THE ROLE OF THE HIPPOCAMPUS AND MATRIX METALOPROTEINASES ON HABITUATION OF THE HEAD-SHAKE RESPONSE TASK/CLASSICAL CONDITIONING PARADIGM

by

ROBERTA V WIEDIGER

**A dissertation submitted in partial fulfillment of
the requirements for the degree of**

DOCTOR OF PHILOSOPHY

**WASHINGTON STATE UNIVERSITY
Department of Psychology**

December 2008

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of ROBERTA V. WIEDIGER find if satisfactory and recommend that it be accepted.

_____
Chair

_____

_____

_____

ACKNOWLEDGMENTS

First and foremost, I would like to thank the incredible mentoring of Dr. Jay Wright. He not only inspired me to search for answers by doing research, but his outstanding guidance and patience encouraged me to look for answers in places that I would otherwise not thought possible. Thank you for being understanding of my needs and not giving up on me, especially during my post-partum depression.

A very special thanks to Dr. Fran McSweeney, who believed in me by opening the doors to her lab, which made all of this possible.

Thanks to Ruth Day and AnnMarie Gooch, whose love and support made WSU feel like home.

Thanks to my parents, who taught me that anything is possible with hard work, patient, and perseverance. Their love and support kept me going during many hard times. Thanks to my dad for teaching me the priorities in life, and thanks to my mom for supporting me during my post-partum depression.

And last but not least, to my wonderful, caring, loving, amazing, very patient husband, whose support and dedication made life in graduate school a dream come true. I not only fell in love, but I was also given the most beautiful and wonderful gift in the world, Melanie, my amazing daughter.

You will always be in my heart. I am where I am in my life because I was fortunate to have crossed paths with all of you. Thank you for believing in me even when I had doubts.

THE ROLE OF THE HIPPOCAMPUS AND MATRIX METALOPROTEINASES ON HABITUATION OF THE HEAD-SHAKE RESPONSE TASK/CLASSICAL CONDITIONING PARADIGM

Abstract

By Roberta V. Wiediger, Ph.D.
Washington State University
December 2008

Chair: John W. Wright

These experiments were designed to find evidence regarding the relationship between the hippocampus and matrix metalloproteinases (MMP) during the head-shake response (HSR) task/classical conditioning paradigm. Habituation is the simplest form of learning, where an organism's response is decreased due to a repeated presentation of a harmless stimulus. The HSR task has been very predictable in showing habituation to repeated air stimulation to a rat's ear and the spontaneous recovery of the habituated response twenty-four hours later. Since the hippocampus and MMPs are implicated in learning and memory, these experiments investigated their role during a HSR task when a classical conditioning paradigm was added. Therefore these studies were designed to investigate the following: 1) Can a tone serve as the conditioned stimulus (CS) during the HSR task? 2) Is the dorsal hippocampus important during the HSR/Classical conditioning paradigm? 3) Are hippocampal MMPs important during the CS-US

(unconditioned stimulus) association? 4) Is hippocampal MMP-3 important during the CS-US association? Findings revealed: 1) the tone presented 1-s prior to the US became a CS. 2) Dorsal hippocampectomized animals were not able to make the CS-US association. 3) Injections of FN-439, a general MMP inhibitor, into the dorsal hippocampus interfered with the CS-US association. 4) Dorsal hippocampus injections of MMP-3 inhibitor also interfered with the CS-US association. Therefore, animals which were not able to form the CS-US association showed similar rates of responding twenty-four hours later. Based on the collective findings of these experiments it is evident that during a HSR/classical conditioning paradigm, the hippocampus plays an important role in consolidating and storing the CS-US association. More specifically dorsal hippocampus MMP-3 was found to be particularly important in the formation of the CS-US association.

TABLE OF CONTENTS

LIST OF FIGURES

Dedication

To my husband and my father, who inspired me to reach high and never give up.

Thank you!

CHAPTER ONE

GENERAL INTRODUCTION

A. General Introduction

1. *Definition of Learning*

Learning has been defined as "a relatively long-term change in behavior that results from experience" (Kosslyn & Rosenberg, 2004). "A process by which experience produces a relatively enduring change in the organism's behavior or capabilities" (Passer & Smith, 2004). "A relatively permanent change in an organism's behavior due to experience" (Myers, 2004). It is known that the hippocampus plays a role in learning and memory and therefore dorsal hippocampectomy was presently employed in an effort to determine its importance in spontaneous recovery and habituation of the head-shake response (HSR). The protocols used included the use of the HSR habituation task, the introduction of a tone (conditioned stimulus – CS) prior to each introduction of the air stimulus (unconditioned stimulus – US), hippocampectomized rats and the infusion of matrix-metalloproteinase inhibitors (MMPIs) injected in the dorsal hippocampus. These paradigms permitted the examination of the role of the dorsal hippocampus during a HSR task combined with a CS-US association, and the role of MMPs during this task.

B. Types of learning

1. *Associative Learning*

Experimental studies concerning learning are usually divided into the categories of **associative** and **non-associative** learning (Eisenstein, Eisenstein, & Bonheim, 1991). However, the majority of introductory psychology textbooks only describe associative learning (Myers, 2004; Weiten, 2004). Associative learning relates to the occurrence of certain events together in time and space (Wieten, 2004). It includes **classical** and **operant** conditioning. The former is a subtype of associative learning characterized by the association of an originally neutral stimulus

with an unconditioned stimulus causing a conditioned response to be emitted that was previously unconditioned. An example of classical conditioning is the use of an aversive stimulus to encourage people to stop smoking. Specifically, giving a smoker a drug (UCS) that causes nausea (UCR), and later introducing cigarette smoking behavior (CS) right before the UCS will decrease cigarette smoking behavior by its association with (CR). Classical conditioning, also referred to as Pavlovian conditioning, alters the way in which animals process events and may produce a variety of consequences (Holland, 1997). Classical conditioning changes the way the animal attends to CSs including the production of various types of CRs, the acquisition of reinforcing power, and the direction of attention toward or away from those stimuli, in addition it also may involve distinct, relatively independent neural circuitry (Holland, 1997).

On the other hand, operant conditioning is a subtype of learning that associates the behavior with the consequence of that behavior making it more or less likely to reoccur in the future. An example of operant conditioning is receiving a ticket for speeding and also having your automobile insurance rate increase. Presumably this will cause the driver to avoid speeding in the future.

2. Non-associative learning

Non-associative learning includes **habituation** and **sensitization** (Eisenstein et al., 1991). A definition of non-associative learning could not be found in the literature. Most behavioral scientists use the term non-associative learning as an antonym to associative learning. This nomenclature has been adopted without providing operational definitions. Habituation is defined as "a decrease in responsiveness to repeated presentations of a stimulus" (Groves & Thompson, 1970), and is considered the simplest form of learning (Harris, 1943; Thorpe, 1966). According to Staddon (2001), habituation is the waning of a reflexive response to repeated stimulation.

Consisted with this definition Zaccardi, and colleagues (2001) have proposed that habituation is the simplest form of learning seen in all animals consisting of a decrease in responsiveness due to a repeatedly applied innocuous stimulus.

Thompson and Spencer (1966) listed nine parametric characteristics proper to habituation. Recently, McSweeney and Murphy (2000) added five more characteristics to this list ranging from, **spontaneous recovery**, "the recovery of a habituated response to a stimulus when that stimulus is not present for a period of time," to **stimulus intensity**, "habituation sometimes is more pronounced and faster for less intense than for more intense stimuli." Two out of these fourteen characteristics are concerned with the phenomenon called sensitization. According to Eisenstein et al. (1991), sensitization has been employed with several different procedural variations and meanings. Groves et al. (1970) have described sensitization as an increase in responsiveness due to early stimulus presentation. Sensitization has also been defined as "an increase in responsiveness due to the presentation of a stimulus from different modality" (Swithers & Hall, 1994). Some researchers have named this phenomenon **dishabituation** (Marcus, Nolen, Rankin, & Carew, 1998). As a result, sensitization has been employed with several different procedural variations and meanings (Eisenstein et al., 1991), but the most commonly used is that of an initial increase in responsiveness due to the repeated presentation of a stimulus (Groves et al., 1970).

McSweeney, Hinson & Cannon (1996) proposed a sensitization-habituation hypothesis. According to this hypothesis early-session increases in responding are primarily due to sensitization, while late-session decreases are primarily produced by habituation to the repeatedly presented reinforcer. This approach is important because it compares the phenomena of sensitization and habituation with empirical characteristics of within-session changes in

operant responding, thus suggesting that the two types are produced by similar variables. Therefore, both phenomena can occur within a wide variety of species, performing a wide variety of responses (McSweeney et al., 1996).

According to Harris (1943) the most ubiquitous phenomenon in animal behavior is that of response decrement as the result of repeated stimulation. This phenomenon has been reported in studies ranging from ameba's locomotion, to humans' reflex mechanism. Marcus et al. (1998), have reported habituation of the siphon withdrawal reflex in the marine mollusk Aplysia. Murphy et al., (2005), have noted habituation in rats during the HSR task. Eisenstein and colleagues, (1990), studied habituation and sensitization of the palmar galvanic skin response (GSR) to shock in college males. Ornitz and Guthrie (1989) showed habituation and sensitization of the acoustical startle response in adult humans. The behaviors described above demonstrate that the animal does not necessarily need to learn to respond to certain stimuli. Inborn reflexive responses are extremely important for survival, therefore requiring little or no time to develop associations.

C. Physiological basis of learning

As mentioned above an organism is constantly exposed to a variety of information that must be learned and stored in memory so that it can be retrieved at a later time. However, little is known about the neural mechanisms underlying learning and memory. Nevertheless, many studies have shown that the hippocampus is involved in learning and memory (Collingridge, Isaac, & Wang, 2004; Lee, Everitt, & Thomas, 2004; Wright, et al., 2004b). One fascinating and exceedingly important aspect of the brain is its capacity to efficiently pass information from one neuron to another and to modify these circuits with experience. This property is called "synaptic plasticity", the ability of the brain to change, reconfigure and in this way consolidate vast

amounts of behavioral information (Collingridge et al., 2004). The neural plasticity underlying memory appears to undergo a continuous pattern of maintenance and reconsolidation made possible by extracellular matrix (ECM) molecules (reviewed in Wright & Harding, 2004a, and described below).

1. *Extracellular Matrix*

Within the Central Nervous System is an extracellular matrix composed of a network of proteins involved in cellular development, migration, connectivity and reorganization (Novak & Kaye, 2000). Many ECM molecules are responsible for maintaining and changing the synaptic configuration presumed to be indispensable for the processes of neural plasticity, memory and learning. Recently, researches have begun to identify those molecules responsible for reconfiguring and storing memory. Much attention has been given to the matrix metalloproteinases (MMPs), enzymes critical to the maintenance and reconstruction of the ECM (Wright et al., 2004b). The MMPs are responsible for ECM degradation, and the mediation of new synaptic reconfiguration. Therefore, MMPs are extremely important in learning, as they are markers of neural plasticity. Several studies have measured changes in MMP expression in the hippocampus after an animal has successfully performed a task, implying that memory consolidation is taking place (Meighan, et al., 2006; and Meighan et al., 2007).

The hippocampus is a region of the limbic cortex located in the temporal lobe, linked to learning and memory (Collingridge et al., 2004; Kandel, Schwartz & Jessel, 2000; Kandel, 2001). The hippocampal formation is composed of the hippocampus proper, dentate gyrus and the subiculum (Carlson, 2001). The major neocortical input of the hippocampal formations is through the entorhinal cortex, a region of the limbic system. Neurons in the entorhinal cortex send information to the granule cells, found in the dentate gyrus, through axons that form the

perforant path. The hippocampus is also composed of subfields (CA1, CA2 and CA3). The CA1 field posses many pyramidal cells, large neurons with pyramid shaped soma, which provide primary output for the hippocampus. They send axons to neurons in the subicular complex, which project out of the hippocampal formation to the entorhinal cortex and to the basal forebrain. The CA3 pyramidal cells branch in two directions. One pathway goes to the CA1 field and the other goes to the fornix and structures in the basal forebrain, including the septum and the mammillary bodies (Carlson, 2001). The CA2 field connects CA3 and CA1 fields thus completing the neural circuit.

There are three major pathways in the hippocampus: 1) The perforant pathway, projecting from the entorhinal cortex to the granule cells of the dentate gyrus. 2) The mossy fiber pathway, containing the axons of granule cells that project to the pyramidal cells in the CA3 field of the hippocampus. 3) The Schaffer collateral pathway, which consists of the excitatory collaterals of the pyramidal cells in the CA3 region and ends on the pyramidal cells in the CA1 region (Carlson, 2001).

2. Long-term potentiation

According to Collingridge et al. (2004), one form of synaptic plasticity, known as long-term potentiation (LTP), has consolidated its status as a synaptic model for investigating the molecular basis of memory. LTP has been identified as the biological substrate for at least some types of memories (Lynch, 2004). LTP has been most frequently studied in the hippocampus, although it is also seen in other CNS structures, such as the amygdala and cerebellum.

Long-term potentiation has two phases, an early and a late phase. The early phase (E-LTP) lasts for a few hours and does not require protein synthesis, while the late phase (L-LTP) can last for at least twenty-four hours and requires protein and RNA synthesis (Kandel et al.,

2000; Kandel, 2001). This requirement for new mRNA and protein synthesis implies that transcriptional regulation represents the principal control point for the consolidation of synaptic plasticity, and translation of new synthesized mRNAs playing a more secondary role (Kelleher, Govindarajan & Tonegawa, 2004). To inhibit L-LTP expression, treatment must occur before protein synthesis has taken place, and before repeated tetanization has occurred. The application of a protein synthesis inhibitor, e.g. anisomycin, after the induction of L-LTP is ineffective. However, the ability of protein synthesis inhibitors to block the enhancement of protein synthesis, without depleting the ones necessary for basal neuronal and synaptic function, seems to be of brief duration (Kelleher et al., 2004). This suggests that anisomycin interferes only with the induction of L-LTP not with its maintenance.

ECM molecules are regulated via expression of MMPs that function to degrade existing matrix, but it is offset by tissue inhibitors of MMPs (TIMPs) that serve to preserve the matrix (Wright et al., 2002). Therefore, the ECM is well suited to mediate the synaptic plasticity requirements assumed to occur during LTP and underlie learning and memory. Degradation of ECM by MMPs is controlled by three mechanisms: 1) Regulation of gene transcription, which occurs via stimulation of growth factors, oncofene products, phorbol esters, cell-to-cell and cell-to-ECM interactions. 2) Regulation of pro-enzyme activation and 3) through the presence of TIMPs.

3. *Importance of calcium ions during learning*

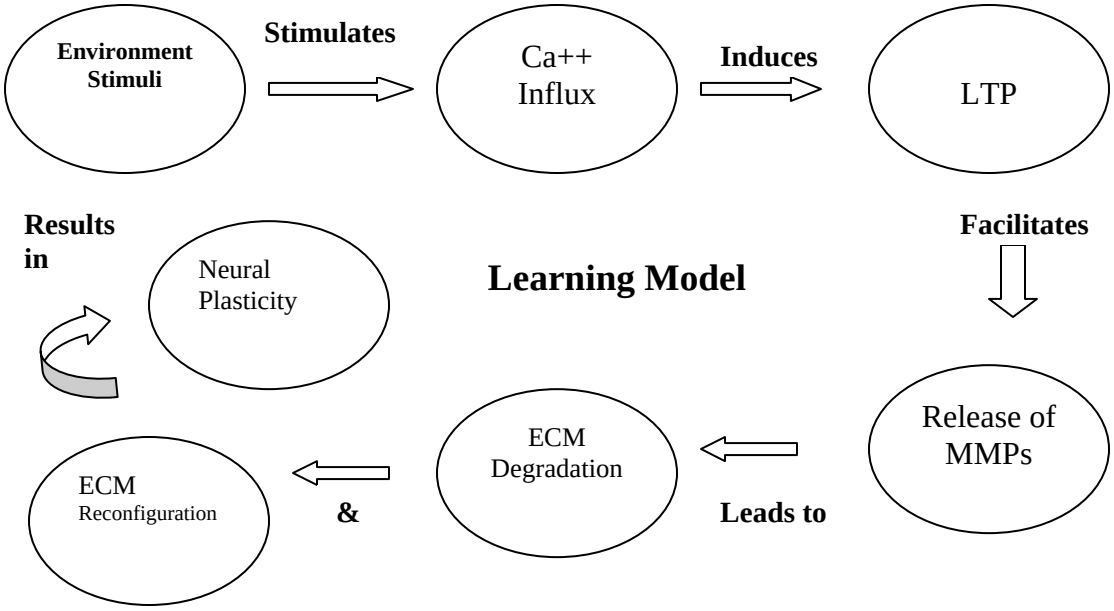
Calcium plays a very important role in the learning process, as a primary component of the signaling process, beginning with the action potential. The flow of calcium ions through the ionotropic receptors into the neuron depolarizes the plasma membrane (Kandel et al., 2000). When the cell reaches threshold, action potentials are generated moving down the axon and

reaching the terminal buttons. The terminal buttons release neurotransmitters, e.g. glutamate, to the next neuron. Therefore, a chemical signal is given to the next neuron which in turn initiates an action potential. Calcium ions are extremely important to the induction of action potentials since they contribute to membrane depolarization resulting in LTP, and new spine formation. Structural changes in the synapses can be due to cell-to-cell rearrangements and extracellular matrix-to-cell interactions (Dityatev, Dityateva, Sytnyk, Delling, Toni, Nikonenko, Muller, & Schachner, 2004). New neuronal spines are formed during memory consolidation. Experiments have demonstrated the role of neurogenesis in long-term memory in rats. Snyder and colleagues (2005), have shown that newly formed dentate granule neurons may be required in order to form long-term spatial memories in rats. Therefore, dynamic changes in structural characteristics of the synapses are thought to underlie synaptic plasticity, thus permitting memory consolidation. Recently it had been reported that rats allowed to explore a compartmentalized environment for 30 min showed 30% more synaptic spines with dense phosphorylated cofilin immunoreactivity in hippocampal field CA1 than rats pretreated with an NMDA receptor antagonist (Fedulov, Rex, Simmons, Palmer, Gall, & Lynch, 2007). The authors interpreted these results to suggest that cellular events associated with LTP such as synaptic spine changes, occur during learning and form the basis of a new memory. If these results are confirmed it will be the first time LTP has been directly linked to a change in synaptic spine induced by experience in a novel environment.

D. Physiological model of learning

1. *A molecular view*

The model below illustrates how learning may take place.



According to this model, learning is formed when an organism is exposed to environmental stimuli leading to calcium influx into neuronal cells resulting in LTP. When LTP is induced MMPs are released into the cytoplasm degrading the extracellular matrix. By allowing degradation of ECM new configurations of neurons are formed. This process of ECM degradation and reconfiguration represents a primary mechanism of neural plasticity. The ability of neurons to work as a network, connecting and disconnecting from adjacent neurons, permits neural plasticity and thus learning and memory to take place.

For example, consider "shaping," a type of associative learning. More specifically operant conditioning, which utilizes reinforcement to closely approximate responses to the desired ones. An example is training a pet to "sit" or "role over," by reinforcing a response that is close to the desired behavior. This leads to the learning of the goal behavior after successive reinforced approximations of the targeted behavior.

How can associative learning be related to the above model? For learning to take place, the environmental stimuli, verbal command "sit" and the reinforced behavior of sitting, need to

be presented contiguously. When the organism is presented this new information calcium influx into the cell occurs. With a sufficient increase in action potential firing rate LTP is induced. In turn, LTP stimulates MMPs to be released leading to ECM degradation and reconfiguration, allowing the neuronal network to reconfigure. Therefore, when the animal learns the meaning of the word "sit" it responds accordingly, and an increase in hippocampal MMP expression occurs accompanied by the formation of new synaptic spines. In this way, new synapses are formed due to the degradation of ECM.

According to Thompson (1990), memory traces are formed in the hippocampus and in the cerebellum during classical conditioning of discrete behavioural responses. Learning and retrieval both activate hippocampal circuits and activate molecular pathways (Power, Berlay, McGaugh, & Steward, 2006). The hippocampus is thought to participate in the processing of contextual and temporal information (Hoehler & Thompson, 1980; Lee & Kim, 2004). The hippocampus also appears to be involved in certain types of conditioned fear memory, i.e. fear to a contextual cue, but not to a tone cue (Kim & Jung, 2006). Although the hippocampus plays a role in certain aspects of conditioning, it is not necessary for learning and memory of the basic conditioned responses. The cerebellum and its associated brain-stem circuitry, on the other hand, do appear to be essential for learning and memory of the conditioned response (Thompson, 1990). According to Lee and Kim (2004), the cerebellum is essential to establish the CS-US association for conditioned eyeblink responses, and the hippocampus on the other hand, is not critical for eyeblink responses but affects eyeblink CRs perhaps by processing contextual information during conditioning. Lesion of the dorsal part of the hippocampus decreases performances in associative spatial tasks like the radial maze or the Morris water maze (Stupien, Florian, & Roullet, 2003).

However, differences in neural substrates have recently been noted between associative and non-associative learning. For example, habituation of the HSR in rats occurs with repeated puffs of air to the ear. Habituation occurs rapidly and is evidenced as a decreasing number of head-shakes during the session. Nonetheless, twenty-four hours later the rat will exhibit spontaneous recovery, presenting nearly the same within-session change in response to the air puff as it did twenty-four hours earlier. If learning represents a permanent change in the organism's behavior, then spontaneous recovery should not occur. Looking at the model, perhaps the ECM was not reconfigured, thus no neural plasticity took place.

Many studies on cellular mechanisms of gill-withdraw reflex in Aplysia, a large marine snail, have shown several forms of learning including, habituation, sensitization, and classical conditioning (Carew, Castellucci, & Kandel, 1971; Carew, Walters, & Kandel, 1981). A recent study done by Hawkins, Clark and Kandel (2006), found that gill withdrawal in Aplysia can also undergo operant conditioning. They showed that the mechanism of operant conditioning of gill withdrawal might be an elaboration of mechanisms that contribute to non-associative effects of the siphon shock. However, the associative and non-associative effects had different time courses, suggesting that they may involve different neural mechanisms of learning (Hawkins et al., 2006).

Wright et al., (2004b) have shown that hippocampectomized rats revealed habituation of the HSR task, demonstrating the ability to develop non-associative learning even though no hippocampus was present. Anisomycin, a protein inhibitor, when infused in the CA1 region of the hippocampus have been found to have no effect on long-term memory formation of spatial habituation (Vianna, Alonso, Viola, Quevedo, Paris, Furman, Stein, Medina, & Izquierdo, 2000). This could indicate that the hippocampus does not play a role in reflexive, non-associative

learning. Perhaps this is a way organisms have to preserve a behavior that is critically important for survival. According to Vienna et al., (2000), different neural mechanisms are involved in memory formation of hippocampal-dependent associative and non-associative memories.

Since habituation of HSR was not affected by hippocampectomy, then where does that non-associative learning take place? Given that the HSR is a reflex, like eye blinking and galvanic skin response, and is important for survival but is not plastic, then mediation could occur in subcortical areas of the brain. Brain stem structures are likely candidates to hold such important processes as non-associative learning. Swithers-Mulvey and Hall (1993), have argued that oral habituation, the opening or closing of jaws and/or movement of tongue, is neurally represented in the brainstem, since habituation continues to be expressed in rat pups after decerebration. According to Kandel et al., (2000), the midbrain controls many sensory and motor functions, including eye movements and the coordination of visual and auditory reflexes, and is important for this type of learning. An animal in its natural habitat habituates to a startle response due to the harmless repeated noise produced by the wind passing through the tree branches. However, the next day that similar noise could be caused by something else, perhaps, a predator. Thus, the startle response is important for survival. As a result the system does not modify this behavior via synaptic plasticity, because in a different situation that startle reflex could indeed be useful. Perhaps that is the reason that such important non-associative learning does not take place in the hippocampus, but elsewhere, (perhaps in the brainstem), sending the information to the neocortex to be processed. And that is why repeated presentation of a puff of air to the rat's ear will decrease the HSR behavior, conserving energy, however twenty-four hours later, the same situation is presented and the rat responds as if it had little prior experience with the stimulus.

However, a recent head-shake experiment in rats, has shown the latter to be inaccurate (Wright et al., in preparation). Sensitization-habituation hypothesis is easily seen in this type of experiment. The rat's response at the beginning of the session is higher, due to sensitization, than at the end of the session. Habituation takes place soon after the session begins with the animal reducing the number of head-shake response. However, in this experiment the rats were subjected to five sessions of twenty-four trials each. The more sessions the animals were exposed to, the less likely spontaneous recovery was to occur. When the inter-session-interval (ISI) was five minutes the animals revealed very little spontaneous recovery. Even when the ISI was twenty-four hours, spontaneous recovery was diminished. In other words, the more exposure to the stimulus the less likely the animal was to present spontaneous recovery on the next session. Some "savings" of habituation was transferred from one session to the next. According to Skinner (1950) the only way to achieve full extinction in the presence of the stimulation of starting an experiment is to start the experiment repeatedly. Meaning, the only way to extinguish spontaneous recovery, in a new session, is to restart the experiment many times. This would consequently end the novelty of the initialization of the session. Perhaps this was the reason for these results.

E. Proposed experiments

Consequently, the question of whether the hippocampus and MMPs are important for the CS-US association to occur during the HSR task was asked. Specifically, chapter two looked at habituation of the HSR, and the influence of a tone (CS) preceding the air-puff (US). Dorsal hippocampectomy was performed on a subset of animals in order to determine whether this structure was necessary for this association to occur. Chapter three consisted of two experiments: The first examined whether a general MMPi inhibitor infused into the dorsal

hippocampus had an effect on spontaneous recovery of the HSR when the CS preceded the US. The second experiment investigated the role of a more specific MMPi inhibitor, MMP-3i delivered into the dorsal hippocampus and its affect on spontaneous recovery of the HSR when a CS precedes a US.

F. References

Carlson, N. R., (2001). Physiology of Behavior (7th ed.). Needham Heights, MA: Allyn & Bacon.

Carew, T. J., Castellucci, V. F., & Kandel, E. R., (1971). An analysis of dishabituation and sensitization of the gill withdrawal reflex in Aplysia. *Int. J. Neurosc.* 2, 79-98.

Carew, T. J., Walters, E. T., & Kandel, E. R., (1981). Associative learning in Aplysia: cellular correlates supporting a conditioned fear hypothesis. *Science*. 211, (4481), 501-504.

Collingridge, G. L., Isaac J. T., & Wang Y. T., (2004). Receptor trafficking and synaptic plasticity. *Nature Reviews Neuroscience,* 5, 952-962.

Dityatev, A., Dityateva, G., Sytnyk, V., Delling, M., Toni, N., Nikonenko, I., Muller, D., & Schachner, M., (2004). Polysialylated Neural Cell Adhesion Molecule Promotes Remodeling and Formation of Hippocampal Synapses. *Journal of Neuroscience*, 24 (42), 9372-9382.

Eisenstein, E. M., Eisenstein, D., & Bonheim, P., (1991). Initial habituation or sensitization of the GSR depends on magnitude of first response. *Physiology and Behavior*, 49 (1), 211-5.

Eisenstein, E. M., Eisenstein, D., Bonheim, P., & Welch, E. A., (1990). Habituation of the galvanic skin response in adult males as a function of age. *Physiology and Behavior*, 48 (1), 169-73.

Fedulov, V., Rex, C. S., Simmons, D. A., Palmer, L., Gall, C. M., & Lynch, G., (2007). Evidence that long-term potentiation occurs within individual hippocampal synapses during learning. J Neuroscience 27(30): 8031-9.

Groves, P. M., & Thompson, R. F., (1970). Habituation: a dual-process theory. *Psychological Review*, 77 (5), 419-50.

Harris, J. D., (1943). Habituatory response decrement in the intact organism. *Psychological Bulletin*, 40, 385-422.

Hawkins, R. D., Clark, G. A., & Kandel, E. R., (2006). Operant conditioning of gill withdrawal in Aplysia. *The Journal of Neuroscience*. 26 (9), 2443–2448

Hoehler, F. K., & Thompson, R. F., (1980). Effect of the interstimulus (CS-UCS) interval on hippocampal unit activity during classical conditioning of the nictitating membrane response of the rabbit (Oryctolagus cuniculus). *J Comp Physiol Psychol*. 94 (2), 201-15.

Holland, P. C., (1997). Brain mechanisms for changes in processing of conditioned stimuli in Pavlovian conditioning: implications for behavior theory. *Anim. Learn. Behav*. 25, 373 – 399.

Kandel, E. R., (2001). The molecular biology of memory storage: a dialog between genes and synapses. *Bioscience Reports*, 21 (5), 565-611.

Kandel, E. R., Schwartz, J. H., & Jessel, T. M., (2000). Principles of neural science (4th ed.). New York, NY: McGraw-Hill.

Kelleher, R. J., Govindarajan, A., & Tonegawa, S., (2004). Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron*, 44, 59–73.

Kim, J. J., & Jung, M. W., (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. *Neurosci. And Biobeh. Reviews.* 30, 188 – 202.

Kosslyn, S. M., & Rosenberg, R. S., (2004). Psychology, the brain, the person the world (2nd ed.). Boston, MA: Allyn & Bacon.

Lee, T., & Kim, J. J., (2004). Differential effects of cerebellar, amygdalar and hippocampal lesions on classical eyeblink conditioning in rats. *J. Neurosci*. 24, 3242 – 3250.

Lee, J. L. C., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science,* 304, 839-843.

Lynch, M. A., (2004). Long-term potentiation and memory. *Physiological Reviews*, 84, 87-136.

Marcus, E. A., Nolen, T. G., Rankin, C. H., & Carew, T. J., (1998). Behavioral dissociation of dishabituation, sensitization, and inhibition in Aplysia. *Science*, 241 (4862), 210–213.

Meighan, S. E., Meighan, P. C., Choudhury, P., Davis, C. J., Olson, M. L., Zornes, P. A., Wright, J. W., & Harding, J. W., (2006). Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *Journal of Neurochemistry*, 96, 1227–1241.

Meighan PC, Meighan SE, Davis CJ, Wright JW, Harding JW. (2007). Effects of matrix metalloproteinase inhibition on short- and longterm plasticity of schaffer collateral/CA1 synapses. *Journal of Neurochemistry, 102,* 2085–2096.

McSweeney, F. K., Hinson, J. M., & Cannon, C. B., (1996). Sensitization-habituation may occur during operant conditioning. *Psychological Bulletin, 120,* 256-271.

McSweeney, F. K., & Murphy, E.S., (2000). Criticisms of the satiety hypothesis as an explanation for within-session decreases in responding. *Journal of the Experimental Analysis of Behavior, 74* (3), 347–361.

Murphy, E. S., Harding, J. W., Muhunthan, K., Holtfreter, K., & Wright, J. W. (2005). Role of mitogen-activated protein kinases during recovery from head-shake response habituation in rats. *Brain Research, 1050,* 170-179.

Myers, D. G., (2004). Psychology (7th ed.). New York, NY: Worth.

Novak, U., & Kaye, A. H., (2000). Extracellular matrix and the brain: components and function. *Journal of Clinical Neurosciensce, 7* (4), 280-290.

Ornitz, E. M., & Guthrie, D., (1989). Long-term habituation and sensitization of the acoustic startle response in the normal adult human. *Psychophysiology, 26* (2), 166-73.

Passer, M. W., & Smith, R. E., (2004). Psychology, the science of mind and behavior (3rd ed.). New York, NY: McGraw-Hill.

Power, A. E., Berlau, D. J., McGaugh, J. L., & Steward, O. (2006) Anisomycin infused into the hippocampus fails to block "reconsolidation" but impairs extinction: the role of re-exposure duration. *Learning and Memory*. 13, 27 – 34.

Skinner, B. F., (1950). Are theories of learning necessary? *Psychological Review*, 57 (4), 193-216.

Snyder, J. S., Hong, N. S., McDonald, R. J., & Wojtowicz, J. M., (2005). A role for adult neurogenesis in spatial long-term memory. *Neuroscience*, 130 (4), 843-52.

Staddon, J. E. R., (2001). The new behaviorism. Philadelphia, PA: Taylor & Francis.

Stupien, G., Florian, C., & Roullet, P. (2003). Involvement of the hippocampal CA3-region in acquisition and in memory consolidation of spatial but not in object information in mice. *Neurobio. of learning and memory*. 80, 32 – 41.

Swithers, S. E., & Hall, W. G., (1994). Does oral experience terminate ingestion? *Appetite*, 23 (2), 113-38.

Swithers-Mulvey, S. E., & Hall, W. G. (1993). Integration of oral habituation and gastric signals in decerebrate rat pups. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 265, 216 – 219.

Thompson, R. F., (1990). Neural mechanism of classical conditioning in mammals. *Philos. Trans. R. Soc. London Ser. B.* 329, 161 – 170.

Thompson, R. F, & Spencer, W. A., (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychological Review*, 73 (1), 16-43.

Thorpe, W. H., (1966). Learning and instinct in animals. Cambridge, MA: Harvard University Press.

Vianna, M. R. M., Alonso, M., Viola, H., Quevedo, J., Paris, F., Furman, M., Stein, M. L., Medina, J. H., & Izquierdo, I. (2000). Role of hippocampal signaling pathways in long-term memory formation of a nonassociative learning task in the rat. *Learning and memory*. 7, 333 – 340.

Weiten, W., (2004). Psychology, themes and variations (6th ed.). Belmont, CA: Wadsworth & Thomson.

Wright, J. W., & Harding, J. W., (2004a). The brain angiotensin system and extracellular matrix molecules in neural plasticity, learning, and memory. *Progress in Neurobiology*, 72, 263-293.

Wright, J.W., Reichert, J. R., Davis, C. J, Harding, J. W., (2002). Neural plasticity and the brain rennin-angiotensis system. *Neuroscience and Biobehavioral Reviews. 26, 529-552.*

Wright, J. W., Murphy, E. S., Elijah, I. E., Holtfreter, K. L., Davis, C. J., Olson, M. L., Muhunthan, K., & Harding, J. W., (2004b). Influence of hippocampectomy on habituation, exploratory behavior, and spatial memory in rats. *Brain Research*, 1023, 1-14.

Zaccardi, M. L., Traina, G., Cataldo, E., Brunelli, M., (2001). Nonassociative learning in the leech Hirudo medicinalis. *Behavioral Brain Research*, 126 (1-2), 81-92.

CHAPTER TWO

INFLUENCE OF DORSAL HIPPOCAMPUS LESIONS ON SPONTANEOUS RECOVERY FOLLOWING HABITUATION OF A HEAD-SHAKE/CLASSICAL CONDITIONING RESPONSE

Editor-in-Chief: Dr. Paul Gold
Neurobiology of Learning and Memory

Influence of dorsal hippocampus lesions on spontaneous recovery following habituation of a head-shake/classical conditioning response

Roberta V. Wiediger[a] and John W. Wright[b]*

[a]Department of Psychology, Lincoln Land Community College

Springfield, IL 62794

[b]Departments of Psychology, Veterinary and

Comparative Anatomy, Pharmacology and Physiology,

Washington State University, Pullman, WA 99164-4820 USA

Running head: Habituation and hippocampectomy

*Corresponding author: Roberta V. Wiediger

Lincoln Land Community College

5250 Shepherd Road

Springfield, IL 62794 USA

Tel: 1 217 786 2394

Fax: 1 217 786 2470

E-mail address: Beth.Wiediger@llcc.edu

Abstract

The present investigation combined a classical conditioning paradigm with a head-shake response (HSR) habituation task in intact and bilateral dorsal hippocampus lesioned rats. HSRs were elicited by applying a mild air stimulus to the ear. In the first experiment animals tested for HSR habituation revealed rapid habituation and an 85% level of spontaneous recovery following a 24-h inter-session-interval. The addition of a tone immediately prior to the air stimulus produced a similar pattern of habituation during the first session; however the level of spontaneous recovery was significantly reduced (44%) during the second session. Random presentation of the tone resulted in a spontaneous recovery level equivalent with the no tone group (87%). In a second experiment dorsal hippocampus lesioned rats placed on the tone/HSR paradigm responded at nearly the same rate during Sessions I and II, as did dorsal hippocampus lesioned rats that did not experience the tone. In contrast, neocortex lesioned rats placed on the tone/HSR paradigm displayed a significantly reduced level of spontaneous recovery (55.4%); while neocortex lesioned-no tone animals revealed a spontaneous recovery level of 83.8%. These results demonstrate the importance of the dorsal hippocampus in the formation of conditioned associations that can impact spontaneous recovery without changing the initial pattern of habituation. It appears that the dorsal hippocampus influences habituation by conserving responses and reducing spontaneous recovery during a second habituation session only if a temporally contingent signaling cue is present.

Keywords: Habituation; Spontaneous recovery; Head-shake response; Dorsal hippocampus lesions; Classical conditioning; Learning

1. Introduction

Habituation is considered a form of nonassociative learning and is characterized by a decrease in the strength of a response to a repeatedly presented stimulus (Harris, 1943; McSweeney & Murphy, 2008; Thompson & Spencer, 1966; Staddon, Chelaru, & Higa, 2002; Thorpe, 1966). This decrement in response strength cannot be attributed to sensory adaptation or motor fatigue, but is thought to involve neural plasticity within the central nervous system (Carew & Kandel, 1973). The hippocampus has been implicated in the control of inhibitory processes, particularly habituation (Douglas 1967; Jarrard & Bunnell, 1968; Kimble, 1968; Leaton, 1965, 1981; Oswald, Yee, Rawlins, Bannerman, Good, & Honey, 2002; Pribram, 1967; Roberts, Dember, & Brodwick, 1962). For example, patients with hippocampal damage show disrupted cortical response to novel stimuli (Yamaguchi & Knight 1991) and altered habituation (Yamaguchi, Hale, D'Esposito, & Knight, 2004). Several researchers have shown that the hippocampus also underlies successful associative learning, including classical and operant conditioning (reviewed in Suzuki, 2007; Thompson, 2005; Vianna, Alonso, Viola, Quevedo, Paris, Furman, Stein, Medina, & Izquierdo, 2000).

Our laboratory recently reported that hippocampectomized rats revealed severe impairment in spatial memory and habituation to objects in an open field; however they retained normal habituation to the head-shake response (HSR) task (Wright, Murphy, Elijah, Holtfreter, Davis, Olson, Muhunthan, & Harding, 2004). The HSR consists of a rapid rotation of the head about the anterior to posterior axis due to a mild air stimulus applied to the ear (Askew, Leibrecht, & Ratner, 1969). This response follows a decreasing negatively accelerated function of stimulus frequency, such that the higher the rate of stimulus presentation the faster the rate of habituation. Following habituation the HSR spontaneously recovers as a function of the inter-session-interval

(ISI), reaching approximately 85-90% of its original response strength following 24-h of rest (Murphy, Harding, Muhunthan, Holtfreter, & Wright, 2005). From these studies we concluded that the hippocampus is important regarding the interpretation and consolidation of spatial information required in open field, radial and Morris water maze tasks, but appears to be much less important to habitation of reflex behaviors such as the HSR, and perhaps startle response and lick suppression.

The present study further explored the role of the hippocampus in habituation by combining classical conditioning and HSR protocols. This was accomplished by presenting a short duration tone (CS) prior to the air stimulus (US) on each habituation trial. We predicted that the introduction of the tone would significantly reduce spontaneous recovery following a 24-h ISI when the hippocampus was intact. In contrast, dorsal hippocampus lesioned animals were expected to loose the ability to make use of the tone as an anticipatory associative learning signal resulting in maximum spontaneous recovery. This latter prediction assumed that a hippocampus-dependent association between the CS and US would be formed in intact animals; while hippocampus lesioned animals would be unable to form this association. We focused on the dorsal hippocampus (and the CA1 field) given recent indications that it is important in the temporal ordering of objects (Hoge & Kesner, 2007), spatial working memory (Dillon, Qu, Marcus, & Dodart, 2008), contextual fear conditioning (Chang, Chen, & Liang, 2008) and conditioned place preference (Meyers, Zavala, & Neusewander, 2003) in laboratory animals. Damage to the CA1 field also results in severe antegrade amnesia in human patients (Rempel-Clower, Zola, Squire, & Amaral, 1996).

2. Materials and methods

The protocols used in this investigation were approved by the Washington State University Institutional Animal Care and Use Committee and conformed to the guidelines for the care and use of laboratory animals as required by the American Psychological Association and the National Institutes of Health (NIH Publication #80-23).

2.1. Subjects

The first experiment utilized twenty-four male Sprague-Dawley rats, ranging in age from 3-4 months (350-450 g, breeding stock derived from Taconic, Germantown, NY) placed on a 12-h light/dark cycle initiated at 06:00-h, and were housed separately with food and water *ad libitum.* The second experiment used thirty-two Sprague-Dawley rats of the same gender and age as above, under the same housing conditions except that food was removed the night prior to surgery.

2.2. HSR habituation

The apparatus was patterned after that used by Leibrecht and Askew (1969) and consisted of an elevated platform measuring 7.5 x 16 cm. This platform was positioned on a 0.9 m tall wooden column that allowed the subject freedom of movement during testing. To discourage escape attempts the surface of the platform was covered with 1 cm square wire mesh cloth surrounded by plywood sloping down and outward thus forming a collar 17 x 24 cm at its extreme. The base of the wooden column pivoted 360° thus permitting the experimenter to compensate for movements by the subject and maintain a face-to-face orientation. The test room was painted black with ambient light set at the minimum level (5.7 Fc) necessary to score responses in order to reduce spatial cues. Head-shake responses were elicited by moving a hand held tube (orifice diameter = 0.5 mm) that provided a continuous stream of air across the center of the subject's left ear at an approximate rate of 3 cycles per s. The air tube was held 1-1.5 cm

from the animal's ear during the entire 15-s trial. The intensity of the air stream was set at 5-6 cm displacement of a 40 cm column of 95% ethanol in a 0.5 mm inside diameter U-shaped manometer. The standard habituation session consisted of a 5-min adaptation period on the test stand, followed by 24, 15-s stimulus presentations separated by a fixed 15-s inter-trial-interval (ITI). An IBM compatible computer signaled the intervals to the experimenter by displaying the 15-s trial in green numbers, and the 15-s ITI in red numbers. The experimenter recorded the number of HSRs following each trial. A tone (1-s duration @ 74 dB) was generated from speakers attached to the IBM compatible computer.

The rats were randomly assigned to one of three groups (n=8 each) and were tested over two habituation sessions separated by a 24-h ISI. Members of Group 1 served as a "no tone group", and received the standard HSR protocol. Members of Group 2, "tone group", received the same protocol as Group 1, however a tone (1-s in duration) was presented 2-s prior to the presentation of the air stimulus on each trial. Group 3, "random tone group", received the same protocol as Group 2; but the tone was randomly presented with an equal probability of occurring during either the 15-s HSR trial or during the 15-s ITI, i.e. "random contingency". In this way the presentation of the CS was non-contingent with the presentation of the US and the occurrence of the CS provided no information about the subsequent occurrence of the US. Thus, this procedure eliminated the CS-US contingency (Rescorla, 1967).

2.3. Surgery

Each member of the four groups (n=8 each) of the second experiment was anesthetized using Ketamine hydrochloride and Xylazine (100 and 2 mg/ml/kg, i.m., respectively; Phoenix Scientific, St. Joseph, MO, and Mobay, Shawnee, KS). Members of two of these groups received bilateral aspiration lesions of the dorsal hippocampus according to procedures described

by Isaacson and Woodruff (1976). Each rat was placed in a stereotaxic frame equipped with a nose clamp rather than ear bars (Model 900, David Kopf Instruments, Tujunga, CA). A previous investigation (Kramer & Wright, 1971) determined that insertion of ear bars resulted in hyper-responsiveness during subsequent HSR habituation trials. A midline incision was made thus exposing the dorsal cranium. Bone was removed between bregma and lambda, and the dura was retracted permitting tissue aspiration using a 21 g x 4 cm length blunt tip stainless steel hypodermic needle attached to a 1 ml syringe barrel inserted into Tygon tubing (ID= 7 mm, Norton, Akron, Ohio) under vacuum. Gelfoam (Upjohn, Kalamazoo, MI) soaked in sterile 0.15 M NaCl with vitamin K (20 mg/ml) added was used to pack the lesion sites. The incision was closed and the animal was placed onto a pre-warmed gel pack (Model 390P, BrainTree Scientific, BrainTree, MA) until fully conscious at which time it was transferred to its home cage. Members of the two remaining groups had the neocortex dorsal to the hippocampus removed by aspiration. Each animal received Buprenorphine hydrochloride analgesia (0.25 mg/kg, i.m.; Rechitt Benckiser Healthcare, Hull, UK) immediately following surgery. All rats were permitted 6 days of recovery prior to testing.

Members of Group 4, no tone hippocampectomized, were tested over two habituation sessions with an ISI of 24-h as described in the first experiment. Members of Group 5, tone hippocampectomized, were treated as described for Group 4 but with the contingent tone as described in the first experiment. Members of Group 6, no tone neocortex lesioned, experienced the same protocol as Group 4. Members of Group 7, tone neocortex lesioned, were treated the same as group 5.

2.4. Histology

Once behavioral testing was completed each member of Groups 4-7 was deeply anesthetized with Equithesin (pentobarbital: 100 mg/kg, i.p., Jensen-Salsbury Labs, Kansas City, MO) and transcardally perfused (0.15 M NaCl followed by 10% formalin). The brains were immediately removed and stored in a 30% sucrose in 10% formalin solution at 4° C for at least 10 days. Each brain was then placed on the stage of a freezing microtome (Spencer Lens, Buffalo, NY) and sectioned in the coronal plane at 40 μm. The sections were mounted on gelatin-coated slides and stained with Cresyl violet. Once the slides were cover-slipped they were viewed using an overhead slide projector (Model X-1000, Ken-A-Vision, Raytown, MO) thus permitting localization of the aspiration lesions in each hemisphere according to the rat brain atlas of Paxinos and Watson (1986).

2.5. Data analyses

The twenty-four trials were grouped into 8 blocks of 3 trials each, and the mean number of HSRs of each trial block was calculated for each animal. The data from Groups 1-3 were analyzed using a 3 (groups) X 8 (trial blocks) analysis of variance (ANOVA), with repeated measures on the second factor, for each of the two sessions. A one-way ANOVA was used to compare the groups on the first trial block of Session II. Significant effects were further analyzed using Newman-Keuls post-hoc tests with a level of significance set at $p < .05$. In addition, *t*-tests for repeated measures were used to analyze the first trial blocks of Sessions I and II for each group. Alpha levels were adjusted according to the Bonferonni technique. The data from Groups 4-7 were analyzed using a 4 (groups) x 8 (trial blocks) ANOVA, with repeated measures on the second factor. Additional analyses were as described above.

3. Results

3.1. HSR habituation and spontaneous recovery

Figure 1 presents the results of HSR habituation comparing the no tone, the tone, and the random tone groups of the first experiment. These groups indicated similar patterns of habituation over trials during Session I. The no tone group revealed a mean (SEM) response level of 6.5 (.5) HSRs on the first trial block of Session I, while 24-h later mean responding decreased to 5.5 (.6) HSRs during the first trial block of Session II, representing a spontaneous recovery level of 84.6%. The tone and random tone groups revealed mean response levels of 6.6 (.8) and 6.9 (.5) HSRs, respectively, on the first trial block of Session I, decreasing to 2.9 (.5) and 6.0 (.5) HSRs, respectively, during the first trial block of Session II, representing spontaneous recovery levels of 43.9 and 86.9%, respectively. Thus, the levels of spontaneous recovery were equivalent for the no tone and random tone groups, but greatly reduced for the contingent tone group.

The statistical analyses to support the above observations concerning Session I indicated a significant effect for trial blocks ($F_{7,147} = 133.52$; $p<.0001$), revealing an expected within-session decrease in responding during the session (i.e. habituation). No groups x trials interaction was found ($F_{14,147} = 1.10$; $p>.10$), and no groups effect ($F_{2,21} = .16$; $p>.10$), suggesting that the overall levels of HSRs during the session were similar across groups. Analyses of Session II data revealed a significant effect for trial blocks ($F_{7,147} = 97.51$; $p<.0001$), suggesting an expected within-session decrease in responding over sessions. A significant interaction was found ($F_{14,147} = 9.00$; $p<.0001$), but no groups effect ($F_{2,21} = .56$; $p>.10$). The significant interaction indicated that the within-session changes in responding varied across the groups. A one-way ANOVA comparing these three groups on the first trial block of Session II was significant ($F_{2,21} = 12.16$; $p<.0001$). Post-hoc tests revealed that the contingent tone group's level of spontaneous recovery was less than the other groups; while the other two groups did not

differ. Finally, *t*-tests for repeated measures indicated that the number of HSRs emitted during the first trail block of Session II was significantly less than that of Session I for the contingent tone group ($t_7 = 6.09$; $p<.001$). The other groups did not differ on this measure.

3.2. Histological results

Figure 2 presents hippocampus lesions in the animals of Groups 4 and 5 extended from -2.3 to -6.3 mm posterior to bregma and impacted the CA1 field and dentate gyrus (Figure 2A). The dorsal hippocampus was spared at its anterior extreme (-1.8 to -2.3 mm) and at medial and lateral extremes from -2.6 to -6.3 posterior to bregma. There was evidence of slight damage to laterodorsal thalamic nuclei in 5 rats (2 in the no tone hippocampus lesioned group and 3 in the tone hippocampus lesioned group). In addition to the dorsal hippocampal damage the corpus callosum, subcortical white matter, and neocortex overlying the hippocampus were extensively damaged in all rats. The lesions in all animals approximated bilateral symmetry. Damage to members of the neocortex lesioned groups (Groups 6 and 7) extended from -2.3 to -6.3 mm posterior to bregma (Figure 2B) and impacted the corpus callosum at its dorsal extreme.

3.3. Influence of dorsal hippocampus lesions on HSRs and spontaneous recovery

Figure 3 presents the behavioral results comparing dorsal hippocampus and neocortex lesioned groups. The no tone hippocampus lesioned group indicted a mean of 8.3 HSRs during the first trial block of Session I, decreasing to 7.1 HSRs on the first trial block of Session II, representing a spontaneous recovery level of 85.7%. The tone hippocampus lesioned group revealed mean responding of 9.1 (.9) HSRs during the first trial block of Session I; 24-h later responding decreased to 7.9 (.9) HSRs on the initial trial block of Session II. This represented a spontaneous recovery level of 86.8%. The no tone neocortex lesioned group evidenced 8.17 (0.4) and 6.85 (0.3) HSRs on the first trial blocks of Sessions I and II, respectively, representing

a spontaneous recovery of 83.8%. In contrast, the tone neocortex lesioned group displayed a mean of 8.3 (.8) HSRs during the first trial block of Session I, decreasing to 4.6 (.9) HSRs on the initial trial block of Session II, representing a spontaneous recovery level of 55.4%. Thus, members of the tone neocortex lesioned group utilized the tone as a cue to the onset of the air stimulus and reduced responding at the initiation of Session II. On the other hand, members of the tone hippocampus lesioned group failed to make use of the signaling value of the tone. These results suggest that an intact dorsal hippocampus is necessary in order for the tone to be utilized to reduce responding during Session II.

The statistical analysis to support these conclusions concerning Session I indicated a significant effect of trial blocks ($F_{7,196} = 240.53$; $p<.001$), suggesting a within-session decrease in responding during session I. No groups x trials interaction ($F_{21,196} = 1.50$; $p>.10$), or groups effect ($F_{3,28} = .67$; $p>.10$), were found. Statistical analysis of Session II suggested a significant effect of trial blocks ($F_{7,196} = 179.34$; $p<.001$), indicating a within-session decrease in responding during the session. A significant interaction of groups x trials was also found ($F_{21,196} = 3.29$; $p<.001$), but no groups effect ($F_{3,28} = 2.67$; $p>.05$). The significant interaction suggested that the within-session changes in responding varied across the groups. A one-way ANOVA comparing the groups on the first trial block was significant ($F_{3,28} = 9.47$; $p<.001$). Finally, *t*-tests for repeated measures indicted that the number of HSRs emitted by the tone neocortex lesioned group during the first trial block of Session II was less than that measured in Session I ($t_7 = 6.85$; $p<.001$). The other groups did not differ on this measure.

4. Discussion

These patterns of HSR habituation and spontaneous recovery are consistent with previous reports in that a decreasing negatively accelerated level of responding was present accompanied

by substantial spontaneous recovery following a 24-h ISI (Murphey, Harding, Muhunthan, Holtfreter, & Wright, 2005; Wright, Meighan, Murphy, Holtfreter, Davis, Olson, Benoist, Muhunthan, & Harding, 2006). The hippocampus has been implicated in the control of inhibitory processes including habituation (Douglas, 1967; Jarrard & Bunnell, 1968; Kimble, 1968; Leaton, 1965; Primbram, 1967; Roberts, Dember, & Brodwick, 1962), fear extinction and spontaneous recovery (Ji & Maren, 2007), and in the formation and utilization of spatial memory (Bures, Fenton, Kaminsky, & Zinyuk, 1997; McNaughton, Barnes, Meltzer, & Sutherland, 1989; Morris, Garrud, Rawlins, & O'Keefe, 1982; Nadel, 1991; O'Keefe & Nadel 1978; Wishaw, 1987). Thus, hippocampal damage has been shown to result in an impaired ability to solve tasks that rely on spatial search strategies in several mammalian species including rat (Goodrich-Hunsaker, Hunsaker, & Kesner, 2008; Jarrard, 1993; Morris, Garrud, Rawlins, & O'Keefe, 1982; Olton, Walker, & Gage, 1978; Sutherland & McDonald, 1990), mouse (Dillon, Qu, Marcus, & Dodart, 2008) and human (Cummings, Tomiyasu, Read, & Benson, 1984; Volpe & Hirst, 1983; Zola-Morgan, Squire, & Amaral, 1986).

Investigations concerned with the influence of brain lesions on HSR determined that frontal cortex aspiration lesions failed to alter the habituation of this response (Kramer & Wright, 1971); as did bilateral ibotenic-acid-induced lesions of the nucleus basalis magnocellularis (Dokla, Parker, & Thal, 1990). Recently, suprachiasmatic nucleus lesions were reported to reduce spontaneous recovery of the HSR following a 24-h ISI, suggesting that "clock genes" located in this nucleus may be involved in resetting responsiveness (Holtfreter, Murphy, Harding, & Wright, 2008). Most relevant to the present findings, reasonably large bilateral hippocampus lesions failed to alter the pattern of HSR habituation and spontaneous recovery (Wright, Murphy, Elijah, Holtfreter, Davis, Olson, Muhunthan, & Harding, 2004). However, the relative

importance of the dorsal hippocampus with regard to signaling cues during habituation had not been previously investigated.

The present study combined a classical conditioning paradigm with HSR habituation in order to examine the influence of the dorsal hippocampus on spontaneous recovery. We predicted that the presentation of a short duration signaling cue prior to the air stimulus to the ear would permit an association to form and be stored in the dorsal hippocampus. The consolidation of this association was expected to reduce the strength of responding, and thus spontaneous recovery, during a second HSR session. We further hypothesized that bilateral dorsal hippocampus lesions would prevent such an association from forming, and maximum spontaneous recovery would occur during the second session despite the contingent presentation of the tone. In other words, the signaling value of the tone would be lost.

The results of the first experiment demonstrated a decreasing negatively accelerated response pattern over trials asymptoting by about the fifth trial block (Figure 1). Following a 24-h ISI the first trial block of Session II compared with that of Session I, indicated a spontaneous recovery level of 85% by the HSR group. The addition of a randomly applied tone failed to change this pattern. A contingent tone presented prior to the onset of the air stimulus on each trial of Session I did not alter the pattern of habituation, however, following a 24-h ISI the response strength was reduced resulting in a 44% level of spontaneous recovery (Figure 1). Thus, the addition of a signaling tone that alerted the animal to the impending air stimulus significantly reduced the number of HSRs during the first trial block of Session II. This suggests that the CS-US association formed during Session I influenced spontaneous recovery of the HSR such that 24-h later these animals responded less when compared with the no tone HSR rats. In contrast, non-contingent presentation of the tone did not affect spontaneous recovery. These results establish

that a CS-US association can be formed during HSR habituation and that this paradigm significantly influenced the subsequent level of spontaneous recovery.

The no tone and tone dorsal hippocampus lesioned groups of the second experiment revealed robust spontaneous recovery following a 24-h ISI (85.7 and 86.8% respectively, Figure 3). The tone neocortex lesioned group showed a similar level of spontaneous recovery as the tone group of the first experiment (55 and 44%, respectively); although the neocortex lesioned animals evidenced some heightened responsiveness. The no tone neocortex lesioned group indicated significant spontaneous recovery during Session II (83.8%), equivalent with the no tone and tone dorsal hippocampus lesioned groups. Thus, when the tone was presented prior to the onset of the air stimulus to dorsal hippocampus damaged animals maximum spontaneous recovery was present following the 24-h ISI. In contrast, when the hippocampus was intact the presentation of the tone prior to the onset of the air stimulus resulted in a significant decrease in spontaneous recovery. These results suggest that the formation of the association during Session I was hippocampal-dependent and illustrate the importance of monitoring and controlling potential environmental cues that could be associated with the eliciting stimulus during habituation trials. These findings further suggest that the addition of a contingent cue to a habituation paradigm involving reflex-like responses engages the dorsal hippocampus.

In summary, this investigation is the first to examine the role of the dorsal hippocampus in the association formed between a signaling cue and the stimulus to induce HSR habituation. The results indicate an important role for the dorsal hippocampus when this combined paradigm includes a contingently placed tone. Under this condition intact animals conserved responses, however dorsal hippocampus damaged animals were unable to make use of this cue forcing a less efficient response pattern with more HSRs emitted than necessary to cope with the

repeatedly applied air stimulus. Taken together these findings suggest a modified hypothesis concerning the role of the hippocampus in habituatory processes. Specifically, the hippocampus may be essential to the associations formed during classical conditioning (Suzuki, 2007; Thompson, 2005; Vianna, Alonso, Viola, Quevedo, Paris, Furman, Stein, Medina, & Izquierdo, 2000) and during the interpretation of spatial information as required in open-field, eight arm radial maze, and Morris water maze tasks. However, the hippocampus may not be as important regarding the habituation of reflex-like behaviors such as lick suppression, startle response, and HSR unless contingent cues are present. Thus, the neural mechanism(s) underlying the habituation that occurs with reflex-like behaviors, and the habituation that occurs in the presence of a signaling cue, appear to be mediated by different anatomical/neurochemical systems.

Acknowledgements

The authors thank Dr. Robert A. Rescorla for valuable advice concerning the random contingency placement of the tone during the training of the "random tone group", Dr. Eric S. Murphy for helpful comments during the writing of this manuscript, and Mr. Mathew D. Wiediger for assisting with computer programs. This study was supported by a Laing Endowment for Alzheimer's disease grant to J.W.W., and funds provided by Washington State University.

References

Askew, H. R., Leibrecht, B. C., & Ratner, S. C. (1969). Effects of stimulus duration and repeated sessions on habituation of the head-shake response in the rat. *Journal of Comparative & Physiological Psychology, 67,* 497–503.

Bures, J., Fenton, A. D., Kaminsky, Y., & Zinyuk, L. (1997). Place cells and place navigation. *Proceedings National Academy of Sciences USA, 94,* 343–350.

Carew, T. J., & Kandel, E. R. (1973). Acquisition and retention of long-term habituation in Aplysia. *Science, 182,* 1158-1160.

Chang, S. D., Chen, D. Y., & Liang, K. C. (2008). Infusion of lidocaine into the dorsal hippocampus before or after the sock training phase impaired conditioned freezing in a two-phase training task of contextual fear conditioning. *Neurobiology of Learning and Memory, 89,* 95-105.

Cummings, J. L., Tomiyasu, U., Read, S., & Benson, D. F. (1984). Amnesia with hippocampal lesions after cardiopulmonary arrest. *Neurology, 34,* 679– 681.

Dillon, G. M., Qu, X., Marcus, J. N., & Dodart, J. C. (2008). Excitotixic lesions restricted to the dorsal CA1 field of the hippocampus impair spatial memory and extinction learning in C57BL/6 mice. *Neurobiology of Learning and Memory, 90,* 426-433.

Dokla, C. P., Parker, S. C., & Thal, L. J. (1990). Habituation and retention of the head-shake response: lack of impairment by nucleus basalis magnocellularis lesions. *Pharmacology Biochemical & Behavior, 35,* 151– 155.

Douglas, R. J. (1967). The hippocampus and behavior. *Psychological Bulletin, 67,* 416 – 422.

Goodrich-Hunsaker, N. J., Hunsaker, M. R., & Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience, 122*(1), 16-26.

Harris, J. D. (1943). Habituatory response decrement in the intact organism. *Psychological Bulletin, 40,* 385-422.

Hoge, J. & Kesner, R. P. (2007). Role of CA3 and CA1 subregions of the dorsal hippocampus on temporal processing of objects. *Neurobiology of Learning and Memory, 88,* 225-231.

Holtfreter, K.L., Murphy, E.S., Harding, J.W., & Wright, J.W. (2008). Effects of suprachiasmatic nucleus lesions on habituation of the head-shake response. *Neuroscience Letters 439,* 203-207.

Isaacson, R. L., & Woodruff, M. L. (1976). Spontaneous alternation and passive avoidance in rats after hippocampal lesions. In: B. L. Hart (Ed.), *Experimental Psychobiology, A Laboratory Manual* (pp. 103-109). New York, NY: Freeman.

Jarrard, L. E. (1993). On the role of the hippocampus in learning and memory in the rat. *Behavioral & Neural Biology, 60,* 9– 26.

Jarrard, L. E., & Bunnell, B. N. (1968). Open-field behavior of hippocampal lesioned rats and hamster. *Journal of Comparative & Physiological Psychology, 66,* 500-502.

Ji, J., & Maren, S. (2007). Hippocampal involvement in contextual modulation of fear extinction. *Hippocampus, 17,* 749-758.

Kimble, D. P. (1968). Hippocampus and internal inhibition. *Psychological Bulletin, 70,* 285 – 295.

Kramer, T. J., & Wright, J. W. (1971). Effects of bilateral frontal lesions on habituation of the head-sake response in the rat. *Physiology & Behavior, 7,* 211 – 214.

Leaton, R. N. (1965). Exploratory behavior in rats with hippocampal lesions. *Journal of Comparative & Physiological Psychology, 59*, 325–330.

Leaton, R. N. (1981). Habituation of startle response, lick suppression, and exploratory behavior in rats with hippocampal lesions. *Journal of Comparative & Physiological Psychology, 95*, 813– 826.

Leibrecht, B. C., & Askew, H. R. (1969). Habituation of the head-shake response in the rat: recovery, transfer, and changes in topography. *Journal of Comparative & Physiological Psychology, 69*, 699 – 708.

McNaughton, B. L., Barnes, C. A., Meltzer, J., & Sutherland, R. J. (1989). Hippocampal granule cells are necessary for normal spatial learning but not for spatially- selective pyramidal cell discharge. *Experimental Brain Research, 76*, 485–496.

McSweeney, F.K., and Murphy, E.S. (2008). Sensitization and habituation regulate reinforce effectiveness. *Neurobiology of Learning and Memory*, Aug 18. [Epub ahead of print].

Meyers, R.A., Zavala, A.R. & Neusewander, J.L. (2003). Dorsal, but not ventral, hippocampal lesions disrupt cocaine place conditioning. *Learning & Memory, 14*, 2127-2131.

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

Murphy, E. S., Harding, J. W., Muhunthan, K., Holtfreter, K., & Wright, J. W. (2005). Role of mitogen-activated protein kinases during recovery from head-shake response habituation in rats. *Brain Research, 1050*, 170-179.

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

O'Keefe, J., & Nadel, L. (1978). *The Hippocampus as a Cognitive Map* (pp. 265-290). Oxford, UK: Oxford University Press.

Olton, D. S., Walker, J. A., & Gage, F. H. (1978). Hippocampal connections and spatial discrimination. *Brain Research, 139,* 295–308.

Oswald, C.J.P., Yee, B.K., Rawlins, M.N.P., Bannerman, D.B., Good, M. & Honey, R.C. (2002). The influence of selective lesions to components of the hippocampal system on the orientating response, habituation and latent inhibition. *European Journal of Neuroscience, 15,* 1983-1990.

Paxinos, G., & Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates* (2nd ed.). New York: Academic Press.

Pribram, K. H. (1967). The limbic systems efferent control of neural inhibition and behavior. *Progress in Brain Research, 27,* 318 – 336.

Rempel-Clower, N. L., Zola, S. M., Squire, L. R. & Amaral, D. G. (1996). Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. *Journal of Neuroscience, 16*(16), 5233-5255.

Rescorla, R. A. (1967). Pavlovian conditioning and its proper control procedures. *Psychological Review, 74,* 71-80.

Roberts, W. W., Dember, W. N., & Brodwick, M. (1962). Alternation and exploration in rats with hippocampal lesions. *Journal of Comparative & Physiological Psychology, 55,* 695–700.

Staddon, J.E.R., Chelaru, I.M., & Higa, J.J. (2002). Habituation, memory and the brain: The dynamics of interval timing. *Behavioural Processes 57,* 71-88.

Sutherland, R. J., & McDonald, R. J. (1990). Hippocampus, amygdala and memory deficits in rats. *Behavioural Brain Research, 37,* 57– 79.

Suzuki, W.A. (2007). Integrating associative learning signals across the brain. *Hippocampus, 17,* 842-850.

Thompson, R.F. (2005). In search of memory traces. *Annual Reviews of Psychology, 56,* 1-23.

Thompson, R.F., & Spencer, W.A. (1966). Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review, 73,* 16-43.

Thorpe, W. H. (1966). *Learning and instinct in animals.* Cambridge, MA: Harvard University Press.

Vianna, M. R. M., Alonso, M., Viola, H., Quevedo, J., Paris, F., Furman, M., Stein, M. L., Medina, J. H., & Izquierdo, I. (2000). Role of hippocampal signaling pathways in long-term memory formation of a nonassociative learning task in the rat. *Learning and Memory, 7,* 333 – 340.

Volpe, B. T., & Hirst, W. (1983). The characterization of an amnesic syndrome following hypoxic ischemic injury. *Archives of Neurology, 40,* 436–440.

Whishaw, I. Q. (1987). Hippocampal granule cell and CA3-4 lesions impair formation of a place learning-set in the rat and induce reflex epilepsy. *Behavioural Brain Research, 24,* 59–72.

Wright, J. W., Meighan, S. E., Murphy, E. S., Holtfreter, K. L., Davis, C. J., Olson, M. L., Benoist, C. C., Muhunthan, K., Harding, J. W. (2006). Habituation of the head-shake response induces changes in brain matrix metalloproteinases-3 (MMP-3) and -9. *Behavioural Brain Research, 174,* 78-85.

Wright, J. W., Murphy, E. S., Elijah, I. E., Holtfreter, K. L., Davis, C. J., Olson, M. L., Muhunthan, K., & Harding, J. W. (2004). Influence of hippocampectomy on habituation, exploratory behavior, and spatial memory in rats. *Brain Research, 1023,* 1-14.

Yamaguchi, S., Hale, L.A., D'Esposito M., & Knight R.T. (2004). Rapid prefrontal-hippocampal habituation to novel events. *Journal of Neuroscience, 24*, 5356-5363.

Yamaguchi, S., Knight, R.T. (1991). Anterior and posterior association cortex contributions to the somatosensory P300. *Journal of Neuroscience, 11*, 2039-2054.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial–temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus, *Journal of Neuroscience, 6*, 2950– 2967.

Figure Captions

Figure 1. Mean (± SEM) group changes in number of head-shake responses per 3-trial blocks during Sessions I and II of habituation trials separated by a 24-h ISI. There were no differences among the groups concerning the pattern of habituation during Session I. There were differences among the groups during the first trial block of Session II with the contingent tone group revealing a significantly reduced level of spontaneous recovery as compared with the other two groups which did not differ. *p<.001.

Figure 2. Schematic reconstruction of the smallest (solid black region) and largest (solid black + hatched regions) aspiration lesions within the dorsal hippocampus (A) and neocortex (B) for members of the groups utilized in Experiment 2. Coordinates for these coronal sections are indicated in mm posterior to bregma with reference to the stereotaxic brain atlas of Paxinos and Watson (1986).

Figure 3. Mean (± SEM) number of head-shake responses per 3-trial blocks for dorsal hippocampectomized (HippX) and neocortex (NeoX) lesioned rats during Sessions I and II of habituation trials separated by a 24-h ISI. These groups did not differ regarding pattern of habituation during Session I; however, members of the tone neocortex lesioned group revealed significantly attenuated levels of spontaneous recovery during the first trial block of Session II. *p<.001.

Figure 1

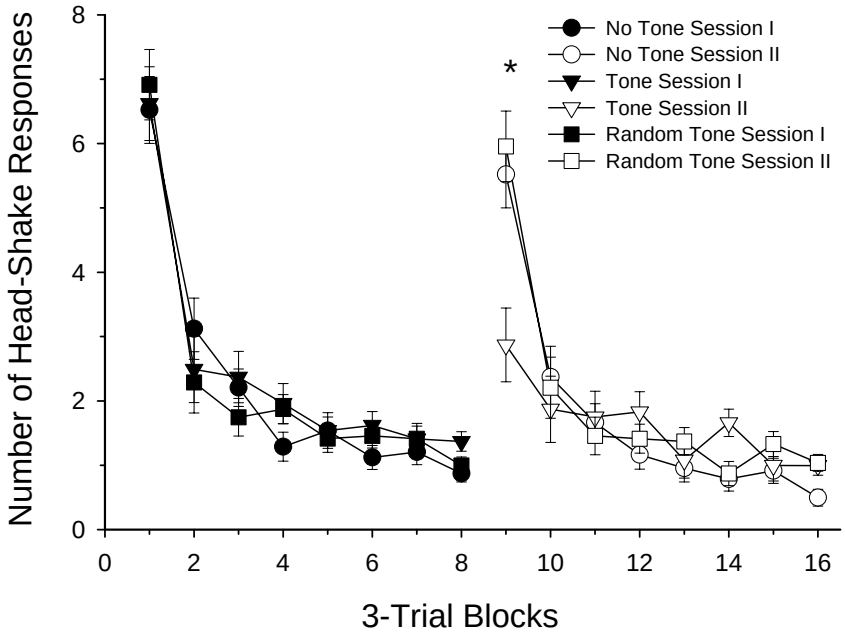


Figure 2

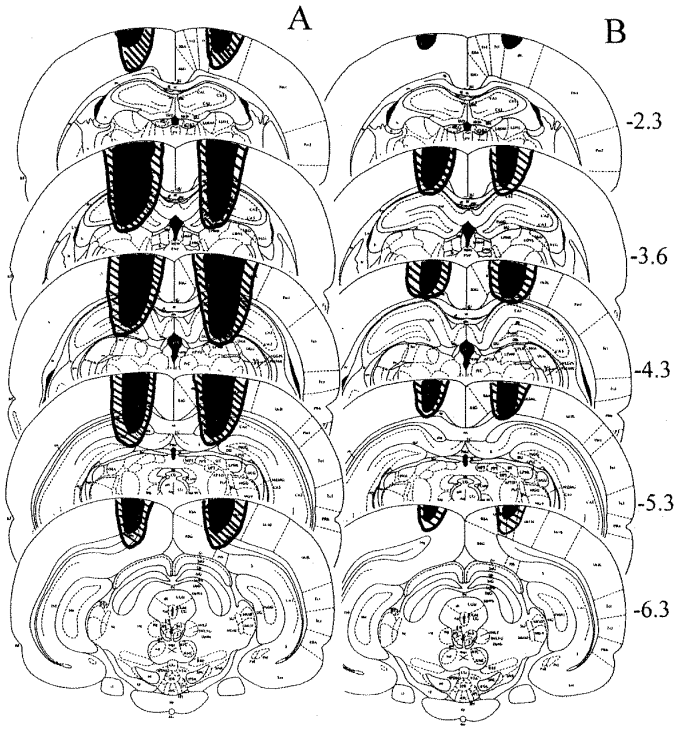
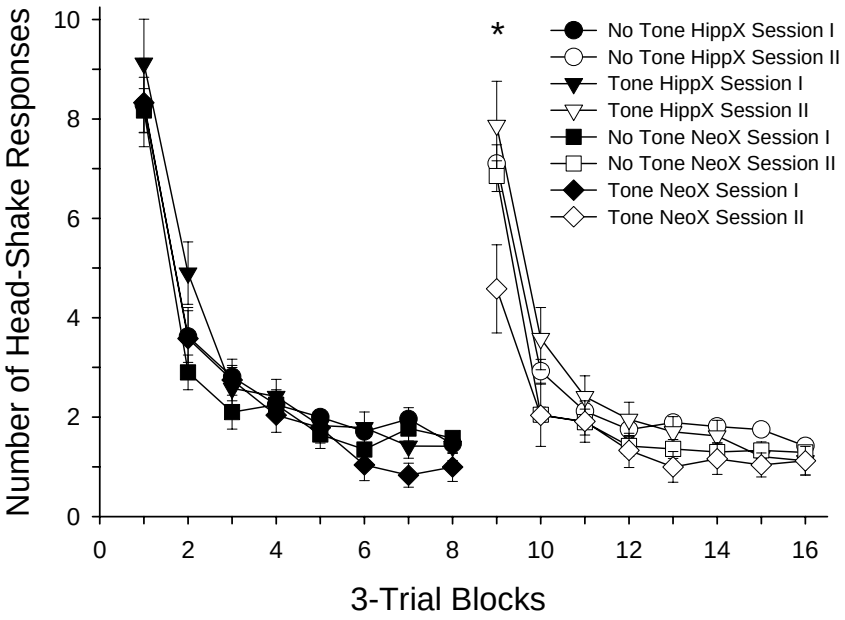


Figure 3


Number of Head-Shake Responses
3-Trial Blocks
*
No Tone HippX Session I
No Tone HippX Session II
Tone HippX Session I
Tone HippX Session II
No Tone NeoX Session I
No Tone NeoX Session II
Tone NeoX Session I
Tone NeoX Session II
0
2
4
6
8
10
12
14
16

CHAPTER 03

THE ROLE OF MATRIX METALLOPROTEINASES ON HABITUATION DURING A HEAD SHAKE RESPONSE TASK

Editor-in-Chief: Dr. F. E. Bloom
Brain Research

The role of matrix metalloproteinases on habituation during a head shake response task

Roberta V. Wiediger[a*] and John W. Wright[b,c,d]

[a]Department of Psychology, Lincoln Land Community College,

Springfield, IL 62794

[b]Department of Psychology, Washington State University, Pullman, WA 99164-4820

[c]Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology,

Washington State University, Pullman, WA 99164-6520

[d]Program in Neuroscience, Washington State University, Pullman, WA 99164-6520

Running head: Head-shake response habituation

*Corresponding author: Roberta V. Wiediger

Lincoln Land Community College

5250 Shepherd Road

Springfield, IL 62794 USA

Tel: 1 217 786 2394

Fax: 1 217 786 2470

E-mail address: Beth.Wiediger@llcc.edu

Abstract

Matrix Metalloproteinases (MMPs) have been implicated as markers of neural plasticity. The present investigation employed a head-shake response (HSR)/classical conditioning paradigm as the habituated response in rats. A tone served as the conditioned stimulus (CS) and an stream of air to the ear as the unconditioned stimulus (US). The bilateral injection of a general MMP inhibitor, FN-439, into the dorsal hippocampus resulted in maximal spontaneous recovery during the second habituation session conducted 24-h following the first session. The control group was injected with artificial cerebrospinal fluid and showed a significant attenuation of spontaneous recovery during the second session. Members of a separate group of rats were injected with a specific MMP-3 inhibitor into the dorsal hippocampus. The results were similar to those animals treated with FN-439. These findings indicate that during this HSR/classical conditioning task when animals were given an MMP inhibitor no association between the CS-US was formed in the dorsal hippocampus, therefore maximum spontaneous recovery was present 24h later. In contrast, when the dorsal hippocampus was functioning this association was formed and spontaneous recovery was reduced thus conserving the number of responses emitted. This experiment suggests that activated MMP-3 is an important enzyme in the formation and consolidation of this association in the hippocampus.

Key words: Habituation; Spontaneous recovery; Head-shake response; Classical conditioning; Matrix metalloproteinases; FN-439; MMP-3 inhibitor; Hippocampus; Learning

1. Introduction

Habituation is characterized by a decrease in the strength of a response to a repeatedly presented stimulus (Harris, 1943; Thompson and Spencer, 1966; Staddon, et al., 2002; Thorpe, 1966) that is not related to sensory adaptation or motor fatigue (McSweeney et al., 2005). This phenomenon is a well documented form of learning (Mackintosh, 1987) that occurs across many species for a number of response systems ranging from the gill-withdrawal reflex in *Aplysia* (Castellucci and Kandel, 1974), tap withdrawal or chemotaxic response in the nematode *Caenorhabditis elegans* (Bernhard and van der Kooy, 200; Rose and Rankin, 2001), to acoustic startle response in rats and mice (Masini et al., 2008; Plappert and Pilz, 2005; Stevenson and Gratton, 2004), and feeding in humans (Ernst and Epstein, 2002). The present investigation focused on the head-shake response (HSR) which consists of a rapid rotation of the head about the anterior to posterior axis due to a mild air stimulus applied to the ear (Askew et al., 1969). This response follows a decreasing negatively accelerating function of stimulus frequency, such that the higher the rate of stimulus presentation the faster the rate of habituation (Murphy et al., 2005). Following habituation the HSR spontaneously recovers as a function of the inter-session-interval (ISI), reaching approximately 85-90% of its original response strength following 24-h of rest.

The hippocampus has been linked to learning and memory storage which appears to be dependent upon synaptic plasticity (Collingridge et al., 2004). Thus, memory consolidation requires synaptic reconfiguration which in turn is dependent upon changes in extracellular matrix (ECM) molecules mediated by matrix metalloproteinases (MMPs; Wright et al., 2002). ECM molecules are composed of proteins involved in cellular development, migration, connectivity and reorganization (Novak and Kaye, 2000). The temporary dissociation of ECM molecules by

MMPs permits the reconfiguration of the brain's synaptic architecture thought to be critical to the processes of neural plasticity, learning and memory (Dityatev and Schachner, 2003). We have recently shown that the hippocampus is involved in the HSR task when a tone (1-s in duration) is presented 2-s prior to the air stimulus (Wiediger and Wright, submitted). Dorsal hippocampectomized rats exposed to the tone revealed high levels of spontaneous recovery after a 24-h ISI; however animals with an intact hippocampus revealed attenuated levels of spontaneous recovery, suggesting that the hippocampus is important during the formation of this CS-US pairing and promotes conservation of responding.

The present study examined the role of MMPs in the dorsal hippocampus during habituation utilizing this combined classical conditioning/HSR habituation protocol. We reasoned that if dorsal hippocampal MMPs are critical to reconfiguration of ECM molecules during learning, then animals treated with an MMP inhibitor (MMPi) should loose the ability to make use of the tone as an anticipatory associative cue. This would result in maximum spontaneous recovery during a second habituation session following a 24-h ISI. In contrast, animals injected with vehicle should make use of this cue and attenuation in spontaneous recovery would be anticipated during the second session. We initially tested this hypothesis with a general MMPi, FN-439, and then with a specific MMP-3i. MMP-3 has been shown to be important to both spatial and associative learning (Wright et a., 2006; Olson et al., 2008; Brown et al., 2007; Meighan et al., 2006; Wright et al., 2004).

2. Results

2.1 Hippocampal infusion of FN-439

Figure 1 presents the results of HSR habituation comparing the high and low dose FN-439 groups, and the aCSF group. Similar patterns of habituation over trials were seen among

these groups during Session I. The group that received the high dose of FN-439 revealed a mean (SEM) response level of 7.6 (.5) HSRs on the first trial block of Session I. The low dose FN-439 and aCSF groups indicated mean response levels of 6.2 (.9) and 6.5 (.5) HSRs, respectively. During the first trial block of Session II the high and low dose FN-439 groups and the aCSF group indicated mean HSR levels of 6.1 (.4), 2.4 (.8) and 2.9 (.4), respectively, representing spontaneous recovery levels of 81.1, 39.2 and 44.5%, respectively. Thus, the levels of spontaneous recovery were attenuated and equivalent for the low dose FN-439 and aCSF groups as compared with the high dose FN-439 group.

The statistical analyses to support the above observations concerning Session I indicated a significant effect for trial blocks, $F(7,112)= 144.32$, $p<.0001$, confirming an expected within-session decrease in responding during the session. No group or interaction of groups x trials was found, $F(2,16)= 2.56$, $p>.10$ and $F(14,112)=1.34$, $p>.10$, respectively, suggesting that the overall levels of HSRs during the session were similar across groups. Statistical analyses of Session II data revealed a significant effect for trial blocks, $F(7,112)=75.44$, $p<.0001$, suggesting an expected within-session decrease in responding over trials. Significant group and interaction effects were also found, $F(2,16)=5.32$ and $F(14,112)=12.33$, $p<.0001$, respectively. The significant interaction suggested that the within-session changes in responding varied across the groups. The significant group effect suggested that the groups differed in responding during session II. A one-way ANOVA comparing these three groups on the first trial block of Session II was significant, $F(2,18)=16.937$, $p<.0001$. Post-hoc tests indicated that the low dose FN-439 and the aCSF groups were each different from the high dose FN-439 group, but the two former groups did not differ. Repeated measures t-test confirmed that the low dose FN-439 and aCSF groups revealed significant decreases in HSRs comparing the first trial blocks of Session I and II,

$t(7)=4.33$ and 9.00 respectively, $p<.01$. The high dose FN-439 group did not differ, $t(7)=1.69$, $p>.05$.

2.2 Hippocampal infusion of MMP-3 inhibitor

Figure 2 presents the results of HSR habituation comparing the MMP-3i treated group and the aCSF control group. Similar patterns of habituation over trials were seen during Session I. The group that received the MMP-3i revealed mean responding of 6.8 (.3) HSRs during the first trial block of Session I. The aCSF group displayed mean responding of 6.9 (.3) HSRs. During the first trial block of Session II the MMP-3i and the aCSF groups indicated mean HSR levels of 5.3 (.2) and 2.8 (.2), respectively, representing spontaneous recovery levels of 77.9 and 40.6%, respectively Thus, the level of spontaneous recovery was low for the aCSF group, but remained high for the MMP-3i group.

The statistical analysis to support the above observations concerning Session I indicated a significant effect of trial blocks, $F(7,98)=175.84$, $p<.001$, suggesting a within-session decrease in responding during session I. No interaction of groups x trials, $F(7,98)=.41$, $p>.10$, and no groups effect, $F(1,14)=.09$, $p>.10$, were found. Statistical analysis of Session II indicated a significant effect of trial blocks, $F(7,98)=134.73$, $p<.001$, indicating a within-session decrease in responding. A significant interaction of groups x trials was found, $F(7,98)=23.89$, $p<.0001$, but no groups effect, $F(1,14)=1.13$, $p>.05$. The significant interaction suggested that the within-session changes in responding varied across the groups. An independent t-test indicated that the aCSF group's level of spontaneous recovery during the first trial block of Session II was significantly less than that of the MMP-3i group, $t(14)=6.609$, $p<.0001$. Repeated measures t-test confirmed that the aCSF group emitted fewer HSRs during the first trial block of Session II

as compared with Session I, $t(7)=8.99$, $p<.001$; while the MMP-3i group did not differ, $t(7)=2.08$, $p>.05$.

3. Discussion

MMPs have been shown to be critical to the maintenance and reconstruction of the ECM (Wright et al., 2004), suggesting that MMPs are important in learning given that they are markers of neural plasticity. Currently over 25 MMPs have been identified. Of these MMP-9 and MMP-3 have been shown to be particularly instrumental regarding synaptic plasticity and memory formation (Olson et al., 2008). Several studies have measured changes in MMP expression in the hippocampus after the animal has successfully solved the Morris water maze task, HSR task, passive avoidance and conditioned place preference implying that memory consolidation is taking place (Wright et al., 2006; Olson et al., 2008; Brown et al., 2007; Meighan et al., 2006; Wright et al., 2004). Thus, MMP activity appears to produce transient alterations in the ECM that may be prerequisite to hippocampal-dependent learning.

Meighan et al. (2006) employed the general MMP inhibitor FN-439 infused intracerebroventricularly (icv) to disrupt the acquisition of the Morris water maze task of spatial memory. FN-439 has also been shown to inhibit the induction and stability of long-term potentiation (LTP) in hippocampal slices (Meighan et al., 2006; Meighan et al., 2007). Recently, Olson et al. (2008), measured high levels of MMP-3 at 1 and 4 hours after the acquisition of a passive avoidance conditioning task. These investigators also showed that the icv infusion of an MMP-3 inhibitor during the acquisition of a passive avoidance conditioning task resulted in dose-dependent learning deficits. Of particular relevance to the present investigation, Wright et al. (2006) measured elevations in MMP-3 protein expression in the hippocampus, pre-frontal cortex and piriform cortex at the 2-h ISI during a HSR task.

The present study combined a classical conditioning paradigm with HSR habituation in order to examine the influence of MMPs on spontaneous recovery. The application of a signaling cue, a tone prior to the air stimulus to the ear, permitted an association to form, presumably stored in the dorsal hippocampus, that reduced the strength of responding and thus spontaneous recovery during a second HSR session. We hypothesized that the injection of an MMP inhibitor into the dorsal hippocampus would prevent such an association from forming and maximum spontaneous recovery would occur during the second session despite the contingent presentation of the tone. In other words, the signaling value of the tone would be lost due to the inhibition of MMPs acting on ECM molecules with a resulting disruption of synaptogenesis.

The results concerned with FN-439 infusion demonstrated a predictable decreasing negatively accelerated response pattern over trials asymptoting by about the fifth trial block (Figure 1). Following a 24-h ISI the first trial block of Session II compared with that of Session I indicated a spontaneous recovery level of 81.1% by the high dose FN-439 group. However the low dose FN-439 group and the control group showed a spontaneous recovery of 39.2 and 44.5%, respectively. Thus, the high dose of FN-439 interfered with the CS-US association and prevented memory consolidation. On the other hand, the group that received the low dose of FN-439 and the control group formed the association resulting in attenuated spontaneous recovery during the second session. These results suggest that dorsal hippocampus MMPs play a role in the formation of such an association.

In the second experiment a predictable decreasing negatively accelerated response pattern was also present over trials (Figure 2). Following a 24-h ISI the first trial block of Session II compared with that of Session I indicated a spontaneous recovery level of 77.9% by the MMP-3i group. The control group showed a level of spontaneous recovery of 40.6%. When MMP-3 was

inhibited no association was formed and a high level of spontaneous recovery was measured during session II. In contrast, animals that received aCSF made use of the tone as a signaling cue and an attenuated level of spontaneous recovery occurred during the second session. Thus, the targeted MMP-3 appears to be an important enzyme involved in the formation of this CS-US association.

In summary, this investigation is the first to examine the impact of MMP inhibitors delivered to the dorsal hippocampus on the association formed between a signaling cue and HSR habituation. The results indicate an important role for MMP-3 in the dorsal hippocampus when a HSR habituation paradigm is combined with a contingently placed tone. Under control conditions the animal conserved responses during the initial trial blocks of the second session, thus reducing spontaneous recovery. However, when an MMP inhibitor was injected bilaterally into the dorsal hippocampus the animals failed to make use of this cue thus forcing a less efficient response pattern with more HSRs emitted than necessary to cope with the repeatedly applied air stimulus. Taken together these findings suggest that MMP-3 may be essential to the forming of the CS-US association during this HSR task.

4. Materials and methods

The protocols used in these studies were approved by the Washington State University Institutional Animal Care and Use Committee and conformed to the guidelines for the care and use of laboratory animals as required by the National Institutes of Health.

4.1 Animals

Male Sprague-Dawley rats (300-350 g, breeding stock derived from Taconic, Germantown, NY) were adapted to a 12-h light/dark cycle initiated at 0600 h in an American Association for the Accreditation of Laboratory Animal Care-approved vivarium at a

temperature of 21 ± 1° C. The animals were housed in pairs and provided water and food (Harlan Teklad F6 Rodent Diet, Madison, WI) *ad libitum*, except the night prior to surgery when food was removed.

4.2. Head-shake habituation

Independent groups of rats (8 per group) were tested over the course of 24 trials during the first habituation session, followed by an additional 24 trials during a second session separated by a 24-h ISI. The apparatus was patterned after that used by Askew et al. (1969) and consisted of an elevated platform measuring 7.5 x 16 cm. This surface was positioned on a 0.9 m tall wooden column that allowed the subject freedom of movement during testing. To discourage escape attempts the surface of the platform was covered with 1 cm square wire mesh cloth surrounded by plywood sloping down and outward, forming a collar 17 x 24 cm at its extreme. The base of the wooden column pivoted 360˚, thus permitting the experimenter to compensate for movements by the animal and maintain a face-to-face orientation. HSRs were elicited by a hand held tube (orifice diameter = 0.5 mm) that provided a continuous stream of air that was oscillated across the center of the subject's left ear at an approximate rate of 3 cycles/s. The air tube was held 1-1.5 cm from the animal's ear. The intensity of the air stream was set at 5-6 cm displacement of a 40 cm column of 95% ethanol in a 0.5 mm inside diameter U-shaped manometer (Askew et al., 1969). The test room was painted black with ambient light set at the minimum level necessary to score responses in order to reduce spatial cues.

4.3. Procedure

The habituation session consisted of a 5-min undisturbed adaptation period on the test stand, followed by 24, 15-s stimulus presentations. Each stimulus presentation was separated by a fixed 15-s inter-trial interval (ITI). An IBM-compatible computer signaled the intervals to the

experimenter by displaying the 15-s trial in green numbers, and the 15-s ITI in red numbers. A tone (1-s in duration) was presented 2-s prior to the air stimulus on each trial. From where the animal was positioned in the room, it received a buzzer sound of 74 dB from speakers of an IBM compatible computer. The experimenter recorded the number of HSRs following each trial. At the end of Session I animals were taken to an adjacent room to receive the designated injections within 5 min post-testing and again at 1-h post-testing. This injections schedule was chosen to ensure that the inhibitor was present at 1-2 h post-conditioning, when elevations in active MMPs have been measured (Olson et al., 2008).

4.4. Surgery

All rats were anesthetized using ketamine hydrochloride and xylazine (100 and 2 mg/ml/kg, i.m., respectively; Phoenix Scientific, St. Joseph, MO, and Mobay, Shawnee, KS). Each rat received bilateral guide cannulas targeting the dorsal hippocampi (flat skull coordinates relative to bregma: posterior: -4.0 mm, lateral: +-2.5 mm from midline). The guide was constructed from PE-60 tubing (Clay Adams, Parsippany, NJ) with a heat bulge that rested on top of the cranium thus serving as a stop to further penetration. The total length of the guide was 2.5 cm with a distance from the beveled tip to the heat bulge of 2.5 mm. All infusions were made using a 30-gauge stainless-steel tubing injector with a beveled end that protruded 2.0 mm beyond the tip of the guide cannula. The injector was attached to a 10 ul Hamilton syringe by PE-20 tubing.

4.5. Hippocampal infusion of FN-439

The first experiment examined whether the general MMP inhibitor, FN-439 (4-Abz-Gly-Pro-D-Leu-D-Ala-OH, mw=490.6; MMP inhibitor 1 #444250, Calbiochem, San Diego, CA), interfered with the CS-US association formed during session I, by looking at the level of

spontaneous recovery during session II. This was accomplished by injecting a low dose (25 µg in 2.5 µl aCSF) or a high dose (50 µg in 2.5 µl aCSF) of FN-439 bilaterally at 5 min and 1 h post-testing after the termination of Session I. Members of the control group received aCSF (2.5 µl) delivered to each side at the same times post-testing.

4.6. Hippocampal infusion of MMP-3 inhibitor

The second experiment examined whether a specific MMP-3 inhibitor (Ac-Arg-Cys-Gly-Val-Pro-Asp-NH_2, mw=686.8; #444218, Calbiochem, San Diego, CA) interfered with the CS-US association formed during session I, by looking at the level of spontaneous recovery during session II. This was accomplished by injecting MMP-3i (50 µg in 2.5 µl aCSF) to each side at 5 min and 1 h post-testing after the termination of Session I. Members of the control group received aCSF (2.5 µl) delivered to each side following the same procedure. Each group consisted of 8 rats.

4.7. Histology

Once behavioral testing was completed each animal was deeply anesthetized with Equithesin (pentobarbital: 100 mg/kg, i.p., Jensen-Salsbury Labs, Kansas City, MO) and transcardally perfused (0.15 M NaCl followed by 10% formalin). The brains were immediately removed and stored in a 30% sucrose in 10% formalin solution at 4° C for at least 30 days. Each brain was then placed on the stage of a freezing microtome (Spencer Lens, Buffalo, NY) and sectioned in the coronal plane at 40 µm. The sections were mounted on gelatin-coated slides and stained with Cresyl violet. Once the slides had been cover-slipped they were viewed using an overhead slide projector (Model X-1000, Ken-A-Vision, Raytown, MO) thus permitting localization of the aspiration lesions in each hemisphere according to the rat brain atlas of Paxinos and Watson (1986).

4.8 Statistical analyses

The twenty-four trials of each session were grouped into 8 blocks of 3 trials each, and the mean of each trial block was calculated for each animal. These data were analyzed using a 3 (groups) x 8 (trial blocks) analysis of variance (ANOVA) with repeated measures on the second factor, for each of the two sessions. A one-way ANOVA was used to compare the groups on the first trial block of Session II. Significant effects were further analyzed using Newman-Keuls post-hoc tests with a level of significance set at $p < .05$. In addition, *t*-tests for repeated measures were used to analyze the first trial blocks of Sessions I and II for each group. Alpha levels were adjusted according to the Bonferonni technique. The data of the second experiment were analyzed using a 2 (groups) x 8 (trial blocks) ANOVA with repeated measures on the second factor. Post-hoc tests were as described above.

Acknowledgements

The authors thank Mr. Mathew D. Wiediger for assisting with computer programs. This study was supported by a Laing Endowment for Alzheimer's disease grant to J.W.W., and funds provided by Washington State University.

References

Askew, H.R., Leibrecht, B.C., Ratner, S.C., 1969. Effects of stimulus duration and repeated sessions on habituation of the head-shake response in the rat. J. Comp. Physiol. Psychol. 67, 497–503.

Bernhard, N., van der Kooy, D., 2000. A behavioral and genetic dissection of two forms of olfactory plasticity in Caenorhabditis elegans: adaptation and habituation. Learn. Mem. 7, 199–212.

Brown, T.E., Forquer, M.R., Cocking, D.L., Jansen, H.T., Harding, J.W., Sorg, B.A., 2007. Role of matrix metalloproteinases in the acquisition and reconsolidation of cocaine-induced conditioned place preference. Learn. Mem. 14, 214–223.

Castellucci, V.F., Kandel, E.R., 1974. A quantal analysis of the synaptic depression underlying habituation of the gill-withdrawal reflex in Aplysia. Proc. Natl. Acad. Sci. U. S. A. 77, 7492–7496.

Collingridge, G.L., Isaac, J.T., Wang, Y.T., 2004. Receptor trafficking and synaptic plasticity. Nat. Rev. Neurosci. 5, 952-962.

Dityatev, A., Schachner, M., 2003. Extracellular matrix molecules and synaptic plasticity. Nat. Rev. Neurosci. 4, 456-468.

Ernst, M.M., Epstein, L.H., 2002. Habituation of responding for food in humans. Appetite 38, 224–234.

Harris, J.D., 1943. Habituatory response decrement in the intact organism. Psychol. Bull. 40, 385-422.

Mackintosh, N.J., 1987. Neurobiology, psychology, and habituation. Behav. Res. Ther. 25, 81-97.

Masini, C.V., Day, H.E.W., Campeau, S., 2008. Long-term habituation to repeated loud noise is impaired by relatively short inter-stressor intervals in rats. Behav. Neurosci. 122, 210-223.

McSweeney, F.K., Kowal, B.P., Murphy, E.S., Wiediger, R.V., 2005. Stimulus change dishabituates operant responding supported by water reinforcers. Behav. Proces. 70, 235-246.

Meighan, S.E., Meighan, P.C., Choudhury, P., Davis, C.J., Olson, M.L., Zornes, P.A., Wright, J.W., Harding, J.W., 2006. Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. J. Neurochem. 96, 1227 – 1241.

Meighan, P.C., Meighan, S.E., Davis, C.J., Wright, J.W., Harding, J.W., 2007. Effects of matrix metalloproteinase inhibition on short- and longterm plasticity of schaffer collateral/CA1 synapses. J. Neurochem. 102, 2085–2096.

Murphy, E.S., Harding, J.W., Muhunthan, K., Holtfreter, K., Wright, J.W., 2005. Role of mitogen-activated protein kinases during recovery from head-shake response habituation in rats. Brain Research. 1050, 170-179.

Novak, U., Kaye, A.H., 2000. Extracellular matrix and the brain: components and function. J. Clin. Neurosci. 7, 280-290.

Olson, M.L, Meighan, P.C, Brown, T.E, Asay, A.L, Benoist, C.C, Harding, J.W, Wright, J.W., 2008. Hippocampal MMP-3 elevation is associated with passive avoidance conditioning. Regul. Pept. 146, 19–25.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates (2nd ed.). New York: Academic Press.

Plappert, C.F., Pilz, P.K., 2005. Long-term habituation of the startle response in mice evoked by acoustic and tactile stimuli. Behav. Brain Res. 162, 307–310.

Rose, J.K., Rankin, C.H., 2001. Analysis of habituation in Caenorhabditis elegans. Lear. Mem. 8, 63-69.

Staddon, J.E.R., Chelaru, I.M., Higa, J.J., 2002. Habituation, memory and the brain: The dynamics of interval timing. Behav. Proces. 57, 71-88.

Stevenson, C.W., Gratton, A., 2004. Basolateral amygdala dopamine receptor antagonism modulates initial reactivity to but not habituation of the acoustic startle response. Behav. Brain Res. 153, 383–387.

Thompson, R.F., Spencer, W.A., 1966. Habituation: A model phenomenon for the study of neuronal substrates of behavior. Psychol. Rev. 73, 16-43.

Thorpe, W.H., 1966. Learning and instinct in animals. Cambridge, MA: Harvard University Press.

Wright, J.W., Reichert, J. R., Davis, C. J, Harding, J. W., 2002. Neural plasticity and the brain rennin-angiotensis system. Neurosci. Biobeh. Rev. 26, 529-552.

Wright, J.W., Meighan, S.E., Murphy, E.S., Holtfreter, K.L., Davis, C.J., Olson, M.L., Benoist, C.C., Muhunthan, K., Harding, J.W., 2006. Habituation of the head-shake response induces changes in brain matrix metalloproteinases-3 (MMP-3) and -9. Behav. Brain Res. 174, 78-85.

Wright, J.W., Murphy, E.S., Elijah, I.E., Holtfreter, K.L., Davis, C.J., Olson, M.L., Muhunthan, K., & Harding, J.W., 2004. Influence of hippocampectomy on habituation, exploratory behavior, and spatial memory in rats. Brain Res. 1023, 1-14.

Figure Captions:

Figure 1. Mean (± SEM) group changes in number of head-shake responses per 3-trial blocks during Sessions I and II of habituation trials separated by a 24-h ISI. There were no differences among the groups concerning the pattern of habituation during Session I. There were differences among the groups during the first trial block of Session II with the high FN439 group revealing a significantly high level of spontaneous recovery as compared with the other two groups which did not differ. *p<.001.

Figure 2. Mean (± SEM) number of head-shake responses per 3-trial blocks for the MMP-3i and aCSF injected rats during Sessions I and II of habituation trials separated by a 24-h ISI. These two groups did not differ regarding pattern of habituation during Session I; however, members of the aCSF group revealed significantly attenuated levels of spontaneous recovery during the first trial block of Session II. *p<.001.

Figure 1

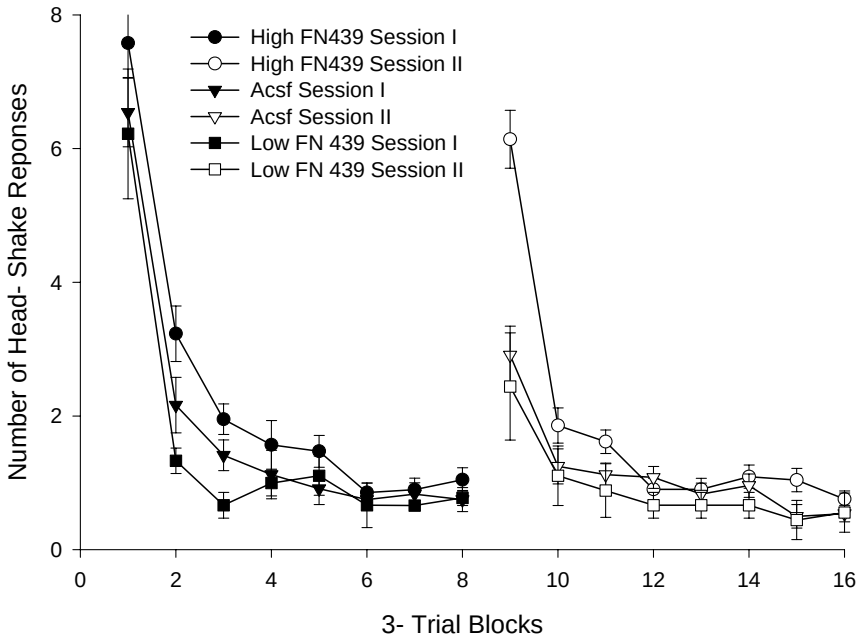
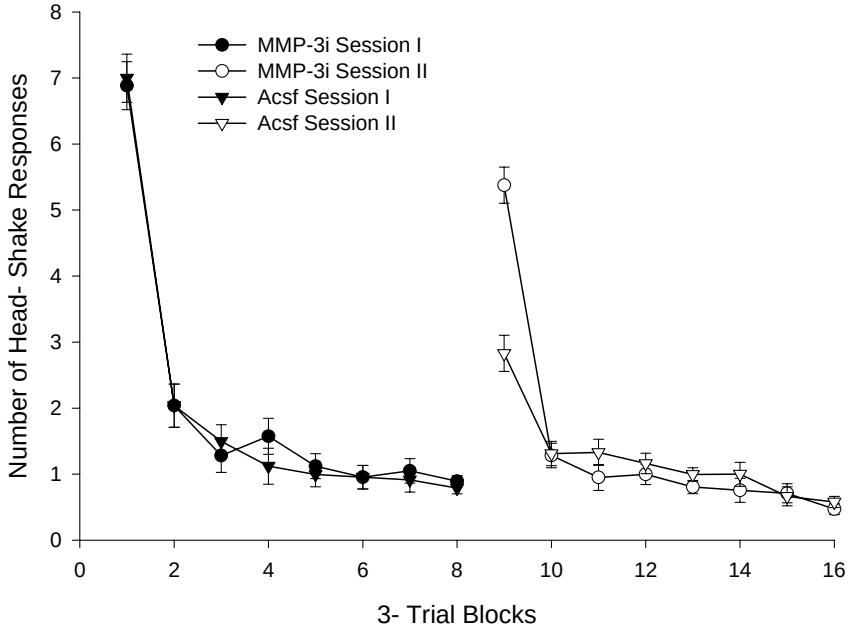


Figure 2



CHAPTER FOUR

GENERAL DISCUSSION

General Discussion

These experiments were designed to provide a better understanding concerning the role of the hippocampus and the matrix metalloproteinases (MMPs) during the head-shake response task combined with a classical conditioning paradigm.

Patterns of the HSR habituation and spontaneous recovery showed a reliable decreasing negatively accelerated response pattern that was followed by a substantial spontaneous recovery following a 24-h ISI (Askew et al., 1969; Leibrecht et al., 1969; Murphy et al., 2005; Wright et al., 2006; McSweeney & Murphy, 2000). The hippocampus has been implicated in the control of inhibitory processes including habituation (Douglas, 1967; Jarrard & Bunnell, 1968; Kimble, 1968; Leaton, 1965; Primbram, 1967; Roberts et al., 1962), fear extinction and spontaneous recovery (Ji & Maren, 2007), and in the formation and utilization of spatial memory (Bures et al., 1997; McNaughton et al., 1989; Morris et al., 1982; Nadel, 1991; O'Keefe and Nadel 1978; Wishaw, 1987).

The hippocampus is also linked to learning and memory storage because of its substantial synaptic plasticity (Collingridge et al., 2004). ECM molecules mediate changes in the brain's synaptic architecture thought to be critical to the processes of neural plasticity, learning and memory (Dityatev and Schachner, 2003). Wright and colleagues (2007) reported a compromised ability to reconfigure ECM molecules by inhibiting MMP activity in the dorsal hippocampus during spatial memory acquisition. Meighan et al. (2006) used the general MMP inhibitor FN-439 to interfere with the late phase of long-term potentiation (LTP), and when FN-439 was infused intracerebroventricularly (icv) it disrupted the acquisition of the Morris water maze task of spatial memory. FN-439 has also been shown to disrupt the induction and stability of long-term potentiation (LTP) in hippocampal slices (Meighan et al., 2006 and Meighan et al., 2007).

Recently, Olson et al, (2008) measured high levels of MMP-3 at 1 and 4 hours after the acquisition of a passive avoidance conditioning task. They also showed that the icv infusion of an MMP-3 inhibitor during the acquisition of a passive avoidance conditioning task resulted in dose-dependent learning deficits. Therefore the overall objective of this dissertation was to answer the following questions: 1) Can a CS-US association be formed during a HSR task, and if so, what is its effect on spontaneous recovery? 2) Is the dorsal hippocampus important during the CS-US association during a HSR task, and if so, what is its effect on spontaneous recovery? 3) What is the role of MMPs on the CS-US association during a HSR task? Will the inhibition of MMPs interfere with the acquisition of the CS-US association? Will the inhibition of MMP-3 interfere with the acquisition of the CS-US association?

Summary of Findings

An association between the tone (CS) and the air stimulus to the ear (US) was formed during the HSR task. Thus, animals that received the CS-US pairing showed reduced spontaneous recovery after a 24-h ISI; while the control group showed high spontaneous recovery. This was interpreted to suggest that the animals were able to recognize the association of the two stimuli and therefore little HSR occurred. The association between the CS-US was confirmed when a random tone control group presented a pattern of responding very similar to the no tone control group. In other words, when the tone was noncontingent, or when no presentation of the tone was made, spontaneous recovery of the HSR 24-h later was high. These findings suggest that when the CS-US association is acquired animals conserve energy by reducing responding during a second HSR session. To further understand this phenomenon the dorsal hippocampus was bilaterally removed. Under this condition the animals were unable to learn the CS-US association, therefore 24-h later they showed the same pattern of responding as

the no tone control group described above. These results strongly suggest that when the general MMP inhibitor, FN439, or the specific MMP-3 inhibitor was infused into the dorsal hippocampus the animals were not able to acquire the CS-US association. Thus, their pattern of responding 24-h later during the second session was very similar to that displayed by members of the no tone control group which never experienced the CS-US pairing.

In conclusion, the dorsal hippocampus appears to play a significant role in mediating learning when a classical conditioning paradigm is combined with the HSR task. Removing the hippocampus, or inhibiting MMPs in this structure, interfered with the learning of the CS-US association. These results highlight the importance of the dorsal hippocampus with respect to the pairing of a signaling cue with the habituation of a reflex-like behavior such as HSR. Once this association was in place the animal's subsequent behavior was changed such that fewer responses were emitted. This represents a much more rapid adaptation to the repeated presentation of an innocuous stimulus than when the association is unavailable to the animal. These results illustrate the usefulness of combining associative and nonassociative learning paradigms in an effort to understand the parameters under which habituation and spontaneous recovery can be modified, and further point out the importance of MMPs to this phenomenon.

References

Askew, H. R., Leibrecht, B. C., & Ratner, S. C. (1969). Effects of stimulus duration and repeated sessions on habituation of the head-shake response in the rat. *Journal of Comparative & Physiological Psychology, 67,* 497–503.

Bures, J., Fenton, A. D., Kaminsky, Y., & Zinyuk, L. (1997). Place cells and place navigation. *Proceedings National Academy of Sciences USA, 94,* 343–350.

Collingridge, G. L., Isaac J. T., & Wang Y. T., (2004). Receptor trafficking and synaptic plasticity. *Nature Reviews Neuroscience, 5*, 952-962.

Dityatev, A., and Schachner, M., (2003). Extracellular matrix molecules and synaptic plasticity. *Nature Reviews Neuroscience.* Vol.4, no.6, pp.456-468.

Douglas, R. J. (1967). The hippocampus and behavior. *Psychological Bulletin, 67,* 416 – 422.

Jarrard, L. E., & Bunnell, B. N. (1968). Open-field behavior of hippocampal lesioned rats and hamster. *Journal of Comparative & Physiological Psychology, 66,* 500-502.

Ji, J., & Maren, S. (2007). Hippocampal involvement in contextual modulation of fear extinction. *Hippocampus, 17*, 749-758.

Kimble, D. P. (1968). Hippocampus and internal inhibition. *Psychological Bulletin, 70,* 285 – 295.

Leaton, R. N. (1965). Exploratory behavior in rats with hippocampal lesions. *Journal of Comparative & Physiological Psychology, 59,* 325–330.

Leibrecht, B. C., & Askew, H. R. (1969). Habituation of the head-shake response in the rat: recovery, transfer, and changes in topography. *Journal of Comparative & Physiological Psychology, 69,* 699 – 708.

McNaughton, B. L., Barnes, C. A., Meltzer, J., & Sutherland, R., J. (1989). Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. *Experimental Brain Research, 73* (3), 1432-1106

McSweeney, F. K., & Murphy, E.S., (2000). Criticisms of the satiety hypothesis as an explanation for within-session decreases in responding. *Journal of the Experimental Analysis of Behavior, 74* (3), 347–361.

Meighan, S. E., Meighan, P. C., Choudhury, P., Davis, C. J., Olson, M. L., Zornes, P. A., Wright, J. W., & Harding, J. W. (2006). Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *Journal of Neurochemistry, 96*, 1227 – 1241.

Meighan PC, Meighan SE, Davis CJ, Wright JW, & Harding JW. (2007). Effects of matrix metalloproteinase inhibition on short- and longterm plasticity of schaffer collateral/CA1 synapses. *Journal of Neurochemistry, 102*, 2085–2096.

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

Murphy, E. S., Harding, J. W., Muhunthan, K., Holtfreter, K., & Wright, J. W. (2005). Role of mitogen-activated protein kinases during recovery from head-shake response habituation in rats. *Brain Research, 1050*, 170-179.

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

O'Keefe, J., & Nadel, L. (1978). *The Hippocampus as a Cognitive Map* (pp. 265-290). Oxford, UK: Oxford University Press.

Olson ML, Meighan PC, Brown TE, Asay AL, Benoist CC, Harding JW, Wright JW. 2008. Hippocampal MMP-3 elevation is associated with passive avoidance conditioning. *Regulatory Peptides, 146,* 19–25.

Pribram, K. H. (1967). The limbic systems efferent control of neural inhibition and behavior. *Progress in Brain Research, 27,* 318 – 336.

Roberts, W. W., Dember, W. N., & Brodwick, M. (1962). Alternation and exploration in rats with hippocampal lesions. *Journal of Comparative & Physiological Psychology, 55,* 695–700.

Whishaw, I. Q. (1987). Hippocampal granule cell and CA3-4 lesions impair formation of a place learning-set in the rat and induce reflex epilepsy. *Behavioural Brain Research, 24,* 59–72.

Wright, J.W., Reichert, J. R., Davis, C. J, Harding, J. W. (2002). Neural plasticity and the brain rennin-angiotensis system. *Neuroscience and Biobehavioral Reviews. 26, 529-552.*

Wright, J. W., Brown, T. E., Harding, J. W. (2007). Inhibition of hippocampal matrix metalloproteinase-3 and -9 disrupts spatial memory. *Neural Plasticity.* 1-8.

Wright, J. W., Meighan, S. E., Murphy, E. S., Holtfreter, K. L., Davis, C. J., Olson, M. L., Benoist, C. C., Muhunthan, K., Harding, J. W. (2006). Habituation of the head-shake response induces changes in brain matrix metalloproteinases-3 (MMP-3) and -9. *Behavioural Brain Research, 174,* 78-85.