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Peripheral bacterial endotoxin administration triggers both memory consolidation and reconsolidation deficits in mice

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ABSTRACT

Peripherally administered inflammatory stimuli, such as lipopolysaccharide (LPS), induce the synthesis and release of proinflammatory cytokines and chemokines in the periphery and the central nervous system, and trigger a variety of neurobiological responses. Indeed, prior reports indicate that peripheral LPS administration in rats disrupts contextual fear memory consolidation processes, potentially due to elevated cytokine expression. We used a similar, but partially olfaction-based, contextual fear conditioning paradigm to examine the effects of LPS on memory consolidation and reconsolidation in mice. Additionally, interleukin-1β (IL-1β), brain-derived neurotrophic factor (BDNF), and zinc finger (Zif)-268 mRNA expression in the hippocampus and the cortex, along with peripheral cytokines and chemokines, were assessed. 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. Additionally, as hypothesized, LPS administered immediately following the fear memory trace reactivation session impaired memory reconsolidation processes. Four hours post-injection, both central cytokine and peripheral cytokine and chemokine levels were heightened in LPS-treated animals, with a simultaneous decrease in BDNF, but not Zif-268, mRNA. Collectively, these data reinforce prior work showing LPS- and cytokine-related effects on memory consolidation, and extend this work to memory reconsolidation.

1. Introduction

The immune system and central nervous system (CNS) have a bidirectional relationship, in which each exerts important regulatory control over the other (Hart, 1988; Exton et al., 2001; Quan and Banks, 2007). Both systems play a major role in the physiological and behavioral responses to pathogen invasion, and their interaction is thought to be generally adaptive (Ziemssen and Kern, 2007). An example of this relationship is an array of behavioral adaptations induced by immune stimulation collectively termed "sickness behaviors" (Dantzer, 2001, 2006). Some of the symptoms of sickness behavior include mood alterations, anhedonia, diminished social exploration, and diminished locomotor activity (Avitsur and Yirmiya, 1999; Castanon et al., 2001; Engeland et al., 2001; de Paiva et al., 2010). Additionally, various proinflammatory cytokines have been shown to mediate fever (Dinarello, 1999; Netea et al., 2000), modulate control of feeding (Kent et al., 1996; Plata-Salamán, 1999, 2001; Luheshi, 1999), and alter sleep regulation (Borbely and Tobler, 1989; Opp et al., 1991; Krueger, 2008). However, along with these adaptive modifications come seemingly non-adaptive immune-related events, including deficits in learning and memory (Oitzl et al., 1993; Gibertini et al., 1995; Pugh et al., 1998, 1999; Barrientos et al., 2002, 2009; Hein et al., 2007; Bilbo et al., 2008). Proinflammatory cytokines, such as interleukin-1β (IL-1β), are present at low levels in the healthy brain, and apparently exert beneficial effects (Yirmiya et al., 2002). However, they are most notable in the neuroimmunology literature for their negative effects, when expressed at higher levels (Maier et al., 1994).

LPS is an endotoxin produced from the degraded cell wall of Gram-negative bacteria that acts through Toll-like receptor 4 (TLR4) as a non-specific activator of proinflammatory cytokine release from macrophages (Borowski et al., 1998) and microglia (Van Dam et al., 1995). LPS administration, and subsequently elevated cytokine levels, can produce noticeable deficits in learning and memory (Pugh et al., 1998; Sparkman et al., 2005a; Thomson and Sutherland, 2005; Tarr et al., 2011), and a portion of these deficits in animals given LPS often resemble those observed when animals are given proinflammatory cytokines such as IL-1β (Barrientos

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et al., 2002). Although IL-1β cannot completely account for all the behavioral effects of LPS (Thomson and Sutherland, 2005), some of the effects of LPS can be blocked through administration of IL-1 receptor antagonist (IL-1ra; Bluthé et al., 1992; Abraham and Johnson, 2009), suggesting a key role for IL-1β in mediating many behavioral effects of LPS.

Most importantly for the current set of studies, it has previously been shown that peripheral LPS administration impairs contextual fear conditioning (Pugh et al., 1998, 1999; Barrientos et al., 2002; Thomson and Sutherland, 2005; Bilbo et al., 2006), spatial learning (Shaw et al., 2001; Sparkman et al., 2005b; Terrando et al., 2010), avoidance learning (Sparkman et al., 2005a; Kohman et al., 2007a,b; Tarr et al., 2011), and conditioned taste aversion/nausea (Cross-Mellor et al., 2009; Chan et al., 2009). For example, Pugh et al. (1998) showed that LPS, administered peripherally immediately after training in rats, inhibits contextual fear memory consolidation, but has no effect on auditory fear conditioning. The reason for this discrepancy may be that contextual fear conditioning is a hippocampus-dependent task (Fanselow, 2000; Antoniadis and McDonald, 2000; Barrientos et al., 2002; Celerier et al., 2004), whereas auditory-cue fear conditioning is dependent primarily on the amygdala (Bailey et al., 1999; Lamprecht et al., 2009). Additionally, it has previously been shown that olfactory contextual fear conditioning, the task used in the current study, also depends on the integrity of the hippocampus (Parsons and Otto, 2008). More specifically, dorsal hippocampal lesions impair olfactory contextual conditioning, but not explicit olfactory or auditory-cued fear conditioning (Otto and Poon, 2006).

The effects of peripheral immune activation on memory consolidation processes are fairly well described (Barrientos et al., 2002, 2009; Bilbo et al., 2008; Hein et al., 2007; Kent et al., 2007; Pugh et al., 1998, 1999; Shaw et al., 2001; Tarr et al., 2011). There has been considerably less inquiry into the effects of immune activation or cytokines on memory reconsolidation (Machado et al., 2010). Only relatively recently has it been demonstrated that once consolidated, long-term memory is in a highly labile phase in which it is susceptible to disruption upon its reactivation, and must be stabilized through a process termed "reconsolidation" (Nader et al., 2000a,b; Sara, 2000; Eisenberg et al., 2003). Reconsolidation has been shown to occur in a broad range of learning paradigms and species (Eisenberg et al., 2003; Suzuki et al., 2004), and the labile or instable period of the reconsolidation persists for several hours post-memory retrieval (Duvarci and Nader, 2004). As was shown previously for memory consolidation (Pugh et al., 2001; Gonzalez et al., 2009), it has very recently been shown that IL-1β infused directly into the hippocampus may also have a detrimental effect on the reconsolidation of contextual fear memory (Machado et al., 2010). Additionally, Barnes et al. (2010) found that the reconsolidation of contextual fear memory relies on IL-1 receptor signaling in the hippocampus.

Although both consolidation and reconsolidation require *de novo* protein synthesis for the memory to persist further (Nader et al., 2000a), it has been reported that the molecular mechanisms underlying these two processes, within the hippocampus, are different (Lee et al., 2004). Indeed, brain-derived neurotrophic factor (BDNF) is selectively required for memory consolidation, whereas the transcription factor zinc finger-268 (Zif-268) is selectively required for reconsolidation of contextual fear memory, and BDNF is not (Lee et al., 2004). Moreover, Zif-268 is activated in the hippocampus by retrieval of a contextual fear memory (Hall et al., 2001), and has been shown to regulate the expression of a variety of plasticity-associated target genes, such as activity-regulated cytoskeleton-associated protein (Arc; Li et al., 2005).

Previous research suggests a link between peripheral immune activation-associated cognitive deficits and alterations in BDNF levels in the hippocampus (Barrientos et al., 2004; Guan and Fang, 2006; Schnydrig et al., 2007; Bilbo et al., 2008). More specifically, Guan and Fang (2006) reported that BDNF protein levels 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 BDNF mRNA levels measured 4, 24, 48, and 72 h post-LPS, while reduced, were not significantly different between timepoints. Furthermore, following a single intracerebroventricular injection of LPS (5 μg/kg) into the lateral ventricle of mice, the expression of Zif-268 was significantly lower in the cortex, but not hippocampus, as compared to the saline-treated mice (Bonow et al., 2009).

The current study 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, BDNF, and Zif-268, along with learning and memory in a contextual fear conditioning paradigm. We first set out to ascertain that conditioned freezing was under selective contextual control, and was not due to non-associative factors such as sensitization or pseudoconditioning. Further, we hypothesized that mice administered LPS 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), a finding demonstrated only one other time in mice (Terrando et al., 2010). Additionally, we hypothesized that mice administered LPS immediately after the reactivation session (Day 2) would show a significant decrease in the percentage of time spent freezing on the test day, approximately 24 h later (Day 3; i.e., impaired reconsolidation). Lastly, based on prior work, we predicted that animals that received LPS would show an increase in both peripheral and central expression of the proinflammatory cytokines and chemokines mentioned above, as well as diminished BDNF and Zif-268 mRNA expression in the hippocampus and the cortex.

2. Materials and methods

2.1. Experimental subjects

Subjects 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 × 20 × 16 cm) at ambient temperature (22 °C), following weaning at one month of age, and allowed access to food and water *ad libitum*. Lights were set to an automated 0600 on and 1800 off light–dark cycle, and all behavioral testing was done between 7 and 9 a.m. All animals received care consistent with the *Guide for the Care and Use of Laboratory Animals*, and the experiments were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee at Texas Christian University.

2.2. Treatment conditions

Animals were randomly divided within each cage into treatment conditions outlined for each of the six experiments (see Table 1). Intraperitoneal (i.p.) injections of LPS (*Escherichia coli* serotype 0111:B4; Sigma, St. Louis, MO) were given at doses of either 0, 250 μg/kg (Experiments 2–4), or 125 μg/kg (Experiments 5–6) in sterile, pyrogen-free 0.9% saline (Baxter, Deerfield, IL; LPS was not used in Experiment 1). In Experiments 2–6, 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 (Experiments 2–4) because prior work has shown that this dose reliably