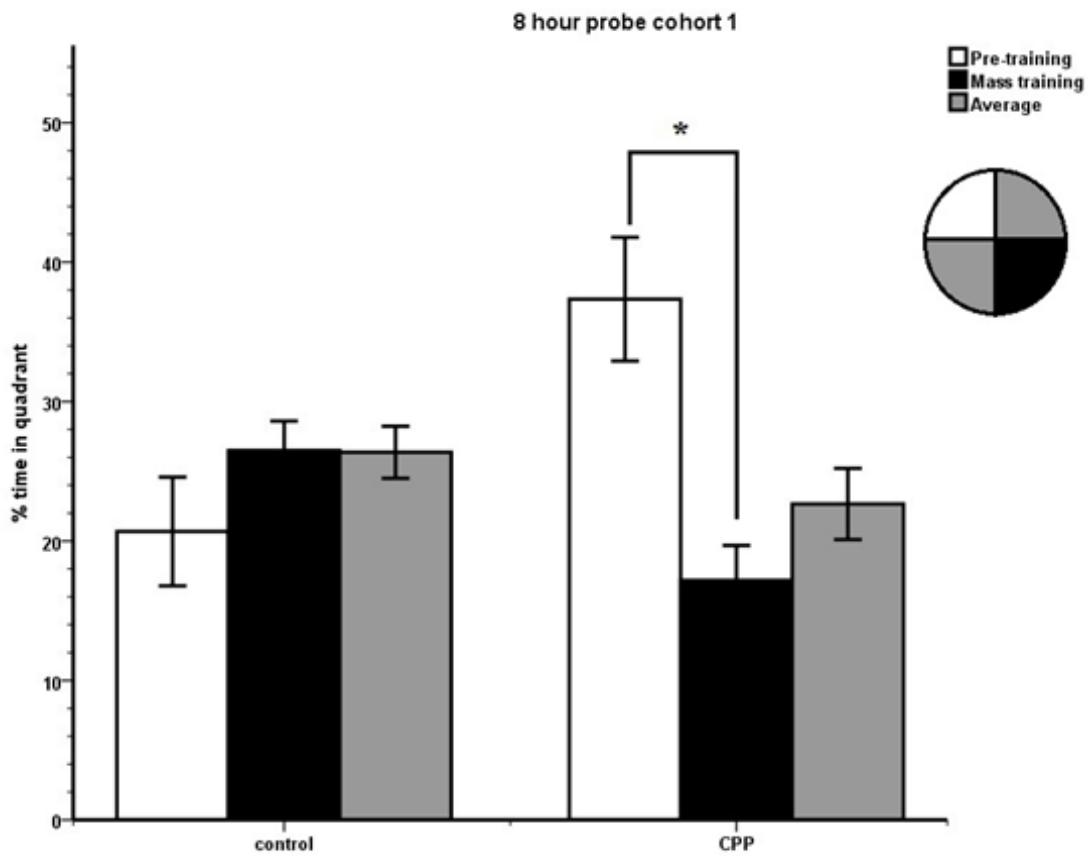


Figure 1.9: 30 second probe test done 8 hours after massed reversal training comparing preference for the pre-trained location, massed reversal trained location in experiment 1, cohort 1. CPP rats display a preference for the pre-trained quadrant, control rats do not. *
 $p < 0.05$, Error bars: ± 1 SE

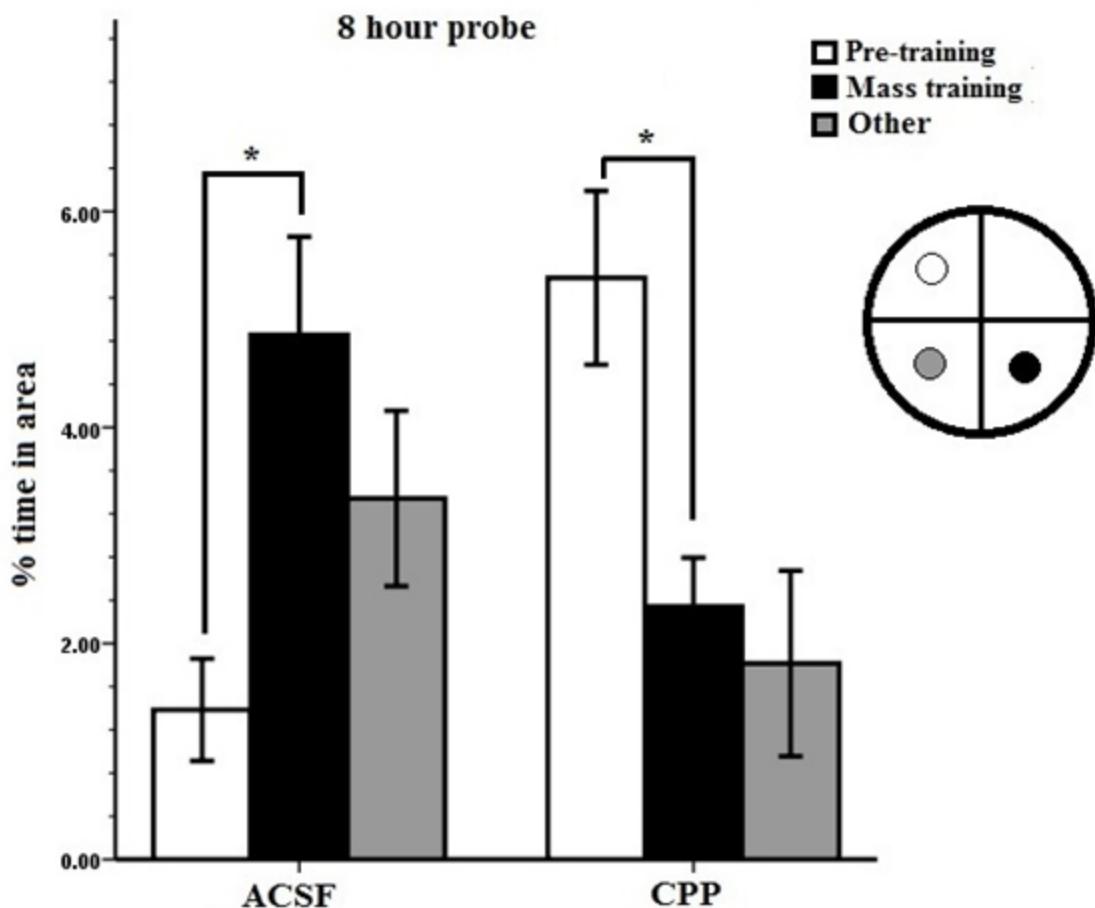


Fig. 1.10: 30 second probe test done 8 hours after massed reversal training comparing time spent in an area immediately surrounding the mass training platform location (2% of total pool surface area) to the pre-training platform location in experiment 1, cohort 1. The CPP rats show preference for the pre-trained location while the control rats show the opposite, a preference for the mass trained location compared to the pre-trained location.
 $* p < 0.05$, Error bars: ± 1 SE

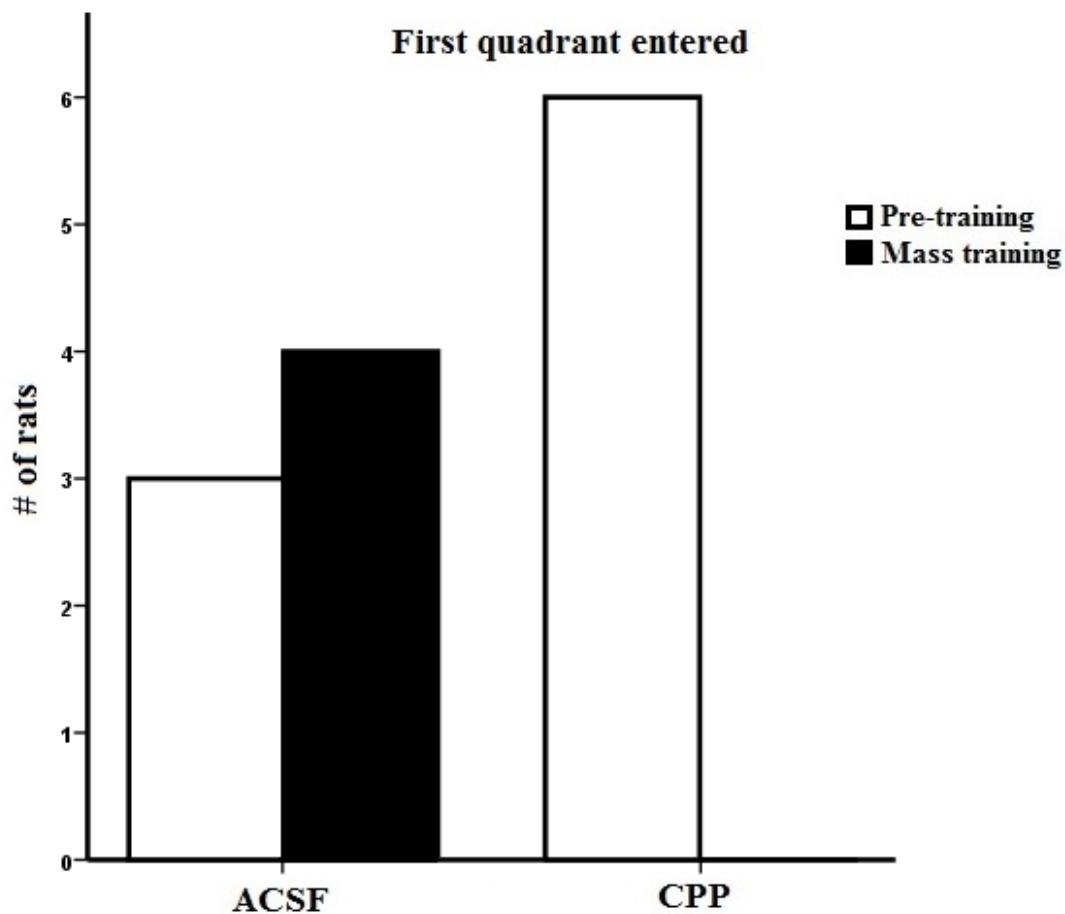


Fig. 1.11: The number of rats that swim to either the mass trained quadrant or pre-trained quadrant first in the 30 second probe done 8 hours after mass training for each group in experiment 1, cohort 1. Most of the rats from the CPP group swim first to the pre-trained quadrant. The control rats split and go to either, displaying no uniform pattern of direction.

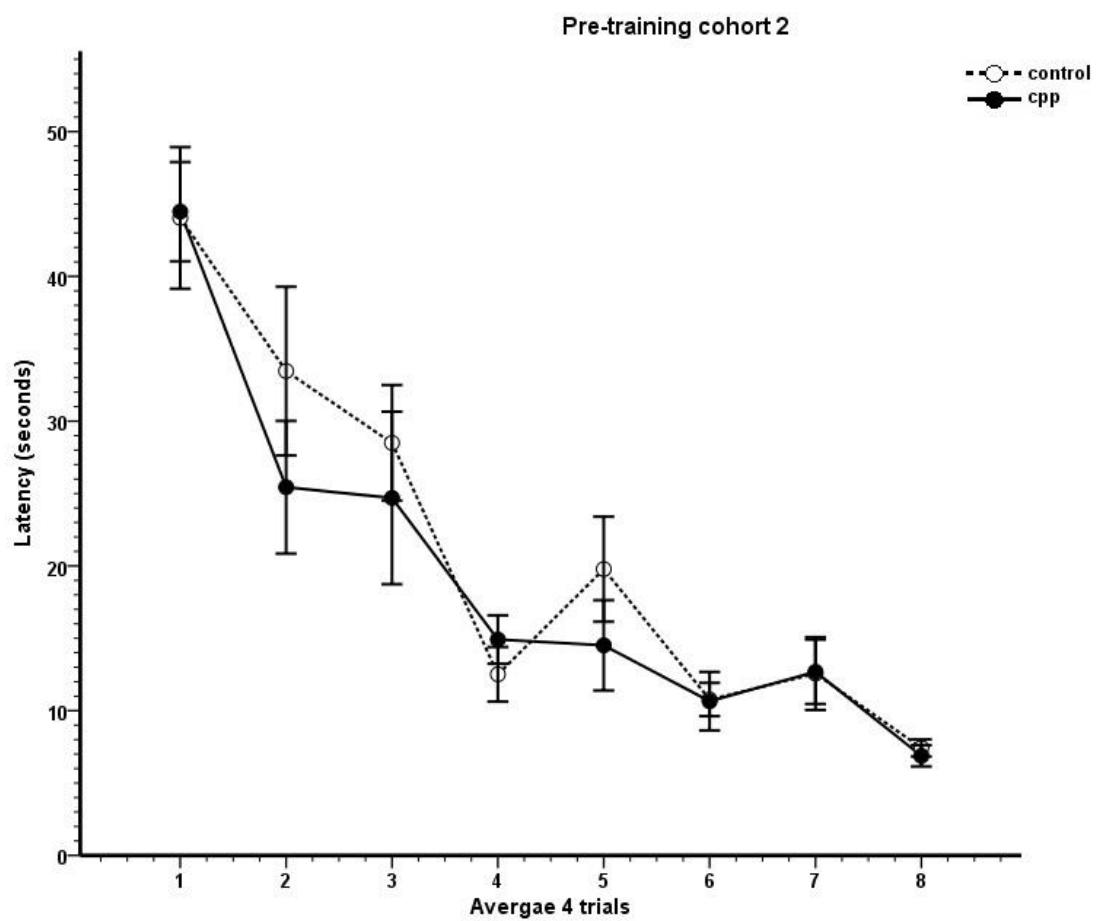


Figure 1.12: Pre-training performance of rats in cohort 2, experiment 1 on the morris water maze- Latency. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

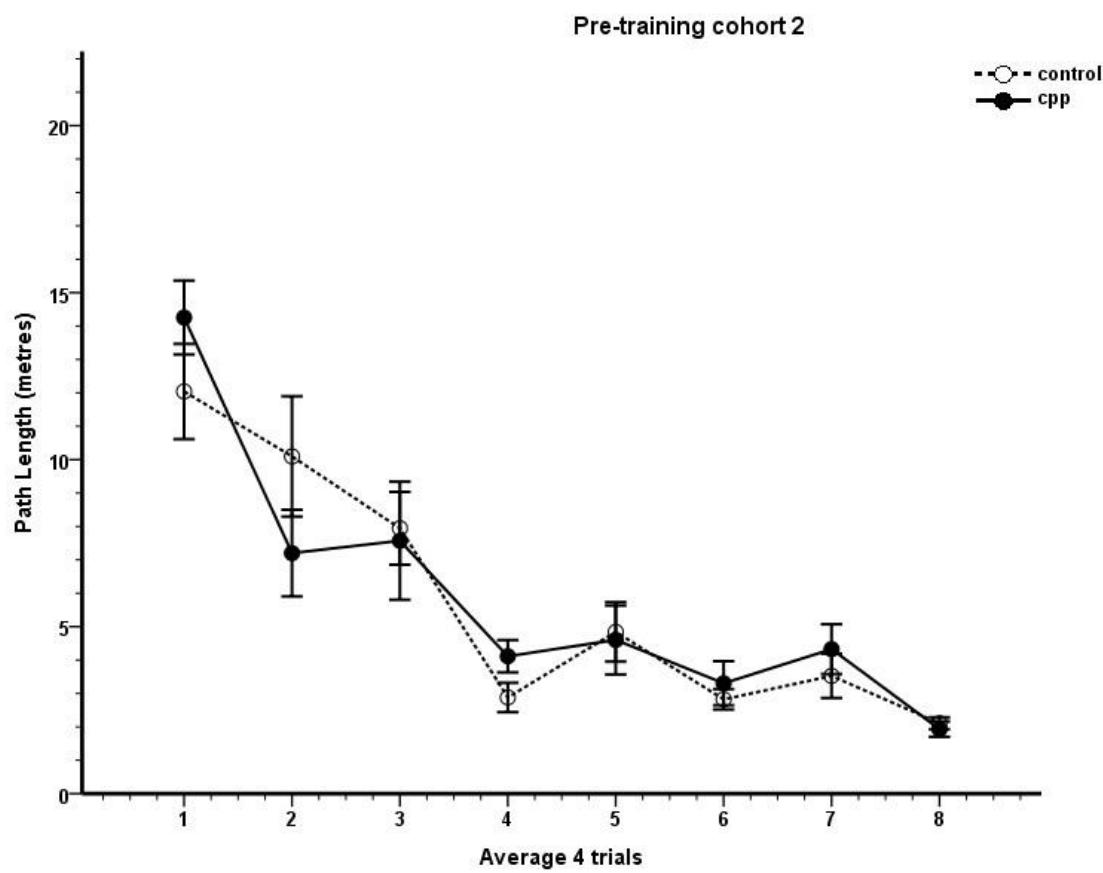


Figure 1.13: Pre-training performance of rats in cohort 2, experiment 1 on the morris water maze- Path length. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

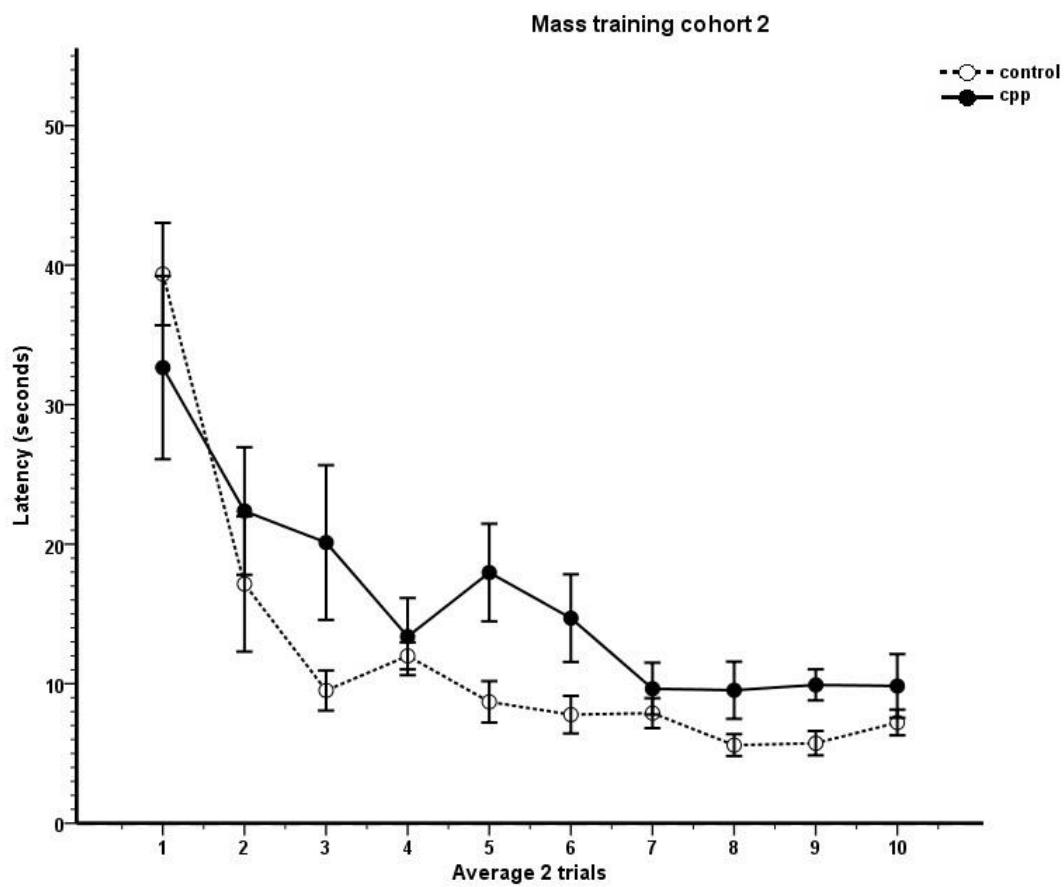


Figure 1.14: Mass training performance of rats in cohort 1, experiment 1 on the morris water maze- Latency. Rats were trained over 2 hours, 20 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE.

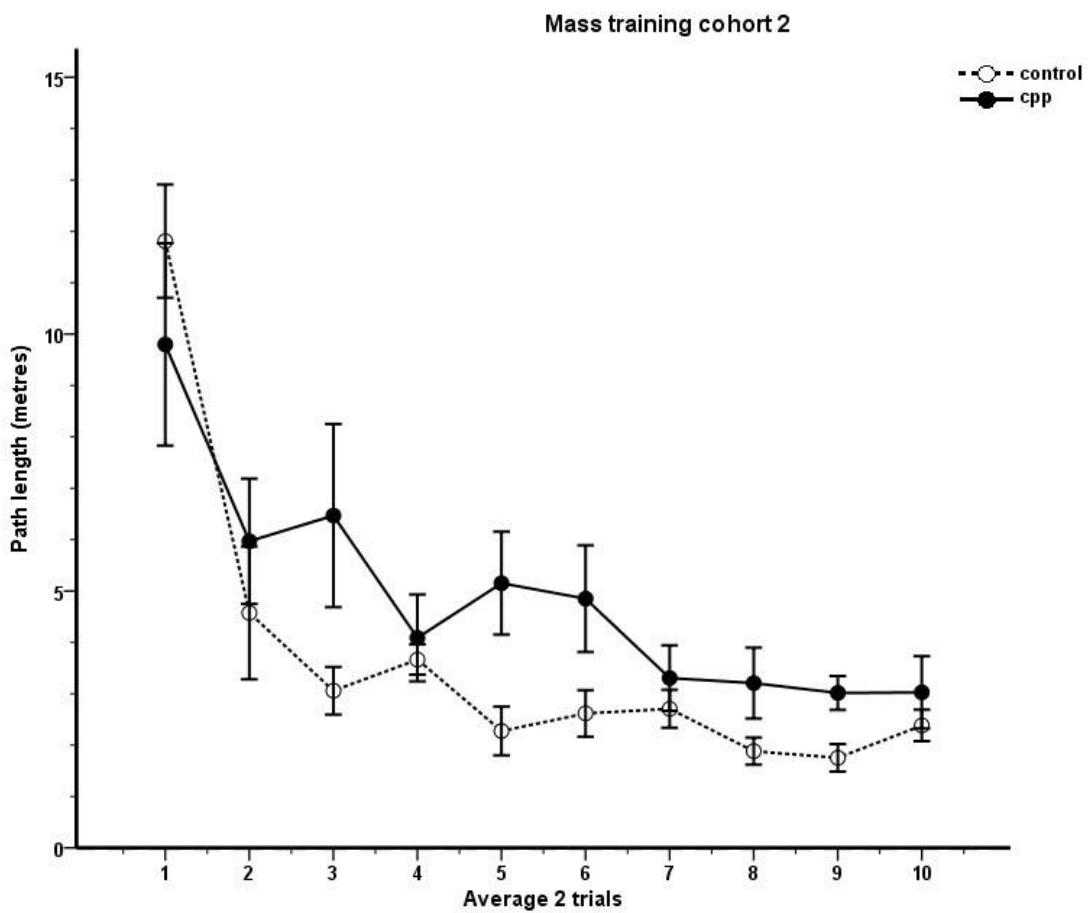


Figure 1.15: Mass training performance of rats in cohort 2, experiment 1 on the morris water maze- Path length. Rats were trained over 2 hours, 20 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

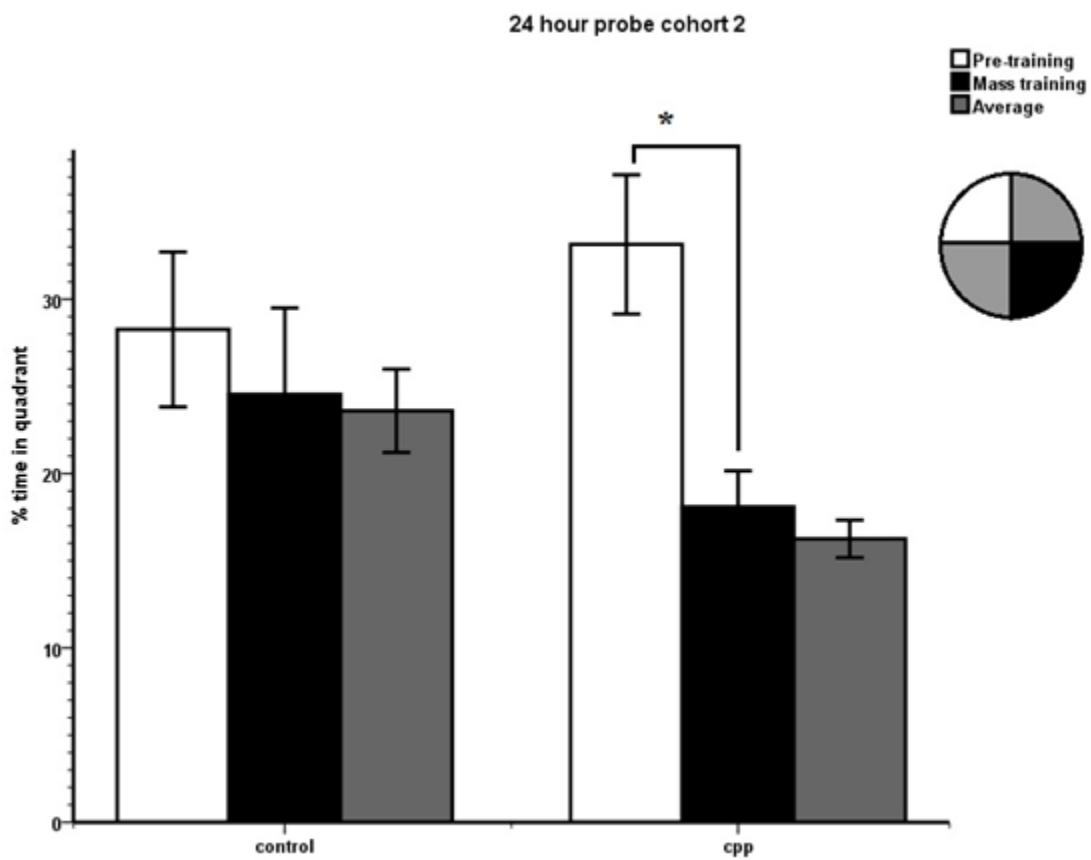


Figure 1.16: 30 second probe test done 24 hours after massed reversal training comparing preference for the pre-trained location, massed reversal trained location in experiment 1, cohort 2. CPP rats display a preference for the pre-trained quadrant, control rats do not. *
 $p < 0.05$, Error bars: ± 1 SE

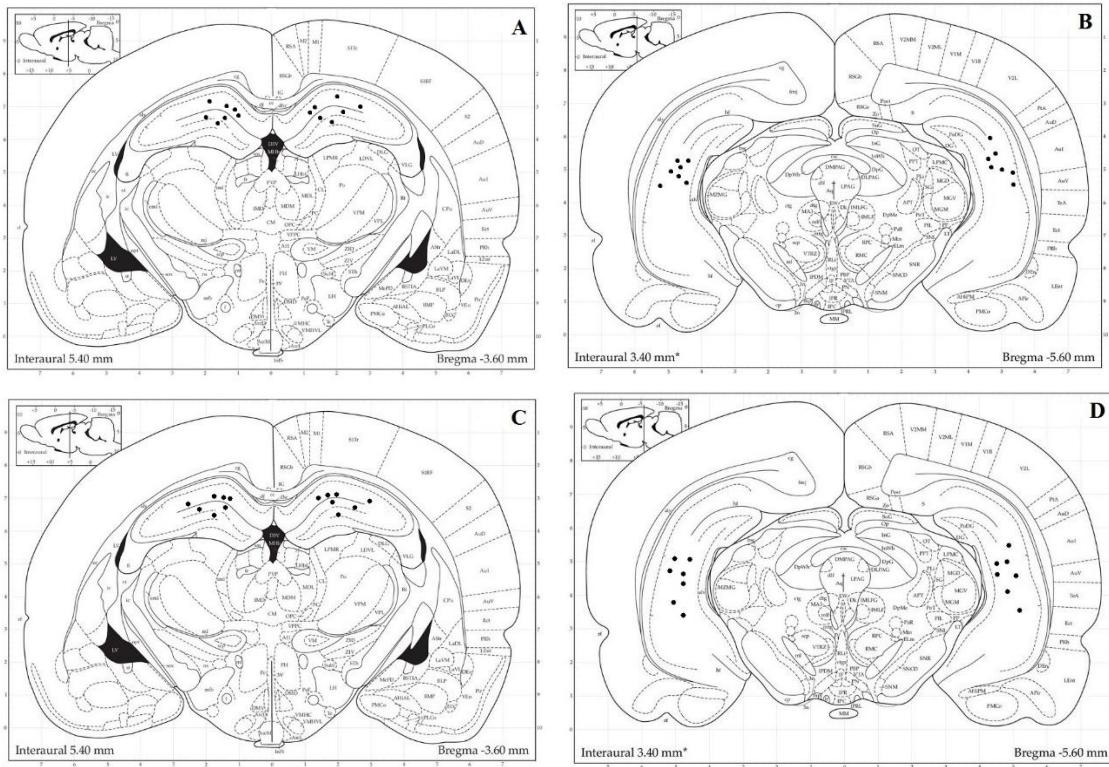


Figure 1.17: Dorsal and ventral hippocampal cannulation placements for CPP group rats, experiment 1. Cannulation end points were taken from hippocampal slices and imposed onto images of the dorsal and ventral hippocampi. **A.** dorsal cohort 1, **B.** ventral cohort 1, **C.** dorsal cohort 2, **D.** ventral cohort 2.

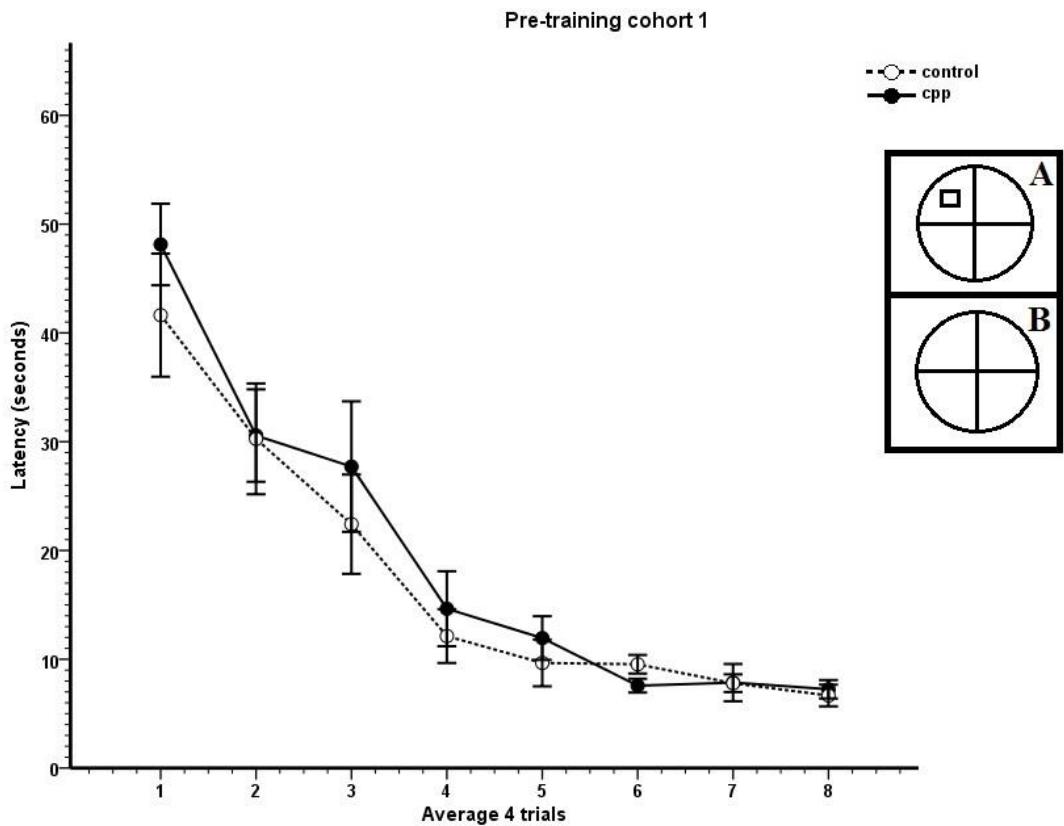


Figure 2.1: Pre-training performance of rats in cohort 1, experiment 2 on the morris water maze in room A- Latency. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

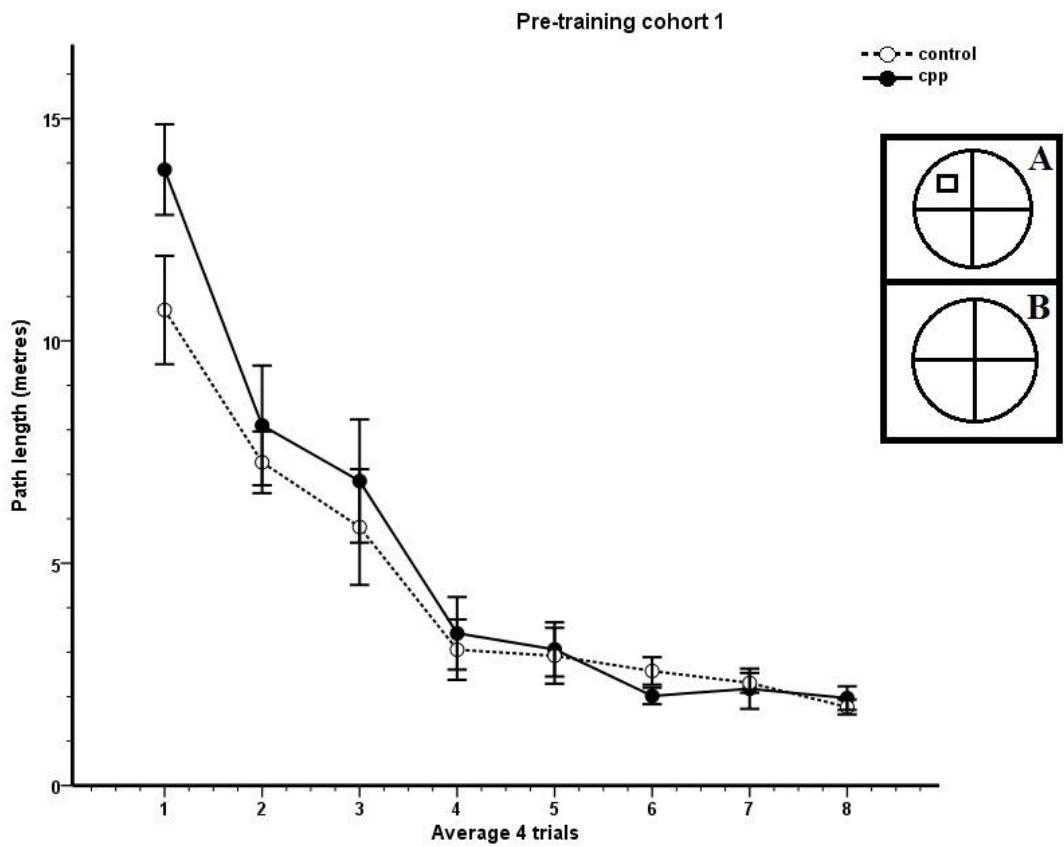


Figure 2.2: Pre-training performance of rats in cohort 1, experiment 2 on the morris water maze in room A- Path length. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

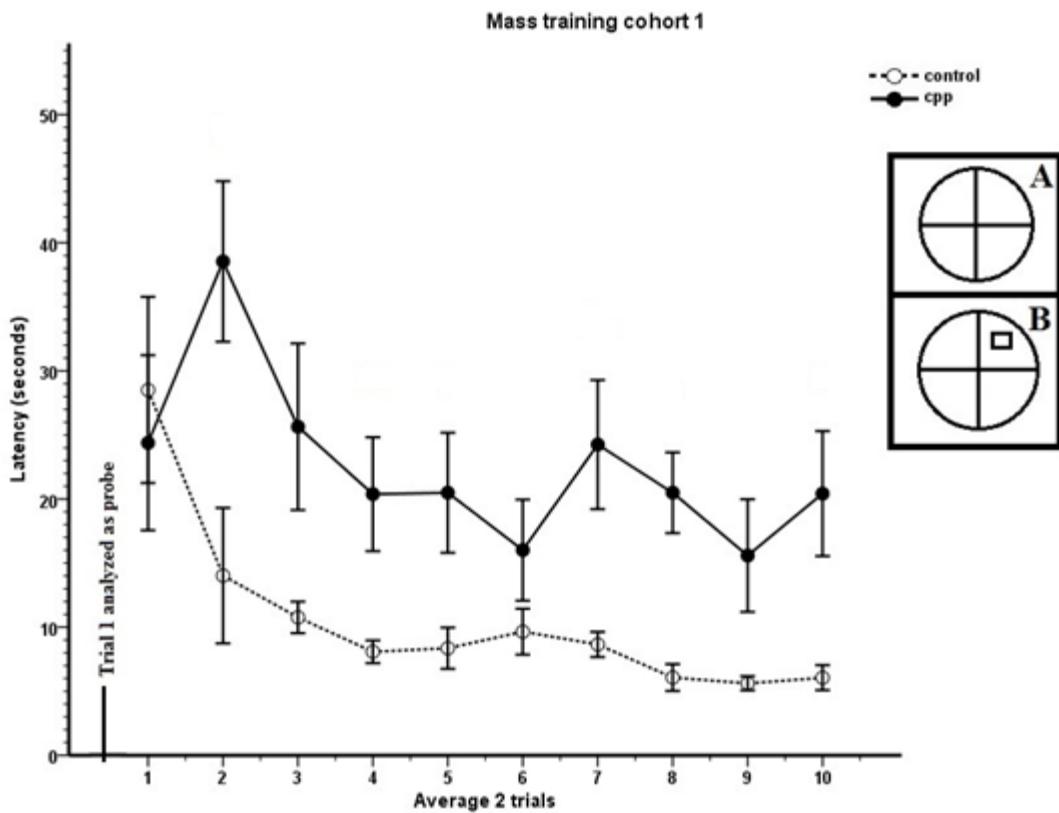


Figure 2.3: Mass training performance of rats in cohort 1, experiment 2 on the morris water maze in room B- Latency. Rats were trained over 2 hours, 20 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE.

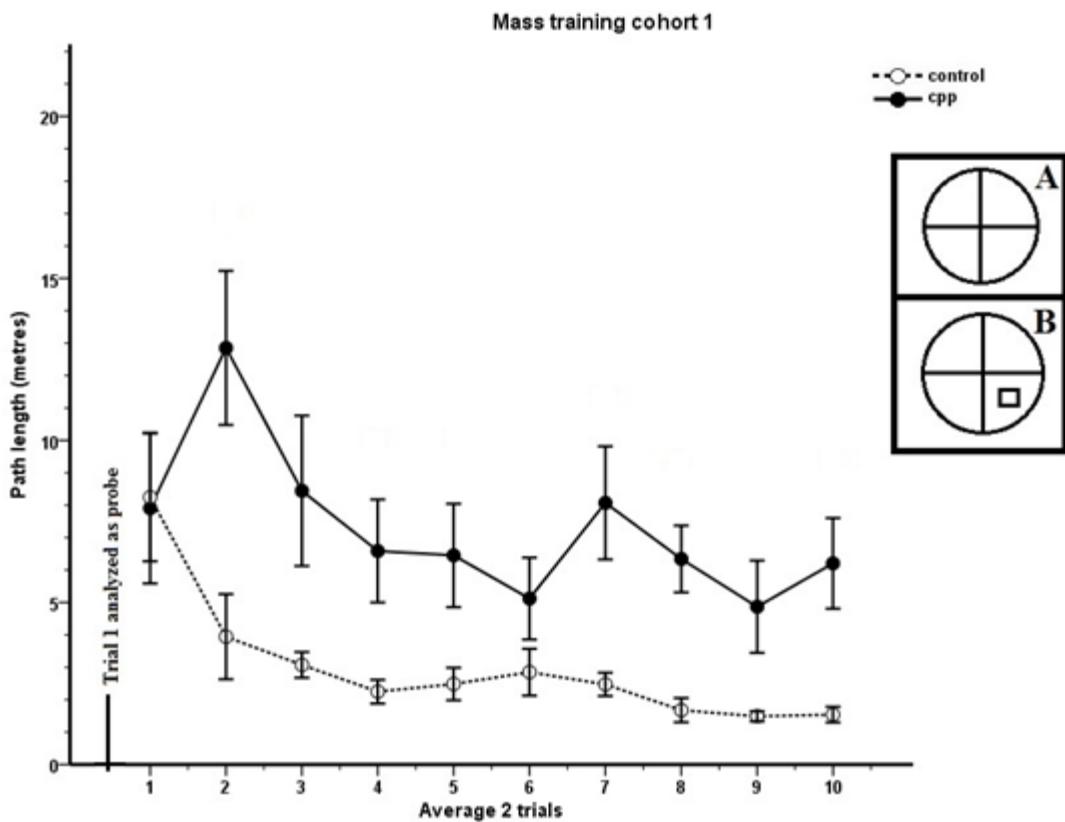


Figure 2.4: Mass training performance of rats in cohort 1, experiment 2 on the morris water maze in room B- Path length. Rats were trained over 2 hours, 20 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

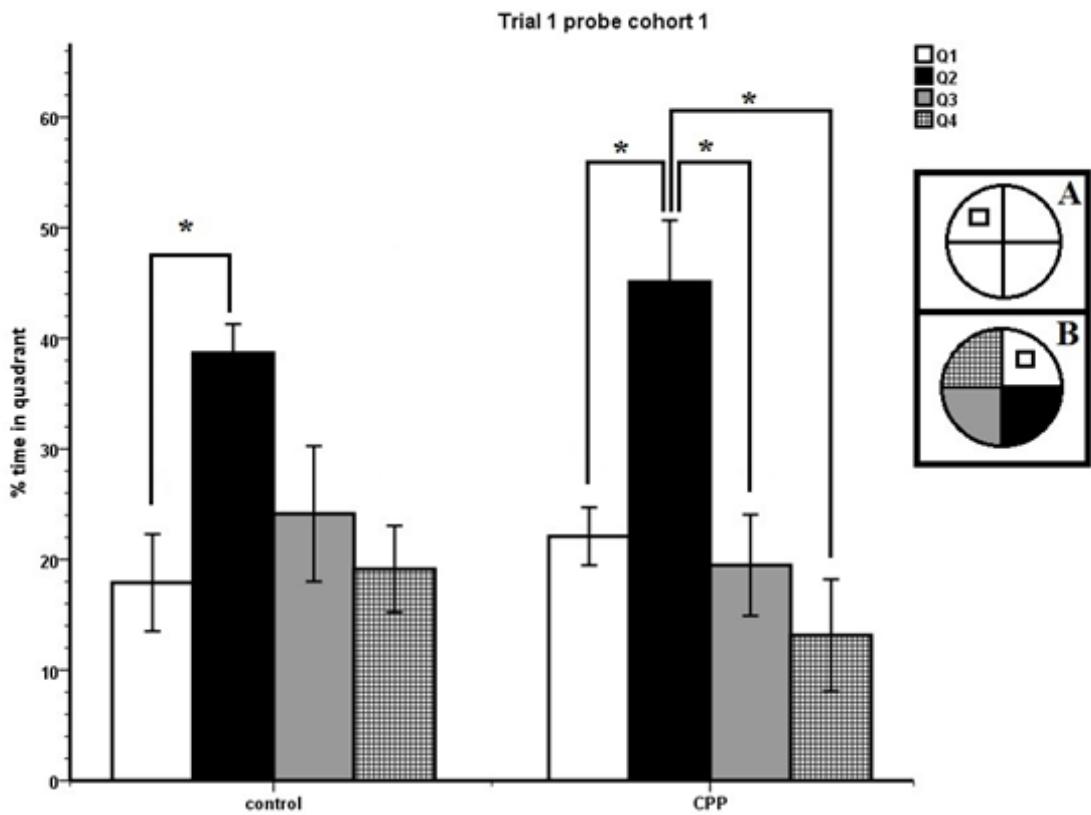


Figure 2.5: Trial 1 of mass training in room B analyzed as a 30 second probe to test transfer of spatial information, cohort 1 in experiment 2. Rats were pre-trained in room A and brought to room B for the first time. The percentage of time spent in each quadrant was measured. During trial 1 of pre-training rat from both groups display preferences for quadrant 2, despite never having been trained in that pool or room before. * $p < 0.05$, Error bars: ± 1 SE

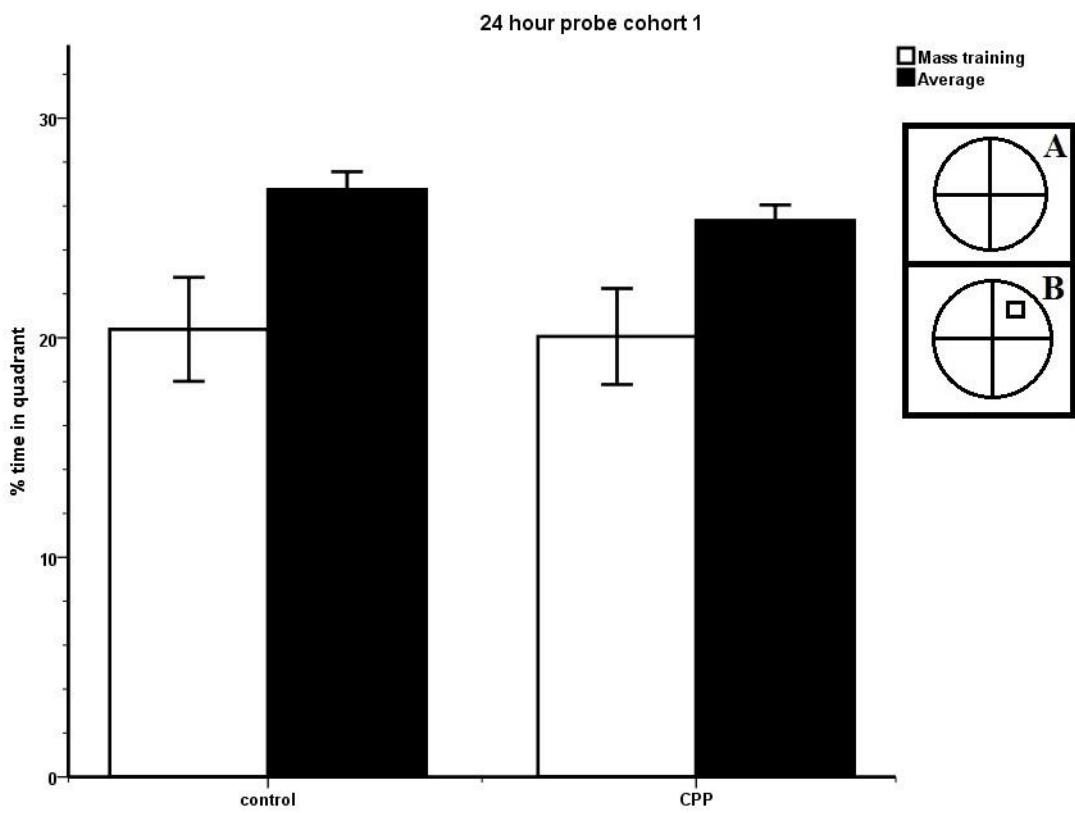


Figure 2.6: 30 second probe test done 24 hours after massed reversal training comparing preference for the mass trained quadrant and an average of the other three quadrants in experiment 2, cohort 1. Neither group of rats maintained a preference for the mass trained quadrant 24 hours after mass training. * $p < 0.05$, Error bars: ± 1 SE

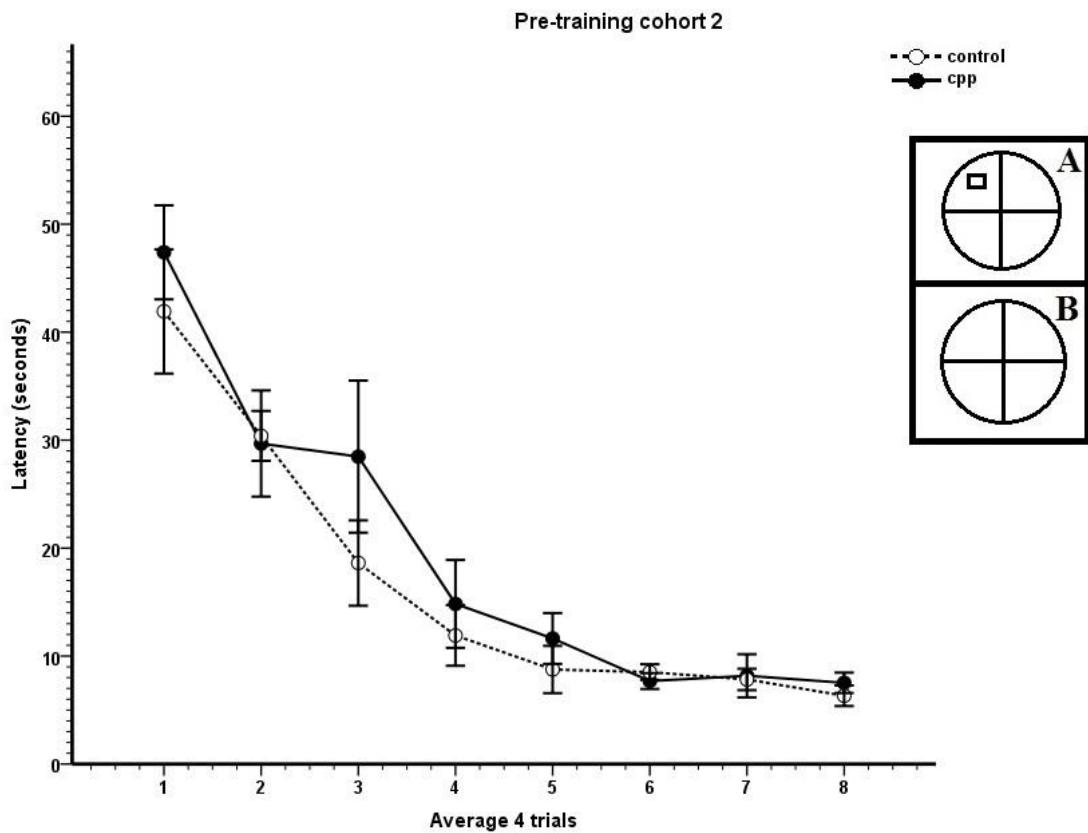


Figure 2.7: Pre-training performance of rats in cohort 2, experiment 2 on the morris water maze in room A- Latency. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

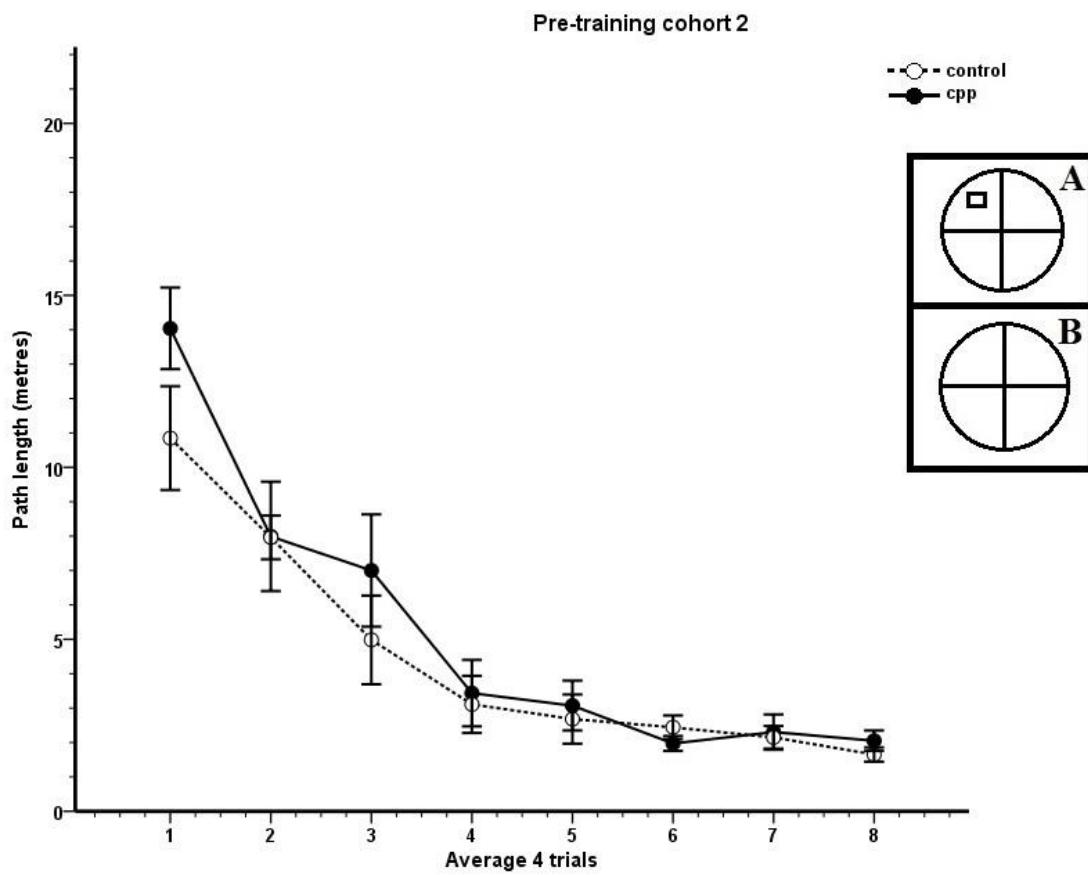


Figure 2.8: Pre-training performance of rats in cohort 1, experiment 2 on the morris water maze in room A- Path length. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

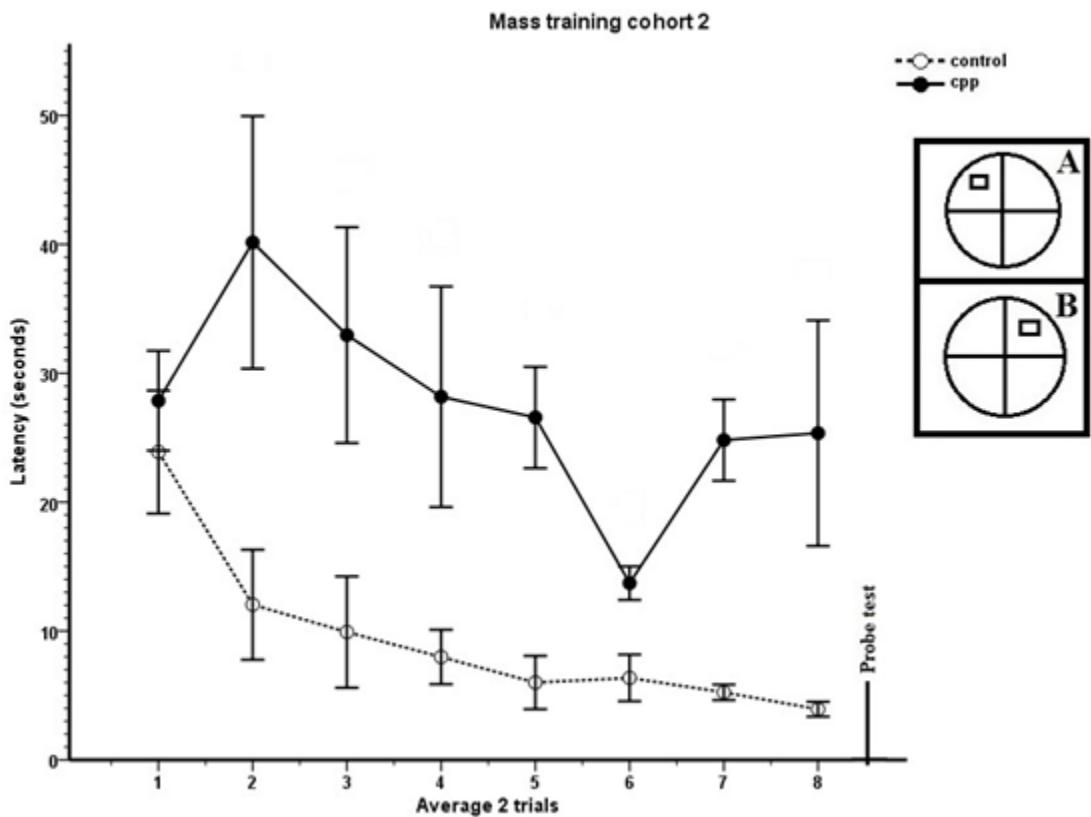


Figure 2.9: Mass training performance of rats in cohort 2, experiment 2 on the morris water maze in room B- Latency. Rats were trained over 2 hours, 16 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE.

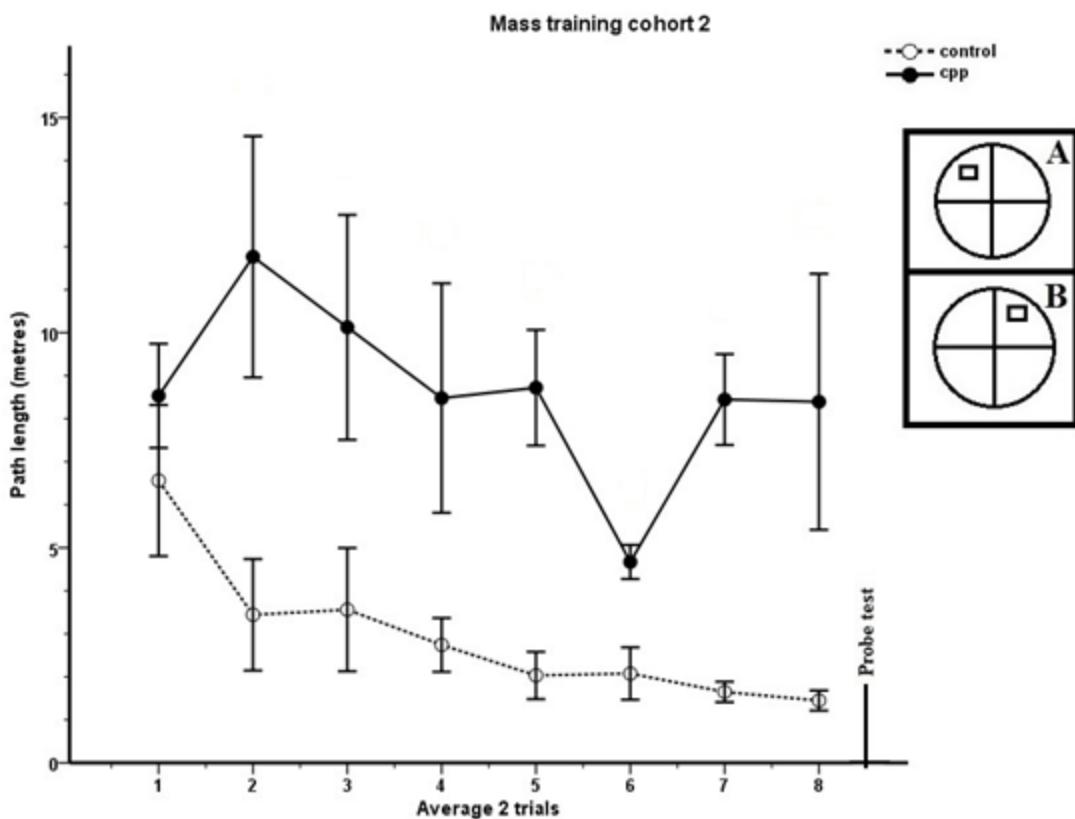


Figure 2.10: Mass training performance of rats in cohort 2, experiment 2 on the morris water maze in room B- Path length. Rats were trained over 2 hours, 16 trials to a platform in the opposite quadrant to that of pre-training. Here is shown 2 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. Both groups successfully learn. Error bars: +/- 1 SE

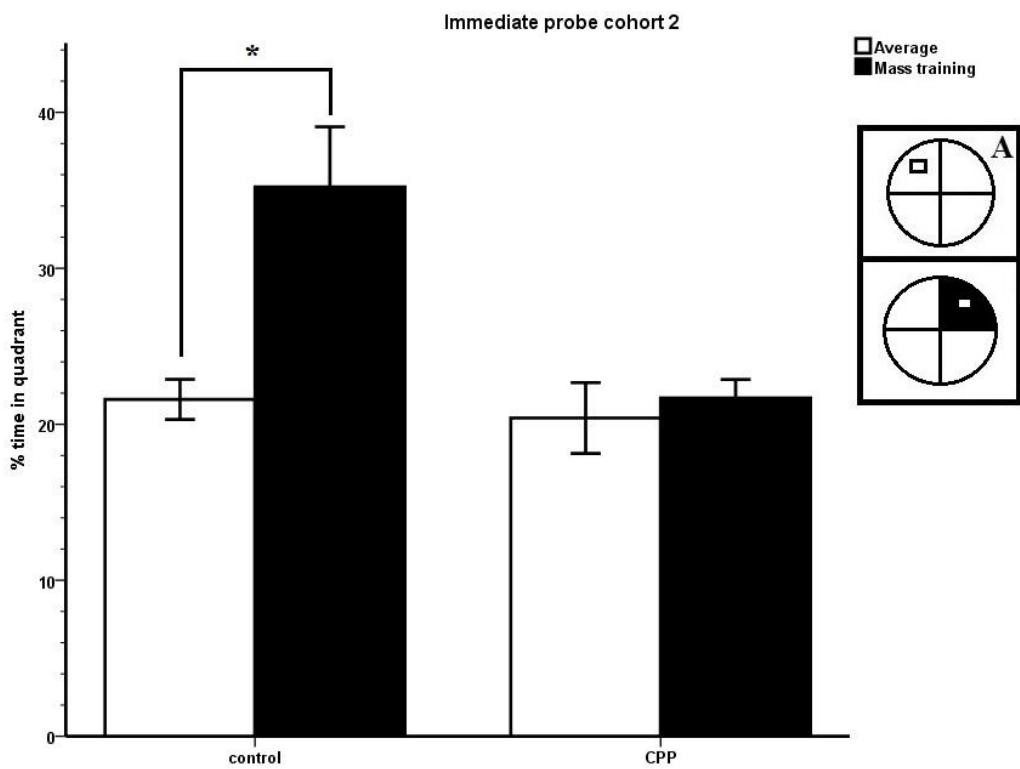


Figure 2.11: 30 second probe test done 24 hours after massed training in room B comparing preference for the mass trained quadrant in experiment 2, cohort 2. Control rats display a preference for the mass trained quadrant, CPP rats do not. * p < 0.05, Error bars: +/- 1 SE

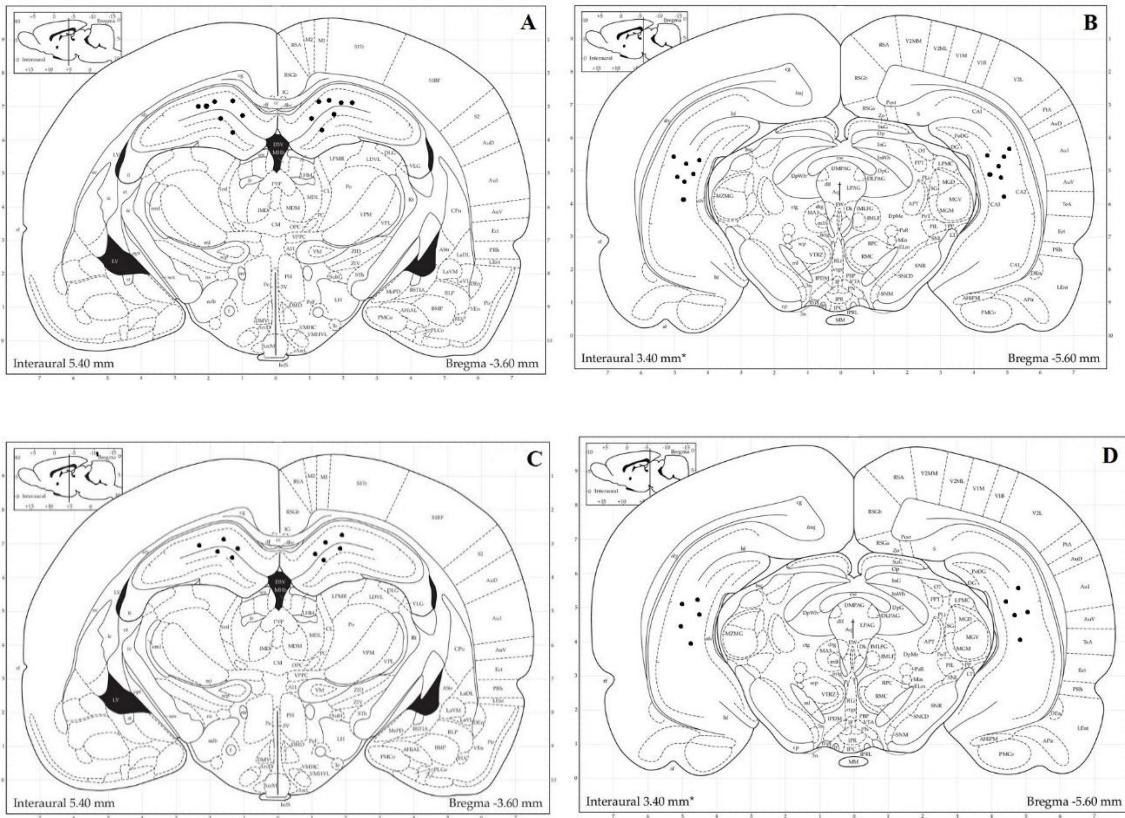


Figure 2.12: Dorsal and ventral hippocampal cannulation placements for CPP group rats, experiment 2. Cannulation end points were taken from hippocampal slices and imposed onto images of the dorsal and ventral hippocampi. **A.** dorsal cohort 1, **B.** ventral cohort 1, **C.** dorsal cohort 2, **D.** ventral cohort 2.

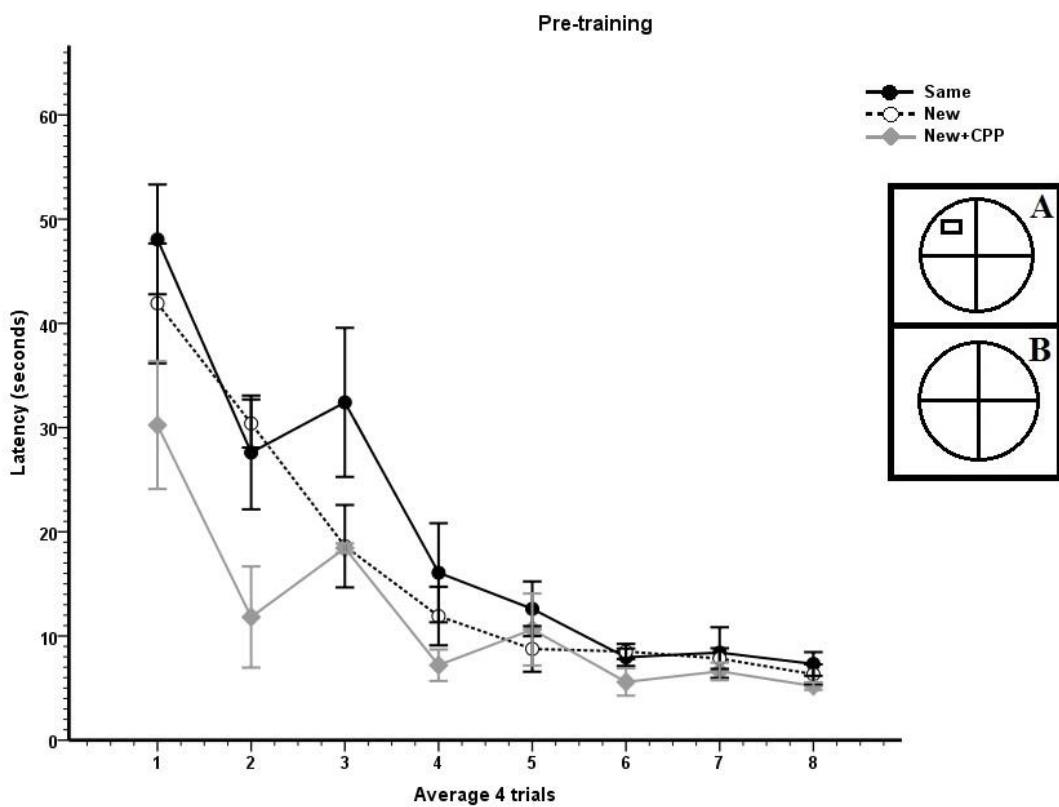


Figure 3.1: Pre-training performance of rats in experiment 3 on the morris water maze in room A- Latency. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. All groups successfully learn. Error bars: ± 1 SE

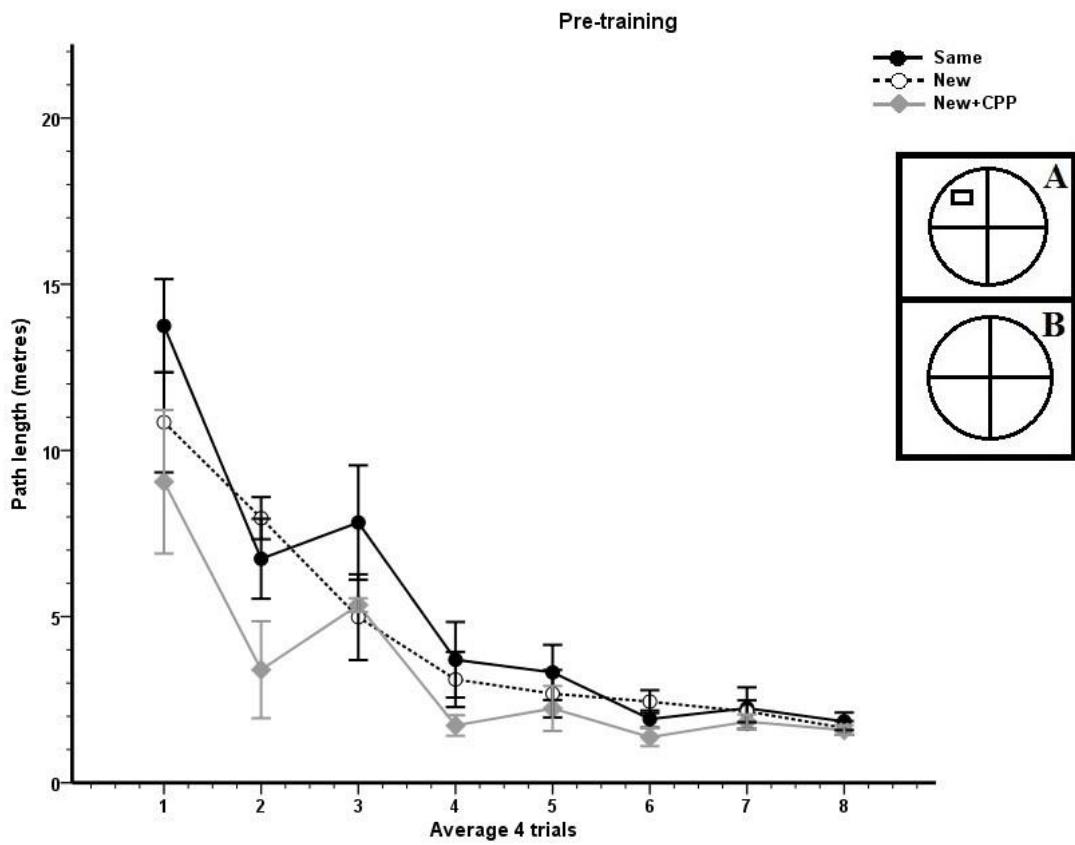


Figure 3.2: Pre-training performance of rats in experiment 3 on the morris water maze in room A- Path length. Rats were trained over 4 days, 8 trials per day. Here is shown 4 trial averages of the length of the swim paths it took the rats from each group to find the hidden platform over the course of training. All groups successfully learn. Error bars: +/- 1 SE

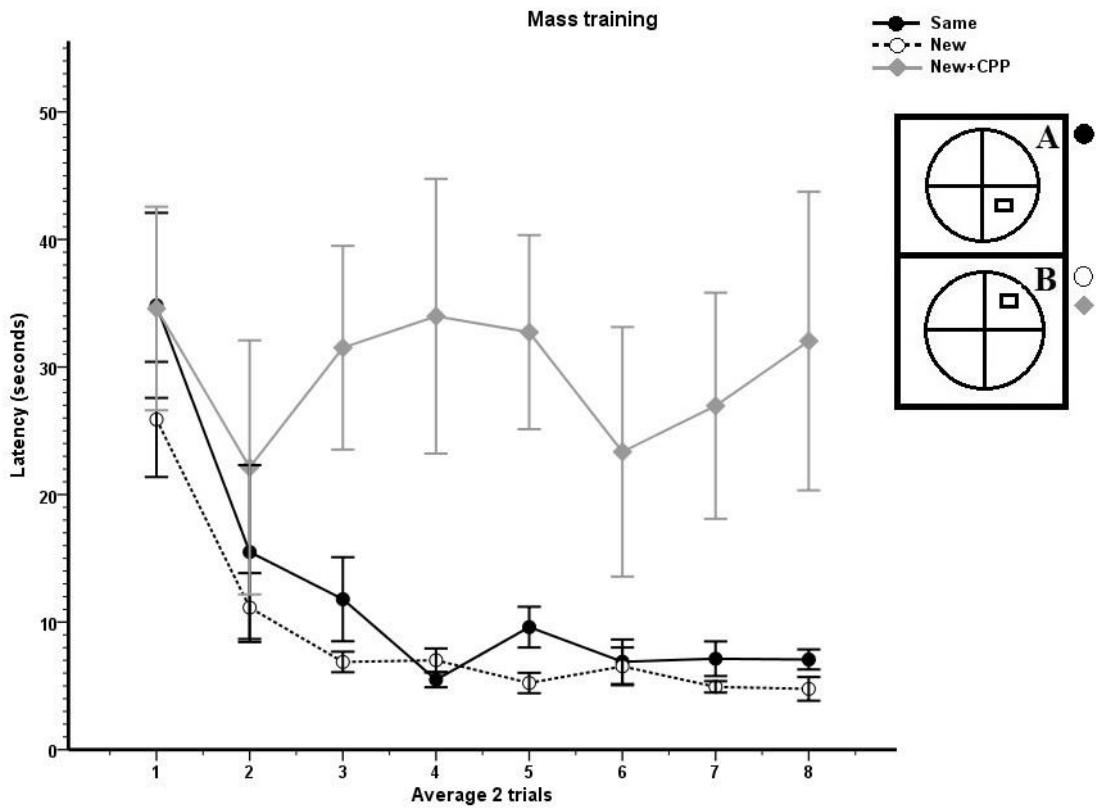


Figure 3.3: Mass training performance of rats in experiment 3 on the morris water maze in room B- Latency. Rats were trained over 2 hours, 16 trials to a platform in one of three conditions: a new platform location in room A, in room B, or in room B with CPP infusion. Here is shown 2 trial averages of the time it took the rats from each group to find the hidden platform over the course of training. Both Same and New groups successfully learned, and CPP+new rats did not. Error bars: +/- 1 SE.

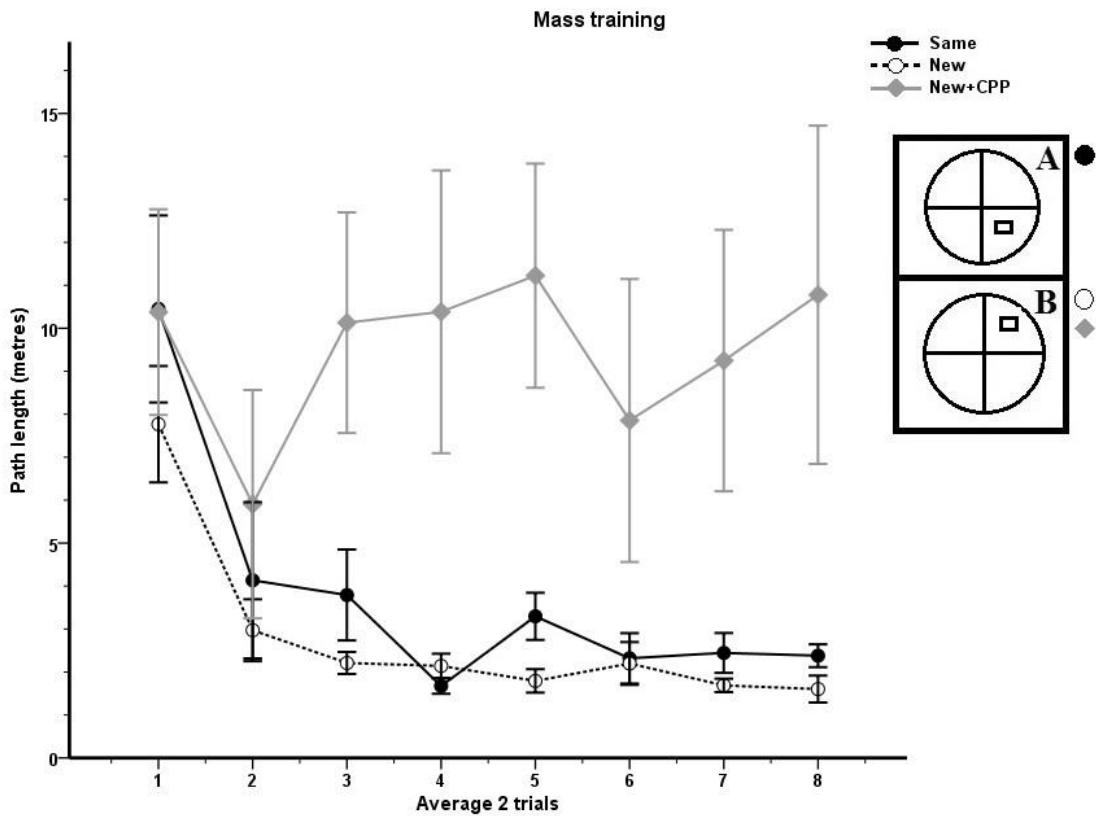


Figure 3.4: Mass training performance of rats in experiment 3 on the morris water maze in room B- Path length. Rats were trained over 2 hours, 16 trials to a platform in one of three conditions: a new platform location in room A, in room B, or in room B with CPP infusion. Here is shown 2 trial averages of the length of the swim path it took the rats from each group to find the hidden platform over the course of training. Both Same and New groups successfully learned, and CPP+new rats did not. Error bars: +/- 1 SE.

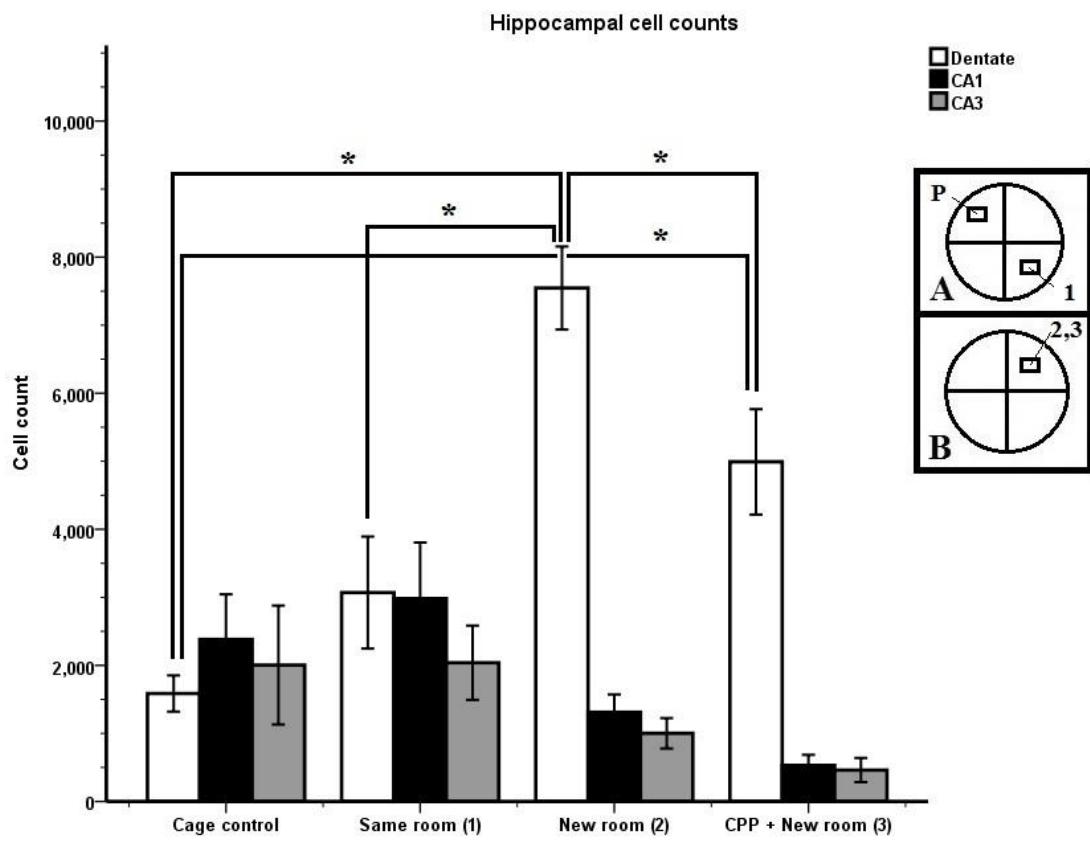


Figure 3.5: Hippocampal cell counts of Arc positive cells in the dentate gyrus, CA1, and CA3, 70-80 minutes after trial 8 of massed training. * $p < 0.05$, Error bars: ± 1 SE

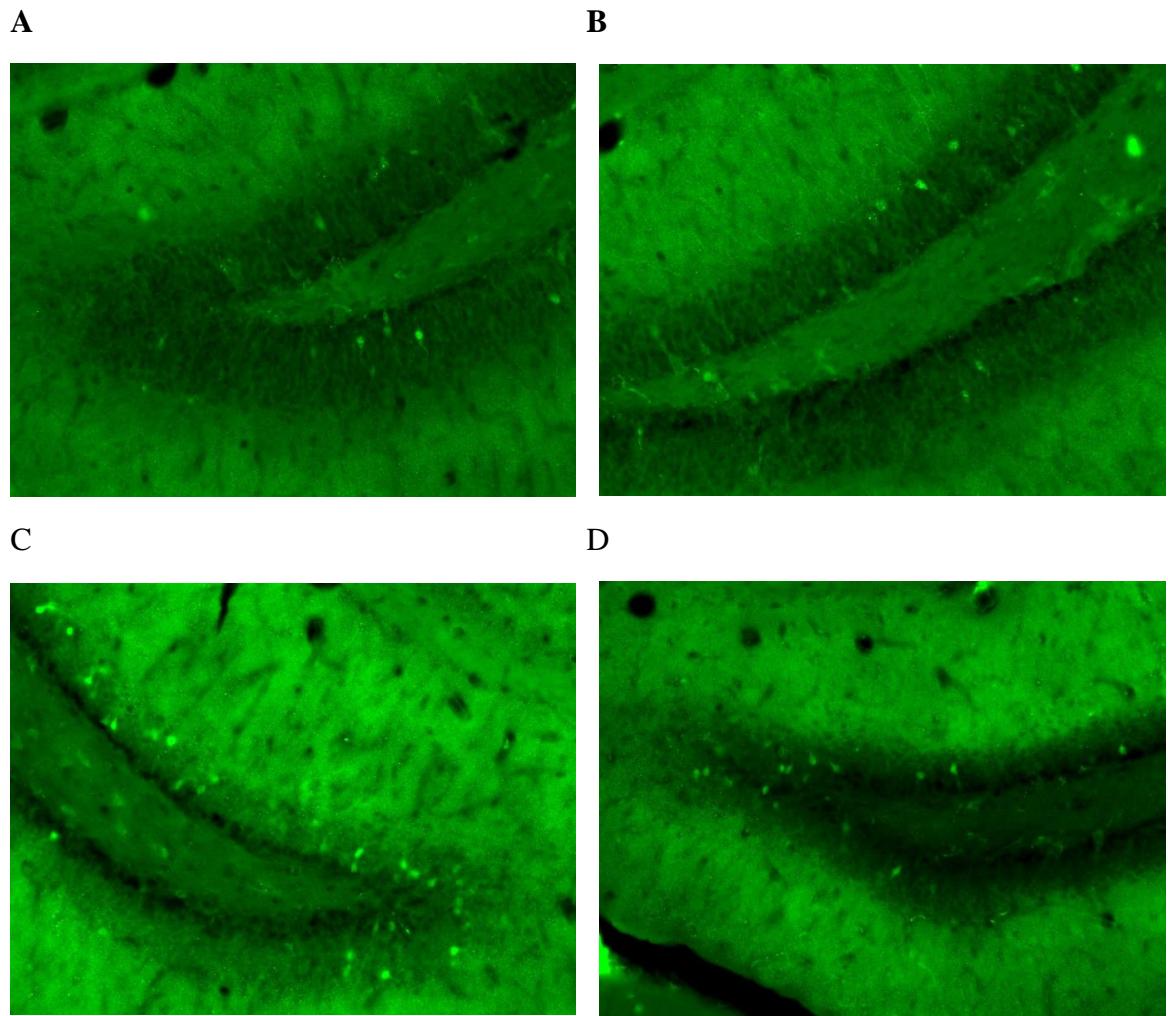


Figure 3.6: Granule cells in the dorsal dentate gyrus stained positive for Arc protein. **A** Cage controls. **B** Same room. **C** New room. **D** New+CPP.

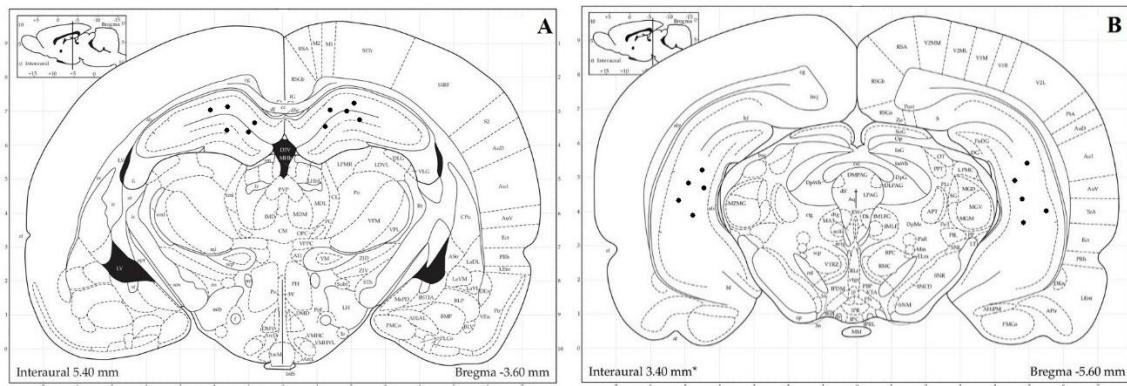


Figure 3.7: Dorsal and ventral hippocampal cannulation placements for CPP group rats, experiment 3. Cannulation end points were taken from hippocampal slices and imposed onto images of the dorsal and ventral hippocampi. **A.** dorsal, **B.** ventral.