2009

Modulation of memory formation following violations of conditioned expectations

Dennis Antonio Amodeo

MODULATION OF MEMORY FORMATION FOLLOWING VIOLATIONS OF CONDITIONED EXPECTATIONS

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

in

Psychology:

General-Experimental Psychology

---

by

Dennis Antonio Amodeo

September 2009

MODULATION OF MEMORY FORMATION FOLLOWING VIOLATIONS OF CONDITIONED EXPECTATIONS

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

by

Dennis Antonio Amodeo

September 2009

Approved by:

[redacted]
Dr. Allen E. Butt, Chair, Psychology

8-3-09
Date

[redacted]
Dr. Sanders A. McDougall

[redacted]
Dr. Matt Riggs

ABSTRACT

The unexpected violation of a previously established association (i.e., prediction error) typically leads to an increase in attention to the conditioned stimulus (CS) that has had its predictive value altered. This increment in attention to the CS thereby leads to an increased rate of acquisition of new associations involving that CS. While the neuroanatomical basis of this phenomenon is largely understood, little is known about the synaptic mechanisms underlying memory formation for prediction error. The current experiment tests the overall hypothesis that this specific form of memory depends on N-methyl-D-aspartate (NMDA) receptor activation. Immediately prior to prediction error conditioning trials, separate groups of rats were administered either saline or one of two different doses of the NMDA receptor antagonist dizocilpine (MK-801; 0.15 or 0.20 mg/kg) or agonist D-cycloserine (DCS; 15.0 or 20.0 mg/kg). NMDA antagonist treatment was expected to disrupt memory for prediction error in a dose-dependent manner, whereas agonist treatment was expected to facilitate memory in a dose-dependent manner. The strength of prediction error memory was assessed the following day by measuring the

rate of new association learning with the affected CS. Impaired memory was expected to retard subsequent conditioning, while enhanced memory was expected to improve subsequent conditioning. Results supported the hypothesis that NMDA receptor blockade would disrupt the formation of memory for unexpected violations of previously learned associations. Treatment with both doses of MK-801 subsequently prevented the expression of enhanced new learning, although the degree of impairment was not dose-dependent. In contrast, results failed to support the hypothesis that NMDA receptor agonist treatment would enhance prediction error memory. New learning performance in the group treated with the low dose of DCS did not differ from the saline-treated control group. Treatment with the high dose of DCS paradoxically impaired new learning. Although the high dose DCS effects were paradoxical, these findings nevertheless demonstrate NMDA receptor involvement in the memory mechanisms underlying enhanced attention to cues whose predictive value has changed. Collectively, these results support the hypothesis that memory for prediction error is NMDA receptor-dependent.

ACKNOWLEDGMENTS

Firstly, I would like to thank my thesis advisor, Dr. Allen Butt for his guidance and support of this thesis. Without him, the present thesis would not have been possible. I am deeply appreciative of the time and dedication he has given me, and the confidence he has demonstrated in my ability to become a scientist. I greatly appreciate his continued support and willingness to help and guide me.

I would also like to thank Dr. Sanders McDougall for his continued support, expertise, and guidance during my development in the program. Additionally, I would like to thank Dr. Matt Riggs for his support and assistance through the course of my education and work on this thesis.

Finally I would like to thanks the Associated Student, Inc. for financial support of my thesis.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE

LONG TERM POTENTIATION AND MEMORY FORMATION

The search for the neurobiological substrates of memory formation has been a focus of behavioral neuroscience since the early research of Karl Lashley (Lashley & Franz, 1917). Contemporary views of neural mechanisms of memory center on experience-dependent changes in synaptic connectivity among ensembles of neurons representing specific experience (Watson, Herbert, & Stanton, 2009). One prominent mechanism of synaptic plasticity is long-term potentiation (LTP). LTP, described as a prolonged augmentation in synaptic efficiency, is viewed as being the most cohesive and accepted model for information storage in the mammalian brain (Kullmann & Lamsa, 2008; Martinez & Derrick, 1996).

The concept of memory formation occurring through the remodeling of synaptic connections among neurons has a long history, beginning with the work of Donald Hebb. Donald Hebb (1949) proposed that networks of neurons store memories in reverberating assemblies of neurons. The Hebbian postulate closely resembles what memory researchers refer to as the "engram", or the physical

representation of memory in the brain. According to Hebb, these reverberating circuits have the ability to modify and reform connections, thus leading to plastic changes in the brain's representation of experience.

A lead candidate for the neurobiological substrate of experience-dependent synaptic plasticity involves the N-methyl-D-aspartate (NMDA) receptor (Martinez & Derrick, 1996; Lynch, 2004). Research has shown that memory formation depends on the ability of neural networks to modify, form, and restructure their connections (Takehara-Nishiuchi, Kawahara, & Kirino, 2005; Takehara-Nishiuchi, Nakao, Kawahara, Masuki, & Kirino, 2006). A promising approach to linking changes in synaptic connectivity to learning and memory involves the study of synaptic plasticity during the comparatively simple form of learning known as Pavlovian conditioning.

This well-known form of associative learning was formalized by Ivan Pavlov, who demonstrated that a previously irrelevant stimulus can come to elicit a behavior normally brought about by the presence of a biologically relevant stimulus (Pavlov, 1927). In Pavlovian or classical conditioning the conditioned stimulus (CS) is initially behaviorally neutral and lacks

biological significance. However, when this neutral CS is paired with an unconditioned stimulus (US), which itself has innate biological significance to the animal, the CS comes to elicit the corresponding reflex-like behavior known as the conditioned response (CR; Pavlov, 1927).

Martin, Grimwood, and Morris (2000) point out the similarities between the acquisition of Pavlovian conditioning and the formation of LTP. These authors illustrate how the CS gradually comes to evoke a CR, and how the process mediating conditioned responding involves increases in either synaptic efficacy, similar to presynaptic facilitation or LTP, or neuronal excitability such as excitatory post-synaptic potential (EPSP) spike potentiation (Martin, Grimwood, & Morris, 2000). Martinez and Derriek (1996) describe three factors suggesting that Pavlovian conditioning resembles LTP: first, repeated presynaptic stimulation can be viewed as being analogous to repeated presentations of the CS during conditioning; second, associative properties are present in both LTP and conditioning; and third, LTP can remain for lengthy periods of time, just as the memory underlying the CR persists across time (Martinez & Derrick, 1996).

Neuronal Mechanisms of Long Term Potentiation

Hebb (1949) was concerned with the underlying process forming experience-dependent connections between neurons. Hebb's axiom of reverberating circuits proposed that if two neurons are co-active, the connection between these neurons would be strengthened (Hebb, 1949). This thinking has been paraphrased in the statement "neurons that fire together wire together." This statement emphasizes the role that co-activity among pre- and post-synaptic neurons plays in the establishment of strengthened or potentiated synaptic connections among neurons.

Bliss and Lomo (1973) demonstrated that high-frequency electrical stimulation of pre-synaptic axons of the perforant pathway increased the post-synaptic response in the target neurons of the dentate gyrus in the hippocampus. The increase in EPSPs following LTP induction lasts at least several hours. This experience-dependent change in synaptic connectivity is argued to be a potential mechanism of memory storage with clear parallels to Hebb's postulate (Hebb, 1949). LTP shares key characteristics with memory, including its longevity, rapid induction, and it is associative.

Associability in LTP is based on the fact that only co-active synapses become strengthened.

Subsequent research has shown that LTP critically depends on the NMDA receptor (Collingridge, Kehl, & McLennan, 1983). Although NMDA receptor-independent LTP does occur, the predominant mode of LTP depends on NMDA receptors (Martin, Grimwood, & Morris, 2000). The NMDA receptor is responsible for detection of co-activity of presynaptic neurons releasing glutamate, and postsynaptic depolarization. When these conditions are met, the NMDA receptor initiates the synaptic changes responsible for LTP. Collingride et al. (1983) demonstrated that while the NMDA receptor is critical for the induction of LTP, it is not necessary for the maintenance of LTP. These researchers infused the competitive NMDA receptor antagonist 2-amino-5-phosphonovaleric (APV) into rat hippocampus and found that applying high-frequency stimulation to the perforant path in the presence of APV prevented the induction of LTP. In contrast, APV infusions made following LTP induction did not prevent the maintenance of LTP (Collingridge et al., 1983).

Unlike other receptors, the NMDA receptor is doubly-gated; in addition to the activation of

glutamate-gated channels, post-synaptic depolarization is also required for the channel to open, thus permitting calcium (Ca^{2+}) influx (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2008). A magnesium (Mg^{2+}) ion is normally bound to the NMDA receptor, blocking Ca^{2+} movement into the intracellular space. Before allowing Ca^{2+} influx, the post-synaptic cell must be depolarized to a point sufficient to eject the Mg^{2+} ion.

In addition to binding to the NMDA receptor, glutamate binds to the amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor, an ionotropic receptor with a channel for Na^{+} (Wang, Hu, & Tsien, 2006). Activation of the AMPA receptor allows Na^{+} influx and thereby depolarizes the post-synaptic cell. The AMPA receptor, which is co-localized with the NMDA receptor, plays a critical role in NMDA receptor activation. Once a sufficient number of AMPA receptors are activated and the cell is depolarized, the Mg^{2+} ion is ejected from the NMDA receptor (Mayer et al., 1984; Nowak et al., 1984). Provided that glutamate is attached to its ligand binding site on the NMDA receptor, the removal of the Mg^{2+} blockade opens the NMDA receptor and permits an influx of

extracellular Ca^{2+}. In addition to admitting Ca^{2+}, Na^{+} also enters into the post-synaptic neuron via the NMDA receptor, thereby causing further depolarization. The influx of Ca^{2+}, however, is the critical element in triggering LTP changes both pre- and post synaptically.

Following Ca^{2+} influx into the post-synaptic neuron, Ca^{2+}-dependent protein kinases are activated, triggering the pre- and post-synaptic events resulting in increased synaptic strength. Calmodulin, a Ca^{2+} binding protein, binds to Ca^{2+} once it enters the dendrites of the activated cell. Activation of Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) enhances LTP by modifying AMPA receptor conformation through phosphorylation, which subsequently leads to significantly more Na^{+} entering the post-synaptic neuron through active AMPA receptors (Alberts et al., 2008; Rudy, 2008). Activation of CaMKII also results in the insertion of AMPA receptors transported from the cytoplasm into the cell membrane (Lisman, Schulman, & Cline, 2002). The resulting increase in the number and sensitivity of AMPA receptors provides the basis for the increased post-synaptic activation characteristic of LTP.

Lisman et al. (2002) showed that CaMKII in the postsynaptic density zone is fundamental for the induction of LTP. Electrophysiological *in vitro* studies using hippocampal slices have demonstrated that mice genetically altered to prohibit CaMKII processes do not exhibit LTP (Giese, Fedorov, Filipkowski, & Silva, 1998). Studies by Lisman et al. (2002) have shown that with direct application of CaMKII, glutamate activation of the post-synaptic neuron is amplified.

The NMDA receptor contains separate subunits including the NR1 and NR2 subunits (Tsien, Huerta, & Tonegawa, 1996). The NR2 subunit is further divided into the NR2A, NR2B, NR2C, and NR2D type subunit (Tsien et al., 1996). The NR1 and NR2 subunits are necessary for the activation of the Ca^{2+} channel within the NMDA receptor. Tsien et al. (1996) demonstrated that genetically altered rats with deletion of the NR1 receptor subtype do not show LTP induction. Tang et al. (1999) found that rats genetically altered to over-express the NR2B subunit demonstrated an enhancement in Morris water maze performance, thus NMDA receptor-dependent synaptic plasticity has been implicated in spatial memory. The ability of the NR2B

subtype to enhance LTP and therefore augment memory formation supports the argument that NMDA receptor-dependent plasticity plays an important role in memory.

CHAPTER TWO

THE N-METHYL-D-ASPARTATE RECEPTOR AND MEMORY FORMATION IN PAVLOVIAN CONDITIONING

Introduction

A growing body of research suggests that the induction of LTP is critical for many forms of learning and memory. According to Hebb's (1949) postulate, memories are stored in reverberating assemblies of neurons that cooperate and reorganize to form long lasting synaptic connections. *In vivo* studies demonstrate that once LTP induction takes place, increased synaptic connectivity can persist for weeks and even months (Barnes, 1979). Rumelhart and McClelland (1986) show that by inhibiting the induction of LTP, the ability to learn a new task is significantly impaired. NMDA receptor-dependent synaptic plasticity (i.e., LTP) is necessary for normal performance in a variety of behavioral tasks including trace eye blink conditioning, contextual fear conditioning, and latent inhibition and extinction of fear conditioning (Morris, Anderson, Lynch, & Baudry, 1986; Gruart, Munoz, & Delgado-Garcia, 2006).

Research supports the view that memory formation and LTP induction are both NMDA receptor-dependent (Highfield, Nixon, & Amsel, 1996; Xu, Boshoven, Lombardo, & Spranger, 1998). Contemporary studies have used pharmacological methods to block or facilitate NMDA receptor function, where conditioned behavior is either disrupted or enhanced as a function of drug treatment. By blocking the induction of LTP with NMDA receptor antagonists such as 5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine (MK-801 or dizocilpine), 2-amino-5-phosphonovaleric (APV/AP5), and phencyclidine (PCP), researchers have demonstrated significant impairments in post-treatment memory formation (Thompson & Disterhoft, 1997). Conversely, by administering NMDA receptor agonists such as D-cycloserine (DCS), researchers have demonstrated significant enhancement of learning and memory (Thompson & Disterhoft, 1997a).

By blocking AMPA/NMDA receptor function, LTP and subsequent plasticity is disrupted (Hölscher, 1999). For example, MK-801 binds to the NMDA receptor and blocks Ca^{2+} influx into the post synaptic neuron. As a non-competitive antagonist of the NMDA receptor, MK-801

does not compete with glutamate sites and therefore allows NMDA receptor activation. However, MK-801 blocks Ca^{2+} from entering the post-synaptic cell, thus inhibiting LTP processes (Woodruff, Foster, Gill, Kemp, Wong, & Iversen, 1987). NMDA receptor antagonist drugs disrupt acquisition in several behavioral paradigms by preventing NMDA receptor-dependent plasticity believed to underlie memory formation (Thompson & Disterhoft, 1997; Watson, Herbert, & Stanton, 2009). Conversely, NMDA receptor agonists such as DCS, a partial agonist at the strychnine-insensitive glycine site of the NMDA receptor, can improve learning performance by enhancing NMDA receptor efficiency and promoting memory consolidation (Norberg, Krystal, & Tolin, 2008).

The hippocampus and amygdala, two regions that are critically involved in learning and memory, depend on NMDA receptor activation for the induction of LTP, and require normal NMDA receptor function in order to support learning and memory formation (Laurent & Westbrook, 2008; Matus-Amat, Higgins, Sprunger, Wright-Hardesty, & Rudy, 2007). The infusion of NMDA antagonists including APV, 2-amino-5-phosphonovaleric acid (AP-5), or 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid

(CPP), produces similar effects across studies; the blockade of NMDA receptors disrupts acquisition in several conditioning tasks while sparing previously learned CRs (Staubli, Thibault, DiLorenzo, & Lynch, 1989) The majority of research on NMDA receptor blockade involves the competitive NMDA receptor antagonist APV. APV occupies receptor sites usually occupied by glutamate (Rudy, 2008). By blocking glutamate from attaching to the appropriate receptor, NMDA receptor function is disrupted.

Blockade of the NMDA receptor disrupts acquisition of trace conditioning. The trace form of Pavlovian conditioning is an attention-demanding (Han, O'Tuathaigh, van Trigt, Quinn, Fanselow, Mongeau, Koch, & Anderson, 2003) hippocampus-dependent form of declarative memory (Manns, Clark, & Squire, 2002) that is more difficult to learn than delay conditioning. In the delay condition, the US is terminated simultaneously with the offset of the CS. In contrast to trace conditioning, delay conditioning does not require the activity of the hippocampus (Seo, Pang, Shin, Kim, & Choi, 2008). Because trace conditioning depends on an intact hippocampus, and because the hippocampus is a site where NMDA

receptor-dependent plasticity occurs, trace conditioning (but not delay conditioning) is especially vulnerable to pharmacological blockade of the NMDA receptor (Seo, Pang, Shin, Kim, & Choi, 2008).

Thompson and Disterhoft (1997) investigated the effects of MK-801 and phencyclidine (PCP) on both trace and delay eyeblink conditioning. Rabbits were systemically injected with MK-801 or PCP either prior to testing, during acquisition, post acquisition, or prior to pseudoconditioning procedures. Rabbits in the MK-801 experiment received daily doses of 0, 10, 40, 80, or 160 μg/kg MK-801 5 min before testing. Subjects in the PCP experiment received a daily dose of either 0, 0.1, or 1.0 mg/kg PCP 5 min before training. Eighty CS-tones lasting 400 ms each were presented in the delay condition, and 100 ms CSs were presented in the trace condition with US-air puff trials administered daily. Trace conditioning incorporated a 500 ms stimulus-free interval after the offset of the CS and before the onset of the US, similar to the studies previously described. The post-acquisition subjects were treated with MK-801 or PCP after CS-US training was over, these subjects were tested for drug

effects on extinction. In the pseudo conditioning group subjects were tested with random CS or US presentations. These groups were included to investigate the non-associative effects of both drugs (Thompson & Disterhoft, 1997).

Results demonstrated that high doses of MK-801 blocked trace conditioning, while delay conditioning was only slightly impaired. When doses of 80 µg/kg were administered, a CR occurred on no more than 30% of the trials. Higher doses of MK-801 caused greater impairments in CR acquisition (Thompson & Disterhoft, 1997).

All doses of PCP caused effects similar to those found in both trace and delay conditions of MK-801-injected subjects. Under both trace and delay conditioning, high doses of PCP impaired extinction but not retention, demonstrating that the NMDA receptor is necessary for new learning but not for the expression of previously acquired learning (Thompson & Disterhoft, 1997).

Sakamoto, Takatsuki, Kawahara, Kirino, Niki, and Mishina (2005) conducted a study investigating the effects of the NMDA receptor antagonism in the

hippocampus on trace eyeblink conditioning. Hippocampal infusions of APV were administered before conditioning in C57BL/6 mice. Consistent with the argument that delay conditioning is hippocampus-independent, APV-treated mice acquired the delay CR eye blink normally. Trace conditioning, however, was profoundly disrupted by NMDA receptor blockade. Sakamoto et al. (2005) point out that NMDA receptor facilitation of LTP is necessary in the modulation of systems needed for trace conditioning.

In addition to the hippocampus, trace conditioning critically depends on the medial prefrontal cortex (mPFC). Takehara-Nishiuchi, Kawahara, and Kirino (2005) implanted rats with bilateral cannulae in the prelimbic area of the mPFC. APV was infused either immediately before, or immediately after training in trace eye blink conditioning. During conditioning, a CS tone was presented followed by a 500 ms trace interval, a shock US was then administered to the left upper eyelid. The blockade of NMDA receptors in the mPFC immediately before testing completely disrupted CR acquisition in the trace conditioning task. APV infusions made immediate post-training, however, had a lesser effect. These data suggest that NMDA receptor activity is essential for

early consolidation of memory in the trace eye blink conditioning paradigm, but that as memory is established (i.e., during the course of the conditioning session), NMDA receptor blockade had progressively less of an effect on subsequent performance (Takehara-Nishiuchi et al., 2005). Weible, McEchron, and Distorhoft (2000) similarly showed that NMDA receptor function in the mPFC is essential for the acquisition and consolidation of trace eye blink conditioning.

In addition to trace conditioning, NMDA receptor activation has also been implicated in fear conditioning. For example, Goosens and Maren (2004) demonstrate that administration of the competitive NMDA receptor antagonist CPP before auditory fear conditioning prevented the development of conditioned single unit activity in the lateral amygdala as well as preventing the acquisition of a behavioral CR of fear. In addition to this behavioral study, Goosens and Maren (2004) also examined the effect of CPP on LTP induction in a separate group of rats. Systemic injections of CPP blocked amygdaloid LTP in anesthetized rats. High frequency stimulation of the ventral angular bundle, which projects to the lateral amygdala, produced robust amygdaloid LTP

in saline-treated rats. This produced an increase in the amplitude and slope of the evoked potential in the lateral amygdala. However, in the CPP-treated rats, LTP was blocked as evidenced by the finding that high frequency stimulation of the ventral angular bundle had no effect on the amplitude or slope of the evoked potential in the lateral amygdala (Goosens & Maren, 2004). These results support the view that NMDA receptor function is crucial in conditioning-related plasticity in amygdaloid regions.

Contextual fear conditioning is similarly affected by NMDA receptor blockade. For example, Sakamoto and colleagues (2005) assessed the effects of intra-hippocampal infusion of the NMDA receptor antagonist APV on contextual fear conditioning. Mice infused with APV shortly before fear conditioning failed to acquire the conditioned fear response (i.e., freezing) to the training context. These results show that hippocampal NMDA receptor activity is necessary for contextual fear conditioning.

The extinction of fear conditioning also requires the NMDA receptor. For example, Walker, Ressler, Lu, and Davis (2002) administered intra-amygdala infusions of

NMDA receptor agonist DCS before and after conditioned fear extinction trials. NMDA receptor agonist administration caused an enhanced rate of extinction. Quartermain et al. (1994) found that administration of DCS enhanced spatial performance in the linear water maze. Rats receiving an acute injection of DCS (3, 10, 20, 40, or 80 mg/kg) immediately post-training, showed significant enhancements in spatial performance 24 h after drug treatment (Quartermain et al., 1994).

Schauz and Koch (2000) demonstrated that NMDA receptor function in the amygdala is crucial in the latent inhibition of fear conditioning. Repeated exposure to a CS without the presentation of the US impairs subsequent learning of the CS-US association (Lubow & Moore, 1959). This is known as latent inhibition. In the Schauz and Koch (2007) study, Wistar rats were implanted with bilateral cannulae in the amygdala. The experimental group was infused with the NMDA antagonist AP-5, while controls received infusions of saline. Infusions of AP-5 or saline were administered before conditioning. In the pre-exposure group rats received several CS only presentations to induce latent inhibition. On the testing day, rats were placed into the same chamber and received

CS-US (shock) pairings for two days. Rats in the pre-exposure (i.e., latent inhibition) group were infused and then tested for conditioned acquisition (Schauz & Koch, 2000). Rats pre-exposed to the CS and then infused with AP-5 did not show latent inhibition. This supports the hypothesis that blocking NMDA receptor function in the amygdala prevents latent inhibition from occurring. An NMDA receptor-dependent system must be responsible for the formation of fear conditioning memories because the lack or disruption of these receptors impairs acquisition of fear conditioning (Schauz & Koch, 2000).

Given that NMDA receptor antagonism disrupts learning and memory in a variety of Pavlovian conditioning tasks, it might be expected that facilitating NMDA receptor function with agonist drug treatments would enhance memory formation in these same tasks. Indeed, Woods and Bouton (2006) demonstrate that NMDA receptor facilitation improves conditioned-fear extinction (i.e., pharmaceutically suppressed the fear response). These researchers administered the NMDA receptor agonist DCS 15 min prior to extinction testing following fear conditioning. Systemic injections of DCS at 15 mg/kg did not facilitate extinction, while 30 mg/kg

DCS significantly enhanced suppression of conditioned fear behaviors during fear-extinction trials (Woods & Bouton, 2006).

CHAPTER THREE

ATTENTION AND PAVLOVIAN CONDITIONING

Introduction

Understanding the neurological mechanisms underlying attention has been a focal point of behavioral neuroscience as early as 1931, with Easley's attempt to isolate the process of attention (Easley, 1931). To understand the neurological mechanisms that drive attention to biologically relevant cues and discount inconsequential signals, cognitive learning theorists emphasize the dynamic nature of attention to the conditioned stimulus (CS) across the learning experience (Paschal, 1941). According to Mackintosh (1975), attention for a given CS increases when that CS gains salience as it becomes a reliable predictor for the unconditioned stimulus (US). Based on the assumption that animals have a limited capacity for processing information, Mackintosh (1975) argues that as the salience of a predictive CS increases, thereby commanding greater levels of attention, the ability of other cues to attract attention is diminished. Thus, a CS that has become a reliable predictor of US occurrence, according

to Mackintosh (1975), will have a high level of salience and will thereby command a great deal of attention.

In contrast, Pearce and Hall (1980) suggest that the amount of attention commanded by the CS reflects the degree to which the CS is followed by unexpected events. For example, early in conditioning when the US is not yet fully predicted by the CS, attention to the CS is increased. This increase in attention to the CS is argued to facilitate learning; therefore, attention to the CS determines the associability (i.e., the ease of conditioning) of that cue. Thus, as learning proceeds and the US becomes better predicted by the CS, the cue actually loses salience and its associability decreases.

Prediction Error

As previously described, the amygdala is pivotal in fear conditioning, but relatively few studies have directly investigated the role of the amygdala in attention. According to Pearce and Hall (1980) a CS loses associability once the US is sufficiently predicted. This theory suggests that once a previously established association is violated, attention to the CS increases. Previous studies have shown that a circuit containing the

central nucleus of the amygdala (CeA), the sublenticular substantia innominata, and the posterior parietal cortex is activated during violations of established predictions, also known as "prediction error" (Bucci, Holland, & Gallagher, 1998; Holland & Gallagher, 1993).

Bucci and MacLeod (2007) conducted a study to investigate the cortical changes that occur during trials in which errors in prediction take place. Previous research has shown that the CeA and the cholinergic substantia innominata/nucleus (SI) are critical for the processing of prediction errors (Holland & Gallagher, 2006). Using a complex Pavlovian conditioning paradigm known as the incremental attention task, originally designed by Wilson, Boumphrey, and Pearce (1992), Bucci and MacLeod (2007) measured brain activity at different time points in the incremental attention task.

In Phase I of the task, rats were given four random 10 s light, 10 s tone, food trials and four light-tone-nothing presentations. In Phase II, one group of rats received (consistent) identical light-tone-food, light-tone-nothing trials presented in Phase I. A second group of rats received (shift group) random light-tone-food and light-no food trials. The shift group

was presented with a violation of a previously established association amongst cues. In Phase III all subjects received only light-food pairings no matter their Phase II grouping. Phase III involved learning of a new, direct association between the light and the food pellet US.

Bucci and MacLeod (2007) hypothesized that Fos expression would be greater in select cortical regions of the cortex and especially in the visual and auditory cortex of the surprise/shift group (Bucci & MacLeod, 2007). Brain regions active during conditioning show a greater concentration of Fos positive nuclei in comparison to brain regions not active during conditioning. Behavior was evaluated by snout entries into a food magazine located in the chamber. Rats were sacrificed after Phase 3 testing and brains were sectioned and stained for Fos positive nuclei. Bilateral sections of the primary auditory cortex, secondary auditory cortex (dorsal, Au2d, ventral, Au2v), cingulate cortex (Cg), frontal association cortex, (FrA), PPC, RSP, primary visual cortex (V1), secondary visual cortex (mediomedial, V2MM, mediolateral, V2ML, and lateral, V2L), substantial innominata, and central nucleus of the

amygdala were analyzed for positively stained nuclei. Overall results showed that concentrated staining was found in middle and deep layers of cortex, while superficial layers lacked positive Fos staining. The PPC of rats in the surprise/shift group showed significantly more Fos positive nuclei than rats in the consistent group; significant differences were not found in any of the other structures. Fos-positive cells were found in greater quantities in the substantia innominata and amygdala of surprise/shift rats. The BLA of rats in the consistent group showed greater staining than those in the surprise/shift group, contrary to researcher expectations.

Bucci and MacLeod (2007) note that the higher expression of Fos in the PPC of rats in the surprise/shift group suggests that the PPC may be a critical component in enhanced attention for violations of previously established associations (Bucci & MacLeod, 2007). These results coincide with findings of Bucci, Holland, and Gallagher (1998), demonstrating that with disruption of cholinergic projections to the PPC, impairments were found in surprise-induced attention. Importantly, the CeA of rats in the shift condition

expressed greater Fos positive nuclei (Bucci & MacLeod, 2007). Although increased amounts of Fos were found in the substantia innominata, the authors propose that this region is active during surprise but is only necessary during the enhanced attention for the light in Phase III.

Holland, Thornton, and Ciali (2000) conducted an experiment in which rats were given bilateral ibotenic lesions of the CeA and were tested in the negative patterning task. In negative patterning, a CS_a alone (white noise) presentation is followed by a US (sucrose); then another separate CS_b (light) presentation is followed by the US. However, when CS_a and CS_b are presented simultaneously, the US is not delivered. Negative patterning is designed to decrease responding to the compound stimulus and increase responding to individual CS presentations.

Holland, Thornton, and Ciali (2000) found that lesions of the CeA impaired the ability to refrain from making non-adaptive responses (responding to non-reinforced CSs; Holland et al., 2000). These findings suggest that the CeA mediates the attentional demands of differentiating between (adaptive) reinforced and (non-adaptive) non-reinforced trials.

Neurobiology of Attention in Pavlovian Conditioning

According to Ledoux (2007) the amygdala is instrumental in regulating and modulating both attention and emotion. The amygdala is central in the processing of emotional stimuli and to determine the significance of environmental events, allows attention to be directed to the appropriate stimulus. Packard, Cahill, and McGaugh (2000) hypothesize that the amygdala enhances memory formation and subsequent storage, but is not the principal mechanism driving memory formation for attended events.

The amygdala is composed of several subnuclei, but research has implicated the BLA in mediating memory formation (McGaugh, Roozendaal, & Cahill, 2000). Gallagher and Holland (1994) illustrate that although the amygdala is central in emotional and fear conditioning, less is known concerning its role in attention. The amygdala is implicated in fear conditioning, eyeblink reflexive conditioning, conditioned changes in heart rate, potentiation of startle, and more recently, in mediating attention (Churchill, Green, & Voss, 2001;

Hardesty & Rudy, 2007; Walker, Ressler, Lu, & Davis, 2002).

Maddux et al. (2007) conducted two experiments to differentiate between "attention in learning" and "attention in action". In Experiment 1, it was hypothesized that rats with lesions of the CeA or medial frontal cortex (MFC) would show impairments in the five-choice serial reaction time (5-CSRT) task compared to intact control and PPC lesioned rats.

Long-Evans rats were tested in the 5-CRST task, under which port illuminations cued subjects to enter the port where reinforcement was provided. Subjects were first presented with a ready light over the port entry; access to reward was indicated by simultaneous illumination of the ready light and the food magazine light. In the first trials rats were consistently reinforced on all correct responses. Once subjects reached proficient levels of responding on the consistent reinforcement schedule, rats were switched to a partial schedule of reinforcement (50%). Two ports were set on a continuous schedule of reinforcement (1:1), two ports were set on a PRF schedule (50% reinforced trials), and one port never delivered reinforcement. To deliver a

range of cues experimenters shortened and dimmed light presentations to manipulate attentional demand. Results from Experiment 1 demonstrated that continuous reinforcement cues produced a greater correct and fewer error responses compared to rats subjected to the PRF schedule. Behavior was measured by a percentage of correct responses to illuminated arm entries of the 5CSRT apparatus.

As predicted, training with the PRF schedule of reinforcement and light CS, overshadowed responding of high tone presentation with the continuous schedule of reinforcement (Maddux et al., 2007). Differential overshadowing was not obtained for rats with lesions of the CeA or cholinergic lesions to the PPC. Results demonstrate that the MFC is not crucial for systems supporting surprise-induced associability (Maddux et. al., 2007).

Maddux et al. (2007) showed that disruption of the cholinergic projections of the MFC, impaired 5-CSRT performance. In contrast, lesions did not disrupt surprise-induced enhancement of learning and cholinergic lesions of the PPC did not impair 5-CSRT performance. The findings of this study support views that the MFC is

crucial for attention in action and the PPC is crucial for attention in new learning.

A study by McGaughy, Dalley, Morisson, Everitt, and Robbins (2002) shows that 192 IgG-saporin, a selective cholinergic immunotoxin, lesioning of the SI/nBM impairs 5-CSRT and reduces acetylcholine (ACh) in the MFC compared to sham-lesioned rats. McGaughy et al. (2002) found that 192 IgG-saporin lesions of the SI/nBM produced both impairments in behavior and reductions in ACh efflux in the MFC during the 5-CSRT task. These findings suggest a relationship between selective damage of the basal forebrain and subsequent decreases in ACh with impairments in attentional function.

Holland (2007) demonstrates how disruptions of the CeA and SI/nBM circuit impairs the ability to perform attentional demanding tasks. The disconnection of these structures deprives the cortex of the cholinergic innervation needed for surprise-induced learning. In Experiment 1, Holland (2007) examined the effects of partially reinforced schedules of reinforcement on attention in a 5-CSRT task, after training with a consistent contingency. In a second experiment a disconnection of the CeA and SI/nBM circuit was tested

under the same conditions as Experiment 1. Rats received unilateral lesions of the CeA and SI/NBM. While other subjects received contralateral lesions, CeA lesion in one hemisphere and SI/NBM lesion on the contralateral hemisphere. Ipsilateral lesions left an intact CeA and SI/NBM circuit in one hemisphere while contralateral lesions disrupted both circuits. This disassociation between lesions is possible due to ipsilateral connections between the CeA and SI/NBM. Rats were subject to identical behavioral procedures conducted in the first experiment.

Holland (2007) found that the neural circuit connecting the CeA and SI/NBM is necessary for mediating the attentional demands of briefly presented cues. Holland (2007) illustrates that a CeA and SI/NBM circuit may function as an early stage facilitator of attention. Holland (2007) proposes that the amygdala triggers the neural circuitry needed to facilitate attention for new learning, and that a connection between the CeA and SI/NBM is, in fact, necessary but not the solitary engine driving attention.

In conclusion, attention is a multifaceted and complex phenomenon. The ability to direct attention to

biologically relevant cues and ignore non-adaptive cues may be modulated by several systems with inputs from various regions. Among these, the amygdala and the SI/NBM are especially important.

CHAPTER FOUR

THESIS EXPERIMENTS

Introduction

The objective of the current research was to investigate the function of the NMDA receptor in memory formation in an attentionally-mediated "prediction error" task. Prediction error refers to a disparity between predicted outcomes and actual outcomes experienced during associative learning. In Pavlovian conditioning, prediction error occurs on early conditioning trials where the unconditioned stimulus (US) is not yet fully predicted by the presentation of the conditioned stimulus (CS). The error in predicting the US based on CS occurrence early in conditioning is argued to enhance attention to the CS and thereby increase the rate of learning for that CS as a predictor for the US. According to Pearce and Hall (1980), the magnitude of the prediction error on a given conditioning trial determines the associability (i.e., the rate of subsequent conditioning the CS will support) of the CS on that trial. Thus, as learning proceeds and the US becomes better predicted by the CS, the associability of that CS,

somewhat paradoxically, decreases. The predictive strength of the CS, however, does progressively increase across training as expected.

Incremental Attention

After conditioning has been established, prediction error can occur if an animal experiences an unexpected violation of previously established predictive relationships among CSs and USs (Pearce & Hall, 1980). The Pavlovian conditioning task known as the incremental attention paradigm involves such a violation of conditioned expectations (Wilson, Boumphrey, & Pearce, 1992). This task is designed such that a surprising prediction error is produced when, after initial training, an expected outcome does not occur following its usual predictive CS signal. As a result of this prediction error, attention to the affected CS is enhanced such that the CS gains in associability and new associations involving that CS are subsequently learned more quickly. This increase in associability can be assessed by measuring the rate with which the affected CS enters into new associations with a US (Holland & Gallagher, 2006; Wilson et al., 1992).

Training in the incremental attention task occurs in three phases, with one group of rats (Consistent Prediction group) exposed to a consistent relationship among cues (leading to a decrease in associability of those cues), and another group of rats (Predictive Shift group) exposed to a surprising shift in the predictive relationship among cues (leading to an increase in associability of the cues). In Phase I of the incremental attention task, rats are presented with serial conditioning trials where a visual CS (light) and an auditory CS (white noise) are presented sequentially. On half of these trials, the light-noise sequence is followed by a food pellet US (light-noise-US), and on the other half of the trials the light-noise sequence is not followed by the US (light-noise). Compared to the light, the noise CS acquires substantial predictive value (i.e., associative strength) due to its close temporal proximity and strong contingent relationship with the US. Moreover, as the relationship between light and the noise becomes better established, animals will pay progressively less attention to the light. A similar decrement in attention to the noise also occurs as the noise becomes an

established predictor of the US (Holland & Gallagher, 2006; Wilson et al., 1992).

In Phase II, the Predictive Shift group experiences "surprising" trials where attention to the light CS is increased by altering its relationship to the noise CS. During this phase, rats in the Predictive Shift group continue to receive the light-noise-US sequence on half of their trials, but the light-noise trials are replaced by light alone trials. This change in the predictive relationship between light and noise results in increased attentional processing of the light. Rats in the Consistent Prediction group simply continue to receive light-noise-US and light-noise trials just as they did during Phase I. Thus, attention to the noise, and especially to the light, continues to diminish in the Consistent Prediction group during Phase II.

Changes in associability resulting from surprising prediction error occurring in Phase II are assessed in Phase III of the incremental attention task. In this phase, the light is paired directly with the US (light-US). Rats in the Predictive Shift group typically show faster conditioning to light compared to rats in the Consistent Prediction group. This is because the

surprising trials experienced in Phase II result in an increase in attention to the light. In contrast, rats in the Consistent Prediction group have not undergone this increase in attention and therefore tend to ignore the light CS and learn more slowly than rats in the Predictive Shift group during Phase III.

Neural Substrates of Incremental Attention

Holland and Gallagher (2006) demonstrate that the CeA is critical for the surprise-induced enhancement of attention and the subsequent facilitation of learning in the incremental attention task. These researchers found that the CeA is necessary at the time of surprise but is not necessary during subsequent assessment of enhanced attention in Phase III of the task. This conclusion is based on the finding that disrupting CeA function with the competitive AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) disrupted Phase III learning if infusions were administered during surprising trials in Phase II, but did not affect performance when injected during Phase III.

Injections of NBQX into the SI/NBM, which send cholinergic projections to the neocortex, during Phase II

did not prevent enhancement of conditioning in Phase III. However, inhibition of the SI/NBM by NQBX during Phase III testing blocked the enhanced attention to the CS and rats in this condition learned no more quickly than rats in the Consistent Prediction condition. Thus, Holland and Gallagher (2006) conclude that the CeA is critical for prediction error processing, and the SI/NBM is critical for expressing the resulting enhanced attention to the affected CS.

Holland and colleagues (Lee, Youn, & Holland, 2008) also demonstrated that the connections between the CeA and substantia nigra (SNc) must be intact for processing prediction error during surprising trials during Phase II. In contrast, communication between CeA and SNc is not necessary for expression of surprise-induced enhancement in later learning during Phase III.

Using C-Fos expression methods, Bucci and Macleod (2007) demonstrated that the CeA and SI/NBM are active at the time of unexpected violations of previously established associations in Phase II. Following surprising trials, the CeA and the SI/nBM showed an increase in C-Fos positive nuclei, while the BLA and substantia nigra pars compacta did not show increased Fos

expression. Thus, although Holland and Gallagher (2006) showed that activity in the SI/NBM is not necessary for prediction error processing, the C-Fos data of Bucci and Macleod (2007) demonstrated that the SI/NBM is nevertheless active during the surprising trials of Phase II. The SI/NBM becomes critically involved, however, in Phase III where enhanced attention to the affected CS translates into a greater rate of conditioning compared to the learning rate in the Consistent Prediction group.

Neural Mechanisms of Memory for Prediction Error

While the neuroanatomy underlying the incremental attention phenomenon is becoming better understood through temporary and permanent lesion experiments (e.g., Bucci & Macleod, 2007; Holland & Gallagher, 2006; Lee, Youn, & Holland, 2008), little is known about the synaptic mechanisms underlying memory formation resulting from the error prediction experience. A likely candidate for memory formation during Phase II surprising trials is NMDA receptor-dependent synaptic plasticity. It has long been established that NMDA receptor activation is necessary for the induction of long-term potentiation (LTP), a form of synaptic plasticity linked to learning

and memory (Lynch, 2004). However, no experiments to date have explored the potential link between NMDA receptor-dependent memory formation and the process of error detection. The proposed experiments examine the possibility that NMDA receptor antagonism or facilitation during the surprising phase (Phase II) of the incremental attention task can disrupt or enhance memory for violations of conditioned expectations, respectively. Such disruption or enhancement of memory for prediction error will be assessed in Phase III of the incremental attention task, in a test of new learning involving the affected CS.

Hypotheses

The current experiment selectively disrupted or facilitated NMDA receptor function during exposure to surprising trials in Phase II of the incremental attention paradigm. Systemic injections of the non-competitive NMDA receptor antagonist MK-801 (0.15, 0.20 mg/kg, i.p.), were administered prior to Phase II training. NMDA receptor blockade was hypothesized to interfere with the memory for the prediction error encountered during surprising trials. This failure to

consolidate memory for the prediction error experience was expected to attenuate the enhanced learning normally observed during Phase III conditioning trials as compared to saline-treated controls.

Conversely, the NMDA receptor agonist D-cycloserine (15.0, 20.0 mg/kg, i.p.), a partial agonist at the strychnine-insensitive glycine binding site on the NMDA receptor, was expected to facilitate memory formation during surprising trials in Phase II. Therefore, subsequent appetitive conditioning using the affected cue was expected to be enhanced in the D-cycloserine-treated rats as compared to saline-treated controls.

The effects of NMDA receptor manipulations on memory consolidation during the error detection phase (Phase II) of the incremental attention paradigm was subsequently assessed in a novel association learning task on the following day in Phase III. Importantly, Phase III testing was conducted in the absence of drug treatment, such that drug effects were limited to memory consolidation following Phase II, rather than reflecting drug-induced changes in performance. Subsequent to conditioning in the surprising or consistent conditions, both groups were trained in Phase III where the light CS

was paired directly with the sucrose pellet US. Surprise-induced enhancement of associability of the light CS is reflected by a greater learning rate than is observed in animals that do not experience prediction error (see Holland & Gallagher, 2006). The MK-801-treated groups in the surprise condition, however, were expected to show reductions in enhanced learning compared to saline-treated controls trained in the same task. It is further expected the higher dose of MK-801 would produce greater deficits than the lower dose in rats trained in the predictive shift condition.

The D-cycloserine-treated group in the surprise condition, conversely, was expected to show even greater levels of enhanced learning in Phase III than saline-treated controls. It was expected that the higher dose of D-cycloserine will result in greater levels of enhancement than the lower dose, but that both D-cycloserine-treated groups will outperform saline-treated rats.

In contrast to predictions for rats in the Predictive Shift condition, NMDA receptor modulation in the Consistent Prediction drug-treated groups was not expected to change subsequent learning in Phase III. The

Consistent Prediction groups do not experience an unexpected violation of predictions during Phase II, unlike the Predictive shift groups. Instead, this group merely undergoes a continuation of training parameters experienced in Phase I of the incremental attention task. Therefore, no new learning takes place in the Consistent Prediction condition during Phase II. Consequently, manipulations of NMDA receptor activity, and the hypothesized effects on memory consolidation during Phase II training were expected to be without effect during subsequent learning in Phase III in these animals.

Methods

Guidelines for Animal Use

The following procedures involving research animals met the requirements set by the Guidelines for Ethical Conduct in the Care and Use of Animals (American Psychological Association, 2005) and the California State University, San Bernardino Animal Care and Use Committee.

Subjects

A total of 80 male Long-Evans rats (appx. weight 300 g upon arrival) were purchased from a commercial research animal vendor (Harlan, Indianapolis, IN). Rats

were individually housed under a 12 hr light/dark cycle (lights on at 18:00 hours) with *ad libitum* water and standard rat chow prior to testing. Beginning one week before testing rats were reduced to and maintained at 85% of their *ad libitum* weights by limiting access to food. Water access was provided *ad libitum*.

Apparatus

Training and testing were conducted in individual computer-controlled, sound-attenuating operant chambers (Coulbourn Instruments, Allentown, PA) equipped with a speaker capable of producing the white noise CS. The US consisted of the delivery of a single sucrose pellet (45 mg; MedAssociates, Lancaster, NH) into a magazine located at floor level. The onset and duration of snout entries into the food magazine during CS presentations and during the 10 s preceding these presentations were recorded using photo-beam response detectors (MedAssociates, Lancaster, NH) located inside the food magazine. A 5 W white light bulb located at the top of the chamber provided ambient illumination. The presentation of white noise and sucrose pellets, as well as response detection and recording were controlled by

computer interface (Coulbourn Instruments, Allentown, PA).

Behavioral Methods

Rats were first pre-exposed to 20 sucrose pellets in their home cage to reduce neophobic responses to the pellets during subsequent testing. The following day, rats were trained to locate sucrose pellets in the food magazine by placing them individually in the operant chambers with 10 sucrose pellets placed in the food cup. Rats were allowed to consume the sucrose pellets and explore the chamber for 1 hr. On the following day, rats began the incremental attention task.

As noted previously, this conditioning task consisted of two experimental conditions trained across three phases of testing. The first of the two main conditions of the task is termed the Predictive Shift condition, where established predictive relationships are violated and therefore lead to an increase in attention to relevant cues. The second condition is termed the Consistent Prediction condition, where a fixed relationship among predictive cues is established and maintained, leading to a decrease in attention to the well-established predictive cues.

Phase I of the task exposes rats in both conditions to identical trial types. In Phase I, all rats were exposed to 60 serial conditioning trials per day for 10 consecutive days. On every trial, an auditory CS (white noise: 10 s) and a visual CS (light: 10 s) were presented sequentially. Half of these trials were reinforced in that the light-white noise sequence was followed immediately by the sucrose pellet US (light-white noise-US), and the other half of trials were reinforced in that no US occurred (light-white noise). A variable inter-trial interval with an average of 40 s (ITI 40 s) separated each trial. Each trial type occurred pseudo-randomly such that no more than three trials of the same type occur consecutively and that an equal number of each trial type occurred within each of the two 30 min intervals per 1 h testing session.

In Phase II, the Predictive Shift group had attention to light manipulated (i.e., increased) by altering its relationship to white noise. These rats continued to receive 30 reinforced trials as in Phase I, but the 30 non-reinforced light-white noise trials were replaced by 30 light alone trials. The Consistent Prediction group simply continued to receive the same

trial types as in Phase I. Trials were again separated by an ITI of 40 s. Each trial type occurred pseudo-randomly such that no more than three trials of the same type occurred consecutively and that an equal number of each trial type occurred within each of the two 30 min intervals per 1 h testing session. Phase II testing took place on only one day.

In Phase III, both the Predictive Shift and Consistent Prediction conditions received 30 trials where the light CS is paired directly with the US (light-US). These trials were separated by a 100 s ITI, with a 1 h total session duration. Phase III testing took place on only one day. The incremental attention task is illustrated schematically in Figure 1.

Rats are trained in a Predictive Shift (SHIFT) condition or a Consistent Prediction (CONSIST). In Phase I of both conditions, animals receive serial conditioning trials where a light (L) is followed by a noise (N) and, on half of all trials, the N is followed by delivery of the unconditioned stimulus (US). For rats in the SHIFT condition, the L N trials are replaced by L only trials during Phase II, while rats in the CONSIST condition

continue to receive trials in the same manner as in Phase I.

Phase III training is identical for the SHIFT and CONSIST conditions and involves learning a new, direct association between L and US. Drug treatment occurred 40 min prior to training in Phase II, with sub groups of rats in the SHIFT or CONSIST training conditions receiving injections of saline (SAL), MK 801 (0.15 or 0.20 mg/kg), or DCS (15 or 20 mg/kg). The critical test of enhanced attention to the L occurred 24 hr later, during Phase III training.

Drug Administration

Forty minutes prior to the Phase II training session, separate groups of rats (n = 8 per group) receive systemic injections of the NMDA receptor antagonist MK-801 (0.15, 0.20 mg/kg dissolved in sterile saline, 1 ml/kg volume; i.p.), D-cycloserine (15.0, 20.0 mg/kg dissolved in sterile saline, 1 ml/kg volume; i.p.), or equivalent volume of physiologic saline (i.p.). Upon completion of Phase II testing, rats were returned to their home cages and behavioral testing in Phase III began 24 hr following Phase II injections.

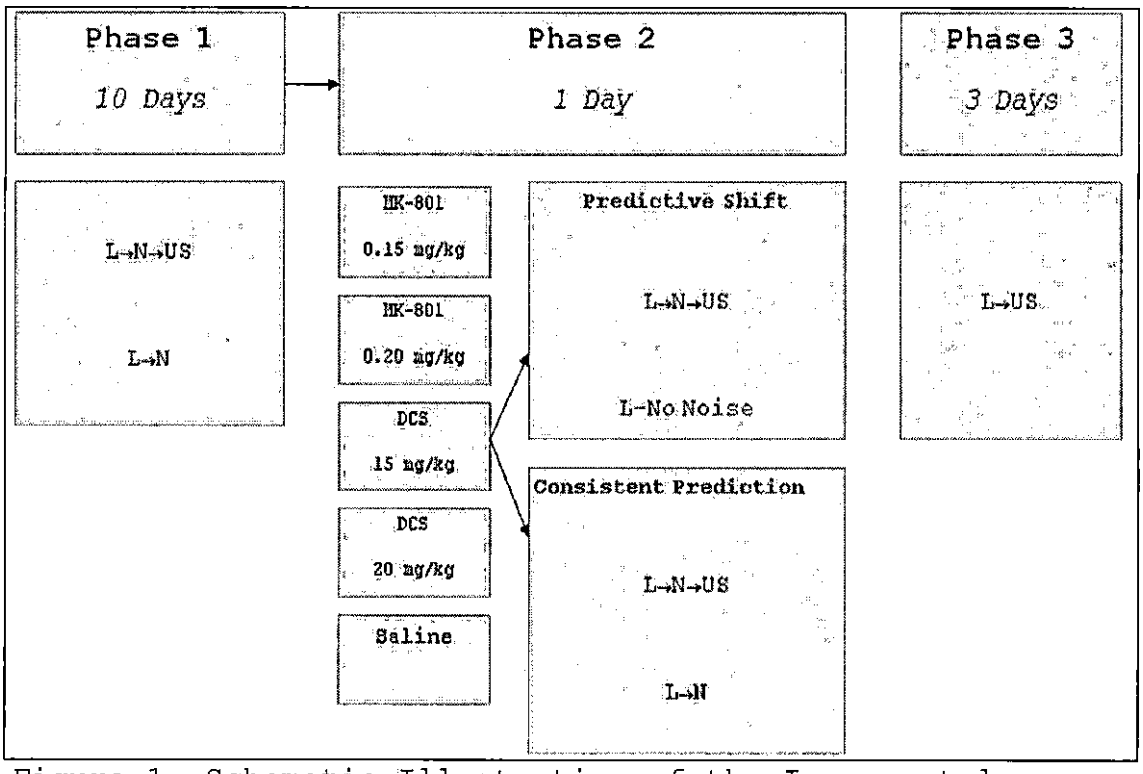

Figure 1. Schematic Illustration of the Incremental Attention Paradigm, Experimental Design, and Timeline

Data Analysis

During all phases of the behavioral task, the number of snout entries into the food magazine during each 10 s pre-CS interval and during the 10 CS intervals (i.e., intervals for light and white noise stimuli) were recorded in 2 s intervals via computer interface (Coulbourn, Allentown, PA).

Previous research has shown that maximal conditioned food cup approach occurs during the latter part of both

visual and auditory CS presentations (Bucci, Holland, & Gallagher, 1998; Holland & Gallagher, 2006). The CR will therefore be defined as the difference between the duration of snout entries during the last 4 s of each 10s CS interval from the duration of snout entries during the comparable pre-CS interval (i.e., mean baseline responding per 4 s during the 10 s immediately preceding CS presentation). These difference scores were analyzed by repeated measures analysis of variance (ANOVA).

The critical test of the hypotheses that drug treatment systematically affects performance was analyzed with comparisons of CR difference scores from Phase III of the incremental attention task. However, comparisons among the all groups in the Predictive Shift and Consistent Prediction conditions on performance during Phases I, where both conditions received identical training, were made in order to rule out the potential pre-existing differences among groups prior to drug treatment (which occurs immediately following Phase II testing). Therefore, omnibus repeated measures ANOVAs were used to compare the CR to the Light CS and Noise CS across the ten 60-trial Blocks in Phase I in the MK801-treated, DCS-treated, and saline-treated groups

tested in the Predictive Shift and Consistent Prediction groups.

Omnibus repeated measures ANOVAs were used to compare the CR to the Light CS across 5-trial Blocks in Phase III in the saline-treated and MK801-treated groups, and in the saline-treated and DCS-treated groups in the SHIFT condition. Ominibus ANOVAs were similarly conducted on data from the saline-treated and MK801-treated groups, and in the saline-treated and DCS-treated groups tested in the CONSIST condition. Significant findings from analysis of data from the SHIFT or CONSIST conditions were followed up with two-way ANOVAs in order to determine potential differences between drug-treated and saline-treated groups or between different doses of the same drug. Comparisons which did not meet the assumption of sphericity were consequently analyzed with Greenhouse-Geisser adjustments for degrees of freedom for within-group, between-group, and within-group interactions tests.

CHAPTER FIVE

RESULTS

Phase I Behavioral Data

Omnibus ANOVAs (CS Type x Group x Block) were performed on data for the CR to the two CS types (Light CS and Noise CS) from each group across ten 60-trial blocks (i.e., ten days). Because the training parameters for animals in the SHIFT and CONSIST groups were identical during Phase I, these data were analyzed together. This analysis yielded a significant main effect of CS Type ($F_{(5,\ 712)} = 81.58$, $p < .001$; Greenhouse-Geisser correction), with the level of CR to the Noise CS exceeding the CR to the Light CS. A significant main effect of Block also occurred ($F_{(5,\ 712)} = 12.05$, $p < .001$, Greenhouse-Geisser corrections) with groups showing an increase in CR to the Light CS and Noise CS across Blocks. No significant between-group or Group by Block interactions were observed (see Figure 2).

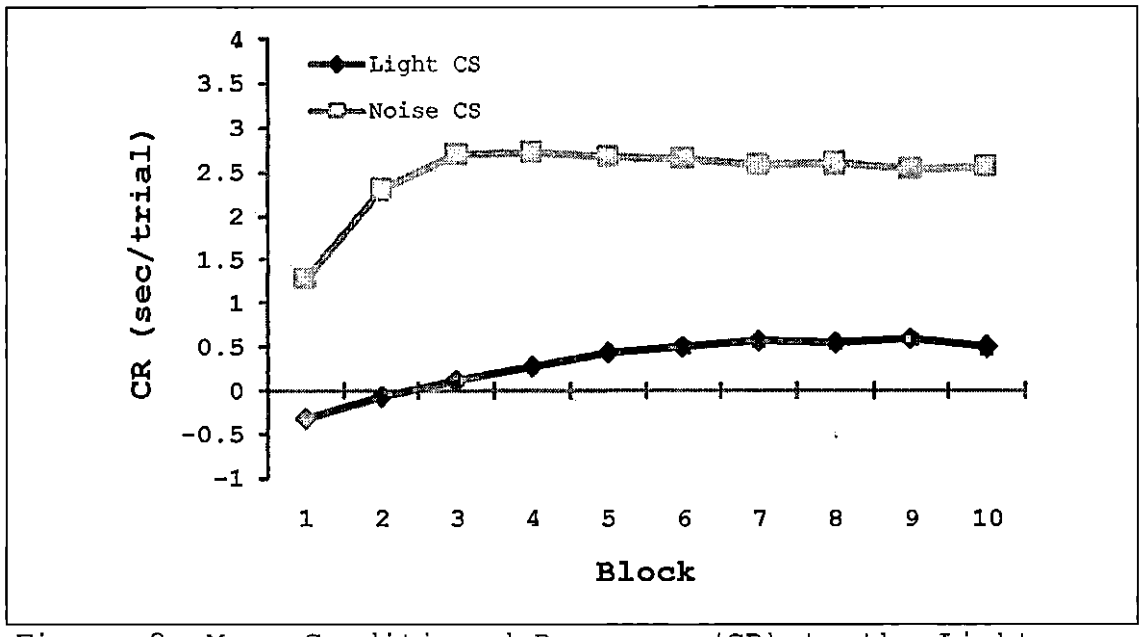

Figure 2. Mean Conditioned Response (CR) to the Light Conditioned Stimulus (CS) and to the Noise Conditioned Stimulus (CS) during Phase I in the Saline-Treated and Drug-Treated Groups Ultimately Tested in the SHIFT and CONSIST Conditions during Phase II. Groups did not Differ on Either the CR to the Light CS or Noise CS. Overall Responding to the Noise CS Exceeded Responding to the Light CS.

The observed pattern of responding to the Noise CS and Light CS, where greater conditioned responding occurred to the Noise CS, is consistent with the argument that animals attend more to the noise than to the light in this phase of the task (Holland & Gallagher, 2006).

The lack of Phase I group differences or Group by Block interactions for either CS allowed subsequent comparisons on Phase III data to be made without concern for potential pre-existing differences between the saline-treated and drug-treated groups.

Phase III Behavioral Data

Omnibus repeated measures ANOVAs were used to compare the CR to the Light CS across 5-trial Blocks in Phase III in the saline-treated and MK801-treated groups, and in the saline-treated and DCS-treated groups in the SHIFT condition. Similar omnibus ANOVAs were conducted on data from the saline-treated and MK801-treated groups, and in the saline-treated and DCS-treated groups tested in the CONSIST condition. When necessary two-way ANOVAs were used in order to determine potential differences between drug-treated and saline-treated groups or between different doses of the same drug.

Predictive Shift Condition

MK-801 Antagonist Drug Effects

Omnibus repeated measures ANOVA for CR scores from the two MK-801-treated groups and the SAL-SHIFT group revealed a within-group main effect ($F_{(5, 130)} = 6.48$,

$p < .001$) and Group by Block interaction ($F_{(10, 130)} = 2.97$, $p < .005$) and no between-group effects (see Figure 3).

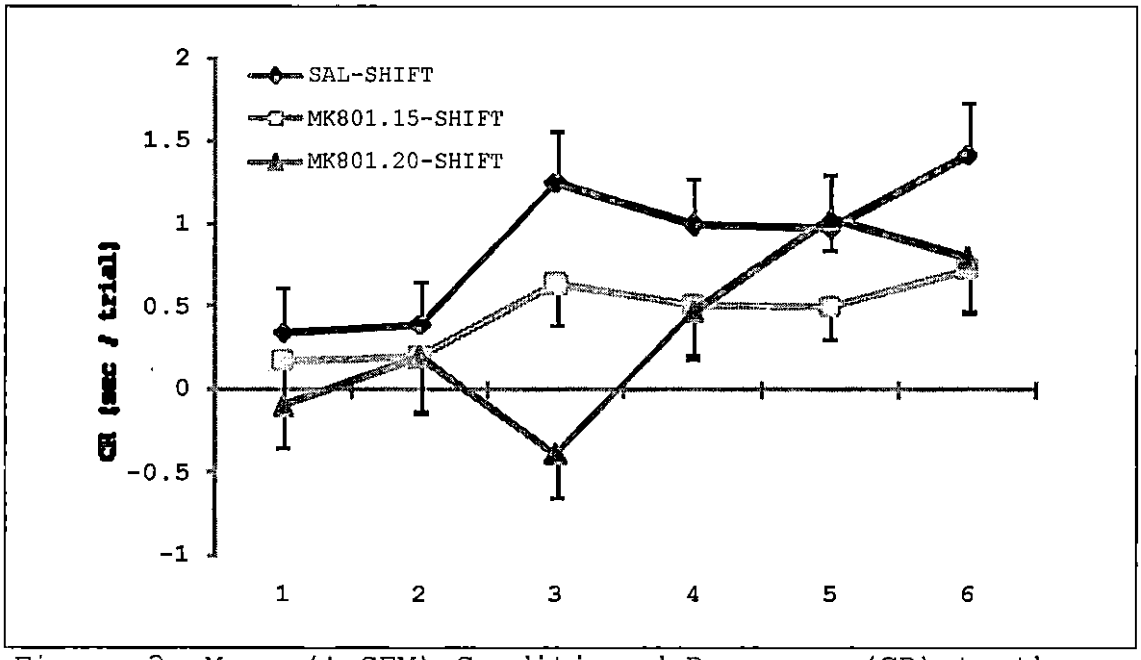

Figure 3. Mean (± SEM) Conditioned Response (CR) to the Light Conditioned Stimulus (CS) in the MK801.15-SHIFT, MK801.20-SHIFT, and SAL-SHIFT Groups. The SAL SHIFT Group Acquired the CR at a Greater Rate than Both the MK801.15 SHIFT ($p < .05$) and MK801.20 SHIFT ($p < .01$) groups.

Subsequent two-way repeated measures ANOVAs were performed on data for the CR to the Light CS and revealed a significant Group by Block interaction between the SAL-SHIFT and MK801.15-SHIFT groups, where the SAL-SHIFT group improved performance across Blocks at a greater

rate than the MK801.15-SHIFT group; $F_{(5, 95)} = 2.88$, $p < .05$. Tukey HSD post hoc analysis revealed that group differences on Blocks 3, 4, and 6 were statistically significant.

Two-way repeated measures ANOVAs on data from the SAL-SHIFT and MK801.20-SHIFT groups yielded a significant Group by Block interaction, again where the SAL-SHIFT group improved performance across Blocks at a greater rate than the MK801.20-SHIFT group; $F_{(5, 100)} = 4.79$, $p < .01$. Tukey HSD post hoc analysis revealed that group differences on Blocks 3 and 6 were statistically significant.

Two-way repeated measures ANOVAs comparing the MK801.15-SHIFT and MK801.20-SHIFT groups yielded a within-group effect of $F_{(5, 75)} = 4.45$, $p < .01$. No significant interaction or between group effects were observed. Thus, the 0.15 mg/kg and 0.20 mg/kg MK801-treated groups did not differ on the CR to the Light CS during Phase III, although both MK801-treated groups in the SHIFT condition showed impaired performance compared to the SAL-SHIFT control group.

D-Cycloserine Agonist Drug Effects

Omnibus repeated measures ANOVA for CR scores from the two DCS-treated groups and the SAL-SHIFT group revealed a within-group main effect ($F_{(5, 130)} = 8.60$, $p < .001$) and Group by Block interaction ($F_{(10, 130)} = 1.96$, $p < .05$) and no main between-group effects (see Figure 4).

Subsequent two-way repeated measures ANOVAs were performed on data for the CR to the Light CS from the SAL-SHIFT and DCS15-SHIFT groups, which revealed a within-group effect of $F_{(5, 95)} = 11.69$, $p < .001$. No significant between-group or Group by Block interaction effects occurred.

In contrast, two-way repeated measures ANOVAs were performed on data for the CR to the Light CS and they revealed a significant Group by Block interaction between the SAL-SHIFT and DCS20-SHIFT groups, where the SAL-SHIFT group improved performance across Blocks at a greater rate than the DCS20-SHIFT group; $F_{(5, 95)} = 2.39$, $p < .05$. Tukey HSD post hoc analysis revealed that group differences on Blocks 3, 5, and 6 were statistically significant.

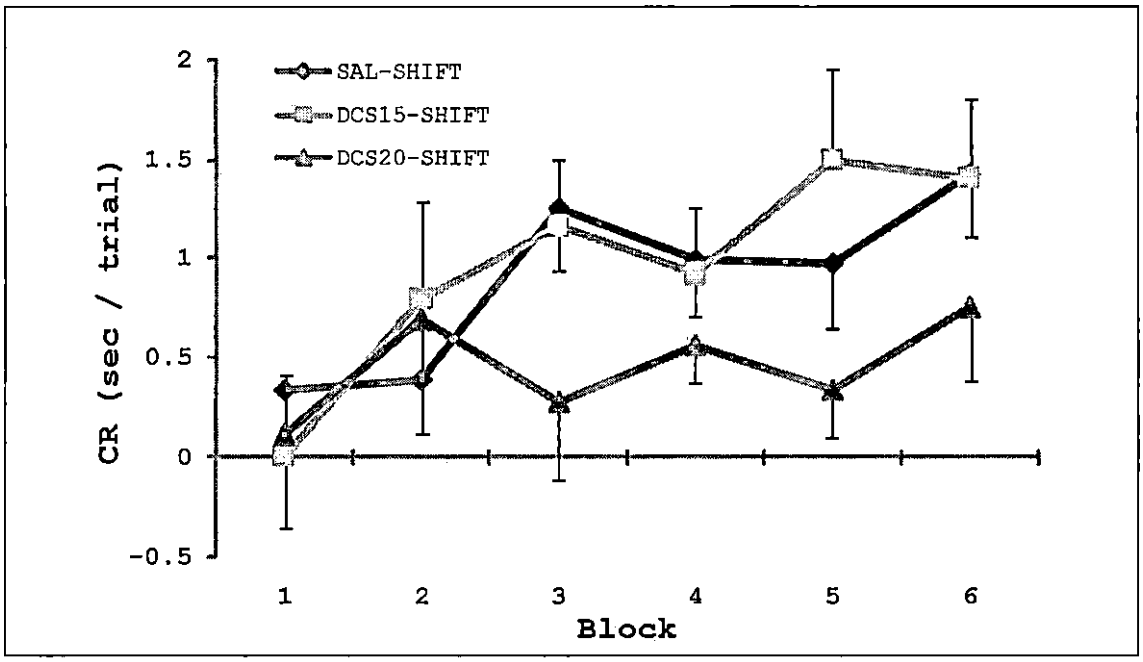

Figure 4. Mean (± SEM) Conditioned Response (CR) to the Light Conditioned Stimulus (CS) in the SAL-SHIFT, DCS15-SHIFT and SAL-SHIFT Groups during Phase III. The SAL SHIFT Group and DCS15 SHIFT Groups did not Differ. Compared to the SAL SHIFT Group, the DCS20 SHIFT Group Acquired the CR at a Significantly Slower Rate ($p < .05$)

Two-way repeated measures ANOVAs between the DCS15-SHIFT and DCS20-SHIFT yielded a within-group effect of $F_{(5, 70)} = 4.31$, $p < .01$. Between-group differences were not significant ($F_{(5, 70)}, = 2.04$, $p = .08$), despite the apparent differences in the means between the DCS15-SHIFT and DCS20-SHIFT. No significant Group by Block interaction effects were observed.

Consistent Prediction Condition

MK-801 Antagonist Drug Effects

As shown in Figure 5, an omnibus repeated measures ANOVA on data for the CR to the Light CS during Phase III revealed a significant main effect of Block ($F_{(3, 74)} = 4.896$, $p < .005$; Greenhouse-Geisser corrections). No significant between-group or Group by Block interaction effects were observed (see Figure 5).

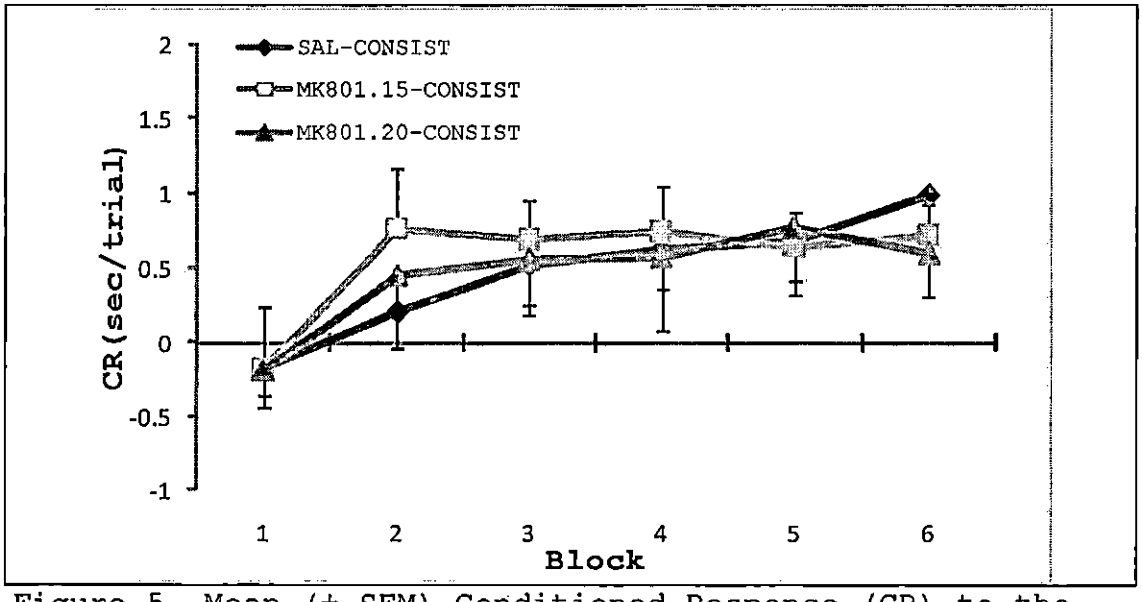

Figure 5. Mean (± SEM) Conditioned Response (CR) to the Light Conditioned Stimulus (CS) in the MK801.15-CONSIST, MK801.20-CONSIST, and SAL-CONSIST Groups during Phase III. Neither the MK801.15-SHIFT nor the MK801.20-SHIFT Groups Differed from the SAL-CONSIST Group.

D-Cycloserine Agonist Drug Effects

As shown in Figure 6, omnibus repeated measures ANOVA comparisons on data for the CR to the Light CS during Phase III revealed a significant main effect of Block ($F_{(5, 115)} = 5.50$, $p < .001$). No significant between-group or Group by Block interaction effects were observed.

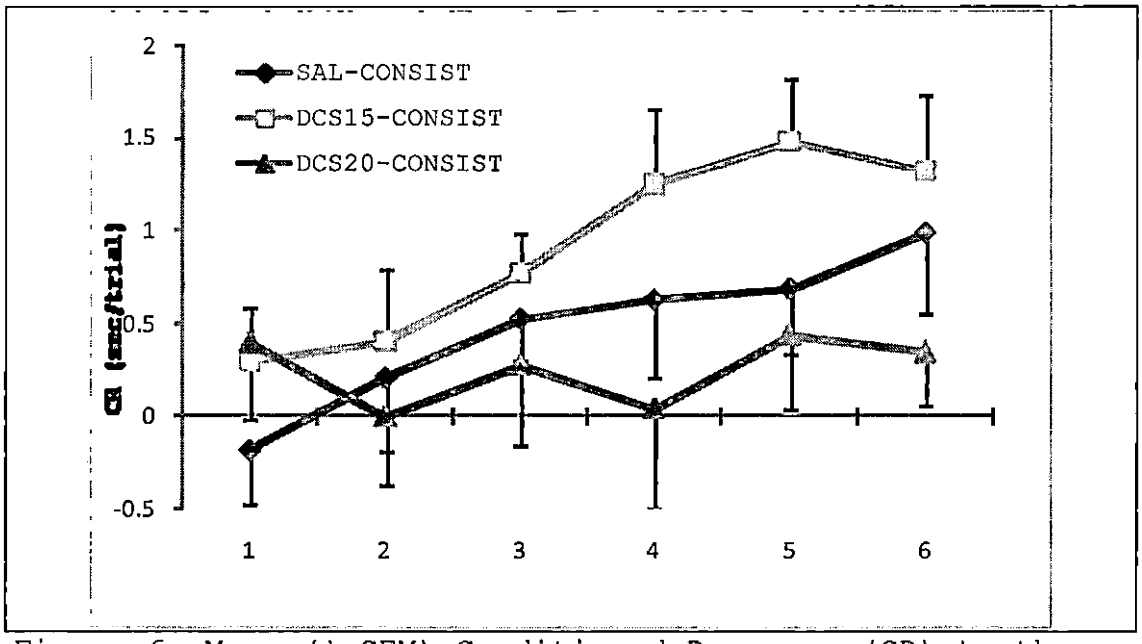

Figure 6. Mean (± SEM) Conditioned Response (CR) to the Light Conditioned Stimulus (CS) in the DCS15-CONSIST, DCS20-CONSIST, and SAL-CONSIST Groups during Phase III. Neither the DCS15-CONSIST nor the DCS20-CONSIST Groups Differed from the SAL-CONSIST Group.

CHAPTER SIX

DISCUSSION

Introduction

The results of the present experiment support the hypothesis that NMDA receptor blockade would disrupt the formation of memory for unexpected violations of previously learned associations. Treatment with both doses of the NMDA receptor antagonist MK-801 (0.15, 0.20 mg/kg), given to rats in the predictive shift condition immediately prior to Phase II prediction error trials, subsequently prevented the expression of enhanced new learning normally observed in Phase III of the incremental attention task. Although memory impairment was observed following treatment with both doses of MK-801, as evidenced by the lack of enhanced acquisition of the CR during Phase III, there were no differences in the rate of learning in the high and low dose MK-801-treated groups in the shift condition as originally anticipated. Nevertheless, these findings demonstrate that blocking NMDA receptor function during surprising trials impairs subsequent enhancements in attention to predictive cues. These results suggest that

NMDA receptor function is necessary for the formation of memories for prediction errors.

The impairments observed in the MK-801-treated groups in the shift condition were not a reflection of potentially long-lasting, non-specific drug effects such as decreased appetite and decreased motor control which are typical symptoms found with MK-801 administration (Gilmour et al., 2009). This argument is based on the lack of differences observed between the MK-801- and saline-treated rats in the consistent condition, where enhanced conditioning was not expected. This outcome supports the hypothesis that NMDA receptor function is necessary for memory formation in Phase II needed to increase responding for Light CS presentations in Phase III. Additionally, post-treatment differences in Phase III acquisition cannot be attributed to pre-existing group differences, as pre-treatment conditioned responding in the saline- and MK-801-treated groups did not differ during Phase I.

The lack of MK-801 dose-dependent impairment in Phase III conditioning may have occurred due to several reasons. It is possible that the behavioral impairment caused by the low dose of MK-801 represents a floor for

acquisition in Phase III such that higher doses would have no further effects on behavior. Another possibility is that the lower dose of 0.15 mg/kg may have reached maximal antagonistic effects at the NMDA receptor (i.e., saturation), limiting the opportunity for higher doses of MK-801 to further block NMDA receptors. Alternatively, there may not have been a sufficient differentiation between the doses used. With a greater range between doses of MK-801, a dose-dependent effect may have occurred in the current study (see Wozniak et al., 1990).

In contrast to NMDA receptor blockade, which was expected to impair memory, NMDA receptor agonist treatment was hypothesized to improve memory and thereby further enhance Phase III conditioning in the incremental attention task. Contrary to this hypothesis, however, rats in the predictive shift condition treated with the NMDA receptor partial agonist DCS at the low dose (15.0 mg/kg) failed to enhance new learning. Specifically, acquisition of the CR in the DCS15-SHIFT group was no more rapid than that observed in the saline-treated controls during Phase III testing.

One possible explanation for the lack of enhanced memory in the low dose DCS group tested in the predictive

shift condition may be that animals in both the DCS-treated and saline-treated groups quickly reached asymptotic performance in Phase III acquisition of the CR. Even if the low dose of DCS facilitated memory for prediction error in Phase II, potential increases in attention to the Light CS in Phase III may have had no measurable effect relative to the already rapid learning rate observed in the saline-treated control group. Indeed, the Light CS - sucrose pellet US conditioning procedure in Phase III is ultimately a very simple association that is readily learned, even in rats trained in the consistent prediction condition.

Surprisingly, Phase II administration of 20.0 mg/kg DCS to rats in the predictive shift condition not only failed to enhance subsequent learning, but instead caused significant impairment in Phase III conditioning. The differences observed between the DCS20-SHIFT and SAL-SHIFT groups cannot be attributed to non-specific drug effects because no such acquisition differences occurred between the DCS 20 mg/kg group and the saline-treated group tested in the consistent prediction condition. Instead, the observed impairment in the DCS20-SHIFT group appears to have resulted from a

paradoxical attenuation of NMDA receptor activity during the surprising trials in Phase II of the incremental attention task. Although the high dose DCS effects were paradoxical, these findings nevertheless demonstrate NMDA receptor involvement in the memory mechanisms underlying enhanced attention to cues whose predictive value has changed.

The impairment caused by the high dose of DCS may be due to the mechanism by which DCS normally facilitates NMDA receptor function. As mentioned previously, DCS is a partial agonist at the glycine site of the of NMDA receptor. Consequently, when endogenous glycine levels are relatively low, DCS administration can indirectly increase glutamatergic activation of the NMDA receptor (Norberg et al., 2008). Conversely, because DCS is less efficacious than endogenous glycine in enhancing NMDA receptor activation, high levels of DCS can interfere with glycine binding and thereby reduce NMDA receptor function by as much as 50% (Norberg et al., 2008). This mechanism of the reduced NMDA receptor activation might account for the memory impairments observed in the DCS20-SHIFT group in the current study.

Possible Anatomical Substrates

The enhanced conditioning that occurs after experiencing surprising events is dependent upon circuitry involving the central nucleus of the amygdala (Holland & Gallagher, 1999, 2006). Because NMDA receptors are found in high concentration on neurons of the central nucleus of the amygdala (de Armentia & Sah, 2007), it is therefore possible that the NMDA agonist and antagonist drug effects on performance during Phase III were due to changes in NMDA receptor function in the amygdala occurring during Phase II prediction error trials.

Amygdala NMDA receptor involvement in memory formation has been demonstrated in a number of studies (Lee & Kim, 1998; Mao, Hsiao, & Gean, 2006; Maren, 1999; Pistell & Falls, 2008). For example, direct infusion of NMDA receptor antagonists into the amygdala interfere with the acquisition of a conditioned fear response (Pistell & Falls, 2008). Although amygdala NMDA receptor involvement in memory for prediction error has not previously been directly studied, Lee and Kim (1998) present results consistent with the view that NMDA receptors on amygdaloid neurons play a role in memory for surprising violations of conditioned expectations. These

researchers exposed rats to fear-conditioning trials with a light CS and shock US. Next, they infused the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV) into the basolateral nucleus of the amygdala and found that NMDA receptor blockade completely prevented subsequent fear conditioning to a novel tone CS. The unexpected change in reliable predictors for the shock US in this paradigm can be seen as an example of prediction error. Therefore, this study provides support for the suggestion that NMDA receptor activity in the amygdala is necessary to store new memories following prediction error experience.

Conclusion

The pharmacological blockade of the NMDA receptor by MK-801 or, paradoxically, by high doses of DCS, during surprising trials results in impaired memory for prediction error. In saline-treated animals, the unexpected violation of a previously established association (i.e., surprise) typically leads to an increase in attention to the CS that has had its predictive value altered. This increment in attention to the CS thereby leads to an increased rate of acquisition

for new associations involving that CS (Holland & Gallagher, 2006). NMDA receptor blockade prevented this enhancement in conditioning.

These results support the hypothesis that memory for prediction error is NMDA receptor-dependent, and further suggests that such memory is necessary for the expression of attention-dependent enhancement of subsequent conditioning. Findings from this experiment demonstrate the interplay between an NMDA receptor-dependent memory system for prediction error, normally active during surprising conditioning trials, and an attention system (e.g., substantia innominata/nucleus basalis magnocellularis), which must subsequently become activated in order to express enhanced conditioning in the incremental conditioning paradigm (see Holland & Gallagher, 2006). These interacting systems may serve as the neural basis for a self-correcting association learning mechanism, where memory influences attention, and attention modifies memory.

REFERENCES

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2008). *Molecular biology of the cell* (2th ed.). New York: Garland Science.

American Psychological Association. (2005). *Guidelines for ethical conduct in the care and use of animals.* Washington DC: Author.

Barnes, C. A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *Journal of Comparative Physiological Psychology, 93,* 74-104.

Bliss, T. V. P., & Collingridge, G. L. (1993). A synaptic model of memory long-term potentiation in the hippocampus. *Nature, 361,* 31-39.

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

Bucci, D. J., Holland, P. C., & Gallagher, P.C. (1998). Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *Journal of Neuroscience, 18,* 8038-8046.

Bucci, D., & MacLeod, J. E. (2007). Changes in neural activity associated with a surprising change in the predictive validity of a conditioned stimulus. *European Journal of Neuroscience,* 26, 2669-2676.

Churchill, J. D., Green, J. T., & Voss, S. E. (2001). Discrimination reversal conditioning of an eyeblink response is impaired by NMDA receptor blockade. *Integrative Physiological and Behavioral Science, 36,* 62-74.

Clark, R. E., Manns, J. R., & Squire, L. R. (2002). Classical conditioning, awareness, and brain systems. *Trends in Cognitive Sciences, 6,* 524-531.

Collingridge, G. L., Kehl, S. J., & McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *Journal of Physiology, 34,* 334-345.

De Armentia, M. L., & Sah (2007). Bidirectional synaptic plasticity at nociceptive afferents in the rat central amygdala. *Journal of Physiology, 581,* 961-970.

De Lima, M. N. M., Laranja, D. C., Bromberg, E., Roesler, R., & Schroder, N. (2005). Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behavioural Brain Research, 156,* 139-143.

Easley, H. (1931). An attempt to isolate the factor of "attention". *American Journal of Psychology, 43,* 202-215.

Elvander-Tottie, E., Eriksson, T. M., Sandin, J., & Ogren, S. O. (2006). *N*-methyl-D-asparate receptors in the medial septal area have a role in spatial and emotional learning in the rat. *Neuroscience, 142,* 963-978.

Gallagher, M., & Holland, P. C. (1994). The amygdala complex: Multiple roles in associative learning and attention. *Proceedings of the National Academy of Sciences of the United States of America, 91,* 11771-11776.

Giese, K. P., Fedorov, N. B., Filipkowski, R. K., & Silva, A. J. (1998). Autophosphorylation at Thr286 of the alpha calcium-modulin kinase II in LTP and learning. *Science, 279,* 870-873.

Gilmour, G., Pioli, E. Y., Dix, S. L., Smith, J. W., Conway, M. W., Jones, W. T., Loomis, S., Mason, R., & Shahabi, S. (2009). Diverse and often opposite behavioural effects of NMDA receptor antagonists in rats: Implications for "NMDA antagonist modeling" of schizophrenia. *Psychopharmacology, 205,* 1530-1537.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learning and Memory, 8,* 148-155.

Goosens, K. A., & Maren, S. (2004). NMDA receptors are essential for the acquisition, but not expression, of conditional fear and associative spike firing in the lateral amygdala. *European Journal of Neuroscience, 20,* 537-548.

Grover, L. M., & Yan, C. (1999). Blockade of $GABA_A$ receptors facilitates induction of NMDA receptor-independent long-term potentiation. *Journal of Neurophysiology, 81,* 2814-2822.

Gruart, A., Munoz, M. D., & Delgado-Garcia, J. M. (2006). Involvement of the CA3-CA1 synapse in the acquisition of associated learning in behaving mice. *The Journal of Neuroscience, 26,* 1077-1087.

Han, C. J., O'Tuathaigh, C. M., van Tright, L., Quinn, J. J., Fanselow, M. S., Mongeau, R., Koch, C., & Anderson, D. J. (2003). Trace but not delay fear conditioning requires attention and the anterior cingulate cortex. *Proceeding of the National Academy of Sciences, United States of America, 100,* 13087-13092.

Hebb, D. O. (1949). *The Organization of Behaviour.* New York: Wiley.

Highfield, D., Nixon, K., & Amsel, A. (1996). The NMDA antagonist MK-801 affects nonspatial learning in preweanling rats. *Behavioral Neuroscience, 110,* 300-304.

Holland, P. C. (2007). Disconnection of the amygdala central nucleus and substantia innominata/nucleus basalis magnocellularis disrupts performance in a sustained attention task. *Behavioral Neuroscience, 121,* 80-89.

Holland, P. C., & Gallagher, M. (1993). Effects of amygdala central nucleus lesions on blocking and unblocking. *Behavioral Neuroscience, 107,* 235-245.

Holland, P. C., & Gallagher, M. (2006). Different roles for amygdala central nucleus and substantia innominata in the surprise-induced enhancement of learning. *Journal of Neuroscience, 20,* 6701-6706.

Holland, P. C., Thornton, J. A., & Ciali, L. (2000). The influence of associability changes in negative patterning and other discriminations. *Journal of Experimental Psychology Animal Behavior Processes, 26,* 462-476.

Hölscher, C. (1999). Synaptic plasticity and learning and memory: LTP and beyond. *Journal of Neuroscience Research, 58,* 62-75.

Kawabe, K., Yoshihara, T., Ichitani, Y., & Iwasaki, T. (1998). Intrahippocampal d-cycloserine improves MK-801-induced memory deficits: radial-arm maze performance in rats. *Brain Research, 814,* 226-230.

Kim, J., Clark, R., & Thompson, R. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience, 109,* 195-203.

Kishimoto, Y., Kawahara, S., Mori, H., Mishina, M., & Kirino, Y. (2001) Long trace interval eyeblink conditioning in impaired in mutant mice lacking the NMDA receptor subunit €1. *European Journal of Neuroscience, 13,* 1221-1227.

Kishimoto, Y., Kawahara, S., Tonegawa, S., Kirino, Y., & Kano, M. (2006). Hippocampal CA3 NMDA receptors are crucial for adaptive timing of trace eyeblink conditioned response. *Journal of Neuroscience, 26,* 1562-1570.

Kolb, B. (1984). Functions of the frontal cortex of the rat: A comparitive review. *Brain Research Review, 8,* 65-98.

Kullmann, D. M., & Lamsa, K. (2008). Roles of distinct glutamate receptors in induction of anti-Hebbian long-term potentiation. *Journal of Physiology, 586,* 1481-1486.

Lashley, K., & Franz, S. (1917). The effects of cerebral destruction upon habit-formation and retention in the albino rat. *Psychobiology, 1,* 71-139.

Laurent, V., & Westbrook, R. F. (2008). Distinct contributions of the basolateral amygdala and the medial prefrontal cortex to learning and relearning extinction of context conditioned fear. *Learning and Memory, 15*, 657-666.

Ledoux, J. (2007). The amygdala. *Current Biology, 17*, 868-874.

Lee, H. J., Youn, J. M., Gallagher, M., & Holland, P. C. (2008). Temporally limited role of substania nigra-central amygdala connections in surprise-induced enhancement of learning. *European Journal of Neuroscience, 27*, 3043-3049.

Lisman, J. E., Schulman, H., & Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioral memory. *Nature Reviews Neuroscience, 3*, 175-190.

Lubow, R., & Moore, A. (1959). Latent inhibition: The effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology, 52*, 415-419.

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

Mackintosh, N. J. (1975). A theory of attention: Variations in the associability of stimuli with reinforcement. *Psychological Review*, 82, 276-298.

Maddux, J., Kerfoot, E. C., Chatterjee, S., & Holland, P. C. (2007). Dissociation of attention in learning and action: effects of lesions of the amygdala central nucleus, medial prefrontal cortex, and posterior parietal cortex. *Behavioral Neuroscience, 121,* 63-79.

Malenka, R.C., & Bear, M.F., (2004). LTP and LTD: an embarrassment of riches. *Neuron, 44,* 5-21.

Manns, J., Clark, R., & Squire, L. (2002). Standard delay eyeblink classical conditioning is independent of awareness. *Journal of Experimental Psychology: Animal Behavior Processes, 28,* 32-37.

Mao, S., Hsiao, Y., & Gean, P. (2006). Extinction training in condjuction with partial agonist of the glycine site on the NMDA receptor erases memory trace. *The Journal of Neuroscience, 26,* 8892-8899.

Maren, S. (1999). Long-term potentiation in the amygdala: A mechanism for emotional learning and memory. *Trends In Neurosciences, 22,* 561-567.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996). N-methyl-D-Aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience, 110*, 1365-1374.

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

Martinez, J. L., & Derrick, B. E. (1996). Long-term potentiation and learning. *Annual Review of Psychology, 47*, 173-203.

Matus-Amat, P., Higgins, E. A., Sprunger, D., Wright-Hardesty, K., & Rudy, J. (2007). The role of the dorsal hippocampus and basolateral amygdala NMDA receptors in the acquisition and retrieval of context and contextual fear memories. *Behavioral Neuroscience, 121*, 721-731.

Mayer, M. C., Westbrook, G.L., & Guthrie, P.B. (1984). Voltage dependent block by magnesium of NMDA responses in spinal cord neurons. *Nature, 309*, 261-267.

McGaugh, J. L., Roozendaal, B., & Cahill, L. (2000). Modulation of memory storage by stress hormones and amygdala complex. In M. S. Gazzaniga (Ed.), *The New Cognitive Neurosciences* (pp. 1981-1998). Cambridge, MA: MIT Press.

McGaughy, J., Dalley, J. W., Morrison, C. H., Everitt, B. J., & Robbins, T. W. (2002). Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192IgG-saporin on attentional performance in a five-choice serial reaction time task. *Journal of Neuroscience, 22,* 1905-1913.

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

Norberg, M. M., Krystal, J. H., & Tolin, D. F. (2008). A meta-analysis of d-cycloserine and the facilitation of fear extinction and exposure therapy. *Biological Psychiatry, 63,* 1118-1126.

Nowak, L., Bregostovski, P., Ascher, P., & Herbert, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurons. *Nature, 307*, 462-465.

Packard, M. G., Cahill, L., & McGaugh, J. L. (1994). Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proceedings of the National Academy of Sciences, United States of America, 91*, 8477-8481.

Paschal, F. (1941). The trend in theories of attention. *Psychological Review, 48*, 383-403.

Pavlov, I. P. (1927). *Conditioned reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex*. London: Oxford Press.

Pierce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review, 87*, 532-552.

Pistell, P. J., & Falls, W. A. (2008). Extended fear conditioning reveals a role for both N-methyl-D-aspartic acid and non-N-methyl-D-aspartic acid receptors in the amygdala in the acquisition of conditioned fear. *Neuroscience, 155*, 1011-1020.

Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature, 307*, 462-465.

Quartermain, D., Mower, J., Rafferty, M. F., Herting, R. L., & Lanthron, T. H. (1994). Acute but not chronic activation of the NMDA-coupled glycine receptor with D-cycloserine facilitates learning and retention. *European Journal of Pharmacology, 12*, 7-12.

Rodriguez-Moreno, A., Carrion, M., & Delgado-Garcia, J. M. (2006). The nicotinic agonist RJR-2403 compensates the impairment of eyeblink conditioning produced by the noncompetitive NMDA-receptor antagonist MK-801. *Neuroscience Letters, 402*, 102-107.

Rudy, J. (2008). *The neurobiology of learning and memory.* Massachusetts: Sinauer Associates, Inc.

Rumelhart, D., & McClelland, J. (1986). *Parallel Distribution of Processing, Vol. 1.* Cambridge: MIT Press.

Sakamoto, T., Takatsuki, K., Kawahara, S., Kirino, Y., Niki, H., & Mishina, M. (2005). Role of hippocampal NMDA receptors in trace eyeblink conditioning. *Brain Research, 1039*, 130-136.

Sanders, M. J., & Fanslow, M. S. (2003) Pre-training context fear conditioning deficits produced by hippocampal NMDA receptor blockade. *Neurobiology of Learning and Memory, 80,* 123-129.

Saucier, D., & Cain, D. P. (1995). Spatial learning without NMDA receptor dependent long-term potentiation. *Nature, 378,* 186-189.

Schauz, C., & Koch, M. (2000). Blockade of NMDA receptors in the amygdala prevents latent inhibition of fear-conditioning. *Learning and Memory,* 7, 393-399.

Schultz, W., & Dickinson, A. (2000). Neuronal coding of prediction errors. *Annual Review of Neuroscience, 23,* 473-500.

Seo, D., Pang, M., Shin, M., Kim, H., & Choi, J. (2008). Hippocampal NMDA receptors are necessary for auditory trace fear conditioning measured with conditioned hypoalgesia in rats. *Behavioural Brain Research, 192,* 264-268.

Stalnaker, T. A., & Craig W. B. (2003). AMPA receptor stimulation within the central nucleus of the amygdala elicits a differential activation of central dopaminergic systems. *Neuropsychopharmachology, 28,* 1923-1934.

Staubli, U., Thibault, O., DiLorenzo, M., & Lynch, G. (1989). Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. *Behavioral Neuroscience, 103,* 54-60.

Takehara-Nishiuchi, K., Kawahara, S., & Kirino, Y. (2005). NMDA receptor-dependent processes in the medial prefrontal cortex are important for acquisition and the early stage of consolidation during trace, but not delay eyeblink conditioning. *Learning & Memory, 12,* 606-614.

Takehara-Nishiuchi, K., & McNaughton, B. L. (2008). Spontaneous changes of neocortical code for associative memory during consolidation. *Science, 322,* 960-963.

Takehara-Nishiuchi, K., Nakao, K., Kawahara, S., Matsuki, N., & Kirino, Y. (2006). Systems consolidation requires postlearning activation of NMDA receptors in the medial prefrontal cortex in trace eyeblink conditioning. *Journal of Neuroscience, 26,* 5049-5058.

Tang, Y. P., Shimizu, E., Dube, G. R., Rampon, C., Kerchner, G. A., Zhuo, M., Liu, G., & Tsien, J. Z. (1990). Genetic enhancement of learning and memory in mice. *Nature, 401,* 63-69.

Thompson, L. T., & Disterhoft, J. F. (1997a). N-Methyl-D-Aspartate receptors in associative eyeblink conditioning: Both MK-801 and phencyclidine produce task-and dose-dependent impairments. *Journal of Pharmacology and Experimental Therapeutics, 281,* 928-940.

Thompson, L. T., & Disterhoft, J. F. (1997b). Age- and dose-dependent facilitation of associative eyeblink conditioning by [d]-cycloserine in rabbits. *Behavioral Neuroscience, 111,* 1303-1312.

Traverso, L. M., Ruiz, G., & De la Casa, L. G. (2003). Latent inhibition disruption by MK-801 in a conditioned taste-aversion paradigm. *Neurobiology of Learning and Memory, 80,* 140-146.

Tsien, J. Z., Huerta, P. T., & Tonegawa, S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell, 87,* 1327-1338.

Walker, D. L., Ressler, K. J., Lu, K. T., & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *Journal of Neuroscience, 22,* 2343-2351.

Wang, H., Hu, Y., & Tsien, J. Z. (2006). Molecular and systems mechanisms of memory consolidation and storage. *Progress in Neurobiology, 79,* 123-135.

Wanish, K., Tang, J., Mederer, A., & Wotjack, C. T. (2005). Trace fear conditioning depends on NMDA receptor activation and protein synthesis within the dorsal hippocampus of mice. *Behavioural Brain Research, 157,* 63-69.

Watson, D., Herbert, M., & Stanton, M. (2009). NMDA receptor involvement in spatial delayed alternation in developing rats. *Behavioural Neuroscience, 123,* 44-53.

Weible, A. P., McEchron, M. D., & Disterhoft, J. F. (2000). Cortical involvement in acquisition and extinction of trace eyeblink conditioning. *Behavioral Neuroscience, 114,* 1058-1067.

Williams, S. H., & Johnston, D. (1996). Actions of endogenous opioids on NMDA receptor-independent long-term potentiation in area CA3 of the hippocampus. *Journal of Neuroscience, 16,* 3652-3660.

Wilson, P. N., Boumphrey, P., & Pearce, J. M. (1992). Restorations of the orienting response to a light by a change in its predictive accuracy. *Quarterly Journal of Experimental Psychology, 44B,* 17-36.

Woodruff, G. N., Foster, A. C., Gill, R., Kemp, J. A., Wong, E. H., & Iversen, L. L. (1987). The interaction between MK-801 and receptors for N-methyl-D-aspartate: functional consequences. *Neuropharmacology, 26,* 903-909.

Woods, A. M., & Bouton, M. E. (2006). D-cycloserine facilitates extinction but does not eliminate renewal of the conditioned emotional response. *Behavioral Neuroscience, 120,* 1159-1162.

Wozniak, D. F., Olney, J. W., Kettinger III, L., Price, M., & Miller, J. P. (1990). Behavioral effects of MK-801 in the rat. *Psychopharmachology, 101,* 47-56.

Xu, X., Boshoven, W., Lombardo, B., & Spranger, J. (1998). Comparison of the amnestic effects on NMDA receptor antagonist MK-801 and nitric oxide synthase inhibitors: L-NAME and L-NOARG in goldfish. *Behavioral Neuroscience, 112,* 892-899.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2005). Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. *European Journal of Neuroscience, 22,* 505-512.

Zhang, X., Wu, L., Gong, B., Ming, R., Li, B., & Zhuo, M. (2008). Induction-and conditioning-protocol dependent involvement of NR2B-conditioning NMDA receptors in synaptic potentiation and contextual fear memory in the hippocampal CA1 region of rats. *Molecular Brain Research, 1,* 1-16.