BEHAVIORAL CONSEQUENCES FOLLOWING

AAV MEDIATED HIPPOCAMPAL EAAC1 KNOCKDOWN

by

Katie Marie Coombs

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Applied Psychology

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2007

APPROVAL

of a thesis submitted by

Katie Marie Coombs

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

Dr. Michael Babcock

Approved for the Department of Psychology

Dr. Richard Block

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Katie Marie Coombs

April, 2007

ACKNOWLEDGEMENTS

I would like to thank my committee members for their support in the completion of my thesis. I would especially like to thank my advisor, Dr. A. Michael Babcock. His dedication and commitment has helped me grow as a researcher. I would also like to express my appreciation to the members of Dr. Babcock's laboratory for their contributions. Specifically, Christy Weeden, Damon McNeil, Khena Bullshields and Denean Standing for their assistance with collecting the behavioral data. Dr. Dave Poulson from the department of Pharmaceutical Sciences at the University of Montana provided the viral vectors for this project. Funds for this project were provided by: R01NS33270 and P20 RR15583.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

The neuronal glutamate transporter EAAC1 (EAAT3) is present in hippocampal neurons to prevent excessive glutamate accumulation. Glutamate receptor-dependent synaptic plasticity is important for learning and memory. The present study investigates behavior associated with blocking the glutamate transporter EAAC1. To manipulate EAAC1 function, rats were intrahippocampally injected with a adeno-associated viral (AAV) vector encoding an EAAC1 antisense mRNA sequence or an AAV empty cassette. Twenty-eight days following surgery, rats were tested in a delayed matching-to-place (DMTP) watermaze task to examine spatial memory, which is hippocampal-dependent. Rats treated with EAAC1 antisense exhibited shorter latencies to locate the target platform relative to controls ($p < 0.05$). These data indicate that microinfusion of AAV encoding EAAC1 antisense significantly altered performance on task involving glutamate transmission and the hippocampus.

INTRODUCTION

Despite controversy surrounding the role of the hippocampus in learning and memory, there is general agreement that it is necessary for spatial memory in rodents (Nadel, 1991). Spatial memory is responsible for recording information about one's environment and its spatial orientation. Research suggests that hippocampal damage impairs spatial memory. Glutamate receptors, in particular NMDA receptors that are concentrated in the hippocampus, have been postulated to be involved in the molecular mechanisms of spatial learning and memory formation.

The aim of this study was to decrease the expression of the glutamate transporter excitatory amino acid carrier-1 (EAAC1) using a viral mediated antisense strategy in order to manipulate glutamate function in the hippocampus. By blocking the reuptake of glutamate in the hippocampus, it is possible to examine the importance of this transmitter in learning and memory processes. In the present study, adeno-associated virus (AAV) vector encoding an antisense mRNA sequence was microinfused into the dorsal hippocampus to knockdown the glutamate transporter EAAC1. The use of AAV construct delivered by this method can be precise and long lasting, providing sufficient time for detailed behavioral analysis. To explore the behavioral effects of EAAC1 suppression, a delayed matching-to-place paradigm was used to examine the flexible use of spatial memory. Delayed matching-to-place is a behavioral task that is sensitive to spatial memory performance (Whishaw, Rod, & Auer, 1994).

BACKGROUND

Neuroanatomy of the Hippocampus

The hippocampal formation is located inside the medial temporal lobe. The term hippocampal formation generally applies to the entorhinal cortex, the hippocampus, and the subicular complex (Gigg, Tan, & Finch, 2004) (Figure 1). The hippocampus is composed of the dentate gyrus (DG) and the *Cornu Ammonis* (Ammon's horn). The Ammon's horn can be further subdivided into four subregions, CA1-CA4, with the CA4 frequently termed the hilus and considered part of the dentate gyrus. The subicular complex includes the subiculum, presubiculum, and parasubiculum (refer to Figure 1).

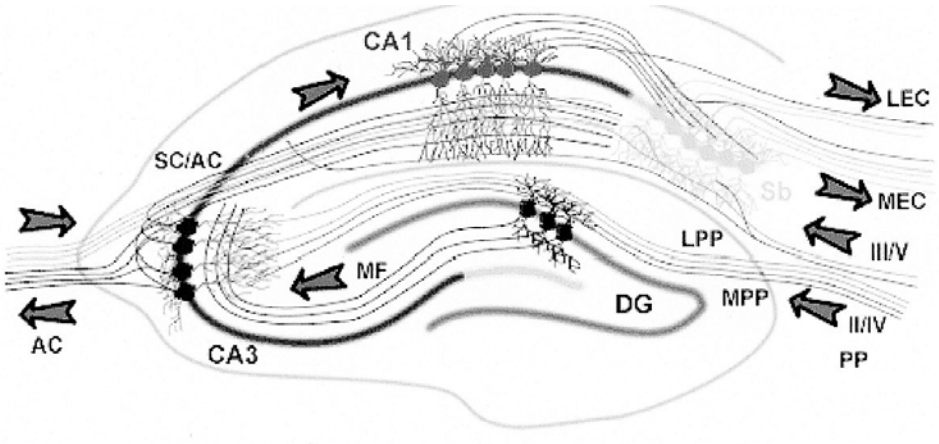

Figure 1. The Hippocampal formation. The hippocampus forms a network with input from the Entorhinal Cortex (EC) that forms connections with the Dentate Gyrus (DG) and CA3 pyramidal neurons via the Perforant Path (PP- split into lateral and medial). CA3 neurons also receive input from the DG via the moss fibers (MF). They send axons to CA1 pyramidal cells via the Schaffer Collateral Pathway (SC). CA1 neurons also receive input from the Perforant Path and send axons to the Subiculum (Sb). These neurons in turn send the main hippocampal output back to the EC, forming a loop. (MRC Centre for Synaptic Plasticity, 2007)

The perforant path, which brings information primarily from the entorhinal cortex (EC), is considered the main source of input to the hippocampus. In rodents, the EC is located at the caudal end of the temporal lobe, with three distinct bands whose connectivity runs perpendicular across the area. The EC layers receive highly integrated projections from many cortical regions, especially associational, perirhinal, and parahippocampal cortices, as well as the prefrontal cortex. The EC also sends information back mainly from the deep layers of this structure (Lavenex & Amaral, 2000). It therefore serves as an interface between the hippocampus and other cortical areas. The deep layers of the EC receive one of three main outputs of the hippocampus. The additional output pathways of the hippocampus are the cingulum bundles and the fimbria/fornix, which arise from field CA1 and the subiculum.

Based on their connectivity, regions of the hippocampal formation represent a circuit sequenced EC-DG-CA3-CA1-subiculum-presubiculum/parasubiculum-EC (Amaral & Witter, 1989). Layer II of EC is the origin of the perforant path, bringing input to the DG and field CA3, while EC layer III projects to field CA1 and the subiculum. Perforant path input from EC layer II enters the dentate gyrus and is relayed to region CA3 through mossy fibers (MF) (Henze, Urban & Barrionuevo, 2000). Region CA3 combines this input with signals from EC layer II which has extensive connections within the region and also sends projections to region CA1 via the Schaffer Collaterals (SC). Region CA1 receives input from the CA3, EC layer III and the nucleus reuniens of the thalamus. In turn, CA1 projects to the subiculum as well as sending information along output paths of the hippocampus. The subiculum is the final stage in the pathway,

combining information from the CA1 and entorhinal layer III to also send information along the output pathways of the hippocampus.

The connections between different regions of the hippocampal formation are typically formed between principal cells, which are excitatory (Lavenex & Amaral, 2000). The principal cells in the DG are granular cells, which are small and densely packed. In the Ammon's horn (CA1-CA4) the principal cells are pyramidal cells (Patton & McNaughton, 1995; Hammond, 2001). There are also various types of locally-connected inhibitory interneurons present in these regions (Jones & Yakel, 1999).

Hippocampal Role in Memory

The hippocampal formation (the CA fields, DG, and subicular complex) is part of a system of structures that are important for mammalian memory. In humans, non-human primates, and rodents, damage to this region impairs performance on a variety of learning and memory tasks (Eichenbaum & Cohen, 2001).

The importance of the hippocampus in memory function became firmly established following the bilateral removal of various medial temporal lobe structures (including ablation of the hippocampi) in a patient who became known as H.M. (Scoville & Milner, 1957). At the time Brenda Milner documented H.M., the anatomy of the medial temporal lobe was poorly understood, and it was not known what specific damage within this large region was responsible for the observed memory impairment. Ultimately, cumulative behavioral work with an animal model of human memory impairment, together with neuroanatomical studies confirmed the role of the

hippocampus as a important component of memory consolidation (Squire & Zola-Morgan, 1983; Lavenex & Amaral, 2000; Suzuki & Amaral, 1994). The specific functional contributions of the medial temporal lobe structures (the hippocampus, the EC, the DG, and the subicular complex) remain a matter of dispute. Rival theories regarding the hippocampus include proposals for a role in cognitive mapping (O'Keefe & Nadel, 1978), declarative memory (Squire, 1992; Eichenbaum & Cohen, 2001), and certain aspects of episodic-like memory (Tulving, 1983). Despite conflicting theories, these data are unequivocal that medial temporal lobe damage produces severe memory impairment and that hippocampal damage, in particular, impairs spatial memory.

Memory impairment following medial temporal lobe damage is characterized by specific features. First, the impairment is multimodal; memory is affected regardless of the sensory modality in which information is presented (Levy, Wu, Greene, & Spellman, 2003; Milner, 1972; Murray & Mishkin, 1984; Squire, Clark, & Knowlton, 2001). This is consistent with the finding that the medial temporal lobe structures are the final stage of convergence of cortical processing, receiving projections from all sensory modalities (Lavenex & Amaral, 2000). Accordingly, there has been interest in the possibility that the hippocampus may be important for tasks that depend on relating or combining information from multiple sources, as in certain spatial memory tasks (O'Keefe & Nadel, 1978). Second, memory impairment following damage to the medial temporal lobe region can occur simultaneously with intact perceptual abilities and intellectual functions. For example, patient H.M. incurred bilateral damage to the hippocampal formation as well as perirhinal cortex, yet scored normally on tests of intelligence, perceptual function

and lexical knowledge (Kensinger, Ullman, & Corkin, 2001). Finally, following damage to the medial temporal lobe, immediate memory is intact, but retention diminishes without adequate rehearsal. For example, rats with hippocampal lesions learned the delayed nonmatching-to-sample task at a normal rate using a short delay (4-s) between sample and choice (Clark, West, Zola, & Squire, 2001). However, performance was impaired when the delay was increased by 1- or 2- min. Further, during delayed testing, performance remained fully intact when 4-s delay trials were introduced intermittently, thereby indicating both retention of the nonmatching rule and intact short-term memory. Even when extended training was given at a 1-min delay, exceeding the training given at the 4-s delay, performance remained intact at the short delay and impaired at the long delay.

Evidence that the temporal lobes play a fundamental role in memory has existed for some time. However, evidence that the hippocampus is specifically involved in memory functioning has taken longer to emerge. Many of the memory deficits seen following hippocampal damage are spatial in nature, which led to the idea of cognitive mapping. Since the discovery of hippocampal place cells in the rodent (O'Keefe & Dostrovsky, 1971), an influential idea has been that the hippocampus creates and uses spatial maps and that its predominant function is to support spatial memory (O'Keefe & Nadel, 1978). When the animal is confronted with a new environment, place cells are activated in relation to the significant features of the environment (Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999). In their early work, O´Keefe and Nadel (1978) suggested that rats can learn the correct path to reach a goal in a maze utilizing a

“true spatial learning” strategy. A rat using this strategy to solve a problem would form a cognitive map of the environment; where the maze is located, and of the specific location of the rewarded goal-arm within that environment. A crucial feature of the O´Keefe and Nadel (1978) perspective is that learning happens in an all-or-nothing way, and that the formation and readjustment of a spatial representation of the environment in the brain occurs automatically. Further, research has demonstrated that activation of cells is often greater during way finding than when following a well-learned path (Hartley, Maguire, Spiers, & Burgess, 2003), and greater during route learning than learning from an aerial view (Shelton & Gabrieli, 2002). Collectively, these studies suggest that spatial memory following damage to the hippocampus is impaired because the hippocampus, which contains place cells, is necessary for an animal to learn the location. However, a number of recent studies have suggested that spatial navigation impairment following hippocampal damage can be attributed to deficits of nonspatial component of navigation (Morris, Garrud, Rawlins, & O’Keefe, 1982; Sutherland, Kolb & Whishaw, 1982).

Morris et al. (1982) and Sutherland et al. (1982) suggest a modified view of the hippocampus. They have shown that place responses can be acquired despite hippocampus damage. A critical observation of place learning following hippocampal damage concludes that although hippocampal lesions do not impair rats' ability to use a localized beacon to find their goal, such lesions have a drastic effect on their ability to swim to the invisible platform in a watermaze (Morris, Garrud, Rawlins & O'Keefe, 1982; Sutherland, Whishaw & Kolb, 1983). The obvious interpretation must be that such lesions have an effect on their ability to use representations of distal visual cues to locate

a goal. In support of this interpretation, Eichenbaum, Stewart and Morris (1990) and Whishaw, Cassel and Jarrard (1995) found that if lesioned rats were given extensive training to swim directly to a visible platform, they continued to perform well when the platform was made invisible, and spent as much time as controls searching in the platform quadrant of the pool on test trials when the platform was absent. Whishaw et al. (1995) interpreted their results as evidence of a dissociation between “getting there” and “knowing where”. They concluded that lesioned rats could use external landmarks to define the location of the platform, but could not learn to swim directly to it. They attributed this failure to an inability to use path integration. Eichenbaum et al. (1990) however, showed that lesioned rats were not using the arrangement of external landmarks to locate the platform in the way that normal rats do, but were relying on one or two prominent landmarks that were directly in line with the platform from the fixed starting point. When these landmarks were moved, the lesioned rats still swam straight towards them, while control rats continued to swim to the platform. The results suggest that lesioned rats use visual cues just like normal rats. However, their spatial representations are inflexible, especially when the task is altered. This suggests that learning and memory deficit resulting from hippocampal damage spares acquisition of procedures, but impairs the flexible use of learned information.

Glutamate

Glutamate is the primary excitatory amino acid neurotransmitter in the central nervous system and is important for hippocampal function. Glutamate acts on several types of receptors, including metabotropic (coupled to intracellular second messengers) and ionotropic (coupled to ion channels). Metabotropic receptors, or mGluRs, respond to glutamate by activating proteins that affect cell metabolism. Ionotropic receptors include those selectively activated by N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainite (Randall, Burggren, & French, 2002). NMDA receptors are most densely concentrated in the cerebral cortex, the CA1 region of the hippocampus, amygdala, and basal ganglia. Activation of glutamate receptors is fundamental to excitatory synaptic transmission. Synaptic transmission refers to the propagation of nerve impulses from one nerve cell to another. This occurs at a specialized cellular structure known as the synapse, a junction at which the axon of the presynaptic neuron terminates at some location upon the postsynaptic neuron. An electrical impulse in one cell causes an influx of calcium ions and the subsequent release of a chemical neurotransmitter. The transmitter diffuses across the synapse and stimulates the subsequent cell in the chain by interacting with receptor proteins. The inflection of this activity occurs by modulation of glutamate receptors and by the removal of glutamate from the synaptic cleft by glutamate transporters (Randall et al., 2002).

Besides being essential for nervous system development, glutamate neurotransmission is necessary for learning and memory formation mediated by the

hippocampus. Glutamate receptors, in particular NMDA, are involved in the molecular mechanisms of learning and memory formation via enhanced synaptic efficacy. NMDA receptors enable alterations of synaptic efficacy because the ion-channel associated with them is voltage-gated and only becomes permeable when the postsynaptic membrane is partially depolarized (Mayer, Westbrook, & Guthrie, 1984). The NMDA receptors also have a high conductance for calcium (Lynch, 1998). Together these characteristics allow NMDA receptors to detect the conjunction of two events; presynaptic release of glutamate, and postsynaptic depolarization. These events produce an influx of calcium ions through voltage-dependent ion channels.

The influx of calcium in to the cell activates several kinases: cyclic AMP (cAMP), dependent protein kinase A (PKA), mitogen-activated protein kinase (MAPK), and calcium/calmodulin protein kinase II (CaMK). Each of these then activates the gene transcription factor cAMP response element binding protein (CREB) which in turn initiates the expression of specific genes to produce new proteins. The proteins are then synthesized and are transported throughout the cell, strengthening synapses. Although the strengthening of synapses is not inevitable, high frequency postsynaptic activity triggers intracellular events which eventually result in alternations in the effectiveness of the synapse (Collingridge and Bliss, 1987). The mechanism underlying enhanced synaptic efficacy is called long-term potentiation (LTP) (Brown, Kairiss, & Keenen,1990). This type of plasticity occurs during and is necessary for hippocampus-mediated memory functions such as spatial memory formation in the rat (Martin, Grimwood & Morris, 2000). Applying a series of short, high-frequency electrical stimuli (tetanic stimulation)

to a synapse can strengthen the synapse for a short time. *In vivo*, LTP occurs naturally and can last from hours to years (Bliss & Lomo, 1973). After a synapse has undergone LTP, subsequent stimuli applied to the presynaptic cell are more likely to elicit action potentials in the postsynaptic cell.

Because changes in synaptic strength are thought to underlie memory formation, LTP is believed to play a critical role in behavioral learning. Although the correlation of LTP and learning and memory is still controversial, research has produced definitive evidence suggesting a link. Research has demonstrated that pharmacological blockade of the NMDA receptor from glutamate activation diminishes LTP and significantly impairs memory formation (Pearce, Cambray-Deakin, & Burgoyne, 1987).

For instance, Brun, Ytterbo, Morris, Moser, and Moser (2001) trained animals in a spatial water maze task and then delivered bursts of high frequency stimulation to the perforant path. The high frequency stimulation induced LTP in the dentate gyrus and caused retrograde amnesia for the task. Interestingly, the ability to learn a new water maze task was not affected in these animals. They also administered the NMDA receptor antagonist CPP blocked the induction of LTP. Blocking the induction of LTP), producing severe deficits in performance of certain learning tasks (Collingridge, Kehl, & McLennan, 1983; Harris, Ganong & Cotman, 1984; Errington, Lynch, & Bliss, 1987; Morris, Anderson, Lynch & Baudry, 1986). Results from Brun et al. (2001) showed that high frequency stimulation impaired memory of where the platform was located in a water maze task, implying an involvement of synaptic strengths in the retention of spatial memory. The data confirm predictions that spatial memory is stored as a distribution of

synaptic strengths in the hippocampal formation, and that performance would deteriorate following any treatment that alters the connection of strengths of the network, such as LTP (McNaughton & Morris, 1987).

McNaughton, Barnes, Rao, Baldwin, and Rasmussen (1986) also examined memory retention vulnerability to LTP. They trained rats to find an escape tunnel in a Barnes circular maze and then induced LTP by tetanic stimulation within the perforant path of the hippocampal formation. Next, the rats were tested for memory of the escape location. They reported that the tetanic stimulation resulted in a persistent deficit in the acquisition of new spatial information and a disruption of recently acquired spatial information. Well-established spatial memory was unaffected as was the acquisition of spatial information. These results suggest that during the formation of a spatial representation, spatial information must be stored temporarily at modifiable synapses associated with LTP. This information is not needed once the representation of the environment is well established in long term memory.

In another study demonstrating a link between memory formation and LTP, Morris et al. (1986a) demonstrated that chronic intraventricular infusion of the NMDA antagonist 2-amino-5-phosphonopnetanoic acid (AP5) impairs spatial learning while leaving visual discrimination learning intact. The distinction between spared and impaired function is highly suggestive of hippocampal involvement. Other studies have utilized mutant mice models to study the relationship between LTP and learning. For example, CaM kinase II-deficient mice exhibit deficient hippocampal LTP and impaired spatial learning (Silva et al. 1992 a, b). NMDA receptor deficient mice also showed a

decrease of hippocampal CA1 LTP and reduced spatial learning and memory (Sakimura et al. 1995).

Other studies demonstrate that hippocampal LTP can be dissociated from learning and memory. Although the characteristics of LTP make it a good candidate for the synaptic substrate of memory, the evidence remains equivocal. Mice with the protein kinase C-γ null mutation showed an absence of hippocampal CA1 LTP but retained normal spatial learning and memory (Abeliovich et al., 1993b). Mice lacking tissue-type plasminogen activator (t-PA) also revealed that a reduction of LTP in the hippocampal CA1 and CA3 regions did not affect hippocampus-dependent learning and memory (Huang et al., 1996). Although there is no clear explanation for these results, the dissociation of LTP and spatial learning may be explained as follows. Fist, the test paradigms that were used for learning and memory may not have been sensitive enough to quantify a minor change in learning and memory in a mutant. Second, hippocampal LTP acts as an on/off switch for learning and memory, and therefore, the amplitude of LTP is not important for learning and memory. Third, it is possible that low frequency LTP may be normal in mutant mice, and it could be the low frequency stimulated LTP that is more closely associated with spatial learning and memory. Finally, hippocampal LTP might be dissociated from spatial learning and memory as in the case of the protein kinase C-γ or t-PA deficient mice (Abeliovich et al. 1993a, b; Huang et al, 1996).

Glutamate Transporters

The presence of excitatory amino acid (EAA) ionotropic and metabotropic receptors is critical to the ability of the neurotransmitter glutamate to contribute to a broad range of functions in the central nervous system (Meldrum, 2000). An important part of the regulation of extracellular glutamate relies on the function of glutamate transporters (Randall, Burggren, & French, 2002). Under normal conditions, glutamate is released into the synaptic cleft and binds to glutamate receptors. The modulation of this synaptic activity is maintained both by glutamate receptors and by the removal of glutamate from the synaptic cleft by glutamate transporters. Glutamatergic neurotransmission is terminated by high-affinity, sodium-dependent glutamate transporters which are present on both neuronal and astroglial plasma membranes (Schousboe, 1981; Nicholls & Attwell, 1990). Glutamate transporters play a crucial role in the efficient removal of glutamate from the extracellular space. In addition, transporter mediated uptake is critical for terminating the actions of glutamate, preventing the sustained activation of receptors that would otherwise disrupt signaling at synapses and potentially lead to excitotoxic neurodegeneration. These transporters mediate the efficient clearance of a number of extracellular excitatory amino acids. Hence, they are termed excitatory amino acid transporters (EAATs) (Bridges, 2001).

Researchers have cloned five human EAATs, designated EAAT 1-5. The nomenclature in rodents is different; the transporters are termed GLutamate/ASpartate Transporter (GLAST), Glutamate Transporter-1 (GLT-1), and Excitatory Amino Acid

Carrier (EAAC1) (Shashidharan and Plaitakis, 1993; Shashidharan, Wittenberg and Plaitakis, 1994; Kanai and Hediger, 1992). Of primary importance to the present study is EAAC1 (analogous to human EAAT3). EAAC1 is highly expressed on neuronal dendrites, especially those in the hippocampus, cerebellum, and basal ganglia (Furuta, Martin, Lin, DykesHoberg, & Rothstein, 1997a). EAAC1 is also expressed at gamma-aminobutyric acid (GABA) terminals (Rothstein et al., 1994), suggesting a relationship between the presynaptic glutamate transporter and the inhibitory transmitter GABA. This suggests that GABAergic cells might take up glutamate via this transporter and refuel inhibitory neurotransmission after conversion to GABA via glutamate decarboxylase. Sepkuty et al. (2002) examined whether perturbed GABA homeostasis might be responsible for the epileptic phenotype shown by rats with EAAC1 knockdown. They found that reduced expression of EAAC1 by antisense treatment led to behavioral abnormalities, including spontaneous epileptic seizure activity and staring spells. Further, thalamocortical and hippocampal-entorhinal cortical slices from knockdown animals both showed increases in spontaneous epileptiform activity compared with controls. Reducing glutamate uptake diminished evoked inhibitory post synaptic currents (IPSC) and miniature inhibitory post synaptic currents (mIPSC) without affecting postsynaptic receptors. This was accompanied by a reduction of total tissue GABA concentration, especially in the hippocampus. These effects required GABA synthesis but not glutamate metabolism, suggesting that glutamate is taken up directly into inhibitory terminals and converted to GABA, which is then packaged into synaptic vesicles. Enhancement of

mIPSCs by exogenous glutamate requires glutamate uptake and GABA synthesis (Mathews & Diamond, 2003).

The findings of Sepkuty et al. (2002) reveal that GABA metabolism rates were decreased by both antisense treatment and glutamate-uptake blockade. They demonstrated that EAAC1 antisense treated rats develop epilepsy and that this hyperexcitability may be attributable, in part, to a reduction in new GABA synthesis in the hippocampus (Sepkuty et al., 2002). This work suggests that glutamate transporters in general and EAAC1 in particular, play an important role in the synthesis and release of GABA in the hippocampus. Following knockdown, the direct transport of glutamate into neurons is diminished, providing evidence that the proepileptic effect generated by antisense EAAC1 administration might derive from the lack of expression of the transporter in either excitatory or inhibitory neurons (Sepkuty et al., 2002).

Recombinant Adeno-Associated Viral Vector

Recombinant adeno-associated viruses (rAAV) are quickly establishing themselves as highly versatile gene delivery agents for gene therapy, and functional genomic studies (Janson, McPhee, Leone, Freese, & During, 2001; Monahan & Samulski, 2000). Adeno-associated virus (AAV), derived from a non-pathogenic virus of the *Parvoviridae* family, is a replication-defective, non-enveloped virus which is not associated with any known disease. The AAV genome encodes two major overlapping polypeptides: Rep and Cap. Rep codes for proteins responsible for viral replication, whereas Cap codes for capsid protein VP1-3 (Hermonat, Labow, Wright, & Berns, 1984).

AAV contains two open reading frames bordered by the inverted terminal repeat (ITR), at the ends. The ITRs serve as initiation sites for synthesis of a complementary strand during viral DNA replication. These terminal repeats are the only essential components of the AAV for chromosomal integration (Hauswirth & Berns, 1977). AAV is good candidate for gene therapy since viral coding sequences can be removed and replaced by the cassette of genes for delivery. Upon infection of a cell, the viruses can insert genetic material at a specific site on chromosome 19 by introducing their genetic material into the host cell as part of their replication cycle (Kotin, Menninger, Ward, & Berns, 1991). The genetic material contains basic instructions of how to produce more copies of the virus. The host cell will carry out these instructions, incorporating the genes of that virus among the genes of the host cell for the life span of the cell. Viral vectors can efficiently transfer genes of interest to a broad range of mammalian cell types leading to high levels of stable and long-term expression after a single application (Hommel, Sears, Georgescu, Simmons, & DiLeone, 2003). The lack of immunogenicity and no known pathogenicity make recombinant AAV arguably the gene therapy vector of choice for clinical trials.

Viral vectors such as rAAV present many advantages for brain studies. First, rAAV vectors can express either single or multiple foreign genes, although they are limited to 4.7 kilobases of exogenous DNA (Flotte, 2000). Second, AAV can be administered at any developmental stage, can be delivered into a wide range of hosts including many different human and non-human cell lines or tissues, allow either short or long-term gene expression, and allow specific spatial targeting of genes in different regions of the brain using stereotaxic surgery (Janson et al., 2001). In theory, any

neurotropic virus can be modified so that its genome is replaced with other genes (Janson et al., 2001). The viral vector system for protein expression can “turn on” genes by overexpression of a gene of interest or alternately can “turn off” genes through expression of antisense or small interfering RNA (Babcock, Standing, Bullshields, Schwartz, Paden, & Poulsen, 2005). AAV viral vectors make it possible to transfer genes to the brain of any mammal to create a disease model, assuming that genes can be isolated and packaged in the vector. Moreover, viral vectors can be introduced to transgenic animals or to any animal lacking expression of a gene in order to demonstrate specific gene function. Additionally, viral vectors provide a method for introducing multiple genes to the brain, including combinations of neuroprotective and harmful genes to asses their relative effects.

In the past, viral vectors were only considered useful for upregulation and overexpression of genes. However, as the interactions among genes are better understood this limitation no longer applies. For example, viral vectors can be engineered to introduce antisense RNA, which is ideal for downregulating genes in the brain (Wahlestedt, Pich, Koob, Yee, & Heilig,1993; Werstuck & Greene, 1998). When a genetic sequence of a particular gene is known, it is possible to synthesize a strand of nucleic acid that will bind to the messenger RNA (mRNA) produced by that gene and inactivate it. The synthesized nucleic acid is termed the antisense oligonucleotide because its base sequence is complementary to the mRNA, which is called the sense sequence. Antisense molecules interact with complementary strands of nucleic acids, modifying the expression of genes. Expression of antisense vectors produces antisense RNA, which can

hybridize with the target mRNA and prevent its translation by either promoting its degradation or preventing its transport from the nucleus to the cytoplasm. Sepkuty et al. (2002) demonstrated effective knockdown of EAAC1 transporter proteins following antisense administration. The reduced expression of EAAC1 led to behavioral abnormalities, suggesting the importance of EAAC1 to both inhibitory and excitatory neuron function.

Behavior

Morris (1981) was the first to demonstrate that rats could locate an object that they were not able to see, hear, or touch. He used a circular pool full of opaque water from which the animals could escape by climbing to a hidden platform that was located beneath the water surface. The platform always maintained a constant relationship with the landmarks of the room. Rats quickly learned to escape from the water by swimming directly to the platform from different points of the pool. Strength of learning was tested afterwards by a probe trial; the hidden platform was removed and the amount of time spent in the former region of the platform was measured. Morris suggested that the animals located the position of the platform by forming a spatial representation of the position that it maintained in context with the room and the objects that it contained. Morris proposed that initial acquisition of the behavioral task is sensitive to hippocampal lesion (Morris, Garrud, Rawlings, & O'Keefe, 1982). They demonstrated that rats with hippocampal lesions were impaired in both encoding and retrieval of spatial memory. This view is supported by deficits seen in additional maze tasks following hippocampal

lesions. Converging evidence suggests that rats with hippocampal lesions are impaired in both working and reference memory versions of spatial tasks (Morris, Garrud, Rawlins, & O'Keefe, 1982; Morris, Schenk, Tweedie, & Jarrard, 1990). Note that a reference memory task assesses the ability to remember an event that remains constant, which in a water maze task, is achieved by maintaining the position of the hidden platform in the same spatial location throughout maze training. Every time the items and their associations are recalled, the associations become stronger. A working memory task taps into a more short-term form of memory, as it requires the ability to remember a consistent response rule, but with a trial specific event determining the correct response for every particular session of training. Repetitive recall of the items and their associations does not help to build a stronger association. On the contrary, old associations must be effectively inhibited.

Sutherland et al. (1983) were some of the first to document deficits in the standard reference memory Morris water maze task using neurotoxin induced lesions. De Bruin, Moita, de Brabander, and Joosten (2001) also examined spatial navigation following lesion. Rat performance was examined in a standard reference memory Morris water maze task. Performance was impaired in rats with lesions of the medial temporal lobe, but not in rats with damage of the medial prefrontal cortex. These findings are indicative of a deficit in spatial navigation produced by hippocampal damage.

Mohapel, Mundt-Petersen, Brundin, and Frielingsdorf (2006) determined that even rats impaired in cognitive capacity due to stress are able to learn the standard reference memory Morris water maze task over the 4-day training period, as revealed by

the progressively shorter escape latencies to find the submerged platform. This suggests that reference memory task may not be sensitive to subtle environmental influences. Mohapel et al. (2006) found that rats undergoing working memory training showed characteristic relearning of the task each time the platform was moved, as demonstrated by the longer escape latencies on trial one of each session.

Research suggests that working memory and reference memory tasks demonstrate different aspects of spatial memory. There is evidence that animals with as much as 80% of the hippocampus damaged can learn a standard watermaze reference memory task (Moser, Moser, & Anderson, 1993; Moser, Moser, Forest, Anderson, & Morris, 1995). This finding questions whether a more difficult spatial task could still be performed effectively following hippocampal damage. An example of such a task is delayed matching-to-place (DMTP) (Morris, 1983; Panakova, Buresova, & Bures, 1984; Steele & Morris, 1999).

The key feature of the DMTP task is the movement of the hidden platform across days. It is described as matching-to-place because the watermaze and surrounding environment consist of a set of visual cues, with the animal attempting to match its memory representation of escape location to the cues it can perceive (Steele & Morris, 1999). In a standard watermaze task, animals can learn to return to a previous location with few training trials, suggesting that returning to the last place where escape was possible is a natural strategy requiring little or no learning (Steele & Morris, 1999). Effective performance in the DMTP task requires either the selective retrieval of recent information (e.g. working memory), or a continuous process of overwriting previous

memory information in such a way that only the most recently visited location is accessible at any one time (Olton, Becker, & Handelmann, 1979).

To explore the demands of memory flexibility, rats in the DMTP are typically given eight trials per day to find a hidden platform whose position varies from day to day, but remains in the same position throughout the trials of a given day. What is stored in the memory when the animal encounters the submerged platform is unclear. It may be a memory of the platform's location (spatial memory), or possibly a memory of escaping from the water at that point in space (episodic memory). The concept of episodic memory in this context was first introduced by Tulving (1983). Numerous aspects of vertebrate behavior are episodic in nature, notably the ability of birds and selected mammals to cache and retrieve food items (Jacobs, 1995; Clayton, 1998). However, neither the retrieval of food caches nor the behavior of returning accurately to the last place where escape was possible in the DMTP task require episodic memory according to Tulving (1983). Simpler associative explanations are possible; some researchers suggest that the hippocampus may be encoding relationships among all elements of an experience into a representation of one event (Maren & Fanselow, 1997).

The DMTP provides the opportunity to explore demands of memory encoding. The animal is placed on the platform and then removed for a designated delay before beginning behavioral trials. Rats must encode a memory of a spatial cues in a familiar environment and, after a delay, retrieve this memory to efficiently locate the hidden escape platform. Variation in the delay before trial 1 affords the opportunity to explore the delay-dependence of spatial memory. Steele and Morris (1999) found that

damage to the hippocampus causes a delay-dependent deficit in memory of the last location visited in a watermaze in animals trained using a delayed matching protocol. Bast, da Silva, and Morris (2005) demonstrated that place memory declined with increasing retention delay.

By placing the animals on the platform initially, the DMTP also affords the examination of learning without direct reinforcement (Tolman, 1932, 1948, 1949). Tolman carried out experiments to demonstrate that learning may occur without immediate consequences on performance and without reward (Tolman & Honzik, 1930). Mere experience in a situation is sufficient to generate learning. In a typical experiment, hungry rats were allowed to run freely in a complex maze for several trials for a few days. On these trials, food was never present in the maze. Later, food was introduced on a certain day and the rats showed an abrupt change of behavior. In the presence of a food reward, rats ran significantly faster and made few errors on their way to the goal. Even on the trial immediately after food was introduced for the first time, animals that had never been rewarded made no more errors than animals that had been rewarded with food from the beginning of training. Therefore, the rats learned the correct trajectory to the goal-box during the unrewarded trials, and this learning was behaviorally silent until they had an appropriate incentive. The learning design of Tolman and Honzik (1930) can be employed in the DMTP water maze task. By placing the rat on the platform before trial 1 of the DMTP, animals are not given the direct reinforcement of finding the hidden platform. Direct reinforcement is introduced when animals are allowed to swim and locate the escape platform on trial 1. Thus, trials without reward are followed by the

introduction of a reward appropriate to the animal's motives. Rats are provided the reward under a strong irrelevant drive followed by a shift to a strong relevant drive to receive the reward. The concept of reward relevance, identified by MacCorquodale and Meehl (1954), demonstrated that escape from water provided an adequate relevant reward. The swimming trials terminated at a platform covered with food which they never ate, sometimes at an empty platform, and sometimes at a platform covered with food which they found following food deprivation. Learning was most efficient when animals are presented with a strong relevant drive to receive the reward. Results suggest that escape from water was reinforcement for learning the correct trajectory to the escape platform. In the DMTP, being placed on the escape platform away from the deep water represents the strong irrelevant drive to receive the reward. Swimming to the submerged platform in order to escape the water represents the switch to a strong relevant drive to receive the reward. In general, latent learning research suggests that rats may learn the DMTP task by mere experience with the platform before trial 1.

INTRODUCTION TO THE CURRENT EXPERIMENT

The goal of the present study was to investigate the behavioral consequences associated with the knockdown of the hippocampal glutamate transporter EAAC1, which is involved in the removal of glutamate from the synapse. Based on the current literature, I hypothesize that blocking the hippocampal glutamate transporter EAAC1 should produce changes in behavior as observed in altered performance in a working memory task. A recombinant AAV vector was used to modulate the expression of EAAC1 transporter proteins in the CA1 region of the hippocampus. Animals were tested using the DMTP water maze task to examine if this manipulation would alter performance in a task that is hippocampal dependent. The DMTP is a useful behavioral task that is sensitive to even relatively mild impairments to the hippocampus (Whishaw et al., 1994). Although previous studies have examined the effects of altered EAAC1 function on behavior, the present experiment utilized an innovative knockdown model isolated to the CA1 region of the hippocampus.

METHOD

Subjects

The subjects were fifteen (11 male, 4 female) three month old Wister rats (Charles River Laboratories, Raleigh, NC). Animals were housed individually in a temperature (23°C) and light (12 hour light/dark cycle) controlled environment, with access to water and commercial rat pellets ad libitum. Experimentation began one week following arrival to the laboratory. Experimental procedures involving these animals were approved by the MSU Institutional Animal Care and Use Committee.

Animals were randomly assigned to one of two conditions. Eight animals (4 male, 4 female) received an AAV vector encoding an EAAT3 antisense mRNA strand. The remaining animals were treated with an AAV vector lacking an expression cassette. The viral vectors were provided by Dr. David Poulsen (University of Montana).

Apparatus

The apparatus consisted of a galvanized circular pool (1.37 m diameter x 60 cm high) filled to a depth of 30 cm with water maintained at 24°C. Powder black tempera paint was added to render the water opaque. A Plexiglas platform (11.43 cm x 11.43 cm) was placed in the center of a quadrant, submerged 5 cm below the surface of the water. Testing was conducted in a room containing numerous distal visual cues that remained constant throughout testing. An overhead digital camera connected to a computer running

the Any-maze™ software program (Wood Dale, IL) was used to collect the behavioral data.

Procedure

Animals were anesthetized with isoflurane and mounted into a stereotaxic frame apparatus. A midline incision was made and small holes were drilled in the skull 4.1 mm posterior to bregma, and ± 2.0 mm from the midline (flat skull). The tip of an injection cannula was lowered 3.7 mm from the skull surface into the dorsal hippocampus. Injections were made using a Hamilton microsyringe mounted in a programmable infusion pump. The cannula was connected to the microsyringe with clear tubing. Rats received infusions of AAV at a rate of 8 µl over a period of 20 min. The injector remained in place for 2 min following injection. This procedure was repeated for the contralateral side. Following infusion, scalp incisions were sutured and animals returned to their home cages.

Behavioral testing began 24-26 days following surgery to allow optimal vector expression (Hommel et al., 2003). Rats were initially trained in a standard Morris water maze hidden platform paradigm followed by the DMTP water maze task. The standard Morris water maze training took place 4 consecutive days consisting of four trials per animal on each training day. During each trial, rats were released into the water facing the wall at four different locations in random order (N, S, E, W). The platform was located in the center of one quadrant, and remained fixed throughout testing. Trials lasted until the animal had found the platform or 60 sec elapsed. If the animal failed to locate

the platform within 60 sec, the trial was terminated and the rat was guided to the platform by the investigator. Animals were left on the platform for 10 sec prior to removal. Probe trials took place on the fifth day of testing, in which the hidden platform was removed and the amount of time spent in the target quadrant was measured as the strength of the learning. Rats were released in the quadrant opposite to the one where the platform was initially located. All distal cues remained constant during test session and probe trial. Data collected included latency to locate the platform, time spent in target quadrant, swim speed, and duration.

Two days following the standard Morris water maze testing, animals were evaluated using the DMTP task. Rats were tested on four consecutive days with eight trials per day divided into morning (approximately 9:00 am) and afternoon (approximately 3:00 pm) sessions. For all trials on a given day, the platform was located in the center of one quadrant, and remained in the same position. The platform was moved in a pseudo-random order to the center of the remaining quadrants on successive days such that each quadrant was used only once. During each training session, rats were initially placed on the platform for 30 sec. Next, animals were removed from the platform and placed in a cage for a pseudorandomly assigned delay of either 30 sec or 10 min. Rats received both delays on any given day of either in the morning or afternoon session. After the assigned delay had elapsed, rats were released into the water facing the wall at four different locations in random order (N, S, E, W). Trials lasted until the animal had found the platform or 60 sec elapsed. If an animal failed to locate the platform within 60 sec, the trial was terminated and the rat was guided to the platform by the investigator.

After the animal reached the platform, they were left for 10 secs prior to removal. Data collected included latency to locate the platform, time spent in target quadrants, swim speed, duration, and the proportion of trial 1 of each day spent in the quadrant that the platform had occupied on the previous day during trial 1.

RESULTS

Throughout testing, no behavioral abnormalities of the EAAC1 knockdown rats were observed, either in swimming or behavior on the platform. Treatment with the EAAC1 antisense did not alter swim speeds, $F(1, 13) = .455$, $p = .51$.

EAAC1 knockdown animals exhibited shorter escape latencies than control animals. On day 1 the mean escape latency (± SEM) in experimental animals declined from 21.19 ± 3.80 s on trial 1 to 10.56 ± 1.53 s on trial 2. Escape latencies decreased by 13.93 s on day 1 from trial 1 to trial 4. In contrast, the performance of control animals exhibited slower latencies to locate the platform on day 1. The mean escape latency in control animals on day 1 declined from 14.69 ± 2.05 s on trial 1 to 11.26 ± 1.31 s on trial 2. Their escape latencies decreased by .88 s from trial 1 to trial 4 (Table 1).

Table 1. DMTP latency data expressed as means (± SEM)

	Trial 1		Trial 2		Trial 3		Trial 4	
	EAAC1	Cont	EAAC1	Cont	EAAC1	Cont	EAAC1	Cont
Day 1	21.19 (± 3.80)	14.69 (± 2.05)	10.56 (± 1.53)	11.26 (± 1.31)	10.46 (± 2.02)	12.68 (± 2.78)	7.26 (± 1.82)	13.81 (± 2.14)
Day 2	14.76 (± 3.65)	20.34 (± 4.99)	8.20 (± 3.19)	15.38 (± 3.94)	13.22 (± 2.88)	12.89 (± 3.79)	7.89 (± 0.99)	8.01 (± 1.01)
Day 3	9.08 (± 3.29)	22.79 (± 4.35)	8.66 (± 2.07)	13.20 (± 3.01)	6.57 (± 1.80)	11.11 (± 2.55)	7.07 (± 0.69)	6.97 (± 0.53)
Day 4	10.6 (± 3.36)	21.55 (± 4.71)	8.84 (± 1.93)	11.61 (± 2.72)	7.89 (± 1.55)	9.69 (± 1.87)	8.84 (± 1.44)	4.80 (± 1.63)

On the latency measure, an overall analysis of variance (ANOVA) revealed main effects of treatment, F (1,13) = 12.14, $p < .01$, and trials, F (3, 39) = 25.8, $p < .01$ were significant. The repeated measures ANOVA indicated that the latency to the platform varied as a function of both the treatment and day interaction (treatment X day interaction), F (9, 117) = 2.24, $p = .02$. The trials x day x treatment interaction, F (3, 39) = 2.41, $p = .081$ which approached significance. To study these interaction, multiple comparisons were conducted and showed that performance improved over days and trials in both control and EAAC1 knockdown conditions. However, latencies in EAAC1 knockdown rats were superior to that of control rats across the 4 days of testing (Figure 2). A sample of the behavioral data in trial 1 is depicted in Figure 3.

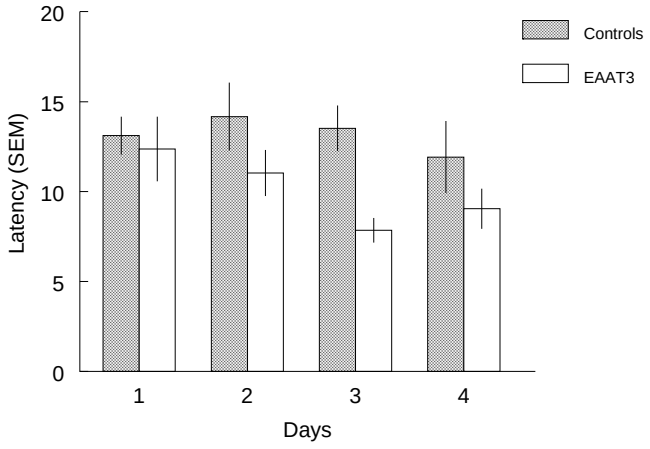

Figure 2. Performance in the DMTP task following microinfusion of AAV viral vector encoding EAAC1 antisense mRNA sequence or an AAV empty cassette. Rats treated with EAAC1 antisense mRNA (n=7) exhibited shorter latencies to locate the target platform relative to the control condition (n = 8) across days, F (1,13) = 12.14, $p = .004$.

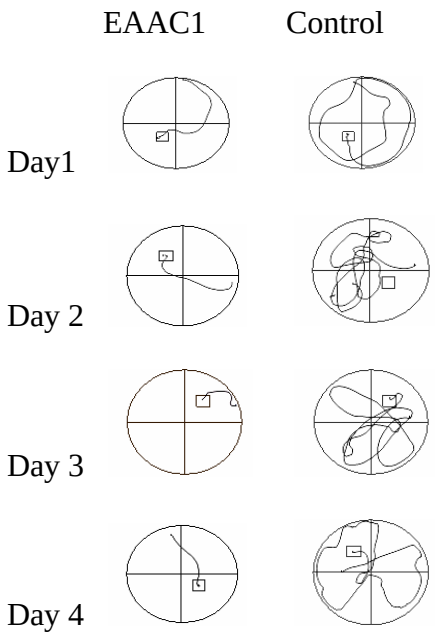

Figure 3. Sample swim paths recorded from EAAC1 knockdown and control conditions during trial 1 on all days. Swim paths demonstrate the relative latencies of the EAAC1 knockdown and control condition. They depict that EAAC1 knockdown animals exhibit significantly shorter latencies to locate the platform on trial 1.

There were no significant differences in latencies between groups as a function of the delay before testing trials ($p > .05$) (Figure 4). Further, whether animals received a short or long day before testing trials did not influence the overall main effect of treatment in this study. The results indicate that EAAC1 knockdown animals exhibited significantly shorter latencies under both delay conditions irrespective of the delay (Figure 5).

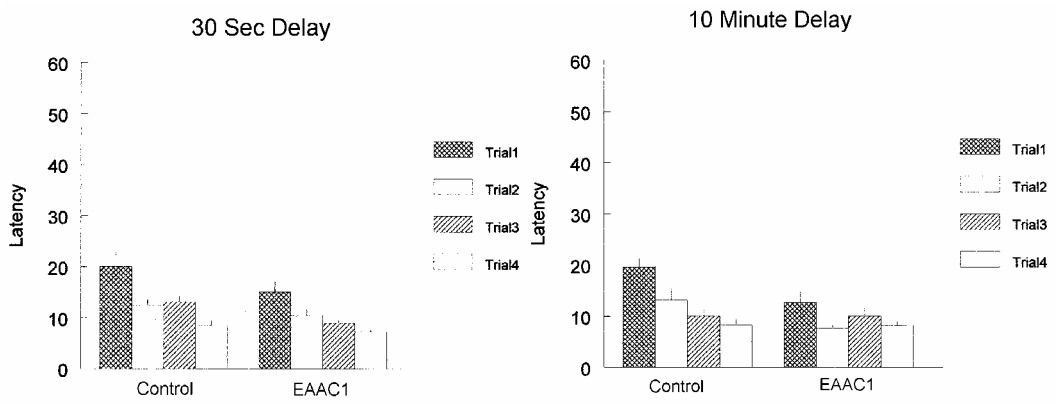

Figure 4. Escape latencies for EAAC1 knockdown and control conditions as a function of delay preceding trial 1. Latency data for each trial was collapsed across days for both conditions. There were no significant differences in latencies between groups as a function of the delay before testing trials ($p > .05$).

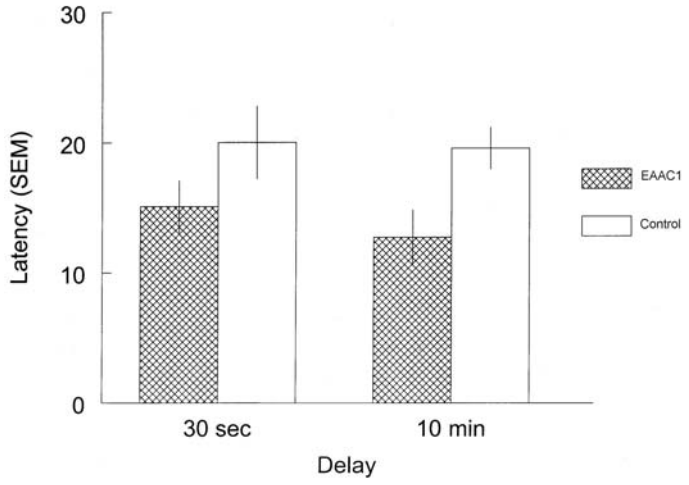

Figure 5. Escape latencies for EAAC1 knockdown and control conditions did not differ as a function of delay. Under both delay conditions EAAC1 knockdown animals exhibited significantly shorter latencies to locate the target platform.

To examine the effects of memory interference from previous days and trials, we conducted analyses of time spent in the quadrant that contained the platform on the previous day. There were two quadrants of concern: the current target quadrant in which the platform was located on any given day and the quadrant where the platform was located on the previous day. The proportion of an animal's trial duration spent in the quadrant where the platform was located on the previous day was compared to the time spent in all four, non-overlapping quadrants. The analysis was conducted for trial 1 of each day only. Results reveal no significant differences between EAAC1 knockdown and control animals in amount of time spent searching in the quadrant where the escape platform was located on the previous day.

DISCUSSION

In the present investigation, the effects of EAAC1 antisense injection into the hippocampus was examined to determine whether or not expression of the hippocampal glutamate transporter EAAC1 had an effect on learning and memory performance. EAAC1 knockdown rats displayed markedly shorter latencies to locate the escape platform in the DMTP water maze task following the infusion of a rAAV vector encoding EAAC1 antisense in the dorsal hippocampus. Both EAAC1 knockdown and control groups learned the DMTP water maze task across days, however, the data revealed that latencies in EAAC1 knockdown rats were superior to those of control rats across the 4 days of testing. Within each day, the EAAC1 knockdown rats consistently improved their escape latencies across trials in the DMTP task under both delay conditions irrespective of the delay employed. Contrary to previous findings, the treatment was not sensitive to the delay before trial 1 (Bast et al., 2005). Rapid escape latencies in trial 2 are reflective of one trial learning. In this case, animals are able to learn the correct trajectory to the escape platform on each day by swimming to the platform on trial 1. The plateau in latencies across trials 2-4 demonstrates that EAAC1 knockdown animals can acquire the concept of escape and adopt effective spatial strategies within trial 1 that are applied in subsequent trials. The control animals also demonstrated one-trial learning, reflected by decreased latencies between trial 1 and trial 2. Results showed that performance improved over days and trials in both control and EAAC1 knockdown conditions. By

trial 4, control animals exhibited escape latencies indistinguishable from EAAC1 knockdown animals.

When the platform location varied between days, making it impossible to predict its precise location on trial 1, the performance of EAAC1 knockdown animals was far superior to their control counterparts. In trial 1 each day, EAAC1 knockdown animals decreased their latency to locate the escape platform. Further in trial 1, the EAAC1 knockdown animals spent a proportionate amount of time searching each of the four quadrants, suggesting that they are successfully overriding the memory of the platform's previous location within the maze. The EAAC1 knockdown animals seemed to acquire the concept of escape and adopted strategies that improved performance (e.g., decreased escape latencies). In contrast, the control animals seemed less able to acquire flexible strategies to improve their escape latencies. Thus, control animals appeared behaviorally less flexible than EAAC1 knockdown animals. Control animals do not improve their latencies in trial 1 across days. It is unclear which, if any, search strategy control animals were using. Control animals also spent a proportionate amount of time searching each of the four quadrants on the first trial, and as a result exhibit much slower escape latencies. A deficit in acquisition might reflect poorer reference memory (i.e., the rat might not recall the platform position from the previous day). Alternatively, it might reflect difficulties in acquiring the concept that a submerged platform exists.

The parsimonious explanation for the results of the present investigation is that the treatment effectively altered the function of the glutamate transporter EAAC1 and further that the excess glutamate may have had advantageous behavioral implications,

which the DMTP task was designed to assess. Despite unequivocal importance of glutamate transporters in modulating extracellular glutamate, there remains no clear evidence highlighting the specific role of the neuronal glutamate transporter EAAC1. Nevertheless, this study demonstrates a relationship between the transporter EAAC1 and learning and memory performance. More specifically, several explanations can be applied to the results of the current investigation.

First, it has been reported that although NMDA receptor-dependent long-term potentiation (LTP) in the hippocampus is important for spatial learning, once the strategies necessary for learning are acquired, performance in spatial tasks progresses readily in the absence of LTP (Moser, Krobert, Moser, Morris, 1998). This evidence suggests that pretraining would diminish behavioral deficits ordinarily provoked by blocking NMDA receptors. Previous studies demonstrate intact spatial learning in pretrained animals when NMDA receptor-dependent LTP is blocked (Bannerman et al., 1995). All animals may have benefited from the transfer of procedural knowledge from the standard Morris water maze task to the DMPT water maze task, such as knowing that there is an escape platform and using the escape platform as an escape from water. Animals lacking pretraining may have been unable to acquire this type of knowledge following the knockdown of the glutamate transporter EAAC1.

In accordance with the findings of the present study however, Otnaess, Brun, Moser, and Moser (1999) found that pretraining is not helpful in a DMTP task. When rats are trained with a new platform position daily, blockade of NMDA receptor dependent LTP prevents retention in a delay-dependent manner despite pretraining (Steele and

Morris, 1999). Steele and Morris noted that an important difference between the DMTP and other tasks is that the target positions used on previous days must be ignored. Successful performance requires the animals to remember both where the platform was positioned and when the platform occupied this position. One function of LTP may be to associate elements of experience in memory. Spatial learning may take place without LTP, but only when the other episodic aspects of the training are very recent.

Further, the robustness of the behavioral differences between groups in the present study may reflect the inherent effectiveness of the DMPT task. In accordance with the methodology of this study, Roitblat and Harley (1988) suggest that duration of the inter-trial interval (ITI) and the type of the trial (matching versus non-matching) affect choice accuracy. Roitblat and Harley (1988) found that increases in the duration of the ITI between non-matching trials resulted in a decrease in the influence of the events of the previous trial. According to this theory, the association between the previous trial and the current trial, that is the intrusion of memories of the previous trial, decline with increase in the ITI duration. The occurrence of intrusions clearly predicts that choice accuracy should be lower when the memory from one trial is the distractor on the next when the intruding memory is competing with a representation of the current trial for control over responding. Sometimes the animal will respond according to the memory of the previous trial, rather than the representation of the present trial. On the contrary, Roitblat and Harley (1988) found that when the representations of two successive trials match, the rat should make a correct response if it recalls the memory of the previous trial. Hence, their hypothesis predicts that choice accuracy should be higher when short

ITI durations separate matching trials than when long ITI durations separate matching trials, as in trials 1-4 of the DMTP water maze task. They further predict that on mismatching trials choice accuracy should be higher when long ITI durations separate the trials, as in the duration between trial 4 on one testing day to trial 1 of the following testing day of the DMTP task.

The findings of Roitblat and Harley (1988) provide strong evidence that the DTMP task employed in the current investigation is effectively eliminating proactive interference effects. Encoding of the spatial representation is poorer when trials are closely space. As a result, choice accuracy on closely spaced trials is poor, even when utilizing the memory of a previous trial leads to accurate performance. In DMTP matching trials within each day, the short ITI durations used could only have aided in producing the correct responses. The rats should have made a correct response by utilizing the memory of the previous trial, or the notion that escape is possible without recollection of the location of the escape platform. Across days, the long durations (~24 hours) between sets of trials should have aided in producing correct responses by reducing the occurrence of memory intrusions from previous non-matching trials. Accordingly, this phenomenon may partially explain the ability of the rats to improve their latency to locate the platform across trials on a given day as well as across days. The superiority performance of the EAAC1 knockdown animals may reflect their ability to more effectively utilize a memory of a previous matching trial during subsequent trials, and further may be more able to override memories of previous non-matching trials.

Finally, the results may be due to changes in synaptic biology due to the absence of the protein transporters. It is well established that abnormalities in glutamate transporter expression produce transporter dysfunction. An important tool in understanding the role of glutamate transporters is the study of glutamate transporter knockout and knockdown. A knockout animal is genetically engineered with one or more of its genes eliminated. A gene knockdown, however, is a genetically modified animal that carries one or more genes in its chromosomes that has had its expression reduced. The importance of glutamate transporters in controlling extracellular levels of glutamate has been demonstrated through studies using antisense knockdown as well as genomic knockout (Rothstein et al., 1996; Tanaka et al., 1997; Watase et al., 1998). For instance, inactivation of the transporters GLAST (EAAT1) or GLT-1 (EAAT2) in rats by antisense oligonucleotide infusion produces increased extracullular glutamate and excitotoxic neurodegeneration (Rothstein et al., 1996). Tanaka et al. (1997) also demonstrated that both antisense knockdown and GLT-1 null mice retain less than 10% of total glutamate transport in the cortex. This suggests that GLT-1 is responsible for the bulk of extracellular glutamate clearance in the central nervous system.

Antisense knockdown of the neuronal transporter EAAC1 (EAAT3), however, did not elevate glutamate levels, produced only mild neurotoxicity, and did not instigate neurodegeneration (Storch et al., 1992; Peghini et al., 1997). Rao and colleagues (2001) examined infarct volume, neuronal death, and neurological deficit in rats subjected to transient middle cerebral artery occlusion (MCAO). They found that antisense knockdown of GLT-1, but not EAAC1, exacerbated the ischemic volume and neuronal

damage in cerebral cortex and striatum. Together these studies provide evidence that although direct transport of glutamate may decrease following EAAC1 knockdown, glutamate accumulation in the synapse is not initiating excitotoxicity nor is it causing neurodegeneration.

Summary and Conclusions

In summary, the behavioral difference between groups in this investigation has a number of conceptual implications. The findings indicate that (1) the EAAC1 knockdown rats can develop a spatial representation of a hidden platform position in this training protocol within a single trial, (2) that rats are able to improve their latency to locate the platform across trials on a given day, and (3) it is possible for rats to develop an effective and flexible search strategy that can be applied to a difficult working memory task. Further, the performance of the control animals in this task suggests that (1) these rats developed a spatial representation of the platform location across trials in a given day when the platform maintained a fixed position, yet (2) were unable to maintain an effective and flexible search strategy that can be applied across days. The results of this investigation demonstrate that microinfusion of rAAV encoding EAAC1 antisense produces significant behavioral consequences in tasks involving glutamate transmission within the hippocampus. The findings suggest that successful performance in the DMTP water maze task requires a flexible spatial memory representation of the specific visual cues necessary for noticing changes in platform position across days. Finally, these

results indicate that the DMTP water maze task demonstrates important effects of treatment.

Although previous studies have demonstrated reliable knockdown of EAAC1 expression after administration of antisense oligonucleotides, the effect of antisense infusion in this investigation should be evaluated by Western blotting to quantify the presence of the proteins within hippocampal tissue. At this time, the effects of the EAAC1 knockdown on hippocampal structure and function are unclear. It is possible that EAAC1 knockdown initiated the accumulation of glutamate in the synapse, leading to an increase in NMDA receptor activation. The more general possibility is that EAAC1 knockdown within the hippocampus initiated changes in the synaptic biology within the hippocampal tissue. The novelty of the present results merits continued research focused on glutamate transporters and their correlation with learning and memory function.

REFERENCES

Abeliovich, A., Chen, C., Goda, Y., Silva, A. J., Stevens, C. F., & Tonegawa, S. (1993a). Modified hippocampal long-term potentiation in PKC γ-mutant mice. *Cell, 75*, 1253-1262.

Abeliovich, A., Paylor, R., Chen, C., Kim, J. J., Wehner, J. M., & Tonegawa, S. (1993b). PKC γ-mutant mice exhibit mild deficits in spatial and contextual learning. *Cell,* 75, 1263-1271.

Amaral, D. G. & Witter, M. P. (1989). The three-dimensional organization of the Hippocampal formation: A review of anatomical data. *Neuroscience, 31(3*), 571–591.

Babcock, A. M., Standing, D., Bullshields, K., Schwartz, E., Paden C. M., & Poulsen, D.J. (2005). In vivo inhibition of hippocampal Ca^{2+}/calmodulin-dependent protein kinase II by RNA interference. *Molecular Therapy*, *11*, 899-905.

Bannerman, D. M., Good, M. A., Butcher, S. P., Ramsay, M., & Morris, R. G. (1995). Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. *Nature, 378*, 182-186.

Bast, T., da Silva, B. M., & Morris, R. G. M. (2005). Distinct contributions of hippocampal NMDA and AMPA receptors to encoding and retrieval of one-trial place memory. *Journal of Neuroscience, 25*, 5845-5856.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology, 232 (2),* 331-356.

Bridges, R. J. (2001). The Ins and Outs of Glutamate Transporter Pharmacology, *Tocris Reviews No. 17.*

Brown, T. H., Kairiss, E. W., & Keenen, C. L. (1990). Hebbian synapses: biophysical mechanisms and algorithms. *Ann Rev Neurosci, 13*, 475-511.

Brun, V. H., Ytterbo, K., Morris, R. G. M., Moser, M., & Moser, E. I. (2001). Retrograde Amnesia for Spatial Memory Induced by NMDA Receptor-Mediated Long-Term Potentiation. *Journal of Neuroscience, (21),* 356-362.

Clark, R. E., West, A. N., Zola, S. M., & Squire, L. R. (2001). Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. *Hippocampus, 11*, 176–186.

Clayton, N. S. (1998). Memory and the hippocampus in food-storing birds: a comparative approach. *Neuropharmacology, 37,* 441-452.

Collingridge, G. L., Kehl, S. J., & McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *Journal of Physiology, 334,* 33-46.

Collingridge, G. L. & Bliss, T. V. P. (1987). NMDA receptors—Their role in long-term potentiation. *Trends in Neuroscience, 10,* 288-293.

de Bruin, J. P., Moita, M. P., de Brabander, H. M., Joosten, R. N. (2001). Place and Response Learning Of Rats In A Morris Water Maze: Differential Effects Of Fimbria Fornix And Medial Prefrontal Cortex Lesions *Neurobiol. Learn. Mem. 75(2),* 164-78.

Eichenbaum, H. & Cohen, N. J. (2001). *From Conditioning to Conscious Recollection: Memory Systems of the Brain* (Oxford University Press, London).

Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., & Tanila, H. (1999). The hippocampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron, 23,* 209-226.

Eichenbaum, H., Stewart, C., Morris, R. G. M. (1990). Hippocampal representation in place learning. *Journal of Neuroscience, 10,* 3531-3542.

Errington, M. L., Lynch, M. A., & Bliss, T. V. P. (1987). Long-term potentiation in the dentate gyrus: induction and increased glutamate release are blocked by d-aminophosphonovalerate. *Neuroscience, 20,* 279-284.

Flotte, T. R. (2000). Size does matter: overcoming the adeno-associated virus packaging limit. *Current Science Ltd, 1(1),* 16-18.

Furuta, A., Martin, L. J., Lin, C. L. G., Dykes-Hoberg, M., & Rothstein, J. D. (1997a). Cellular and synaptic localization of the neuronal glutamate transporters excitatory amino acid transporter 3 and 4. *Neuroscience, 81,* 1031-1042.

Gigg, J., Tan, A. M., & Finch, D. M. (2004). Glutamatergic hippocampal formation projections to prefrontal cortex in the rat are regulated by GABAergic inhibition and show convergence with glutamatergic projections from the limbic thalamus. *Hippocampus, 4 (2),* 189-198.

Hammond, C. (2001). Cellular and molecular neurobiology. Academic Press, San Diego.

Harris, E. W., Ganong, A. H., & Cotman, C. W. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Research, (323),* 132-137.

Hartley, T., Maguire, E. A., Spiers, H. J., & Burgess, N. (2003). The well-worn route and the path less traveled: distinct neural bases of route following and wayfinding in humans. *Neuron, 37*, 877–88.

Hauswirth, W. W. & Berns, K. I. (1977) Origin and termination of adeno-associated virus DNA replication. *Virology*, *78*, 488-499.

Henze, D. A., Urban, N. N., & Barrionuevo, G. (2000). The multifarious hippocampal mossy fiber pathway: a review. *Neuroscience, 98(3),* 407–427.

Hermonat, P. L., Labow, M. A., Wright, R. & Berns, K. I. (1984). Genetics of adeno-associated virus: isolation and preliminary characterization of adeno-associated virus type 2 mutants. *Journal of Virology, 51,* 329-339.

Hommel, J. D., Sears, R. M., Georgescu, D., Simmons, D. L., & DiLeone, R. J. (2003). Local gene knockdown in the brain using viral-mediated RNA interference. *Nature Medicine, 9(12),* 1539-1544.

Huang, Y.Y., Bach, M. E., Lipp, H. P., Zhou, M., Wolfer, D. P., Hawkins, R. D., Schoonjans, L., Kandel, E. R, Godfraind, J. M., Mulligan, R., Collen, D., & Carmeliet, P. (1996). Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways. *Proc. Natl. Acad. Sci., 93,* 8699-8704.

Jacobs, L. F. (1995). The ecology of spatial cognition. In. Alleva E (Ed.). Behavioral brain research in naturalistic and semi-naturalistic settings (pp. 301-322). Amsterdam: Kluwer Academic.

Janson, C. G., McPhee, S. W., Leone, P., Freese, A., & During, M. J. (2001). Viral-based gene transfer to the mammalian CNS for functional genomic studies. Trends Neurosci, 24(12), 706-12.

Jones, S. & Yakel, J. L. (1999). Inhibitory interneurons in hippocampus. Cell Biochem. Biophys., *31(2),* 207–218.

Kanai, Y. & Hediger, M. A. (1992). Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature, 360*, 467-471.

Kensinger, E. A., Ullman, M. T., & Corkin, S. (2001). Bilateral medial temporal lobe damage does not affect lexical or grammatical processing: Evidence from amnesic patient H.M. *Hippocampus, 11*, 337-346.

Kotin, R. M., Menninger, J. C., Ward, D. C., & Berns, K. I. (1991) Mapping and direct visualization of a region-specific viral DNA integration site on chromosome. *Genomics, 10*, 831-834.

Lavenex, P. & Amaral, D. G. (2000). Hippocampal-neocortical interaction: a hierarchy of associativity. *Hippocampus, 10(4)*, 420–430.

Levy, W. B, Wu, X. B., Greene, A. J., & Spellman, B. A. (2003). A source of individual variation. *Neurocomputing, 52–54*, 165–168.

Lynch, M. A. (1998). Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? *Prog Neurobiol, 56*, 571-589.

Mac Corquodale & Meehl, P. E. (1948): On a distinction between hypothetical constructs and intervening variables. *Psychology Review*, 55.

Maren, S., & Fanselow, M. S. (1997). Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiology of Learning and Memory, 67*, 142-149.

Martin, S. J., Grimwood, P. D., & Morris, R. G. M. (2000). Synaptic plasticity and memory: and evaluation of the hypothesis. *Annual Review of Neuroscience, 23*, 649-711.

Mathews, G., & Diamond, J. (2003). Neuronal glutamate uptake contributes to GABA synthesis and inhibitory synaptic strength. *J Neurosci, 23*, 2040–2048.

Mayer, M. L., Westbrook, G. L., & Guthrie, P. B. (1984). Voltage dependent block by Mg2+ of NMDA response in spinal cord neurons. *Nature, London, 309*, 261-263.

McNaugton, B. L., Barnes, C. A., Rao, G., Balwin, J., & Rasmussen, M. (1986). Long-term enhancement and the acquisition of spatial information. *Journal of Neuroscience, 6*, 563-571.

McNaugton, B. L., & Morris, R. G. M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends in Neuroscience, 10,* 408-415.

Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *Journal of Nutrition, 130,* 1007-1015.

Milner, B. (1972). Disorders of learning and memory after temporal lobe lesions in man. *Clinical Neurosurgery, 19,* 421–446.

Mohapel, P., Mundt-Petersen, K., Brundin, P., Frielingsdorf, H. (2006). Working memory training decreases hippocampal neurogenesis. *Neuroscience, 142,* 609–613.

Monahan, P. E, & Samulski, R. J. (2000). Adeno-associated virus vectors for gene therapy: more pros than cons? *Mol Med Today., 6(11),* 433-40.

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation, 12,* 239-261.

Morris, R. G. M. (1983). An attempt to dissociate 'spatial mapping' and 'working-memory' theories of hippocampal function. In Siefert W (Ed.), Neurobiology of the hippocampus (pp. 405-432). London: Academic Press

Morris, R. G. M., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist: AP5. *Nature, 319,* 774-776.

Morris, R.G.M., Garrud, P., Rawlings, J., & O'Keefe, L. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297,* 681-683.

Morris, R. G. M., Schenk, F., Tweedie, F., & Jarrard, L. E. (1990). Ibotenate Lesions of Hippocampus and/or Subiculum: Dissociating Components of Allocentric Spatial Learning. *European Journal of Neuroscience, 2,* 1016-1028.

Moser, E. I., Krobert, K. A., Moser, M. B., Morris, R.G. M. (1998). Impaired spatial learning after saturation of long-term potentiation. *Science, 281,* 2038-3042.

Moser, E., Moser, M. B., & Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesion, but is hardly present following ventral lesions. *Journal of Neuroscience, 13,* 3916-3925.

Moser, M. B., Moser, E. I., Forest, E., Andersen, P., & Morris, R. G. M. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Porc. National Academy of Science USA, 92,* 9697-9701.

MRC Centre for Synaptic Plasticity, School of Medical Sciences, Department of Anatomy, University Walk, Bristol, BS8 1 TD, UK. (2007). Hippocampal Pathways. Retrieved February 28, 2007, from www.bris.ac.uk/synaptic.

Murray, E. A., & Mishkin, M. (1984). Severe tactual as well as visual memory deficits follow combined removal of the amygdala and hippocampus in monkeys. *Journal of Neuroscience, 4,* 2565-2580.

Nadel, L. (1991). The hippocampus and space revisited. *Hippocampus, 1,* 221-229.

Nicholls, D., & Attwell, D. (1990) The release and uptake of excitatory amino acids. *Trends in Pharmacological Science, 11(11),* 462-468.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res., 34,* 171-175.

O'Keefe, J. A., & Nadel, L. (1978). *The hippocampus as a cognitive map.* Oxford, England: Oxford University Press.

Olton, D. S., Becker, J. T. & Handelman, G. H. (1979). Hippocampus, space and memory. *Behavioral and Brain Sciences, 2,* 313-365.

Otnaess, M. K., Brun, V. H., Moser, M. B., & Moser, E. I. (1999). Pretraining Prevents Spatial Learning Impairment after Saturation of Hippocampal Long-Term Potentiation. *Journal of Neuroscience, 19.*

Panakhova, E., Buresova, O., Bures, J. (1984). Persistence of spatial memory in the Morris water maze tank task. *International Journal of Psychophysiology, 2,* 5-10.

Patton, P. E. & McNaughton, B. L. (1995). Connection matrix of the hippocampal formation: I. The dentate gyrus. *Hippocampus, 5(4),* 245–286.

Pearce, I. A., Cambray-Deakin, M. A., & Burgoyne, R. D. (1987). Glutamate acting on NMDA receptors stimulates neurite outgrowth from cerebellar granule cell. *FEBS Letter, 223,* 143-147.

Peghini, P., Janzen, & Joffel, W. (1997). Glutamate transporter EAAC-1 deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. *EMBO Journal, 16,* 3822–3832.

Randall, D., Burggren, W., & French, K. (2002). Eckert Animal Physiology: Mechanisms and Adaptations (5th ed.). New York: W. H. Freeman and Company.

Rao, V. L. R., Baskaya, M. K., Dogan, A., Rothstein, J. D., Dempsey, R. J. (1998) Traumatic brain injury down-regulates glial glutamate transporter (GLT-1 and GLAST) proteins in rat brain. *Journal of Neurochemistry, 70,* 2020-2027.

Roitblat, H. L. & Harley, H. E. (1988). Rat spatial delayed matching-to-sample performance: Acquisition and retention. *Journal of Experimental Psychology: Animal Behavior Processes,* 14, 71-82.

Rothstein, J. D., Martin, L., Levey, A. I., Dykes-Hoberg, M., Jin, L., Wu, D., Nash, N., & Kuncl, R. W. (1994). Localization of neuronal and glial glutamate transporters. *Neuron, 13,* 713-725.

Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., Kanai, Y., Hediger, M. A., Wang, Y., Schielke, J. P., & Welty, D. F. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron, 16,* 675-686.

Sakimura, K., Tatsuya, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., Sugiyama, H., & Mishina, M. (1995). Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ε1 subunit. *Nature, 373,* 151-155.

Schousboe, A. (1981). Transport and metabolism of glutamate and GABA in neurons are glial cells. *Int Rev Neurobiology, 22,* 1-45.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurol Neurosurg Psychiatry, 20 (1),* 11-21.

Sepkuty, J. P., Cohen, A. S., Eccles, C., Rafiq, A., Behar, K., Ganel, R., Coulter, D. A., & Rothstein, J. D. (2002). A Neuronal Glutamate Transporter Contributes to Neurotransmitter GABA Synthesis and Epilepsy. *The Journal of Neuroscience, 22(15),* 6372-6379.

Shashidharan, P., & Plaitakis, A. (1993). Cloning and characterization of a glutamate transporter cDNA from human cerebellum. *Biochem. Biophys. Acta, 1216,* 161-164.

Shashidharan, P., Wittenberg, I., & Plaitakis, A. (1994). Molecular cloning of human brain glutamate/aspartate transporter II. *Biochem. Biophys. Acta, 1191,* 393-396.

Shelton, A., & Gabrieli, J. D. E. (2002). Neural correlates of encoding space from route and survey perspectives. *Journal of Neuroscience, 22*, 2711–17.

Silva, A. J., Stevens, C. F., Tonegawa, S., & Wang, Y. (1992a). Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science, 257,* 201-206.

Silva, A. J., Paylor, R., Wehner, J. M., & Tonegawa, S. (1992b). Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science, 257*, 206-211.

Squire, L. R., & Zola-Morgan, S. (1983). The neurology of memory: The case for correspondence between the findings for man and nonhuman primate. In J. A. Deutsch (Ed.), The Physiological Basis of Memory, 2nd ed, (pp. 199-268) Academic, New York.

Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychological Review, 99*, 195-231.

Squire, L. R., Clark, R. E., Knowlton, B. J. (2001). Retrograde amnesia. *Hippocampus, 11*, 50-55.

Sutherland, R. J., Kolb, B., & Whishaw, I. Q. (1982). Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat. *Neuroscience Letters, 31*, 271-276.

Sutherland, R. J., Whishaw, I. Q., & Kolb, B. (1983). A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat. *Behavioural Brain Research, 7*, 133-153.

Suzuki, W., & Amaral, D. G. (1990). Cortical inputs to the CA1 field of the monkey hippocampus originate from the perirhinal and parahippocampal cortex but not from area TE. *Neurosci Letter, 115*, 43–48.

Steele, R. J., & Morris, R. G. M. (1999). Delay-Dependent Impairment of a Matching-to-Place Task With Chronic and Intrahippocampal Infusion of the NMDA-Antagonist D-AP5. *Hippocampus, 9,* 118-136.

Storck, T., Schulte, S., Hofmann, K., & Stoffel, W. (1992). Structure, expression, and functional analysis of a Na(+)-dependent glutamate/aspartate transporter from rat brain. *Proc Natl Acad Sci USA, 89,* 10955-10959.

Tanaka, K., Watase, K., Manabe, T., Yamada, K., Watanabe, M., Takahashi, K.,Iwama, H., Nishikawa, T., Ichihara, N., Hori, S., Takimoto, M. & Wada, K. (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT1. *Science, 276,* 1699–1702.

Tolman, E. C. (1932). *Purposive behavior in animals and men*. New York: Appleton-Century-Crofts.

Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychological Review, 55*, 189-208.

Tolman, E. C. (1949). There is more than one kind of learning. *Psychological Review, 56*, 144-155.

Tolman, E. C., & C. H. Honzik. (1930). Degrees of hunger, reward and nonreward, and maze learning in rats. *University of California Publications in Psychology, 4*, 241-275.

Tulving, E. (1983). Elements of episodic memory. Oxford: Clarendon Press.

Wahlestedt, C., Pich, E. M., Koob, G. F., Yee, F., Heilig, M. (1993). Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. *Science, 259,* 528–531.

Watase, K., Hashimoto, K., Kano, M., Yamada, K., Watanabe, M., Inoue, Y., Okuyama, S., Sakagawa, T., Ogawa, S., Kawashima, N., Hori, S., Takimoto, M., Wada, K., & Kohichi, T. (1998) Motor discoordination and increased susceptibility to cerebellar injury in GLAST mutant mice. *Eur J Neurosci , 10*, 976-88.

Werstuck, G. H., & Green, M. R. (1998). Controlling gene expression in living cells through small molecule-RNA interactions. *Science 282*, 296-298.

Whishaw, I. Q., Cassel, J. C., Jarrard, L. E. (1995b). Rats with fimbria–fornix lesions display a place response in a swimming pool: a dissociation between getting there and knowing where. *J Neuroscience, 15,* 5779 –5788.

Whishaw, I. Q., Rod, M. R., & Auer, R. N. (1994). Behavioral Deficits Revealed by Multiple Tests in Rats with Ischemic Damage Limited to Half of the CA1 Sector of the Hippocampus. *Brain Research Bulletin, 34 (3),* 283-289.