The Acquisition and Expression of Remote Spatial Memories Formed During the
Juvenile Period are Impaired by AMPAr Blockade

by

Nikolaos Tzakis

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Carleton University

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Nikolaos Tzakis

Abstract

The current work investigated the effect of AMPAr blockade on the emergence and expression of spatial function and the neural activity patterns in the hippocampus and anterior cingulate cortex (ACC) during remote memory retrieval. Systemic administration of the AMPAr antagonist, NBQX, (0, 5 or 10 mg/kg) prior to training impaired the ability to learn the location of a hidden platform. Administration of NBQX immediately posttraining had no effect on the day-to-day performance. When given a probe test 3 weeks later, the saline group across all conditions spent more time in the target quadrant. The pretraining and pretesting 5 mg/kg NBQX group showed a preference for the target quadrant whereas the posttraining and pretraining/pretesting 5 mg/kg NBQX groups did not. Pretraining, posttraining, and pretraining/pretesting, but not pretesting, groups injected with 10 mg/kg of NBQX did not show a preference for the target quadrant. c-Fos labeling in the hippocampus reflected these differences in probe performance while ACC c-Fos labeling reflected acquisition data. Results indicate that AMPAr activation is necessary for the acquisition and expression of remote spatial memories and that hippocampus activation is required for the expression of those memories.

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List of Abbreviations

AMPAr : α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor

NMDAr : N-methyl-D-aspartate receptors

CA : Cornu ammonis

LTP : Long-term potentiation

P : Postnatal day

GluR : Glutamate receptor subunit

ACC : Anterior cingulate cortex

Introduction

Overview of consolidation

Synaptic consolidation involves neural and biochemical processes that work to reorganize synapses once learning has taken place. Consolidation involving cellular events, known as cellular consolidation, has been hypothesized to be responsible for transforming an initially labile memory to a more permanent representation (McGaugh, 2000). Once this has taken place, memories undergo what is classified as systems consolidation whereby a memory, for example, initially begins as being hippocampus-dependent to one that is hippocampus-independent (Meeter & Murre, 2004; Rudy & Sutherland, 2008). In this model, systems consolidation theory is based on the idea that the hippocampus only serves as a temporary repository of memories (Frankland & Bontempi, 2005). The systems consolidation model posits that after some time has elapsed (months to years), the memory initially processed and stored within the hippocampus is “transferred” to the neocortex via hippocampal-neocortical connections. Although no exact timeframe is known outlining the amount of time required for a memory to transfer from one brain region to another, it appears that remotely stored memories come to be stored in and retrieved by neural circuits in the frontal lobe (Frankland & Bontempi, 2005). Within the frontal lobe circuits, cellular consolidation stabilizes the memory and strengthens both the memory representation within the frontal lobe circuits and connections made with the hippocampus in order to facilitate retrieval of the memory (Meeter & Murre, 2004). As the memory stored in the neocortical region strengthens, the memory representation weakens within the hippocampus (Meeter & Murre, 2004).

Cellular consolidation

Cellular consolidation, also known as synaptic consolidation, is a process through which synapses are strengthened and memory representations are stabilized by initializing cellular and molecular processes within the hippocampus and other brain regions (Mednick *et al.*, 2011; Rudy & Sutherland, 2008). The conversion of short-term memories into long-term ones involves the synthesis of new proteins, which allow for persistent modifications in synaptic transmissions (Debiec *et al.*, 2002). Dudai (2004) explains synaptic consolidation as being triggered by receptor activation leading to a series of intracellular signal transduction cascades such as the activation of adenylate cyclase that initiates cyclic adenosine monophosphate (cAMP) cascade. The initiation of intracellular signal transduction cascades that lead to the expression of certain transcription factors occurs during and immediately after training, which corresponds to synaptic consolidation (Dudai, 2004). cAMP-dependent kinases initiate phosphorylating and activating isoforms of cAMP-response element binding proteins (CREBs). CREBs then allow for a modification of the cAMP response element (CRE) –regulated genes, such as transcription factors that regulate the expression of genes whose products are induced after a few hours, known as late-response genes (Dudai, 2004). In general, these cascades modulate long-term synaptic proteins and synaptic modeling and growth, along with regulating gene expression through the activation of transcription factors (Dudai, 2004).

In addition to modifications of the synapse, the synthesis of neuronal proteins is also required for functional changes to the synapse. Newly modified synaptic proteins tag the activated synapse, which attract new proteins from the cell through a reorganization

of the synapse (Dudai, 2004). In addition, proteins synthesized in the synapse may function to enhance and fortify the synaptic tagging, while acting as retrograde messages that inform the nucleus of the change. The tagged synapses also receive newly synthesized messenger RNAs and proteins via modulation of gene expression in the nucleus. This coordination between the synapse and the nucleus is thought to enhance the specificity of the consolidation process (Dudai, 2004).

Systems consolidation

As previously mentioned, systems consolidation involves processes that mediate the transformation of memories from being hippocampus-dependent to hippocampus-independent. Specifically, systems consolidation posits that, initially, new memories are temporarily stored in the hippocampus and neocortical regions that it projects to. The hippocampus strengthens the initially weak connections within the hippocampal-neocortical network, and after continuous activation of those connections, the new memory trace integrates with pre-existing memories within the neocortex, and the memory storage or retrieval becomes independent of the hippocampus (Mednick *et al.*, 2011; Wang & Morris, 2010; Rudy & Sutherland, 2007). As opposed to cellular consolidation, which is achieved within minutes to hours, systems consolidation processes are achieved over a period of weeks, months, or even years (Dudai, 2004). Research supporting the systems consolidation theory includes functional magnetic resonance imaging studies, which have shown that blood flow to the hippocampus appears to be higher for semantic memory recall of news events within 3 years of the study when compared to older news events (Smith & Squire, 2009; Wang & Morris, 2010). Additionally, Takashima *et al.* (2006) found reduced hippocampal activity during

memory recall tasks for memories that were 90 days old compared to memories that were 1 day old (Wang & Morris, 2010). These results indicate that the hippocampus is responsible for the storage and/or retrieval of the initial memory, but then storage/retrieval is mediated by other brain structures.

Systems consolidation begins with the initial encoding of the memory within the hippocampal and neocortical system, with the hippocampal system possessing the stronger initial memory trace. Through memory recall, the hippocampal-neocortical traces become successively activated and reactivated, which leads to the strengthening of existing neocortical memory, either through adjustments of neocortical connections involving cellular consolidation, or through the establishment of new connections within the neocortex. This strengthening allows the memory trace to integrate with pre-existing memories within the neocortex (Dudai, 2004; Frankland & Bontempi, 2005).

Concurrently, the memory traces within the hippocampal system gradually degrade, resulting in the traces becoming independent of the hippocampus. In this view of systems consolidation, the hippocampus can be seen as ‘teaching’ the neocortex about the information stored in the memory, while freeing up space through degradation to allow for rapid learning of new information without disrupting the memory reservoirs within the neocortex (Dudai, 2004).

Memory retrieval

Memory retrieval involves the expression and reactivation of a memory trace, usually triggered by some external stimulus or cue (Cai, 1990; Lopez *et al.*, 2015). It has previously been assumed that memory retrieval was a passive process, but a recent study has shown that it is, in fact, a very dynamic process (Lopez *et al.*, 2015). Memory

retrieval has been shown to be dependent on the activation of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and metabotropic glutamate (GluR) receptors, but not N-methyl-D-aspartate (NMDA) receptors. Evidently, inactivating AMPA receptors before undergoing a memory retrieval task has shown to diminish recall. In addition to AMPA receptor blockade, the use of GluR antagonists has also prevented the retrieval of contextual fear memories (Szapiro *et al.*, 2002). Systemically injecting rapamycin or anisomycin, which act to inhibit protein synthesis, impaired auditory fear memory recall, and decreased postsynaptic AMPA receptor GluR1 levels. This suggests that, in addition to glutamatergic receptor activation, ongoing protein synthesis is required for memory recall (Lopez *et al.*, 2015).

From a systems view, memory retrieval involves the coordination and activation of different regions in the brain. Positron emission tomography (PET) studies have revealed the involvement of several brain regions including the right prefrontal cortex, the hippocampus and parahippocampus, the anterior and posterior cingulate cortices, the inferior parietal cortex, and the left side of the cerebellum during an episodic retrieval word recognition task (Cabeza *et al.*, 1997; Nyberg *et al.*, 1995). These results suggest the presence of a general episodic retrieval network, composed of the aforementioned brain regions (Nyberg *et al.*, 1995). Although the specific contribution that each of these regions make to the retrieval of memory is unclear, the role of certain brain regions has been postulated. The right prefrontal cortex has been shown to contribute to retrieval attempts, while the hippocampus allows for conscious recollection, and the cerebellum plays a role in self-initiated retrieval (Cabeza *et al.*, 1997).

Spatial memory

The ability for humans and animals to navigate their way around their environment, record information about their spatial orientation, and remember any event that may have occurred within that environment has been credited to spatial memory. Integrating and processing sensory cues from the environment to facilitate navigation through that environment requires spatial working memory processes. A number of factors contribute to spatial memory and successful navigation. Factors such as dimensionality (knowledge of the 3-dimensional elements of the environment), orientation, and self-motion, which informs the individual of any changes in the orientation of spatial representation of the environment all need to be integrated for successful navigation (Burgess *et al.*, 2002). Though a resilient and dynamic process, spatial memory is not impervious to impairment. In fact, one of the first symptoms presented by patients with pathologies such as Alzheimer's has been shown to be impairments of spatial and episodic memory, brought about by cumulative damage to medial temporal lobe structures (Burgess *et al.*, 2002).

One of the more popular approaches to measuring spatial memory and spatial navigation in rats and humans has been the Morris Water Maze. Conceived by Richard Morris in the 1980s, the water maze procedure was used to understand the fundamental basis of spatial and working memory and to differentiate spatial learning from other forms of associative learning (Morris, 1984). Implementing the water maze has given new insight into the neurobiology of spatial learning and memory. Although rodents are natural swimmers, the motivation to escape from water was implemented as an incentive for learning, a technique that has been used for years (Morris, 1984). The basic procedure

of the water maze involves an acquisition training phase, where the spatial memory is acquired, and a testing phase, which tests the retention of that memory. The training phase begins with a circular pool filled with water and a platform hidden just beneath the surface of the water, enough so that the surface of the platform is accessible. The subject is then placed into the pool and allowed to swim freely about the pool. The goal is for the subject to locate the platform by learning the spatial position of the platform relative to distal cues. Although the subject initially finds the platform ‘accidentally’ by swimming randomly, learning becomes evident when the subject is able to swim directly towards the platform from any starting position (Morris, 1984). The testing phase implements the same procedure as the training phase, with the exception that the platform is removed from the pool. Retention of memory is assessed based on the spatial accuracy of the subject, represented by the amount of time the subject spends in the area associated with the platform location (Morris, 1984; D’Hooge & De Deyn, 2001). A subject that has successfully acquired and retained the spatial memory will show a high preference for the area associated with the platform, spending an average of 50% or more of its swimming time in the target area (D’Hooge & De Deyn, 2001). The use of more sophisticated tracking devices allows for a measurement and quantification of thigmotaxis, speed, latency, and direction.

Hippocampal contribution to spatial memory

While many brain regions have been implicated in spatial memory acquisition and retention, the hippocampus appears to be the region most involved in those processes. O’Keefe & Nadel (1978) postulated the existence of a cognitive map and identified it with the hippocampus. According to them, the cognitive map is made up of a place

system, which contains information about places and objects in the environmental surroundings of the organism, along with their spatial relation within that environment, and a misplace system, which signals changes within the environment. The place system allows an organism to explore and locate itself within a familiar environment independent of particular cues, while the misplace system builds maps of new environments and incorporates new information into existing maps (O'Keefe & Nadel, 1978). This model suggests that animals formulate and test hypotheses to find solutions to a problem, which may involve approaching or avoiding places in their environment based on information processed in their hippocampal cognitive mapping system (O'Keefe & Nadel, 1978).

The hippocampus possesses specialized pyramidal cells, known as place cells, which encode the location of an organism within their environment (Muir & Bilkey, 2001). These place cells increase their firing rate when the individual enters a particular place in the environment, known as the place field. The location of the place field of a place cell remains stable in a familiar environment, even when the individual is removed from that environment for extended periods of time. Thus, activation of place cells may indicate that the individual perceives the environment as familiar (Muir & Bilkey, 2001).

Place cells were first discovered by O'Keefe & Dostrovsky (1971) while recording hippocampal cells. They found that the only time the cells elicited any activity was when they were pointing in a particular direction, and that both the orientation and specific stimuli in that direction were required to produce a response. Any other stimulus in that same direction produced no cellular response (O'Keefe & Dostrovsky, 1971). Rats with hippocampal damage showed difficulty navigating their way around mazes and tasks which required alteration of responses on successive trials, which may be due to the

loss of these places cells (O'Keefe & Dostrovsky, 1971). They concluded that the hippocampus provided the rest of the brain with a spatial reference map, and that the activity of the place cells would specify the orientation that the subject was in based on the landmarks that were present in the environment and the presentation of stimuli while facing in that direction. Further, they postulated that the spatial memory deficits experienced by rats with hippocampal lesions were attributed to the loss of their spatial reference map (O'Keefe & Dostrovsky, 1971).

In addition to place cells, hippocampal-dependent spatial memory requires the activation of c-Fos, an immediate early gene that is part of the activator protein-1 transcription factor (Fleischmann *et al.*, 2003). c-Fos has been shown to be involved in the molecular mechanisms of learning and memory, such as synaptic plasticity (Fleischmann *et al.*, 2003). These genes also play a role in the consolidation of short-term memory into long-term stores based on the fact that they are expressed during acquisition and the early phases of memory consolidation (Tischmeyer & Grimm, 1999). Several pharmacological studies have further strengthened the correlation between c-Fos expression and memory formation. One study implementing a post-training application of apamine, a toxin that facilitates memory processing, showed an increase in training-induced c-Fos expression (Tischmeyer & Grimm, 1999; Messier *et al.*, 1991). Similarly, Zhou *et al.* (1995) saw enhanced c-Fos expression in the hippocampus of rats after injecting them with AVP (4–8), a metabolite of arginine-vasopressin that facilitates the acquisition of memory. Conversely, mice with a nervous system-specific c-Fos knock-out showed impairments in various hippocampal-dependent spatial memory tasks, which

were correlated with a reduction of long-term potentiation (LTP). These results suggest a role for c-Fos in spatial memory formation (Fleischmann *et al.*, 2003).

Further evidence for hippocampal contribution to spatial memory can be found in hippocampal lesion studies. Morris *et al.* (1982) performed a study where they removed the entire dorsal and ventral hippocampus by aspiration lesion in rats then subjected them to the Morris water maze task. In doing so, they discovered that the hippocampal-lesioned rats showed a highly significant impairment in the task measured by longer routes to find the hidden platform, compared to control rats. They concluded that hippocampal-lesions caused a severe place-navigational impairment (Morris *et al.*, 1982). Additionally, Lavenex *et al.* (2006) performed bilateral hippocampal ibotenic acid lesions on macaque monkeys, who then performed various spatial relational tasks. They discovered that monkeys that received hippocampal lesions were unable to discriminate between different food locations in the absence of local cues. This indicated that the hippocampal lesion impaired their ability to use spatial relational representations of their environment to discriminate between the different food locations (Lavenex *et al.*, 2006).

Studies involving hippocampal inactivation can also give insight into the hippocampal contribution to spatial memory. Poe *et al.* (2000) used tetracaine, a potent local anesthetic, to inactivate one or both hippocampal hemispheres in aged and young rats, before putting them through the radial arm maze task. They discovered that bilateral and unilateral left hippocampal inactivation impaired the performance of the rats on the task, while unilateral right hippocampal inactivation had no effect on performance (Poe *et al.*, 2000). Because inactivation of both hippocampal hemispheres caused a dysfunction in spatial memory processing, it suggested that the hippocampus plays a significant role

in those processes. Additionally, the asymmetrical response of the hippocampal impairment indicates that the left hippocampus may be more involved in spatial working memory processes (Poe *et al.*, 2000).

Anatomy of the hippocampus

When it comes to the process of learning and remembering, the hippocampal structure as a whole does not work as one cohesive unit, but instead may be split up into different subunits with various functions. The two major units of the hippocampus are the dorsal hippocampus and the ventral hippocampus. There remains some debate, however, as to which part of the hippocampus is more important for memories, the dorsal or the ventral. One argument suggests that the dorsal hippocampus is more involved in the memory process due to the fact that the place fields in this region are more confined, as opposed to the ventral hippocampus, where the place fields are more dispersed (Jung *et al.*, 1994; Faneslow & Dong, 2010). A study completed by Moser *et al.* (1993) supports this theory. After performing localized hippocampal aspiration lesions and testing rats in the Morris water maze, they observed an impaired acquisition rate and precision on the probe test in the rats that received lesions in their dorsal hippocampus, ultimately leading to diminished spatial learning. The rats that received lesions in their ventral hippocampus, however, experienced no such impedance in their spatial learning abilities. Based on these results, they concluded that the dorsal hippocampus is more important for the encoding of new spatial information than the ventral aspect. (Moser *et al.*, 1993; Riedel & Micheau, 2001). Further evidence to support this theory comes from studies completed by Moser *et al.* (1995) and Pothuizen *et al.* (2004), who observed that hippocampal-dependent learning is impeded if the dorsal portion of the hippocampus is lesioned, and,

interestingly, that dorsal hippocampal lesions were just as effective as complete hippocampal lesions in disrupting working memory, as opposed to lesions in the ventral hippocampus, which showed no effect on working memory. It is for these reasons that the present thesis examined the dorsal hippocampus as opposed to the ventral hippocampus.

Subregions of the dorsal hippocampus

The dorsal hippocampus consists of three major regions; the dentate gyrus, the cornu ammonis (CA) fields, which consist of pyramidal cells and are subdivided into four regions (CA1 – CA4), and the parahippocampal gyrus (Amaral, 1993; Cohen *et al.*, 1999; Wible, 2013). The parahippocampal gyrus can be further broken down into three subregions, which include the subiculum, the entorhinal cortex, and the perirhinal cortex. The perirhinal and parahippocampal cortices are the first structures to receive input, generally from the temporal, frontal, and parietal lobes (Lavenex & Amaral, 2000). They then integrate and process the sensory information and transmit it to the second stage of processing, in the entorhinal cortex. The entorhinal cortex is thought to process sensory information with a higher level of associativity, allowing for an increase in the level of integration of information (Lavenex & Amaral, 2000). The perforant pathway begins with the entorhinal cortex sending glutamatergic projections to the dentate gyrus, which has an abundance of both NMDA and AMPA receptors, resulting in the activation of the trisynaptic pathway in the hippocampus. The first synapse in this pathway bypasses the dentate gyrus and sends direct inputs from the entorhinal cortex to the CA3 region, which contains numerous kainate receptors (Naber *et al.*, 1999). The second synapse in the pathway projects from the dentate gyrus to the dendrites of CA3 pyramidal cells via

mossy fibres. The third synapse projects glutamatergic inputs from pyramidal cells in the CA3 region to pyramidal cells in the CA1 region via Schaffer collaterals (Neves *et al.*, 2008). In addition to receiving input from cortical regions, the entorhinal cortex receives output from the CA1 layer via the subiculum, which then project to other brain regions, such as the mamillary nuclei, the thalamus, and the nucleus accumbens (Amaral, 1993; Wible, 2013).

The occurrence of LTP in the hippocampus

Some neurons are able to continuously form new, stronger connections while simultaneously eliminating weaker connections in response to information processing demands. As a result of these constant changes, neurons must garner the ability to adapt, but also remain somewhat stable, in response to intrinsic and extrinsic signals, a process known as neural plasticity (Wainwright & Galea, 2013). This plasticity permits adaptability in a network, regulates future experience-dependent plasticity, and, most importantly for memory, contributes to the formation of a memory trace within the brain (Sehgal *et al.*, 2013). Santiago Ramon y Cajal was the first to postulate that the changes in the strength of synaptic connections between active neurons are the basis of the coding and storage of memories (Lynch, 2004). Later, this came to be known as “Hebbian plasticity”, which suggests that memory formation results from strengthening synaptic connections between pre- and post-synaptic neurons that are activated simultaneously (Hebb, 1949; Bliss & Collingridge, 1993; Johansen *et al.*, 2014).

One important cellular mechanism of learning and memory, and a feature of neural plasticity, is LTP. The occurrence of LTP has been observed in several brain

regions, from subcortical to cortical regions, and from brain regions involved in basic survival mechanisms, to those underlying higher cognitive and perceptual processes (Lynch, 2004). First described in 1973, LTP describes how increased efficiency of synaptic transmissions results from the concurrent activation of both pre- and post-synaptic elements (Bliss & Lomo, 1973; Bliss & Collingridge, 1993; Cooke & Bliss, 2006). LTP is usually induced by a series of high-frequency stimuli, known as tetani, at 100 Hz or more. There is an intensity threshold that must be reached in order to induce LTP; failure to reach that threshold will not trigger LTP. However, Kelso *et al.* (1986), found that a weak stimulus can induce LTP as long as it is active at the same time as a strong tetanus, and as long as they converge on the same input. Gustaffson *et al.* (1987) also performed an experiment whereby they paired an excitatory postsynaptic potential (EPSP) formed by a weak stimulus (0.15 – 0.1 Hz) in the stratum radiatum with depolarizing current pulses. They found that potentiation occurred, as long as the EPSP and current pulse occurred no more than 100 milliseconds apart from each other and that no potentiation occurred if the EPSP came after the pulse. This property of LTP, known as the ‘associative’ property, suggests that LTP is a neuronal underpinning for learning and memory, as it allows associations to be made at the cellular level (Gustaffson *et al.*, 1987; Cooke & Bliss, 2006).

LTP is known to have two phases; an early phase, which lasts 2 to 3 hours and is independent of protein synthesis, and a long-lasting phase, which can last several hours *in vitro* and weeks *in vivo* and requires protein synthesis. Interestingly, it has been shown that the retention of long-term memories requires gene expression, protein synthesis, and new synaptic connections (Lynch, 2004).

Studies focusing on LTP in the developing brain are somewhat scarce; one of the reasons being that the constant requirements of LTP at different ages are varied and, for the most part, uncertain (Teyler *et al.*, 1989). In the rat barrel cortex, LTP has been induced as early as postnatal day (P) 0, with the strongest expression of LTP occurring between P3 and P5 (An *et al.*, 2012). In the CA1 region of the hippocampus, the first occurrence of LTP occurs around P5 and can be seen consistently by P7 – 8. As the organism develops, the threshold to induce LTP increases until it reaches its peak at around P15 then declines in adults (Teyler *et al.*, 1989). Other areas of the brain show a similar pattern of development of LTP as is found within the hippocampus. LTP ontogeny in the neocortex, for example, first occurs around P6 and can be seen consistently by P11 – 20. Again, as in the hippocampus, the threshold to induce LTP reaches its peak around P16 – 20 then declines in adults (Teyler *et al.*, 1989).

The developmental emergence of LTP within the hippocampus has been a focal point of various studies, although the definite developmental time point of its emergence has been debated. Bekenstein and Lothman (1991) calculated responses elicited in the CA1 region and in the dentate gyrus in developing rats. They discovered that LTP ontogeny and development occurred at different rates in the two regions, with LTP reaching adult values during the second postnatal week in the hippocampal CA1 region and in the dentate gyrus during the third postnatal week. Based on these results, they postulated that the later neurogenesis of dentate granule cells and later functional maturation of NMDA receptor-channel complexes in the dentate gyrus might account for the differences (Bekenstein & Lothman, 1991). Durand *et al.* (1996) combined whole-cell recordings and confocal microscopy to investigate hippocampal glutamatergic synapses

during development and found that LTP can be induced as early as P2, conflicting with the results reported by Bekenstein & Lothman (1991). The difference in results may come from the fact that Durand *et al.* (1996) paired postsynaptic depolarization with afferent stimulation, which may have been strong enough to induce LTP earlier in development. A consensus view on LTP development is that LTP does not occur before P5, and that the basic mechanism for LTP is seen between P10 and P12, which is based on an increase in AMPA receptor responsiveness that activates silent synapses (Durand *et al.*, 1996).

Excitatory Connections in Hippocampal Subregions

The importance of the associative property becomes evident when examining the role of NMDA receptors in the induction of LTP. One feature of NMDA receptors is that their channels are blocked by a voltage-dependent Mg^{+2} ion. When a single, weak stimulus is applied, it is typically not sufficient to depolarize the membrane and displace the Mg^{+2} block. If, however, a strong stimulus is applied via activation of multiple inputs, there will be a depolarization of the membrane releasing the Mg^{+2} block. The coincident release of the Mg^{+2} block and glutamate binding causes Ca^{+2} to enter the cell. The subsequent influx of Ca^{+2} induces either LTP or long-term depression (LTD), depending on the neuron and type of NMDAr expressed, and activates a series of intracellular signaling cascades. Those cascades lead to the activation of immediate early genes, such as c-Fos, which, among other processes, regulate synaptic protein synthesis (Fleischmann *et al.*, 2003).

Using the competitive NMDA receptor antagonist DL-2-amino-5-phosphonovalerate (AP5) lead to the discovery of the involvement of the NMDA receptor in the induction of LTP (Collingridge *et al.*, 1983; Harris *et al.*, 1984; Monaghan *et al.*, 1989). A study completed by Morris *et al.* (1986) blocked NMDA receptors in the hippocampus using AP5, which ultimately prevented the induction of hippocampal LTP. They discovered that preventing LTP impaired place learning, adding further support to the involvement of LTP in learning (Morris *et al.*, 1986). Additional studies have confirmed these findings by inhibiting NMDA receptors with noncompetitive antagonists, such as phencyclidine (PCP) and ketamine (Stringer *et al.*, 1983; Monaghan *et al.*, 1989).

In order to assess the potential role of NMDA receptors in memory expression, Steele & Morris (1999) infused the hippocampus of rats with the NMDA receptor antagonist, D-AP5, before a retrieval test on a Morris Water Maze. They discovered that NMDA receptor blockade did not seem to impede memory retrieval, indicating that, although there is strong evidence for the involvement of NMDA receptors in acquiring and consolidating memories, they do not seem to be involved in memory retrieval (Steele & Morris, 1999; Szapiro *et al.*, 2002).

The CA1 region of the hippocampus possesses the highest concentration of NMDA receptors in the brain (Wible, 2013). These receptors are permeable to calcium, sodium, and potassium ions, but it is the increase in intracellular calcium ions that is thought to be responsible for NMDA receptor-induced plasticity in the brain (Monaghan *et al.*, 1989). Although NMDA receptors are extensively studied for their role in learning and memory, they are not the only receptor types that contribute to those processes.

Kainate receptors, as well as quisqualate receptors, which are now known as AMPA receptors, also have an important function in mediating information processing in the hippocampus. NMDA and AMPA receptors are colocalized, and thus like NMDA receptors, one of the highest areas of concentration of AMPA receptor binding sites is in the CA1 stratum radiatum region of the hippocampus, an area that contains Schaffer collaterals and is one of the main sites for observing LTP (Cotman *et al.*, 1988; Monaghan *et al.*, 1989).

AMPA Contribution to LTP

While NMDA receptors are important during the induction phase of LTP, activation of AMPA receptors seem to be critical for not only the induction phase but also the expression phase of LTP. As previously mentioned, the influx of Ca^{+2} through the NMDA receptors induces LTP and activates a series of intracellular signaling cascades, which act as secondary messengers. Those secondary messengers include Ca^{+2} -calmodulin-dependent protein kinase II (CAMKII), which function to phosphorylate the AMPA receptors, increasing their sensitivity to glutamate, while inserting more AMPA receptors into the post-synaptic membrane. In mature hippocampal pyramidal cells, AMPA receptors are made up of four homologous subunits, from GluR1 to GluR4, with GluR1 and GluR2 or GluR2 and GluR3 being the predominate combination of subunits (Song & Huganir, 2002; Malinow & Malenka, 2002). Of the four different subunits, studies have provided evidence to suggest that the GluR1 subunit is the most important in terms of the expression of LTP. Studies that have used genetic knockout mice lacking the GluR1 subunit have shown that, while glutamatergic synaptic transmission is unaffected,

these mice do not express LTP in the Schaffer collateral region of the hippocampus, indicating the importance of AMPA receptors in LTP expression (Jensen *et al.*, 2003). Additional evidence supporting this finding comes from a study by Sanderson *et al.* (2010), who found that spatial working memory was hindered in knockout mice lacking the GluR1 subunit, suggesting that hippocampal synaptic plasticity was impaired and that the GluR1 subunit of the AMPA receptor is required for short-term spatial working memory tasks.

Szapiro *et al.* (2000) antagonized AMPA receptors 10 minutes before testing subjects on a memory recall task. They found that the pre-test blocking of AMPA receptors resulted in impaired retrieval, suggesting that AMPA receptors played a role in the expression of long-term memories. Similarly, Riedel *et al.* (1999) antagonized AMPA receptors during training and before testing, or before testing alone on the Morris Water Maze. They discovered that rats whose AMPA receptors were blocked during training and before testing performed poorly during both occasions and the rats whose receptors were blocked before testing spent less time in the quadrant associated with the platform, even though they were trained with fully functioning AMPA receptors. These results indicate that the AMPA receptors play a role in the encoding, consolidating, and retrieval of memories (Meeter & Murre, 2004; Szapiro *et al.*, 2002; Riedel *et al.*, 1999).

As previously mentioned, the underlying mechanism that mediates AMPA GluR1 subunit function and importance in terms of generating LTP lies in its ability to be phosphorylated, which enhances the function of the AMPA receptor ion channel. The GluR1 subunit possesses two sites that can be phosphorylated, Serine 831 (Ser831) and Serine 845 (Ser845), which are found at the intracellular C-terminus. Ser831 is

phosphorylated by CaMKII and protein kinase C (PKC), while Ser845 is phosphorylated by cAMP-dependent protein kinase A (PKA). Once the receptor sites are phosphorylated, the AMPA receptor shows increased conductance during LTP expression (Lee *et al.*, 2003; Song & Huganir, 2002; Malinow & Malenka, 2002). Out of the two phosphorylation sites, an increase in Ser831 phosphorylation is most probably associated with LTP expression, while LTD is associated more so with the dephosphorylation of the Ser845 site. In addition, Lee *et al.* (2000) observed that, upon expression of LTP, there was a significant increase in Ser831 phosphorylation, while there was no significant increase in Ser845 phosphorylation. These findings are consistent with the notion that GluR1 phosphorylation is responsible for the expression of LTP and LTD (Lee *et al.*, 2000; Lee *et al.*, 2003).

Not only does the phosphorylation of the AMPA GluR1 receptor subunit mediate LTP and LTD expression, but it has also been found to contribute to the retention of spatial memories. Genetically substituting the Ser831 and Ser845 sites with alanine in mice prevented phosphorylation of these sites, which inhibited both LTD and LTP. However, in addition to blocking LTP and LTD expression, these mutations interrupted the retention of a spatial memory task; the mice were able to remember the new location of the platform in the Morris water maze task after a short delay, but were unable to retain that memory in the long-term. These results suggest that the phosphorylation of the GluR1 subunit may be necessary for the retention and consolidation of spatial memories (Lee *et al.*, 2003).

The AMPA receptor subunit GluR2, which plays a role in the induction of LTP, begins to develop from the day of birth (P0). From there, it is up regulated by 132% on

P7, followed by 163% on P15 and 301% on P45 (Pandey *et al*, 2015). Similarly, the expression levels of the GluR1 and GluR3 subunits begin to rise during the second postnatal week, reaching levels seen in adults by the third postnatal week (Lohmann & Kessels, 2013).

Hippocampal contribution to remote memory stores

One of the first, and most famous studies linking the importance of the hippocampus to memory stores was that of patient Henry Molaison (H. M.). After a long history of seizures, H. M. underwent a bilateral medial temporal-lobe resection, which ultimately lead to the destruction of the anterior two thirds of the hippocampus and hippocampal gyrus bilaterally (Scoville & Milner, 1957). Following the surgery, it became apparent that H. M. had lost all recent memory and was unable to form new memories, while retaining his earlier memories. After observing the case of H. M., Scoville and Milner (1957) proceeded to collect data from other patients who underwent similar bilateral medial-temporal resections. In almost all of the patients, most of the areas removed included a portion of the anterior hippocampus and the hippocampal gyrus. They discovered that the patients whose hippocampus was either damaged or removed had severe short-term memory deficits similar to those of H. M., while the patients whose hippocampus remained intact experienced no such deficit (Scoville & Milner, 1957). It became apparent that removal of the hippocampus impinged on the ability to retain short-term memories, while concurrently impeding the consolidation of short-term-memories into long-term stores. The memories that had been transferred into long-term stores prior to the resection, however, remain unaffected and intact. These studies suggested that the hippocampus was responsible for the storage and recall of

short-term memories and played a role in mediating the consolidation of short-term memories into long-term stores, but had little influence on memories that had been previously integrated into long-term stores. If the storage of remote memories is hippocampus-independent, then long-term memory reservoirs must lie within other cortical structures.

Memory storage in the anterior cingulate cortex

In the aforementioned study of patient H. M., following the medial temporal-lobe resection, he was unable to retain any of his recent memories, suggesting that the hippocampus played a role in the acquisition and retention of recent short-term memories, yet he was able to remember his earlier remote memories, those that were encoded a few years prior to his surgery. This indicates that there is some hippocampus-independent mechanism that allows for the retention, storage, and reconsolidation of "remote" memories. To examine this hypothesis, Izquierdo *et al.* (1997) trained rats in an inhibitory avoidance task and tested them after 1 day, 30 days, or 60 days. They used CNQX to antagonize AMPA receptors in the hippocampus and amygdala, the entorhinal cortex, or the parietal lobe prior to testing and found that AMPA receptor blockade in all of the three regions at the 1-day interval impeded performance, while blockade only in the entorhinal and parietal cortices after 30 days affected performance. After 60-days, however, only injections in the parietal cortex impeded performance, suggesting that, initially, the memories were stored in and retrieved from the neocortex and hippocampus, however as the time increased after learning, the neocortex sustained retrieval on its own (Izquierdo *et al.* (1997); Meeter & Murre, 2004).

To complement these findings, Bontempi *et al.* (1999) infused a (^{14}C)2-deoxy-glucose tracer into mouse brains to assess metabolic activity in different brain regions over time during a spatial working memory task. They discovered an inverse relationship between the interval between training and testing and metabolic activation in the hippocampus. As the interval between training and testing increased, the metabolic activity in the hippocampus decreased. This indicated that the hippocampus was no longer being activated to recall those memories. Conversely, the metabolic activity in other cortical areas, specifically the anterior cingulate cortex, increased during recollection of those memories, suggesting that the long-term storage lies in that area of the brain (Bontempi *et al.*, 1999). Damage to the cingulate cortex has also proven to impede the retention of long-term remote memories, further demonstrating the role of the cingulate cortex in the long-term memory storage process (Insel & Takehara-Nishiuchi, 2013; Wartman *et al.*, 2014).

It appears as though once memories are received and processed in the hippocampus, they undergo a process whereby those recently encoded memories solidify and stabilize in the brain in order to persist, a process known as memory consolidation. Memory consolidation involves two levels of processing: the cellular level, which involves regulating gene expression and protein synthesis in localized circuits through intracellular signaling cascades, and the brain systems level, which involves more gradual changes in brain structures spanning over a much longer time frame (Einarsson & Nader, 2012; Wartman *et al.*, 2014). The long-term memory consolidation process is mediated by various intricate and complex neural connections between the cingulate cortex, the entorhinal cortices, and the hippocampus (Insel & Takehara-Nishiuchi, 2013).

The anatomical connectivity between the hippocampus and anterior cingulate cortex may support the transfer of memories initially encoded by the hippocampus for long-term storage in the anterior cingulate cortex. One pathway that has been observed is that of the superficial layer of the entorhinal cortex projecting input from the sensory and association cortices to areas of the hippocampus and the cingulate cortex. From there, the hippocampus and the cingulate cortex send output to the deep layer of the entorhinal cortex, which then projects back to the sensory and association cortices (Insel & Takehara-Nishiuchi, 2013; Takehara-Nishiuchi, 2014). A more direct pathway between the hippocampus and cingulate cortex has also been observed, whereby neurons in the ventral CA1 region project to the rostral cingulate, while neurons in the dorsal CA1 region project to the retrosplenial granular A cortex, a posterior region of the cingulate cortex (Insel & Takehara-Nishiuchi, 2013). The subiculum has the same projections as those in the CA1 region, while also projecting to the retrosplenial granular cortex B, a more anterior, caudal region of the cingulate cortex. Activation of both pathways simultaneously has been shown to strengthen synaptic connections in both the cingulate cortex and the entorhinal cortex, which in turn increases the capacity for long-term memory storage capabilities (Insel & Takehara-Nishiuchi, 2013).

During the early stages of recent memory formation, spine density on hippocampal cell dendrites increases, allowing for rapid synaptic rearrangement, the underlying factor of synaptic plasticity. It has been demonstrated that these same neuronal structural changes, specifically dendritic spine growth, develop in the anterior cingulate cortex during remote memory acquisition (Restivo *et al.*, 2009). In mice, recent memory formation was associated with an increase in spine density on hippocampal

neurons, but not on cortical neurons. During remote memory formation, however, the inverse was seen, where the spine density on anterior cingulate cortical neurons, but not on hippocampal neurons, increased (Restivo *et al.*, 2009). These differences in temporal changes suggest that the formation and expression of remote memories is a result of gradual structural changes in hippocampal-cortical network connectivity (Restivo *et al.*, 2009).

As in the hippocampus, activation of c-Fos can be used as a marker of neuronal activity and as evidence that learning and memory has occurred in the anterior cingulate cortex. Teixeira *et al.* (2006) assessed the retention of spatial memory in mice either 1 day (recent test) or 30 days (remote test) after their last day of training on the Morris water maze. They found that the mice in both groups spent more time in the area associated with the removed platform compared to other areas, indicating that mice in both groups retained that memory. They compared their behavioral results to c-Fos expression in the anterior cingulate cortex and found there to be significantly higher c-Fos expression in the remote test group compared to the recent test group, suggesting that the anterior cingulate cortex was preferentially involved in the storage of remote spatial memories (Teixeira *et al.*, 2006).

Inactivation of the anterior cingulate cortex also seems to impair the expression of remote spatial memories. Mice treated with infusions of lidocaine directly into their anterior cingulate cortex showed no difference in time spent in the area associated with the removed platform versus other areas during a remote (30 days post-training) probe test using the Morris water maze (Teixeira *et al.*, 2006). This disruption in performance was not seen in mice that underwent the same pharmacological treatment, but were tested

more recently (1 day post-training), which would indicate that the anterior cingulate cortex plays an important role in the storage of remote, but not recent, spatial memories and its activation impedes the expression of those memories (Teixeira *et al.*, 2006).

Development of the hippocampus

In humans, volumetric MRI studies have indicated that the hippocampus undergoes rapid development during late gestation/postnatal life, with continued rapid growth up to 7 years of age (Thompson, *et al.*, 2014; Lavenex & Lavenex, 2013). It is also around this time, especially at the end of gestation, that the hippocampus is most sensitive to toxic insults that could interfere with neurogenesis and other cellular processes in the hippocampus (Rice & Barone Jr., 2000). Afterwards, development begins to decelerate up to 14 years and continues until about 25 years (Thompson, *et al.*, 2014; Tamnes *et al.*, 2014; Lavenex & Lavenex, 2013).

Although the hippocampus develops rapidly during early postnatal life, the behaviours mediated by the hippocampus, including learning and memory capabilities, have not yet emerged. Ribordy *et al.* (2013) completed a study on the development of allocentric spatial memory abilities in children of various ages and found that children under 24 months of age were not capable of forming and remembering allocentric spatial memories, while children older than 25 months possessed the ability to complete the task. One possible explanation for this result may be that during the first year of development, neurons in the CA regions of the hippocampus undergo rapid enlargement and maturation. Those neurons then begin to myelinate and this myelination increases into childhood until adolescence (Arnold & Trojanowski, 1996).

The development of the hippocampus in rodents parallels that of humans. Bayer (1980b) examined the development of the rat hippocampus during the early embryonic stage up to P21. According to her, the characteristic fold of the hippocampus is first seen at embryonic day (E) 16, followed by the entorhinal cortex at E16 – E22 and the subicular and CA region pyramidal cells at E18 – E19 and E22 – P1, respectively. The most rapid growth rate in the hippocampus was observed to be from E16 to E17, when the hippocampal volume increased by 1900%. Hippocampal volume continues to increase daily by 66% between E18 and E19, 29% between E19 and E20, 26% between P1 and P7, and 12% between P7 and P21 (Bayer, 1980b).

There is evidence that synaptic connections within the hippocampus that mediate spatial learning and memory undergo remodeling between p18 and p24 (Wartman *et al.*, 2012). Holahan *et al.* (2006) observed increased mossy fiber projections to the stratum lucidum during p18 and p21, which continued to grow, and eventually proliferated into the CA3 pyramidal layer and terminated at the dorsal stratum oriens by p24. The connectivity pattern seen by this time remained stable into adulthood, which would indicate the existence of a crucial hippocampal developmental period between p18 and p24 (Holahan *et al.*, 2006). Blockade of NMDA receptors between p18 and p24 resulted in a reduction of mossy fiber innervation throughout the connection pathway, suggesting that hippocampal development and remodeling are particularly sensitive to pharmacological insults during this timeframe (Holahan *et al.*, 2007). This hippocampal remodeling has also coincided with improved performance on cognitive tasks. Moderate improvements in spatial memory were observed in rats that were assessed in a Morris water maze task between p16 and p19, while dramatic improvements in water maze

performance, similar to those seen in adults, were observed in rats assessed between p18 and p21 (Holahan *et al.*, 2006; Keeley *et al.*, 2010; Wartman *et al.*, 2012). It can then be inferred that pharmacological insults administered during the sensitive developmental period may disrupt the remodeling process, thus impairing the subsequent spatial memory enhancements that may come as a result.

The cellular mechanisms that underlie LTP and synaptic plasticity correlate with the different developmental time points of the rat hippocampus. Bayer (1980a) injected pregnant female rats with ^3H -thymidine in order to trace the development of hippocampal neurogenesis in the offspring. She observed that different hippocampal regions undergo cell proliferation during various time points; in the entorhinal cortex and the CA region, for example, neurons begin to proliferate between E15 and E17, while the subiculum undergoes neurogenesis between E16 and E18. Although neuron proliferation occurs in developmental ‘waves’, the hippocampal structure as whole undergoes neurogenesis between E15 and E20 (Bayer, 1980a; Rice & Barone Jr., 2000).

Although the number of synapses is initially low at birth, synaptogenesis can be observed at that time, with most synapses located on dendritic shafts or small protrusions called filopodia. The synapses continue to gradually develop, especially during the third postnatal week, where a spike in the number of synapses is observed, with most of them found on dendritic spines (Lohmann & Kessels, 2013). Synaptogenesis begins to decelerate during the fourth postnatal week and reaches mature levels in the hippocampus. Interestingly, after the first postnatal week, the absolute number of hippocampal shaft synapses does not change much, allowing for only a small number of excitatory synapses to develop on the shafts of mature hippocampal neurons, which may

indicate that synaptic plasticity and pruning occurs shortly after the onset of synaptogenesis (Lohmann & Kessels, 2013).

As an individual ages, the synaptic input to the pyramidal cells in the CA1 region begin to decline, leading to a loss of synaptic contacts. The loss of these CA1 synapses may account for the impairments seen in older individuals when undergoing a spatial memory task (Poe *et al*, 2000). In fact, the associative neural network theory postulates that the loss of synaptic connections during aging will deteriorate hippocampal-dependent spatial memory abilities. The graceful degradation hypothesis complements this theory, attributing the increased probability of mistakes made during spatial memory tasks in older rats compared to younger rats to impaired functional connectivity within the hippocampus caused by a critical level of synaptic loss (Poe *et al*, 2000).

Purpose of the study

The current study implemented a multimodal approach to investigate the effect of AMPA receptor blockade on the emergence and expression of spatial function during remote memory retrieval. NBQX, a potent AMPA receptor antagonist, was administered to rats at different time points during training and/or testing.

The rats were split into four conditions, with each rat receiving either saline or NBQX injection at different time points across all conditions. The rats received their injections before being trained, after being trained, before being tested, or before being trained and tested on the Morris water maze task. This allowed understanding into the effect of AMPA receptor blockade on both the initial expression and acquisition of memory in juvenile rats, and on the retrieval of remote spatial memories in mature rats. It was hypothesized that AMPA receptor blockade before being trained and before being

trained and tested would result in an impairment of acquisition and expression of remote memories, while AMPAr blockade after being trained would result in an impairment of consolidation of the acquired memories, and AMPAr blockade before being tested would result in an impairment of expression of the acquired memories. In addition, AMPA receptor blockade was postulated to disrupt the consolidation process, thereby resulting in a decrease in c-Fos expression in the brain regions associated with remote memory storage and retrieval, indicative of reduced neuronal activation in those regions.

Materials and methods

Subjects

Seventy-nine male Long-Evans rats (LERs) were used over all experiments. All rats were born in the animal-care facility at Carleton University. The day the pups were born was marked as p0. Pups were weaned on their first day of training. For all experiments, the animals were group-housed. All animals were housed in a temperature-controlled vivarium in polycarbonate cages with a 12-hour light-dark cycle: lights on 8h00, lights off 20h00. Food and water were provided *ad libitum*. All experiments were conducted in accordance with the Canadian Council on Animal Care (CCAC) guidelines and approved by the Carleton University Animal Care Committee.

Apparatus

Water maze

The pool was opaque, white polypropylene with a submerged platform 2 cm below the water surface. Water temperature averaged $25 \pm 1^\circ\text{C}$ throughout behavioural testing. The water maze was a white, circular, polypropylene pool measuring 124 cm in diameter, 31 cm in height and filled with water to a depth of 25 cm. The platform was made of clear Plexiglas and measured 11 cm in diameter. Distal visual cues were present during all trials. Rat movements in the pool were tracked using the HVS Image 2100 Tracking System (HVS Image, Buckingham, UK). The water was quickly cleaned after each trial and the pool was drained and refilled every other day.

AMPA receptor blockade and its effect on the acquisition and expression of spatial memories

Water maze training

All subjects were given eight one-minute trials a day from P18 to P20. Each trial began from a different start point on the perimeter of the pool and the same sequence of start locations was used each day. The platform remained in the same quadrant for all eight trials during each of the three training days. Latency, speed, and pathlength to reach the platform were recorded for all trials. If an animal did not reach the platform within 60 seconds, they were guided to it. After each trial, the animals remained on the platform for 15 seconds before being removed, dried, and placed back into the water maze for the next trial. Following the final trial on each day, every animal was dried with a towel and placed in another holding cage on a heating pad in the housing room for 15 to 20 minutes before being returned to their home cage.

Water maze testing

Three weeks following the last training day (P41), the rats underwent a probe test in the water maze. Each rat was subject to a single 60-second trial, and each trial began from a different start point on the perimeter of the pool. For all probe tests, the platform was removed from the water maze. The amount of time spent in the different quadrants and predefined platform locations was recorded for all trials. After each probe test, the rats were returned to their home cage. Each rat was then injected with 1 mL Sodium Pentobarbital approximately one hour following their probe until they were no longer responsive to a toe pinch and then euthanized by decapitation.

Pharmacological conditions

The rats were sorted into 4 different groups, with each rat receiving a different concentration of the AMPA receptor antagonist, NBQX. All rats were trained on the water maze from P18 to P20 and tested on the water maze at P41. The groups differed based on the timing each rat received their injection relative to when they were trained and/or tested on the water maze.

Pretraining condition

In order to assess the effect of AMPA receptor blockade on spatial memory acquisition in juveniles during a critical period for hippocampal development, eighteen rats were treated with a 0.1 ml intraperitoneal injection of either saline (n = 6), 5 mg/kg NBQX (n = 6), or 10 mg/kg NBQX (n = 6) 5 minutes before being trained on the water maze. The rats were then tested on the water maze at P41, but received no injection prior to being tested.

Posttraining condition

Twenty-one rats were treated with a 0.1 ml intraperitoneal injection of either saline (n = 7), 5 mg/kg NBQX (n = 7), or 10 mg/kg NBQX (n = 7) immediately after each training day on the water maze. This condition was implemented to assess the effect of AMPA receptor blockade on the consolidation of spatial memory. Additionally, this condition controlled for any state-dependent learning and any possible locomotor deficits

caused by either the drug and/or the injection itself. The rats were then tested on the water maze at P41, but received no injection.

Pretesting condition

In order to assess the effect of AMPA receptor blockade on remote spatial memory expression, eighteen rats were treated with a 0.2 ml intraperitoneal injection of either saline (n = 6), 5 mg/kg NBQX (n = 6), or 10 mg/kg NBQX (n = 6) 5 minutes before their probe test on the water maze. The rats were trained on the water maze in the same manner as the rats in the other conditions from P18 – P20, but received no injections on their training days.

Pretraining/pretesting condition

Twenty-two rats were treated with a 0.1 ml intraperitoneal injection of either saline (n = 9), 5 mg/kg NBQX (n = 7), or 10 mg/kg NBQX (n = 6) 5 minutes before being trained on the water maze every day from P18 – P20. They also received a 0.2 ml intraperitoneal injection of the same concentration of NBQX that they had received during the training phase 5 minutes before their probe test on the water maze. This condition was used to examine the effect of AMPA receptor blockade on spatial memory acquisition in juveniles, and on spatial memory expression during a later developmental period (state-dependent learning).

Immunohistochemistry

Brains were immersion fixed in 4%-paraformaldehyde in 0.1M phosphate-buffered saline (PBS) overnight at 4°C. This solution was replaced with 30% sucrose in 0.1M PBS the following day and brains were stored at 4°C until sectioning. Brains were sectioned through the anterior cingulate cortex and the dorsal hippocampus at 60µm on a Leica CM1900 cryostat (Weztler, Germany). Sections were stored in a 0.1% sodium azide solution in 0.1M phosphate buffer (PB) at 4°C.

For immunohistochemistry, sections were placed in phosphate-buffered saline with Triton X (PBS-TX) for three, five-minute washes. They were then incubated in 0.3% hydrogen peroxide (H₂O₂) in PBS-TX for 15 minutes, followed by three, five-minute washes in PBS-TX. Sections were transferred to 1x animal free blocker (AFB; Vector) in PBS-TX for 30 minutes at room temperature. Incubation in the primary antibody (rabbit anti-c-Fos from Abcam, 1:5000) occurred overnight at room temperature. The following day, tissue was washed in PBS-TX for three, 10 minute washes followed by a two-hour incubation in the secondary antibody (anti-rabbit biotinylated from Vector Laboratories, 1:500). Tissue was washed for three, 10-minute washes in PBS-TX before being placed into an ABC solution (Vector) for one hour. The tissue was rinsed in three, five-minute washes using PBS before being placed into a nickel-enhanced DAB solution. Sections were then mounted on glass slides, dehydrated, and cover slipped.

Quantification

For unbiased stereology, the CA1 and anterior cingulate areas were traced at 2.5X magnification. Cell counts were performed at 60X magnification using parameters of 60 μ m by 60 μ m and an optical fractionator of 20 μ m. Two sections from each rat were counted and the estimated total by mean measured thickness was analyzed.

Results

Water Maze Acquisition

Juvenile rats were assigned to one of three pharmacological conditions and trained on the Morris water maze from P18 to P20. Each rat received an injection of either saline (0 mg/kg NBQX), 5 mg/kg NBQX, or 10 mg/kg NBQX during the water maze training and/or testing days. Latency and pathlength used to reach the hidden platform, as well as thigmotaxis, were measured and analyzed.

Trial-by-Trial Latency Results

Pretraining Injections

Trial-by-trial latency data for the pretraining groups are shown in Figure 1A. These data reveal that latencies in the saline group decreased more so over the three training days compared to the two NBQX groups. Additionally, it is evident that, on each training day, the initial latencies for the saline group were consistently lower than the previous day and, with each subsequent trial, those latencies rapidly decreased then plateaued (see Fig 1A). The latencies in the two NBQX groups, however, remained stagnant over the three days and across individual trials (see Fig 1A). A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed main effects of day ($F_{(2, 30)} = 13.465, p < 0.001$) and drug dose ($F_{(2, 15)} = 11.007, p < 0.01$), but no main effect of trial ($F_{(7, 105)} = 0.777, p = 0.608$). The analysis also revealed a significant day by drug dose interaction ($F_{(4, 30)} = 5.866, p < 0.01$), but no significant trial by drug dose interaction ($F_{(14, 105)} = 1.024, p = 0.435$), no

day by trial interaction ($F_{(14, 210)} = 0.618, p = 0.849$), and no day by trial by drug dose interaction ($F_{(28, 210)} = 0.601, p = 0.945$).

The significant day x drug dose interaction was further analyzed to determine on which days there were group (0, 5, 10 mg/kg NBQX) differences. One-way ANOVAs (drug dose as the independent variable and average daily latencies as the dependent variable) with Tukey's post-hoc tests were performed to examine the day by drug dose interaction (Figure 1B). The analyses revealed no significant differences on P18 ($F_{(2, 15)} = 1.681, p = 0.219$), but significant differences were observed on P19 ($F_{(2, 15)} = 5.070, p < 0.05$), where the average latencies of the saline group were significantly lower compared to the 5 mg/kg NBQX group ($p < 0.05$) and the 10 mg/kg NBQX group ($p < 0.05$). Significant differences were also observed on P20 ($F_{(2, 15)} = 15.289, p < 0.001$), where the average latencies of the saline group were significantly lower compared to the 5 and 10 mg/kg NBQX groups ($p < 0.01$). The main effect of drug dose provides evidence that the saline group performed better than the two NBQX groups.

A.

B.

Figure 1. Latency to reach the hidden platform (A) averaged across eight trials of daily training and (B) averaged across three training days for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20.

(***) indicates main effect of training day ($p < 0.001$); (##) indicates main effect of drug dose ($p < 0.01$). (+) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.05$); (++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!) indicates significant differences between the saline group and the 10 mg/kg NBQX group ($p < 0.05$); (!!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.001$).

Posttraining Injections

Trial-by-trial latency data for the posttraining groups are shown in Figure 2. These data revealed no discernible differences in latencies between the three drug dose groups over the three training days. The latencies of each of the three drug groups, however, showed consistent improvement with each successive training day. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 36)} = 54.582, p < 0.001$), but no main effect of drug dose ($F_{(2, 18)} = 0.166, p = 0.848$), or trial ($F_{(7, 126)} = 1.343, p = 0.236$). No significant day by drug dose interaction ($F_{(4, 36)} = 0.710, p = 0.591$), no trial by drug dose interaction ($F_{(14, 126)} = 1.422, p = 0.152$), no day by trial interaction ($F_{(14, 252)} = 1.657, p = 0.065$), and no day by trial by drug dose interaction ($F_{(28, 252)} = 0.946, p = 0.547$) were observed. These data indicate that, while the time it takes to learn the location of the hidden platform during water maze training may ameliorate with age, AMPA receptor blockade after each successive training day does not impede performance. Additionally, the lack of a significant effect of drug dose suggests that neither the injection nor the drug itself impaired locomotor/swimming ability and that no carry-over effect occurred.

Figure 2. Latency to reach the hidden platform averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated after being trained on the water maze from P18 to P20

(***) indicates main effect of training day ($p < 0.001$).

Pretesting Injections

Trial-by-trial latency data for the pretesting groups are shown in Figure 3. The data revealed a uniform decrease in latencies for each of the three drug groups with each training day, with a marked decrease in latencies on P20. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 30)} = 36.087, p < 0.001$) and trial ($F_{(7, 105)} = 3.110, p < 0.01$), but no main effect of drug dose ($F_{(2, 15)} = 0.916, p = 0.422$). No significant day by drug dose interaction ($F_{(4, 30)} = 0.564, p = 0.691$), trial by drug dose interaction ($F_{(14, 105)} = 1.417, p = 0.158$), day by trial interaction ($F_{(14, 210)} = 0.807, p = 0.661$), or day by trial by drug dose interaction ($F_{(28, 210)} = 0.987, p = 0.490$) were observed. The lack of significance between the drug dose groups was self-evident given that the drug was not administered during the training phase.

Figure 3. Latency to reach the hidden platform averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being tested on the water maze at P41.

(***) indicates main effect of training day ($p < 0.001$).

Pretraining/Pretesting Injections

Trial-by-trial latency data for the pretraining/pretesting groups are shown in Figure 4A. These data revealed that latencies in the saline group decreased more so over the three training days compared to the two NBQX dose groups, with a pronounced decrease in latencies on P20. Similar to what was observed with the pretraining group latency results, the initial latencies on each day for the saline group were consistently lower than the previous day and, with each subsequent trial, those latencies rapidly decreased then plateaued. The latencies in the two NBQX dose groups, however, showed little variation throughout each trial and day. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed main effects of day ($F_{(2, 38)} = 26.012, p < 0.001$) and drug dose ($F_{(2, 19)} = 14.709, p < 0.001$), but no main effect of trial ($F_{(7, 133)} = 0.316, p = 0.946$). The analysis revealed a significant day by drug dose interaction ($F_{(4, 38)} = 4.669, p < 0.01$), but no significant trial by drug dose interaction ($F_{(14, 133)} = 0.608, p = 0.855$), day by trial interaction ($F_{(14, 266)} = 0.738, p = 0.735$), nor day by trial by drug dose interaction ($F_{(28, 266)} = 0.933, p = 0.566$).

One-way ANOVAs (drug dose as the independent variable and average daily latencies as the dependent variable) and Tukey's post-hoc tests were then performed to further examine the day by drug dose interaction (Figure 4B). These analyses revealed significant differences on P18 ($F_{(2, 19)} = 6.228, p < 0.01$), where the average latencies of the saline group were significantly lower compared to the 5 and 10 mg/kg NBQX groups ($p < 0.05$). Significant differences were also observed on P19 ($F_{(2, 19)} = 7.191, p < 0.01$), where the average latencies of the saline group were significantly lower compared to the 5 ($p < 0.05$) and 10 ($p < 0.01$) mg/kg NBQX groups. Significant differences were also

observed on P20 ($F_{(2, 19)} = 14.076, p < 0.001$), where the average latencies of the saline group were significantly lower compared to the 5 and 10 mg/kg NBQX groups ($p < 0.001$). The main effect of drug dose provides evidence that the saline group performed better than the two NBQX dose groups.

A. **B.**

Figure 4. Latency to reach the hidden platform (A) averaged across eight trials of daily training and (B) averaged across three training days for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20 and prior to being tested on P41.

(***) indicates main effect of training day ($p < 0.001$); (###) indicates main effect of drug dose ($p < 0.001$). (+) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.05$); (++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!) indicates significant differences between the saline group and the 10 mg/kg NBQX group ($p < 0.05$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$).

Trial-by-Trial Pathlength Results

Pretraining Injections

Trial-by-trial pathlength data for the pretraining group are shown in Figure 5A. The data revealed that, on P18, the pathlength for the 10 mg/kg NBQX dose group was shorter than the other two groups. Subsequently, the pathlength for the 10 mg/kg NBQX group increased over the next two training days while the pathlength for the saline group decreased with each training day. The pathlength for the 5mg/kg NBQX group remained somewhat constant over the three training days. For the most part, the pathlength for the saline group decreased with each successive trial within a training day, while the pathlength for the other two groups showed little variation across each trial. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measure) revealed no main effect of day ($F_{(2, 30)} = 0.077, p = 0.926$) or trial ($F_{(7, 105)} = 0.701, p = 0.671$), but did reveal a main effect of drug dose ($F_{(2, 15)} = 9.047, p < 0.01$). Analysis also revealed a significant day by drug dose interaction ($F_{(4, 30)} = 6.800, p < 0.01$), but no significant trial by drug dose interaction ($F_{(14, 105)} = 0.966, p = 0.493$), no day by trial interaction ($F_{(14, 210)} = 0.965, p = 0.490$), and no day by trial by drug dose interaction ($F_{(28, 210)} = 0.655, p = 0.909$).

One-way ANOVAs (drug dose as the independent variable and average daily pathlength as the dependent variable) and Tukey's post-hoc tests were then performed to further examine the day by drug dose interaction (Figure 5B). The analyses revealed significant differences on P18 ($F_{(2, 15)} = 7.093, p < 0.01$), where the average pathlength of the 10 mg/kg NBQX dose group was significantly lower than the 5 mg/kg NBQX dose group ($p < 0.01$). Significant differences were observed on P19 ($F_{(2, 15)} = 4.865, p < 0.05$),

where the average pathlength of the saline group was significantly lower compared to the 5 and 10 mg/kg groups ($p < 0.05$). Significant differences were observed on P20 ($F_{(2, 15)} = 9.258, p < 0.01$), where the average pathlength of the saline group was significantly lower compared to the 5 and 10 mg/kg NBQX groups ($p < 0.01$).

A.

B.

Figure 5. Pathlength to reach the hidden platform (A) averaged across eight trials of daily training and (B) averaged across three training days for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20.

(+) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.05$); (++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.05$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$); (@@) indicates significant differences between the 5 mg/kg NBQX dose group and 10 mg/kg NBQX group dose group ($p < 0.01$).

Posttraining Injections

Trial-by-trial pathlength data for the posttraining groups are shown in Figure 6. These data revealed no differences in pathlength between the three drug dose groups on the three training days. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 36)} = 12.202, p < 0.001$) and trial ($F_{(7, 126)} = 2.437, p < 0.05$), but no main effect of drug dose ($F_{(2, 18)} = 0.244, p = 0.786$). No significant day by drug dose interaction ($F_{(4, 36)} = 0.122, p = 0.974$), trial by drug dose interaction ($F_{(14, 126)} = 1.424, p = 0.152$), day by trial interaction ($F_{(14, 252)} = 1.452, p = 0.130$), or day by trial by drug dose interaction ($F_{(28, 252)} = 1.194, p = 0.237$) was observed.

Figure 6. Pathlength to reach the hidden platform averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated after being trained on the water maze from P18 to P20

(***) indicates main effect of training day ($p < 0.001$).

Pretesting Injections

Trial-by-trial pathlength data for the pretesting groups are shown in Figure 7. The data revealed a pronounced decrease in pathlength for each of the three drug groups over the three training days. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 30)} = 25.548, p < 0.001$) and trial ($F_{(7, 105)} = 2.533, p < 0.05$), but no main effect of drug dose ($F_{(2, 15)} = 0.399, p = 0.678$). No significant day by drug dose interaction ($F_{(4, 30)} = 0.657, p = 0.626$), trial by drug dose interaction ($F_{(14, 105)} = 1.544, p = 0.108$), day by trial interaction ($F_{(14, 210)} = 0.490, p = 0.937$), or day by trial by drug dose interaction ($F_{(28, 210)} = 1.007, p = 0.461$) was observed.

Figure 7. Pathlength to reach the hidden platform averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being tested on the water maze at P41.

(***) indicates main effect of training day ($p < 0.001$).

Pretraining/Pretesting Injections

Trial-by-trial pathlength data for the pretraining/pretesting groups are shown in Figure 8A. The data revealed a similar pattern as seen in the pathlength data for the pretraining groups, where the pathlength for the 10 mg/kg NBQX was shorter than the other two groups on P18. The pathlength for the 10 mg/kg NBQX dose group then increased over the next two training days while the pathlength for the saline group decreased with each training day. The saline group showed a considerable decrease in pathlength by P20. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 38)} = 6.022, p < 0.01$), but no main effect of drug dose ($F_{(2, 19)} = 2.122, p = 0.147$) or trial ($F_{(7, 133)} = 1.807, p = 0.091$). The analysis revealed a significant day by drug dose interaction ($F_{(4, 38)} = 5.103, p < 0.01$), but no significant trial by drug dose interaction ($F_{(14, 133)} = 0.465, p = 0.948$), day by trial interaction ($F_{(14, 266)} = 0.667, p = 0.806$), or day by trial by drug dose interaction ($F_{(28, 266)} = 1.050, p = 0.401$).

One-way ANOVAs (drug dose as the independent variable and average daily pathlength as the dependent variable) and Tukey's post-hoc tests were performed to further examine the day by drug dose interaction (Figure 8B). The analyses revealed significant differences on P18 ($F_{(2, 19)} = 4.089, p < 0.05$), where the average pathlength of the 10 mg/kg NBQX group was significantly lower than the saline group ($p < 0.05$). No significant differences were observed on P19 ($F_{(2, 19)} = 0.359, p = 0.703$). Significant differences were observed on P20 ($F_{(2, 19)} = 6.489, p < 0.01$), where the average pathlength of the saline group was significantly lower than the 5 mg/kg NBQX group ($p < 0.01$).

A.

B.

Figure 8. Pathlength to reach the hidden platform (A) averaged across eight trials of daily training and (B) averaged across three training days for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20 and prior to being tested on P41.

(**) indicates main effect of training day ($p < 0.01$). (+) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.05$); (!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.05$).

Trial-by-Trial Thigmotaxis Results

Pretraining Injections

Trial-by-trial thigmotaxis data for the pretraining groups are shown in Figure 9A. The data revealed that thigmotaxis for the saline group decreased with each training day, while thigmotaxis for the two NBQX groups remained elevated across the training days. Thigmotaxis data for the saline group decreased, on average, with each successive trial within the training day, while thigmotaxis for the other two groups fluctuated throughout each trial on P18 then plateaued around the second trial on P19 and, for the most part, remained unchanged into P20. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measure) revealed no main effect of day ($F_{(2, 30)} = 3.113, p = 0.059$) or trial ($F_{(7, 105)} = 0.909, p = 0.502$), but did reveal a main effect of drug dose ($F_{(2, 15)} = 9.812, p < 0.01$). The analysis revealed a significant day by drug dose interaction ($F_{(4, 30)} = 4.982, p < 0.01$) and trial by drug dose interaction ($F_{(14, 105)} = 2.003, p < 0.05$), but no significant day by trial interaction ($F_{(14, 210)} = 0.768, p = 0.703$) or day by trial by drug dose interaction ($F_{(28, 210)} = 0.823, p = 0.724$) was evident.

One-way ANOVAs (drug dose as the independent variable and average daily thigmotaxis as the dependent variable) and Tukey's post-hoc tests were performed to further examine the day by drug dose interaction (Figure 9B). The analyses revealed no significant differences on P18 ($F_{(2, 15)} = 2.149, p = 0.151$). Significant differences were observed on P19 ($F_{(2, 15)} = 7.081, p < 0.01$) where the average thigmotaxis of the saline group was significantly lower than the 10 mg/kg NBQX group ($p < 0.01$). Significant differences were also observed on P20 ($F_{(2, 15)} = 13.865, p < 0.001$), where the average

thigmotaxis of the saline group was significantly lower than the 5 and 10 mg/kg NBQX groups ($p < 0.01$).

A.

B.

Figure 9. Thigmotaxis (A) averaged across eight trials of daily training and (B) averaged across three training days for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20.

(##) indicates main effect of drug dose ($p < 0.01$). (++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$); (!!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.001$).

Posttraining Injections

Trial-by-trial thigmotaxis data for the posttraining groups are shown in Figure 10. Thigmotaxis data from each of the three drug groups showed a slight decrease with each successive training day. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 36)} = 25.681, p < 0.001$), but no main effect of drug dose ($F_{(2, 18)} = 0.256, p = 0.777$), or trial ($F_{(7, 126)} = 1.192, p = 0.312$). No significant day by drug dose interaction ($F_{(4, 36)} = 0.118, p = 0.975$), trial by drug dose interaction ($F_{(14, 126)} = 0.987, p = 0.471$), day by trial interaction ($F_{(14, 252)} = 1.387, p = 0.159$), or day by trial by drug dose interaction ($F_{(28, 252)} = 1.172, p = 0.258$) was observed.

Figure 10. Thigmotaxis averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated after being trained on the water maze from P18 to P20

(***) indicates main effect of training day ($p < 0.001$).

Pretesting Injections

Trial-by-trial thigmotaxis data for the pretesting groups are shown in Figure 11. The data revealed a decrease in thigmotaxis for each of the three drug groups from P18 to P20. There appeared to be no consistency in thigmotaxis data on a trial-by-trial basis, nor any identifiable differences in thigmotaxis between the three drug groups. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measures) revealed a main effect of day ($F_{(2, 30)} = 16.538, p < 0.001$) and trial ($F_{(7, 105)} = 2.435, p < 0.05$), but no main effect of drug dose ($F_{(2, 15)} = 0.059, p = 0.943$). No significant day by drug dose interaction ($F_{(4, 30)} = 0.364, p = 0.832$), trial by drug dose interaction ($F_{(14, 105)} = 0.828, p = 0.638$), day by trial interaction ($F_{(14, 210)} = 0.702, p = 0.771$), or day by trial by drug dose interaction ($F_{(28, 210)} = 0.848, p = 0.689$) was observed.

Figure 11. Thigmotaxis averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being tested on the water maze at P41.

(***) indicates main effect of training day ($p < 0.001$).

Pretraining/Pretesting Injections

Trial-by-trial thigmotaxis data for the pretraining/pretesting group are shown in Figure 12. The data revealed that thigmotaxis for the saline dose group decreased with each training day, while thigmotaxis for the two NBQX dose groups remained somewhat elevated across the training days, with a slight decrease in thigmotaxis on P20.

Thigmotaxis data for the saline group showed, on average, a constant decrease with each successive trial during P18 and P19, with a pronounced decrease in thigmotaxis on the seventh and eighth trial on P19, which plateaued into P20. There appeared to be no consistency in thigmotaxis data on a trial-by-trial basis for the two NBQX dose groups. A three-way repeated-measures ANOVA (drug dose as the between-subjects factor, trial and day as the repeated measure) revealed a main effect of day ($F_{(2, 38)} = 8.209, p < 0.01$), and drug dose ($F_{(2, 19)} = 16.116, p < 0.001$), but no main effect of trial ($F_{(7, 133)} = 0.525, p = 0.815$). The analysis revealed no significant day by drug dose interaction ($F_{(4, 38)} = 0.367, p = 0.831$), trial by drug dose interaction ($F_{(14, 133)} = 0.722, p = 0.749$), day by trial interaction ($F_{(14, 266)} = 1.165, p = 0.302$), or day by trial by drug dose interaction ($F_{(28, 266)} = 0.844, p = 0.696$).

Figure 12. Thigmotaxis averaged across eight trials of daily training for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20 and prior to being tested on P41.

(**) indicates main effect of training day ($p < 0.01$); (###) indicates main effect of drug dose ($p < 0.001$).

Single-Trial Probe Test Results

Three weeks following the last training day (P41), the rats underwent a one-minute probe test in the water maze. Each rat assigned to the pretesting or pretraining/pretesting groups received an injection of saline, 5 mg/kg NBQX, or 10 mg/kg NBQX 5 minutes prior to the probe test. The percent of time (split into two 30-second timepoints) spent in the quadrant associated with the platform location was measured and analyzed.

Pretraining Injections

Probe test data for the pretraining groups are shown in Figure 13. The data revealed that the percentage of time spent in the quadrant associated with the former platform location increased for all three pharmacological conditions from the first to the second timepoint. A two-way repeated-measures ANOVA (drug dose as the between-subjects factor, timepoint as the repeated measure) revealed no main effect of timepoint ($F_{(1, 14)} = 2.540, p = 0.133$) but did reveal a main effect of drug dose ($F_{(2, 14)} = 4.414, p < 0.05$). The analysis revealed no significant timepoint by drug dose interaction ($F_{(2, 14)} = 0.136, p = 0.874$).

One-way ANOVAs (drug dose as the independent variable and timepoints as the dependent variables) and Tukey's post-hoc tests revealed significant differences during the first timepoint ($F_{(2, 18)} = 4.122, p < 0.05$) where the percentage of time spent in the quadrant for the saline group was significantly higher than the 10 mg/kg NBQX group ($p < 0.05$).

Figure 13. Percentage of time spent in the quadrant associated with the former platform location during the probe test for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20.

(!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.05$).

Posttraining Injections

Probe test data for the posttraining groups are shown in Figure 14. The data revealed that the percentage of time spent in the quadrant associated with the former platform location increased for the 5 and 10 mg/kg NBQX groups, but decreased for the saline group, from the first to the second timepoint. A two-way repeated-measures ANOVA (drug dose as the between-subjects factor, timepoint as the repeated measure) revealed no main effect of timepoint ($F_{(1, 18)} = 0.460, p = 0.506$), but did reveal a main effect of drug dose ($F_{(2, 18)} = 4.589, p < 0.05$), and a significant timepoint by drug dose interaction ($F_{(2, 18)} = 4.000, p < 0.05$).

One-way ANOVAs (drug dose as the independent variable and timepoints as the dependent variable) and Tukey's post-hoc tests were performed to further examine the timepoint by drug dose interaction. Significant differences were observed during the first timepoint ($F_{(2, 18)} = 8.350, p < 0.01$) where the percentage of time spent in the quadrant for the saline group was significantly higher than the 5 mg/kg NBQX group ($p < 0.01$) and the 10 mg/kg NBQX group ($p < 0.05$).

Figure 14. Percentage of time spent in the quadrant associated with the former platform location during the probe test for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated after being trained on the water maze from P18 to P20.

(++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.05$).

Pretesting Injections

Probe test data for the pretesting groups are shown in Figure 15. The data revealed that the percentage of time spent in the quadrant associated with the former platform location increased for the saline and 5 mg/kg NBQX group, but decreased for the 10 mg/kg NBQX group, from the first to the second timepoint. A two-way repeated-measures ANOVA (drug dose as the between-subjects factor, timepoint as the repeated measure) revealed no main effect of timepoint ($F_{(1, 15)} = 0.825, p = 0.378$) or drug dose ($F_{(2, 15)} = 0.744, p = 0.492$). The analysis also revealed no significant timepoint by drug dose interaction ($F_{(2, 15)} = 2.642, p = 0.104$).

Figure 15. Percentage of time spent in the quadrant associated with the former platform location during the probe test for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being tested on the water maze at P41.

Pretraining/Pretesting Injections

Probe test data for the pretraining/pretesting groups are shown in Figure 16. The data revealed that the percentage of time spent in the quadrant associated with the former platform location increased for the 5 and 10 mg/kg NBQX groups, but slightly decreased for the saline group, from the first to the second timepoint. A two-way repeated-measures ANOVA (drug dose as the between-subjects factor, timepoint as the repeated measure) revealed a main effect of timepoint ($F_{(1, 19)} = 5.776, p < 0.05$) and drug dose ($F_{(2, 19)} = 4.181, p < 0.05$). The analysis revealed no significant timepoint by drug dose interaction ($F_{(2, 19)} = 2.296, p = 0.128$).

One-way ANOVAs (drug dose as the independent variable and timepoints as the dependent variables) and Tukey's post-hoc tests revealed significant differences during the first timepoint ($F_{(2, 19)} = 10.163, p < 0.01$) where the percentage of time spent in the quadrant for the saline group was significantly higher than the 5 and 10 mg/kg NBQX group ($p < 0.01$).

Pretraining/Pretesting NBQX: Target

Figure 16. Percentage of time spent in the quadrant associated with the former platform location during the probe test for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20 and after being trained on the water maze from P18 to P20.

(^) indicates main effect of timepoint ($p < 0.05$). (++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$).

Immunohistochemistry

In order to examine the effect of AMPAr blockade during water maze training and/or testing on neural circuit changes, immunohistochemical techniques were used to label immunopositive cells in the anterior cingulate cortex (ACC) and the CA1 region of the hippocampus.

Immunohistochemistry Results

Pretraining Injections

Mean cell density for the CA1 region of the hippocampus for the pretraining group is shown in Figure 17A. A one-way ANOVA revealed a significant difference between the three pharmacological conditions ($F_{(2, 21)} = 7.923, p < 0.01$). A Tukey post-hoc test further revealed that the saline group had significantly more activation in the CA1 region compared to the 10 mg/kg NBQX group ($p < 0.01$).

Mean cell density for the ACC of the pretraining group is shown in Figure 17B. A one-way ANOVA revealed a significant difference between the three pharmacological conditions ($F_{(2, 21)} = 15.714, p < 0.001$). A Tukey post-hoc test further revealed that the saline group had significantly more activation in the ACC compared to the 5 mg/kg NBQX group ($p < 0.01$) and the 10 mg/kg NBQX group ($p < 0.001$).

A.

Pretraining HPC

B.

Pretraining ACC

Figure 17. Quantification of c-Fos staining in the (A) CA1 region of the hippocampus and the (B) ACC region for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20.

(++) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.01$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$); (!!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.001$).

Posttraining Injections: ACC

Mean cell density for the CA1 region of the hippocampus for the posttraining group is shown in Figure 18A. A one-way ANOVA revealed a significant difference between the three pharmacological conditions ($F_{(2, 21)} = 8.336, p < 0.01$). A Tukey post-hoc test further revealed that the saline group had significantly more activation in the CA1 region compared to the 5 mg/kg NBQX group ($p < 0.05$) and the 10 mg/kg NBQX group ($p < 0.01$).

Mean cell density for the ACC of the posttraining group is shown in Figure 18B. A one-way ANOVA revealed no significant difference between the three pharmacological conditions ($F_{(2, 21)} = 0.042, p = 0.959$).

A.

Posttraining HPC

B.

Posttraining ACC

Figure 18. Quantification of c-Fos staining in the (A) CA1 region of the hippocampus and the (B) ACC region for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated after being trained on the water maze from P18 to P20.

(+) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.05$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$).

Pretesting Injections: ACC

Mean cell density for the CA1 region of the hippocampus for the pretesting group is shown in Figure 19A. A one-way ANOVA revealed a significant difference between the three pharmacological conditions ($F_{(2, 21)} = 4.787, p < 0.05$). A Tukey post-hoc test further revealed that the 10 mg/kg NBQX group had significantly more activation in the CA1 region compared to the saline group ($p < 0.05$).

Mean cell density for the ACC of the pretesting group is shown in Figure 19B. A one-way ANOVA revealed no significant difference between the three pharmacological conditions ($F_{(2, 21)} = 2.308, p = 0.124$).

A.

Pretesting HPC

B.

Pretesting ACC

Figure 19. Quantification of c-Fos staining in the (A) CA1 region of the hippocampus and the (B) ACC region for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being tested on the water maze at P41.

(\$) indicates significant differences between the 10 mg/kg NBQX dose group and the saline group ($p < 0.05$).

Pretraining/Pretesting Injections

Mean cell density for the CA1 region of the hippocampus for the pretraining/pretesting group is shown in Figure 20A. A one-way ANOVA revealed a significant difference between the three pharmacological conditions ($F_{(2, 21)} = 7.363$, $p < 0.01$). A Tukey post-hoc test further revealed that the saline group had significantly more activation in the CA1 region compared to the 10 mg/kg NBQX group ($p < 0.01$).

Mean cell density for the ACC of the pretraining/pretesting group is shown in Figure 20B. A one-way ANOVA revealed a significant difference between the three pharmacological conditions ($F_{(2, 24)} = 6.479$, $p < 0.01$). Tukey post-hoc test further revealed that the saline group had significantly more activation in the ACC compared to the 5 and 10 mg/kg NBQX group ($p < 0.05$).

A.

Pretraining/Pretesting HPC

B.

Pretraining/Pretesting ACC

Figure 20. Quantification of c-Fos staining in the (A) CA1 region of the hippocampus and the (B) ACC region for saline, 5 mg/kg NBQX, and 10 mg/kg NBQX dose groups treated prior to being trained on the water maze from P18 to P20, and after being tested on the water maze at P41.

(+) indicates significant differences between the saline group and the 5 mg/kg NBQX dose group ($p < 0.05$); (!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.05$); (!!) indicates significant differences between the saline group and the 10 mg/kg NBQX dose group ($p < 0.01$).

Discussion

This thesis investigated the effect of AMPA receptor blockade on the acquisition of spatial information during the juvenile period and the expression of this spatial information during a remote memory test. To this end, rats were split into four pharmacological treatment conditions with three doses (0, 5 or 10 mg/kg) of the AMPA receptor antagonist, NBQX. The treatment conditions consisted of rats receiving saline or NBQX injections 1) every day before water maze training during the juvenile period (pretraining condition), 2) every day after water maze training during the juvenile period (posttraining condition), 3) once before the remote memory test (pretesting condition), or 4) every day before being trained during the juvenile period and once before the remote memory test (pretraining/pretesting condition). Along with behavioral measures to assess the acquisition and retention of spatial information, c-Fos labeling was assessed to determine the contribution of the hippocampus and anterior cingulate cortex during the remote memory test.

The pretraining condition was used to examine the effect of blocking AMPA receptors on the acquisition of the water maze task and how this would affect the long-term storage of spatial information. Due to potential performance deficits (i.e., impaired motor ability) produced by the drug in the pretraining condition, the posttraining condition was employed to allow the rats to perform the task while not under the influence of the drug. Giving the drug immediately after training was hypothesized to interfere with a memory consolidation process thereby ruling out any performance deficits on task acquisition and retrieval. This condition would control for any motor deficits caused by the drug that may not have been apparent during the pretraining or

pretraining/pretesting conditions. The pretesting condition was used to assess the effect of AMPAR blockade on the expression of a remote spatial memory when the acquisition of the task was allowed to proceed normally. The pretraining/pretesting condition was employed mainly as a pharmacological control to assess state-dependent learning and retrieval. Hence, if the rats performed poorly in the pretesting condition, this could be due to either a memory retrieval deficit or an incongruent “state” during the test. The pretraining/pretesting condition would assess the state interpretation.

Three measurements were recorded and analyzed during the water maze acquisition trials: latency in seconds to reach the platform, pathlength in meters, and thigmotaxis (percent pathlength). Pathlength has been widely used as a measure of performance on learning trials in the Morris water maze as it has the advantage of being immune to swimming speed (Vorhees & Williams, 2006). Swimming a shorter path to reach the platform is indicative of learning, as the animal demonstrates that it remembers the platform location from previous trials (Voorhees & Williams, 2006). Memory impairment would therefore manifest as a longer pathlength, brought about by the inability of the animal to use spatial memory acquired from previous trials to navigate a direct path to the platform.

One criticism over using pathlength as a measure of performance is that not all start locations are equidistant from the platform, creating short and long paths to the goal. Unfortunately, there is no true solution to this problem, because using the same start position for every trial may create skewed results as the rats might memorize specific routes rather than use distal cues to try and remember the platform location (Voorhees & Williams, 2006). Some drugs have been known to interfere with sensorimotor functions,

which are required in order for the animal to recognize that the platform is the goal (Cain *et al.*, 1996; Voorhees & Williams, 2006). In order to assess that the animal understands the goal of the task, and the degree of spatial memory acquisition, thigmotaxis is measured. Once the animal has learned that there is no escape located around the perimeter of the tank, they swim away from the wall and look for alternate means of escape. If, however, the animal has not gained an adequate awareness of its surroundings and memory impairment has resulted in an inability to recognize that the goal is to find the platform located closer to the inside of the pool, then excessive thigmotaxic swimming will result (Cain *et al.*, 1996; Voorhees & Williams, 2006).

Water Maze Training

Both the pretraining and pretraining/pretesting groups showed the same pattern of results across all three measurements. In these groups, performance of the saline and the two NBQX groups did not differ on the initial training day. As the days progressed, the saline group showed a marked increase in performance (decrease in all three measures), while both NBQX groups showed no improvement. These observations suggest that the saline group was able to acquire the task but the two NBQX groups were impaired.

One possibility that accounts for the impaired performance in the NBQX groups during training suggests that AMPAr are required for the acquisition of short-term spatial memories and blocking them interferes with the short-term processing of spatial information leading to impaired performance on this spatial memory task. These results parallel those observed by Filliat *et al.* (1997) and Liang *et al.* (1997), who reported that infusion of AMPAr antagonist before training on the water maze resulted in longer

escape latencies compared to their control groups. Liang *et al.* (1997) suggested that these results demonstrate a role of AMPAr in acquiring spatial information and that AMPAr blockade prevents the membrane depolarization necessary for opening NMDA channels, which, in turn, impedes LTP initiation. Ferretti *et al.* (2014) also found that spatial training in the water maze induces an increase in phosphorylation levels at the Ser845 or Ser831 sites of the GluR1 subunit, which modulates AMPAr activity, which is highest immediately after training. Thus, blocking AMPAr before training may impair spatial memory acquisition by preventing phosphorylation of the GluR1 subunit.

An alternate explanation to the impaired performance in the NBQX groups is that the drug resulted in a motor deficit or some other confound that disrupted task completion. Saab *et al.* (2012) observed that AMPAr inactivation in the cerebellum resulted in impairments in fine motor coordination. As such, the pathlength data in the 10 mg/kg NBQX groups (both pretraining and pretraining/pretesting) on P18 was significantly lower than the saline group. Because this was the first day of training, it suggests that the 10 mg/kg dose may have impeded swimming ability. Based on the fact that the other measures showed no such discrepancy, it is conceivable that the rats spent little time swimming and possibly remained immobile for much of the trial, which would have resulted in a shorter recorded pathlength. However, this was short-lived as the pathlengths in these groups increased over days suggesting a minimal effect of the drug on motor performance.

To be able to assess the memory-altering properties of NBQX while controlling for motor impact, posttraining injections of NBQX were used. In addition to examining

the effect of AMPAr blockade on the consolidation process, the posttraining condition served to control for any locomotor deficits that the injection and/or drug may cause, as well as to control for any drug carry-over effects. Because memory consolidation processes continue after training, injections of NBQX during this time were hypothesized to specifically affect the memory process while allowing the rats to swim unencumbered by the drug and not interfering with motor function. Both NBQX groups in the posttraining condition showed similar performance as the saline group on each of the three measures. The lack of significant differences between the saline and the two NBQX groups indicate that the drug did not cause any locomotor deficits. The two NBQX groups in the posttraining condition received their injections after being trained on the water maze and showed no observable lag in performance on the training day after being administered the drug. This indicates that the washout period for the drug is quick and that its effects on performance did not carry over into the next day of training. This also suggests that the differences observed from P18 to P19 and from P19 to P20 for the pretraining and pretraining/pretesting group were a result of AMPAr blockade impairing their memory on the training day, and were not caused by a systemic accumulation of the drug from one training day to the next.

AMPAr blockade did not seem to affect the day-to-day water maze performance as evident by the similarities in performance on the first trial on P19 and P20. Since the rats were treated with the drug after being trained on each of the three days, an impairment in the short-term processes as a result of AMPAr blockade would have resulted in significant differences in the initial trial of each successive day. No discernible

lag in performance during the initial trial was observed on P19 and P20, suggesting that AMPAr blockade immediately after training caused no deficit in short-term processing.

The lack of significant differences observed in the pretesting groups was expected as none of the rats were treated with the AMPAr antagonist during the training phase, allowing them to acquire the memory under normal conditions. This condition examined the effect of AMPAr blockade on retention or retrieval of the spatial information during the probe trial.

Water Maze Testing

Three weeks after the last training day (P41), all rats underwent a single, one-minute probe test in the water maze with the platform removed. Only the rats in the pretesting and pretraining/pretesting conditions received an injection 5 minutes prior to the probe test. The percentage of time spent in the quadrant associated with the platform was measured, split into two 30-second timebins, and analyzed to assess how the various drug manipulations during the training or testing phases affected the integrity of the spatial memory representation.

Both the saline and 5 mg/kg NBQX groups in the pretraining condition showed a preference for the target quadrant, spending significantly more time in that quadrant than chance and the 10 mg/kg group. The 10 mg/kg group spent less than 25% of their time in the target quadrant. The preference shown by the saline and 5 mg/kg groups continued into the second 30-second timebin, indicating that both of these groups showed an intact spatial memory representation. Although the 10 mg/kg NBQX group showed an increase

from below to slightly above chance during the second timebin, results suggest that this group did not have an intact spatial memory representation. These results parallel those observed by Liang *et al.* (1997), who found that AMPA blockade during training resulted in less time spent in the target quadrant during the probe test, and more time in the adjacent quadrant, suggesting that the memory of the platform was less precise than that of the saline treated group. In the present work, 10 mg/kg NBQX administered during the juvenile period impaired a memory that was retrieved 3 weeks later. This indicates that AMPA receptors are required for spatial memory acquisition and that blocking them during a sensitive developmental period leads to long-lasting memory impairments.

It was interesting to note that the 5 mg/kg NBQX group showed a similar target quadrant preference as the saline group, despite evidence from the training phase that indicated they were not learning. This may indicate that the dose was enough to impair most, but not all, memory acquisition at a younger age, allowing the memory that was acquired to be consolidated, stored, and expressed during the probe test. Filliat *et al.* (1998) observed similar results, where adult rats treated with 30 mg/kg NBQX during water maze training showed significantly higher latencies compared to the control group. Data from the probe test, however, showed that both the control group and the 30 mg/kg NBQX group swam preferentially in the target quadrant. They concluded that the effects of repeated injections of 30 mg/kg NBQX were reversible, and that the retention of those rats was not affected (Filliat *et al.*, 1998).

The saline group in the posttraining condition spent more time in the target quadrant than both NBQX groups despite showing no discernible differences during the training phase. The saline posttraining group spent close to 50% of their time in the target quadrant during the first 30 second timebin and showed a slight decrease in time (to about 30%) spent in the target quadrant during the second timebin. One possible explanation for this is that after unsuccessful attempts to find the platform in the target quadrant, the saline group simply altered their search pattern and searched for it in the other quadrants. The percentage of time spent in the target quadrant for the saline group was still above chance during the second timebin, indicating that, despite their exploratory behavior to try and find the platform, they still showed a preference for the target quadrant. Since both NBQX groups showed significantly less than chance time spent in the target quadrant, it suggests that their spatial memory representation was not intact, despite being able to swim to and locate the platform during the training phase. These results indicate that, while blocking AMPAR has no effect on short-term recall after memory acquisition has occurred under normal conditions, AMPAR blockade may disrupt the memory consolidation process such that the memory that was acquired cannot be expressed after some time has passed.

Immediately after training, various second messengers mediate the activation of biochemical cascades, which facilitate the memory consolidation process through the activation of immediate early and late genes, which create lasting synaptic alterations (Barros *et al.*, 1999; Dudai, 1996). One such second messenger, CaMKII, has been shown to be involved in both consolidation and LTP. Within the first 30 minutes after training, these complexes increase in the CA1 and phosphorylate the GluR1 subunit of the

AMPAr. This CaMKII-mediated phosphorylation correlates with increased AMPAr binding within the first 1 – 3 hours after training, and has been observed to be necessary for memory formation (Barros *et al.*, 1999). Infusion of a CaMKII inhibitor immediately after training has been shown to impair performance in a probe test, alluding to the involvement of CaMKII in the consolidation process, which happens immediately after training (Barros *et al.*, 1999). Referring back to the results of the current study, AMPAr blockade may have lead to the inability of CaMKII to phosphorylate AMPAr, thus impeding the consolidation process, resulting in the impaired performance observed during the probe trial.

Some discrepancies were noted between the NBQX groups in the pretraining and posttraining conditions. On average, it appeared that the pretraining NBQX groups spent more time in the target quadrant than the NBQX groups in the posttraining condition. This may, in part, be due to the timing of the injections and the time course of pharmacological activity of the NBQX. It is possible that AMPAr activation is required more so during the immediate post-consolidation period, once the memory has been acquired, than it is before memory acquisition. This is evident by the fact that the pretraining group showed a significant impairment during training, yet showed some memory expression during the probe test, to the extent that there were no longer any significant differences in performance between the saline and 5 mg/kg NBQX group. The posttraining group, however, showed no significant differences in performance during training, yet both NBQX groups showed a significant impairment in memory expression.

In terms of the time course of NBQX, if it were active for a particular period of time, for example 30 minutes, then there would have been functional blockade of AMPAr during training, followed by 15 minutes or so after training for the pretraining group. Functional AMPAr blockade for the posttraining group would have lasted for the full 30 minutes of NBQX activity immediately after training. As hypothesized, it would be during this time, immediately after training, that AMPAr activation would be critical for the memory consolidation and storage processes. If NBQX were active for twice as long immediately following training in the posttraining condition, then it is conceivable that any memory acquired was unable to be consolidated into long-term stores and thus expressed during the probe test. Any memory fragments acquired during training by the pretraining group, however, may have had the opportunity to be consolidated and stored under normal conditions, and expressed during the probe test, given that AMPAr blockade occurred for less time after training.

In the pretesting condition, there were no differences in the percentage of time spent in the target quadrant between both NBQX groups and the saline group despite receiving the AMPAr antagonist 5 minutes prior to the probe test. The 5 mg/kg NBQX group showed an increase in the time spent in the target quadrant from the first to the second timebin similar to that of the saline group. This pattern may indicate that AMPAr blockade resulted in a slower retrieval process, but with time, the memory was able to be retrieved either independently from AMPAr activation, or occurred with fewer active receptors.

The 10 mg/kg NBQX group spent the same percentage of time in the target quadrant as the saline group during the initial timebin, but then decreased their time spent in the target quadrant during the second timebin. This observation may be explained in a similar manner as the saline group in the posttraining condition, where the rats remembered where the platform was, but after unsuccessful attempts to find it, searched for it in the other quadrants.

When compared to the pretraining and posttraining conditions, the pretesting NBQX treatment produced the smallest deficit in memory expression. AMPAr play a role in memory retrieval by mediating fast excitatory transmission through hippocampal synapses required to activate stored memories (Bast *et al.*, 2005). AMPAr blockade has been shown to impair memory expression, as well as a reduction in fast excitatory transmission at perforant-path synapses onto dentate granule cells in the dorsal hippocampus (Bast *et al.*, 2005; Izquierdo *et al.*, 1993). Liang *et al.* (1997) found that rats treated with an AMPAr antagonist prior to being tested showed impaired memory retrieval, spending less time in the target quadrant than the controls. They suggested that AMPAr play a role in utilization of spatial information that was previously acquired under normal conditions. Szapiro *et al.* (2000) observed similar results upon administration of an AMPAr antagonist prior to a probe test and suggested that AMPAr play a role in retrieval by activating signaling cascades involving PKA and MAPK, which are required for remote memory retrieval. They further indicated that low concentrations of the drug were ineffective (Szapiro *et al.*, 2000). In the present, pretesting condition, it is conceivable that the concentration of the drug was too low to

show an effect. The drug dose was chosen based on the younger rats, and may not be as effective during adolescence. This may be due to the size of the rat on P41, or possibly an increase in the number of AMPAr.

Both NBQX groups in the pretraining/pretesting condition showed less of a preference for the target quadrant than the saline group, as is evident by the fact that they spent a lower percentage of time in the target quadrant; less than chance during the first timebin and at chance during the second timebin. A lack of state-dependent memory is also apparent from these results. A strong performance on the probe test may have been indicative of a more efficient memory retrieval process, resulting from being in a state similar to when the memory was acquired, and not truly representative of the ability to express the memory. The resulting impaired probe test performance, however, discounts the presence of any state-dependent memory, and ascertains the pretraining and pretesting effects observed.

There were some discrepancies in the pretraining and pretraining/pretesting 5 mg/kg NBQX groups even though they showed identical performance during training. The pretraining 5 mg/kg NBQX group showed intact memory, while the pretraining/pretesting 5 mg/kg showed impaired memory expression. The main difference between the two conditions was the administration of the drug prior to the probe test in the pretraining/pretesting condition. Therefore, the combined effect of AMPAr blockade during training and testing appears to be required to impair memory expression with this 5 mg/kg dose.

A plausible explanation for the discrepancies observed between the pretesting and pretraining/pretesting groups may be that training initiates AMPAr- and NMDAr-mediated LTP. This LTP initiation clearly continues into the posttraining consolidation period, during which new AMPAr are inserted into the membrane. When the memory is reactivated during the probe test, the increase in AMPAr makes it easier to activate neurons and complete the memory. As previously mentioned, the drug dose was not high enough to completely impair memory expression in the pretesting condition. In the pretraining/pretesting condition, some AMPAr are blocked during training, resulting in a weakened LTP process. Although the pretraining 5 mg/kg NBQX group showed no impairment in memory expression, likely due to the aforementioned functional properties of the drug, it is possible that the pretraining manipulation decreased the total number of new AMPAr that were inserted into the membrane. Thus, NBQX administration prior to the probe test would result in AMPAr blockade and impaired memory expression.

c-Fos Stereology

Immediate early gene proteins regulate the transcription of additional genes directing the cellular response to a variety of environmental stimuli (Sheng *et al.*, 1990). These regulatory proteins, such as c-Fos, control downstream gene expression and are thought to translate environmental signals into neuronal function (Goelet *et al.*, 1986; Kaczmarek & Chaudhuri, 1997; Sheng *et al.*, 1990). It has been suggested that when these regulatory proteins are translated, they return to the nucleus to regulate the expression of late response genes whose products are thought to directly subserve changes responsible for synaptic function (Carew, 1996). If this view were correct, it

would mean that expression of the c-Fos protein might provide a marker for cells that have recently been activated.

In the present thesis, expression of the c-Fos protein was used as a marker for recently activated cells. The function of the c-Fos protein depends on its binding with Jun proteins (Sheng & Greenberg, 1990). These Fos/Jun protein complexes stimulate transcription (c-Fos/c-Jun dimer) or repress transcription (Fos/JunB dimer) (Sheng & Greenberg, 1990). Therefore, the c-Fos protein may activate or repress intracellular direct effector proteins leading to enhanced or depressed cellular function (Kaczmarek, 1993; Kaczmarek & Nikolajew, 1990). In the present thesis, the c-Fos protein was used as a measure of cellular activity.

An elevation in c-Fos expression has been shown to occur in the hippocampus during retrieval of remote memory in animals after being trained on the water maze (Wartman *et al.*, 2014). Increased activity and structural changes have been observed in the ACC during remote memory tests, indicative of remote memory storage (Goshen *et al.*, 2011; Wartman *et al.*, 2014). Inactivation of the ACC has been shown to impair performance on remote memory tests (Wartman *et al.*, 2014). These findings indicate the existence of a hippocampal-cortical network that becomes active during remote spatial memory testing and therefore, contributes to the memory retrieval process (Clark *et al.*, 2007; Teixeira *et al.*, 2006; Wartman *et al.*, 2014).

Successful performance in the water maze requires the individual to be able to locate itself in the environment and specify the goal of the task. A network of place cells within the hippocampus, which serve to support accurate spatial navigation, mediates the former (Hok *et al.*, 2005). The latter is mediated by place fields in the medial prefrontal

cortex (mPFC), including the ACC, which encode the goal of the task and location of the goal in order to plan a path to achieve the goal (Insel & Barnes, 2014; Hok *et al.*, 2005). Inactivation of the mPFC has resulted in altered hippocampal place cell activity, suggesting a modulatory role of the mPFC in spatial responses in the hippocampus (Kyd & Bilkey, 2003). Therefore, the activation of both the hippocampus and ACC are required to successfully complete the water maze task.

Compared to the saline group, both NBQX group in the posttraining condition showed impaired memory expression during the probe test, which coincided with reduced hippocampal activation in that group. The two NBQX groups of the posttraining condition showed normal memory acquisition during the training phase, despite being treated with NBQX after each training day, which, based on previous findings, should have resulted in increased hippocampal activation and unhindered performance during the probe test (Wartman *et al.*, 2014). Instead, reduced hippocampal activation was observed in conjunction with impaired memory expression. As previously discussed, this indicates that AMPAR blockade impaired the memory consolidation process into long-term stores such that the memory acquired could not be retained or expressed. From a systems level, the acquired spatial memory remained in the short-term hippocampal stores, as reflected in the training data, however, treatment with NBQX impaired the ability of the hippocampus to consolidate that memory into long-term stores. The hippocampus, then, was unable to retrieve that memory, preventing the rats from being able to navigate around the pool, resulting in decreased activation and impaired performance on the probe test.

There was no difference in ACC activation in the posttraining condition between the saline and NBQX groups, even though both NBQX groups showed impaired performance on the probe test. The reason for this may be that the memory of the goal was unaffected by AMPAr blockade after it was acquired and was successfully consolidated into long-term stores. During the probe test, the ACC was activated in order to express the memory for the goal and to plan a path to reach the goal. Since, however, the spatial memory was impaired from AMPAr blockade, it could not be expressed, as evident from the decreased hippocampal activation, and thus the rats were unable to complete the task. This suggests that activation of the ACC to express the memory of the goal is not sufficient to complete the task, but that activation of both the hippocampus (to express the spatial memory) and the ACC are required to complete the task.

The 5 mg/kg NBQX group in the pretraining condition showed no difference in hippocampal activation relative to saline and subsequent unhindered performance during the probe test. ACC activation for this group, however, was significantly lower than saline. This may indicate that, in spite of AMPAr blockade, some spatial memories were acquired by the hippocampus during training and consolidated into long-term stores and, once the opportunity to express those memories arose, the hippocampus was reactivated and retrieved the memories during the probe test. It is possible that any fragment of the spatial memory expressed in combination with the expression of the goal memory may have been sufficient to successfully complete the task. The reduced ACC activation may have been a reflection of the impaired spatial memory retention, and any activation observed could have served to solely express the goal memory. The 10 mg/kg NBQX

group showed impaired memory expression during the probe test, which coincided with reduced hippocampal activation. The explanation provided for the decreased hippocampal activation in the posttraining condition may account for these results. Unlike the posttraining condition, which was allowed to acquire the spatial memory under normal conditions, treatment with NBQX prior to training impaired memory acquisition, as evidenced by the impaired performance during the training phase. Therefore, there was no short-term memory for the hippocampus to retain and consolidate into long-term stores. Since there was no memory to retrieve from long-term stores, the hippocampus remained relatively inactive, resulting in the impaired performance observed. The decreased ACC activation observed further indicated the degree of impairment in spatial memory acquisition caused by AMPAR blockade, and any activation observed served to express the goal memory, which, as previously stated, is not sufficient to complete the task.

The 5 mg/kg group in the pretraining/pretesting condition showed increased hippocampal activation and decreased ACC activation, which coincided with impaired probe test performance. These results differ from the 5 mg/kg NBQX group probe test data, despite undergoing the same pretraining manipulation and showing similar patterns of activity, suggesting that the pretesting NBQX treatment was enough to cause an impairment in memory expression. Since the hippocampus showed no difference in activation from saline, it is more than likely that the impairment comes from a decreased activation in the ACC. It is possible that the memory fragments were consolidated and stored, as in the pretraining condition; however, re-administration of the drug was enough

to impair the ACC from expressing that memory. The hippocampus, however, was actively trying to retrieve the abolished memory, resulting in increased activation, to no avail as the trial indicated a complete impairment in memory expression. The 10 mg/kg group showed decreased activation in both the hippocampus and ACC, along with impaired performance on the probe test, as was observed in the 10 mg/kg group of the pretraining condition. Similar to the pretraining condition, it is possible that treatment with NBQX prior to training impaired memory acquisition. Therefore, there was no short-term memory for the hippocampus to retain and consolidate into long-term stores. Since there was no memory to retrieve from long-term stores, the hippocampus remained inactive, resulting in the impaired probe test performance. The re-administration of the drug would have resulted in the abolishment of any memory that may have been consolidated into long-term stores, and therefore, could not have been expressed, thus explaining the decreased ACC activation.

The pretesting group showed some discrepancies, with greater hippocampal activation being observed in the 10 mg/kg NBQX group relative to saline. The only plausible explanation for these results is an error in the cell count. The pretesting saline group showed a significantly lower cell density compared to the other saline groups, which is highly unlikely given that the saline groups received no pharmacological treatment. This further confirms the likelihood of a cell count error, and thus might not be representative of the actual hippocampal activation that may have occurred during the probe test. The ACC showed no difference in activation between the saline and the two NBQX groups. This suggests that the memories formed during training remained intact,

and that the ACC activation was unaffected by either drug doses, perhaps as a result of the drug dose not being high enough, as previously discussed, allowing it to express the memory unencumbered. The performance on the probe test further confirms this by showing that the performance for the NBQX did not differ from saline, indicating successful expression of the memory.

Conclusion

The results from this study suggest that AMPAr play a significant role in learning and memory processes. AMPAr blockade resulted in impaired memory acquisition and remote expression (pretraining; pretraining/pretesting), along with impaired memory consolidation (posttraining). Decreased c-Fos staining in the hippocampus and ACC further confirm the role of AMPAr in the acquisition and remote expression of memories. The stereology data also suggests the presence of multiple memory fragments encoded during training that must be expressed during testing in order to successfully complete the task. Therefore, the combination of both behavioural and neuroanatomical results confirms the hypothesis that AMPAr are required for the acquisition and expression of remote spatial memories.

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