THE EFFECTS OF MICROGLIAL INACTIVATION AND DECREASED PROSTAGLANDIN SYNTHESIS ON LIPOPOLYSACCHARIDE-INDUCED LEARNING DEFICITS

by

ANDREW JUSTIN TARR

Associates in Liberal Arts, 2002
University of Southern Maine
Portland, Maine

Bachelor of Arts, 2003
University of Southern Maine
Portland, Maine

Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
in partial fulfillment of requirements
for the degree of

Master of Science

April 2007

ACKNOWLEDGEMENTS

With a deep sense of gratitude, I would like to thank all the people that helped make this thesis possible. First and foremost, I would like to extend my deepest thanks to my mentor Dr. Gary W. Boehm. Under his expert tutelage, I have grown not only as a student, but as a person in the scientific community. His guidance and support will never be overlooked by me or anyone else for that matter. I would like to also extend special thanks to Rachel Kohman, my laboratory mate and moreover my friend, who helped me through the awkward years as a new graduate student. Furthermore, I would like to thank my committee, Dr. Timothy Barth and Dr. Timothy Hubbard for their insightful comments and guidance throughout the thesis process, and as a graduate student as well. Lastly, I would like to thank my parents Michael Tarr and Mary Parsons, brother James Tarr, sister Sarah Tarr and daughter Zoë Tarr, for the continuous love and support that I receive from you throughout my career and life in general.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE EFFECTS OF MICROGLIAL INACTIVATION AND DECREASED PROSTAGLANDIN SYNTHESIS ON LIPOPOLYSACCHARIDE-INDUCED LEARNING DEFICITS

1. INTRODUCTION

1.1. Neural-immune Interaction

Researchers long believed that the immune system and the central nervous system (CNS) operate autonomously to exert their effects on the body, but in recent years this has been proven not to be the case (Dantzer & Kelly, 1989; Maier et al., 1994; Cohen & Kinney, 2001). These systems have a bidirectional relationship, in which both have important regulatory control over each other. Both systems play a major role in the physiological and behavioral responses to pathogen invasion. This interaction between the immune system and the CNS is thought to be highly adaptive, in keeping us aware of our body's needs, such as promoting increased rest during an infected state, to allow for recuperation. Given this relationship between the immune system and the CNS, it is important to focus on both systems, to obtain a better understanding of the intricacies of their interaction. So far, a comprehensive understanding of the mechanisms behind the communication between them remains elusive. Influence of the immune system by the CNS has been shown to be largely due to the autonomic nervous system (SNS) effects upon immune organs, such as the thymus, bone marrow, spleen and even lymph nodes, by the release of catecholamines (Felten & Felten, 1991). The neuroendocrine system is also involved, by the means of activation of the hypothalamic pituitary adrenal (HPA) axis and release of glucocorticoids by the adrenal cortex (Black, 1994; Maier et al., 1994). Recently, research showed that cytokines (soluble proteins released primarily by macrophages in the periphery and microglia in the CNS in response to an antigen), previously known only to be involved in the communication between immune cells in the periphery to regulate a immune response, are the main signals that notify the CNS of immune activation in the periphery. Moreover, the CNS

helps coordinate portions of the immune response. For example, certain behaviors that have been associated with illness are due to the central release of cytokines. Still not widely acknowledged by scientists are the cognitive deficits that sometimes develop during acute infection or other conditions that involve elevated cytokine levels.

1.2. Cytokines

In addition to other cells, macrophages in the periphery release substances such as cytokines, nitric oxide, and chemokines (Maier & Watkins, 1998) following contact with a pathogen. Cytokines help coordinate and stimulate immune cells to secrete antibodies and produce memory cells to offer a faster defense in the future. Cytokines can also directly stimulate phagocytic cells both centrally and peripherally. Furthermore, they are the major pathway of communication between immune cells in the CNS and peripheral nervous system (PNS). Cytokines enter the brain via two main pathways, 1) the neuronal route, in which vagal afferents conduct information from the site of the infection, and 2) humoral pathways that involve production of cytokines near the circumventricular organs (CVOs) and the choroid plexus or via active transport across the BBB (Konsman et al., 2002). Many examples of these cytokines are named "interleukins", because of their role in the communication between leukocytes (white blood cells).

The primary cytokines released by macrophages after a bacterial infection are interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) (Konsman et al., 2002). These proteins are shown to help coordinate the inflammatory response at the site of infection by sequestering other immune cells that will aid in the inflammation and healing process, as well as signaling other immune cells to join the process (Maier & Watkins, 1998). Cytokines can either be classified as proinflammatory cytokines, which promote the inflammatory response, or anti-inflammatory cytokines, which inhibit the inflammatory response

via regulation of proinflammatory cytokines. Proinflammatory cytokines regulate a complex cascade of changes in the organism by enlisting the help of T-cells and B-cells (producers of antibodies) which are also important in the specific defense against infection and injury (Maier & Watkins, 1998; Kent et al., 1992). Furthermore, cytokine production can lead to behavioral changes. The concurrent set of behavioral effects is believed to be an adaptive response, not a sign of debilitation. They are viewed as an "evolved strategy" to combat infection and possible injury (Hart, 1988; Maier & Watkins, 1998). Some of these events that signal pathogen invasion are anorexia (decreased feeding), adipsia (decreased drinking), increased slow-wave sleep, anhedonia (decreased pleasure), decreased social exploration, decreased sexual vigor, mood alterations and altered pain threshold, which all have been termed "sickness behavior" (Hart, 1988; Kent et al., 1995; Konsman et al., 2002; Maier & Watkins, 1998).

There are several ways that the body wards off infections. One of major ways is via the inflammatory response. With inflammation comes a fever response (potential aspect of "sickness behavior") marked by an increase in temperature. The reason why this is so adaptive is that many microbial pathogens cannot reproduce effectively at elevated temperatures. Moreover, inflammation and fever also facilitate the body's immunological response (Kluger, 1978; Long et al., 1990; Maier & Watkins, 1998). Along with this adaptive state, there are also immune-related non-adaptive events that occur in the organism, including alterations of learning and memory (Kent et al., 1995; Pugh et al., 1998). Even though there is much evidence to support the notion that cytokines (particularly IL-1β) affect behavior and cognitive abilities in organisms with infections, the physiological mechanisms that explain these decrements remain mostly unexplored.

1.3. Lipopolysaccharide and the Induction of Cytokines

Lipopolysaccharide (LPS) is a non-specific activator of proinflammatory cytokine release from macrophages and microglia. LPS is an endotoxin that is produced from the degraded cell wall of Gram-negative bacteria, and is a potent stimulator of the immune system (Borowski et al., 1998). The primary reason that researchers use LPS to model infection in animal models is that working with live bacteria and viruses represents a potential confound in cytokine-related pathology. LPS reduces this risk by allowing investigators to examine effects of the immune response per se rather than the more variable effects of a live, replicating pathogen. LPS has been shown to act on toll-like receptor-4 (TLR-4) molecules on macrophages to induce the release of cytokines, namely IL-1β, IL-6, and TNF-α (Borowski et al., 1998; Larson & Dunn, 2001). As mentioned earlier, LPS induces "sickness behavior." (Kent, et al., 1995; Hart, 1988; Lacosta et al., 1999; Konsman et al., 2002; Borowski et al., 1998;Yirmiya, 1996). These sickness behaviors have been thought to be an adaptive response to acute sickness induced by a pathogen and thought to aid in the restoration of homeostatic equilibrium (Kent, et al., 1995; Hart, 1988). The behavioral changes in animals that are given LPS imitate the behavioral changes when animals are given cytokines (e.g., IL-1β). It is believed that these two pathways share the same physiological mechanisms to induce sickness behavior (Bluthé et al., 1992; Borowski et al., 1998).

1.4. Immune System Influence on Learning and Memory Processes

Infections, along with many neurodegenerative diseases, are often associated with disruptions in cognitive functions (Larson & Dunn, 2001). Moreover, it has been shown that disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and depression have been associated with the over-expression of cytokines. As noted previously, proinflammatory cytokines also often mediate a behavioral change (i.e., sickness behavior). Although it has not

been determined what mechanisms are behind the cognitive deficits (Bluthé et al., 1992; Barrientos et al., 2002), there is some evidence for decreased brain derived neurotrophic factor (BDNF), decreased long term potentiation (LTP), and decreased AMPA receptors. Commonalities among the cognitive impairments indicate that cytokines, as a result of an infectious substance, may be part of the cognitive deficits underlying cause. In humans it has been shown that LPS/IL-1β administration leads to learning/memory problems (e.g., Spath-Schwalbe et al., 1998; Reichenberg et al., 2001); it has also been shown to be detrimental in animal models (e.g., Aubert et al., 1995; Pugh et al., 1998; Sparkman et al, 2005; Barrientos et al., 2002). For example, a number of studies showed that rodents that were injected with IL-1β or LPS showed LPS-induced impairments in the Morris Water maze (MWM), a common test for spatial learning/memory (Gibertini, 1996; Oitzl et al., 1993; Shaw et al., 2001; Sparkman et al., 2005).

In other studies, LPS and IL-1β have been shown to hinder contextual fear conditioning, which reinforces the evidence that they might have learning/memory effects. For example, Pugh et al. (1998) showed that LPS given peripherally inhibits contextual fear conditioning, but had no effect on auditory fear conditioning. Their methods included putting experimental subjects into a chamber in which they received two, 2-sec shocks with an inter-trial interval (ITI) of 120 sec, given during a single trial. To evaluate memory, half the rats were placed into chambers, in which they were presented with a conditioned auditory stimulus (CS) for 20 sec, followed by a mild shock (US); in the contextual fear paradigm, no auditory cue was presented. Upon the presentation of the US, the CS was terminated. Immediately after this procedure, subjects were divided into four groups that were given intraperitoneal injections of LPS at doses of 0, 0.125, 0.25 or 0.5 mg/kg. The subjects were assessed 48 hours later, in the auditory fear conditioning and contextual fear conditioning paradigm, for either a fear response (i.e., freezing) or an active

response. The rats that were in the contextual conditioning group went back into the same chambers that were used to establish the conditioned fear response, and were assessed for freezing behavior every 10 sec. The rats in the auditory fear conditioning paradigm were given 3 min without CS presentation. This allowed the researchers to assess any un-cued freezing behavior. After this 3 min period, only the CS was presented. Fear was determined by freezing, a response that a rat presumably makes when it thinks that it is in immediate danger. Freezing was assessed in the same manner as the contextual-fear paradigm, every 10 sec. In the conditioned fear procedure, freezing is therefore an indicator of memory (i.e., rats freeze because they have learned that a shock is soon to follow the CS). The results of this experiment showed that LPS selectively impairs contextual fear conditioning but has no effect on auditory-fear conditioning. Doses of 0.125 and 0.25 mg/kg significantly interfered with the contextual fear conditioning while the 0.5 dose did not. Furthermore, as in the tone group and the saline control group, LPS showed no effect on freezing in the altered context or when the auditory cue was paired with the shock.

It is important to note that because that it was the LPS-treated animals that *failed* to freeze, there appear to be no motor impairments or altered motivation due to LPS injections. As noted earlier, injections of LPS were presumably given after a fear response was established, and the results therefore suggest that LPS impairs some aspect of post-trial consolidation or memory representation of the context in which the fear was established. Results also suggest that LPS selectively impairs only certain kinds of memory processes. For example, contextual fear conditioning (shown to be disrupted by LPS) is thought to be a hippocampus-dependent task, whereas auditory fear conditioning (which is not disrupted by LPS) does not involve the hippocampus (Pugh et al., 1998).

Given that contextual fear is developed by the association of context to the US (i.e., footshock in this case), Pugh et al. (1998) argue that if LPS interferes with contextual fear conditioning by interfering with memory consolidation of the context, then preexposure to the context before conditioning and LPS injections should eliminate the impairment of contextual fear conditioning shown in LPS administration. On the other hand, if LPS disrupts the association of contextual memory representation to the shock, then preexposure to the context should have no effect. To experimentally examine this hypothesis, Pugh et al. (1998) preexposed rats to the context for two minutes 24 hours prior to conditioning, and the controls were handled for the same amount of time. At this point, the groups were not given LPS. After preexposure was finished, the conditioning session occurred and then half the rats in both groups were given LPS doses of 0.125 mg/kg. After analysis, LPS again produced a large detrimental effect on contextual fear conditioning when LPS was given immediately after the consolidation phase, but preexposure eliminated this effect. Therefore, the authors argued that preexposure to the context diminished the effects of LPS on the mental representation of the context. Coinciding with these LPS studies, Barrientos et al. (2002) showed that IL-1β administration impedes contextual fear conditioning by interrupting the mental representation of the context when given directly after conditioning. They found that infusion of IL-1β bilaterally into the dorsal hippocampus (the brain structure important in long term potentiation and memory consolidation) reduced the effects that preexposure to the context usually has.

A study that provides additional evidence that LPS produces learning/memory decrements was conducted by Sparkman et al. (2005). Four–month old experimentally naïve C57BL/6 mice were injected with LPS intraperitoneally or saline on day 1 of testing four hours prior to testing in a two-active avoidance conditioning procedure. Animals received 50 trials for five consecutive days. Each trial consisted of a CS (house light) for 5 sec, followed by a mild

footshock (0.4mA) for up to 5 sec. Each trial was separated by a 20 sec inter-trial interval (ITI). Animals could learn to avoid the shock (i.e., the US) by crossing to the other side of the conditioning chamber while the CS was being presented and before the presentation of the US. This was scored an avoidance response. If the animal did not cross while the CS was presented, the US was then presented. If the animal crossed when the US was presented, the response was scored as an escape response. If the animal did not cross when either the CS or the US was being presented (total of 10 sec) the response was scored as a null response. The results of this study showed that LPS interrupted the acquisition of this task, as evidenced by the LPS-treated animals making fewer avoidance responses than did control animals. To ensure that these data were not due to motor impairments, latencies to cross to the other side of the chamber (a good indicator of motor impairment in two-way active avoidance) was recorded, and there was no significant difference between treatment groups. These findings suggest that failure to avoid by LPS-treated animals was not due to motor impairment, but rather poor acquisition or a diminished ability to learn to avoid the footshock when presented with the CS (optimal response). The authors argued that the LPS-treated subjects may be less able to learn the CS-US relationship.

These data illustrate one important facet of the relationship between the immune system and CNS, in which cytokines are the primary mediators (Dantzer & Kelly, 1989). Furthermore, it has been shown that IL-1β administration inhibits long term potentiation (LTP), an effect that may have physiological relevance to hippocampus-dependent learning (Kelly et al., 2001). Knowing that pro-inflammatory cytokines act as mediators for deficits in learning and memory in some human populations, (e.g., people afflicted by inflammatory disease or people receiving cytokine treatment for cancer or hepatitis C infections), it seems important to look more closely at this relationship, to perhaps alleviate some of these deficits.

1.5. The Role of Microglia in the CNS

Microglial cells are known to play an important role in the CNS as immunocompetent and phagocytic cells, capable of releasing substances such as proinflammatory cytokines, in the event of CNS compromise by injury or infection (Kim & de Vellis, 2005). They have also been shown to play important roles in neuronal and glial cell degeneration in neurological disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis (Bo et al., 1994; McGeer et al., 1993; McGeer & McGeer, 1995). Microglia act as antigen presenting cells in the CNS, and are very important in the immune function of the CNS, through their expression of major histocompability complex (MHC) class II molecules and their phagocytic abilities (McGeer & McGeer, 1995). Peripheral administration of LPS is known to stimulate the immune system by activating macrophages in the periphery. Along with immune activation in the periphery, LPS has also been shown to act in the CNS to stimulate microglia, the brain's resident "immune cell." Indeed most scientists theorize that microglial cells arise from monocyte lineage from cells born in the bone marrow (just as peripheral macrophages are), and become fixed in the brain in early development, where they differentiate into glial cells (Rock et al., 2004). It is therefore not surprising that microglia release pro-inflammatory cytokines such as IL-1β and TNF-α in the hippocampus, as well as in other parts of the brain (Quan et al., 1994; Tanaka et al, 2006). It has been well established that microglia are the primary releasers of cytokines in the brain and possess receptors for many cytokines, and are thus able to regulate an inflammatory reaction in the CNS.

In addition to their role in cytokine release in the brain, microglia utilize a variety of other molecules, including some that promote inflammation through converting arachidonic acid (AA) to prostaglandins. These molecules are named cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), and are expressed most notably by neurons and glial cells in the

frontal cortex and the hippocampus (Breder et al., 1992; Yamagata et al., 1993). These isoforms convert free AA precursors to prostaglandins. Prostaglandins play a role in many CNS activities, such as the fever response, pain processing, inflammation, and blood flow (Yermakova & O'Banion, 2000). It is well known that the febrile response (i.e., fever response) is accompanied by prostaglandin production, and can be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs). Prostaglandins, particularly PGE2, influence expression of inflammation-related molecules such as IL-6, which causes proliferation of glial cells through its actions. Moreover, upregulation of COX-2 by proinflammatory cytokines occur in many peripheral cell types and in glia (O'Banion, 1999). Further, COX-2 is induced in microglia by LPS and the levels can be modulated by a variety of inflammation-related factors (Hoozmans et al., 2002). Neurons express cytokine receptors and show morphologic and phenotypic changes in response to IL-1β or other proinflammatory cytokines. The connection between inflammatory states and neuronal function may be provided, at least partially, by the cytokine-dependent regulation of COX in neurons. Lastly, prostaglandins themselves may mediate decreased food intake and somnolence behaviors that are expressed after LPS and cytokine administration, and NSAIDs such as indomethacin and ibuprofen are able to block this effect (Hellerstein et al., 1989).

1.6. Effects of Minocycline on Brain Physiology, Cytokines, and Learning

Minocycline is a second-generation semi-synthetic tetracycline derivative that crosses readily into most tissues (Aronson, 1980; O'Neil, 1999), and inhibits the activation of microglia, diminishing cytokine, COX, and PGE2 production (Kim et al., 2004; Yrjanheikki et al., 1999). In addition to being an antibiotic, minocycline has also been shown to act as a neuro-protective drug, by inhibiting microglial activation in many areas of the brain (Tikka et al., 2001). For example, Thomas-Cardiel et al. (2004) showed that minocycline treatment protected nigral dopaminergic neurons (an area that is substantially affected by Parkinson's disease) after LPS

insult, by inactivating microglial cells. Along with these results, minocycline partially prevented LPS-induced increases in mRNA for IL-1β and TNFα, as evidenced by reverse transcriptase polymerase chain reaction (RT-PCR) analysis. LPS is also known to have a detrimental effect on the neurogenesis of basal hippocampal neurons in rats, an area that, even as in adults, still shows signs of neuronal proliferation. A study done by Ekdahl et al. (2003) showed that administration of minocycline restored LPS-impaired neurogenesis, but did not effect neurogenesis in control animals. Inactivation of microglia contributed to the recovery of neurogenesis. Decreased neurogenesis in the hippocampus has been suggested to be involved in memory impairment (Shors et al., 2001) and depression (Thomas and Peterson, 2003). Ekdahl et al. (2003) concluded that the suppression of hippocampal neurogenesis by activated microglia contributes to the decreased cognitive function shown in aging, dementia, and other diseases in which inflammation frequently occurs.

The inactivation of microglia may have an effect on neurogenesis in other parts of the brain in addition to the hippocampus. For example, overproduction of cytokines also leads to neuronal loss in basal forebrain cholinergic neurons (BFCNs), which provide important input to hippocampal and cortical areas and are believed to be linked to memory and attentional processes (Kasa et al. 1997). LPS has been shown by many researchers to induce cholinergic loss in the basal forebrain, and this mimics many of the same symptoms that are seen in AD (Willard et al., 1999). Hunter et al. (2004) showed that long-term minocycline treatment inhibits microglial activation, prevents BFCN degeneration, and improves cognitive performance. To do this, they utilized a mouse model for Down's syndrome (DS) that showed many of the same behavioral symptoms as an AD model, and treated it chronically with minocycline. Subjects were tested for their performance in a water-escape radial-arm maze procedure, and their brains were later examined by immunohistochemistry for BFCN loss. Reduced cholinergic phenotypic

mice were accompanied by inflammation in the basal forebrain and limbic cortex, as evidenced by the increased activation of microglia in these regions. Minocycline treatment also reduced the cholinergic loss in the BFCN compared to control animals. Furthermore, minocycline reduced the severity of cognitive impairment of the DS mouse model, as evidenced by the reduced numbers of reference memory (RM) errors in the water radial-arm maze. Results from this study have shown that long-term minocycline prevents cholinergic loss and improves cognitive performance in a Down's syndrome mouse model.

1.7. Effects of Indomethacin on Brain Physiology, Cytokines, and Learning

The non-steroidal anti-inflammatory drug indomethacin, which causes the inhibition of cyclooxygenase (COX1 and COX2) and thereby prevents arachidonic acid from being converted into prostaglandins, has been shown to reduce inflammation (Vane & Blotting, 1998). Peripheral administration of LPS has been shown to increase expression of not only cytokines, but cyclooxygenases in the brain, which may contribute to observed cognitive deficits and may also contribute to pathological cell death via COX activity. Shaw et al. (2005) showed that blocking the LPS-induced COX activity produced a reversal in the learning deficits produced by LPS. Ibuprofen, a broad-spectrum COX inhibitor, was co-administered with LPS 4 hrs before testing in the Morris water maze. Swim rate analysis suggested that in fact the LPS/ibuprofen group learned the task better than the LPS only group, as evidenced by swim route analysis. Furthermore, Shaw et al. suggested that this effect can be explained by the inhibition of COX, through the administration of ibuprofen, disrupting the AA-PG pathway.

Along with the alleviation of the effects of LPS on spatial learning in the Morris water maze via ibuprofen, the administration of indomethacin, another non-selective COX inhibitor, has been shown to reduce the behavioral effects shown by IL-1β administration on schedule-controlled behavior and social exploration. A study conducted by Crestani et al. (1992)

investigated the possibility that the behavioral effects of IL-1β are mediated by prostaglandins. They injected IL-1β and indomethacin into subjects and they showed that pretreatment with indomethacin abolished the reduction in both schedule-controlled behavior and social exploration that was induced by IL-1β itself. They concluded that this effect was due to indomethacin-induced blocking of prostaglandin production, and that prostaglandins mediate the behavioral effects of peripherally injected IL-1β. They also concluded that prostaglandins are involved in the mediation of the behavioral components of the febrile response when the CNS is exposed to an endotoxin. Further, indomethacin-induced reduction in behavioral effects of LPS administration in one-way active avoidance, another task with clear hippocampus involvement, has also previously been reported. Ma and Zhu (1997) examined the effects of administration of indomethacin on LPS-induced impairment in one-way active avoidance and IL-6-induced increase in PGE2 release. Bilateral infusion of LPS into the hippocampus resulted in significant decrements in acquisition and retention (beginning 10 days after LPS infusion) by lengthening the latency to avoid in the shuttle-box. Intraperitoneal injections of indomethacin (10mg/kg; administered 24 hrs. post surgery, and for the next seven days) significantly shortened the latency to cross to the other side of the chamber, thus improving the deficit. The authors also showed that infusion of IL-6 increased PGE2 release in the hippocampus and this was suppressed by the infusion of indomethacin in the hippocampus for 1 hr.

In conjunction with behavioral/cognitive deficits, neuronal cell loss has also been shown following high doses of LPS infused directly into the brain, but when NSAID's are given, this loss is substantially reduced (Stéphan et al., 2003). As addressed in the previous section, microglia are the primary releasers of proinflammatory agents in the CNS. Furthermore, microglial activation due to inflammatory events is the primary means of progressive neurodegeneration and synaptic plasticity in AD. Stéphan et al. (2003) illustrated the

effectiveness of NSAID treatment on the restoration of working memory and impaired long-term potentiation in the dentate gyrus. Rats were injected with amyloid fragments (Aβ40/43), in order to produce aggregated amyloid deposits, cell loss, and inflammation in tissue, much like the natural progression of AD. When given indomethacin, subjects showed no deficit in working memory or reference memory relative to controls on an 8 arm radial maze (another indicator of spatial learning/memory). Furthermore, LTP in the dentate gyrus had no effect when animals were treated with indomethacin prior to injection of amyloid fragments. Lastly, their results suggest that the inflammatory reaction itself is an important pathological element of amyloid-induced deficits in synaptic function, which ultimately lead to working and reference memory errors.

As noted previously, there is considerable evidence suggesting that microglial inhibitors, such as minocycline, and non-selective COX inhibitors, such as indomethacin, reduce LPS-induced cytokine production, but the behavioral effects of minocycline and indomethacin on LPS-induced behavioral effects have not yet been adequately explored. The following experiments were designed to extend previous research to test the hypothesis that indomethacin and minocycline attenuate the behavioral effects of LPS administration. It was hypothesized that minocycline and indomethacin would reduce or eliminate the LPS-induced learning/memory deficits reported by Sparkman et al. (2005). Further, it was hypothesized that attenuation achieved via minocycline will be larger in magnitude than that produced by indomethacin administration, since prostaglandin synthesis is but one of the means by which microglia cells promote CNS inflammation. These findings might further provide new insights relevant to treatment of the negative effects that are often experienced by people suffering from autoimmune/inflammatory diseases, AD, PD, MS, and may help provide a portion of the theoretical framework for a more finely tuned therapeutic regimen.

2. METHODS

2.1. Experimental Subjects

Experiment 1 consisted of 101 and Experiment 2 consisted of 89 four-month old experimentally-naïve male C57BL/6J mice, that were bred in the Texas Christian University vivarium from a breeding stock obtained from The Jackson Laboratory (Bar Harbor, ME). All animals were housed in groups of 3–4 in standard polycarbonate mouse cages and allowed access to food and water *ad libitum*. Lights were set to an automated 0600 on and 1800 off light-dark cycle. All animals received care consistent with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and the experiment was conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Texas Christian University.

2.2.1. Experiment 1 Treatment Groups: Minocycline Pretreatment and LPS-induced Learning Decrement

The design for Experiment 1 was a 2 x 3 factorial design with two between factors (i.e., Minocycline treatment at doses of 0, 20, 40mg/kg and LPS treatment at doses of 0, 250μg/kg) for a total of six treatment groups (see Table 1 below). Minocycline hydrochloride (Sigma, St. Louis, MO) was diluted in a small volume of deionized water. Minocycline was co-administered intraperitoneally with LPS on day 1 only. Intraperitoneal injections of LPS (*Escherichia coli* serotype 0111:B4, Sigma, St. Louis, MO) was diluted in sterile 0.09% saline and given four hours prior to testing, in conjunction with the minocycline. For treatment controls, sterile 0.09% saline (vehicle for LPS; close to physiological cellular fluid) and deionized water (vehicle for minocycline; used for solubility purposes) were used and control animals received volume-equivalent injections at the same time(s) (i.e., all experimental subjects received injections of either minocycline, LPS, deionized water, saline, or a combination). All mice were visually

examined for common manifestations of "sickness behavior" (e.g., hunched posture, piloerection, decreased motor activity, etc.) and weighed daily, directly prior to testing.

N=101	VEHICLE (DIH_2O)	MINOCYCLINE (20MG/KG)	MINOCYCLINE (40MG/KG)
Saline	*n*=16	*n*=17	*n*=17
LPS (250μg/kg)	*n*=16	*n*=18	*n*=17

Table 1. Experimental design for Experiment 1 using minocycline and LPS.

2.2.2. Experiment 2 Treatment Groups: Indomethacin Pretreatment and LPS-induced Learning Decrement

As in Experiment 1, the design for Experiment 2 was a 2 x 3 factorial design with the two between factors (this time, Indomethacin treatment [Sigma, St. Louis, MO] at doses of 0, 1, 10mg/kg and LPS treatment at doses of 0, 250μg/kg) for a total of six treatment group (see Table 2 below for experimental design for Experiment 2). Indomethacin was diluted in 0.1M $NaHCO_3$ and then gently heated before injections, in order to keep the drug in solution.

N=89	VEHICLE ($NAHCO_3$)	INDOMETHACIN (1MG/KG)	INDOMETHACIN (10MG/KG)
Saline	*n*=15	*n*=15	*n*=13
LPS (250μg/kg)	*n*=14	*n*=16	*n*=16

Table 2. Experimental design for Experiment 2 using indomethacin and LPS.

2.3. Testing Procedures for Experiments 1&2

All instruments and testing procedures described below, was used in both experiments. The testing order is shown in Table 3 below.

Table 3. Timeline for both Experiments 1 & 2 on day 1 of testing only.

2.3.1. Testing Procedure: Elevated Plus Maze

The elevated plus maze is a black Plexiglas platform that is shaped like a plus symbol, with arms measuring 5 cm wide at all points and standing 50 cm off of the floor (File et al., 1993). Two arms located at opposite sides of the maze have translucent Plexiglas walls, while the other "open" arms have none. Each of the experimental subjects was tested only once, one hour before the start of two-way active avoidance and 3 hours after LPS and drug administration on day 1. The trial was recorded by a computerized animal tracking system containing a template of the maze (Accuscan Instruments, Columbus, OH) for 5 minutes. Number of entries into the open arms (*without* Plexiglas walls), number of entries into closed arms (*with* Plexiglas walls), time spent in center square, entries into the distal portion of open arms, and number of rears were recorded.

2.3.2. Testing Procedure: Two-way Active Avoidance

Fully automated Gemini II shuttlebox units (San Diego Instruments; San Diego, CA) were used to assess two-way active avoidance learning (Sparkman et al., 2005). Within each shuttlebox, a barrier with a square hole (3 3/8in W X 2 3/8in L) at floor-level divides the box into two separate but equal chambers (8 1/8in W X 9 7/8in L X 6 3/4in H), in which infrared photocells sense the location of the animal within the chamber. At the beginning of each day of testing, there was a 5 min acclimation period during which the subject was able to move freely between the chambers. At the end of the acclimation period, the conditioned stimulus (CS) was presented for a CS interval of 5 s. The unconditioned stimulus (US), a footshock mild(0.4mA), was administered through an electronic scrambler, for up to 5 s. The CS remained on when the US was delivered. If the animal crossed to the other chamber before the end of the CS interval, the US was not presented and a 20 s inter-trial interval (ITI) began. During the ITI, the animal was able to move back and forth between the two chambers, without affecting the beginning of the next trial. Each mouse was given 50 trials a day, for 5 days. Each trial was scored as an avoidance response (crossing to the other chamber before shock initiation), an escape response (crossing to the other chamber during the shock), or a null response (remaining in the original chamber and receiving 5 s of shock). In addition, latency to perform avoidance and escape responses was recorded, to uncover any freezing behavior or locomotor impairments. Lastly, the number of ITI crossings was recorded as a measure of non-cued, or random, crossing activity. Between testing sessions, the shuttleboxes were cleaned with Odormute™ (Ryter Corp., Madelia, MN) to reduce odor transfer between each experimental subject. The lighting within the testing room was minimized to make the CS more prominent, and a white noise generator was placed in the room to minimize superfluous sounds and sound transfer between boxes.

2.4. Statistical Analyses

The data for the weight and elevated plus maze were analyzed using standard factorial analysis of variance (ANOVA) procedures (Statview 5.0, SAS Institute Inc., Cary, NC), with Drug (minocycline or indomethacin, Sigma, St. Louis, MO) and LPS condition as the between-subject variables. These data from two-way active avoidance were analyzed using repeated-measures ANOVAs, with LPS administration (LPS 250μg/kg vs. Saline) and either minocycline (20mg/kg vs. 40mg/kg) or indomethacin (1mg/kg vs. 10mg/kg) as the between-subjects variables and Testing Day (days 1–5) as the within-subjects and repeated measures variables. Significant main effects and interaction effects were subjected to post-hoc analysis to determine significant differences between treatment groups. The criterion for rejecting the null hypothesis was set at an alpha level of $p < 0.05$.

3. RESULTS

3.1. Weight (Experiments 1 & 2)

Each day following behavioral testing, animals were visually inspected and weighed. For Experiment 1, there was a LPS treatment x Day interaction for weight ($F(4,380)=12.21$; $p<0.0001$; see Table 4), with LPS-treated subjects animals weighing less than saline control animals on days 2, 3, and 4 of testing. For Experiment 2, there was also a significant LPS x Day interaction, with LPS-treated subjects weighing less than saline control animals on days 2, 3 and 4 of testing ($F(4,364)=21.81$; $p<0.0001$; see Table 5), and a significant Indomethacin x Day interaction ($F(8,364)=7.07$; $p<0.0001$; see Table 6).

	Day 1	Day 2	Day 3	Day 4	Day 5
Saline	28.43 (0.31)	27.07 (0.52)	27.45 (0.31)	27.47 (0.32)	27.39 (0.33)
LPS	29.11 (0.28)	26.04 (0.29)	26.37 (0.28)	26.98 (0.30)	27.05 (0.29)

Table 4. Mean weight for saline- and LPS-treated animals across testing days for Experiment 1. The standard error of the mean is indicated in parentheses.

	Day 1	Day 2	Day 3	Day 4	Day 5
Saline	27.95 (0.32)	27.33 (0.33)	27.04 (0.33)	26.70 (0.33)	26.90 (0.31)
LPS	28.89 (0.27)	26.85 (0.28)	26.94 (0.29)	27.01 (0.26)	27.32 (0.32)

Table 5. Mean weight for saline- and LPS-treated animals across testing days for Experiment 2. The standard error of the mean is indicated in parentheses.

	Day 1	Day 2	Day 3	Day 4	Day 5
Vehicle	28.17 (0.33)	26.62 (0.32)	26.75 (0.34)	26.82 (0.34)	26.94 (0.33)
Indo 1	28.97 (0.40)	27.47 (0.35)	27.55 (0.38)	27.53 (0.42)	27.87 (0.36)
Indo 10	28.25 (0.37)	27.15 (0.36)	26.66 (0.38)	26.20 (0.33)	26.51 (0.31)

Table 6. Mean weight for vehicle, indomethacin (1 and 10 mg/kg) treated animals across testing days for Experiment 2. The standard error of the mean is indicated in parentheses.

3.2. Experiment 1: Elevated Plus Maze

In Experiment 1, there were no significant main effects of LPS or minocycline, nor were there any LPS x Minocycline interactions for percent time spent in closed arms, center of maze, and open arms (see Figure 1). LPS-treated animals spent more time in the closed arms, open arms and then the center of the maze respectively, but this was not significantly different than observed for saline-treated animals. However, there was a significant main effect of LPS for total distance traveled in the elevated plus maze ($F(1,127)=0.78$; $p<0.05$; See Figure 2), in which the LPS-treated animals moved less in the maze than did the saline-treated animals.

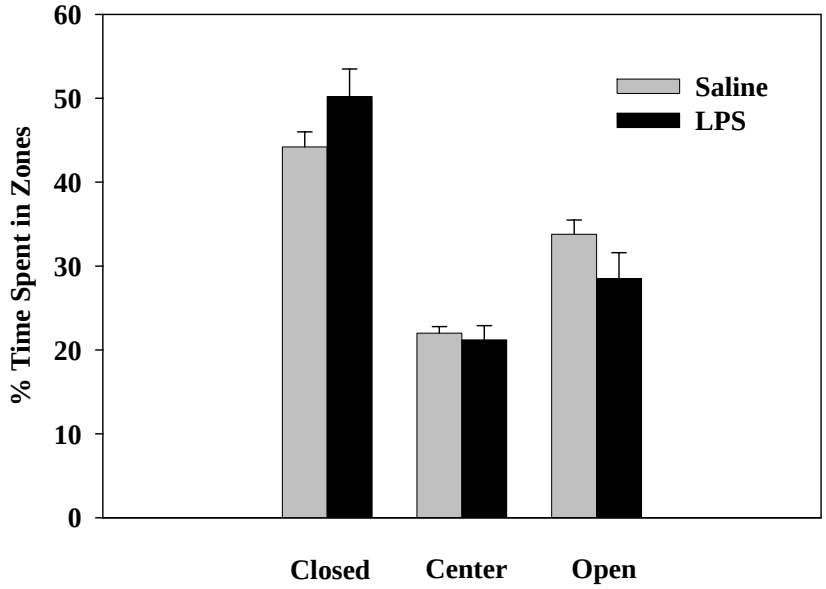

Figure 1. LPS-treated animals did not show the hypothesized decrease in percentage of time spent in open arms and center of the maze or the hypothesized increase in percentage of time spent in closed arms of the elevated plus maze as compared to saline-treated animals. Error bars reflect the standard error of the mean.

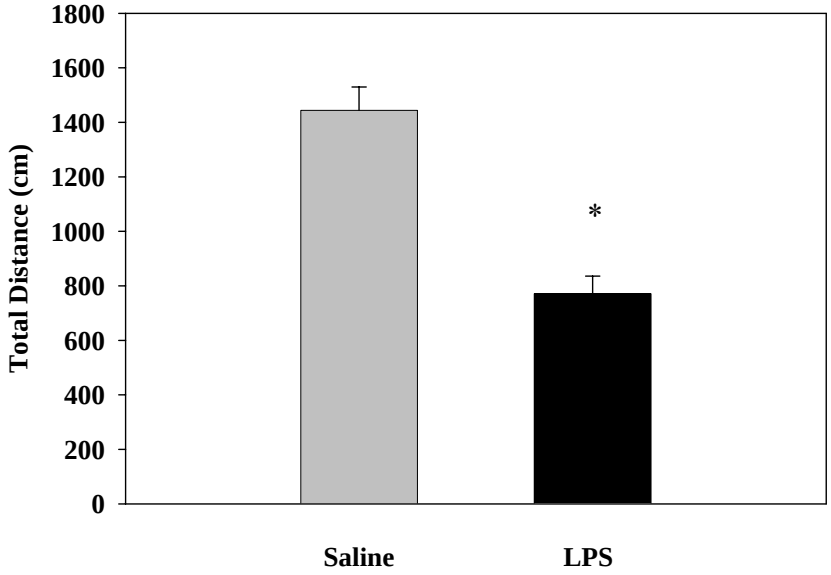

Figure 2. LPS-treated animals showed diminished locomotor activity in elevated plus maze as compared to saline-treated animals as measured by the total distance traveled.* Significantly different from saline (p<0.05). Error bars reflect standard error of the mean.

3.3. Experiment 1: Two-way Active Avoidance

In Experiment 1, two-way active avoidance data showed no significant main effect of LPS or minocycline alone, but there was a significant LPS x Minocycline interaction for avoidance responses (F(2,95)=4.78; p<0.05; see Figure 3). Post-hoc Fisher's PLSD (probable least-squares difference) revealed that the difference was between Saline/Minocycline 40 mg/kg vs. Saline/Vehicle (p<0.05; see Figure 3), indicating that when saline animals are given minocycline 40 mg/kg they show a greater number of avoids compared to vehicle control group. Oddly, animals given LPS showed a greater number of avoidance responses than saline-treated animals when both were co-administered vehicle (p<0.05; see Figure 3). Finally, there was a difference between the LPS/Minocycline 40 mg/kg vs. Saline/Minocycline 40 mg/kg groups (p<0.05; see Figure 3), showing that the group given LPS in conjunction with minocycline 40 mg/kg showed a decreased number of avoidance responses compared to animals given saline with minocycline 40 mg/kg. Additionally, there was a significant LPS x Minocycline interaction for escape responses (F(2,95)=4.94; p<0.005; see Figure 4). Fisher's PLSD again revealed that

the difference was between Saline/Minocycline 40 mg/kg vs. Saline/Vehicle ($p<0.05$; see Figure 4), indicating that saline animals given minocycline 40 mg/kg made fewer escape responses compared to animals given saline with vehicle. Also, there was a difference between LPS/Vehicle vs. Saline/Vehicle groups ($p<0.05$; see Figure 4), indicating that animals given LPS performed fewer escape responses than saline animals when both were co-administered with vehicle. Additionally, a difference between LPS/Minocycline 40 mg/kg vs. Saline/Minocycline 40 mg/kg groups ($p<0.05$; see Figure 4) was found, indicating that LPS given in conjunction with minocycline 40 mg/kg increased the number of escape responses compared to animals given saline and minocycline 40 mg/kg.

Finally, there was a significant LPS x Minocycline interaction ($F(2,95)=3.83$; $p<0.05$) for Response Efficiency (see Figure 5). Fisher's PLSD showed that differences were between Saline/Minocycline 40 mg/kg vs. Saline/vehicle ($p<0.05$; see Figure 5), indicating that saline animals given minocycline 40 mg/kg showed more response efficiency compared to animals given saline with vehicle. Post-hoc testing once again showed a difference between the LPS/Minocycline 40 mg/kg vs. Saline/Minocycline 40 mg/kg groups ($p<0.05$; see Figure 5), indicating that minocycline 40 mg/kg given in conjunction with LPS decreased the response efficiency, compared to animals given minocycline 40 mg/kg with saline. Co-treatment with LPS and either 20 or 40 mg/kg of minocycline did not significantly alter performance on two-way active avoidance conditioning, as illustrated by the LPS x Minocycline x Day interaction ($F(8,380)=1.02$;) for avoidance responses ($p=0.42$ n.s.; see Figure 6), escape responses ($p=0.51$ n.s.; see Figure 7), ITI crossings ($p=0.50$; see Figure 8), or response efficiency ($p=0.26$ n.s.; see Figure 9).

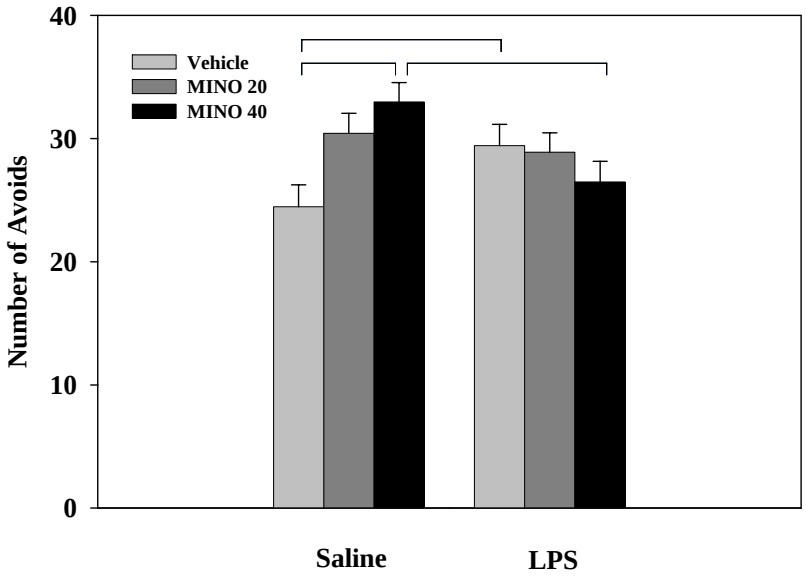

Figure 3. Mean differences between treatment groups for avoidance responses. Collapsed across days. Bars indicate significant differences between groups ($p<0.05$). Error bars reflect the standard error of the mean.

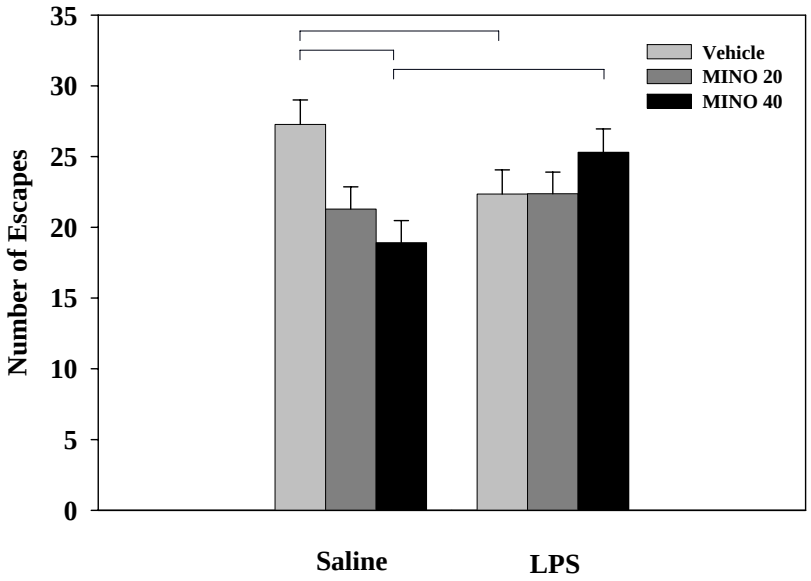

Figure 4. Mean differences between treatment groups for escape responses. Collapsed across days. Bars indicate significant differences between groups ($p<0.05$). Error bars reflect the standard error of the mean.

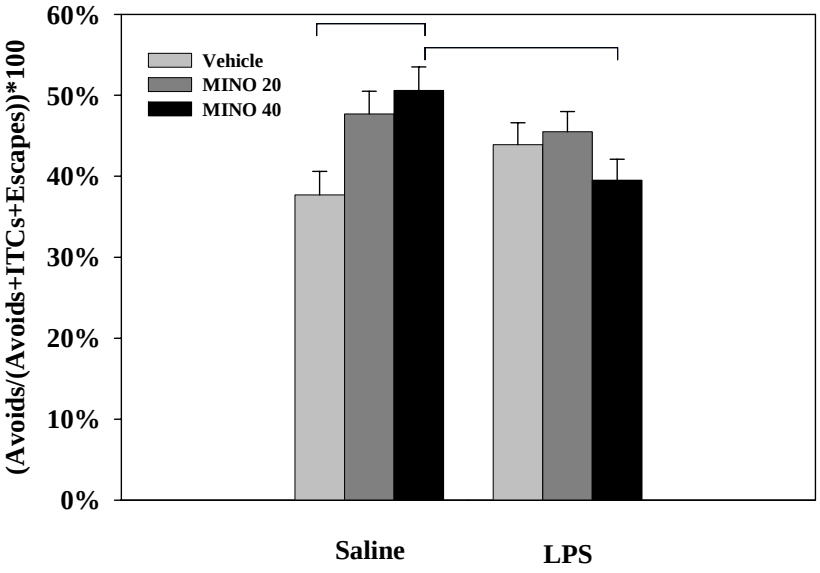

Figure 5. Mean differences between treatment groups for response efficiency. Collapsed across days. Bars indicate significant differences between groups (p<0.05). Error bars reflect the standard error of the mean.

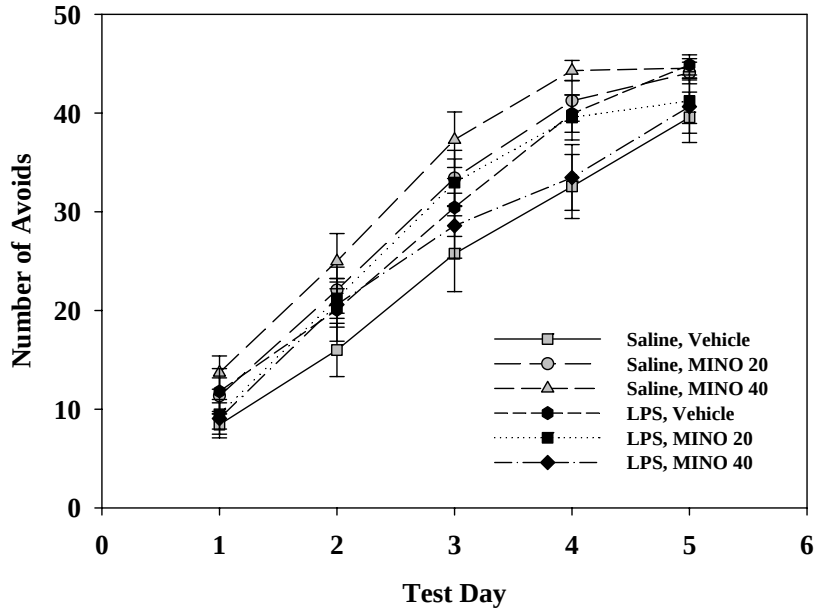

Figure 6. Effects of co-administration of LPS and minocycline on the number of avoidance responses across testing days. Error bars reflect the standard error of the mean.

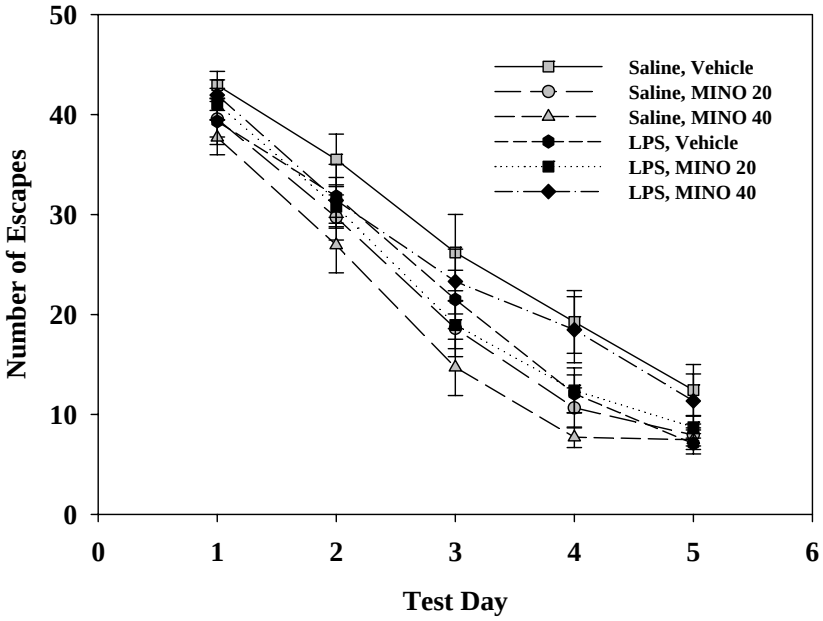

Figure 7. Effects of co-administration of LPS and minocycline on the number of escape responses across testing days. Error bars reflect the standard error of the mean.

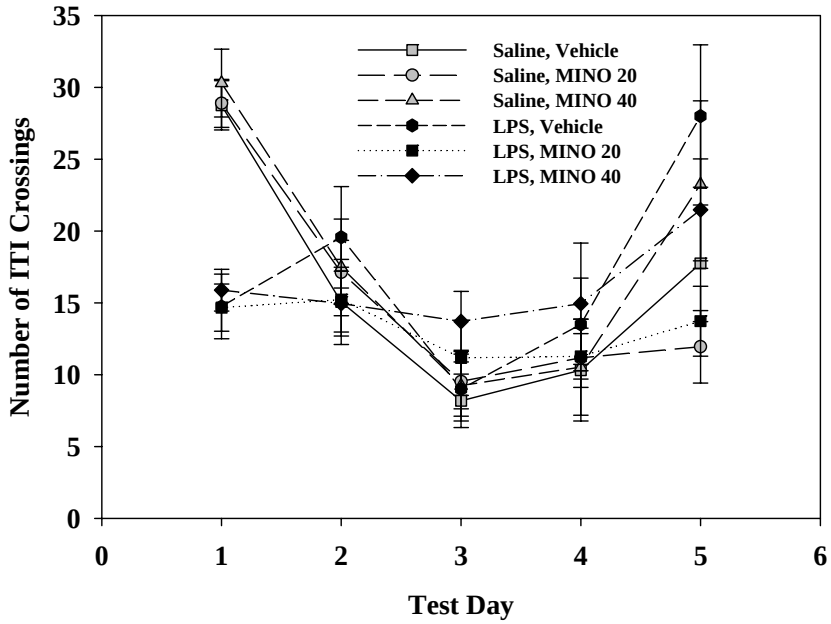

Figure 8. Effects of co-administration of LPS and minocycline on the number of inter-trial interval crossings across testing days. Error bars reflect the standard error of the mean.

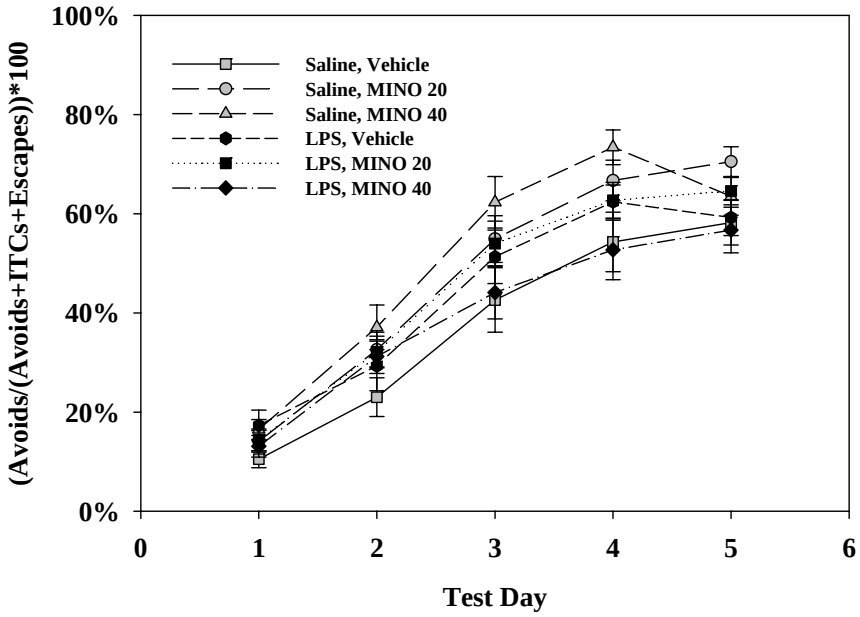

Figure 9. Effects of co-administration of LPS and minocycline on response efficiency across testing days. Error bars reflect the standard error of the mean.

3.4. Experiment 2: Elevated Plus Maze

In Experiment 2, there were significant LPS main effects for percent time spent in closed arms ($F(1,93)=4.47$; $p<0.05$; see Figure 10), and percent time spent in center of maze ($F(1,93)=5.27$; $p<0.05$; see Figure 10), but not for percent time spent in open arms ($F(1,93)=1.03$; $p=0.31$ n.s.; see Figure 10). Overall, LPS-treated animals spent more time in the closed arms versus the open arms. Moreover, indomethacin had no significant impact on LPS-induced anxiety-like behavior, evidenced by the lack of significant LPS x Indomethacin interactions for the percent time spent in closed arms ($F(2,93)=0.29$; $p=0.75$ n.s.; data not shown), percent time in center ($F(2,93)=0.37$; $p=0.69$ n.s.; data not shown), and percent time in open arms ($F(2,93)=0.47$; $p=0.63$ n.s.; data not shown).

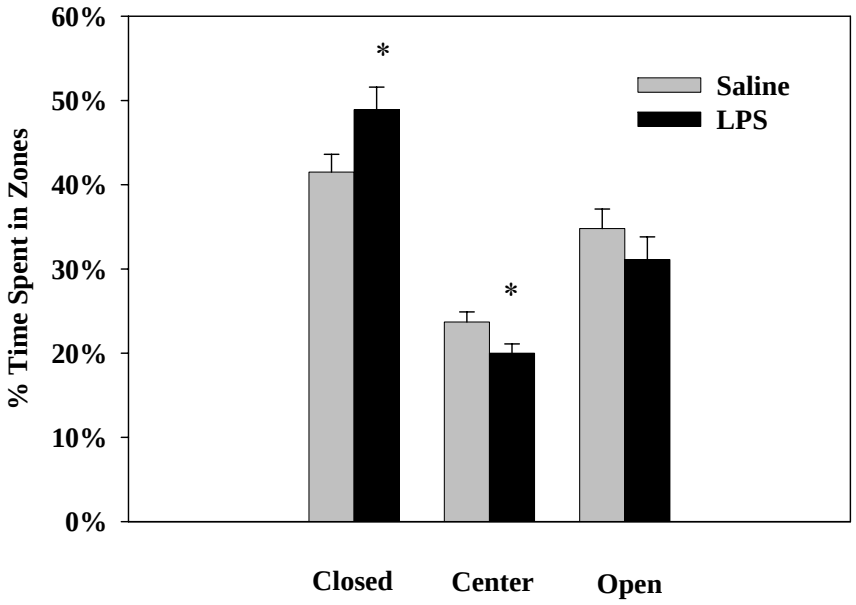

Figure 10. LPS-treated animals showed the hypothesized increase in the percentage of time spent in the closed arms and center of the elevated plus maze as compared to saline-treated animals. There was no difference in the percent time spent in the open arms of the maze. * Significantly different from saline ($p<0.05$). Error bars reflect the standard error of the mean.

3.5. Experiment 2: Two-way Active Avoidance

In Experiment 2, two-way active avoidance data revealed that there were no significant effects of LPS, Indomethacin, or LPS x Indomethacin interactions. Co-administration of LPS and either 1 or 10 mg/kg of indomethacin also did not significantly alter performance on two-way active avoidance conditioning, as illustrated by the lack of significant LPS x Indomethacin x Day interactions for avoidance responses (p=0.41 n.s.; see Figure 11), escape responses (p=0.49 n.s.; see Figure 12), inter-trail crossings (p=0.29 n.s.; see Figure 13), and for response efficiency (p=0.82 n.s.; see Figure 14) in two-way active avoidance.

.

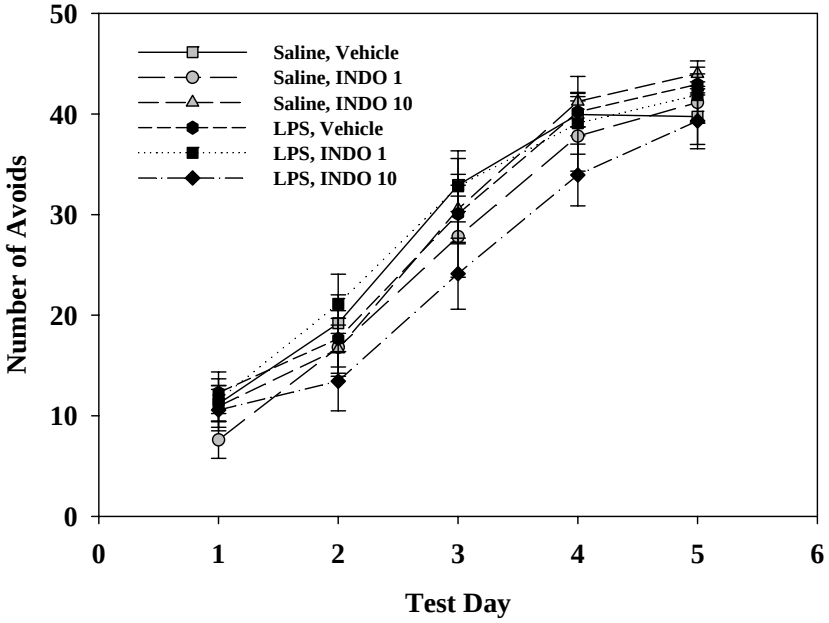

Figure 11. Effects of co-administration of LPS and indomethacin on the number of avoidance responses in across testing days. Error bars reflect the standard error of the mean.

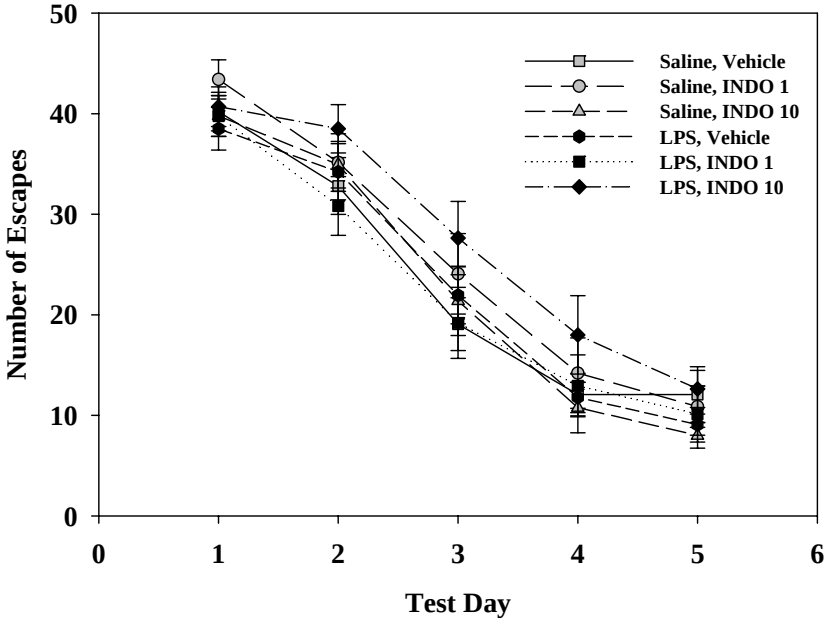

Figure 12. Effects of co-administration of LPS and indomethacin on the number of escape responses in across testing days. Error bars reflect standard error of the mean.

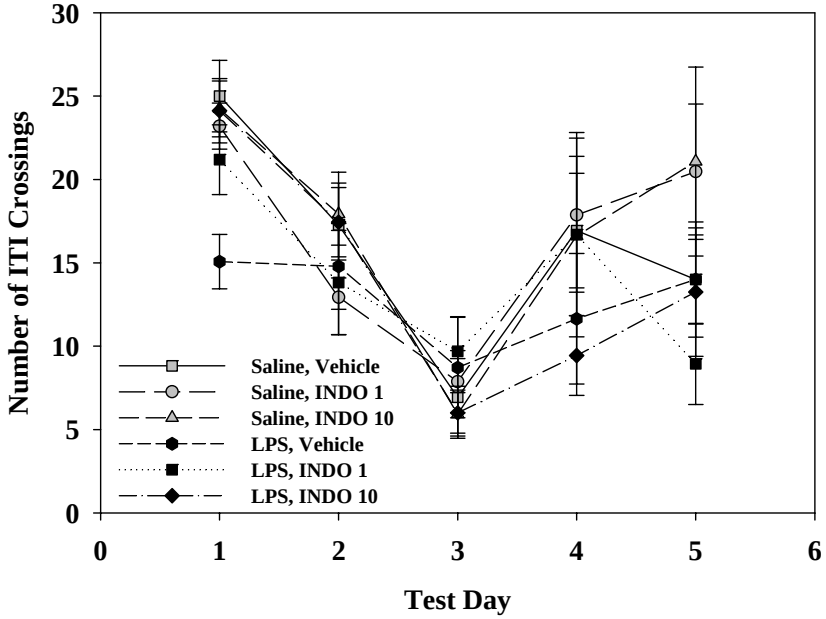

Figure 13. Effects of co-administration of LPS and indomethacin on the number of inter-trial interval crossings across testing days. Error bars reflect the standard error of the mean.

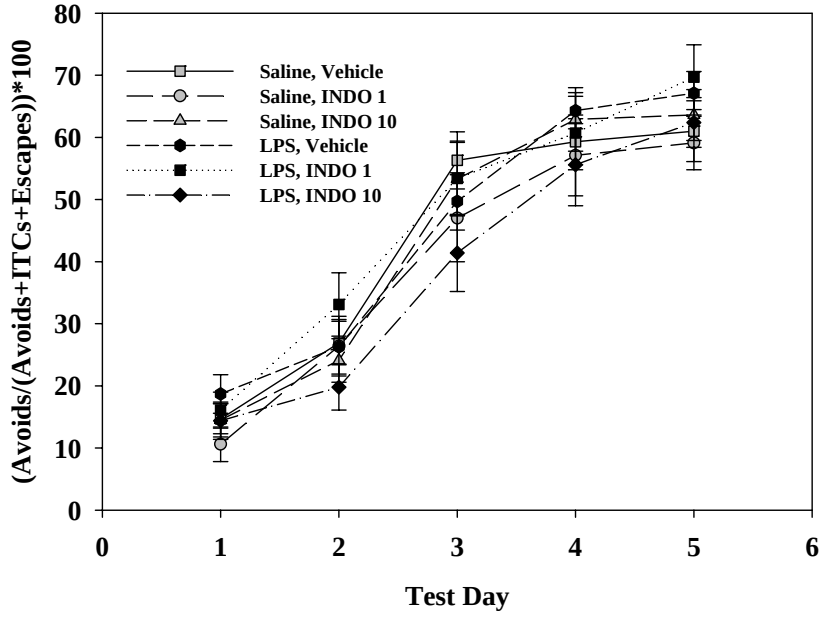

Figure 14. Effects of co-administration of LPS and indomethacin on response efficiency across testing days. Error bars reflect the standard error of the mean.

4. DISCUSSION

The current experiments were designed to investigate the role of microglial activation and prostaglandin production on LPS-induced learning impairment, by determining whether minocycline and/or indomethacin would attenuate the behavioral effects of LPS administration. Experiment 1 addressed the hypothesis that minocycline, through microglial inhibition, would attenuate the behavioral effects seen after LPS administration via reduction of central cytokine production. It was hypothesized that indomethacin, through COX and prostaglandin inhibition would produce a smaller attenuation of behavioral sequellae of following LPS administration. Analyses revealed that data from both experiments failed to support the respective hypotheses.

4.1. LPS and Anxiety-Like Behavior

Previous research has shown that LPS, presumably via cytokine pathways, produces anxiety-like effects in the elevated plus maze by increasing the overall time spent in the closed arms verses the open arms (Lacosta et al., 1999). An anxious animal spends more time in the closed arms as it is thought to feel more secure than when in a wide open space. In Experiment 1, it was hypothesized that the inactivation of microglia by minocycline (an antibiotic and potent microglia inhibitor) would attenuate the predicted effects of LPS on anxiety-like behavior. As expected, LPS decreased the overall distance traveled (a measure of sickness behavior) in the elevated plus maze, indicating LPS treatment decreased locomotor activity. Minocycline given in conjunction with LPS had no effect on this LPS-induced decrease in locomotor activity. LPS did not significantly effect anxiety levels when compared to the saline control group, as evidenced by no differences between the two groups in the percent time spent in the closed, center, or open zones of the maze. Furthermore, minocycline given with LPS or saline showed no significant effect on the percent time spent in the closed, open, and center zones of the maze.

These data fail to support the hypothesis that minocycline would attenuate the LPS-induced behavioral effects in the elevated plus maze.

In contrast to the pure performance effects observed in experiment 1, there was modest evidence of LPS-induced anxiety in Experiment 2. LPS administration significantly increased anxiety-like behavior, as evidenced by LPS-treated animals spending more time in the closed arms and less time in the center of the maze compared to saline-treated animals. These findings support previous research by Lacosta et al. (1999). This effect cannot be attenuated to deficits in locomotor activity, given that LPS produced no effect on the total distance traveled in the elevated plus maze when compared to saline-treated animals. Given that prostaglandin production ultimately leads to overproduction of cytokines in the CNS, it was hypothesized the reduction of prostaglandin synthesis produced by cyclooxygenase inactivation, that indomethacin would attenuate the behavioral effects of LPS administration. However, the data failed to support that hypothesis. Further, indomethacin administration showed no statistically significant effects on either LPS- or saline-treated animals. This indicates that indomethacin failed to attenuate the LPS-induced anxiety-like behavior that has been reported by Lacosta et al.

4.2. LPS and Learning/Memory

Proinflammatory cytokines are thought to be the main contributors to cognitive impairments resulting from immune activation. Past research has shown that LPS and cytokine administration interfere with learning/memory processes in a variety of paradigms (Aubert et al., 1995; Barrientos et al., 2002; Pugh et al., 1998; Shaw et al., 2001; Sparkman et al., 2005). Typically, in a two-way active avoidance paradigm, LPS-treated animals perform fewer of the optimal avoidance responses and display decreased response efficiency, relative to saline control animals. As microglial cells are the primary releasers of cytokines in the CNS, we hypothesized that blocking microglia activation with minocycline would attenuate the LPS-induced learning

and memory deficits in a dose-dependent manner. Another pathway that leads to cytokine overproduction is via prostaglandins synthesized by COX enzymes. Therefore, it was also hypothesized that indomethacin, through inhibition of prostaglandin synthesis, would also attenuate LPS-induced learning/memory in a dose-dependent manner. Neither hypothesis was confirmed.

In these rounds of two-way active avoidance, surprisingly, LPS administration did not alter learning. In fact, oddly, data showed animals given LPS and vehicle made significantly *greater* number of avoidance responses, and fewer escape responses when compared to animals given saline and the vehicle, seemingly indicating that LPS-treated animals learned the task better than the control animals. However, these data directly contradict *a number* of previous studies from our lab. We attribute this effect to the altered performance in the control group, as they performed at levels that were significantly lower than we normally observe (i.e., it appears that something was wrong with our control group). Further, minocycline administration modestly, but significantly, altered behavior both in LPS- and saline-treated animals. Data revealed that saline animals given high-dose minocycline showed a significantly greater number of avoidance responses, made fewer escape responses, and showed greater response efficiency than saline vehicle animals, implying that they learned the task of shuttling when presented with the CS. It appears that giving high-dose minocycline actually improved learning in two-way active avoidance in saline animals relative to vehicle (control). Finally, the data indicated that animals administered LPS and the high-dose minocycline showed a decrease in the number of avoidance responses, a corresponding increase in the number of escape responses, and a decrease in response efficiency when compared to animals that received saline and high-dose minocycline, indicating that they failed to adequately learn the task. These data failed to support the original hypothesis that minocycline would attenuate LPS-induced learning deficits that have

been previously reported by our lab. Low-dose minocycline did not show any significant effects in two-way active avoidance.

Two-way active avoidance results for Experiment 2, as for Experiment 1, showed no significant effects for LPS treatment, indomethacin treatment (1 or 10 mg/kg), or any interactions between these variables. These results did not replicate findings seen previously in our lab, results that have consistently shown that LPS impairs learning in two-way active avoidance conditioning. Therefore, because we were unable to replicate our basic LPS findings, it is not surprising that these data do not support the hypothesis that indomethacin would attenuate LPS-learning deficits in two-way active avoidance paradigm (i.e., there were no deficits to attenuate). Further, indomethacin failed to exert any effect whatsoever on any dependent variables analyzed.

The present data of Experiment 1 & 2 failed to show the expected effect of LPS on learning. Therefore, indomethacin and minocycline might have worked, if LPS results were consistent with prior results that LPS impairs learning and memory processes. This study, as it stands, lacks confirming evidence of the potential benefits of minocycline and indomethacin administration in attenuating LPS learning/memory deficits seen previously in our lab.

4.3. Summary, Possible Explanations, and Future Directions

In summary, data from this study were disappointing and did not support our primary hypotheses. For Experiment 1, LPS-treated animals traveled less than saline-treated animals in the elevated plus maze as expected, showing sickness behavior. However, there were no clear effects on anxiety levels. In contrast to Experiment 1, LPS-treated subjects in Experiment 2 showed increased anxiety-like behavior in the elevated plus maze. The data revealed that LPS-treated animals spent significantly more time in the closed and center of the maze, when compared to saline-treated animals. These data support previous work by Lacosta et al. (1999),

showing that LPS-treated animals spend more time in the closed arms, when compared to saline-treated animals, showing anxiety-like effects. However, indomethacin or minocycline did not significantly alter the LPS-induced effects, disconfirming our hypothesis.

Results from both experiments failed to show the expected LPS-induced learning deficits. Oddly, the data indicate that the LPS/vehicle group learned marginally better than the Saline/Vehicle animals, as they performed a greater number of avoidance responses, compared to saline-treated animals, when they were both co-administered with vehicle. Furthermore, high-dose minocycline given with either LPS or saline, altered performance. Mice that received saline and high-dose minocycline showed a greater number of avoidance responses than animals that received saline and vehicle. These data were unexpected, and suggested that high-dose minocycline itself actually improves learning. However, giving high dose minocycline with LPS impairs learning when compared to saline/high dose minocycline animals. This odd pattern of findings might indicate the absence of a trustworthy LPS control group. The lower dose of minocycline did not alter behavior. Indomethacin at either dose levels (20 or 40 mg/kg) did not significantly alter performance, nor did it interact with LPS administration for the number of avoids, number of escape responses, inter-trial interval crossings or response efficiency in two-way active avoidance.

The reasons for these irregular results are currently unclear. Potential reasons that the data failed to support our hypotheses may include, among other possibilities, the following: 1) LPS-treated animals failed to show normal deficits in learning, so there was no chance of confirming the hypotheses that minocycline/indomethacin would abrogate the learning effects of LPS; 2) even when other effects of LPS were present (e.g., weight loss, locomotor effects on Day 1, etc.) dose levels of indomethacin and minocycline were not within the right range to attenuate these effects of LPS; 3) despite correct dose levels, pharmacokinetic variables may not have

allowed minocycline/indomethacin to reach sufficient plasma levels/central levels to block these effects; 4) there was a low-level infection in the colony when data was being collected ; 5) experimental error (e.g., undergraduate helpers, fluctuating vivarium conditions, other nuisance variables, etc.) disrupted the studies; or 6) the null hypothesis is correct (i.e., these drugs simply do not affect at least the sickness behavior aspects of LPS administration).

Considering some of the potential reasons that these studies failed to support the hypotheses, some future directions are clear. One is to do an animal health check prior to any future experiments. Secondly, an attempt to replicate these studies needs to be done, in an effort to obtain the LPS effects on learning typically observed, so that drug administration has a chance to exert effects. Also, manipulation of drug doses to determine the optimal dose for behavioral effectiveness. Along similar lines, we plan to examine chronic doses of both minocycline and indomethacin to increase both plasma and central levels of the drugs before LPS administration. Lastly, assessing fever in experimental animals might also provide a beneficial means by which to show the possible blockade of the sickness response to LPS administration. These directions could prove beneficial in supplementing the current experimental design, in order to address the hypotheses that minocycline and indomethacin attenuate LPS-induced learning/memory deficits. Potential benefits from these studies could help provide a theoretical basis for treatment of various progressive neurodegenerative and chronic inflammatory disorders.

REFERENCES

Aronson, A.L. (1980). Pharmacotherapeutics of the newer tetracyclines. *Journal of the American Veterinary Medical Association,* 176, 1061–1068.

Aubert, A., Vega, C., Dantzer, R., and Goodall, G. (1995). Pyrogens specifically disrupt acquisition of a task involving cognitive processing in the rat. *Brain, Behavior, and Immunity,* 9, 129–148.

Barrientos, R.M., Higgins, E.A., Sprunger, D.B., Watkins, L.R., Rudy, J.W., and Maier, S.F. (2002). Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus. *Behavioural Brain Research,* 134, 291–298.

Black, P.H. (1994). Central nervous system-immune system interactions: psychoneuroendocrinology of stress and its immune consequences. *Antimicrobial Agents and Chemotherapy,* 38, 1–6.

Bluthé, R.M., Dantzer, R., and Kelly, K.W. (1992), Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rats. *Brain Research,* 573, 318–320.

Bo, L., Monk, S., Kong, P., Nyland, H., Pardo, C., and Trapp, B. (1994). Detection of MHC class II antigens on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis lesions. *Journal of Neuroimmunology,* 51, 135–146.

Borowski, T., Kokkinidis, L., Merali, Z., and Anisman, H. (1998). Lipopolysaccharide, central *in vivo* biogenic amine variations, and anhedonia. *NeuroReport,* 9, 3797–3802.

Breder, C.D., Smith, W.L., Raz, A., Masferrer, J., Siebert, K., Needleman, P., and Saper, C.B. (1992). Distribution and characterization of cyclooxygenase immunoreactivity in the brain. *Journal of Comparative Neurology,* 322, 409–438.

Cohen, N., and Kinney, K.S. (2001). Exploring the phylogenic history of neural-immune system interactions. In R. Ader, D.L. Felton, & N. Cohen (Eds.), *Psychoneuroimmunology* (3rd ed., pp. 21–54). New York: Academic Press.

Crestani, F., Seguy, F., and Dantzer, R. (1990). Behavioural effects of peripherally injected interleukin-1: role of prostaglandins. *Brain Research,* 542, 330–335.

Dantzer, R., and Kelly, K.W. (1989). Stress and immunity: An integrated view of relationships between the brain and the immune system. *Life Sciences,* 44, 1995–2008.

Ekdahl, C.T., Claasen, J., Bonde, S., Kokaia, Z., and Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proceedings of the National Academy of Sciences,* 100, 13632–13637.

Felten, S.Y. and Felten, D.L. (1991). *Innervation of lymphoid tissue.* (2nd Ed.). San Diego, CA: Academic Press.

File, S.E. (1993). The interplay of learning and anxiety in the elevated plus-maze. *Behavioural Brain Research,* 58, 199–202.

Gibertini, M. (1996). IL-1 beta impairs relational but not procedural rodent: learning in a water maze task. *Advanced Experimental Medical Biology,* 402, 207–217.

Hart, B.L. (1988). Biological basis of behavior sick animals. *Neuroscience and Biobehavioral Reviews,* 12, 123–137.

Hellerstein, M.K., Meydani, S.N., Meydani, M., Wu, K., and Dinarello, C.A. (1989). Interleukin-1 induced anorexia in the rat: influence of prostaglandins. *Journal of Clinical Investigation,* 84, 228–235.

Hoozemans, J.M., Veerhuis, R., Janssen, I., van Elk, E., Annemieke, J. M., Rozemuller and Eikelenboom, P. (2002). The role of cyclo-oxygenase 1 and 2 activity in prostaglandin E_2

secretion by cultured human adult microglia: Implications for Alzheimer's disease. *Brain Research,* 951, 218–226.

Hunter, C.L., Bachman, D., and Granholm, A. (2004). Minocycline prevents cholinergic loss in a mouse model of down's syndrome. *Annals of Neurology,* 56, 675–688.

Kasa, P., Rakonczay, Z., and Gulya, K. (1997). The cholinergic system in Alzheimer's disease. *Progress in Neurobiology,* 52, 511–535.

Kelly, A., Lynch, A., Vereker, E., Nolan, Y., Queenan, P., Whittaker, E., O'Neill, L.A.J. and Lynch, M. (2001). The anti-inflammatory cytokines, interleukin (IL)-10, blocks the inhibitory effect of IL-1β on long term potentiation. *The Journal of Biological Chemistry,* 276, 45564–45572.

Kent, S., Bluthé, R.M., Kelley, K.W., and Dantzer, R. (1992). Sickness behavior as a new target for drug development. *Trends in Pharmacological Sciences,* 13, 24–28.

Kent, S., Bret-Dibat, J.L., Kelley, K.W., and Dantzer, R. (1995). Mechanisms of sickness-induced decreases in food motivated-behavior. *Neuroscience and Biobehavioral Reviews,* 20, 171–175.

Kim, S.S., Kong, P.J., Kim, B.S., Sheen, D.H., Nam, S.Y., and Chun, W. (2004). Inhibitory action of minocycline on lipopolysaccharide-induced release of nitric oxide and prostaglandin E2 in BV2 microglial cells. *Archival Pharmacology Research,* 27, 314–318.

Kim, S.U. & de Vellis, J. (2005). Microglia in health and disease. *Journal of Neuroscience Research,* 81, 302–313.

Kluger, M.J. (1978). The evolution and adaptive role of fever. *American Scientist,* 66, 38–43.

Konsman, J.P., Parnet, P., and Dantzer, R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. *Trends in Neuroscience,* 25, 154–159.

Lacosta, S., Merali, Z., and Anisman, H. (1999). Behavioral and neurochemical consequences of lipopolysaccharide in mice: anxiogenic-like effects. *Brain Research,* 818, 291–303.

Larson, S.J. and Dunn, A.J. (2001). Behavioral Effects of cytokines. *Brain, Behavior, and Immunity,* 15, 371–387.

Long , N.C., Otterness, I., Kunkel, S.L., Vander, A.J., and Kluger, M.J. (1990). Roles on interleukin 1 β and tumor necrosis factor in lipopolysacharide fever in rats. *American Journal of Physiology,* 259, R724–R728.

Ma, T. and Zhu, X. (1997). Suppression of lipopolysaccharide-induced impairment of active avoidance and interleukin-6-induced increase of prostaglandin E_2 release in rats by indomethacin. *Arzneimittel-Forschung/Drug Research,* 47, 595–597.

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

Maier, S.F., Watkins, L.R., and Fleshner, M. (1994). Psychoneuroimmunology: The interface between behavior, brain, and immunity. *American Psychologist,* 49, 1004–1017.

McGeer, P.L., Kwamata, T., Walker, D., Akiyama, H., Tooyama, I., and McGeer, E.G. (1993). Microglia in degenerative neurological disease. *Glia,* 7, 84–92.

McGeer, P.L., and McGeer, E.G. (1995). The inflammatory response system of brain: implication for therapy of Alzheimer and other neurodegenerative diseases. *Brain Research Reviews,* 21, 195–218.

O'Banion, M.K. (1999). Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. *Critical Reviews in Neurobiology,* 13, 45-82.

O'Neil, M.J. (1999). *Merck Manual of Diagnosis Therapy*. (17th ed., pp. 2833) New Jersey: Merck.

Oitzl, M., van Oers, H., Schöbitz, B., and de Kloet, E. (1993). Interleukin-1β, but not interleukin-6, impairs spatial navigation learning. *Brain Research,* 613, 160–163.

Quan, N., Sundar, S.K., and Weiss, J.M. (1994). Induction of interleukin-1 in various brain regions after peripheral and central injections of lipopolysaccharide. *Journal of Immunology,* 49, 129–134.

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

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M, Morag, A., and Pollmacher, T. (2001). Cytokine-associated emotional and cognitive disturbances in humans. *Archives of Geriatric Psychiatry,* 58, 445–452.

Rock, B.R., Gekker, G., Hu, S., Sheng, W.S., Cheeran, M., Lokensgard, J.R. and Peterson, P.K. (2004). Role of microglia in central nervous system infections. *Clinical Microbiology Reviews,* 17, 942–964.

Shaw, K., Commins, S., and O'Mara, S. (2005). Cyclooxygenase inhibition attenuates endotoxin-induced spatial learning deficits, but not an endotoxin-induced blockade of long-term potentiation. *Brain Research,* 1038, 231–237.

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

Shors, T.J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., and Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature,* 410, 372–376.

Sparkman, N.L., Kohman, R.A., Calvert, W.S., Martin, L.A., Byler, S.L., Garcia, A.K., and Boehm, G.W (2005). Peripheral lipopolysaccharide administration disrupts two-way active avoidance conditioning in male and female C57BL/6J mice. *Physiology and Behavior,* 85, 278–288.

Sparkman, N.L., Kohman, R.A. Scott, V.J., and Boehm, G.W. (2005). Bacterial endotoxin-induced behavioral alterations in two variations of the Morris water maze. *Physiology and Behavior,* 86, 244–251.

Spath-Schwalbe, E., Hansen, K., Schimidt, F., Schrezenmeier, H., Marshall, L., Burger, K., Fehm, H., and Born, J. (1998). Acute effects of recombinant human interleukin-6 on endocrine and central nervous sleep functions in healthy men. *Journal of Clinical Endocrinology and Metabolism,* 83, 1573–1579.

Stéphan, A., Laroche, S., and Davis, S. (2001). Generation of aggregated β-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. *Journal of Neuroscience,* 21, 5703–5714.

Stéphan, A., Laroche, S., and Davis, S. (2003). Learning deficits and dysfunctional synaptic plasticity induced by aggregated amyloid deposits in the dentate gyrus are reduced by chronic treatment with indomethacin. *European Journal of Neuroscience,* 17, 1921–1927.

Tanaka, S., Ide, M., Shibutani, T., Ohtaki, H., Numazawa, S., Shioda, S., and Yoshida, T. (2006). Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. *Journal of Neuroscience Research,* 83, 557–566.

Tikka, T., Fiebich, B.L., Goldstins, G., Keinanen, R., and Koistinaho, J. (2001). Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *Journal of Neuroscience,* 21, 2580–2588.

Thomas, R.M. and Peterson, D.A. (2003). A neurogenic theory of depression gains momentum. *Molecular Interventions,* 3, 441–444.

Tomás-Camardiel, M., Rite, I., Herrera, A.J., de Pablos, R.M., Cano, J., Machado, A., and Venero, J.L. (2004). Minocycline reduces lipopolysaccharide-induced inflammatory reaction, peroxynitrite-mediated nitration of proteins, disruption of the blood-brain barrier, and damage in the nigral dopaminergic system. *Neurobiology of Disease,* 16, 190–201.

Vane, J.R. and Botting, R.M. (1998). Anti-inflammatory drugs and their mechanisms of action. *Inflammation Research,* 47, S78–S87.

Willard, L., Hauss-Wegrzniak, B., and Wenk, G. (1999). Pathological and biochemical consequences of acute and chronic neuroinflammation within the basal forebrain cholinergic system of rats. *Neuroscience,* 88, 193–200.

Yamagata, K., Andreasson, K.I., Kaufmann, W.I., Barnes, C.A., and Worley, P.F. (1993). Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron,* 11, 371–386.

Yirmira, R. (1996). Endotoxin produces a depressive-like episode in rats. *Brain Research,* 711, 163–174.

Yermakova, A., and O'Banion, M.K. (2000). Cyclooxygenases in the central nervous system: implications for treatment of neurological disorders. *Current Pharmaceutical Design,* 6, 1755–1776.

Yrjanheikki, J., Tikka, T., Keinanen, R., Goldsteins, G., Chan, P.H. and Koistinaho, J. (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proceedings of the National Academy of Sciences,* 96, 13496–13500.

VITA

Personal	Andrew Justin Tarr
Background	Son of Michael James Tarr and Mary Lee Parsons One daughter: Zoë Francesca Tarr
Education	Diploma, Washburn District High School, Washburn Maine, 1995 Associates, Liberal Arts, University of Southern Maine, Portland, Maine, 2002 Bachelor of Arts, Major: Psychology; Minor: Biology, University of Southern Maine, Maine, 2003 Master of Science, Experimental Psychology Texas Christian University, Fort Worth, 2007
Experience	Research Assistant in Psychology/Environmental Toxicology, University of Southern Maine 2001-2004 Research Assistant in Psychiatry, Southern Maine Medical Center, 2003-2004 TCU Fellow, Texas Christian University, 2004-Present Teaching Assistantship, Texas Christian University, 2004-Present
Professional Memberships	Society for Neuroscience Psychoneuroimmunology Research Society (PNIRS) American Psychological Association Psi Chi (National Honors Society in Psychology) Golden Key International Honors Society

ABSTRACT

THE EFFECTS OF MICROGLIAL INACTIVATION AND DECREASED PROSTAGLANDIN SYNTHESIS ON LIPOPOLYSACCHARIDE-INDUCED LEARNING DEFICITS

by Andrew Justin Tarr, MS., 2007
Department of Psychology
Texas Christian University

Thesis Advisor: Dr. Gary W. Boehm, Professor of Psychology

Microglial inhibitors and non-selective COX inhibitors maybe possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis by reducing cytokine overexpression. Experiments 1 & 2 attempted to further the understanding of the mechanisms of how proinflammatory cytokines contribute to learning/memory deficits seen after lipopolysaccharide administration and to the extent to which microglial cells play a role in this. Specifically, the present study investigated the role of prostaglandin production and microglial activation in the development of LPS-induced cognitive impairments. Experiment 1 consisted of a co-administration of minocycline and LPS to C57BL/6J male mice. Experiment 2 consisted of a co-administration of indomethacin and LPS also to C57BL/6J male mice. We hypothesized that minocycline and indomethacin would reduce central cytokine levels and reduce or eliminate the effects of LPS-induced anxiety and cognitive deficits in both elevated plus maze and 2-way active avoidance conditioning paradigms.