INFLUENCE OF PRENATAL STRESS ON BEHAVIORAL, ENDOCRINE, AND CYTOKINE RESPONSES TO ADULT ENDOTOXIN EXPOSURE

by

RACHEL ANN KOHMAN

Bachelor of Arts, 2000
Augsburg College
Minneapolis, Minnesota

Master of Science, 2004
Texas Christian University
Fort Worth, Texas

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

Doctor of Philosophy

14 August 2007

ACKNOWLEDGEMENTS

I would like to express my appreciation to my mentor Dr. Gary Boehm who has successfully guided me through my graduate career by providing me with continuous support and encouragement. While I am excited to move onto the next stage of my career, I am saddened to know I will no longer be working with Dr. Boehm. Gary's passion for science and commitment to his students is inspiring; I leave TCU in hope that I may one-day match up to the example set by Dr. Boehm. In addition, I would like to thank my committee members, Dr. David Cross, Dr. Mauricio Papini, Dr. Giri Akkaraju and Dr. Timothy Barth, for providing me with insightful comments and suggestions on my dissertation project and for their support throughout my graduate career. Special thanks to my fellow graduate student Andrew Tarr for his contributions to my project and friendship. Last, but not least, I would like to thank my family for always believing in me and for their support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Early experiences can have a profound effect in shaping an organism's physiological and psychological health. It is of no surprise that during fetal development, a time of substantial growth and neural development, alterations in the uterine environment can produce lifelong effects. The psychological wellbeing of the mother during pregnancy also has the potential to alter the development of a growing fetus. For example, stress experienced during pregnancy has been correlated with increased risk of miscarriage and low birth weight (for review, see Knackstedt et al., 2005). In addition, stress during pregnancy may increase the risk of developing a mood disorder (Phillips et al., 2005), may lead to cognitive deficits (for review, see Chapillon et al., 2002; O'Connor et al., 2002), and may increase susceptibility to disease (e.g., asthma, atopy, and heart disease) later in life (for review, see Barker, 1995; Knackstedt et al., 2005; von Hertzen, 2002). Although a number of studies have evaluated the effects of prenatal stress on immunity or on cognitive abilities separately, an area currently unexplored is whether prenatal stress could lead to cognitive deficits by altering the interactions between the immune system and the central nervous system.

1.1. Neural-immune interactions

Although the elimination of bacteria, viruses, or other pathogens is primarily the responsibility of the immune system, research has shown that changes in immune function can be induced by the central nervous system (CNS) or activation of the hypothalamic-pituitary-adrenal (HPA) axis (for review, see Boomershine et al., 2001), suggesting that an effective defense against an infection may require coordination of

multiple systems within the body. Of particular interest is the interaction between the CNS and the immune system. Fairly recently, scientists have abandoned the notion that the immune system and the central nervous system function independently, as numerous regulatory links between these systems have been discovered (Cohen & Kinney, 2001; Dantzer & Kelly, 1989; Maier & Watkins, 2003). One of the initial findings to suggest the connection between the CNS and the immune system was the discovery that, through classical conditioning, the presentation of a conditioned stimulus, previously paired with an immunosuppressant drug, can decrease the activity of the immune system, an effect known as conditioned immunosuppression (Ader & Cohen, 1975). The relationship between the CNS and immune system is characterized by bidirectional communication, in which activation of the immune system can produce alterations in neural function, and the CNS can alter immune function.

The nervous system alters immune function primarily through the activation of the neuroendocrine and autonomic nervous systems. In reciprocal fashion, the immune system informs the CNS about immune activity in the periphery primarily through the activity of cytokines (For review, see Maier & Watkins, 1998). Cytokines are chemical messengers that help orchestrate the body's response to infection by signaling and activating a variety of immune cells. In general, cytokines are classified based on whether they promote an inflammatory response (i.e., proinflammatory cytokines) or act to inhibit an inflammatory response by regulating the activity of the proinflammatory cytokines (i.e., anti-inflammatory cytokines) (Maier & Watkins, 1998). Three of the major proinflammatory cytokines, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are released from macrophages following bacterial exposure.

Research has shown that the production of cytokines within the peripheral nervous system can upregulate cytokine production within the central nervous system, alter neuronal function by influencing neurotransmitter levels, alter long-term potentiation, decrease levels of brain-derived neurotrophic factor (BDNF), and augment the activity of the HPA-axis (Barrientos et al., 2004; Bellinger et al., 1993; Berkennosch et al., 1987; Borowski et al., 1998; Dunn, 2001; Lapchak et al., 1993; Murray et al., 1998).

The discovery of the immune system's ability to communicate with the brain through cytokines begged the question of how blood-borne cytokines reach the CNS. Currently, research suggests there are several routes of access, each occurring under different circumstances. One such route is entry at the sites of the circumventricular organs (CVOs) (Katsuura et al., 1990; Rivest, 2003). The CVOs are a collection of structures lacking the blood-brain barrier (BBB) that provide a way for blood-borne substances to communicate with the CNS. After entering the brain via the CVOs, the cytokines may induce central cytokine production by activating microglial cells. Although cytokines can gain entry to the CNS through the CVOs, the amount that enters this way is very limited (Rivest, 2003). An additional route was discovered by Banks et al. (2002), who demonstrated that cytokines are actively transported across the BBB. In addition to studies showing evidence of peripherally administered radiolabeled cytokines getting into the brain, Banks and his colleagues found that cognitive deficits induced by a peripheral IL-1α injection were blocked by central administration of hIL-1α antibody, proving that the IL-1α was crossing the BBB to influence the CNS (Banks et al., 2001). Another proposed route occurs via peripheral cytokine interaction with the vagus nerve; activation of the vagus nerve stimulates central cytokine production (Konsman et al.,

2000). Research by Bluthé et al. (1996) demonstrated that the vagus nerve does in fact mediate the majority of the effects of cytokines on the CNS, as vagotomized animals did not development sickness behaviors when injected with peripheral IL-1. However, this effect was specific to intraperitoneal injections, as the vagotomy had no effect when IL-1 was injected either subcutaneously or intravenously. These findings indicate the vagus nerve only communicates the presence of cytokines in the abdominal cavity (Bluthé et al., 1996). Although the route is variable, it is clear that cytokines can influence the CNS, through either a direct route (e.g., diffusion at the CVOs or active transport across the BBB) or an indirect route (e.g., vagal afferent activation that induces central expression).

1.2. LPS and cytokine effects on behavior and learning/memory

To explore the affects of immune activation, researchers often administer the endotoxin lipopolysaccharide (LPS), a component of Gram-negative bacterial cell wall (Borowski et al., 1998). Through its actions on toll-like receptor-4 (TRL-4) and CD-14 molecules, LPS stimulates the production and release of the three major proinflammatory cytokines, IL-1β, IL-6, and TNF-α (Agelaki et al., 2002; Nomura et al., 2000; Zuckerman et al., 1989). One advantage of activating the immune system with LPS rather than exposing an organism to an infectious agent is that LPS stimulation allows the researcher to study the effects of immune activation itself without having to attempt to separate effects due to the actual pathogen versus the effects of the immune system. Following a single LPS injection, an organism will display a constellation of symptoms that have been termed “sickness behavior”; these include fever, anhedonia, anorexia, weight loss, decreased social and sexual behavior, and decreased exploration and

locomotor activity (Borowski et al., 1998; Crestani et al., 1991; Lacosta et al., 1999; Uehara et al., 1989; Yirmiya, 1996). The development of sickness behavior is thought to reflect an adaptive shift in an organism's motivation in order to encourage recovery from an infection (Hart, 1988; Kent et al., 1995). The release of cytokines, particularly IL-1β, primarily mediates the appearance of sickness behavior, as cytokine administration mimics the effects of LPS administration. Furthermore, administration of IL-1 receptor antagonist (IL-1ra) can prevent the development of LPS-induced sickness behavior (Bluthé et al., 1992; Borowski et al., 1998; Uehara et al., 1989).

Repeated administration of LPS, a model of chronic inflammation, leads initially to an exaggerated display of sickness behavior, which is then followed by the development of tolerance-like behavioral effects and diminished peripheral cytokine production (Chen et al., 2005; Sparkman et al., 2004; Sparkman et al., 2005a; Sparkman et al., 2005b). The development of tolerance to repeated exposures to endotoxin is an adaptive response that prevents endotoxic shock (a potentially lethal drop in blood pressure due to widespread vascular permeability and tissue edema, mediated primarily by TNF-α). Although peripheral cytokine release is diminished, consecutive injections of LPS (up to nine days) elicit the same significant elevation in central cytokine production as the initial injection (Chen et al., 2005). These findings suggest that while there is evidence of tolerance within the periphery, tolerance to repeated exposure to LPS does not occur within the brain, as central cytokine levels remain elevated. Currently, it is unclear why, in such a situation, animals do not display overt sickness behaviors, which are mediated by the activity of central cytokines, following repeated exposure to LPS, when central levels are still elevated. Although LPS-induced sickness behavior and

peripheral cytokine release are decreased by consecutive administrations of LPS, there is evidence to suggest that re-exposing an animal to TNF-α can sensitize the behavioral, neurochemical, and endocrine responses if the exposures are spaced 14–28 days apart (Hayley et al., 1999). Schmidt et al. (1995) report similar sensitization effects on endocrine function following repeated IL-1β administration. These findings suggest that even when some time has passed, successive pathogen exposures may elicit more severe sickness behavior.

Activation of the immune system, in addition to the development of sickness behavior, has been shown to produce cognitive deficits. A number of studies utilizing laboratory animals have reported that administration of LPS or cytokines leads to deficits in learning/memory (Aubert et al., 1995; Barrientos et al., 2002; Gibertini et al., 1995; Kent et al., 1996; Oitzl et al., 1993; Pugh et al., 1998; Shaw et al., 2001; Sparkman et al., 2005a; Sparkman 2005b). The release of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α also appears to produce cognitive disturbances in humans (Riechenberg et al., 2001; Spath-Schwalbe et al., 1998). Research conducted by Sparkman et al. (2005a) provides an example of the learning deficits observed in animal studies following LPS administration. On day 1 of testing, four month-old male C57BL/6J mice were administered a single intraperitoneal injection of LPS four hours prior to testing on two-way active avoidance conditioning. Within this paradigm, animals could learn to avoid the footshock by crossing to the other side of the chamber during the presentation of the light (avoidance response), reduce the duration of the shock by crossing during the shock (escape response), or could fail to cross. Each subject received 50 trials per session for five consecutive days. Results demonstrated that LPS-treated animals gave significantly

fewer avoidance responses overall than control animals, indicating a learning deficit in LPS-treated animals. To ensure the behavior observed was not due to a motor impairment, the latency to make a response, and the frequency of intertrial-interval (ITI) crossings was recorded. There was no significant difference in latency to escape or avoid between treatment groups. Additionally, LPS-treated animals had significantly more ITI crossings than controls and less efficient crossing behavior, potentially indicating that LPS-treated animals may have formed a relatively weak association between the discriminative stimulus and the footshock. Taken together, these behavioral measures suggest that the LPS-treated animals, although physically capable of making the response, failed to learn the association between the light and the footshock adequately (Sparkman et al., 2005a). Although this and other experiments (Pugh et al., 1998; Barrientos et al., 2002) provide clear evidence of LPS- and cytokine-induced learning decrements, others studies have only found evidence of performance effects (Gahtan & Overmeir, 2001; Sparkman et al., 2004), and still others actually report facilitation of learning/memory following low-dose IL-1β administration (Yirmiya et al., 2002). Although LPS and cytokine administration generally impair learning/memory, the disparity in findings underscores the potential influence of sex, age, strain, route of administration, and dose on behavioral outcome. Additionally, the results highlight the need to use behavioral tests that allow discrimination of performance and/or motivational deficits from true learning decrements.

1.3. Stress and immune function

The discovery that the immune system can influence neural functioning and can lead to cognitive impairments has produced a need to explore possible factors that may exacerbate the development of cytokine-induced cognitive deficits. Factors that lead to altered immune function may have relevance to the onset of cognitive deficits, particularly in a developing organism. One likely mediating factor of immunocompetence for an organism is stress (Bauer et al., 2001; Cohen et al., 1991; Esterling & Rabin, 1987; Keller et al., 1983; Palermo-Neto et al., 2003; for review, see Moynihan, 2003). Societies throughout history have proposed that, in one form or another, challenging life events and the personality reacting to them can influence an individual's wellbeing (Reiser, 1985). Some of the first experimental evidence for physiological manifestation of stress was provided by Hans Selye (1936) who, by accident, discovered that exposure to stress can lead to enlargement of adrenal glands, development of ulcers, and shrinking of immune tissues. Current evidence indicates that the effects of stress on immune function are tremendously complex, as a stressful experience can lead to either suppression or enhancement of immune function, dependent upon a number of factors.

When an individual encounters a stressor, the brain releases neurotransmitters (e.g., norepinephrine and epinephrine) and hormones (e.g., glucocorticoids) to prepare the body for handling the stressor. Stressful events lead to activation of the sympathetic nervous system and the parvocellular neurosecretory cells, located in the paraventricular hypothalamus, causing the release of corticotropin-releasing hormone (CRH) into the anterior pituitary (for review, see Rivier, 2001). The presence of CRH in the pituitary initiates the release of adrenocorticotropic hormone (ACTH) into the bloodstream. When

ACTH reaches the adrenal cortex, it in turn triggers the release of cortisol or corticosterone (the primary glucocorticoid in rodents). Once released into the bloodstream, the circulating cortisol will increase blood glucose levels and alter metabolic processes (and is shown to impact immune function). This sequence of events (in conjunction with epinephrine and norepinephrine release) is an important part of the stress response, and some of the structures involved make up the hypothalamic-pituitary-adrenal (HPA) axis (Rivier, 2001). The activation of the HPA axis is thought to help the body cope with a threatening stimulus or event by focusing attention and energy resources towards potentially dangerous stimuli (Schurmeyer & Wickings, 1999) or recovery from energy depletion in the wake of a stressor.

Activation of the sympathetic nervous system and the HPA-axis have been shown to have biphasic effects on certain parameters of immune function (for review, see Dhabhar & McEwen, 2001; Bauer et al., 2001). For example, exposure to an acute stressor has been found to increase the number of blood leukocytes initially. Through the activity of epinephrine and norepinephrine, leukocytes previously stored in the lungs, spleen, and thymus are drawn out into the blood. This increase in the number of leukocytes in the blood is transient, as these cells are then distributed to the skin, lymph nodes, and other organs in preparation for a potential assault (Dhabhar & McEwen, 2001). Therefore, alterations in blood leukocyte numbers apparently reflect a redistribution of the cells rather than a change in the total number of cells. One to four hours following the stressful event, the number of blood leukocytes returns to the pre-stress levels (Dhabhar & McEwen, 2001).

Although some stress-induced immunosuppression may reflect a redistribution of cells rather than a decrease in the total number of cells, a host of studies, indicate that stress can depress immune function. For example, research has shown that following a stressful experience, humans are more susceptible to the common cold (Cohen et al., 1991). In animal research, a number of studies have reported exposure to stress decreases lymphocyte proliferation in response to a mitogen (Bauer et al., 2001; Keller et al., 1981; Laudenslager et al., 1983; Mormede et al., 1988). Furthermore, a study by Palmermo-Neto et al. (2003) found that stress decreases activity of macrophages and increases growth of tumor cells. To evaluate the effects of stress on macrophage activity, mice were subjected to either a physical (escapable shock ES or inescapable shock IS) or a purely psychological stressor (PS; being exposed to the responses of animals in IS condition) and macrophage spreading and phagocytic activity was measured from cultured peritoneal macrophages. Results showed that ES and IS stress decreased both spreading and phagocytic activity in comparison to control animals, though PS had no effect on either measure. To evaluate the effects of stress on tumor growth, mice were injected intraperitoneally with Ehrlich tumor cells. Results showed that exposure to IS or ES stress lead to a greater number of tumor cells in comparison to controls, and again PS had no effect on tumor growth. Although Palmermo-Neto et al. (2003) found that exposure to a psychological stressor did not alter activity of macrophages cells, a number of others have reported that exposure to psychological stress induces central cytokine production, suppresses T cell proliferation, and alters lymphocyte distribution (Bauer et al., 2001; Esterling & Rabin, 1987; Minami et al., 1991; Morméde et al., 2002; Nguyen et al., 1998). Collectively, these data indicate that stress can alter the activity of the immune

system, potentially leaving an organism vulnerable to infection.

Activation of the HPA axis is believed to partially mediate the immunosuppressive effects stress. For example, through feedback on activated macrophages and monocytes, glucocorticoids decrease the synthesis and release of IL-1β and other proinflammatory cytokines (high levels of cortisol may also induce apoptosis and other effects in immune cells). Through the negative feedback loop of HPA activation, IL-1β triggers glucocorticoid production and inhibits additional cytokine release (Knudsen et al., 1987; Zuckerman et al., 1989). Supporting this notion that glucocorticoid secretion will suppress immune function, research has shown that adrenalectomized animals produce an exaggerated response to infection (e.g., excessive release of proinflammatory cytokines), as removal of the adrenal cortex eliminates this route of negative feedback on the immune system. Furthermore, supplementary replacement of corticosterone reverses this effect (Bertini et al., 1998; Morrow et al., 1996). Moreover, Auphan et al. (1995) have demonstrated that glucocorticoids in culture may suppress immune function by inhibiting the activity of the immunoregulatory transcription factor NF-κB. NF-κB is involved in the production of many of the cytokines and immune cell receptors. Glucocorticoids apparently inhibit immune function by inactivating NF-κB, via increasing levels of IκBα, thereby preventing transcription of immunoregulatory genes (Auphan et al., 1995). Although the HPA axis clearly plays a role in regulating the immune system, evidence suggests that other mechanisms also exist, including mechanisms involving sympathetic activation (for review, see Moynihan, 2003). For example, a number of studies have shown that stress-induced changes in immune function can occur in the absence of any changes in corticosterone levels

(Moynihan et al., 2000), and adrenalectomized animals still show evidence of altered immune function following stress (Esterling et al., 1987; Keller et al., 1983). Furthermore, Mormede et al. (1988) found that animals exposed to predictable or unpredictable footshocks had similar corticosterone responses, but only the animals in the unpredictable condition showed evidence of suppressed immune function. These data suggest that corticosterone production cannot completely account for all stress-induced changes in immune function.

The effects of stress on immune function are not completely straightforward (for review, see Moynihan, 2003). For example, exposure to some kinds of stressors can enhance immune function (for review, see Dhabhar & McEwen, 2001). Consistent with this notion, research suggests that stress can sensitize the physiological response to LPS administration. Rats injected with LPS 24 hours after exposure to inescapable tailshock show increased peripheral and central levels of IL-1β and TNF-α 1 hour after LPS administration, relative to LPS-treated non-stressed controls. Exposure to tailshock sensitized the LPS-induced cytokine production up to 4 days following stress exposure, however no differences were observed 10 days after shock administration (Johnson et al., 2002). In addition, stressed animals administered LPS exhibit a higher fever response during the light, but not dark phase, relative to controls (Johnson et al., 2003). These findings indicate that pre-exposure to stress may lead to enhancement of the response of the innate immune system for several days following the stressor. Furthermore, stressful events have been found to induce IL-1β and IL-6 expression in the brain in the absence of an immune stimulus (Minami et al., 1991; Morméde et al., 2002; Nguyen et al., 1998). A study by Bauer et al. (2001) demonstrated that rats exposed to acute stress showed

increased phytohemagglutinin-induced splenocyte proliferation, but a reduced proliferation response in the blood, relative to non-stressed controls. Together, these findings show that exposure to stress does not strictly depress all aspects of immune function, but rather that stress may exaggerate some aspects of immune function.

Clearly, stressful experiences can lead to imbalances in immune function, though the direction of the effect is dependent on a number of influential variables such as the type of the stressor, the state of the animal, or the type of immune function/immune compartment assayed (Laudenslager et al., 1983). For example, research by Mormede et al. (1988) demonstrated the exposure to inescapable footshock reduced splenocyte proliferation. However, there was no difference in the proliferation response if the shock was preceded by a warning signal. The duration of the stressor has also been found be a mediating factor. Exposure to acute stress is often followed by an enhancement of immune function, whereas exposure to chronic stress is generally followed by suppression of immune function (for review, see Dhabhar & McEwen, 2001). Collectively, these data show that characteristics of the stressor are important factors (e.g., physical, psychological, predictability, duration, intensity, etc.) in determining the effects of stress on immunity (Laudenslager et al., 1983; Mormede et al., 1988; Palermo-Neto et al., 2003). Findings suggest that although stress does not necessarily lead to illness through suppression or exaggeration of certain parameters of immune function, an organism may be more vulnerable to develop an autoimmune disease, cancer, or show an increased susceptibility to infection (for review, see Elenkov & Chrousos, 2002; Madden, 2003; Moynihan, 2003). Moreover, these stress-induced alterations of immune function may have potential implications for the neural effects of immune activation.

1.4. Perinatal stress and immune function

In part, the responsiveness of the immune system can be determined by events occurring during early development. For example, Kennedy et al. (1994) found that separating pups from their mother for 24 hours suppressed splenocyte proliferation in response to the mitogen concanavalin A at 16 and 20 days of age, however no differences were found in younger pups. This research indicates that, as in adulthood, exposure to stress can produce alterations in immune function in youth.

Evidence for prenatal programming of adulthood immunity has come from a number of studies that have shown application of a variety of stressors to mothers during pregnancy can affect both the humoral response and the cell-mediated response of the adaptive immune system. Humoral immunity is mediated by the production of antibodies from B-cells, and allows for a quicker and more effective response to an infection on subsequent exposures (Fearon & Locksley, 1996). Kay et al. (1998) found that exposure to bright lights and noise throughout pregnancy led to a decreased B-cell proliferation response to a mitogen in 2-month-old offspring. The reduced B-cell response was not due to alterations in lymphocyte distribution, since decreases were observed in multiple compartments. In addition, research has shown that prenatally stressed animals show altered antibody levels in comparison to controls (Klein & Rager, 1995; Sobrain et al., 1992; Tuchsherer et al., 2002). For example, Sobrain et al. (1992) evaluated the effects of daily prenatal environmental stress (PES; exposure to unsignaled, inescapable footshocks) and prenatal psychological stress (PPS; watching, hearing, and smelling females in the PES condition) on gestational days 15–21. On postnatal days 0, 7, 14, 21,

and 28, serum basal levels of IgG (immunoglobin G) were determined from trunk blood samples. PPS decreased basal IgG levels only on postnatal day 0, where as environmental stress decreased basal IgG levels on postnatal days 0, 7, and 28, suggesting that environmental stress may produce prolonged alterations in antibody production.

Exposure to prenatal stress has also been found to decrease the cell-mediated response (immune response mediated by T cells) of the adaptive immune system. Research suggests that, in humans, prenatal stress may further increase the risk of developing asthma by inhibiting the shift towards T_H1-dominated immunity via the activity of glucocorticoids (for review, see von Hertzen et al., 2002), which inhibition may diminish the cell-mediated immune response and increase susceptibility to viruses and intracellular bacteria. In rodent models, exposing pregnant rats to three 45-minute sessions of restraint stress on gestational days 11–21 decreased the offspring's basal levels of circulating lymphocytes. Laviola et al. (2004) collected tail vein blood samples from the offspring of prenatally stressed and control animals on postnatal day 36, and the sample were analyzed for levels of CD4+ and CD8+ cells (T helper cells and T cytotoxic cells, respectively) by using fluorescent-labeled antibodies. Results showed that prenatally stressed animals had 80 percent fewer circulating CD4+ T cells, but prenatal stress had no effect on the number of CD8+ T cells relative to controls. In addition, rats from disturbed pregnancies had a decreased CD4:CD8 ratios in comparison to control rats, suggesting prenatal stress can lead to alterations in the cell-mediated immune response (Laviola et al., 2004). The effect of a reduction in cell-mediated immunity can clearly been seen in individuals suffering from AIDS (acquired immune deficiency syndrome), a disease which destroys CD4+ T cells, leaving the individual highly

susceptible to infections caused by intracellular pathogens (for review, see Fauci, 1988). Although, the effects of prenatal stress are certainly not as devastating as the effects of AIDS, the reduction in the activity of CD4+ T cells caused by prenatal stress may make an individual more susceptible to disease.

In addition to the effects on the adaptive immune system, a few studies have shown that prenatal stress alters the innate immune system, the body's first line of defense against infection (Fearon & Locksley, 1996). The action of cells of the innate immune system, such as macrophage and neurtrophils, typically precedes any action by B- and T cells. Fonseca et al. (2002) administered daily footshock to pregnant dams from gestational day 15 to 19, and then measured the activity of peritoneal macrophage cells, collected after an intraperitoneal injection of *Mycobacterium bovis*, in the male offspring at postnatal days 30 and 60. Exposure to prenatal stress decreased macrophage spreading in adult (i.e., 60 day old), but not juvenile (i.e., 30 day old) subjects. Although neither prenatal nor postnatal stress alone had an effect on phagocytosis, measured by counting the number of cells with phagocytized particles of zymosan, exposure to both pre- *and* post-natal stress led to decreased phagocytic activity (Fonseca et al., 2002). Prenatal stress has also been found to alter the activity of NK (natural killer) cells (Kay et al., 1998; Klein & Rager, 1995; Tuchscherer et al., 2001). Kay et al. (1998) reported suppressed NK cytotoxicity in 2-month-old prenatally stressed animals, and similar results have been reported in offspring of prenatally stressed sows up to 35 days of age (Tuchscherer et al., 2001). However, other researchers have reported an initial suppression of NK activity followed by a trend towards heightened NK activity with age (i.e., 90 days of age; Klein & Rager, 1995).

Although the literature predominantly shows that prenatal stress suppresses the offspring's immune system, a few papers have reported that prenatal stress can exaggerate certain aspects of the immune system. For example, Laviola et al. (2004) showed that exposure to prenatal stress can enhance central and peripheral cytokine levels, though they showed a significant decrease in the circulating number of CD4+ T-helper cells relative to controls. Cultured splenocytes, from the offspring of mothers exposed daily to three 45-minute sessions of restraint stress, produced significantly higher levels of IL-1β in response to the mitogen phytohaemagglutinin than non-stressed controls. In addition, prenatally stressed subjects showed increased IL-1β levels in the cortex, but not in the hypothalamus, relative to non-stressed controls. Prenatally stressed subjects also showed enhanced effects of cyclophophamide (an immunosuppressive agent) administration, as indicated by a decrease in the percentage of T cells. Moreover, a study by Hashimoto et al. (2001) demonstrated that prenatally stressed adult rats showed an altered fever response to LPS administration. On gestational days 15−17, pregnant rats were exposed to 30 or 240 minutes of restraint stress. Offspring were then evaluated for changes in body temperature, corticosterone, epinephrine, and norepinephrine levels following 60 minutes of restraint stress or LPS administration (10μg/kg) at 9−10 weeks of age. Adulthood exposure to restraint stress produced opposite effects on fever response relative to the LPS-treated subjects. Offspring from the 240 minute prenatal stress condition showed a decrease in body temperature following adulthood restraint stress. However, they developed significantly higher fevers in response to LPS administration relative to non-stressed controls. Although the offspring from the 30 minute prenatal stress condition showed the same pattern of results following exposure to LPS or restraint

stress in adulthood, they were not significantly different from control animals. The duration of maternal stress also had different effects on corticosterone and catecholamine production for the offspring exposed to restraint stress as adults. Prenatally stressed subjects exposed to 240 minutes of restraint stress, but not those exposed only for 30 minutes, showed significantly increased levels of corticosterone relative to controls. The reverse was true for catecholamine release; significantly higher levels were only found in the subjects from the 30-minute maternal stress condition, whereas both groups of prenatally stressed subjects showed a significant increase in corticosterone and no effect on catecholamine release following LPS administration. These results suggest that prenatal stress may lead to an exaggerated response to low doses of LPS (i.e., 10μg/kg), and that the febrile response induced by an immune stressor is mediated by a different mechanism than the febrile response to restraint stress.

Collectively, these findings suggest the immunocompetence of an individual may be partially determined prior to birth. As with adulthood exposure to stress, prenatal stress may produce different effects on immune function, depending on which parameter of the immune system is measured. These findings have clear implications for whether an organism can mount an appropriate immune response against an infection. As both depression and exaggeration of the immune system can have potentially detrimental health effects, further research is needed to better characterize the changes in immune function induced by prenatal stress, and how such changes may alter neural-immune interactions more generally.

1.5. Behavioral and endocrine effects of prenatal stress

Exposure to stress during pregnancy may be sufficient to induce lifelong alterations in emotionality, cognition, neuroendocrine response, and behavior. However, these effects are not reported consistently, and some differences can be partially explained by a gender difference, as there is evidence to suggest that the effects of prenatal stress may differ based on the gender of the offspring. Prenatally stressed females appear to exhibit greater deficits in learning and alterations in the reactivity of their HPA axis than do their prenatally stressed male counterparts.

Several studies report that prenatal stress can lead to alterations in learning/memory in adulthood. For example, Meek et al. (2000) showed that exposing pregnant CD-1 mice to bright light and noise during the last week of pregnancy impaired learning in the female, but not male offspring. Prenatally stressed females had significantly longer latencies to locate the platform in the MWM, whereas prenatally stressed males were not different from non-stressed controls. This lack of a spatial learning deficit in prenatally stressed males in the MWM, and in the radial arm maze has been reported by multiple researchers (Aleksandrov et al., 2001; Bowman et al., 2004; Valleé et al., 1999). However Valleé et al. (1999) reported that although no deficits were evident in younger males, by 21-months of age, the prenatally stressed male subjects performed a significantly greater number of errors on the Y-maze and the radial arm maze relative to controls. Sex differences in learning ability in prenatally stressed subjects was also found in a passive avoidance conditioning paradigm, in which female subjects had significantly shorter (i.e., worse) step-through latencies to enter the dark compartment than males (Gué et al., 2004). However, both male and female prenatally

stressed subjects showed deficits in the T-maze (Gué et al., 2004). The learning differences between males and females may not be surprising since the effects of prenatal stress on endocrine and neurodevelopment are known to be gender-specific (McCormick et al., 1995; Szuran et al., 2000). Exposure to prenatal stress may actually facilitate learning in fearful situations, as prenatally stressed subjects performed more avoidance responses in a two-way active avoidance conditioning paradigm than non-stressed controls (Fride et al., 1986). This facilitation in avoidance conditioning may reflect a behavioral supersensitivity to adulthood stress, as documented by Rimondini et al. (2003) in the elevated plus maze. At best, the literature on the effects of prenatal stress exposure on learning are inconsistent. As noted, although some researchers report prenatal stress exposure alters learning (Fride et al., 1986; Gué et al., 2004; Lordi et al., 1997; Louvart et al., 2005; Meek et al., 2000; Valleé et al., 1999), others found that prenatal stress has no effect on learning, or that the deficits are gender-specific (Aleksandrov et al., 2001; Bowman et al., 2004; Fride et al., 1986; Gué et al., 2004; Meek et al., 2000; Valleé et al., 1999).

In humans, exposure to early stress appears to increase the likelihood of developing a mood disorder such as anxiety or depression (Heim et al., 2002). Similar effects have been reported in the animal literature. For example, prenatally stressed offspring are more responsive to adulthood stress exposure, as indicated by alterations in behavior in the elevated plus maze. A number of studies report that prenatally stressed animals have longer latencies to enter the open arms and spend less time in the open arms when exposed to restraint stress as adults (Estanislau et al., 2005; Rimondini et al., 2003). This anxiety-like behavior was not observed in the subjects that were only exposed to

restraint stress in adulthood; this adulthood stressor only affected the behavior of the prenatally stressed subjects. A few studies have reported that offspring from stressful pregnancies show increased anxiety-like behavior relative to non-stressed controls in the absence of any adulthood stressor. Laviola et al. (2004) reported that prenatally stressed subjects, had significantly longer latencies to approach a novel object in an open field unit. Furthermore, Ward et al. (2000) found that prenatally stressed subjects had exaggerated behavioral and neuroendocrine responses to novel and aversive stimuli, relative to controls. Based on these experiments, it appears that exposure to prenatal stress may have lifelong anxiogenic effects. However, it seems that exposing the subject to a stressor in adulthood is sometimes necessary to produce an increase in anxiety greater than observed in controls.

In addition to the behavioral effects, exposure to prenatal stress alters the activity of the offspring's HPA axis. Previous reports have shown that prenatal stress exposure increased females', but not males' basal corticosterone levels (Szuran et al., 2000; Valleé et al., 1999). This suggests that female offspring may be more vulnerable, in terms of effects on the HPA axis, to the effects of prenatal stress (McCormick et al., 1995). In general, findings show that prenatally stressed females produce higher levels of ACTH and corticosterone in response to adulthood stress than control animals. Prenatally stressed males do not show an increased corticosterone response to stress (McCormick et al., 1995). Although most studies found that prenatal stress does not alter basal levels or the amount of corticosterone produced in male offspring, there is evidence to suggest that prenatally stressed males, as well as females, take longer to return to baseline levels following adulthood stress exposure (Barbazanges et al., 1996; Kinnunen et al., 2003;

Koenig et al., 2005; Maccari et al., 1995; Valleé et al., 1999). This slowed return to basal levels likely reflects impairment in the offspring's negative-feedback loop, as prenatally stressed subjects express fewer type I and type II corticosterone receptors within the hippocampus than non-stressed controls (Barbazanges et al., 1996; Koehl et al., 1999; Maccari et al., 1995). Prenatally stressed subjects also fail to adapt to repeated exposure to a novel stimulus, as they continue to show elevated levels of corticosterone in response to a stressor whereas, non-stressed controls show habituation in their HPA axis response to re-exposure to a stressor (Fride et al., 1986). Collectively, these findings indicate that exposing pregnant dams to stressful events leads to alterations in their offsprings' HPA axis, although the nature and severity of the abnormality is dependent on the gender of the offspring.

1.6. Mechanisms of the effects of prenatal stress

Activation of the mother's HPA axis is believed to be the primary cause of the developmental effects of maternal stress. Maternal corticosterone can readily cross the placenta and reach the fetus (Zarrow et al., 1970). Research has shown that blocking maternal corticosterone secretion, via adrenalectomy, prior to stress, prevents the prenatal stress-induced changes in the offspring's HPA axis and glucocorticoid receptor density (Barbazanges et al., 1996). Furthermore, if during the prenatal stress procedure, adrenalectomized mothers were given injections of corticosterone, the effects of prenatal stress were reinstated in the offspring (Barbazanges et al., 1996). In addition, Cratty et al. (1995) reported that prenatally stressed offspring showed elevated levels of CRH within the amygdala relative to non-stressed controls. Similar increases in amygdala mRNA

levels of CRH were found in the offspring of mothers that were administered the synthetic glucocorticoid dexamethasone during pregnancy, suggesting that exposing a fetus to corticosterone in utero leads to increased CRH production (Welberg et al., 1995). As CRH plays a role in modulating behavioral responses to stress (Dunn and Berridge, 1990), corticosterone exposure, via increased amygdala levels of CRH, may be the primary cause of the alterations in anxiety and the behavior response to stress frequently observed in prenatally stressed subjects (for review, see Chapillon et al., 2002). These findings suggest that maternal corticosterone secretion is an important mechanism for the developmental effects of prenatal stress.

Altered HPA activity in pups receiving prenatal stress, may lead to long-term alterations in the offspring's immune system. As previously discussed, corticosterone production plays a significant role in stressed-induced alterations in certain aspects of the immune system (for review, see Moynihan, 2003). However, the effects of prenatal stress on the offspring's immune system could also be the result of alteration in their catecholamine levels. Exposure to prenatal stress has been show to increase epinephrine and norepinephrine turnover rates in the offspring (Huttunen, 1971; Moyer et al., 1977; Takahashi et al., 1992). These neurotransmitters have been found enhance or depress immune function depending upon the immune parameter measured, and often act in conjunction with the HPA axis to modulate immune function during stressful events (for review, see Madden, 2003). Although the specific mechanisms of prenatal stress-induced immune alterations have not been determined, the direct application of a stressor to a pregnant female indirectly alters the offspring's immune system, which may have implications for how an offspring responds to exposure to pathogens in adulthood.

Clearly, exposure to prenatal stress can lead to developmental alterations that can have implication for the offspring's health, endocrine function, and emotional wellbeing. Although a number of researchers have focused on the effects of prenatal stress on immune function, many important questions remain. For instance, to date, no one has explored the possibility that prenatally stressed subjects may be more vulnerable to cognitive deficits following an infection. This possibility seems likely, as previous research has found that prenatally stressed subject's show an exaggerated fever response to adulthood LPS administration (Hashimoto et al., 2001). Furthermore, offspring from stressful pregnancies show enhanced cytokine levels, the primary mediators of LPS-induced learning/memory deficits, in both the periphery and the brain (Laviola et al., 2004).

The proposed experiments were designed to explore the behavioral ramifications of adulthood LPS exposure following unusual stress during early development. To test our hypothesis that prenatal stress would enhance susceptibility to LPS-induced learning deficits and that these behavioral effects would coincide with increased IL-1β expression, we evaluated performance in multiple behavioral tasks and explored concomitant IL-1β expression in the brain and periphery. We hypothesized that the prenatally stressed animals would be hypersensitive to endotoxin exposure and would therefore show evidence of learning impairments at a subthreshold dose of LPS, and/or show exaggerated deficits in learning and increased anxiety following endotoxin administration in adulthood. Regardless of the adulthood treatment condition, we predicted that prenatally stressed subjects would show evidence of anxiety-like behavior in the elevated plus maze relative to non-stressed controls. Furthermore, we anticipated that males and

females would respond differently to prenatal stress exposure in terms of their behavior and corticosterone response. Specifically, we hypothesized that females would show a higher peak corticosterone response to adulthood LPS administration, and that prenatally stressed females will perform worse in the MWM in comparison to males. In addition, we expected to replicate the findings of Hashimoto et al. (2001) who showed that prenatally stressed subjects show an enhanced fever response and display greater corticosterone production. Furthermore, we hypothesized that exposure to prenatal stress would enhance central and peripheral cytokine production in response to LPS administration in adulthood. Finally, based on previous research, we predicted that regardless of prenatal treatment, re-exposure to LPS after a recovery period would sensitize behavioral and physiological responses to endotoxin exposure.

2. METHODS

2.1. *Subjects*

Subjects were 307 male and female C57BL/6J mice bred at the TCU vivarium from breeding stock obtained from The Jackson Laboratory (Bar Harbor, ME). Forty mice were used as breeders, 227 of their offspring were used as subjects in Experiment 1, and 40 mice were used to complete Experiment 2. Animals were housed, with free access to food and water under a 12:12 light/dark cycle, in standard polycarbonate mouse cages, in groups of 3–4. All animals were housed and treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996), and in accordance with a protocol approved by the TCU Institutional Animal Care and Use Committee.

2.2. Prenatal stress procedure

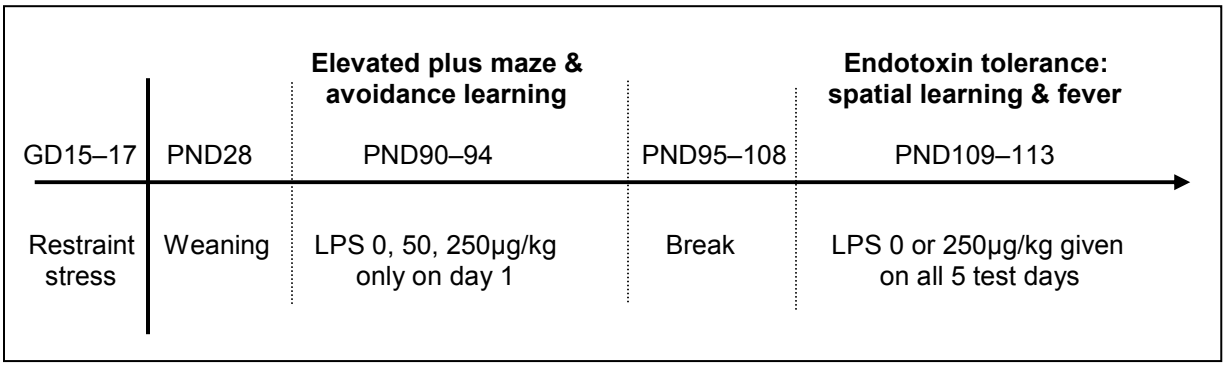
Pregnancy was determined by inspecting females 2–3 times daily until the appearance of a vaginal plug was noted (designated as gestation day [GD] 0). Pregnant females were randomly assigned to either the prenatal stress (PS) or control group. The subjects in the PS groups were exposed daily to 240 min of restraint stress in a brightly lit room, on GD 15–17 (Hashimoto et al., 2001). Females in the control group were left undisturbed in their home cages. Only litters consisting of 4–6 pups were used in the two experiments; litters were culled to six pups if necessary. To diminish potential litter effects, no more than 2 mice of the same sex from each litter were assigned to an experimental condition (Chapman & Stern, 1977). At 4 weeks of age, pups were weaned and housed in groups of 3–4 per cage. Behavioral testing began on postnatal day 90, a time point by which the neuroendocrine and cathecholaminergic system have already reached maturity (Choi & Kellogg, 1996; Kellog et al., 1998; Laviola et al., 2002). See Table 1 for experimental time-line.

2.3. Evaluation of maternal care

To assess whether maternal stress altered the mother-pup interaction, mothers were observed twice a day for alterations in nursing on postnatal days 0–3 and for changes in pup retrieval on postnatal day 2. The frequency of mothers nursing their litters was assessed twice a day, once in the morning and early evening. Pup retrieval was evaluated by gently removing the pups from the nest and placing them on the opposite end of the cage. The time for the mother to return all of the pups to the nest was recorded; data are expressed as the average latency per pup by taking the total time divided by the number

of pups in the litter, to control for differences in litter size. Although it is possible that any prenatal manipulation can affect maternal care (Denenberg 1977; Meaney, 2001), a number of studies have reported that prenatal stress produced no differences in maternal care of pups (Meek et al., 2001; Pardon et al., 2000; Sobrian et al., 1992).

Table 1. Experimental time-line. Table shows the age of the animals (in days) at time of testing, timing of LPS administration, and order of behavioral tests for Experiment 1



2.4. Treatment conditions and experimental design

Our design is a 2 x 3 factorial with two between-subject factors (i.e., Prenatal Treatment and LPS Condition; see Table 2). At postnatal day (PND) 90, two-thirds of the prenatally stressed and control groups received a single intraperitoneal (ip) injection of lipopolysaccharide at a dose of 50 or 250µg/kg; the remaining animals in the prenatal stress group and control group received an equivalent volume of sterile saline (see Table 2), and then tested for five consecutive days on two-way active avoidance conditioning. The high dose of LPS (i.e., 250µg/kg) is a dose commonly used in the literature, and has produced consistent learning deficits in our lab. Based on our previous experience with these mice, we selected the lower dose of LPS (i.e., 50µg/kg), as it is not sufficient to produce learning deficits in the unstressed control animals. Following avoidance testing

and a subsequent fourteeen day break, subjects were administered either repeated ip LPS (250μg/kg) or sterile saline injections for five days to evaluate physiological and behavioral manifestations of endotoxin tolerance. This was measured via evaluation of the fever response and performance in a spatial learning task (see Table 3). A detailed description of the behavioral testing paradigms and procedures are discussed in sections below. Due to the experimental design, counter balancing the order of the behavioral tests, to eliminate possible carryover effects, was not possible. To minimize carryover effects the least aversive test, the elevated plus maze, was conducted first followed by active avoidance training. Subjects were allowed a two-week rest period between avoidance training and the water maze. Although we cannot completely rule out the possibility, training in the active avoidance paradigm is unlikely to influence perform in the water maze as the tests evaluate different types of learning (i.e., associative versus spatial) and are conducted in separate apparatuses in different testing rooms.

Table 2. Effects of prenatal stress and adulthood LPS administration on anxiety and avoidance learning

N=104 females N=119 males	Saline		LPS low (50μg/kg)		LPS high (250μg/kg)	
Prenatal stress	Males n=23	Females n=19	Males n=19	Females n=19	Males n=20	Females n=16
Control (no-stress)	Males n=20	Females n=17	Males n=17	Females n=16	Males n=20	Females n=17

2.5. Experiment 1A: testing procedures

On PND 90, subjects were tested in the elevated plus maze three hours after a single ip injection of LPS (0, 50, 250μg/kg) injection (see Table 1) and then in two-way active avoidance, four hours post-injection. The elevated plus maze is a simple plus-shaped, black Plexiglas platform, with arms 10 cm wide, lifted 50 cm off the floor. Two

of the four arms are enclosed with translucent Plexiglas walls, while the other two arms have no walls. Each animal was tested only once; the trial is recorded by a computerized animal tracking system (Accuscan Instruments, Columbus, OH) for 5 minutes. Measures recorded include time spent in closed arms, time spent in open arms, time spent in the central square, and total distance traveled.

Approximately one hour after testing in the elevated plus maze, subjects then began testing in a two-way active avoidance conditioning paradigm, which occurred in six identical Gemini II shuttlebox units manufactured by San Diego Instruments (San Diego, CA). The apparatus contain two equal compartments divided by a partition, with an opening at floor level. To assess avoidance learning, all subjects received a 50-trial session per day, for five consecutive days. At the beginning of each testing session, a mouse is placed in the right chamber of the shuttlebox. The room lights were turned off, to ensure the salience of the conditioned stimulus, and the program began. Subjects received a 5-minute acclimatization period, during which they can move freely between the compartments. Following the acclimatization period, the light (the conditioned stimulus; CS) was presented for 5-seconds. If the mouse crossed to the other chamber within 5-seconds (avoidance response), the light was turned off and the 20-second inter-trial interval (ITI) began. If the mouse did not cross within 5-seconds, a mild foot shock (0.4mA) for up to 5-seconds (the unconditioned stimulus; US) was delivered through an electronic scrambler. Following the presentation of the shock, the mouse could terminate the shock by crossing to the other side (escape response), or fail to cross (null response). Following a 20-second ITI, the next trial began. The number of avoidance responses, Escape responses, and null responses was recorded to assess learning. Additionally, the

number of ITI crossings was recorded as a measure of non-cued crossing behavior. To evaluate overall performance, each subject's discrimination index score in performing the task was calculated (i.e., (number of Avoidances/number of Avoidances + number of Escapes + number of ITI crossings) x 100). The discrimination index score provides information on the subject's ability to discriminate between when a crossing response is necessary to avoid the presentation of the US from when it is not. The measure provides valuable information on whether the subject has learned the association between the US and CS, by distinguishing cued crossings from random crossing behavior. After completing the session, the animals were visually inspected, weighed, and then returned to their home cage. To limit noise transfer, 1-inch acoustic foam (Auralex Acoustics, Indianapolis, IN) was placed around each chamber and a white noise generator was used (Gold Line, West Redding, CT). The apparatus were cleaned with Odormute™ (Ryter Corp., Madelia, MN) between subjects. Following the five days of avoidance learning testing subjects were given a fourteen-day rest period.

Experiment 1B: testing procedures

After the fourteen-day rest period following activate avoidance training, subjects were subsequently evaluated for alterations in the development endotoxin tolerance by recording rectal temperature and looking at performance and learning alterations in a spatial learning task. On postnatal days 109–113 subjects received daily ip injections of LPS (250μg/kg; see Table 3) or saline (each subject received five injections). Approximately two hours after LPS or saline administration, rectal temperatures were obtained (Thermalert TH-5, Physitemp Instruments Inc., Clifton, NJ). Two hours after

measuring fever response, subjects were tested in the Morris water maze (MWM) for six consecutive days. In this task, a mouse was placed into a white circular tub (d=123 cm) of water from any of four locations, and has to utilize extramaze cues to find a submerged platform (d=10 cm) set 23 cm from the wall. At the end of each trial, the mouse remained on the platform for 10–15 seconds, and then placed in a cage under a red light. After each animal in the testing squad (4–6 mice) completed trial 1, the next trial began. Each mouse was given four trials (maximum time of 60 seconds per trial) a day, one from each of four positions, with the order determined semi-randomly. Subjects were tested for five days with the platform in a fixed position. On the sixth day of testing, a "probe trial" was conducted, in which the platform was removed from the maze and each subject received two 60-second trials. The mouse's path was recorded via our EZ Video animal tracking system (Accuscan Instruments, Columbus, OH) that includes a camera mounted on the ceiling, which sends data to a computer containing a template of the maze. The computer program computed the following measures: latency to locate the platform, distance traveled (cm), and swim speed (cm/sec). For the probe trials, the percent of time spent in the portion (i.e., quadrant 1) of the maze where the platform was previously located was recorded.

Table 3. Alterations in spatial learning and fever response following repeated LPS injections and LPS re-exposure (a further division of treatment groups shown in Table 2)

N=107 females N=106 males	Saline			LPS (250μg/kg)		
Initial Treatment Condition	Saline	LPS low	LPS high	Saline	LPS low	LPS High
Prenatal stress	Male n=8	Male n=9	Male N=8	Male n=9	Male n=9	Male n=8
	Female n=10	Female n=9	Female N=8	Female n=11	Female n=10	Female n=9
Control (no-stress)	Male n=8	Male n=8	Male N=9	Male n=10	Male n=7	Male n=11
	Female n=8	Female n=8	Female N=8	Female n=9	Female n=8	Female n=9

2.7. Experiment 2

A new batch of 40 (5 males & 5 females per treatment condition) C57BL/6J mice following prenatal treatment were given an ip injection of LPS (0 or 250μg/kg) on PND 90 (see Table 4). Four hours following LPS administration, subjects were rapidly euthanized, and trunk blood, spleens, and brains were collected. Using an RNase-free sample corer, tissue punches were dissected on a chilled glass plate from the hippocampus and cortex. The amount of specific mRNA transcript present in each sample was determined by quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR), since this procedure is more sensitive than other methods of measuring central cytokine expression. The RNA was first isolated (RNeasy Micro kits, Qiagen, Valencia, CA), then quantified and assessed for purity using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). The amount of IL-1β RNA present was determined by utilizing TaqMan™ probe and primer chemistry (Applied Biosystems, Foster City, CA) specifically designed to bind to reverse-

transcribed IL-1β cDNA. The relative amount present was then compared across the treatment conditions. Serum levels of IL-1β and corticosterone were measured by an ELISA (enzyme-linked immunosorbent assay), according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Using standards of known concentrations, an individual standard curve was created for each plate. The value of each unknown sample well was then quantified based on the standard curve, to determine the amount of IL-1β or corticosterone present.

Table 4. Effects of prenatal stress and endotoxin administration on central and peripheral IL-1β and corticosterone production.

N=20 females N=20 males	Saline	LPS 250μg/kg
Prenatal stress	N=10	n=10
Control (no-stress)	N=10	n=10

2.8. Statistical procedures

Elevated plus maze data were analyzed by standard factorial ANOVAs, with Prenatal Treatment, Gender, and LPS Treatment as the between-subject variables. Data from the two-way active avoidance paradigm were analyzed using repeated-measures ANOVAs, with Prenatal Treatment, Gender, and LPS Treatment (0, 50, 250μg/kg) as the between-subjects variables, and Test Day (days 1–5) as the within-subjects (i.e., repeated-measures) variable. Morris water maze and rectal temperature data were also analyzed using repeated-measures ANOVAs, with Prenatal Treatment, Gender, LPS Treatment, and Repeated LPS Treatment as between-subject variables, and Test Day as the within-subjects variable. Significant effects were submitted to Fisher's PLSD post-hoc test to analyze significant differences between treatment groups and/or days. An alpha level of 0.05 was the criterion for rejection of the null hypothesis. Central IL-1β gene expression

was calculated by normalizing the amplification efficiency of IL-1β expression against the amplification efficiency of β-actin (the endogenous control gene), which has equal expression across treatment conditions, using the DART-PCR method (Peirson et al., 2003). Standard factorial ANOVAs were then used to compare differences in IL-1β expression levels across treatment conditions. Corticosterone and peripheral IL-1β production were analyzed by standard factorial ANOVAs, with Prenatal Treatment, Gender, and LPS Treatment as the between-subject variables.

3. RESULTS

3.1. Maternal care

As an attempt to determine whether restraint stress during pregnancy altered the dams care of their offspring, nursing behavior, pup retrieval latency, and litter size were measured. Results showed that there were no significant effects of Stress Exposure on litter size ($F(1,67)=0.30$, $p=0.58$ns, see Figure 1), as stressed and control dams both had an average litter size of five pups. In addition, there were no significant effects of Stress Exposure on latency to retrieve pups ($F(1,67)=1.15$, $p=0.29$ns, see Figure 1). Furthermore, there no significant differences between control and stressed dams on frequency of nursing their pups over three days of observation ($F(1,67)=0.50$, $p=0.47$ns, see Figure 1).

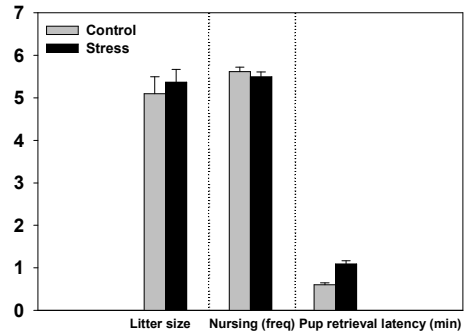


Figure 1. Exposure to restraint stress during pregnancy did not significantly influence litter size, nursing behavior, nor latency to retrieve pups. Bars represent mean ± standard error of the mean (SEM).

3.2. Weight of offspring in adulthood

Administration of LPS, at either dose, lead to a significant, albeit slight, reduction in weight relative to saline controls ($F(2,215)=7.92$, $p<0.0005$). A significant main effect of Gender ($F(1,215)=461.43$, $p<0.0001$), showed, as expected, that male subjects weigh more than female subjects. A significant Prenatal Treatment X LPS Treatment X Day interaction ($F(8,860)=3.18$, $p<0.005$) showed that non-stressed controls subjects treated with the high LPS dose showed a significant reduction in weight on day 2 relative to saline controls (*p's*<0.05), whereas prenatally stressed subjects administered LPS showed a significant reduction in weight on days 2–3 relative to saline-treated subjects (*p's*<0.05).

3.3. Experiment 1A: Elevated plus maze

Prenatally stressed subjects spent significantly less time in the open arms of the maze and significantly more time in the closed arms relative to non-stressed controls ($F(1,210)=4.0$, $p<0.05$; $F(1,210)=6.61$, $p<0.05$, respectively, see Figures 2A and 2B). Administration of LPS significantly increase the amount of time spent in closed arms ($F(2,210)=3.08$, $p<0.05$, see Figure 2B), as subjects that received the low or high dose of

LPS spent significantly more time in the closed arms than saline-treated subjects (*p's*<0.05). Subjects administered the high dose of LPS spent significantly less time in the center of the maze than saline-treated subjects ($F(2,210)=3.83$, $p<0.05$, see Figure 2C). As expected, administration of LPS significantly reduced distance traveled in a dose-dependent manner ($F(2,210)=89.04$, $p<0.0001$, see Figure 2D), as both LPS treatment groups traveled a shorter distance than saline-treated subjects (*p's*<0.0001), and subjects given the high LPS dose traveled a significantly shorter distance than subjects given the low LPS dose ($p<0.0001$). There was a significant Prenatal Treatment X LPS Treatment interaction for time spent in the closed arms ($F(2,210)=3.28$, $p<0.05$, see Figure 2B). While there was no significant difference between prenatally stressed and non-stressed subjects administered saline, the prenatally stressed subjects administered the high dose of LPS spent significantly more time in the closed arms of the maze than prenatally stressed subjects administered saline and non-stressed controls administered the high dose of LPS (*p's*<0.05). Significant main effects of Gender for the percent time spent in the center of the maze and distance traveled ($F(1,210)=6.85$, $p<0.01$; $F(1,210)=25.91$, $p<0.0001$, respectively, see Figures 2C and 2D), showed that female subjects spent significantly less time in the center of the maze and traveled a longer distance than males subjects. However, there were no significant Prenatal Treatment X Gender nor LPS Treatment X Gender interactions for any of the dependent measures.

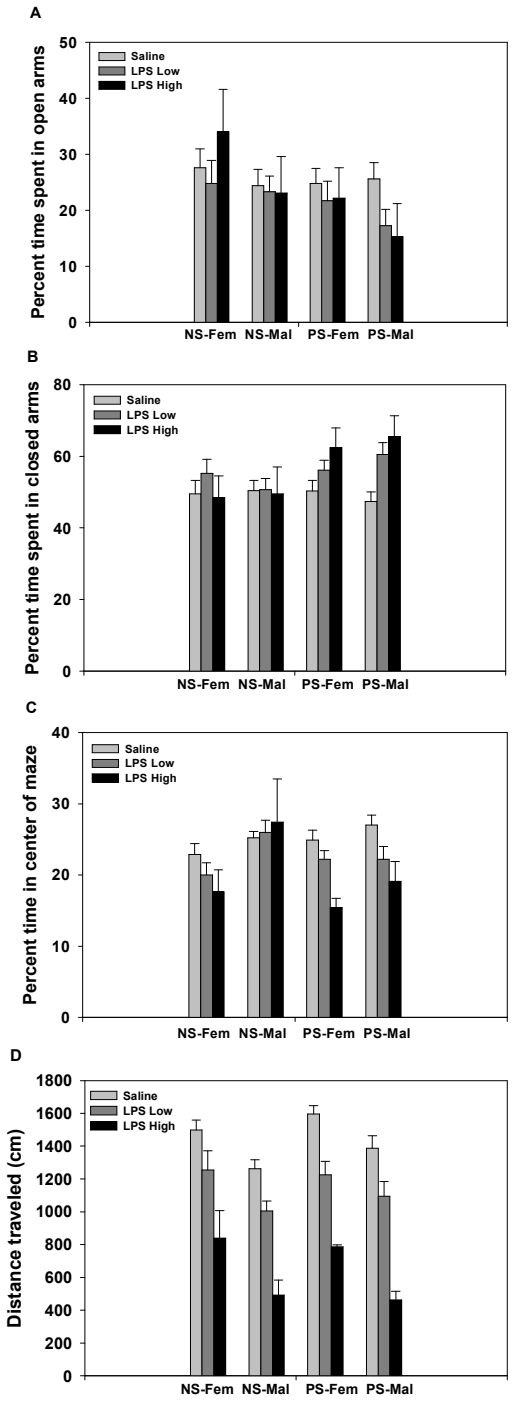


Figure 2. Prenatally stressed subjects spent significantly less time in the open arms (A) and more time in the closed arms (B); Prenatally stressed subjects administered the high dose of LPS spent significantly more time in the closed arms than saline-treated subject. LPS administration significantly reduced time spent in the center of the maze (C) and distance traveled (D). NS=non-stressed controls, PS=prenatal stress, Fem=Female subjects, Mal=Male subjects. Bars represent mean ± standard error of the mean (SEM).

3.4. Experiment 1A: Two-way active avoidance conditioning

There were significant main effects of Gender for the number of avoidance and escape responses ($F(1,211)=5.44$, $p<0.05$; $F(1,211)=7.19$, $p<0.05$), which showed female subjects performed significantly more avoids and fewer escape response than male subjects. Furthermore, significant Gender X Day interactions for the number of avoidance responses, escape responses, null responses, and the discrimination index score ($F(4,844)=28.9$, $p<0.0001$; $F(4,844)=19.7$, $p<0.0001$; $F(4,844)=2.52$, $p<0.05$; $F(4,844)=14.59$, $p<0.0001$, respectively, see Figure 3), showed that on day 1 of testing female subjects performed fewer avoidance responses and more escape response and null responses than males (*p's*<0.05). However, on days 3–5 of testing, female subjects performed significantly more avoidance responses and fewer escape responses than male subjects (*p's*<0.05). There were no significant differences between males and females for the number of null responses on days 2–5 of testing. Similarly, female subjects had significantly reduced discrimination index scores on day 1 of testing relative to male subjects ($p<0.05$), however, on days 3 and 4, females subjects had significantly higher discrimination scores than male subjects (*p's*<0.05). In addition, there were significant Prenatal Treatment X Gender interactions for the number of avoidance responses, escape responses, and the discrimination index score ($F(1,211)=4.29$, $p<0.05$; $F(1,211)=3.96$, $p<0.05$; $F(1,211)=7.70$, $p<0.01$, respectively, see Figures 3A–3F). Prenatally stressed male subjects performed significantly fewer avoidance responses, more escape responses, and had significantly reduced discrimination index scores than prenatally stressed females (*p's*<0.05) and non-stressed control male and female subjects (*p's*<0.05). A significant main effect of LPS Treatment and a LPS Treatment X Day interaction for the

number of ITI crossing ($F(2,211)=5.29$, $p<0.01$; $F(8,884)=8.08$, $p<0.0001$, respectively, see Figure 4A and 4B), revealed that both LPS treatment groups performed significantly fewer ITI crossings than saline-treated subjects on day 1 (*p's*<0.0001). In addition, subjects given the high LPS dose performed fewer ITI crossings than those given the low LPS dose ($p<0.0001$). A significant LPS Treatment X Gender interaction for the number ITI crossings ($F(2,211)=4.51$, $p<0.05$), showed that saline-treated females performed significantly more ITI crossings than male subjects. Furthermore, females given LPS performed fewer ITI crossings than saline-treated females *(p's*<0.0001) and females given the low LPS dose ($p<0.0001$); however, there were no significant differences between males administered LPS or saline. Finally, a significant Prenatal Treatment X LPS Treatment X Gender X Day interaction for the number of null responses ($F(8,844)=1.97$, $p<0.05$, see Figures 4C and 4D), showed that on day 1 of testing prenatally stressed female subjects administered the high dose of LPS had significantly more null responses than prenatally stressed females administered the low dose of LPS or saline (*p's*<0.05).

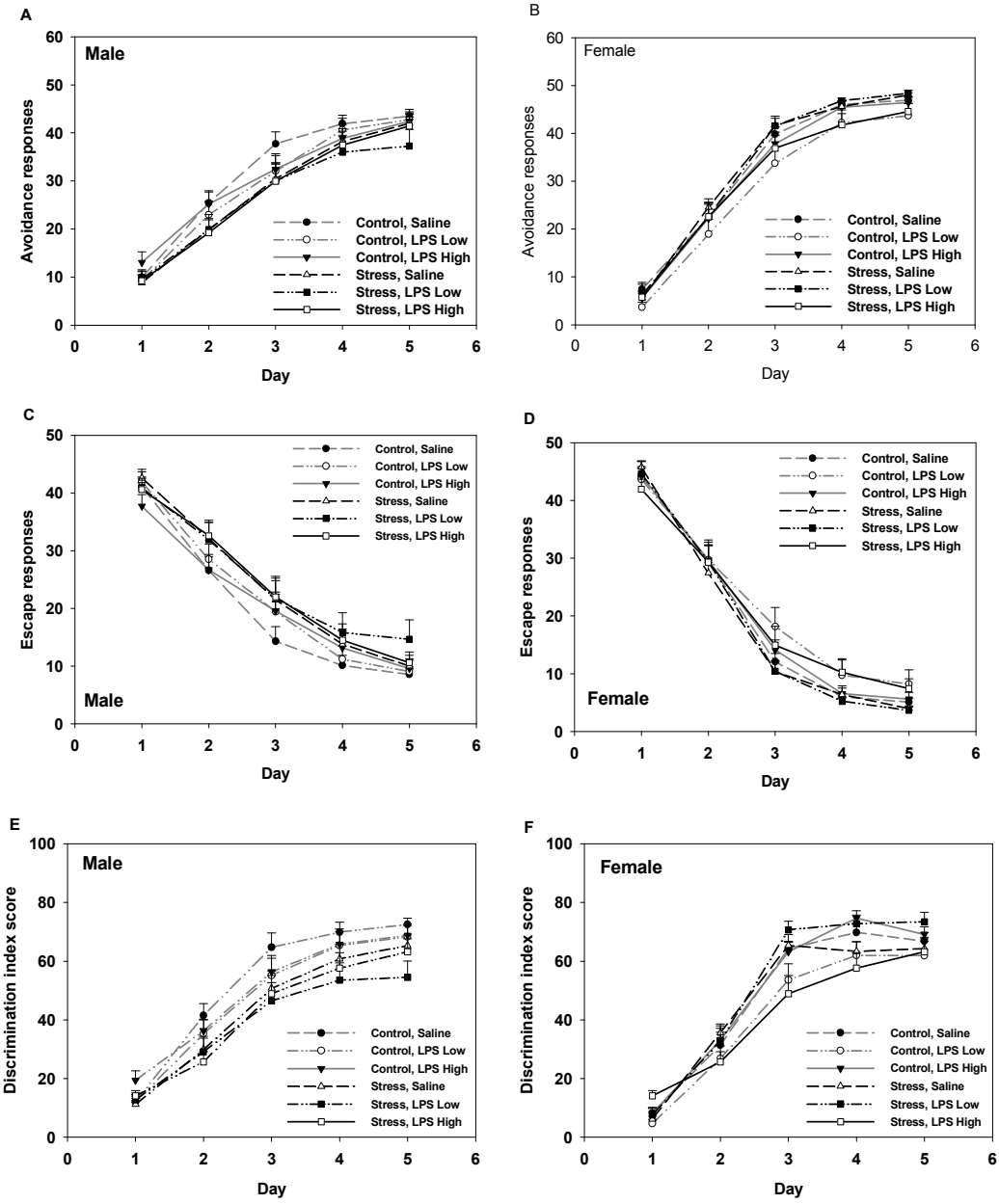


Figure 3. Prenatally stressed male subjects performed significantly fewer avoidance responses (A, B), had significantly reduced discrimination scores (E, F), and performed significantly more escape responses (C, D) than non-stressed controls. Grey line represent non-stressed controls (i.e., Control). Black lines represent prenatally stressed (i.e., Stress) subjects. Lines represent mean ± standard error of the mean (SEM).

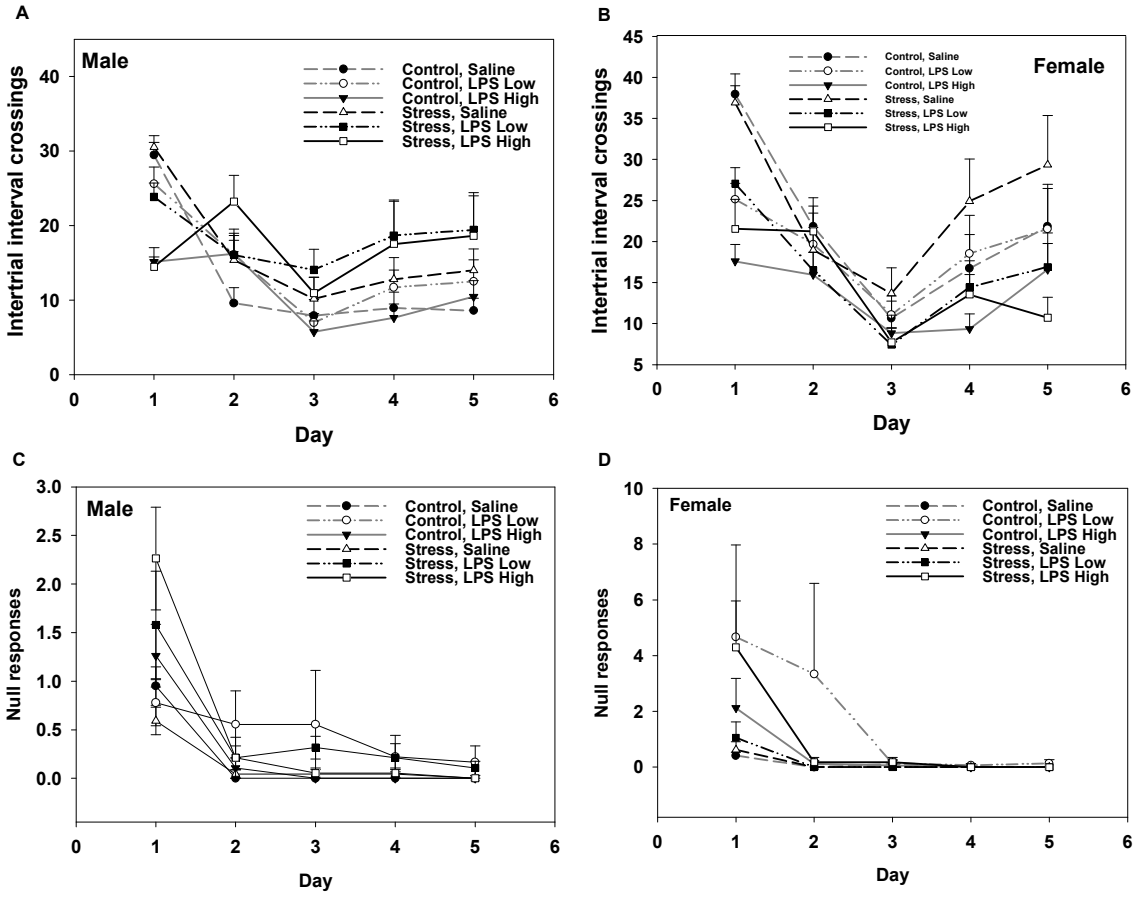


Figure 4. Prenatally stressed subjects performed significantly more ITI crossings than non-stressed control (A, B). LPS-treated subjects performed significantly fewer ITI crossings relative to saline-treated subjects (A, B). Prenatally stressed female subjects administered the high dose of LPS performed significantly more null responses on day 1 (C) than those given the low dose of LPS or saline. Lines represent mean ± standard error of the mean (SEM).

3.5. Experiment 1B: Body temperature

Following a fourteen-day break after testing in the two-way active avoidance conditioning paradigm, subjects received five consecutive injections of LPS or saline and were evaluated for changes in temperature, followed two hours later by testing in the MWM. There was a significant main effect of Repeated LPS Treatment and a Repeated LPS Treatment x Day interaction for temperature ($F(1,197)=170.85$, $p<0.0001$; $F(4,788)=6.13$, $p<0.0001$, respectively; see Figures 5A and 5B). On days 1–5,

administration of LPS led to a significant increase in core body temperature relative to saline-treated subjects (*p's*<0.05). In addition, subjects administered repeated LPS injections showed a larger increase in temperature on day 2 relative to all other days of LPS administration (*p's*<0.05). There was a significant main effect of Gender and a Gender x Day interaction for temperature ($F(1,197)=224.28$, $p<0.0001$; $F(4,788)=3.52$, $p<0.01$, respectively; see Figures 5A and 5B). On days 1–5, female subjects had significantly higher temperatures than male subjects (*p's*<0.05). Furthermore, there was a significant Repeated LPS Treatment X Gender X Day interaction for temperature ($F(4,788)=5.3$, $p<0.0005$, see Figures 5A and 5B). Saline-treated females had significantly higher temperatures than saline-treated males on days 1–5 (*p's*<0.05). LPS-treated females mounted a significantly higher fever response than LPS-treated males on days 1–5 (*p's*<0.05). In addition, LPS-treated males had a significantly lower temperature than saline-treated females on days 1 and 3 (*p's*<0.05); however, LPS-treated males had a higher temperature on day 2 ($p<0.05$). There were no significant effects of Prenatal Treatment nor any Prenatal Treatment X Repeated LPS Treatment interactions.

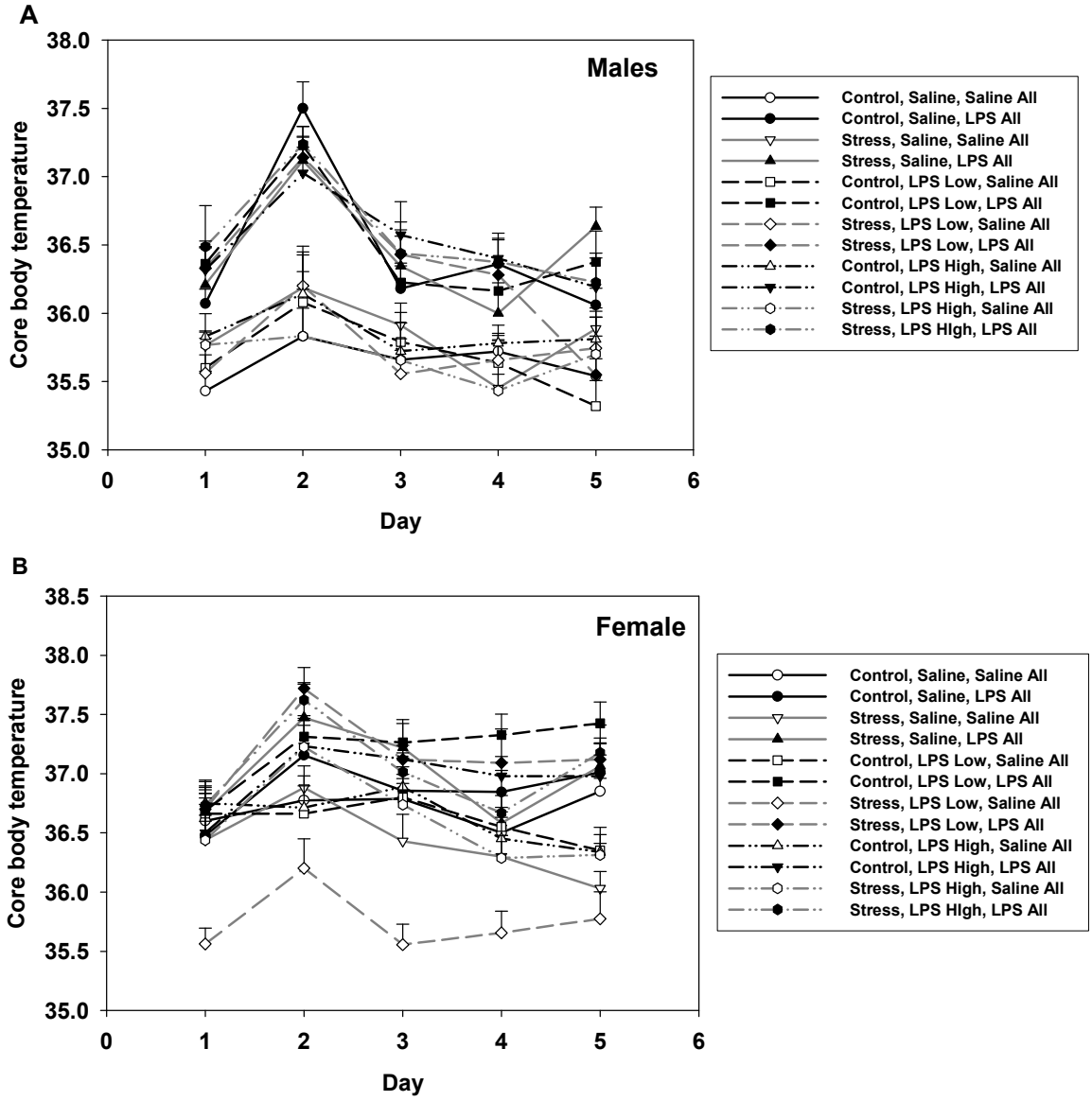


Figure 5. Administration of LPS significantly increased core body temperature in both male (A) and female (B) saline-treated subjects. Subjects displayed a fever response to LPS administration on days 1–5 of treatment, with a significantly higher fever on day 2. Grey lines are non-stressed control subjects and black lines are prenatally stressed subjects. Open symbols represent subjects given of repeated saline injections, while closed symbols represent subjects given repeated LPS injections on postnatal days 109–113. Lines represent mean ± standard error of the mean (SEM).

3.6. Experiment 1B: Morris water maze

Prenatally stressed subjects overall swam significantly more slowly than non-stressed controls, as shown by a significant main effect of Prenatal Treatment

($F(1,192)=4.45$, $p<0.05$, see Figures 6E and 6F). However, Prenatal Treatment had no effect on any or the other dependent measures collected. In addition, there were no significant main effects or interaction effects of Gender for any of the dependent variables during the 5 days of MWM training.

Repeated administration of LPS led to a significant increase in subject's latency to locate the platform relative to saline-treated subjects ($F(1,192)=6.64$, $p<0.05$, see Figures 6C and 6D). A significant Repeated LPS Treatment X Day interaction for distance swam ($F(4,768)=6.61$, $p<0.0001$, see Figures 6A and 6B) showed that LPS-treated subjects swam a significantly shorter distance to locate the platform on day 1 ($p<0.05$) of testing and a significantly longer distance on days 3–5 of testing relative to saline controls (*p's*<0.05). There was also a significant Repeated LPS Treatment X Day interaction for swim speed ($F(4,768)=43.61$, $p<0.0001$, see Figures 6E and 6F); on days 1 and 2 of testing, the LPS-treated subjects swam significantly slower than the saline-treated subjects (*p's*<0.0001), whereas on days 4 and 5 of testing, the LPS treated animals swam faster than the saline controls (*p's*<0.0001). There was a significant LPS Treatment X Repeated LPS Treatment interactions observed for swim speed ($F(8,768)=3.76$, $p<0.0005$), which showed administration of LPS two weeks prior during active avoidance testing modulated their response to the repeated LPS administration during MWM testing. On day 1, subjects given repeated LPS injections swam significantly more slowly than saline-treated subjects (*p's*<0.05). However, on day 2, subjects that received the high or low dose of LPS two weeks prior (during active avoidance training) and then repeated LPS injections during MWM testing swam significantly faster than subjects that received a saline injection two weeks ago followed by repeated LPS injections (*p's*<0.05).

Analysis of the probe trial data revealed there was a significant Repeated LPS Treatment X Gender interaction ($F(1,196)=5.91$, $p<0.05$, see Figures 7A and 7B). Female, but not male, subjects given repeated LPS injections spent significantly less time in the quadrant of the maze where the platform was previously located relative to saline-treated female subjects ($p<0.01$). In addition, saline-treated females spent significantly more time in the quadrant of the maze where the platform was previously located than saline-treated males ($p<0.05$). There were no significant Prenatal Treatment effects on performance in the probe trials.

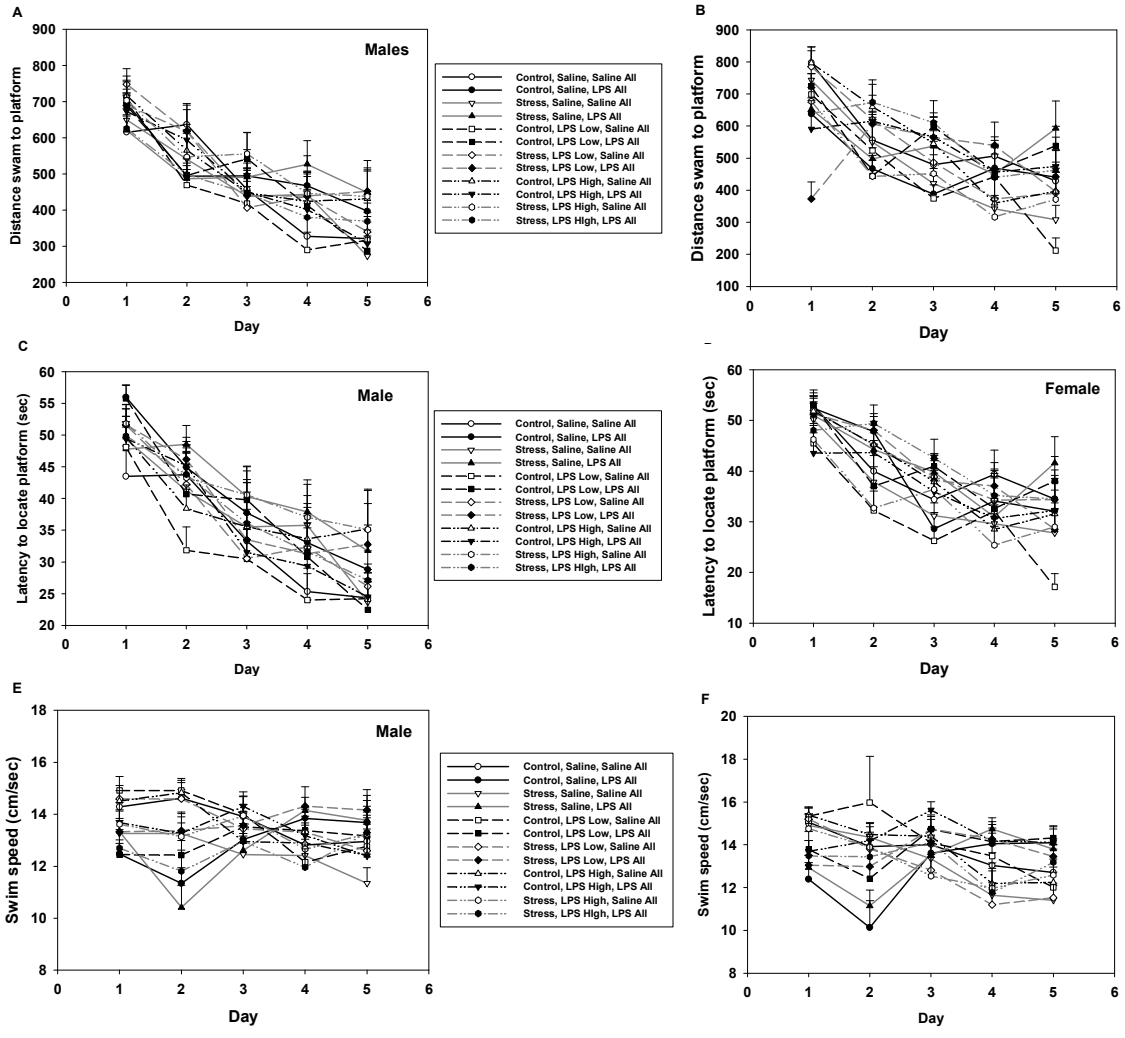


Figure 6. LPS-treated subjects swam a shorter distance on day 1 and longer distance on days 3–5 to locate platform than saline-treated subjects (A, B). LPS administration significantly increased latency to locate the

platform (C, D). LPS administration significantly reduced swim speed on days 1 and 2, however, LPS-treated subjects swam significantly faster than saline-treated subjects on days 3–5 of testing (E, F). Prior LPS administration modulated the response to repeated injections during MWM testing, however, as LPS-treated subjects, previously exposed to LPS, swam significantly faster than subjects previously administered saline. Lines represent mean ± standard error of the mean (SEM).

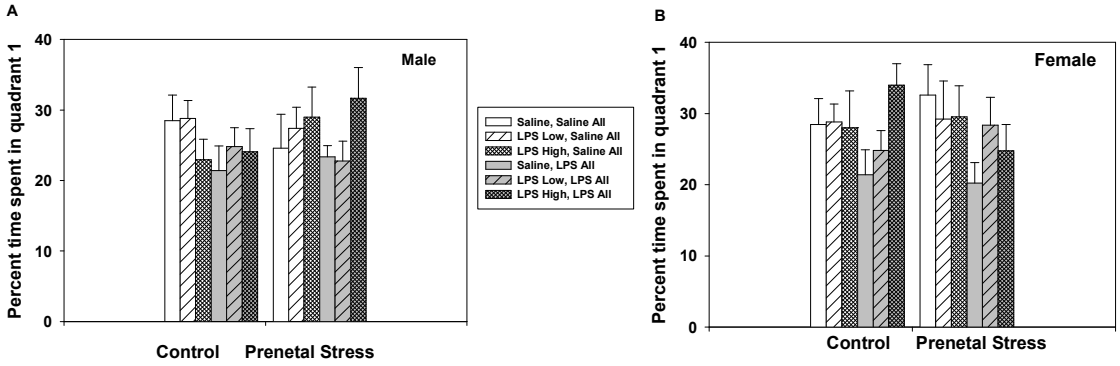


Figure 7. Percent time spent in the quadrant of the maze where the platform was previously located, male subjects (A) and female subjects (B). During probe trails, the LPS-treated female subjects spent significantly less time in the quadrant that previously contained the platform than saline-treated subjects. Bars represent mean ± standard error of the mean (SEM).

3.8. Experiment 2: Serum levels of corticosterone

Analysis of serum corticosterone levels, as expected, revealed that LPS-treated subjects (both males and females) had significantly higher levels of corticosterone than saline-treated subjects ($F(1,35)=144.61$, $p<0.0001$, see Figure 8). Further, a significant main effect of Gender and a significant LPS Treatment X Gender interaction showed that female subjects produced significantly more corticosterone in response to LPS administration than male subjects administered LPS ($F(1,35)=19.39$, $p<0.0001$; $F(1,35)=14.12$, $p<0.001$, respectively; see Figure 8). There were no significant effects or interaction effects of Prenatal Treatment.

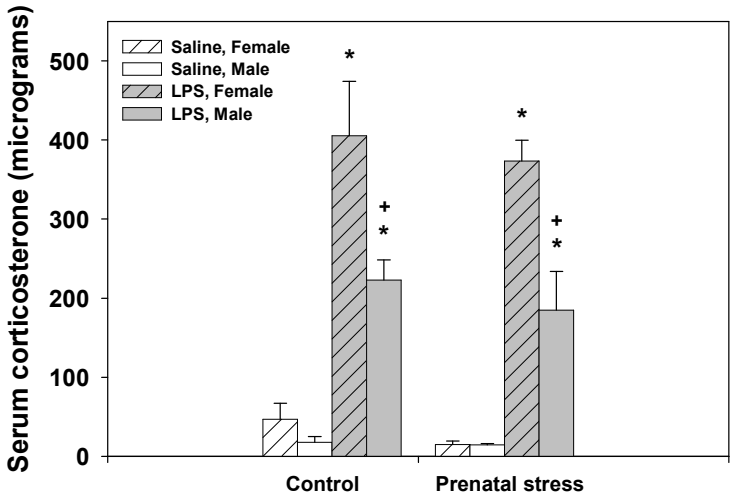


Figure 8. Administration of LPS significantly increased corticosterone production relative to saline-treated subjects (p<0.0001). Female mice released significantly more corticosterone in response to LPS administration relative to LPS-treated male mice (p<0.001). Prenatal treatment had no effect on corticosterone production. * indicates a significant difference from saline-treated subjects. + indicates a significant difference from female subjects. Bars represent mean ± standard error of the mean (SEM).

3.9. Experiment 2: Serum and splenic levels of IL-1β

Analysis of peripheral IL-1β production showed that administration of LPS significantly increased IL-1β in both the serum and spleen relative to saline-treated subjects (F(1,35)=145.08, p<0.0001; F(1,35)=34.45, p<0.0001, respectively; see Figures 9A and 9B). There were no significant main effects or interaction effects of Gender or Prenatal Treatment.

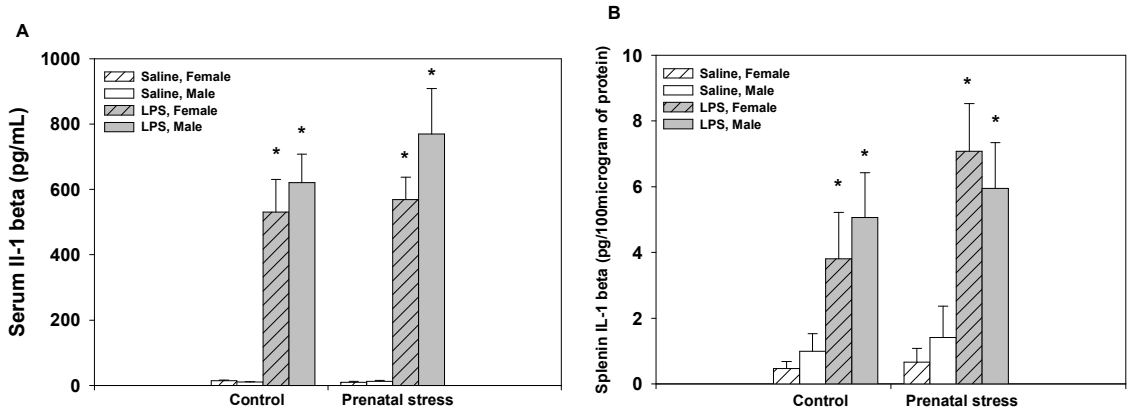


Figure 9. Administration of LPS significantly increased IL-1β production in both the serum (A) and the spleen (B) relative to saline-treated subjects (*p's*<0.05). Prenatal treatment had no effect on IL-1β production. * indicates a significant difference from saline-treated subjects. Bars represent mean ± standard error of the mean (SEM).

3.10. Experiment 2: Hippocampal and cortical IL-1β gene expression

Administration of LPS significantly increased IL-1β mRNA expression in the hippocampus and the cortex relative to saline-treated subjects ($F(1,34)=78.97$, $p<0.0001$; $F(1,35)=46.83$, $p<0.0001$, respectively; see Figures 10A and 10B). Furthermore, a significant main effect of Gender showed that male subjects show increased expression of IL-1β in both the hippocampus and the cortex relative to female subjects ($F(1,34)=14.12$, $p<0.001$; $F(1,35)=5.6$, $p<0.05$, respectively; see Figures 10A and 10B). There was a significant LPS Treatment X Gender interaction for IL-1β expression in the hippocampus, but not for the cortex ($F(1,34)=13.74$, $p<0.001$, see Figure 10B). Expression of IL-1β following LPS administration was significantly higher in male subjects than in female subjects treated with LPS ($p<0.05$). No significant main effects or interaction effects of Prenatal Treatment were found.

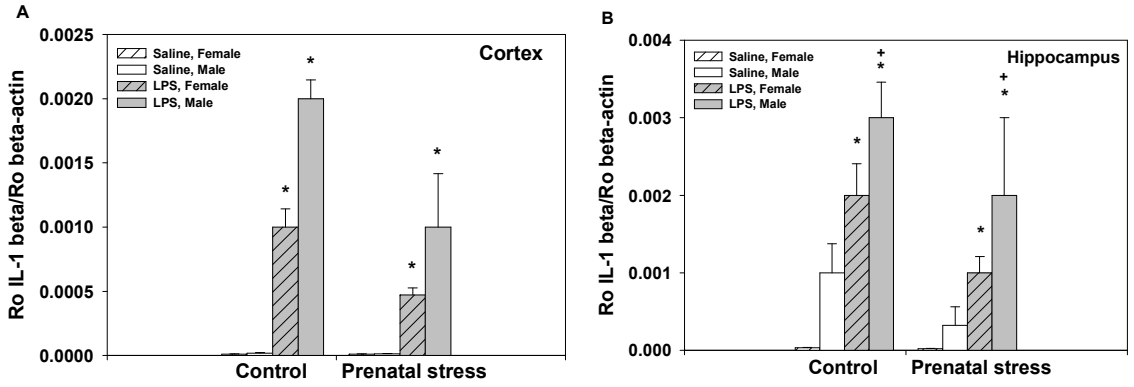


Figure 10. Administration of LPS significantly increased IL-1β expression in both the cortex (A), and the hippocampus (B) relative to saline-treated subjects (*p's*<0.05). Analysis of IL-1β expression in the cortex showed that female mice, regardless of their LPS treatment condition, showed significantly less IL-1β expression relative to male subjects (p<0.05). For hippocampal samples, male subjects expressed significantly higher levels of IL-1β following LPS administration than female subjects given LPS (p<0.05). * indicates a significant difference from saline-treated subjects. + indicates a significant difference from female subjects. Bars represent mean ± standard error of the mean (SEM).

4. DISCUSSION

The proposed experiments were designed to explore the behavioral ramifications of unusual stress during prenatal development, both with and without endotoxin exposure in adulthood. We hypothesized that, regardless of the adulthood LPS treatment condition, prenatally stressed subjects would show evidence of increased anxiety-like behavior relative to non-stressed controls. Furthermore, we anticipated that males and females would respond differently to prenatal stress exposure in terms of their behavior and corticosterone response. Based on previous research, we predicted that, regardless of prenatal treatment, endotoxin administration would result in behavioral deficits and increased corticosterone and increased peripheral and central IL-1β production relative to saline controls. Moreover, we hypothesized that re-exposure to LPS after a recovery period would sensitize the subject to the behavioral and physiological responses associated with endotoxin administration. Finally, we hypothesized that there would be

synergistic effects of prenatal stress and adulthood endotoxin exposure. Specifically, we predicted that prenatal stress exposure would enhance susceptibility to LPS-induced performance and learning deficits, and that these behavioral effects would coincide with increased peripheral and central IL-1β expression.

The results obtained partially supported our hypotheses, as prenatal stress exposure impaired performance in the active avoidance conditioning paradigm, and did so in a gender-dependent manner. Furthermore, as expected, endotoxin administration led to performance deficits, deficits in spatial learning, a fever response, and increased corticosterone and IL-1β production. However, these data failed to confirm the primary hypothesis that prenatal stress exposure would exaggerate cognitive deficits induce by immune activation. Despite this, the data provide evidence that the prenatal environment may modulate the anxiety-related behavioral response induced by endotoxin exposure.

In both human and non-human animals, prenatal stress exposure has been associated with increased anxiety and a propensity to develop depressive disorders (Estanislau et al., 2005; Heim et al., 2002; Laviola et al., 2004; Rimondini et al., 2003). However, previous studies suggest that often exposure to a stressor in adulthood is required before prenatally stressed subjects will exhibit behavior related to increased anxiety (Estanislau et al., 2005; Rimondini et al., 2003). The present study hypothesized that prenatal stress exposure would lead to increased anxiety levels, and that prenatally stressed subjects would show an exaggerated anxiety response following LPS administration. The data suggest that prenatal stress exposure increased behavior associated with anxiety in the elevated plus maze. Prenatally stressed subjects displayed behavior reflective of increased anxiety, as they spent significantly less time in the open

arms and significantly more time in the closed arms of the maze than non-stressed controls. These findings are consistent with the work of Laviola et al. (2004), who showed prenatally stressed subjects spend less time in the open arms and more time in the closed arms of the elevated plus maze, suggestive of increased anxiety levels in the prenatally stressed subjects. The present data confirmed that exposure to stress during prenatal development increases the propensity for higher levels of anxiety, as indicated by behavior alterations, and that these effects are gender-dependent.

There is some evidence to suggest that activation of the immune system can increase anxiety-like behavior in the elevated plus maze (Lacosta et al., 1999), however, the results are limited, and are complicated by the performance decrements induced by endotoxin administration. The current results showed that LPS administration significantly increased time spent in the closed arms, potentially indicating an anxiety-like response to immune activation. However, the most prominent finding was a performance deficit unrelated to anxiety. For both male and female subjects, administration of LPS significantly reduced the distance traveled in the elevated plus maze in a dose-dependent manner. Subjects administered the low or high dose of LPS (i.e., 50µg/kg and 250µg/kg, respectively) traveled a significantly shorter distance than saline-treated subjects, and those given the high dose traveled a shorter distance than subjects given the low dose of LPS. The present results show modest evidence of behavioral changes possibly reflective of elevated anxiety levels following immune activation. Our results suggest that prenatal stress exposure augmented this response to LPS administration. Prenatally stressed male subjects treated with LPS spent significantly more time in the closed arms relative to stressed subjects given saline, whereas the LPS

administration had no effect on the non-stressed control subjects. Although similar trends were observed for the percentage of time spent in the center and open arms of the maze, the differences were not significant. These data provide preliminary evidence that an early stressor may lead to an exaggerated response to immune activation.

The alterations in anxiety associated with prenatal stress are believed to result, in part, from changes in the reactivity of the offspring's HPA axis. Previous research has shown that prenatally stressed females, but not males, show increased basal corticosterone levels, while both sexes show increased corticosterone release in response to an adulthood stressor (McCormick et al., 1995; Szuran et al., 2000; Valleé et al., 1999). In addition, prenatal stress exposure has been shown to impair the offspring's HPA negative feedback loop, resulting in a delayed corticosterone response to stress exposure (Barbazanges et al., 1996; Koehl et al., 1999; Maccari et al., 1995). Based on previous reports, we evaluated whether prenatal stress exposure would alter basal and LPS-induced corticosterone production. The present study failed to find evidence of altered basal corticosterone levels in either male or female prenatally stressed subjects. Although there is some evidence to suggest prenatally stressed subjects, particularly females, have higher basal corticosterone levels than non-stressed controls, others have reported no alterations in basal levels (Hashimoto et al., 2001; Laviola et al.,McCormick et al., 1995; Szuran et al., 2000; Takahashi et al., 1992; Valleé, et al., 1999), matching the results of the present study. Furthermore, research by Koehl et al. (1999) suggests that prenatal stress exposure alters the circadian activity of the HPA axis, and that prenatally stressed subjects only show significant increases in basal corticosterone levels relative to non-stressed controls four hours prior to the onset of their dark cycle. The discrepancy in

the findings on basal corticosterone production in prenatally stressed and non-stressed controls may therefore result from variations in the timing of serum collection. The present study did not evaluate alterations in corticosterone release following a psychological stressor, but rather after an "immune stressor" (i.e., LPS). Results showed, as expected, that LPS administration significantly increased corticosterone production, though prenatal stress exposure did not modulate this response. However, the gender of the offspring did modulate corticosterone production following LPS administration, as female subjects produced significantly more corticosterone following LPS administration than did male subjects. This finding confirms research conducted by Frederic et al. (1993), who found that females produce more corticosterone in response to ndotoxin exposure. The present results failed to replicate the findings of Hashimoto et al. (2001), who found that prenatally stressed subjects produce significantly higher levels of corticosterone in response to LPS than non-stressed controls. However, their dose of LPS was significantly smaller (i.e., 10μg/kg) than the dose employed in the present study. Although the present study did not assess this possibility, prenatally stressed subjects, as seen following adulthood stress exposure, may display prolonged corticosterone release (rather than elevated corticosterone release at a given timpoint) following LPS administration. The current findings suggest that prenatal stress exposure does not alter the reactivity of the HPA axis following an immune stimulus, but confirms that females release more corticosterone than males following LPS administration. Collectively, the results suggest that the increased anxiety levels in the prenatally stressed male subjects is unlikely to be due to alterations in the reactivity of the HPA axis, although we cannot rule

out the possibility of prolonged corticosterone production following LPS administration that might affect their behavior.

In addition to alterations in anxiety-related behavior, there is evidence to suggest that prenatal stress exposure can induce learning deficits in the offspring. However, the data are inconsistent, as some reports such that prenatal stress impairs learning, whereas others have found no effects, or that the learning deficits are limited to one gender (Aleksandrov et al., 2001; Bowman et al., 2004; Fride et al., 1986; Gué et al., 2004; Lordi et al., 1997; Louvart et al., 2005; Meek et al., 2000; Valleé et al., 1999). Despite the ambiguity of these reports, we predicted that prenatally stressed subjects would show learning impairments, but that the effects might be gender-dependent. Two paradigms were used to assess alterations in learning, two-way active avoidance and the Morris water maze (MWM), a test of spatial learning. The present findings confirm that prenatal stress exposure can disrupt learning. However, the deficits were limited to male offspring and were dependent upon the test of learning employed. Prenatally stressed male subjects showed deficits in performance in the two-way active avoidance conditioning paradigm, as they performed significantly fewer avoidance responses, more escape responses, and had significantly reduced discrimination index scores relative to non-stressed controls. Female mice showed no evidence of prenatal stress-induced learning decrements, as avoidance responses and discrimination scores were equivalent between prenatally stressed and non-stressed controls. Previous work by Fride et al. (1986) reported that prenatal stress exposure actually facilitated learning in the two-way active avoidance conditioning paradigm. However, the effects reported by Fride et al. (1986) may reflect behavioral supersensitivity to the footshock used as the unconditioned stimulus in the

paradigm, potentially resulting in an increase in random crossing behavior. In terms of spatial learning deficits, prior research suggests that prenatally stressed females, but not males, have spatial learning deficits relative to non-stressed control females (Aleksandrov et al., 2001; Bowman et al., 2004; Meek et al., 2000; Valleé et al., 1999). The present results found no evidence of spatial learning deficits in either male or female subjects that were prenatally stressed. While prenatally stressed subjects swim more slowly than non-stressed controls, they swam an equivalent distance to locate the platform. These results suggest that prenatal stress exposure alone had no effects on spatial learning in either male or female subjects. Together, these findings suggest that prenatal stress exposure, at least in male offspring, may impair learning in certain paradigms, but not in others.

One topic that has received a good deal of attention is the idea that an individual's immune competence can be determined, in part, by perinatal events. Previous research suggests that prenatal stress exposure leads to alterations in the offspring's immune system (Fonseca et al., 2002; Kay et al., 1998; Klein & Rager, 1995; Laviola et al., 2004; Sorbrain et al., 1992; Tuchsherer et al., 2002). Ultimately, these findings suggest that prenatal stress exposure may have significant consequences for an individual's ability to mount an appropriate immune response, and defend against infection. Over the years, it has become evident that the immune response is not simply limited to the activity of immune cells, but, rather, the immune system induces neural, endocrine, behavioral, and even motivational changes to fight off an infection effectively. Cytokine production is one of the primary ways in which the immune system can communicate with and alter the activity of the central nervous system (CNS) and the endocrine system (Dantzer, 2004). As prenatal stress exposure has been shown to alter cytokine production (Laviola et al.,

2004), prenatal stress may dysregulate the communication between the immune system, the endocrine system, and the CNS. Disrupting the communication between the immune system and the rest of the body may make an organism more vulnerable to the negative effects of immune activation, such as increased anxiety, lethargy, and cognitive deficits. Based on previous findings, we hypothesized that the prenatally stressed animals would show an exaggerated response endotoxin administration, due to an increase in peripheral and central cytokine production. We hypothesized that this may surface as increases in the LPS-induced fever response, in exaggerated behavioral responses, and in elevated cytokine production.

Fever is a typical response to infection, as many pathogens cannot proliferate in elevated temperatures (Kluger, 1978; Long et al., 1990). As expected, we found that administration of LPS significantly increased the subject's core body temperature. In addition, the present data shows that both males and females, when exposed to repeated injections of LPS, mount the highest fever following the second consecutive injection. Based by prior work by Hashimoto et al. (2001), we predicted that prenatally stressed subjects would develop a higher fever following endotoxin administration relative to non-stressed controls. However, we failed to find any evidence to support this hypothesis, as prenatally stressed and non-stressed control subjects showed an equivalent fever response to LPS administration. As previously noted, one possible reason we failed to replicate the results of Hashimoto et al. (2001) is that they used a much smaller dose of LPS (i.e., 10μg/kg), whereas we administered repeated injections of a 250μg/kg dose. It may be that prenatally stressed subjects are responsive to what is a subthreshold dose of LPS for a

normal animal, whereas at higher doses, prenatally stressed and non-stressed subjects display similar reactions.

In addition to alterations in the physiological response to immune activation, we hypothesized that prenatally stressed subjects would show exaggerated LPS-induced learning deficits due to increased cytokine production, as cytokines are believed to be the primary mediators of the cognitive deficits associated with immune activation (Dantzer, 2004). Although we found that prenatally stressed males showed evidence of impaired acquisition of the active avoidance conditioning task, as they performed fewer avoidance responses and had reduced discrimination scores, this effect was not influenced by whether the subjects received an LPS or saline injection. The lack of an LPS-induced learning deficit in the two-way active avoidance paradigm is inconsistent with prior reports from our laboratory (Sparkman et al., 2005a; Kohman et al., 2007a; Kohman et al., 2007b). However, the difference in findings may result from the use of younger subjects in the present study (i.e., 3-month-old subjects) versus those used in our prior studies (i.e., 4- to 12-month-old subjects), as it has been our experience that four-month-old or older subjects give us consistent evidence of LPS-induced learning deficits, whereas results are variable with younger subjects. We found evidence of LPS-induced spatial learning deficits in the MWM maze, as subjects given repeated injections of LPS swam significantly longer distances to locate the platform relative to saline-treated subjects. However, the deficit was independent of their prenatal treatment, as prenatally stressed and non-stressed controls showed similar spatial learning deficits following LPS administration. Collectively, these data suggest that although prenatal stress exposure and

immune activation, under some conditions, can independently induced learning impairments, there do not appear to be any synergistic effects.

As an attempt to replicate previous research by Laviola et al. (2004), who showed that prenatally stressed subjects produce more IL-1β in the cortex and the periphery, we evaluated serum and splenic production of IL-1β and IL-1β gene expression in the hippocampus and cortex following LPS administration in prenatally stressed and non-stressed controls. Our results showed, as expected, that administration of LPS significantly increased IL-1β production within the periphery and increased central gene expression of IL-1β in the hippocampus and cortex. However, we failed to find any evidence that prenatal stress exposure altered basal levels of IL-1β, or that prenatally stressed subjects produce more IL-1β in either the periphery or the CNS following immune activation. Differences in findings may results from procedural variations between the current study and the work done by Laviola et al. (2004), as Laviola et al. (2004) restrained pregnant rats for three 45-minute sessions a day on gestation days 11–21, versus three 4-hours sessions on days 15–17 employed in the current study. Additionally, Laviola et al. (2004) used cultured phytohaemagglutin-stimulated splenic cells to assess alteration in peripheral cytokine production and measured basal protein levels of IL-1β in the brain, whereas peripheral IL-1β levels and central IL-1β gene expression alterations were assessed after LPS administration in the current study. Another possibility, as seen with the fever and corticosterone response, is that cytokine production may be enhanced in prenatally stressed subjects if a subthreshold dose of LPS is administered. However, the present data suggest that prenatally stressed and non-

stressed controls have similar peripheral and central cytokine production profiles in responses to LPS administration.

Finally, the present experiment investigated whether or not prenatal stress exposure would alter the development of endotoxin tolerance, an adaptive response in which the immune response becomes attenuated following repeated endotoxin administration to prevent the development of endotoxic shock. Based on previous research (Hayley et al., 1999; Schmidt et al., 1995) that showed subjects become sensitized to the effects of TNF-α and IL-1β administration when the administrations were separated by 14–28 days, we had hypothesized that re-exposing animals to LPS after a two week break would lead to sensitization of the behavioral response. To test the hypothesis, subject received a single injection of saline, a low dose of LPS (i.e., 50μg/kg), or a high dose of LPS (i.e., 250μg/kg) and were tested for alterations in avoidance conditioning. Then, after a two-week break, subjects then received either five consecutive LPS (250μg/kg) or saline injections while being tested in the water maze. Results suggest that previous exposure to endotoxin led to faster onset of endotoxin tolerance, as measured by behavioral correlates of tolerance in the MWM, rather than sensitization. Subjects, regardless of their prenatal treatment condition, that received the high or low dose of LPS, and then repeated LPS injections, swam significantly faster than subjects that received a saline injection followed by repeated LPS injections. While these data confirm that prior endotoxin exposure can modulate the response to subsequent endotoxin administration, in our hands, the results suggest that the subjects will become less responsive to subsequent administrations rather than becoming sensitized. The differences in results may be due to the administration of LPS rather than administration

of a single cytokine such as IL-1β or TNF-α, as LPS administration is likely to induce a larger and more diverse inflammatory reaction than administration of an individual cytokine. Furthermore, the dosage of the subsequent challenge may explain the difference in findings, as Hayley et al. (1999) pre-exposed subjects to a low IL-1β dose (i.e., 1.0μg/mouse, approximately 3.33μg/kg), whereas the present study administered a much larger dose of LPS (i.e., 250μg/kg).

In summary, the present findings confirm that prenatal stress exposure may induce long-term alterations in the offspring's cognitive abilities as well as emotional reactivity. However, these effects appear to be dependent on the gender of the offspring and the testing paradigm employed. Additionally, these data confirm, as expected, that immune activation significantly increases peripheral IL-1β and corticosterone production and upregulates IL-1β mRNA expression within the cortex and the hippocampus. Further, these data highlight that males and females respond differently to endotoxin exposure, as LPS administration leads to greater corticosterone production in females and significantly higher central IL-1β expression in males. The findings provide preliminary evidence that prenatally stressed subjects may display exaggerated behavioral response to LPS relative to non-stressed controls. In conjunction with prior reports (Hashimoto et al., 2001), these results suggest that prenatally stressed subjects may be more responsive to LPS. However, the present study failed to support the general hypothesis that prenatally stressed subjects would show exaggerated responses to endotoxin exposure, in terms of either LPS-induced learning deficits or LPS-induced cytokine production within periphery and the CNS.

REFERENCES

Ader, R., & Cohen, N. (1975). Behaviorally conditioned immunosuppression. *Psychosomatic Medicine,* 37, 333–340.

Agelaki, S., Tsatanis, C., Gravanis, A., & Margioris, A. N. (2002). Corticotropin-releasing hormone augments proinflammatory cytokine production from macrophages in vitro and in lipopolysaccharide-induced endotoxin shock in mice. *Infection and Immunity,* 70, 6068–6074.

Aleksandrov, A. A., Polyakova, O. N., & Batuev, A. S. (2001). The effects of prenatal stress on learning in rats in a morris maze. *Neuroscience and Behavioral Physiology,* 31, 71–74.

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

Auphan, N., DiDonato, J. A., Rosette, C., Helmbergm A., & Karin, M. (1995). Immunosuppression by glucocorticoids: inhibition of NK-κB activity through induction of IκB synthesis. *Science,* 270, 286–290.

Banks, W. A., Farr, S. A., La Scola, M. E., & Morley, J. E. (2001). Intravenous human interleukin-1α impairs memory processing in mice: dependence on blood-brain barrier transport into posterior division of the septum. *The Journal of Pharmacology and Experimental Therapeutics,* 299, 536–5411.

Banks, W. A., Farr, S. A., & Morley, J. E. (2002). Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation,* 10, 319–327.

Barbazanges, A., Piazza, P. V., Le Moal, M., & Maccari, S. (1996). Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *The Journal of Neuroscience,* 16, 3943–3949.

Barker, D. J. P. (1995). Intrauterine programming of adult disease. *Molecular Medicine Today,* 1, 418–423.

Bauer, M. E., Perks, P., Lightman, S., & Shanks, N. (2001). Restraint stress is associated with changes in glucocorticoid immunoregulation. *Physiology & Behavior,* 73, 525–532.

Barrientos, L. G., Sprunger, D. B., Campeau, S., Watkins, L.R., Rudy, J. W., & Maier, S.F. (2004). BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. *Journal of Neuroimmunology,* 155, 119–26.

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

Bellinger, F. P., Madamba, S., & Siggins, G. R. (1993). Interleukin-1β inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Research,* 628, 227–234.

Berkenbosch, F., Oers, J., Del Rey, A., Tilders, F., & Besedovsky, H. (1987). Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science,* 238, 524–526.

Bertini, R., Bianchi, M., & Ghezzi, P. (1998). Adrenalectomy sensitizes mice to the lethal effects of interleukin-1 and tumor necrosis factor. *Journal of Experimental Medicine,* 167, 1708–1712.

Bluthé, R. M., Michaud, B., Kelley, K. W., & Dantzer, R. (1996). Vagotomy attenuates behavioral effects of interluekin-1 injected peripherally but not centrally. *Neuroreport,* 7, 1485–1488.

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

Boomershine, C. S., Wang, T., & Zwilling, B. S. (2001). Neuroendocrine regulation of macrophage and neutrophil function. In R. Ader, D. L. Felten, & N. Cohen (Eds.) *Psychoneuroimmunology* (3rd ed., pp. 289–300). San Diego, CA: Academic Press.

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

Bowman, R. E., Maclusky, N. J., Sarmiento, Y., Frankfurt, M., Gordon, M., & Luine, V. N. (2004). Sexually dimorphic effects of prenatal stress on cognition, hormonal response, and central neurotransmitters. *Endocrinology,* 145, 3778–3787.

Chapillon, P., Patin, V., Roy, V., Vincent, A., & Caston, J. (2002). Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. *Developmental Psychobiology,* 41, 373–387.

Chapman, R. H. & Stern, J. M. (1977). Failure of severe maternal stress of ACTH during pregnancy to affect emotionality of male rat offspring: implications of litter effects for prenatal studies. *Developmental Psychobiology,* 12, 255–267.

Chen, R., Zhou, H., Beltran, J., Malellari, L., & Chang, S. L. (2005). Differential expression of cytokines in the brain and serum during endotoxin tolerance. *Journal of Neuroimmunology,* 163, 53–72.

Choi, S. J. & Kellogg, C. J. (1996). Adolescent development influences functional responsiveness of noradrenergic projections to the hypothalamus in male rats. *Developmental Brain Research,* 94, 144–151.

Cohen, S., Tyrrell, D. A. J., & Smith, A. P. (1991). Psychological stress and susceptibility to the common cold. *The New England Journal of Medicine,* 325, 606–612.

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

Cratty, M. S., Ward, H. E., Johnson, E. A., Azzaro, A. J., & Birkle, D. L. (1995). Prenatal stress increases corticotropin-releasing factor (CRF) content and release in rat amygdala minces. *Brain Research,* 27, 297–302.

Crestani, F., Seguy, F., & Dantzer, R. (1991). Behavioral effects of peripherally injected interleukin-1: role of prostaglandins. *Brain Research,* 542, 330–335.

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

Dantzer, R. (2004). Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *European Journal of Pharmacology*, 500, 399–411.

Denenberg, V. H. (1977). Assessing the effects of early experience. In R. D. Myers (Ed.) *Methods in Psychobiology* (Vol 3, pp. 127–147). New York, NY: Academic Press.

Denenberg, V. H. (1984). Some statistical and experimental considerations in the use of the analysis-of-variance procedure. *American Journal of Physiology,* 246, R403–R408.

Dhabhar, F. S. & McEwen, B. S. (2001). Bidirectional effects of stress and glucocorticoid hormones on immune function: possible explanation for paradoxical observations. In R. Ader, D.L. Felten, & N. Cohen (Eds.) *Psychoneuroimmunology* (3rd ed., pp. 649–666). New York: Academic Press.

Dunn, A. J. (2001). Effects of cytokines and infections on brain neurochemistry. In R. Ader, D.L. Felten, & N. Cohen (Eds.) *Psychoneuroimmunology* (3rd ed., pp. 649–666). New York: Academic Press.

Dunn, A. J. & Berridge, C. W. (1990). Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety of stress responses? *Brain Research Reviews,* 15, 71–100.

Elenkov, I. J. & Chrousos, G. P. (2002). Stress hormones, proinflammatory and anti-inflammatory cytokines, and autoimmunity. *Annuals of New York Academy of Science,* 966, 290–303.

Esterling, B. & Rabin, B. S. (1987). Stress-induced alteration of T-lymphocyte subsets and humoral immunity in mice. *Behavioral Neuroscience,* 101, 115–119.

Estanislau, C. & Morato, S. (2005). Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. *Behavioural Brain Research,* 163, 70–77.

Fauci, A. S. (1988). The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science*, 239, 617–622.

Fearon, D. T. & Locksley, R. M. (1996). The instructive role of innate immunity in the acquired immune response. *Science,* 272, 50–54.

Fonseca, E. S. M., Massoco, C. O., & Palermo-Neto, J. (2002). Effects of prenatal stress-induced changes in behavior and macrophage activity of mice. *Physiology & Behavior,* 77, 205–215.

Frederic, F., Oliver, C., Wollman, E., Delhaye-Bouchaud, N., & Mariani, J. (1993). IL-1 and LPS induce a sexually dimorphic response of the hypothalamo-pituitary-adrenal axis in several mouse strains. *European Cytokine Network,* 4, 321–329.

Fride, E., Dan, Y., Feldon, J., Halevy, G., & Weinstock, M. (1986). Effects of prenatal stress on vulnerability to stress in prepubertal and adult rats. *Physiology & Behavior,* 37, 681–687.

Gahtan, E. & Overmier, B. J. (2001). Performance more than working memory disrupted by acute systemic inflammation in rats in appetitive tasks. *Physiology & Behavior*, 73, 201–210.

Gibertini, M., Newton, C., Friedman, H., & Klent, T. W. (1995). Spatial learning impairments in mice infected with legionella pnuemophila or administered exogenous interleukin-1β. *Brain, Behavior, and Immunity,* 9, 113–128.

Gué, M., Bravard, A., Meunier, J., Veyier, R., Gaillet, S., Recasens, M., & Maurice, T. (2004). Sex differences in learning deficits induced by prenatal stress in juvenile rats. *Behavioural Brain Research,* 150, 149–157.

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

Hashimoto, M., Watanabe, T., Fujioka, T., Tan, N., Yamashita, H., & Nakamura, S. (2001). Modulating effects of prenatal stress on hyperthermia induced in adult offspring by restraint or LPS-induced stress. *Physiology & Behavior,* 73, 125–132.

Hayley, S., Brebner, K., Lacost, S., Merali, Z., & Anisman, H. (1999). Sensitization to the effects of tumor-necrosis factor-α: Neuroendocrine, central monoamine, and behavioral variations. *The Journal of Neuroscience,* 19, 5654–5665.

Heim, C., Newport, J. D., Wagner, D., Wilcox, M. M., Miller, A. H., & Nemeroff, C. B. (2002). The role of early adverse experience and adulthood stress in the prediction of neuroendocrine stress reactivity in women: a multiple regression analysis. *Depression and Anxiety,* 15, 117–125.

Huttunen, M. O. (1971). Persistent alteration of turnover of brain noradrenaline in the offspring of rats subjected to stress during pregnancy. *Nature,* 230, 53–55.

Johnson, J. D., O'Connor, K. A., Deal, T., Stark, M., Watkins, L. R., & Maier, S. F. (2002). Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain, Behavior, and Immunity,* 16, 461–476.

Johnson, J. D., O'Connor, K. A., Hansen, M. K., Watkins, L. R., & Maier, S. F. (2003). Effects of prior stress on LPS-induced cytokine and sickness responses. *American Journal of Physiology Regulatory Integrated Comparative Physiology,* 284, R422–R432.

Katsuura, G., Arimura, A., Koves, K., & Gottschall, P. E. (1990). Involvement of organum vasculosum of lamina terminalis and preoptic area in interleukin 1β-induced ACTH release. *American Journal of Physiology,* 258, E163–E171.

Kay, G., Tarcic, N., Poltyrev, T., & Weinstock, M. (1998). Prenatal stress depresses immune function in rats. *Physiology & Behavior,* 63, 397–402.

Kennedy, S. & Collier, A. C. (1994). Stress-induced modulation of the immune response in the developing rat pup. *Physiology & Behavior,* 56, 825–828.

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

Keller, S. E., Weiss, J. M., Schleifer, S. J., Miller, N. E., & Stein, M. (1981). Suppression on immunity by stress: effects of a graded series of stressors on lymphocyte stimulation in the rat. *Science,* 213, 1397–1400.

Keller, S. E., Weiss, J. M., Schleifer, S. J., Miller, N. E., & Stein, M. (1983). Stress-induced suppression of immunity in adrenalectomized rats. *Science,* 23, 1301–1304.

Kellog, C. K., Awatramani, G. B., & Piekut, D. T. (1998). Adolescent development alters stressor-induced FOS immunoreactivity in rat brain. *Neuroscience*, 83, 681–689.

Kinnunen, A. K., Koenig, J. I., & Bilbe, G. (2003). Repeated variable prenatal stress alters pre- and postsynaptic gene expression in the rat frontal pole. *Journal of Neurochemistry,* 86, 297–302.

Klein, S. L. & Rager, D. R. (1995). Prenatal stress alters immune function in the offspring of rats. *Developmental Psychobiology,* 28, 321–336.

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

Knackstedt, M. K., Hamelmann, E., & Arck, P.C. (2005). Mothers in stress: consequences for the offspring. *American Journal of Reproductive Immunology,* 54, 63–69.

Knudsen, P. J., Dinarello, C. A., & Strom, T. B. (1987). Glucocorticoids inhibit transcriptional and post-transcriptional expression of interleukin 1 in U937 cells. *The Journal of Immunology,* 139, 4129–4134.

Koehl, M., Darnaudéry, M., Dulluc, J., Van Reeth, O., Le Moal, M., & Maccari, S. (1999). Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both genders. *Journal of Neurobiology,* 40, 302–315.

Koenig, J. I., Elmer, G. I., Shepard, P. D., Lee, P. R., Mayo, C., Joy, B., Hercher, E., & Brady, D. L. (2005). Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. *Behavioural Brain Research,* 156, 251–261.

Kohman, R. A., Tarr, A. J., Sparkman, N. L., Day, C. E., Paquet, A., Akkaraju, G. R., & Boehm, G. W. (2007a). Alleviation of the effects of endotoxin exposure on behavior and hippocampal IL-1beta by a selective non-peptide antagonist of corticotropin-releasing factor receptors. *Brain, Behavior, and Immunity*, 21, 824–835.

Kohman, R. A., Tarr, A. J., Byler, S. L., & Boehm, G. W. (2007b). Age increases vulnerability to bacterial endotoxin-induced behavioral decrements. *Physiology & Behavior,* in press.

Konsman, J. P., Luheshi, G. N., Bluthé, R. M., & Dantzer, R. (2000). The vagus nerve mediates behavioral depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *European Journal of Neuroscience,* 12, 4434–4446.

Lacosta, S., Merali, Z., & Amisman, H. (1999). Behavioral and neurochemical consequences of lipopolysaccharide in mice. *Brain Research,* 818, 291–303.

Lapchak, P. A., Beck, K. D., Araujo, D. M., Irwin, I., Langston, J. W., & Hefti, F. (1993). Systemic interleukin-1 beta decreases brain-derived neurotrophic factor messenger RNA expression in the rat hippocampal formation. *Neuroscience*, 53, 297–301.

Laudenslager, M. L., Ryan, S. M., Drugan, R. C., Hyson, R. L., & Maier, S. F. (1983). Coping and immunosuppression: inescapable but not escapable shock suppresses lymphocyte proliferation. *Science,* 221, 568–570.

Laviola, G., Adriani, W., Morley-Fletcher, S., & Terranova, M. L. (2002). Peculiar response of adolescent mice to acute and chronic stress and to amphetamine: evidence of sex differences. *Behavioural Brain Research*, 130, 117–125.

Laviola, G., Rea, M., Morley-Fletcher, S., Di Carlo, S., Bacosi, A., De Simone, R., Bertini, M., & Pacifici, R. (2004). Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. *European Journal of Neuroscience,* 20, 1655–1664.

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

Lordi, B., Protais, P., Mellier, D., & Caston, J. (1997). Acute stress in pregnant rats: effects on growth rate, learning, and memory capabilities of the offspring. *Physiology & Behavior,* 62, 1087–1092.

Louvart, H., Maccari, S., & Darnaudéry, M. (2005). Prenatal stress affects behavioral reactivity to an intense stress in adult female rats. *Brain Research*, 1031, 67–71.

Maccari, S., Piazza, P. V., Kabbaj, M., Barbazanges, A., Simon, H., & Le Moal, M. (1995). Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *The Journal of Neuroscience,* 15, 110–116.

Madden, K. S. (2003). Catecholamines, sympathetic innervation, and immunity. *Brain, Behavior, and Immunity,* 17, S5–S10.

Maier, S. F. & Watkins, L. R. (2003). Immune-to-central nervous system communication and its role in modulating pain and cognition: implications for cancer and cancer treatment. *Brain, Behavior, and Immunity,* 17, S125–S131.

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.

McCormick, C. M., Smythe, J. W., Sharma, S., & Meaney, M. J. (1995). Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density. *Developmental Brain Research,* 84, 55–61.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience*, 24, 1161–1192.

Meek, L. R., Burda, K. M., & Paster, E. (2000). Effects of prenatal stress on development in mice: maturation and learning. *Physiology & Behavior,* 71, 543–549.

Minami, M., Kuraishi, Y., Yamaguchi, T., Nakai, S., Hirai, Y., & Satoh, M. (1991). Immobilization stress induces interleukin-1β mRNA in the rats hypothalamus. *Neuroscience Letter,* 123, 254–256.

Morméde, C., Castanon, N., Médina, C., Moze, E., Lestage, J., Neveu, P. J., & Dantzer, R. (2002). Chronic mild stress in mice decreases peripheral cytokine and increases central cytokine expressions independently of IL-10 regulation of the cytokine network. *Neuroimmunomodulation,* 10, 359–366.

Mormede, P., Dantzer, R., Michaud, B., Kelley, K. W., & Le Moal, M. (1988). Influence of stressor predictability and behavioral control on lymphocyte reactivity, antibody responses and neuroendocrine activation in rats. *Physiology & Behavior,* 43, 577–583.

Morrow, L. E., McClellan, J. L., Klir, J. J., & Kluger, M. J. (1996). The CNS site of glucocorticoid negative feedback during LPS-and psychological stress-induced fevers. *American Journal of Physiology,* 271, R732–R737.

Moyer, J. A., Herrenkohl, L. R., & Jacobowitz, D. M. (1977). Effects of stress during pregnancy on catecholamines in discrete brain regions. *Brain Research,* 121, 385–393.

Moynihan, J. A., Karp, J. D., Cohen, N., & Ader, R. (2000). Immune deviation following stress odor exposure: role of endogenous opioids. *Journal of Neuroimmunology,* 54, 51–58.

Moynihan, J. A. (2003). Mechanisms of stress-induced modulation of immunity. *Brain, Behavior, and Immunity,* 17, S11–S16.

Murray, C. A. & Lynch, M. A. (1998). Evidence that increased hippocampal expression of the cytokines interleukin-1β is a common trigger for age- and stress-induced impairments in long-term potentiation. *The Journal of Neuroscience,* 18, 2974–2981.

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-1β protein in rats. *The Journal of Neuroscience,* 18, 2239–2246.

Nomura, F., Akashi, S., Sakao, Y., Sato, S., Kawai, T., Matsumoto, M., Nakanishi, K., Kimoto, M., Miyake, K., Takeda, K., & Akira, S. (2000). Cutting edge: Endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. *The American Association of Immunologists,* 164, 3476–3479.

O'Connor, T. G., Heron, J., Golding, J., Beveridge, M., & Glover, V. (2002). Maternal antennal anxiety and children's behavioral/emotional problems at 4 years. *British Journal of Psychiatry,* 180, 502–508.

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

Phillips, N. K., Hammen, C. L., Brennan, P. A., Najman, J. N., & Bor, W. (2005). Early adversity and the prospective prediction of depressive and anxiety disorders in adolescents. *Journal of Abnormal Child Psychology,* 33, 13–24.

Pardon, M. C., Gérardin, P., Joubert, C., Pérez-Diaz, F., & Cohen-Salmon, C. (2000). Influence of prepartum chronic ultramild stress on maternal pup care behavior in mice. *Biological Psychiatry,* 47, 858–863.

Palermo-Neto, J., Massoco, C. O., & de Souza, W. R. (2003). Effects of physical and psychological stressors on behavior, macrophage activity, and Ehrlich tumor growth. *Brain, Behavior, and Immunity,* 17, 43–54.

Peirson, S. N., Butler, J. N., & Foster, R. G. (2003) Experimental validation of novel and conventional approaches to quantitative real-time PCR data. *Nucleic Acid Research,* 31, e73–79.

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

Reiser, S. J. (1985). Responsibility for personal health: a historical perspective. *The Journal of Medicine and Philosophy,* 10, 7–17.

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

Rimondini, R., Agren, G., Börjesson, S., Sommer, W., & Heilig, M. (2003). Persistent behavioral and autonomic supersensitivity to stress following prenatal stress exposure in rats. *Behavioural Brain Research,* 140, 75–80.

Rivier, U. K. (2001). The hypothalamic-pituitary-adrenal axis response to immune signals. In R. Ader, D.L. Felten, & N. Cohen (Eds.) *Psychoneuroimmunology* (3rd ed., pp. 633–648). New York: Academic Press.

Rivest, S. (2003). Molecular insights on the cerebral innate immune system. *Brain, Behavior, and Immunity,* 17, 13–19.

Schmidt, E. D., Janszen, A. W. J. W., Wouterlood, F. G., & Tilders, F. J. H. (1995). Interleukin-1–induced long-lasting changes in hypothalamic corticotropin-releasing hormone (CRH)–neurons and hyperresponsiveness of the hypothalamic–pituitary–adrenal axis. *The Journal of Neuroscience,* 15, 7417–7426.

Schurmeyer, T. H. & Wickings, J. E. (1999). Principles of endocrinology. In M. Schedlowski, & U. Tewes (Eds.), *Psychoneuroimmunology: an interdisciplinary introduction* (pp. 113–124). New York: Kluwer Academic/Plenum Publishers.

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature,* 138, 32.

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 rat denate gyrus. *Behavioural Brain Research,* 124, 47–54.

Sobrian, S. K., Vaughn, V. T., Bloch, E. F., & Burton, L. E. (1992). Influence of prenatal maternal stress on the immunocompetence of the offspring. *Pharmacology Biochemistry and Behavior,* 43, 537–547.

Sparkman, N. L., Martin, L. A., Calvert, W. S., & Boehm, G. W. (2004). Effects of intraperitoneal lipopolysaccharide on Morris maze performance in year-old and 2-month-old female C57BL/6J mice. *Behavioral Brain Res*earch, 159, 145–151.

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

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

Spath-Schwalbe, E., Hansen, K., Schimidt, F., Schrezenmeier, H., Marshall, L., Burger, K., Fehm, H., & 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.

Szuran, T. F., Pliška, V., Pokorny, J., & Welzl, H. (2000). Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance. *Physiology & Behavior,* 71, 353–362.

Takahashi, L. K., Baker, E. W., & Kalin, N. H. (1992). Ontogeny of behavioral and hormonal responses to stress in prenatally stressed male rat pups. *Physiology & Behavior,* 47, 357–364.

Tuchscherer, M., Kanitz, E., Otten, W., & Tuchscherer, A. (2002). Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. *Veterinary Immunology and Immunopathology,* 86, 195–203.

Uehara, A., Sekiya, C., Takasugi, Y., Namkik, M., & Arimura, A. (1989). Anorexia induced by interleukin 1: involvement of corticotropin-releasing factor. *American Journal of Physiology,* 257, R613–R617.

Valleé, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M., & Mayo, W. (1999). Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. *European Journal of Neuroscience,* 11, 2906–2916.

von Hertzen, L. C. (2002). Maternal stress and T cell differentiation of the developing immune system: possible implications for the development of asthma and atopy. *Journal of Allergy and Clinical Immunology,* 109, 923–928.

Yirmiya, R., Tio, D. L., & Taylor, A. N. (1996). Effects of fetal alcohol exposure on fever, sickness behavior, and pituitary-adrenal activation induced by interleukin-1β in young adult rats. *Brain, Behavior, and Immunity,* 10, 205–220.

Yirmiya, R., Winocur, G., & Goshen, I. (2002). Brain interleukin-1 is involved in spatial memory and passive avoidance conditioning. *Neurobiology of Learning and Mem*ory, 78, 379–389.

Ward, H. E., Johnson, E. A., Salm, A. K., & Birkle, D. L. (2000). Effects of prenatal stress on defensive withdrawal behavior and corticotropin releasing factor systems in rat brain. *Physiology & Behavior,* 70, 359–366.

Welberg, L. A. M., Seckl, J. R., & Holmes, M. C. (1995). Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behavior. *Neuroscience,* 104, 71–79.

Zarrow, M. X., Philpott, J. E., & Denenberg, V. H. (1970). Passage of ^{14}C-4-corticosterone from the rat mother to the fetus and neonate. *Nature*, 226, 1058–1059.

Zuckerman, S. H., Shellhaas, J., & Butler, L. D. (1989). Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. *European Journal of Immunology,* 19, 301–305.

ABSTRACT

INFLUENCE OF PRENATAL STRESS ON BEHAVIORAL, ENDOCRINE, AND CYTOKINE RESPONSES TO ADULT ENDOTOXIN EXPOSURE

By Rachel Kohman, MS, 2004
Department of Psychology
Texas Christian University

Dissertation Advisor: Dr. Gary W. Boehm, Assistant Professor of Psychology

Exposure to stress early in development can have lifelong effects on an organism's physiological and psychological health. Prior research suggests that prenatal stress exposure, among other effects, can lead to hyper-reactivity of the offspring's HPA axis and alterations in immune function. These stress-induced changes have been linked to a greater propensity to develop depression or an anxiety disorder in both human and non-human animals. Furthermore, prenatally stressed offspring have been found to be more susceptible to certain diseases, relative to non-stressed controls. The immune alterations induced by prenatal stress exposure may disrupt the normal communication between the immune system, endocrine system, and central nervous system, potentially making prenatally stressed individuals more vulnerable to the negative aspects of immune activation, namely cytokine-induced cognitive deficits and increased anxiety. The present study investigated whether prenatal stress exposure would exaggerate these detrimental effects of immune activation. Specifically, we hypothesized that prenatally stressed subjects would be hypersensitive to endotoxin administration and would therefore show exaggerated learning deficits, increased anxiety-like behavior, and increased peripheral and central interleukin-1β production. The observed results only partially supported our

hypotheses, as prenatally stressed subjects showed evidence, albeit modest, of increased anxiety-like behavior following endotoxin administration relative to non-stressed controls. However, the data failed to support the primary hypothesis that prenatally stressed subjects would show exaggerated cognitive deficits, engendered via enhanced peripheral and central IL-1β production, following immune activation. Collectively, these data suggest that while prenatal stress exposure may lead to increases in anxiety-like behavior following a subthreshold dose of endotoxin, it does not appear to produce greater susceptibility to LPS-induced cognitive decline or elevations in proinflammatory cytokine production.

VITA

Personal Background

Rachel Ann Kohman
Dayton, Minnesota
E-mail: r.a.kohman@tcu.edu

Education

Bachelor of Arts, Psychology, Augsburg College, Minneapolis MN, 2000
Master of Science, Psychology, Texas Christian University, Fort Worth Texas, December, 2004
Doctor of Philosophy, Psychology, Texas Christian University, Fort Worth Texas, August, 2007

Peer Reviewed Publications

Kohman, RA., Sparkman, NL., Paquet, A. & Boehm, GW. (2007). Attenuation of endotoxin-induced cognitive deficits by a selective non-peptide antagonist of corticotropin-releasing factor receptor. *Brain, Behavior, and Immunity,* 21, 824–835.

Kohman, RA., Tarr, AJ., Blyer, SL., Lincoln, LK., Boehm, GW (2007). Age affects sensitivity to LPS-induced decrements in shuttlebox learning. *Physiology & Behavior,* in press.

Kohman, RA., Leising, K., Shaffer, M., Higa, JJ. (2006). Effects of Breaks in the Interval Cycle on Temporal Tracking in Pigeons. *Behavioural Processes*, 71; 126–134.

Sparkman, NL., **Kohman, RA.**, Garcia, AK., & Boehm, GW. (2005). Peripheral immune activation alters two-way active avoidance conditioning in male C5BL/6J mice. *Physiology & Behavior* 85; 278–288.

Sparkman, NL., **Kohman, RA.**, Scott, VJ. & Boehm, GW. (2005). Lipopolysaccharide induces behavioral alterations in two water maze test variants. *Physiology & Behavior*, 86; 244–251.

Teaching Experience

Lab Instructor- Texas Christian University, courses: Learning Lab (Spring 2007)
Lab Instructor- Texas Christian University, courses: Principles of Behavior Lab (Fall 2002, 2005, and 2006)
Teaching Assistant- Texas Christian University, courses: Physiology (Fall 2003 and 2004)