DEFICITS IN MEMORY CONSOLIDATION INDUCED BY BACTERIAL ENDOTOXIN IN MICE COINCIDE WITH ELEVATED CYTOKINE AND CHEMOKINE LEVELS, BUT DIMINISHED EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR

by

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INTRODUCTION

Neural-immune interactions

A considerable amount of evidence indicates that the immune system and the central nervous system (CNS) operate in concert to exert their effects on the body (Dantzer & Kelley, 2007; Maier et al., 1994; Wilson et al., 2002). The classical view that the brain is isolated from the immune system, except during the state of disease or trauma, is now outdated, and the blood brain barrier (BBB) was considered impervious to immune mediators produced in the periphery (Schultzberg, 2007). Today, it is well established that the CNS and the immune system influence one another through a multifaceted, generally adaptive, bidirectional interaction (Exton et al., 2001; Quan & Banks, 2007). As but a single example, during infection, neural-immune interaction promotes prolonged sleep/rest periods to allow for revitalization of the organism (Fang, 1998).

The CNS regulates the immune system through both the hormonal and neural pathways. An example of hormonal regulation of the immune system by the CNS is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, and the subsequent release of glucocorticoids from the adrenal cortex (Elenkov & Chrousos, 1999). Glucocorticoids inhibit the production of pro-inflammatory cytokines, but upregulate the production of anti-inflammatory cytokines (Elenkov & Chrousos, 1999). On the other hand, the activation of the autonomic nervous system (ANS), via subsequent release of catecholamines (e.g., dopamine, epinephrine, norepinephrine), which, acting on the immune organs such as the thymus, bone marrow, spleen, and lymph nodes, generally inhibit the production of pro-inflammatory mediators, is considered a neural pathway

(Eskandari et al., 2003). Another distinct neural pathway involves opioid-induced suppression of the immune response, acting through the desensitization of chemokine receptors on neutrophils, monocytes, and lymphocytes (Rogers et al., 2000).

Moreover, the immune system helps coordinate portions of the CNS activity. For example, behavioral signs of sickness (e.g., malaise, fatigue, reduced appetite), following peripheral bacterial infection, are due to the central action of soluble proteins called cytokines, secreted primarily by macrophages in the periphery and microglia in the CNS in response to a pathogen-associated molecular patterns (Eskandari et al., 2003).

Cytokines

Cytokines are immunomodulating agents released by resident macrophages in the periphery following contact with a pathogen. For example, following bacterial infection, pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are released (Maier & Watkins, 1998). These proteins regulate a cascade of changes in the organism and are also important in the specific defense against infection and injury (Kent et al., 1992; Maier & Watkins, 1998). In addition, cytokines are important mediators of maladaptive chronic inflammation, as they may trigger a subsequent release of other pro-inflammatory cytokines (Maier & Watkins, 1998).

As previously stated, cytokines are important modulating factors in the communication between the immune system and the CNS. There are two major ways through which cytokines influence the brain. First is the neuronal pathway in which cytokines act on peripheral sensory afferents that project to the brain, such as found in the vagus nerve (Eskandari et al., 2003). Second are the humoral pathways, in which cytokines, after stimulating the modification of hormone release in the blood by peripheral tissues, such as stomach or adipose tissue, directly

affect the brain activity at the level of the circumventricular organs (CVOs), or can enter the brain directly through active transport across the BBB (Konsman et al., 2002).

Following entry into the CNS, cytokine production can lead both to behavioral and biological changes (Konsman et al, 2002). Behavioral alterations coincide with the expression of pro-inflammatory cytokines in the brain and are thought to be an organism's way of coping with illness (Dantzer, 1989; Maier & Watkins, 1998). Examples of these, so-called "sickness behaviors", are adipsia (i.e., decreased drinking), anhedonia (i.e., inability to experience pleasure), mood alterations, altered pain threshold, decreased sexual vigor, and diminished social exploration (Konsman et al., 2002). Additionally, various pro-inflammatory cytokines have been shown to modulate feeding (Kent et al., 1996; Plata-Salaman, 1999, 2001; Luheshi et al., 1999) and alter sleep regulation (Borbély et al., 1989; Opp et al., 1991; Krueger, 2008). All of these adaptations aid rest and recovery. Another way for the body to defend itself against an infection is through the fever response (Dinarello, 1999; Netea et al., 2000). This is helpful, and fever assists the organism's immune response, as bacteria and viruses often cannot replicate efficiently at increased temperatures (Maier & Watkins, 1998).

However, along with the expression of this large set of adaptive behavioral and neural responses, emerge immune system-related, seemingly non-adaptive events, including cognitive alterations (Barrientos et al., 2002, 2009; Baier et al., 2009; Bilbo et al., 2009; Hein et al., 2007; Kohman et al., 2007a,b; Koo & Duman, 2008; Pugh et al., 1998, 1999; Shaw et al., 2001; Sparkman et al., 2005a,b). More specifically, there is considerable amount of evidence to support the hypothesis that pro-inflammatory cytokines may adversely affect learning and memory processes, such as acquisition and consolidation (Barrientos et al., 2002, 2004, 2009; Cibelli et al., 2010; Cunningham & Sanderson, 2008; Moore et al., 2009).

Interleukin-1β

Several effects of IL-1β on CNS function have been described, but as mentioned previously, one area of the brain in which striking changes have been reported is the hippocampus. Among these changes is IL-1β-induced inhibition of long-term potentiation (LTP) in perforant path-granule cell synapses (Vereker et al., 2000). Increased levels of IL-1β interfere with LTP, a neurobiological phenomenon believed to be an electrophysiological correlate of learning and memory (O'Connor, 1999). LTP is described as repeated activation of a synaptic pathway that leads to a persistent increase in synaptic efficacy (Normann & Berger, 2008). Although LTP remains somewhat controversial as a model for learning, learning correlates with increased LTP in the hippocampus, and learning can be impaired by inhibition of LTP in the hippocampus (Whitlock et al., 2006). Further, support for the role of IL-1β in LTP comes from the findings that the anti-inflammatory cytokine, IL-10, antagonizes the IL-1β-induced inhibition of LTP (Kelley et al., 2001). Kelley et al. (2001) suggest that this is likely a consequence of the ability of IL-10 to induce shedding of the IL-1 type 1 receptor. Likewise, it was previously reported that the injection of IL-10 reduces LPS (lipopolysaccharide)-induced fever (Conti, 2004) as well as the behavioral effects induced by LPS administration (Krzysztone et al., 2008).

IL-1β is present at low levels in the healthy brain and apparently exerts beneficial effects (Yirmiya et al., 2002). Contrary to the previously described negative effects of IL-1β on the induction of LTP, Yirmiya et al. (2002) found that the blockade of IL-1 receptors with IL-1 receptor antagonist (IL-1ra) produced memory deficits in the passive avoidance test and in the spatial, but not the non-spatial, version of the Morris water maze test. Furthermore, Goshen et al. (2001) reported that mice with a genetic deficit in IL-1 signaling show signs of profound memory disturbances, thus, supporting the hypothesis that IL-1 signaling is involved in normal memory function. These findings seemingly contradict previous reports that indicate that IL-1β

has a damaging effect on memory (Gibertini et al., 1995; Pugh et al., 2001), but this difference is likely explained by the doses of IL-1β administered among the experiments. Indeed, Yirmiya et al. (2002) suggest that the basal physiological levels of IL-1 are essential for memory, but that higher doses can be damaging.

Lipopolysaccharide and cytokine/chemokine production

All species have the ability to recognize the presence of infection and to instantaneously take action by generating innate immune responses (Rivest, 2003). Following systemic bacterial endotoxin (i.e., LPS) administration, a wide variety of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, are released from macrophages in the periphery and microglia in the CNS (Borowski et al., 1998). LPS is a component of the outer membrane of the cell wall of Gram-negative bacteria and is frequently used to model bacterial infection in experimental animals (Bluthe et al, 1992). Exploring the mechanisms of cytokine production and action in the periphery and in the brain, after a peripheral immune challenge, has often been conducted using intraperitoneal (i.p.) administration of LPS in rats and mice (Holden et al., 2004, 2008; Gahtan & Overmier, 2001; Kohman et al., 2007a,b; Neveu & Liege, 2000; Pugh et al., 1998; Sparkman et al., 2005a,b). Once present in the organism, LPS acts through Toll-like receptor 4 (TLR4), and immune cells respond to LPS by releasing cytokines, as well as nitric oxide and chemotactic cytokines (i.e., chemokines), such as monocyte chemoattractant protein-1 (MCP-1; also called CCL2) and macrophage inflammatory protein-1α (MIP-1α; also called CCL3; Borowski et al., 1998; Gourmala et al., 1997, 1999; Larson & Dunn, 2001; Thompson et al., 2008).

Cytokine levels generally peak around 4 h and following peripheral administration of LPS, for the most part, return to baseline roughly 24 h later in young adult rats (Richwine et al., 2008). For the duration of this period, cytokines acting in the brain may lead to

underperformance in a wide range of tasks designed to assess learning and memory in rodents, such as contextual fear conditioning, Morris water maze, two-way active avoidance, passive avoidance, and novel object recognition (Gahtan & Overmier, 2001; Holden et al., 2004, 2008; Jacewicz et al., 2005; Kohman et al., 2007a,b; Pugh et al., 1998, 1999; Sparkman et al., 2005a,b). Further, it is worth noting that the cognitive and the behavioral alterations, observed in animals given LPS or pro-inflammatory cytokines such as IL-1β, are strikingly similar (Barrientos et al., 2002).

In addition to the neurobiological effects of systemic inflammation mentioned above, there are many other functional consequences of peripheral LPS administration on host immune defense mechanisms, including an increase in serum and brain pro-inflammatory cytokines (i.e., IL-1β, IL-6, TNF-α; Godbout et al., 2005; Richwine et al., 2008; Schletter et al., 1995), and chemokines, such as MCP-1and MIP-1α (Gourmala et al., 1997, 1999; Thompson et al., 2008). The literature suggests that these chemokines are necessary in cell recruitment and trafficking during inflammation (Rossi & Zlotnik, 2000), are involved in regulating leukocyte movement into the CNS during pathology (Cardona et al., 2008), and may also affect BBB permeability (Stamatovic et al., 2005). More specifically, Thompson et al. (2008) reported that MCP-$1^{-/-}$ mice injected with LPS exhibited higher levels of serum IL-1β and TNF-α compared to LPS-treated MCP-$1^{+/+}$ mice, but, in contrast, MCP-$1^{-/-}$ mice showed significantly lower central expression of pro-inflammatory cytokines and chemokines, and fewer activated microglia. These data indicate a vital role for MCP-1 in regulation of brain inflammation after peripheral LPS administration.

Role of immune activation and cytokine activity in learning and memory processes

There are two distinct parts of any standard learning paradigm, and they are acquisition and retention testing. Pharmacological and neurobiological interventions, either before or after

the acquisition session, can potentially affect a range of sensory, perceptual, attentional, motivational, and/or motor performance factors, in addition to the actual learning and memory processes (Cunningham & Sanderson, 2008). However, when a pre-training manipulation engenders an effect on retention processes, this generally indicates a specific effect on learning- or memory-related processes such as encoding or memory consolidation, as opposed to a non-specific effect on some performance factor, such as changes in attention.

Consolidation is the process by which a short-term memory trace is made more stable and permanent, leading to long-term memory storage (Dudai, 2004; Robbins & Murphy, 2006). The manipulations performed post-acquisition trial cannot affect simple performance factors during the acquisition trial, but can affect memory consolidation, as indicated by performance in the retention test, typically 1–7 days later (Robbins & Murphy, 2006). Also, the memory retrieval processes are potentially affected by the post-trial manipulation (Robbins & Murphy, 2006).

Moreover, many inflammatory diseases and infections are often associated with disruptions in cognitive function (Ader & Kelley, 2007; Perry, 2005). Recent research showed that disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), HIV-related dementia, prion diseases, and depression may be linked with the over-expression of pro-inflammatory cytokines (Ader & Kelley, 2007; Perry, 2005). Pro-inflammatory cytokines are present at low levels in the healthy brain and may exert positive effects (Yirmiya, 2000), but are most notable in the neuroscience literature for their negative effects (Maier, 1994). Indeed, cytokine therapy is used to treat hepatitis C, various cancers, and human immunodeficiency virus (HIV), but the side effect of this kind of therapy, specifically therapy with IL-6 or IL-2, is major depression (Anisman & Merali, 2003).

Brain-derived neurotrophic factor expression and immune activation

Brain-derived neurotrophic factor (BDNF) appears to be involved in synaptic plasticity and there is an interest in its role in learning and memory processes (Binder, 2004; Lipsky & Marini, 2007; Lista & Sorrentino, 2010). Furthermore, Korte (1995) showed that hippocampal LTP is impaired in mice lacking BDNF, and Minichiello (2009) demonstrated that electrical stimuli that induce LTP in the hippocampus simultaneously increase hippocampal BDNF expression. The hippocampus, which is required for some types of long-term memory formation, appears to be an important site of BDNF action (Binder, 2004). Alonso (2002) reported that BDNF is required for both short- and long-term memory formation of inhibitory avoidance learning. It is also worth noting that Hall (2000) reported increased hippocampal BDNF expression during contextual fear conditioning.

Previous research suggests a link between the peripheral immune activation-associated cognitive deficits and alterations in BDNF levels in the hippocampus (Barrientos et al., 2004; Bilbo et al., 2008; Guan & Fang, 2006; Schnydrig et al., 2007). Moreover, Schnydrig (2007) reported that peripheral inflammation (LPS) leads to a reduction of trophic supply in the brain, including BDNF, at synaptic sites. More specifically, Guan & Fang (2006) reported that BDNF protein levels in the hippocampus were significantly reduced 7 h after the highest dose (1 mg/kg, i.p.), but not after the lower dose (0.3 mg/kg, i.p.) of LPS. Additionally, Richwine et al. (2008) found that hippocampal BDNF mRNA levels measured 4, 24, 48, and 72 h post-LPS, while reduced, were not significantly different between timepoints.

Contextual fear conditioning

Fear conditioning is a form of learning in which mild foot shock is associated with an initially neutral environment or context (e.g., the conditioning chamber), or neutral stimulus

(e.g., the light). This is done by pairing the neutral stimulus, or the context, with an aversive stimulus (e.g., the shock). Eventually, the neutral stimulus, or the context, alone can elicit the state of fear (Phillips & LeDoux, 1992). In the parlance of classical conditioning, the neutral discrete stimulus is the "conditioned stimulus" (CS), the aversive stimulus is the "unconditioned stimulus" (US), and the freezing induced by the CS is the "conditioned response" (CR). Fear conditioning has been studied in numerous species, including *Drosophila*, snails, mice, rats, and humans (Maren, 2008). In humans, conditioned fear is often measured with verbal report, galvanic skin response, and potentiated startle (Maren, 2008). In rodents, however, conditioned fear is often measured by their learned fear-induced freezing response, which is formally defined as a cessation of all bodily movement, aside from respiration (Blanchard, 2001).

Most importantly, for this set of studies, it has been shown that both, LPS and IL-1β, impair contextual fear conditioning. For example, Pugh et al. (1998) showed that LPS administered peripherally, in rats, inhibits contextual fear conditioning, but has no effect on auditory-cue fear conditioning. This might be the case because contextual fear conditioning involves both amygdala and the hippocampus (Antoniadis & McDonald, 2000; Barrientos et al., 2002; Celerier et al., 2004; Fanselow, 2000), whereas auditory fear conditioning is dependent primarily on the amygdala (Bailey et al., 1999; Lamprecht et al., 2009). Further, the data of Pugh et al. (1998) show that the effects of LPS were dose-dependent; doses of 125 and 250 μg/kg disrupted contextual fear memory consolidation processes, whereas the highest dose (500 μg/kg) did not have an effect on freezing behavior during testing. One possible explanation, given by the authors, is that the higher doses of LPS stimulate prolonged release of corticosterone that may last longer into the period of memory consolidation. This is important considering that it has been shown that corticosterone may enhance performance in a variety of learning and memory paradigms, including fear conditioning (Bowman et al., 2001; Cordero et al., 2003; Luine, 1994).

As the LPS-treated animals moved more than the saline-treated controls during testing, we can discern that LPS injections did not cause motor impairments. Also, since LPS was administered following the training session, it is reasonable to conclude that LPS impaired memory consolidation processes of the context and foot-shock associations.

Central hypotheses

The current set of experiments sought to further elucidate the effects of the systemic LPS challenge on the expression of serum cytokines and chemokines (i.e., IL-1β, IL-6, TNF-α, MCP-1, and MIP-1α), as well as brain IL-β mRNA and BDNF mRNA, and their downstream effects on learning and memory in a contextual fear conditioning paradigm. Unlike previous studies in the neural-immune literature, we first set out to ascertain that freezing was under selective contextual control, and was not due to non-associative factors such as sensitization or pseudoconditioning. Further, we hypothesized that mice that received LPS (250 μg/kg) immediately or 2 h after the training session (Day 1) would show a significant decrease in the percentage of time spent freezing on the test day, approximately 48 h later (Day 3), as compared to the groups of animals that received either saline or LPS 12 h after training. Lastly, we hypothesized that animals that received LPS would show an increase in both peripheral (i.e., IL-1β, IL-6, TNF-α, MCP-1, and MIP-1α) and central (i.e., IL-1β) expression of cytokines and chemokines, as well as diminished BDNF mRNA expression in the hippocampus and the cerebral cortex.

METHODS

Experimental subjects

Subjects used in all experiments were experimentally naïve 4–6 month-old male C57BL/6J mice bred in the Texas Christian University vivarium from a breeding stock obtained from the Jackson Laboratory (Bar Harbor, ME). Animals were housed in groups of 3–4 in standard polycarbonate mouse cages (30 x 20 x 16 cm), and allowed access to food and water *ad libitum*. Lights were set to an automated 0700 on and 1900 off light-dark cycle. All animals received care consistent with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), and the experiments were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Texas Christian University.

Treatment conditions

Animals were randomly divided within each cage into treatment groups outlined for each of the four experiments (Table 1). Intraperitoneal (i.p.) injections of LPS (*Escherichia coli* serotype 0111:B4; Sigma, St. Louis, MO) were given at doses of either 0 or 250 µg/kg in sterile, pyrogen-free 0.9% saline (Baxter, Deerfield, IL; LPS was not used in Experiment 1). In Experiments 2, 3, 4a, and 4b, control animals received volume-equivalent injections of sterile saline at the same time(s) to rule out effects based upon the stress of the injection procedure itself. We used LPS at the dose of 250 µg/kg because prior work has shown that this dose reliably induces sickness behavior in the C57BL/6J mice, and is one of the doses most commonly used by other laboratories for studies involving behavioral measures in LPS-treated rodents (Gahtan & Overmier, 2001; Kohman et al., 2007a,b; Konsman et al., 2000; Pugh et al., 1998; Sparkman et al., 2005a,b). The experiment was designed to assess the effects of peripheral

LPS administration on contextual fear memory consolidation. In the second experiment the injections were administered immediately after training, whereas in the third experiment injections were administered immediately, 2 h, and 12 h after training. All mice were visually examined for signs of sickness behavior and weighed daily. Table 1 shows only the main differences in experimental procedures and conditions between experiments.

Table 1. Outline of experiments

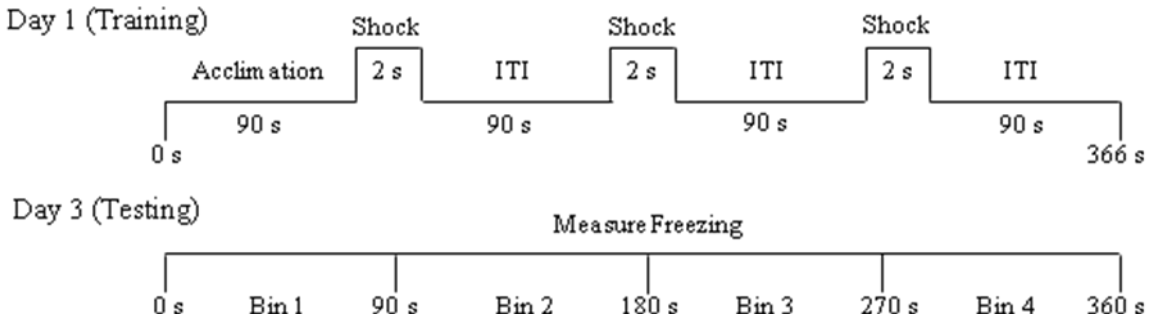
Experiment	*N*	Number of groups	Dose(s) LPS (µg/kg)	Brain tissue/ Serum Collection Time(s)	Timing of injection(s)	Context Shift
1	24	2	n/a	n/a	n/a	Yes
2	56	4	0, 250	n/a	Immediately after training (Day 1)	Yes
3	35	4	0, 250	n/a	Immediately, 2 h, and 12 h after training (Day 1)	No
4a/4b	40	4	0, 250	4 h and 48 h after injection	Immediately after training (Day 1)	Yes

Behavioral testing apparatus

Two fully automated Freeze Monitor System units (San Diego Instruments, San Diego, CA) were used to assess conditioned contextual fear learning. Each of the units has an electrified grid floor (bars 0.3 cm apart), through which an electric shock (0.7 mA) is delivered. At the floor level in each of the units (26.5 x 26.5 x 17.8 cm) are infrared photo beams that detect the movement of the animal. A 24 VCD light bulb was mounted on the ceiling of the units. Each of the units was connected with the Freeze Monitor System software (San Diego Instruments, San Diego, CA) that recorded and analyzed freezing behavior. Movement of the animal was recorded continuously and freezing was defined as no interruptions of the photo beam for at least 2 s at a

time. Between individual testing sessions, the apparatus were cleaned with Odormute™ (Ryter Corp., Madelia, MN). The light levels within the outer room were minimized to make the context more salient. To limit noise transfer, 1 inch acoustic foam (Auralex Acoustics, Indianapolis, IN) was placed around each chamber and a white noise generator was used throughout testing/acclimation (GoldLine, West Redding, CT). The addition of olfactory cues (i.e., context A; corn cob bedding odor/striped pattern walls, and context B; peppermint odor/dotted pattern walls) increased freezing behavior, compared to the visual cues alone. Peppermint oil (Now Foods®, Bloomingdale, IL) was mixed with water (1:10 ratio), and placed underneath the electrified grid floor in a plastic container for the duration of the session. The mixture was replaced after every session.

Figure 1. Contextual Fear Conditioning Protocol (Experiments 1−4).



Experiment 1: Context discrimination

The first experiment was designed to demonstrate selective contextual control of conditioned freezing behavior under conditions similar to those to be used in subsequent experiments, and our study is the first in the pro-inflammatory cytokine literature to include such an experiment. The design for Experiment 1 consisted of two between-subjects factors (i.e., Training Context [A or B], and Testing Context [A or B]), for a total of four treatment groups

(Table 2). Two different sets of cues were used in order to show that the animals were able to distinguish between different contexts. Chamber A had a corn cob bedding odor and striped pattern walls, while chamber B had a peppermint odor and dotted pattern walls. On Day 1 (i.e., training), two groups of animals were placed either into context A or context B and were given a 90 s acclimation period. The light in the chamber was on for the duration of the experiment. After the initial 90 s, a 2-s 0.7 mA shock was delivered. A 90 s inter-trial interval (ITI) period followed. In addition, the shock was delivered for the second time at 182 s, and for the last time at 274 s (Figure 1). Freezing behavior was measured for 90 s before the first shock was administered in order to account for individual baseline differences ($p > 0.05$, *ns*, data not shown). The total time spent in the chamber for one animal per session on Day 1 was 366 s. The testing phase started approximately 48 h after the training session was completed. On Day 3 (i.e., testing), the shock was not administered at any time during the session. Half of the animals from each of the two original treatment groups were tested in the context that was either the same as on Day 1 (A:A or B:B) or in the different context (A:B or B:A). On Day 3, the session was 360 s in length and the light was on during the entire experiment.

Table 2. Experimental design of Experiment 1.

Context Discrimination N=24	Group	Condition	Context	Day 1	Day 2	Day 3
n = 6	#1	Saline	SAME	A:+	/	A:?
n = 6	#2	Saline	DIFF.	A:+	/	B:?
n = 6	#3	Saline	DIFF.	B:+	/	A:?
n = 6	#4	Saline	SAME	B:+	/	B:?

Note: A = Corn cob bedding odor/Striped pattern walls; B = Peppermint odor/Doted pattern walls; (+) = 0.7 mA shock; (?) = test

Experiment 2: Contextual fear memory consolidation and LPS

After we had determined that conditioned freezing was under selective contextual control, the next step was to determine the effects of peripheral LPS administration on fear memory consolidation, as measured by the subjects' freezing response. We hypothesized that i.p. administration of LPS (250 µg/kg) immediately after Day 1 (i.e., training), would impair conditioned contextual fear memory consolidation in our experimental subjects. The design of the second experiment consisted of two between-subject factors (i.e., Context Shift [Same or Different], and LPS Treatment [0 or 250 µg/kg]), for a total of four treatment conditions (Table 3). The experimental procedure was the same as in Experiment 1 (Figure 1), with the exception that i.p. injections of either LPS or saline were given immediately after training on Day 1.

Table 3. Experimental design of Experiment 2.

Contextual Fear Conditioning N=56	Group	Condition	Context	Day 1	Day 2	Day 3
n = 7	#1	LPS	SAME	A:+	/	A:?
n = 7	#2	Saline	SAME	A:+	/	A:?
n = 7	#3	LPS	DIFF.	A:+	/	B:?
n = 7	#4	Saline	DIFF.	A:+	/	B:?
n = 7	#5	LPS	DIFF.	B:+	/	A:?
n = 7	#6	Saline	DIFF.	B:+	/	A:?
n = 7	#7	LPS	SAME	B:+	/	B:?
n = 7	#8	Saline	SAME	B:+	/	B:?

Note: A = Corn cob bedding odor/Striped pattern walls; B = Peppermint odor/Doted pattern walls; (+) = 0.7 mA shock; (?) = test

Experiment 3: Immediate and delayed administration of LPS

To study the time course of LPS-induced memory consolidation impairments, LPS was administered either immediately, 2 h, or 12 h post-training. We hypothesized that LPS administered immediately or 2 h post-training would impair conditioned contextual fear memory

consolidation in our experimental subjects. Although subjects were able to adequately discriminate between both contexts in Experiments 1 and 2, mice re-exposed to the peppermint odor context (context B), while not significantly, exhibited more freezing, both initially and throughout testing, when compared to the animals re-exposed to the corn cob bedding odor context (Context A; $p > 0.05$, data not shown). Therefore, in this experiment, all groups of animals were placed in the same context (i.e., conditioning context B) on the training day (Day 1) and testing day (Day 3) of the fear conditioning procedure. Injections of LPS (250 µg/kg, i.p.) were given either immediately [(LPS (0)], 2 h [(LPS (2)], or 12 h [(LPS (12)] after training on Day 1 (Table 4). The experimental procedure was as previously described (Experiment 1 and Figure 1), except that immediately after the end of the session, animals were injected with either saline (Saline, LPS (2), and LPS (12) groups), or with LPS (LPS (0) group), and were placed back into their respective home cages. Two hours after the acquisition training, Saline, LPS (0) and LPS (12) groups received saline, while the LPS (2) group received LPS. Again, 12 h after the acquisition training, the Saline, LPS (0) and LPS (2) groups received a saline injection, while the LPS (12) group received LPS. This procedure ensured that all animals received the same number of injections in the same temporal pattern. Animals were then given approximately 36 h (i.e., 48 h since the first injection) before the testing phase started on Day 3.

Table 4. Experimental design of Experiment 3.

Contextual Fear Conditioning N = 35	Group	Context	Condition	Day 1	Day 2	Day 3
n = 9	#1	SAME	Saline	B:+	/	B:?
n = 9	#2	SAME	LPS (0 h)	B:+	/	B:?
n = 9	#3	SAME	LPS (2 h)	B:+	/	B:?
n = 8	#4	SAME	LPS (12 h)	B:+	/	B:?

Note: B = Peppermint odor/Doted pattern walls; (+) = 0.7 mA shock; (?) = test

Experiment 4a: Quantitative real-time reverse transcription polymerase chain reaction

For Experiment 4a, two groups of animals were placed either into context A or context B (Table 5). On Day 1, the training session was the same and was previously described (Experiment 1). LPS (250 µg/kg) injections were administered immediately after training. Four and forty-eight h after the injection, subjects were rapidly euthanized via CO_2 inhalation, and, using an RNase-free sample corer, brain tissue punches were obtained. Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was used to assess the levels of IL-1β and BDNF mRNA present in the hippocampus and the parietal cortex at the time of tissue harvesting using a 7500 Real-Time PCR Thermal Cycling System (Applied Biosystems, Foster City, CA). The samples were immersed in a nuclease-free tube containing RNAlater® (Ambion, Austin, TX). The RNA was first isolated (RNeasy Micro kits, Qiagen, Valencia, CA), and measured utilizing a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, NanoDrop products, Wilmington, DE). The amount of 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 and BDNF cDNA, and this amount was compared to levels for an endogenous control gene, β-actin. Samples were run in triplicate, and the relative amount present from the raw fluorescence data was then compared across the treatment conditions, using the DART-PCR method of quantification (Pierson et al., 2003).

Experiment 4b: Cytometric bead array

The animals used in Experiment 4a were also utilized in this experiment. Four and forty-eight hours after the injection of LPS, subjects were rapidly euthanized as described above, whole blood was collected, and serum was frozen at –80 ºC until use. The concentrations of IL-1β, TNF-α, IL-6, MCP-1, and MIP-1α in the serum were quantified using a cytometric bead

array (CBA). The cytokine standard solutions included with the BD™ CBA Flex Sets (BD Biosciences, San Jose, CA) were serially diluted to the following concentrations: 2,560 pg/ml, 1,280 pg/ml, 640 pg/ml, 320 pg/ml, 160 pg/ml, 80 pg/ml, 40 pg/ml, 20 pg/ml, and 0 pg/ml. The upper limit of detection was set by the upper limit of the standard curve, which was preset by the manufacturer at 2,560 pg/ml, while the theoretical lower limit of detection, also preset by the manufacturer was 1.9 pg/ml (IL-1β), 2.8 pg/ml (IL-6), 2.5 pg/ml (TNF-α), 2.7 pg/ml (MCP-1), and 2.3 pg/ml (MIP-1α). Further, a total of 20 µl of serum was mixed with 60 µl of capture beads and 50 µl of PE-labeled detection reagent. After a two hour incubation period, samples were washed and resuspended in 300 µl of wash buffer. Results were obtained using BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and BD CellQuest software (BD Biosciences), and analyzed with FCAP Array Software (Soft Flow, Inc., New Brighton, MN).

Table 5. Experimental design of Experiments 4a/b.

qRT-PCR/CBA N=40	LPS (250 µg/kg)	Saline
Peppermint (Context B) Time point 1	n=5	n=5
Ø Peppermint (Context A) Time point 1	n=5	n=5
Peppermint (Context B) Time point 2	n=5	n=5
Ø Peppermint (Context A) Time point 2	n=5	n=5

Statistical analyses

Data obtained from the behavioral testing and weights were analyzed using mixed-factor ANOVAs, with Treatment [LPS or Saline; Experiment 2 & 3] and Context Shift [Same or Different; Experiment 1, 2, & 4a/b] as the between-subject's variables, and Bin [Bins 1-4; Experiment 1, 2, & 3] as the within-subjects (i.e., repeated-measures) variable. Data were analyzed using StatView 5.0.1 software (SAS Institute Inc., Cary, NC). Central gene expression

was calculated by normalizing the amplification efficiency of the respective gene (i.e., IL-1β and BDNF) expression against the amplification efficiency of β-actin (the endogenous control gene), that had equal expression across treatment conditions, using the DART-PCR method of quantification (Experiment 4a). Standard factorial ANOVAs were then used to compare differences in the respective gene expression levels between treatment conditions. Cytometric Bead Array (CBA) data (Experiment 4b) were analyzed using ANOVA with Treatment [LPS vs. Saline], Timepoint [4 h vs. 48 h], and Context [A vs. B] as the between-subject variables, performed using StatView 5.0.1 software (SAS Institute Inc., Cary, NC). All data were expressed in figures as the mean ±SEM. The alpha level used for all statistical analyses was 0.05, and only significant omnibus effects were followed by Fisher's PLSD post hoc tests.

RESULTS

General appearance and weight loss

All mice were visually inspected for signs of sickness behavior and weighed daily during a three day procedure. Mice given LPS exhibited classic signs of sickness behavior, including decreased locomotion, hunched posture, and piloerection. Additionally, mice given LPS showed minimal, but statistically significant, weight loss. For the second experiment, repeated-measures ANOVA (Treatment [Saline vs. LPS] x Time [Day 1 vs. Day 2 vs. Day 3]) revealed a significant effect for Treatment, F (1,48) = 16.836, $p < 0.001$, a significant effect for Time, F (2,96) = 26.297, $p < 0.0001$, and a significant Treatment x Time interaction, F (2,96) = 8.855, $p < 0.001$ (n = 8−10; graph not shown).

Experiment 1: Context discrimination

The purpose of Experiment 1 was to determine whether fear conditioning is selectively controlled by the training context and not via sensitization or some other non-associative learning process. On Day 1 animals were exposed to one of two contexts. Approximately 48 h later (Day 3), half of the animals trained in context A were tested in the same context and the rest were tested in a different context (i.e., context B). Similarly, half the animals trained in context B were tested in the same context and the rest were tested in context A. Thus, two groups were trained and tested in the same context (A:A and B:B) and two were trained in one context and tested in the other (A:B and B:A). As there were no significant differences in freezing during testing between the A:A and B:B (i.e., Same Context), or between the A:B and B:A (i.e., Different Context) groups, the data were collapsed across context shift for further analysis ($p > 0.05$; data not shown). Repeated-measures ANOVA (Context Shift x Bin) revealed a significant effect for Context Shift, $F(1,22) = 13.363$, $p < 0.01$, a significant effect for Bin, $F(1,22) = 12.325$, $p < 0.0001$, and a significant Bin x Context Shift interaction, $F(3,66) = 7.041$, $p < 0.0005$. Post-hoc analysis revealed that the Same Context group was significantly different from the Different Context group during the initial 270 s of the testing session ($p < 0.05$). Further, as hypothesized, the percentage of time spent freezing decreased throughout the session in the group Same Context, because no shock was administered during this time (i.e., extinction of fear; Figure 2).

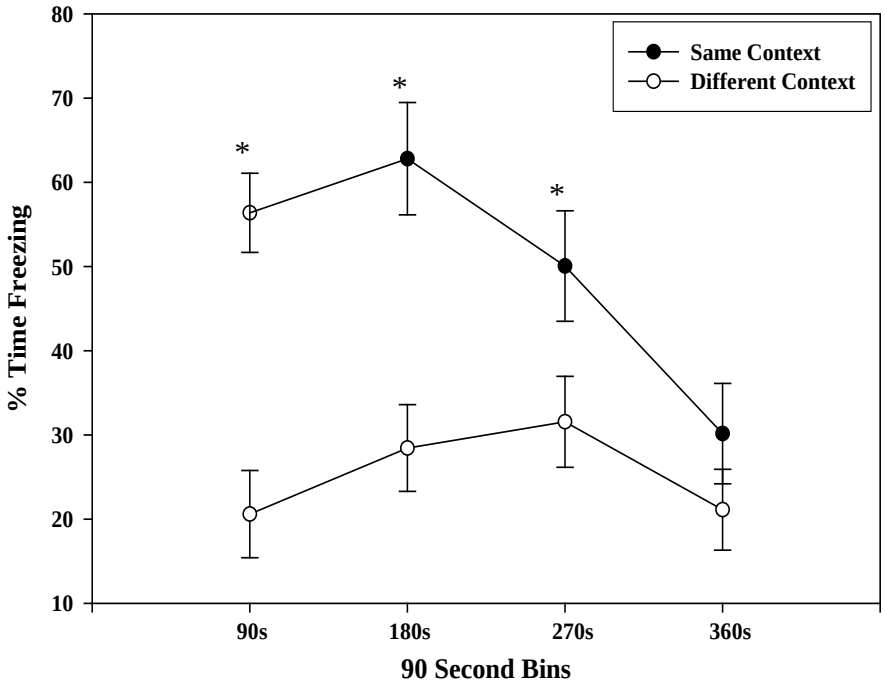


Figure 2. The effects of context shift on percent time freezing during the contextual fear test (i.e., Day 3). C57BL/6J mice placed in the same context on both Day 1 (i.e., training) and Day 3 (i.e., testing) showed significantly more freezing on Day 3 than the group placed in a different context on Day 1 and Day 3. Levels of freezing in the group Same Context decreased over the session (i.e., extinction). ***** indicates $p < 0.05$ when group Same Context is compared to group Different Context, n = 12. Symbols represent mean ±SEM.

Experiment 2: Contextual fear memory consolidation and LPS

The purpose of Experiment 2 was to determine whether peripheral LPS, administered immediately after the training session (i.e., Day 1), would disrupt memory consolidation. There were no significant differences in freezing between animals exposed to the same context on training and testing days, regardless of LPS treatment, and the groups were collapsed for further analysis. The same was true for animals exposed to a context shift. Repeated-measures ANOVA (Context Shift x Treatment x Bin) revealed a significant main effect for Context Shift, F (3,48) = 30.008, $p < 0.0001$, a significant main effect for Treatment, F (1,48) = 14.278, $p < 0.0005$, a significant Context Shift x Treatment interaction, F (9,144) = 8.961, $p < 0.005$, a significant

main effect for Bin, F (3,144) = 14.781, $p < 0.0001$, and a significant Bin x Context Shift interaction, F (9,144) = 12.491, $p < 0.0001$. Neither the Treatment x Bin interaction nor the Context Shift x Treatment x Bin interaction were significant.

Post-hoc analysis revealed that mice that were administered saline, and were placed in the same context on both days (i.e., group Same/Saline) froze significantly more, during the initial 180 s of the testing session, than did mice that were administered LPS and placed in either the same (i.e., group Same/LPS), or a different context (i.e., group Different/ LPS), or mice that received saline and were placed in a different context (i.e., group Different/Saline; Figure 3).

These results indicate that the animals that were given LPS immediately after the conditioning procedure, and were placed in the same context on both Day 1 (i.e., training) and Day 3 (i.e., testing), show impairment of fear memory consolidation. Additionally, control animals showed fear memory extinction, as the percent time spent freezing decreased throughout the session (group Same/Saline) because shock was not administered at this time.

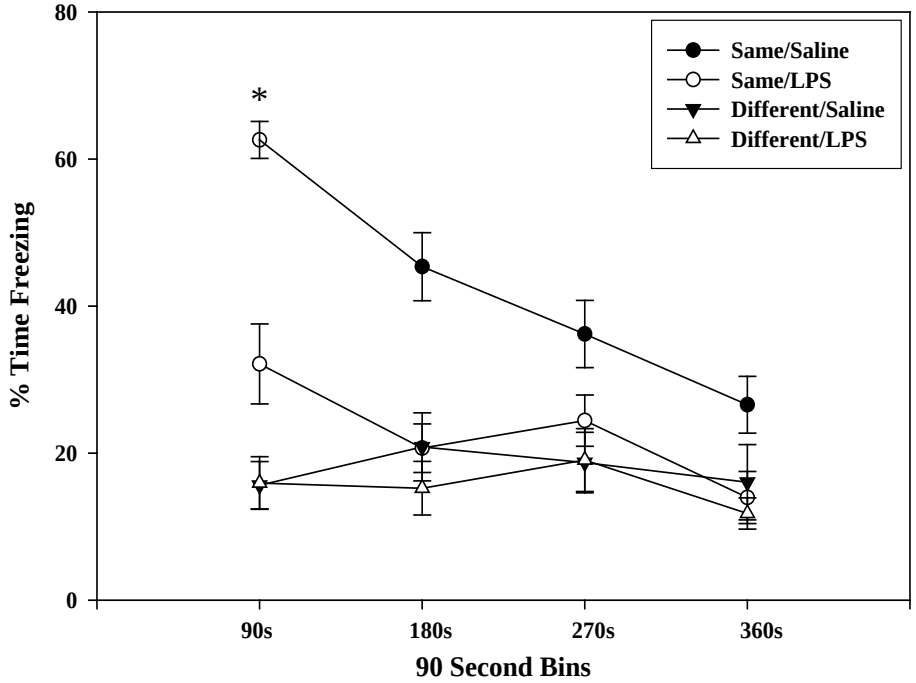


Figure 3. The effects of LPS (250 µg/kg, i.p.) on percent time freezing during the contextual fear test (i.e., Day 3). C57BL/6J mice that received saline and were placed in the same context froze significantly more than the other three groups during the initial 180 s. ***** indicates $p < 0.0005$ when group Same/Saline is compared to groups Same/LPS, Different/Saline, and Different/LPS, n = 14. Symbols represent mean ±SEM.

Experiment 3: Immediate and delayed administration of LPS

The purpose of this experiment was to determine the time course for the fear memory consolidation impairments following peripheral administration of LPS. Mice were injected with either saline or LPS immediately, LPS 2 h, or LPS 12 h after training. Repeated-measures ANOVA (Treatment x Bin) revealed a significant effect for Bin, F (3, 31) = 3.861, $p < 0.05$ (Figure 4). Neither the Treatment nor the Treatment x Bin interaction were significant. Post-hoc analysis revealed that the group that received saline displayed significantly more freezing than the groups that received LPS injection immediately [LPS (0)] or 2 h [LPS (2)] after training ($p < 0.05$), but was not significantly different from the group that received LPS 12 h [LPS (12)] post-training, during the initial 90 s of the Day 3 (i.e., test) session (Figure 4). Also, post-hoc analysis revealed that the LPS (12) group was significantly different from the LPS (2) group during the initial 90 s of the testing session ($p < 0.05$; Figure 4).

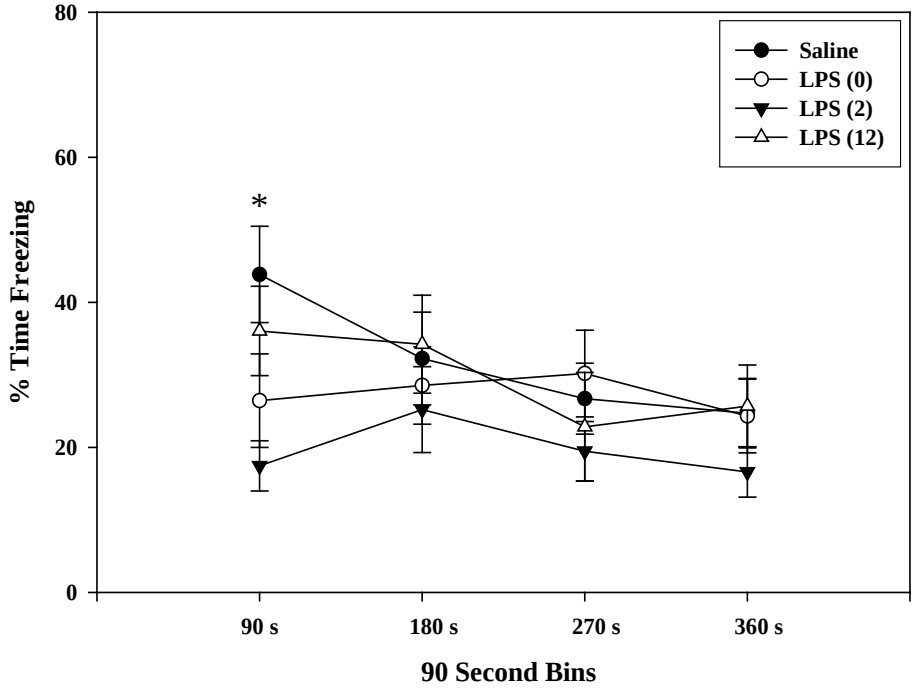


Figure 4. The effects of immediate and delayed LPS (250 µg/kg, i.p.) on percent time freezing during the contextual fear test (i.e., Day 3). C57BL/6J mice given saline displayed significantly more freezing than LPS (0) and LPS (2) groups during the initial 90 s of the testing session. Further, LPS (12) group was significantly different from the LPS (2) group, but not significantly different from the Saline and LPS (0) group, n = 8−9. ∗ indicates $p < 0.05$. Bars represent mean ±SEM.

Experiment 4a: Quantitative real-time reverse transcription polymerase chain reaction

Administration of LPS significantly increased IL-1β mRNA in the hippocampus and the parietal cortex, relative to saline-treated subjects, 4 hours post-injection (F (1,20) = 7.966, $p < 0.05$; F (1,14) = 9.028, $p < 0.01$, respectively, Figure 5A), and simultaneously decreased BDNF mRNA in the hippocampus and the parietal cortex, relative to saline controls (F (1,16) = 54.777, $p < 0.0001$; F (1,16) = 83.120, $p < 0.0001$, respectively, Figure 5B). Further, at 48 hours post-injection, IL-1β mRNA returned toward baseline, but was still significantly elevated in both the hippocampus and the parietal cortex, respectively, relative to saline-treated subjects (F (1,15) = 20.280, $p < 0.0005$; F (1,15) = 19.362, $p < 0.0005$, Figure 5A), but BDNF mRNA levels were no

longer significantly different from the saline controls (F (1,15) = 2.73, p = 0.1193, *ns*; (F (1,15) = 0.018, p = 0.8945, for the hippocampus and the parietal cortex, respectively, Figure 5B).

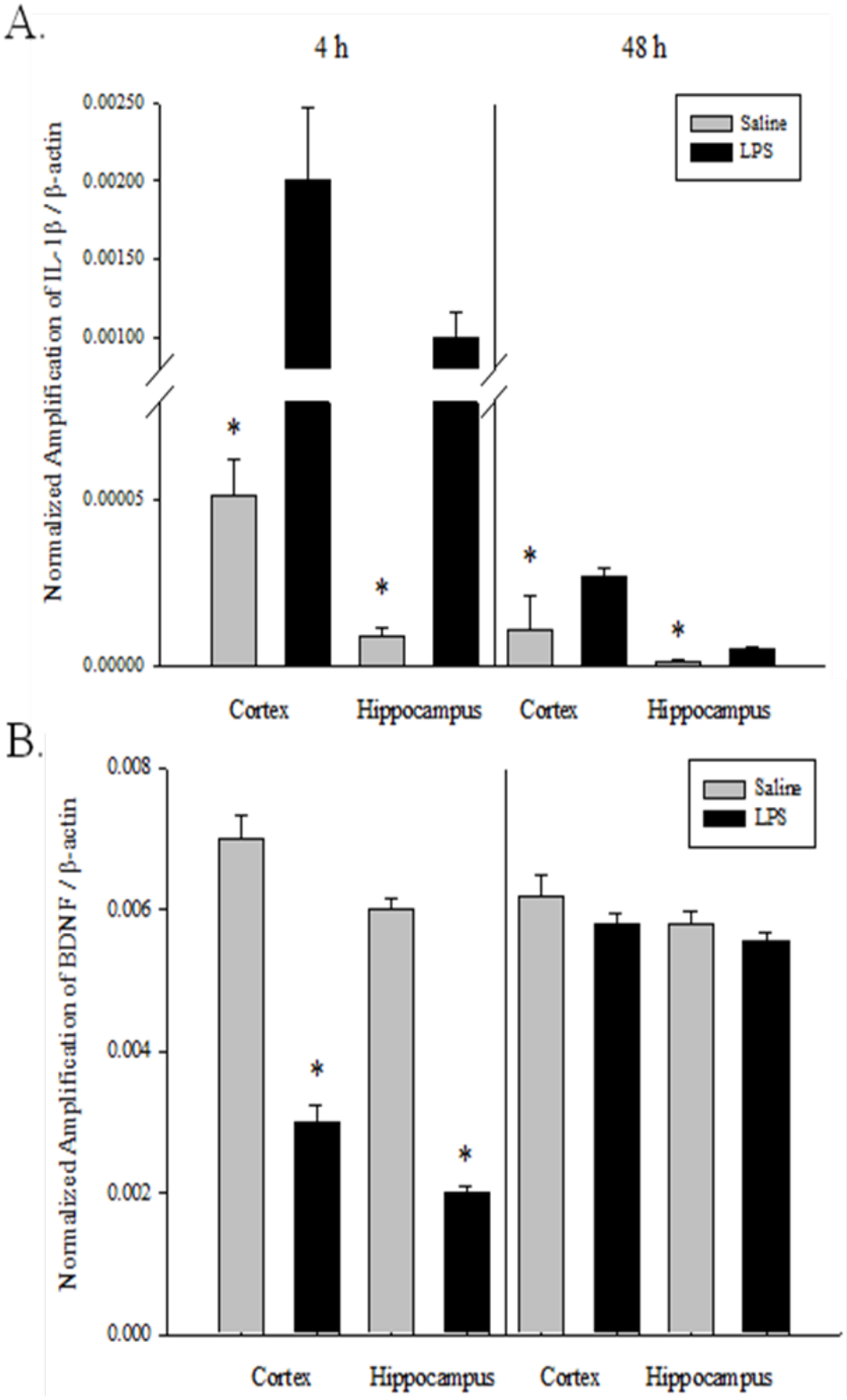


Figure 5. The effects of peripheral LPS (250 µg/kg) on hippocampal and cortical IL-1β and BDNF mRNA expression 4 and 48 hours post-injection. Four hours post-LPS, IL-1β mRNA expression was significantly increased in both the hippocampus and the parietal cortex **(A)**, relative to saline-treated subjects, while BDNF mRNA expression was significantly decreased **(B)**, relative to saline-treated subjects. Forty-eight hours post-LPS, IL-1β mRNA expression returned toward baseline, but was still elevated in both the hippocampus and the parietal cortex **(A)**, relative to saline-treated subjects, while BDNF mRNA expression returned toward baseline **(B)**. * indicates a significant difference from saline-treated subjects, $p < 0.05$; $n = 7-10$. Bars represent mean ±SEM.

Experiment 4b: Cytometric bead array

The concentrations of IL-1β, TNF-α, and MIP-1α in the serum of animals in different experimental groups, at the first time point (i.e., 4 h after LPS injection), are shown in Figures 6A−C. As expected, no significant differences in the IL-1β, TNF-α, and MIP-1α concentrations, respectively, were found between the two different experimental contexts (i.e., A and B; *ns*, data not shown), and the data were collapsed across context for further analysis. As hypothesized, 4 hours after the injection of LPS (250 µg/kg), the concentrations of IL-1β, TNF-α, and MIP-1α were significantly increased, compared to saline-treated controls (F (1,18) = 8.047, $p < 0.05$; (F (1,18) = 13.057, $p < 0.005$; (F (1,17) = 14.806, $p < 0.005$, respectively, Figures 6A-C). At 48 hours, IL-1β, TNF-α, and MIP-1α concentrations, respectively, returned to basal levels (F (1,17) = 1.118, $p = 0.3051$, *ns*; (F (1,17) = 0.234, $p = 0.6346$, *ns*; (F (1,17) = 0.211, $p = 0.6519$, *ns*, Figures 6A-C).

In addition to the cytokines and chemokine noted above, we also included IL-6 and MCP-1, but the serum concentrations for the animals given LPS were well above the highest value of the standard curve for the analysis. For this reason, we were unable to include these data, but it is safe to conclude that IL-6 and MCP-1 concentrations were dramatically elevated in animals given LPS, at 4 hours post-injection. As expected, there was no main effect of Context

for either IL-6 or MCP-1, 48 hours post-injection, respectively, and the data were collapsed across context for further analysis (F (1,15) = 2.688, $p > 0.05$, *ns*; (F (1,15) = 2.281, $p > 0.05$, *ns*, figures not shown). In contrast to IL-1β, TNF-α, and MIP-1α, concentrations of serum IL-6 and MCP-1 were still elevated 48 hours after the LPS injection (F (1,17) = 7.326, $p < 0.05$; (F (1,17) = 6.433, $p < 0.05$, respectively; Figures 7A and B).

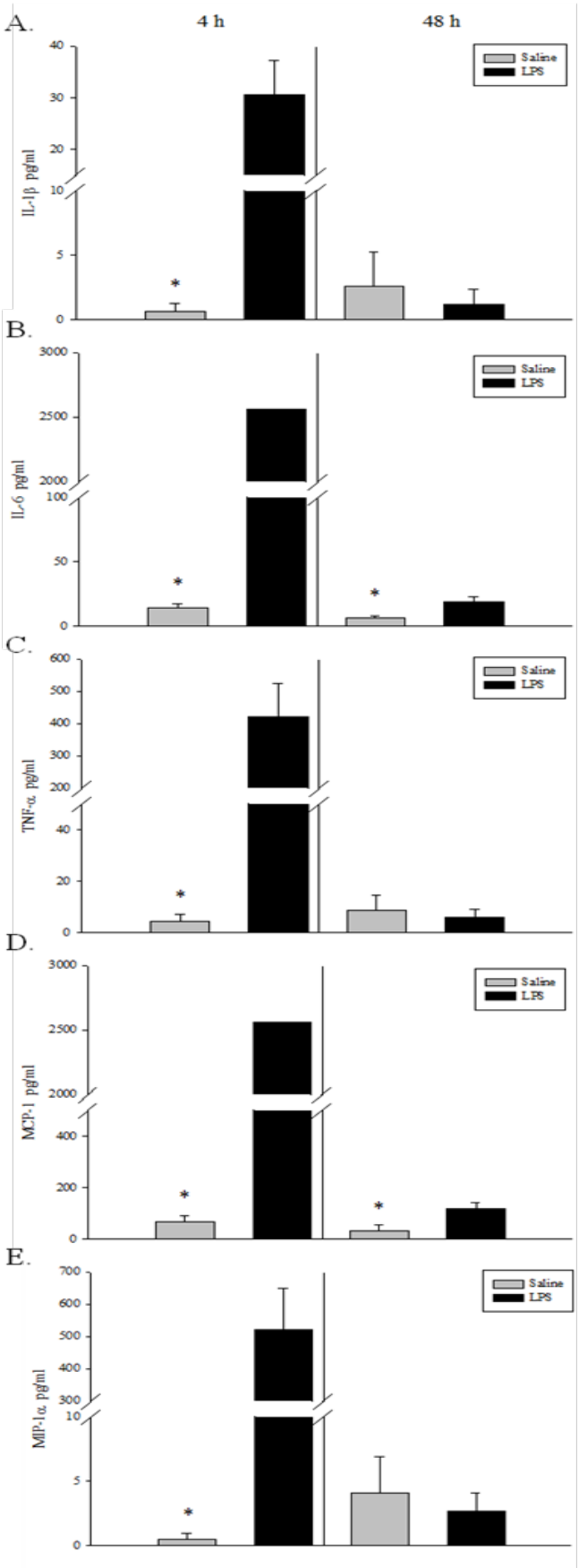

A.
4 h
48 h
Saline
LPS
IL-1β pg/ml
B.
IL-6 pg/ml
C.
TNF-α pg/ml
D.
MCP-1 pg/ml
E.
MIP-1α pg/ml

Figure 6. The effects of peripheral LPS (250 µg/kg) on serum concentrations of cytokines and chemokines 4 and 48 hours post-injection. Four hours post-LPS, serum IL-1β **(A)**, IL-6 **(B),** TNF-α **(C)**, MCP-1 **(D)** and MIP-1α **(E)** concentrations were significantly increased relative to saline-treated subjects. Forty-eight hours post-LPS, serum IL-1β **(A)**, TNF-α **(B)**, and MIP-1α **(C)** concentrations had returned to basal levels, while serum IL-6 **(B)** and MCP-1 **(D)** concentrations were still significantly increased relative to saline-treated subjects. * indicates a significant difference from saline-treated subjects, n = 7−10. Bars represent mean ±SEM.

DISCUSSION

The present experiments were designed to further evaluate the effects of peripheral immune activation on memory consolidation in the contextual fear conditioning paradigm. We used olfactory and visual cues to differentiate the contexts and a context-shift manipulation to control for the potential contribution of non-associative factors to freezing (e.g., sensitization). Moreover, we hypothesized that a single systemic injection of LPS at 250 µg/kg, given immediately or 2 h after training, but not 12 h post-training, would impair the processes of contextual fear memory consolidation. Moreover, based on previous research, we hypothesized that cytokine and chemokine levels measured 4 h post-injection, both centrally and peripherally, would be increased as compared to saline controls. Finally, we hypothesized that BDNF mRNA levels in the hippocampus and the parietal cortex would be diminished when measured four and 48 h after LPS injection.

The results obtained in the current study generally supported the original hypotheses. A single systemic injection of LPS at 250 µg/kg, given immediately or 2 h after training, but not 12 h post-training, impaired contextual fear memory consolidation in the testing phase for the mice that were placed in the same context on both days. To the best of the author's knowledge, this is the first time that such an effect has been demonstrated in mice. The finding that peripheral immune activation impaired contextual fear memory consolidation processes in mice

is in agreement with previously published studies using rats (Pugh et al., 1998; Thomson & Sutherland, 2005).

It is well known that immune activation, such as that induced by LPS or IL-1β, may trigger deficits in learning and memory, and this effect has been shown in several behavioral testing paradigms (Barrientos et al., 2002, 2004, 2009; Bilbo et al., 2006; Gonzalez et al., 2009; Hein et al., 2007; Kohman et al., 2007a,b; Pugh et al., 1998, 1999; Thomson & Sutherland, 2005; Sparkman et al., 2005a,b). More specifically, it has been shown that peripheral immune activation may negatively influence hippocampus-dependent memory consolidation processes (Pugh et al., 2001). One well established and popular task designed to study hippocampus-dependent learning and memory processes is contextual fear conditioning (Barrientos et al., 2002; Bilbo et al., 2006; Gonzalez et al., 2009; Hein et al., 2007; Phillips & LeDoux, 1992; Pugh et al., 1998; Thomas & Sutherland, 2005). There are key advantages in using contextual fear conditioning, as opposed to most other widely accepted learning and memory paradigms. For example, the possible confound of decreased locomotor activity cannot be mistaken for learning and memory impairments, as freezing is a learned response in a fear conditioning task, and LPS-treated animals tend to freeze less (i.e., move more) than saline-treated controls. However, in our experience, mice may show substantial difficulties in adequately learning this task. To address this issue, as olfaction is a dominant sense in rodents, the addition of a salient olfactory stimulus to the conditioned context proved efficacious.

In our initial experiment, unlike any prior experiments utilizing LPS or IL-1β, we first established that conditioned freezing was under contextual control as opposed to a variety of possible non-associative factors (i.e., sensitization or pseudoconditioning). As hypothesized, subjects placed in the same context on both Day 1 (i.e., training) and Day 3 (i.e., testing), 48 h apart, showed higher levels of freezing behavior than animals shifted across contexts from

training to testing. Furthermore, in the second experiment, saline-treated subjects placed in the same context on both days froze significantly more than those administered LPS, indicating that endotoxin, most likely, interfered with memory consolidation. Albeit central IL-1β levels were still elevated at the time of testing, it is unlikely that memory retrieval processes have been disrupted since mice that received LPS 12 hours after training did not differ in their freezing behavior when compared to the saline-treated animals. Moreover, as hypothesized, the difference in freezing between saline- and LPS-treated animals was not significant by the midpoint of the extinction phase, as shock was not administered at any time during testing.

A number of studies have shown that peripheral LPS administration causes an increase in pro-inflammatory cytokines in the brain (Beishuizen, 2003; Gabellec et al., 1995; Hansen et al., 2000; Turrin et al., 2001), and a transient decrease in BDNF mRNA expression (Guan & Fang, 2006; Schnydrig et al., 2007), which are both presumably, in part, responsible for the impaired processes of memory consolidation. In the current study, we found that in LPS-treated animals, central IL-1β mRNA levels, while returning toward baseline, were still elevated 48 hours post-injection, both in the hippocampus and the parietal cortex, though peripheral IL-1β had returned to basal levels. Additionally, LPS administration initially led to decreased BDNF mRNA levels both in the hippocampus and the parietal cortex, and this effect dissipated by the second time-point (i.e., 48 hours post-LPS injection).These results support a link between cognitive deficits following peripheral immune activation and alterations in central IL-1β and BDNF mRNA levels. Guan and Fang (2006) found that BDNF protein levels, measured 7 h post-injection, were decreased only after the administration of the highest dose of LPS (1 mg/kg, i.p. vs. 250 μg/kg), but the discrepancy may potentially be explained by the difference in species (mice vs. rats). Additionally, Richwine et al. (2008) reported that BDNF mRNA levels, measured 72 h post-LPS injection, were still decreased compared to saline controls, but the discrepancy between these

results and our findings could potentially be explained in that Richwine et al. (2008) used a different strain of mice (BALBc vs. C57BL/6J) and a higher dose of LPS (330 µg/kg vs. 250 µg/kg).

Because experimental subjects were given only one post-training injection, the observed LPS-induced effects are mediated, presumably, by cytokine-induced disruption of the processes of memory consolidation, potentially via diminished BDNF production, with no influence on acquisition. In contrast to our behavioral findings that fear memory consolidation is not disrupted when LPS is administered 12 h after conditioning, Barrientos et al. (2002) reported that the storage of the contextual fear memory is affected when IL-1β is injected into dorsal hippocampus either immediately, 3 h, or 24 h, but not 48 h post-training. A possible explanation for this discrepancy between the two studies may be the variation in species (mice vs. rats), immune stimulant (LPS vs. IL-1β), and/or route of administration (i.p. vs. i.c.v.).

It is well documented that a number of chemokines, in addition to cytokines, are upregulated in the periphery and brain after a peripheral injection of LPS (Gourmala et al., 1997, 1999; Thibeault et al., 2001; Zisman et al., 1997). More specifically, Thompson et al. (2008) reported a significant increase in MCP-1 serum levels 1.5 h post-LPS injection (5 mg/kg, i.p.). This elevation, compared to saline-treated controls, was still significant when measured 24 h later, and these findings are consistent with our results. Additionally, Zisman et al. (1997) showed that MCP-1 plasma levels in CD-1 mice challenged with LPS (750 µg/kg, i.p.) resulted in a rapid increase, and then returned toward baseline levels by 48 h after LPS. Our results indicate that concentrations of serum MCP-1 were still significantly elevated 48 h after injection, even with a much lower LPS dose (250 µg/kg), but this difference can potentially be explained in that Zisman et al. (1997) used CD-1 mice, as opposed to C57BL/6J mice utilized in this study.

In summary, the present findings confirm that a single systemic LPS injection impairs contextual fear memory consolidation in mice, while simultaneously increasing peripheral cytokine and chemokine, and central cytokine levels, and decreasing BDNF mRNA in the hippocampus and the parietal cortex. Furthermore, these findings complement the existing literature, and confirm that the cognitive deficits induced by peripheral immune activation appear to be similar across rodent species.

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ABSTRACT

DEFICITS IN MEMORY CONSOLIDATION INDUCED BY BACTERIAL ENDOTOXIN IN MICE COINCIDE WITH ELEVATED CYTOKINE AND CHEMOKINE LEVELS, BUT DIMINISHED EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR

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Lipopolysaccharide induces the synthesis and release of pro-inflammatory cytokines and chemokines in the periphery and the central nervous system. It has previously been reported that peripheral LPS administration in rats disrupts contextual fear memory consolidation processes, potentially due to elevated cytokine expression. We used a similar, but predominantly olfactory based, paradigm to characterize the behavioral effects of LPS administration in mice. As hypothesized, LPS administered immediately or 2 h, but not 12 h, post-training impaired memory consolidation processes that support the storage of the conditioned contextual fear memory. Consistent with the behavioral findings, 4 h post-injection, cytokine and chemokine levels were heightened in LPS-treated animals, with a simultaneous decrease in brain-derived neurotrophic factor (BDNF) mRNA. Collectively, these data reinforce and extend prior work, and suggest that contextual fear conditioning, with the addition of olfactory cues, offers a promising way to study cognitive deficits induced by peripheral immune activation in mice.