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Dorsal hippocampal neural immune signaling regulates heroin-conditioned immunomodulation but not heroin-conditioned place preference

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ABSTRACT

Repeated pairings of heroin and a context results in Pavlovian associations which manifest as heroin-conditioned appetitive responses and peripheral immunomodulation upon re-exposure to heroin-paired conditioned stimuli (CS). The dorsal hippocampus (DH) plays a key role in the neurocircuitry governing these context-heroin associations. Within the DH, expression of the pro-inflammatory cytokine interleukin-1β (IL-1β) is required for heroin-conditioned peripheral immunomodulation to occur. However, the role of signaling via IL-1 receptor type 1 (IL-1R1) has not been examined. Furthermore, it has not been evaluated whether the involvement of IL-1 in associative learning extends to classically conditioned appetitive behaviors, such as conditioned place preference (CPP). The first set of experiments investigated whether DH IL-1R1 signaling during CS re-exposure modulates heroin-conditioned immunomodulation and heroin-CPP. The second set of experiments employed chemogenetic techniques to examine whether DH astroglial signaling during CS re-exposure alters the same Pavlovian responses. This line of investigation is based on previous research indicating that astrocytes support hippocampal-dependent learning and memory through the expression of IL-1β protein and IL-1R1. Interestingly, IL-1R1 antagonism disrupted heroin-conditioned suppression of peripheral immune parameters but failed to alter heroin-CPP. Similarly, chemogenetic stimulation of G_i-signaling in DH astrocytes attenuated heroin-conditioned peripheral immunomodulation but failed to alter heroin-CPP. Collectively our data show that both IL-1R1 stimulation and astrocyte signaling in the DH are critically involved in the expression of heroin-conditioned immunomodulation but not heroin-CPP. As such these findings strongly suggest hippocampal neuroimmune signaling differentially regulates Pavlovian immunomodulatory and appetitive behaviors.

1. Introduction

Repeated pairings between environmental stimuli and the subjective and physiological effects of heroin result in robust associative learning. The consequent stimulus control over physiology and behavior is integral to heroin addiction and has detrimental health consequences that represent a growing public health concern. Heroin-associated contextual stimuli can act as conditioned stimuli (CS) that trigger Pavlovian appetitive conditioned responses, including conditioned place preference (CPP) (Tzschentke, 1998). Additionally, drug-paired contextual stimuli can act as discriminative stimuli or occasion setters that signal drug availability and thus engender drug-seeking behavior in instrumental paradigms (Crombag et al., 2008; Fuchs et al., 2008). Regardless of the specific role of the contextual stimulus, the hippocampus is essential for context-drug associative learning (Kutlu and Gould, 2016). In particular, the dorsal hippocampus (DH) plays a critical role in drug-induced CPP (Corrigall and Linseman, 1988; Meyers et al., 2003; Xia et al., 2017) as well as context-induced drug-seeking behaviors (Fuchs et al., 2005; Fuchs et al., 2007; Ge et al., 2017; Xie et al., 2010).

In addition to heroin-conditioned appetitive responses, heroin-associated contextual stimuli can elicit the immunomodulatory effects induced by opioids (Lysle and Ijames, 2002). Heroin and other opioids negatively alter host immunity (McCarthy et al., 2001; Wang et al., 2011). Following repeated context-heroin pairings, exposure to the heroin-paired CS is sufficient to evoke heroin-conditioned suppression of lipopolysaccharide (LPS)-induced peripheral immune parameters (Lysle and Ijames, 2002). We have characterized this heroin-conditioned peripheral immunomodulation as a classically conditioned response that follows the principles of learning (Szczytkowski and Lysle,

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2007). Consequently, it is mediated through DH-dependent processes. GABA agonist-induced DH inactivation during CS exposure significantly disrupts heroin-conditioned suppression of LPS-induced peripheral indices of nitric oxide (NO) production (Szczytkowski et al., 2013). Thus, the DH is an essential component of the neural circuitry governing the retrieval or utilization of the context-heroin association that controls host immunity.

The role of the pro-inflammatory cytokine interleukin-1β (IL-1β) in hippocampal-dependent learning and memory has been well established (Goshen et al., 2007; Jones et al., 2015), with evidence to suggest its involvement in the development and maintenance of long-term potentiation (Donzis and Tronson, 2014; Yirmiya and Goshen, 2011). We have determined IL-1β within the DH is required for the expression of heroin-conditioned immunomodulation. siRNA-mediated knockdown of DH IL-1β during CS exposure blocked heroin-conditioned suppression of LPS-induced peripheral immune measures (Szczytkowski et al., 2013). Interestingly, similar to IL-1β itself, stimulation of IL-1 receptor type 1 (IL-1R1) has been implicated in hippocampal-dependent learning and memory. Genetic knockouts of hippocampal IL-1R1 show profound deficits in learning tasks and long-term potentiation (Ben Menachem-Zidon et al., 2011). Thus, it is likely that IL-1β-dependent memory mechanisms occur through IL-1R1 stimulation and subsequent signaling cascades. Furthermore, we have shown that acquisition of the context-heroin association required for conditioned immunomodulation is mediated through DH IL-1R1 (Lebonville et al., 2016). However, it is unclear whether DH IL-1R1 signaling is involved in the expression of heroin-conditioned immunomodulation. Additionally, it is yet to be determined if heroin-conditioned appetitive responses are governed through DH IL-1-dependent mechanisms. To further our understanding, the first set of experiments in the present study examined the effects of DH IL-1R1 antagonism during CS exposure on the expression of heroin-conditioned suppression of peripheral indices of NO production and heroin-CPP.

The neuroimmune system is a vastly complex network involving multiple cell types and signaling molecules. These components function in concert to produce persistent adaptations in neural communication (Yirmiya and Goshen, 2011). Relevant to our model, astrocyte activity has been implicated in both mechanisms of learning and memory (Ben Achour and Pascual, 2010; Ota et al., 2013) and substance use disorders (Lacagnina et al., 2018; Miguel-Hidalgo, 2009; Scofield and Kalivas, 2014). Astrocytes can directly alter neuronal function and synaptic plasticity through the release of gliotransmitters (Haydon and Carmignoto, 2006) and cytokines (Lacagnina et al., 2018; Santello and Volterra, 2012). Interestingly, astroglia have been shown to support hippocampal-dependent learning and memory through the expression of IL-1β (Jones et al., 2018) and IL-1R1 (Ben Menachem-Zidon et al., 2011). While a mechanistic link between astrocyte activity and subsequent IL-1β release has not yet been confirmed, astrocytes may be a critical cell population involved in mediating heroin-conditioned immunomodulation. Moreover, the role of hippocampal astroglia in heroin-conditioned appetitive responses is presently unknown. Thus, the second set of experiments in the present study examined the role of DH astroglial activity in heroin-conditioned immunomodulatory and appetitive responses. We employed chemogenetic techniques to evaluate the importance of DH astroglial signaling during exposure to heroin-paired contextual stimuli. An adeno-associated viral construct was used to selectively target DH astroglia and express G_i-coupled designer receptors exclusively activated by designer drugs (DREADDs) in this cell population. DREADDs are mutated muscarinic receptors that no longer respond to endogenous ligands and instead are activated by clozapine-N-oxide (CNO) (Roth, 2016). CNO-induced stimulation of astroglial G_i-signaling will attenuate induction of cyclic adenosine monophosphate (cAMP) (Jones et al., 2018) and have distinct functional outcomes for cellular activity. Overall, the present study investigated hippocampal neuroimmune signaling, by way of both IL-1R1 and astroglial signaling, in two Pavlovian procedures: heroin-conditioned immunomodulation and heroin-CPP.

2. Materials and methods

2.1. Animals

Male Lewis rats (~225–250 g) were purchased from Charles River Laboratories (Kingston, NY). All rats were individually housed on a 12-hour reversed light-dark cycle. Animals were handled regularly prior to and throughout experimental procedures. Animals received *ad libitum* home cage access to food and water. All procedures were conducted in compliance with regulations by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

2.2. Drug administration

Heroin (diacetylmorphine, National Institute on Drug Abuse, Drug Supply Program, Bethesda, MD) was dissolved in 0.9% sterile saline. Heroin was stored at 4 °C until use at room temperature. In all experiments, heroin was administered subcutaneously at a dose of 1 mg/kg. This dose was selected based on prior research showing that it induces conditioning and alters endotoxin-induced indices of NO production (Lysle and How, 2000; Lysle and Ijames, 2002; Szczytkowski and Lysle, 2007). Human recombinant interleukin-1 receptor antagonist (IL-1RA; Genscript, Piscataway, NJ) was reconstituted in 0.9% sterile saline vehicle to a concentration of 2.5 μg/μL and stored at −20 °C until use at room temperature. In Experiments 1 and 2, intra-DH IL-1RA (1.25 μg/0.5–0.6 μL per hemisphere) was infused bilaterally at a rate of 0.25 μL/min. Clozapine-*N*-oxide (CNO; Sigma, St. Louis, MO or the National Institutes of Health, Bethesda, MD) was dissolved in a vehicle of 0.9% sterile saline with 0.5% dimethyl sulfoxide (DMSO). In Experiments 3 and 4, CNO (3 mg/kg) or vehicle was administered subcutaneously. Lipopolysaccharide (LPS; derived from *E. coli,* serotype O55:B5, Sigma) was dissolved in 0.9% sterile, pyrogen-free saline. In Experiments 1 and 3, LPS (1 mg/kg) was administered subcutaneously. This LPS dose produces sickness behavior and induces measures of NO production.

2.3. Surgical procedures

Animals were fully anesthetized with a 1 mg/kg intraperitoneal injection of ketamine hydrochloride (100 mg/mL) mixed with xylazine (100 mg/mL) in a 9:1 (vol:vol) ratio.

2.3.1. Cannulation surgeries for IL-1RA experiments

Guide cannulae (26 gauge, Plastics One, Roanoke, VA) were directed bilaterally at the DH (AP −3.4 mm, ML ± 3.1 mm, DV −2.2 mm, relative to bregma, 15° angle laterally, (Paxinos and Watson, 2006)). Cannulae were secured to the skull with screws, cyanoacrylate adhesive gel, and dental acrylic. Dummy injectors (0.008/0.2 mm no projection, Plastics One) were inserted into the guide cannulae to prevent occlusion. Animals were given one week for post-operative recovery and were handled regularly during this time.

2.3.2. Virus infusion surgeries for the GFAP-hM4D(G_i) experiments

An astroglial G_i-coupled DREADD virus (AAV8-GFAP-hM4D(G_i)-mCherry) was infused into the DH. The DREADD construct was packaged into an adeno-associated virus (AAV) by the University of North Carolina at Chapel Hill Vector Core (Chapel Hill, North Carolina). Injectors (33 Gauge, Plastics One) were directed bilaterally at the DH (AP −3.4 mm, ML ± 3.1 mm, DV −3.2 mm, relative to bregma, 15° angle laterally, (Paxinos and Watson, 2006)). Purified viruses were obtained pre-dialyzed (350 mM NaCl, 5% D-sorbitol in PBS) and were microinjected at a viral titer of 2.0×10^{12} particles/mL (Experiment 3) or 9.8×10^{12} particles/mL (Experiment 4). Virus infusions of 0.7 μL per hemisphere were delivered bilaterally at a rate of 0.05–0.1 μL/min. At the end of the infusion, injectors were left in place for 10–15 min to