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Region-specific contribution of the ventral tegmental area to heroin-induced conditioned immunomodulation

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ABSTRACT

Dopamine receptor stimulation is critical for heroin-conditioned immunomodulation; however, it is unclear whether the ventral tegmental area (VTA) contributes to this phenomenon. Hence, rats received repeated pairings of heroin with placement into a distinct environmental context. At test, they were re-exposed to the previously heroin-paired environment followed by systemic lipopolysaccharide treatment to induce an immune response. Bilateral GABA agonist-induced neural inactivation of the anterior, but not the posterior VTA, prior to context re-exposure inhibited the ability of the heroin-paired environment to suppress peripheral nitric oxide and tumor necrosis factor-α expression, suggesting a role for the anterior VTA in heroin-conditioned immunomodulation.

1. Introduction

Opioid administration has detrimental health consequences in addition to the possible development of addictive behaviors and dependence. Clinical studies have revealed abnormalities in basic immune parameters in heroin users, including a decrease in circulating lymphocytes, natural killer cell activity, cytokine production, and antibody-dependent cellular cytotoxicity (Govitrapong et al., 1998; Nair et al., 1986; Olson et al., 2005; Yardeni et al., 2008). Several immune parameters that are critical for innate immune responses are altered by opioid use, such as the expression of inducible nitric oxide synthase (iNOS) (Lysle and How, 2000) and the production of the proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (Chao et al., 1993; Pacifici et al., 2000). These studies suggest that chronic opioid administration results in an impaired ability to defend against infectious disease (Theodorou and Haber, 2005).

Interestingly, opioid-induced immunosuppression has been shown to be mediated by the central nervous system (Fecho et al., 1996; Lysle et al., 1996; Shavit et al., 1986), and the immunosuppressive effects of exogenous opioid administration can be conditioned to the context in which the drug is delivered. Indeed, research from our laboratory has shown that the immune altering effects of opioids, including those of morphine and heroin, can be conditioned to environmental stimuli by pairing opioid administration with exposure to a distinct environmental context. As a result, a morphine-paired context can acquire immune altering effects. For example, following conditioning sessions during which morphine injections were paired with a distinct context, rats exhibited significant reductions in mitogenic responsiveness of lymphocytes, natural killer cell activity, and interleukin-2 production when re-exposed to the distinct context in a drug free state, demonstrating for the first time morphine-conditioned immunosuppression (Coussons et al., 1992). These findings are commensurate with other studies exploring the conditioned effects of opiates on other physiological and behavioral processes. For example, the administration of morphine has been shown to result in the development of conditioned hyperthermia (Broadbent and Cunningham, 1996; Schwarz-Stevens and Cunningham, 1993; Schwarz and Cunningham, 1990). Moreover, exposure to stimuli associated with heroin use has been shown to induce craving for heroin (Daglish et al., 2001; Sell et al., 2000; Zijlstra et al., 2009). Furthermore, contextual stimuli associated with drug self-administration have been shown to reinstate heroin-seeking behavior (Bossert et al., 2004, 2012; Fuchs and See, 2002). Thus, the study of conditioning processes has implications for the health consequences of drug use and drug seeking behavior and addiction.

Early evidence suggested that that dopamine and glutamate are involved in conditioned immunomodulation (Hsueh et al., 1999; Kuo et al., 2001). Findings from our laboratory demonstrated that dopamine receptor activity was necessary for the expression of

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opioid-conditioned immune alterations, as administration of a dopamine D_1-like receptor antagonist prior to re-exposure to a morphine-conditioned stimulus prevented the suppression of natural killer cell activity (Saurer et al., 2008a). Similar immunomodulatory effects have also been demonstrated with heroin (Fecho and Lysle, 2000; Lysle and Ijames, 2002; Saurer et al., 2008a).

Emerging evidence suggests that a limbic neural circuit mediates the expression of heroin-induced conditioned immune alterations, and this circuit likely includes the ventral tegmental area (VTA). In support of this, either GABA agonist-induced neural inactivation of, or dopamine D_1-like receptor antagonism in, the basolateral amygdala (BLA) blocks heroin-induced conditioned immunosuppression (Szczytkowski and Lysle, 2008, 2010). Moreover, unilateral dopamine D_1-like receptor antagonism in the BLA coupled with contralateral NMDA glutamate receptor antagonism in the nucleus accumbens (NAc) shell significantly attenuates the expression of heroin-conditioned immunosuppression (Szczytkowski et al., 2011). In contrast, ipsilateral manipulation of the same brain regions fails to disrupt heroin-conditioned immunomodulation (Szczytkowski et al., 2011). These findings suggest that dopamine in the BLA, via the stimulation of D_1-like receptors, is necessary for obligatory intrahemispheric interactions between the BLA and the NAc in the control of heroin-conditioned immune alterations.

The critical source of dopamine involved in heroin-conditioned immunomodulation has not been investigated even though the VTA is a likely candidate. Opioid administration has been long believed to result in the disinhibition of dopaminergic projection neurons and the subsequent release of dopamine at terminal regions via the stimulation of μ opioid receptors on VTA GABAergic inter- and projection neurons (Johnson and North, 1992a). However, recently a revised model has been proposed, in which opioids directly inhibit GABAergic projections from the rostromedial tegmental nucleus to VTA dopamine neurons, thereby suppressing tonic inhibition and resulting in enhanced phasic dopamine activity (Bourdy and Barrot, 2012). Similarly, heroin-associated stimuli increase the activity of VTA neurons (Kiyatkin and Rebec, 2001). Recent, investigations have focused on elucidating the function of various subregions of the VTA, and the findings have suggested that the VTA is a heterogeneous structure with distinct subregions differentially affecting drug-induced behaviors. For example, μ opioid receptor antagonism in the anterior, but not posterior, VTA prevents the acquisition of cocaine-induced conditioned place preference (Soderman and Unterwald, 2008). Furthermore, rats self-administer the $GABA_A$ antagonist, picrotoxin, into the anterior, but not the posterior, VTA (Ikemoto et al., 1997a). A study utilizing retrograde tracing techniques determined that dopaminergic efferents from the VTA to BLA originate in the anterior VTA (Ford et al., 2006), whereas a large proportion of dopaminergic projections from the posterior VTA terminate in the NAc (Ikemoto, 2007). Based on these findings and the importance of dopamine signaling in the BLA in conditioned immunosuppression (Szczytkowski et al., 2011; Szczytkowski and Lysle, 2008, 2010), we hypothesized that the functional integrity of the anterior, but not posterior, VTA is necessary for the expression of heroin-conditioned immunomodulation.

To test this hypothesis, the present study selectively targeted the anterior or posterior VTA in order to evaluate the distinct contributions of these subregions to the expression of heroin-induced conditioned immunomodulation. Rats underwent a conditioning procedure which consisted of repeated pairings of heroin administration with placement into a distinct environment. Following the conditioning regimen, rats received microinfusions of saline vehicle or a cocktail of the $GABA_B$/$GABA_A$ agonists, baclofen/muscimol (B/M), into the anterior or posterior VTA to temporarily inactivate these VTA subregions. Rats were then re-exposed to the previously heroin-paired environment in a drug free state. Six hours following re-exposure, rats received a subcutaneous injection of lipopolysaccharide (LPS) to induce an immune response. LPS is a component of the outer cell membrane of Gram-negative bacteria, which activates the innate immune response via the CD14/TLR4/MD-2 complex, resulting in a robust proinflammatory response (Fujihara et al., 2003). To assess context-induced alterations in immune status, the effects of these manipulations were examined on the expression of the proinflammatory mediators, iNOS and TNF-α, in the spleen and/or plasma.

2. Materials and methods

2.1. Animals

Male Lewis rats, weighing 225–250 g, were purchased from Charles River Laboratories (Raleigh, NC, USA). Upon arrival, animals were housed individually in plastic cages in a colony room with a reversed light–dark (12-h) cycle maintained through artificial illumination. Animals were allowed access to food and water *ad libitum* throughout the experiment except for the time spent in the conditioning chambers when food and water were not available. All animals were given a 2-week habituation period before the start of experimental manipulations and were handled regularly during this time. All procedures described were approved by the IACUC of the University of North Carolina at Chapel Hill and conformed to National Institutes of Health (NIH) Guidelines on the Care and Use of Laboratory Animals.

2.2. Drug administration

Heroin (diacetylmorphine) was obtained from NIDA (Bethesda, MD, USA) and dissolved in 0.9% sterile saline. Heroin was administered subcutaneously at a dose of 1 mg/kg. This dose was selected based on prior experiments in our laboratory showing that it induces conditioning and alters LPS-induced iNOS and TNF-α mRNA expression in spleen tissue (Lysle and How, 2000; Lysle and Ijames, 2002; Szczytkowski and Lysle, 2007).

2.3. Surgical procedures

Animals were fully anesthetized with 0.35 mL intramuscular injections of 1:1 (vol:vol) ketamine hydrochloride (100 mg/mL) mixed with xylazine (20 mg/mL) and placed into the stereotaxic apparatus. Animals were implanted bilaterally with 26-gauge guide cannulae (Plastics One, Roanoke, VA, USA). The cannulae were angled at 10° and directed towards the anterior VTA (AP −5.0, ML ± 2.2, DV −6.1 mm, relative to bregma) or posterior VTA (AP −6.0, ML ± 2.1, DV −6.3 mm, relative to bregma). Animals were given a 2-week post-surgical recovery period before the start of conditioning trials.

2.4. Conditioning procedure

All animals received five conditioning sessions in standard conditioning chambers (BRS/LVE, Laurel, MD, USA). Chambers were fitted with a metal grid floor design and cedar bedding to create an environment distinct from that of the home cage and to provide both olfactory and tactile cues for conditioning. Artificial noise machines were used to minimize background noise. All conditioning took place during the dark phase of the light cycle in a room separate from the animal colony and the conditioning chambers were kept dark to minimize effects on circadian rhythms. On each conditioning day, a subcutaneous injection of heroin (1 mg/kg) was