THE EFFECTS OF NOREPINEPHRINE AND LIPOPOLYSACCHHARIDE ADMINISTRATION ON MEMORY CONSOLIDATION

By

Kara Gault

Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Psychology

Texas Christian University

Fort Worth, Texas

May 2, 2014

THE EFFECTS OF NOREPINEPHRINE AND LIPOPOLYSACCHHARIDE ADMINISTRATION ON MEMORY CONSOLIDATION

Project Approved:

Supervising Professor: Gary Boehm, Ph.D.

Department of Psychology

Brenton Cooper, Ph.D.

Department of Psychology

Michael Chumley, Ph.D.

Department of Biology

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Gary Boehm for the time he spent encouraging and pushing me to reach my highest academic potential. Without his encouragement and drive, I would not have accomplished as many goals or broadened my academic horizons during my undergraduate career.

Thank you to Dr. Brenton Cooper and Dr. Michael Chumley for supporting my honor's thesis and acting as my committee board.

I would like to thank my parents, Glenn and Rebecca Gault, and sister Lauren Gault, for always seeing the best in me and encouraging me to dream. Without their guidance, love, support, and dedication I would not be the woman I am today.

INTRODUCTION

The brain is an organ that, in addition to the spinal cord, comprises the Central Nervous System (CNS). The brain interacts with the body on many levels, such as coordinating movement by the relay of tactile messages from nerves to the brain. However, recent studies show that the brain also interacts with the immune system in a bidirectional manner. Recent research demonstrates that three of the body's major systems—nervous, endocrine, and immune—work together in a symbiotic relationship to maintain homeostasis (Quan & Banks, 2007).

Decades prior to the neural-immune interaction breakthrough, researchers began to question how the CNS, a system in isolation, could influence and be influenced by the immune system. A recently uncovered example is the endocrine system's activation of the Hypothalamic-Pituitary-Adrenal Axis, a negative feedback loop that communicates with the brain and body through hormonal release (Eskandari *et al.*, 2003). The HPA axis functions to support homeostasis in the body and regulate inflammation through the release of glucocorticoids from the adrenal cortex.

In addition to different pathways, such as the HPA axis, which impact the relationship between the immune system and the CNS, cytokines, endogenous protein molecules within the body, directly impact the immune system by modulating inflammation (Haddad, 2008). When an antigen is detected in the body, different cells, depending on the location of the antigen, release cytokines. When an antigen is detected in the periphery, macrophages and neutriphils release cytokines, which act to change brain function; however, when an antigen is detected in the CNS, microglia release cytokines (Eskandari *et al*., 2003). Upon antigen detection, three pro-inflammatory cytokines are released in concert within the brain:

interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα) (Pugh *et al.,* 2000; Maier & Watkins, 1998).

The purpose of cytokine release after detection of antigens is to cause a pro-inflammatory or anti-inflammatory reaction by the surrounding cells through communication with the brain (Dinarello, 2000). Cytokines also induce sickness behavior, or acute –phase response through the facilitation of pain signaling (Maier & Watkins, 1998; Eskandari *et al.*, 2003). Sickness behavior manifestations in animal models include lessening of food and water intake, less physical activity and exploration, less social and sexual behavior, as well as less aggression, increase in pain sensitivity, depression, and cognitive alterations (Kelley *et al*., 2003; Maier & Watkins, 1998). Cytokine-induced sickness behavior is adaptive to restore the animal to its original health by hindering the continuance of behaviors that require high-energy to be sustained. Instead of foraging for food, a sick animal will rest to help sustain it while the body works to kill-off pathogens (Kelley *et al.*, 2003).

Once activated in the periphery, cytokines act upon two pathways to communicate the presence of pathogens to the brain. One is through the vagus nerve, which innervates visceral organs, such as the stomach. Roughly 70% of the vagus nerve's afferent fibers communicate directly with the brain most of they way down the spinal cord (Maier & Watkins, 1998). When endotoxins and IL-1 are administered to the vagus nerve, Fos expression is induced, which modulates gene expression (Eskandari *et al.,* 2003). When the vagus nerve is severed, intraperitoneal injected cytokines no longer elicit sickness behavior within the animal (Maier & Watkins, 1998).

The body's second means of cytokine transport is through the blood-brain barrier by active transport or through circumventricular organs (Eskandari *et al.,* 2003).

Circumventricular organs are cellular regions within the cerebral ventricles with fenestrated capillaries, which means an absence of the blood-brain barrier (Haines, 2013). Circumventricular organs include the subfornical organ, the organum vasculosum of the lamina terminalis, the subcommissural organ, and the pineal gland (Haines, 2013). Both of these processes provide a means for cytokines to overcome the blood-brain barrier to communicate pathogen presence to the brain.

To induce pro-inflammatory cytokine release, researchers inject lipopolysaccharide (LPS), which is a component of the bacterial coat from gram-negative bacteria that acts as an exogenous pyrogen, into animals (Kelley *et al*., 2003). The proinflammatory cytokines typically released are tumor necrosis factor-alpha (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6) (Maier & Watkins, 1998). These cytokines work to increase activation of immune cells and sickness behavior, which eliminates harmful pathogens through activation of lymphocytes and increase in fever, respectively.

However, these adaptive mechanisms elicited by the activation of pro-inflammatory cytokines can also have detrimental effects for the organism, such as memory loss and lack of memory consolidation. Specifically, pro-inflammatory cytokines work to hinder the function of hippocampus-dependent memory (Pugh *et al*., 1998; Sparkman *et al*., 2005; Kranjac *et al*., 2012a).

Hippocampus-dependent memory systems are modulated by IL-1β expression. Over-expression of IL-1β has been shown to hinder subject ability to process or complete active avoidance training, passive avoidance conditioning, water maze, and contextual fear conditioning due to the impairments induced by neural inflammation (Kranjac *et al*., 2012a; Pugh *et al*., 1998; Shaw *et al*., 2001; Sparkman *et al*., 2005; Thomson & Sutherland, 2005).

Pro-inflammatory cytokines are found within many brain structures, such as the cerebral cortex, hypothalamus, cerebellum, striatum, and the hippocampus, but the highest density of Interleukin 1-β and Interleukin-1r are in the hippocampus (Haas & Schauenstein, 1996).

Interleukin-1β triggers pro-inflammatory cytokine release through the administration of multiple exogenous substances, such as LPS, which is a component of the bacterial coat of gram-negative bacteria from the cell wall (Nguyen *et al*., 1998). LPS interaction with the immune system causes a release of tumor necrosis factor-alpha (TNFα), interleukin-1(IL-1), and interleukin-6 (IL-6) within the periphery and in the CNS (Takeuchi & Akira, 2010). Therefore, LPS is used in research to mimic the innate immune response.

The TLR family is a well-mapped Pattern Recognition Receptor (PRR) group, which is directly involved in the sensing of intracellular endosomes and lysosomes, as well as the detection of invading pathogens (Akira *et al*., 2006). In this way, activation of the innate immune response is facilitated through the use of TLR pathways. LPS works directly on Toll-like receptor 4 (TLR4) (Takeuchi & Akira, 2010).

After LPS administration, the levels of interleukin-1β are typically highest about 4 h later and maintain elevation throughout a 24-48 h period (Kranjac *et al*., 2012a; Richwine *et al*., 2008). For this reason, within a 48 h period hippocampus-dependent memory can be impaired the most through neural inflammation. Deficiencies can be exhibited in experimental animals through different scientific paradigms, such as contextual fear conditioning (Pugh *et al*., 1998; Thomson & Sutherland, 2005; Kranjac *et al*., 2012a), spatial learning (Gibertini *et al*., 1995; Shaw *et al*., 2001; Sparkman *et al*., 2005), avoidance learning (Sparkman *et al*., 2005), novel object recognition (Jacewicz *et al*., 2005), and trace fear conditioning (unpublished data). Therefore, LPS can be utilized to activate the innate

immune response and increase IL-1β levels within the hippocampus, inducing hippocampus-dependent memory deficits. However, to ensure that sickness behavior is not a confounding variable, and that learning and memory consolidation processes are impaired, performance effects must be taken into account. For this reason, we utilized the contextual fear-conditioning paradigm to prevent the confusion of lessened locomotor activity with learning since freezing behavior is elicited by mice within the paradigm because of innate fear (Phillips & LeDoux, 1992). Due to the learned nature of the paradigm, we expect to see increased movement in LPS-administered subjects compared to the control group.

Contextual aversive conditioning is a basic Pavlovian paradigm that is commonly used to assess the learning-related deficits in memory from hippocampal inflammation (Pugh *et al*., 1998; Maren, 2001; Kranjac *et al*., 2012a). Contextual aversive conditioning is dorsal hippocampus (DH) dependent. The idea is that rodent subjects will associate a context (e.g, aversive conditioning apartment) with an aversive stimulus (e.g., footshock), which will induce a learned freezing behavior in the trial portion of the test (Maren, 2001; Chang & Liang, 2012).

In addition to contextual aversive conditioning, researchers utilize auditory-cue conditioning to assess the neurobiological effects of memory consolidation in rodents. Also known as the delayed-cue aversive conditioning, the paradigm is comprised of the pairing of a conditioned stimulus (CS; e.g., tone) with an aversive unconditioned stimulus (US; e.g., footshock). After continued research, it has been found that the amygdala modulates delayed-cue aversive conditioning, while the hippocampus is utilized in contextual aversive conditioning (Kim *et al*., 1993; Maren, 1998).

Lastly, trace-cued aversive conditioning has been found to be hippocampus-dependent without mediation by the amygdala. As previously discussed, LPS administration not only mimics neural inflammatory deficits in memory consolidation within the hippocampus, but also does not affect the amygdala (Pugh *et al*., 1998; Kranjac *et al*., 2012a). The importance of this can be found in that neuroimmune activation by LPS can hinder learning and memory in rodents within the trace-cued aversive conditioning paradigm, but not in animals with neural lesions of the amygdala (Kranjac *et al*., unpublished data). Therefore, trace-cued fear conditioning is a preferable memory paradigm to assess the effects of LPS on the hippocampus.

Since the discovery of brain-derived neurotrophic factors (BDNF), extensive research has been performed to discover the effects and the underlying mechanisms of BDNF of different regions of the brain. Yamada and Nabeshima (2003) discovered the core reliance of the hippocampus on BDNF modulation for neural plasticity and memory consolidation. Recent research found that memory consolidation and acquisition correlate with an increase in BDNF ligand expression of mRNA on the TrkB receptor site (Yamada & Nabeshima, 2003). BDNF is predominantly found in the hippocampus. Research has found a significant increase in BDNF levels within the hippocampus after subjects participated in the contextual aversive conditioning paradigm (Hall *et al*., 2000). In addition to this intriguing research, Liu *et al*. (2004) found that BDNF+/- heterozygote mice did not show learning-implied freezing behavior within the contextual aversive conditioning paradigm, but did within the auditory-cue aversive conditioning paradigm. BDNF synaptic infusion rescues memory impairments caused by the absence of BDNF. This exhibits the role of BDNF modulation of hippocampus-dependent memory, but not amygdala-dependent memory.

The binding of BDNF to TrkB activates cytoplasmic signaling pathways such as mitogen-activated protein kinase (MAPK), phospholipase C-γ (PLC-γ), and phosphatidylinositol-3 kinase (PI3-K) (Yamada & Nabeshima, 2003). Activation of MAPK then activates phosphorylation of cyclic-AMP-response element binding protein (CREB), a transcription factor responsible for the formation of fear memories (Kida *et al*., 2002; Segal, 2003).

BDNF also binds to low-affinity p75 neurotrophin receptor (p75NTR), a member of the tumor-necrosis factor (TNF) family. Low-affinity p75NTR is associated with intracellular signal transduction of Nf-κB (Dechant & Barde, 2002). Despite this, BDNF predominantly facilitates synaptic plasticity and learning through the TrkB receptor site (Nagappan & Lu, 2005). NF-κB within the CNS also facilitates protein kinase A (PKA)/CREB signal transduction pathways that work to consolidate a range of memory forms (Kandel, 2001).

As previously described, LPS administration increases IL-1β levels within the hippocampus, activating the innate immune response and hindering hippocampus-dependent memory consolidation by rodents. Recent studies have found a diminished amount of BDNF mRNA levels within the hippocampus 4 h following LPS administration (Richwine *et al*.,2008; Kranjac *et al*., 2012a). An decrease in BDNF protein levels were also discovered 7 h post-LPS administration (Guan & Fang, 2006). However, Shaw *et al*. (2001) did not see diminished levels of BDNF mRNA after LPS administration within the hippocampus. This difference could be attributed to the lessened amount of LPS utilized by Shaw *et al*. (2001). Also, research has found that after continuous LPS administration over a five-day period, BDNF levels and TrkB activation levels are decreased in a dose-dependent fashion (Tanaka *et al*., 2006).

Over the previous years, researchers have focused on the presence of ionotropic glutamate receptors, specifically α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and *N*-methyl-*D*-aspartate (NMDA) receptors involved in memory consolidation. AMPA receptors are present and distributed across different brain structures, however, four AMPA receptro subunits (GluR1-4; Wenthold *et al*., 1996) have been shown to be involved in neural learning modifications, including those involved in the acquisition of aversion conditioning paradigms (Lissen *et al*., 1999; Shi *et al*., 1999; Yang *et al*., 2008). Recent research has uncovered the specific neural mechanisms underlying aversion conditioning, which is the calcium/calmodulin-dependent protein kinase (CaMKII), and the previously mentioned PKA and MAP kinase (Rodrigues *et al*., 2004). Hippocampus-dependent aversion conditioning learning and memory is directly modulated by the dendritic recruitment of the GluR1-containing AMPA receptors (Matsuo *et al*., 2008). Some believe that the synaptic strengthening is from the addition of AMPA receptors to CA1 synapses (Kessels & Malinow, 2009). Also, synaptic strengthening involves the phosphorylation of Ser831 sites of the GluR1 subunit by protein kinase C (PKC) and CaMKII (Esteban *et al*., 2003; Derkach *et al*., 2007). After administration of IL-1β, Ser831 at GluR1 of the AMPA receptor is down-regulated, but not NMDA receptor NR1 subunit; however, neither is down-regulated by the administration of IL-10 or TNFα (Lai *et al*., 2006). Also, AMPA receptor GluR1 subunit sites are not affected by the administration of IL-1β if IL-1 receptors are blockaded by IL-1β antibody or IL-1ra. Lastly, TNFα upregulates GluR1 Ser831 expression, while IL-1β downregulates GluR1 Ser831 expression; therefore, memory impairments from downregulation of AMPA receptor site GluR1 Ser831 is exclusive to IL-1β interaction (Lai *et al*., 2006). In this way, memory modifications in hippocampus-dependent tasks, such as

trace fear conditioning, may be caused by the hindered expression of AMPA receptor subunit GluR1 site Ser831 caused by innate immune response activation by IL-1β.

However, AMPA receptor sites can also be modulated by Norepinephrine (NE). Norepinephrine, a stress hormone secreted by the adrenal cortex, is involved in emotional memory consolidation, and thought to be involved in Post-Traumatic Stress Disorder (PTSD) (Hu *et al*., 2007). Norepinephrine also works to regulate the sympathetic division ("fight or flight") of the autonomic nervous system. Norepinephrine is derived from tyrosine after a three-step translation process and predominantly found in the locus coeruleus, a paired structure near the fourth ventricle (Haines, 2013). Noradrenergic projections form the locus coeruleus go predominantly to the hippocampus, which is a liaison for exterior threats to the amygdala, and the prefrontal cortex (PFC), a part of the limbic system, which is involved in alertness, arousal and attention, intelligence, and the acquisition of memory (Vermetten & Bremner, 2002; Haines, 2013).

Previous research has shown that the emotional weight of experiences can have a bearing on the ability of the hippocampus to consolidate experiences—essentially, the heavier the load of the emotional arousal during an event, the greater the memory consolidation of said event (Rodrigues *et al*., 2009). Norepinephrine binds either to α-1 or β-adrenergic receptors, which are involved in vasoconstriction and relaxation, both emotionally mediated responses, respectively (Haines, 2013). Pre-training or post-training stress exposure increased NE levels within subjects and improved memory performance (Ferry & McGaugh, 1999). However, a blockade of β-ARs by β-adrenergic antagonists, such as propranolol, or neural lesions to the locus coeruleus, negates the positive increase in memory consolidation caused by NE (McGaugh, 2000). In addition to fear memory disruption in rodents after

propranolol administration, humans also experience a fear memory consolidation deficit after propranolol administration (Grillon *et al*., 2004).

Also, with great bearing on our current study, the systemic administration of norepinephrine (NE) increases NE levels within the CNS. The increased level of NE concentration within the CNS has been shown to facilitate long-term contextual fear memory (Hu *et al*., 2007). Hu *et al*. (2007) have also found that an increase through hippocampal insertion of AMPA receptors within the CNS after peripheral NE administration increase the available synaptic sites for NE binding, which increases the ability for fear memory consolidation. Hu *et al*. (2007), also found that once NE binds to β-ARs, the PKA and CamKII pathways are activated. Once these pathways are activated, a cascade effect phosphorylates Ser845 and Ser831 of the GluR1 subunit of AMPA receptors. Ultimately, this study supports the hypothesis that emotion facilitates enhanced fear memory consolidation through the molecular cascade mechanisms initiated by norepinephrine.

Hypotheses

We hypothesize that LPS (125μg/kg) administration immediately following trace-cued aversion conditioning (tFC) paradigm, but not following delayed-cue aversion-conditioning (dFC) paradigm will impair conditioned fear memory consolidation in our mice. Lastly, we hypothesize that NE administration will rescue cognitive function that is hindered by systemic post-training LPS (125μg/kg) exposure.

METHODS

Research Participants

Participants were 4-6 month-old, experimentally inexperienced, male C57BL/6J mice bred at the Texas Christian University (TCU) vivarium from foundation stock attained from

The Jackson Laboratory (Bar Harbor, ME). After the mice were weaned at one month old, the mice were housed in standard polycarbonate mouse cages (30 x 20 x 16 cm) in groups of 3-4. Subjects were kept at ambient temperatures (22°C) and given food and water *ad libitum*. Vivarium lights for the colony were kept at a 0700h on and 1900h off, light-dark cycle. Learning and memory tests were performed between 1100h and 1500h. Animals were utilized in adherence to the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), and the experimental trials were performed in conformity to the protocol approved by the Institutional Animal Care and Use Committee (IACUC) at TCU. In Experiment 1, thirty-nine mice were used. In Experiment 2, forty-seven mice were used. In Experiment 3, twenty mice were used.

Treatment Conditions

Within each cage, animals were randomly assigned to different treatment groups for each of the three experiments. Thirty minutes prior to aversion conditioning intraperitoneal (i.p.) injections of NE (Sigma, St. Louis, MO) were administered at the dose of 0.5 mg/kg in sterile, pyrogen-free 0.9% saline (Baxter, Deerfield, IL). Immediately following aversion conditioning, 125μg/kg LPS (*Escherichia coli*, sterotype 0111:B4; Sigma, St. Louis, MO) injections were administered i.p. Drug measurement ratios were selected from previous publications (NE: Frankland *et al.,* 2004; Hu *et al.*, 2007, and LPS: Pugh *et al.*, 1998, Kranjac *et al.*, 2012). Throughout the two-day procedure, subjects were weighed and visually inspected daily.

Apparatus and Behavior Measurement

Fully automated units (FreezeFrame, Coulbourn Instruments, Whitehall, PA, USA) with electrified grid floors that emit an electric shock (0.7mA) were used to analyze

conditioned fear learning. Freezing behavior was observed through FreezeFrame Software (Coulbourn Instruments, Whitehall PA, USA) to analyze and record data for each subject. Freezing behavior is classified as below a 10 (the company's default setting) on the motion detection sensitivity scale with a range from 0 to 1000.

Experiment 1: Post-training LPS administration effects on memory

Delay-cued aversion conditioning paradigm and trace-cued aversion conditioning paradigm were utilized to the effects of LPS administration on the hippocampus-independent and hippocampus-dependent memory consolidation, respectively. On training day, each mouse was placed in the conditioning apartment with dotted pattern walls and a peppermint odor for both paradigms (Figure 1) and after a 60-second acclimation period, subjects were presented with a 65dB tone for 30 seconds. In the delay-cued experiment, a 2-second 0.7mA mild shock was emitted immediately after the ending of the tone. In the trace-cued experiment, 30-second tone was followed by a 30-second trace interval, and a 2-second 0.7mA mild shock was delivered immediately after the trace interval terminated.

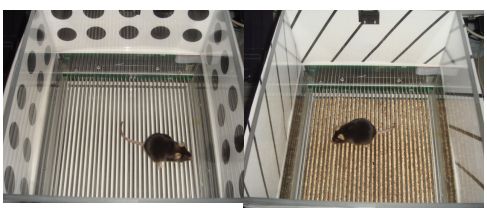
Figure 1: Two contextual-aversion conditioning contexts utilized in the current experiment.

A) Dotted wall context with peppermint odor for heightened context pairing

B) Stripe white-walled context with no added odors for reduced context pairing

A) Left

B) Right



After the training sessions, mice were immediately placed into their home cages and were allowed a 2-minute rest period. Following the rest period, mice were intraperitoneal (i.p.)

injected with sterile saline or LPS (125μg/kg) dependent upon their previously described randomly selected experimental groups. Twenty-four hours post-training, subjects were placed into a novel context for 120 seconds consisting of a white wall backdrop (Figure 1B). For the first 60 seconds, the auditory cue was unavailable ("pre-CS" period), but became present in the last 60 seconds ("CS" period) of the paradigm. Freezing behavior was measured during this time. We hypothesized there would be no difference between saline-treated mice and LPS-treated mice during the first 60 seconds of the treatment, both trace-cued aversion and delay-cued aversion conditioning. However, we hypothesized that there would be a difference shown in LPS-treated subjects freezing behavior during the CS period of the treatment in the trace-cued treatment, but not the delay-cued treatment, in contrast to the saline-treated control mice.

Experiment 2: Peripheral pre-training NE administration effects on memory

To understand the effects of peripherally administered pre-training NE on impaired memory from systemic LPS-injection post-training we employed a trace-cued aversion conditioning paradigm. On training day, each group of mice 30 minutes prior to testing received either a sterile saline or NE (0.5 mg/kg) injection. At the beginning of the training session, mice were put in the conditioning apartment with a white wall backdrop (Figure 1B). After a 60-second adjustment period, a 65 dB, 30-second tone was emitted. After the 30-second tone, a 30-second trace interval followed and a 2-second 0.7 mA shock was emitted at the end of the trace interval (Figure 2A). After the shock, a 30-second rest period followed before termination of the trial.

Promptly following termination of the trial period, mice were returned to their home cages for a 2-minute rest period. Following the 2-minute rest period, subjects were

administered intraperitoneal (i.p.) injection of sterile saline or LPS (125μg/kg) respective of their randomly assigned training groups. In contrast to the prior experiment where subjects were put into a novel context 24-hour post-training, subjects were placed into the same conditioning apartment and context for 120 seconds. Freezing behavior was recorded. Similar to the prior experiment, the first 60-seconds of training were void of the auditory cue ("pre-CS" period), but the last 60-seconds the auditory cue was emitted ("CS" period). We hypothesized their would be a significant decrease in freezing behavior of the LPS-administered subject group during pre-CS (i.e., initial 60-second context) and CS (i.e., 60-second tone emission) periods of testing compared to LPS-treated subject groups that were co-administered NE, saline-treated control subjects, or NE-treated subjects.

Experiment 3: Effects on freezing behavior in the absence of mild shock

To assess the effects of systemic NE and LPS co-administration on sickness behavior, we implemented the trace-cued aversion-conditioning paradigm. On training day, each mouse received either a sterile saline injection or NE (0.5 mg/kg) 30-minutes prior to commencement of the experiment. At the beginning of the session, each subject was placed in the white-walled conditioning apartment (Figure 1B). After a 60-second adjustment period, a 65dB tone was emitted for 30 seconds. Then, after the 30-second tone, a 60-second rest period followed; however, unlike experiment 1 and 2, no shock was ever emitted during the session. Following the 60-second rest period, subjects were placed in their home cages for a 2-minute rest period. After the 2-minute rest period, subjects were administered either an intraperitoneal (i.p.) injection of either LPS (125 μg/kg) or sterile saline.

On testing day, each subject was placed in the white-walled apartment (same context) for 120 seconds and a measurement of freezing behavior was measured. The auditory cue

remained absent the first 60-seconds ("pre-CS" period) of the testing period, but was emitted the last 60-seconds ("CS" period) of testing. We hypothesized no difference in freezing behavior among different treatment groups both during pre-CS and CS phase of the testing session.

Analysis

Experiments 1, 2, and 3 data were analyzed via mixed-factor, repeated-measures ANOVAs, with Condition [tFC and dFC] and Treatment [Saline and LPS] as the between subjects variables, and Period [pre-CS and CS] as the within-subjects variable in Experiment 1, or Condition [Saline and LPS] and Treatment [Saline and NE] as the between-subjects variables, and Period [pre-CS and CS] as the within-subjects variable in Experiments 2 and 3. A 2 x 2 standard factorial ANOVA was implemented to calculate and analyze the weight difference between Days 1 and 2 for Experiments 1, 2, and 3, with Condition [Saline and LPS] and Treatment [Saline and NE] as the between-subjects variables. To assess differences between groups, significant omnibus effects were subjected to Fisher's PLSD post hoc tests. Data analysis processed through StatView 5.0.1 software (SAS Institute Inc., Carry, NC), and were formulated as the mean ± standard error of the mean (SEM).

RESULTS

Weight and appearance

Statistically significant weight loss was found across LPS treatment groups, notwithstanding NE administration. Statistical analysis of Experiment 1 exhibited a significant main effect for Treatment, $F(1,35) = 13.342$, $p < 0.001$, and indicated that, on average, LPS-administered subjects lost 1.3 g; however, saline-administered control subjects gained, on average, 0.8 g. Statistical analysis of Experiment 2 found a significant main effect for Condition, $F(1,43) = 170.891$, $p < 0.0001$. Also, LPS-administered subjects, regardless of NE treatment, gained an average of 0.6 g. Statistical analysis of Experiment 3 uncovered a significant main effect for Condition, $F(1,16) = 21.345$, $p < 0.0001$. LPS-administrated subjects, despite NE treatment, gained an average of 0.1 g. Initial body weights did not differ between the treatment groups ($p > 0.05$; *ns*, data not shown).

Experiment 1: Deficits in hippocampus-dependent memory processing

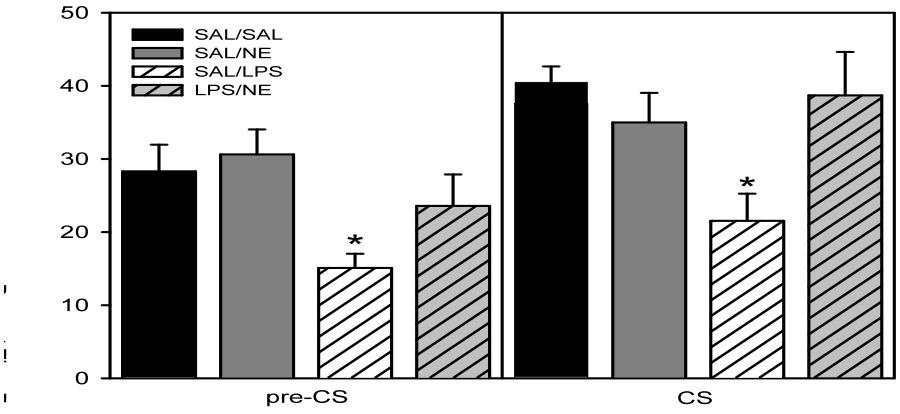
In Experiment 1, we used both delay-cued and trace-cued models to evaluate if systemic administration of LPS post-training elicited memory deficiencies. No variation in freezing behavior was observed during the training session prior to the shock being emitted ($p > 0.05$; *ns*, data not shown) or during the pre-CS period of the testing session ($p > 0.05$; *ns*). A significant main effect for Period, $F(1,35) = 66.794$, $p < 0.0001$, was observed during the CS time-span of the testing period (24 hours post-training). Also, a significant effect of Period x Treatment, $F(1,35) = 5.909$, $p < 0.05$, and Period x Condition x Treatment interaction, $F(1,35) = 5.385$, $p < 0.05$ were uncovered through analysis. However, the Period x Condition interaction only came close to significance, $F(1,35) = 3.796$, $p = 0.059$. As hypothesized, LPS-administered subjects directly following trace-cued but not delay-cued,

inadequately associated the CS (auditory tone) with the US (foot shock). This was demonstrated by lessened freezing behavior in the LPS-administrated groups during CS testing phase, compared to the three other treatment groups.

Experiment 2: Peripheral pre-training NE combats negative memory consolidation

In Experiment 2, we determined if peripheral pre-training administration of NE could restore cognitive deficits in subjects with systemic post-training administration of LPS by using a trace-cued model. No significant difference was found in freezing behavior of subjects before the emission of shock ($p > 0.05$; *ns*, data not shown). Significant effects during the pre-CS and the CS phases of the testing period (24 hours post-training, Figure 2) were discovered for Period, $F(1,43) = 28.672$, $p < 0.0001$, Condition, $F(1,43) = 4.892$, $p < 0.05$, and Period x Condition x Treatment interaction, $F(1,43) = 4.264$, $p < 0.05$. Context ("pre CS" period) and auditory CS ("CS" period) in concert with foot shock associations were poor for mice administered LPS-only. However, LPS and NE-treated mice appeared to have greater success with associating the stimuli, both context and shock-induced fear and the auditory cue (CS) and shock-induced fear (Figure 2).

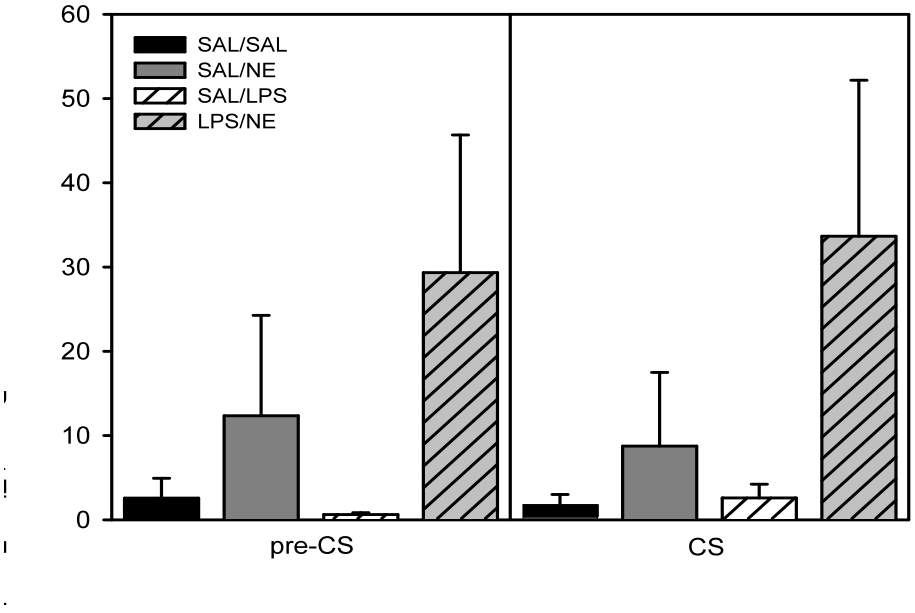
Figure 2: NE appears to rescue memory abilities after LPS administration



Experiment 3: Elevated levels of freezing during testing without mild shock

In Experiment 3, we determined whether NE adminstration, regardless of post-training Saline or LPS, induces sickness behavior. Freezing behavior remained constant over all groups during training before the emission of shock ($p > 0.05$; *ns*, data not shown). A main effect for Treatment, $F(1,16) = 3.553$, $p = 0.0778$, was found during the testing phase (24 hours post-training, Figure 3) for both the pre-CS and CS periods. Also, there was a main effect for Period x Condition interaction, $F(1,16) = 4.111$, $p = 0.0596$, which came close to statistical significance. Elevated levels of freezing without shock were found in mice who were administered NE, and were unaffected by Saline or LPS administration. Due to the lack of behavioral results, we did not run biological experiments.

Figure 3: NE increases freezing behavior regardless of saline or LPS administration.



DISCUSSION

In the current study, using a hippocampus-dependent paradigm to assess whether NE could restore cognitive deficits initiated by systemic bacterial endotoxin post-training administration to elucidate if there is a connection between AMPAR GluR1-related mechanisms and cognitive recovery after innate immune initiation. To uncover the hippocampus basis of the interaction, we used the trace-cued aversion-conditioning model, which is well documented as hippocampus-dependent in function, to assess if NE can restore the function of the hippocampus. To the best of the authors' knowledge, the peripheral administration of norepinephrine to assess the restorative qualities to the hippocampus after bacterial endotoxin exposure has disrupted cognition has not been previously studied.

In the first experiment, we assessed the dependence of the trace-cued aversion-conditioning model on the hippocampus in opposition to the hippocampus-independent delayed-cued aversion-conditioning model. We hypothesized that cognitive function would be impaired by systemic administration of LPS post-training to memory cognition in the trace-cued model subjects, but not in the delay-cued model subjects. In the second experiment, we assessed the effect of peripherally administered NE on hippocampus-dependant cognitive function in the trace-cued aversion-conditioning model in systemic post-training LPS administered subjects. To further evaluate our findings, in Experiment 3 we assessed the effect of peripherally administered NE on freezing behavior in the absence of shock, regardless of saline or LPS administration, during trace-cued aversion conditioning model.

The Experiment 1 data concurred with our hypothesis: that systemic administration post-training of LPS would hinder memory consolidation in trace-cued aversion conditioning

model, but not in the delayed-cue aversion conditioning model. This agreed with previous research that found that LPS administration hinders contextual aversion conditioning learning, while delayed-cue aversion conditioning is unaffected (Pugh *et al*., 1998). However, to the authors' knowledge, this is the first time contextual aversion conditioning has been demonstrated with systemic post-training LPS administration assessed by trace-cued aversion conditioning.

A considerable amount of research has revealed that hippocampus-dependent tasks, such as water maze, contextual aversion conditioning, and active and passive avoidance conditioning are hindered the activation of the innate immune response (Cunningham *et al*., 2009; Huang & Sheng, 2010; Kranjac *et al*., 2012a; Pugh *et al*., 1998; Sparkman *et al*., 2005). Following LPS administration, pro-inflammatory cytokine levels, such as interleukin1-β, TNFα, and interleukin-6, are higher in inflammatory models within the dorsal hippocampus. The specificity of the innate immune response within certain brain regions, such as the dorsal hippocampus, means the inflammatory negative effects only impair learning and memory within that region; therefore, cognitive deficits from LPS are only found in paradigms where the specific pro-inflammatory cytokines are predominantly released (Kranjac *et al*., 2012a; Pugh *et al*., 1998).

BDNF expression has been found to modulate long-term potentiation (LTP) and synaptic plasticity within the dorsal hippocampus. In addition to BDNF's plasticity action, BDNF also is expressed during fear conditioning. Mice lacking the BDNF receptor, TrkB show deficits in hippocampus-dependent learning tasks (Hall *et al*., 2000). Also, BDNF +/- mice show deficits in contextual fear conditioning, but not in auditory cue aversion conditioning. Contextual auditory conditioning is hippocampus-dependent. Infusion of

recombinant BDNF protein partially rescued the contextual aversion-conditioning deficit (Liu *et al*., 2004). Therefore, contextual-auditory conditioning is mediated by BDNF expression. For this reason, in Experiment 1, we can readily assume, without directly testing for it, that systemic post-training LPS administration activated and increased IL-1β levels, as well as decreased BDNF mRNA expression during trace-cued aversion conditioning (tFC).

As mentioned previously, IL-1β release is mediated by the innate immune response, which can be activated by systemic LPS administration. IL-1β acts to cause inflammation and hinder learning and memory within the hippocampus. Recent research has found that IL-1β down-regulated Ser831 phosphorylation and surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit GluR1. These effects were eradicated with the administration of agents that block IL-1β. In addition to modulation of AMPA, IL-1β regulates *N*-methyl-D-aspartate (NMDA) receptor NR1 through depletion of extracellular calcium, which blocks the dephosporylating effects of IL-1β and diminished surface expression of GluR1. Therefore, IL-1β modulates GluR1 expression at Ser831 through extracellular calcium and the NMDA receptor (Lai *et al*., 2006).

Most relevant to our study is the prior research that demonstrates that NE release within the brain processes and facilitates storage of emotional memories. NE activates adenylyl cyclase, cAMP-dependent kinase (PKA), and calcium-calmodulin-dependent protein kinase II (CaMKII) via β-adrenergic receptors (Hu *et al*., 2007). β-Adrenergic receptors are directly involved in the modulation of long-term potentiation (LTP), which is thought to be a part of synaptic plasticity. Also, another study found that NE treatment caused an increase in phosphorylation of GluR1 at Ser845 and Ser831, which are both subunits of AMPAR (Hu *et al*., 2007). After administering NE on organotypic hippocampal

slices, research found that β-AR activation coupled to PKA phosphorylation of GluR1 at subunit Ser845; however, Ser831 phosphorylation of GluR1 is dependent upon CamKII activity and β-AR signaling (Vanhoose & Winder, 2003). Also, AMPAR placement at the synapse in the hippocampus facilitates long-term potentiation of contextual aversion conditioning through its interaction with systemic, pre-training administration of NE (Hu *et al*., 2007).

Further, research has been published describing the acceleration process of fear conditioning through the increase of voltage through AMPAR channels with the use of BDP-12, an ampakine-related drug (Rogan *et al*., 1997). Also, inhibitory avoidance long-term memory is hindered by the presence of anti-BDNF antibodies in CA1 of the hippocampus and blocked GluR1 expression (Slipczuk *et al*., 2009). Lastly, the increase in AMPA receptors present in a synapse within the CA1 portion of the hippocampus increases the contextual aversion conditioning ability of the organism (Mitsushima *et al*., 2011).

However, in opposition to our original hypothesis, Experiments 2 and 3 did not corroborate the aforementioned data. Peripheral co-administration of NE with systemic post-training LPS appeared to increase freezing behavior, but, after testing the effect of NE in the testing paradigm without shock, we found that NE *generally* increases freezing behavior in mice. Therefore, NE did not directly affect learning mechanisms, but affected the motor mechanisms within the subjects. We did not specifically examine the mechanisms utilized by NE; however, through the comparison of research we can draw some conclusions. For example, Huang *et al*. (2011) found NE facilitated the secretion of inflammatory factors by phosphorylation of MAPKs through an α receptor-dependent pathway after activation by LPS-induced macrophage action.

Therefore, in the current experiments, the possibility remains that NE and LPS administration work in concordance with the activated pro-inflammatory cytokines to prolong sickness behavior within the brain parenchyma/periphery, which reduced locomotor activity during testing. In essence, Experiment 3 reinforced the importance of running different tests to assess if there are any unidentified confounding variables.

In conclusion, peripheral administration of pre-training NE fails to restore cognitive function that is impaired by systemic post-training administration of LPS. In contrast, NE and LPS co-administration increased freezing levels of subjects, even without the presence of a shock. When NE was assessed, the same freezing behavior was observed in saline- and LPS-treated subjects. Therefore, NE does not lessen cognitive deficits caused by LPS administration. The mechanisms by which NE worked these effects are currently unclear.

REFERENCES

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell, 124*, 783-801.

Chang, S.D. & Liang, K.C. (2012). Roles of hippocampal GABAa and muscarinic receptors in consolidation of context memory and context-shock association in contextual fear conditioning: A double dissociation study. *Neurobiology of Learning and Memory, 98*, 17-24.

Cunningham, C., Campion, S., Lunnon, K., Murray, C.L., Woods, J.F., Deacon, R.M., Rawlins, J.N., & Perry, V.H. (2009). Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry, 65*, 304-312.

Dechant, G. & Barde, Y.A. (2002). The neurotrophin receptor p75NTR: Novel functions and implications for diseases of the nervous system. *Nature Neuroscience, 5*, 1131-1136.

Derkach, V.A., Oh, M.C., Guire, E.S., & Soderling, T.R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nature Publishing Group, 8*, 101-113.

Dinarello, C.A. (2000). Proinflammatory cytokines. *Chest, 118*, 503-508.

Eskandari, F., Webster, J.I., & Sternberg., E.M. (2003). Neural immune pathways and their connection to inflammatory diseases. *Arthritis Research and Therapy, 5*, 252-265.

Esteban, J.A., Shi, S.H., Wilson, C., Nuriya, M., Huganir, R.L., & Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nature Neuroscience, 6*, 136-143.

Ferry, B. & McGaugh, J.L. (1999). Clenbuterol administration into the basolateral amygdala post-training enhances retention in an inhibitory avoidance task. *Neurobiology of Learning and Memory, 72*, 8-12.

Gibertini, M., Newton, C., Friedman, H., & Klein, T.W. (1995). Spatial learning impairment in mice infected with Legionella pneumophila or administered exogenous interleukin-1-β. *Brain, Behavior, and Immunity, 9,* 113-128.

Grillon, C., Cordova, J., Morgan, C.A., Charney, D.S., & Davis, M. (2004). Effects of the beta-blocker propranolol on cued and contextual fear conditioning in humans. *Psychopharmacology, 175*, 342-352.

Guan, Z., & Fang, J. (2005). Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats. *Brain, Behavior, and Immunity, 20*, 64-71.

Haas, H.S. & Schauenstein, K. (1996). Neuroimmunomodulation via limbic structures—The neuroanatomy of psychoimmunology. *Progress in Neurobiology, 51*, 195-222.

Haddad, J.J. (2008). On the mechanisms and putative pathways involving neuroimmune interactions. *Biochemical and Biophysical Research Communications, 370*, 531-535.

Haines, D.E. (2013). *Fundamental neuroscience for basic and clinical appliations* (4th ed.) Philadelphia, PA: Elsevier.

Hall, J., Kerrie, L.T., & Everitt, B.J. (2000). Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nature Neuroscience*, 3, 533-535.

Hu, H., Real, E., Takamiya, K., Kang, M.G., & LeDoux, J. (2007). Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell, 131,* 160-173.

Huang, Z.B. & Sheng, G.Q. (2010). Interleukin-1β with learning and memory. *Neurosci Bull,* 26, 456.

Jacewicz, M., Czapski, G.A., Katkowska, I., & Strosznajder, R.P. (2009). Systemic administration of lipopolysaccharide impairs glutathione redox state and object recognition in male mice. The effect of PARP-1 inhibitor. *Folia Neuropathologica, 47*, 321-328.

Kandel, E.R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science, 294*, 1030-1038.

Kelley, K.W., Bluthe, R.M., Dantzer, R., Zhou, J.H., Shen, W.H., Johnson, R.W., & Broussard, S.R. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17*, S112-S118.

Kessels, H.W. & Malinow, R. (2009). Synaptic AMPA receptor plasticity and behavior. *Neuron, 61*, 340-350.

Kida, S., Josselyn, S.A., de Ortiz, S.P., Kogan, J.H., Chevere, I., Masushige, S., & Silva, A.J. (2002). CREB required for the stability of new and reactivated fear memories. *Nature Neuroscience, 5*, 348-355.

Kim, J.J., Rison, R.A., & Fanselow, M.S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 107,* 1093-1098.

Kranjac, D., McLinden, K.A., Deodati, L.E., Papini, M.R., Chumbley, M.J., & Boehm, G.W. (2012a). Peripheral bacterial endotoxin administration triggers both memory consolidation and reconsolidation deficits in mice. *Brain, Behavior, and Immunity, 26*, 109-121.

Lai, A.Y., Swayze, R.D., El-Husseini, A., & Song, C. (2006) Interleukin-1 beta modulations AMPA receptor expression and phosphorylation in hippocampal neurons. *Journal of Neuroimmunology, 175*, 91-106.

Lissin, D.V., Carroll, R.C., Nicoll, R.A., Malenka, R.C., & von Zastrow, M. (1999). Rapid, activation-induced redistribution of ionotropic glutamate receptors in cultured hippocampal neurons. *The Journal of Neuroscience, 19*, 1263-1272.

Liu, I.Y.C., Lyons, W.E., Mamounas, L.A., & Thompson, R.F. (2004). Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. *The Journal of Neuroscience, 24,* 7958-7963.

Maier, S.F. & Watkins, L.R. (1998). Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychological Reviews, 105*, 83-107.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *The Journal of Neuroscience, 18*, 3088-3097.

Maren, S. (2001). Neurobiology of pavlovian fear conditioning. *Annual Review of Neuroscience, 24*, 897-931.

Matsuo, N., Reijmers, L., & Mayford, M. (2008). Spine-type: Specific recruitment of newly synthesized AMPA receptors with learning. *Science, 319*, 1104-1107.

McGaugh, J.L. (2000). Memory—a century of consolidation. *Science, 287*, 248-251.

Mitsushima, D., Ishihara, K., Sano, A., Kessels, H.W., & Takahashi, T. (2011). Contextual learning requires synaptic AMPA receptor delivery in the hippocampus. *PNAS, 108*, 12503-12508.

Nagappan, G. & Lu, Bai. (2005). Activity-dependent modulation of the BDNF receptor TrkB: Mechanisms and implications. *TRENDS in Neuroscience, 28*, 464-471.

Nguyen, K.T., Deak, T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., & Maier, S.F. (1998). Exposure to acute stress induces brain interleukin-1beta protein in the rat. *Journal of Neuroscience, 18*, 2239-2246.

Phillips, R.G. & LeDoux, J.E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106*, 274-285.

Pugh, C.R., Kumagawa, K., Fleshner, M., Watkins, L.R., Maier, S.F., & Rudy, J.W. (1998). Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning *Brain, Behavior, and Immunity, 12*, 212-229.

Pugh, C.R., Fleshner, M., Watkins, L.R., Maier, S.F., & Rudy, J.W. (2000). The immune system and memory consolidation: A role for the cytokine Il-1β. *Neuroscience and Behavioral Review, 25*, 29-41.

Quan, N. & Banks, W.A. (2007). Brain-immune communication pathways. *Brain, Behavior, and Immunity, 21*, 727-735.

Richwine, A.F., Parkin, A.O., Buchanan, J.B., Chen, J., Markham, J.A., Juraska, J.M., & Johnson, R.W. (2008). Architectural changes to CA1 pyramidal neurons in adult and aged mice after peripheral immune stimulation. *Neuroendocrinology, 33*, 1369-1377.

Rodrigues, S.M., Schafe, G.E., & LeDoux, J.E. (2004). Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. *Neuron, 44*, 75-91.

Rogan, M.T., Staubli, U.V., & LeDoux, J.E. (1997). AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. *J Neuroscience, 17*, 5928-5935.

Segal, R.A. (2003). Selectivity in neurotrophin signaling: theme and variations. *Annual Review of Neuroscience, 26*, 299-330.

Shaw, K.N., Commins, S., & O'Mara, S.M. (2001). Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rate dentate gyrus. *Behavioral Brain Research, 124*, 47-54.

Shi, S.H., Hayashi, Y., Petralia, R.S., Zaman, S.H., Wenthold, R.J., Svoboda, K., & Malinow, R. (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science, 284*, 1811-1816.

Slipczuk, L. (2009). BDNF activates mTOR to regulate GluR1 expression required for memory formation. *PLoS ONE, 4*, e6007.

Sparkman, N.L., Kohman, R.A., Garcia, A.K., & Boehm, G.W. (2005). Peripheral lipopolysaccharide administration impairs two-way active avoidance conditioning in C57BL/6J mice. *Physiological Behavior, 85*, 278-288.

Takeuchi, O. & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell, 140*, 805-820.

Tanaka, S., Masatoshi, I., Toshiomi, S., HIrokazu, O., Satoshi, N., Seiji, S., & Takemi, Y. (2006). Lipopolysaccharide-induced mircroglial activation induces learning and

memory deficits without neuronal cell death in rats. *Journal of Neuroscience Research, 83*, 557-566.

Thomson, L.M. & Sutherland, R.J. (2005). Systemic administration of lipopolysaccharide and interleukin-1beta have different effects on memory consolidation. *Brain Research Bulletin, 67*, 24-29.

Wenthold, R.J., Petralia, R.S., Blahos II, J. & Niedzielski, A.S. (1996). Evidence of multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *The Journal of Neuroscience, 16*, 1982-1989.

Vanhoose, A.M. & Winder, D.G. (2003). NMDA and beta1-adrenergic receptors differentially signal phosphorylation of glutamate receptor type 1 in area CA1 of hippocampus. *Journal of Neuroscience*. *23,* 5827-5834.

Vermetten, E. & Bremner, J. (2002). Circuits and systems in stress. I. Preclinical studies. *Depression and Anxiety, 15*, 126-147.

Yamada, K. & Nabeshima, T. (2003). Brain-derived neurotrophic factor/TrkB signaling in memory processes. *Journal of Pharmacological Sciences, 91*, 267-270.

Yang, Y., Wang, X.B., Frerking, M., & Zhou, Q. (2008). Delivery of AMPA receptors to perisynaptic sites precedes the full expression of long-term potentiation. *PNAS, 105*, 11388-11393.