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Research report

Peripheral administration of D-cycloserine rescues memory consolidation following bacterial endotoxin exposure

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HIGHLIGHTS

- D-Cycloserine rescued memory consolidation following systemic LPS exposure.
- D-Cycloserine failed to restore BDNF levels that were diminished following LPS.
- D-Cycloserine did not alter NR1 or NR2C NMDA receptor subunit expression.

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ABSTRACT

In the current study, the partial NMDA receptor agonist D-cycloserine (DCS) rescued memory consolidation following systemic bacterial endotoxin exposure. DCS failed, however, to restore hippocampal BDNF mRNA levels that were diminished following a systemic administration of LPS, and did not alter NR1 or NR2C NMDA receptor subunit expression. These results extend prior research into the role of DCS in neural-immune interactions, and indicate that the detrimental effects of peripheral LPS administration on consolidation of contextual fear memory may be ameliorated with DCS treatment, though the mechanisms underlying these effects are currently unclear.

1. Introduction

Research models examining the effects of cytokine action in the central nervous system suggest that appropriate cytokine levels are essential for normal molecular, cellular, and cognitive function [1–4]. The pro-inflammatory cytokines, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF)-α modulate synaptic plasticity, long-term potentiation (LTP), neurogenesis, and learning and memory [5]. At low levels of expression, these cytokines are required for, or may enhance, certain learning and memory processes, including consolidation of newly acquired memories that depend on the integrity of the hippocampus [6–11]. Rapidly accumulating evidence indicates that IL-1β is induced during learning, and that hippocampus-dependent learning and memory processes may be facilitated by exogenous administration of IL-1β [12,13]. Moreover, IL-1 receptor knock-out (IL-1rKO) and IL-1 receptor antagonist (IL-1ra)-overexpressing mice display learning impairments compared to control animals, and exogenous administration of IL-1ra induces learning deficits [14–16].

Alternately, elevated levels of the pro-inflammatory cytokines, following stimulant-induced innate immune system activation, trigger learning and memory deficits in a variety of tasks that require normal hippocampal function, including contextual fear conditioning [17–23].

Systemic administration of the Toll-like receptor (TLR)-4 agonist lipopolysaccharide (LPS) stimulates the innate immune system, and results in cognitive deficits in hippocampus-dependent memory acquisition, consolidation, and reconsolidation [22–24,18,25]. The exact mechanisms for LPS-induced learning and memory deficits remain unknown. However, one pathway implicates a decline in brain-derived neurotrophic factor (BDNF) mRNA and protein, triggered by elevated levels of hippocampal IL-1β [26,18,27].

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BDNF is critically involved in learning and memory [28,29], and diminished BDNF signaling due to inflammatory cascades may impair hippocampus-dependent cognitive processes [23,26,18,27]. In addition to suppressing transcriptional and translational expression of BDNF, elevated hippocampal IL-1β may also directly interfere with either the activation of the primary BDNF receptor, TrkB [30], or with BDNF-induced activation of the cAMP response element-binding (CREB) transcription factor [31], and result in learning and memory deficits. However, Tong et al. [31] concluded that IL-1β does not affect the activation of TrkB receptors.

Accumulating evidence indicates that the *N*-methyl-D-aspartate receptors (NMDARs) are critically involved in learning and memory processes [32–35]. Inflammation attenuates NMDA-dependent long-term potentiation, (LTP [36]), and leads to both a diminished hippocampal NMDAR NR1 subunit mRNA expression [37] and a decrease in the number of NMDARs within the dentate gyrus [38]. The opposite effect for both LTP and learning/memory processes is true following the administration of NMDAR agonists [39]. For example, D-cycloserine (DCS), a partial agonist at the glycine$_B$ recognition site (NR1/NR2B) of the NMDAR [40], may enhance various forms and phases of learning and memory, including memory consolidation [41–46]. Glycine, the endogenous ligand at this binding site, has greater affinity compared to DCS; thus, DCS displays about 40–70% of the efficacy of glycine, and is characterized as a partial agonist [47]. However, DCS was recently shown to have a higher relative efficacy than glycine at the NR1/NR2C binding site [47].

DCS was employed in the current study, in an effort to ameliorate memory consolidation deficits observed following LPS, because previous research demonstrated that pre- and post-training administration of DCS either reverses memory deficits or improves memory retention in both human [48] and non-human animal studies [49–52]. More specifically, post-training DCS administration enhances consolidation in the plus-maze retest paradigm [45], footshock avoidance [41], spontaneous place recognition [44], and linear maze task [46].

Importantly, earlier studies have shown that, under conditions of inflammation, NMDAR activation may lead to increased or restored expression of BDNF [53,39,54]. In a mouse model of closed head injury, a single systemic injection of DCS facilitated a faster recovery of object recognition memory function and reinstated traumatic brain injury-induced reduction of BDNF in the CA1 subregion of the hippocampus [39]. In addition, NMDAR activation leads to an increased neuronal, but not astrocytic, release of BDNF [55,56].

For the current study, we administered DCS to explore the involvement of NMDAR/glycine$_B$ mechanisms in a contextual fear memory consolidation task following activation of the innate immune system by post-training peripheral LPS administration. A contextual fear conditioning paradigm employing olfactory, visual, and tactile cues was utilized. This cognitive task has been described in detail previously, and successfully used to investigate the effects of systemic LPS administration on hippocampus-dependent learning and memory processes in mice [17,18]. To the best of the authors' knowledge, the effects of DCS administration on memory consolidation following a peripheral injection of LPS have not been previously examined.

In the first experiment, we tested the hypothesis that post-training DCS administration would rescue hippocampus-dependent fear memory consolidation following LPS injection. In the second experiment, we examined the effects of DCS and LPS on mRNA expression of IL-1β, BDNF, NR1, and NR2C within the dorsal hippocampus. Although a single systemic injection of DCS was previously shown to restore BDNF levels in the context of brain injury [39], it is currently unknown whether systemic administration of DCS can rescue LPS-induced memory consolidation deficits by restoring hippocampal BDNF levels.

2. Materials and methods

2.1. Experimental subjects

Subjects were 4–6 month-old, experimentally naïve, male C57BL/6J mice bred at the Texas Christian University (TCU) vivarium from breeding stock purchased from The Jackson Laboratory (Bar Harbor, ME). Following weaning at one month of age, animals were housed in groups of 3–4 in standard polycarbonate mouse cages (30 cm × 20 cm × 16 cm), at ambient temperature (22 °C), and allowed access to food and water ad libitum. Lights were set to an automated 0700 h on and 1900 h off light-dark cycle, and tests of learning and memory were done between 0900 h and 1100 h. Animals were treated in compliance 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 (IACUC) at TCU. Thirty-six animals were used in Experiment 1, and 40 animals were used in Experiment 2.

2.2. Treatment conditions

Intraperitoneal (i.p.) injections of LPS (*Escherichia coli*, serotype 0111:B4; Sigma, St. Louis, MO) were given at the dose of 250 μg/kg, and i.p. injections of DCS (Sigma, St. Louis, MO) were given at the dose of 15 mg/kg in sterile, pyrogen-free 0.9% saline (Baxter, Deerfield, IL). Doses for both LPS and DCS were derived from previously published literature (LPS [18,57,58,20,59,60,61,44,45]). More specifically, we utilized LPS at the dose of 250 μg/kg as prior work has shown that this dose reliably induces sickness behavior and learning deficits in rodents (e.g., [18]), and we utilized DCS at the dose of 15 mg/kg as previously published literature indicates that this dose reliably facilitates various forms and phases of learning and memory, including memory consolidation (e.g., [44]).

As we wanted to assess the effects of systemic LPS and DCS co-administration on fear memory consolidation, our injection timepoint was immediately following the conditioning session. All mice were visually inspected and weighed daily during the three-day procedure.

2.3. Behavior testing apparatus

Fully automated units (FreezeFrame, Coulbourn Instruments, Whitehall, PA, USA) were used to assess conditioned contextual fear learning. Each of the units had an electrified grid floor, through which an electric shock (0.7 mA) was delivered. Each of the units was connected with the FreezeFrame Software (Coulbourn Instruments, Whitehall, PA, USA) that enabled recording and analysis of freezing behavior. Movement of the animal was recorded continuously and, in order for the behavior to count as freezing, the animal needed to stay below level 10 (the company's default setting) on a motion detection sensitivity scale (scale range: 0–1000).

2.4. Experiment 1: contextual fear memory consolidation

As noted above, we utilized a contextual fear conditioning paradigm validated and discussed in detail previously [18]. Briefly, on the training day, mice were placed into the conditioning chambers with dotted pattern walls and a peppermint odor. After a 90-s acclimation period, mice received three 2-s 0.7 mA shocks, each 90 s apart. Immediately following the conditioning session, mice were administered intraperitoneal injections of either sterile saline or LPS (250 μg/kg); then, mice in each of these groups received either sterile saline or DCS (15 mg/kg). Forty-eight hours after post-training injections, mice were placed into the same conditioning chamber and context for 90 s, and freezing behavior was measured. DCS has a half-life of 180 min [62], and behavioral testing was delayed 48 h to ensure that DCS would not affect memory retrieval processes. We hypothesized that mice administered LPS would poorly associate the context with footshock, whereas LPS-treated mice that are co-administered DCS would more successfully form an association between the context and shock-induced fear.

2.5. Experiment 2: qPCR (hippocampal IL-1β, BDNF, NR1, and NR2C expression)

In the second experiment, the effects of DCS and/or LPS administration on the expression of IL-1β, BDNF, NR1, and NR2C mRNA in the dorsal hippocampus were examined. Following a contextual fear conditioning training session to ensure treatment is identical to what subjects in the behavioral experiment had experienced, mice were first injected with either saline or LPS (250 μg/kg), and were then also injected with either saline or DCS (15 mg/kg). Four and 48 h later, subjects were rapidly euthanized via CO_2 inhalation. Using an RNase-free sample corer, brain tissue punches were obtained, rinsed, placed in a nuclease-free tube containing RNAlater® (Ambion, Austin, TX), and frozen until use. Hippocampal RNA was first isolated (RNeasy Micro kits, Qiagen, Valencia, CA), and measured utilizing a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, NanoDrop products, Wilmington, DE). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to assess the levels of IL-1β, BDNF, NR1, and NR2C mRNA present in the hippocampus at the time of tissue harvesting, utilizing a 7500 Real-Time PCR Thermal