Neurobiology of Learning and Memory 137 (2017) 83–91

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Ethanol-seeking behavior is expressed directly through an extended amygdala to midbrain neural circuit

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ARTICLE INFO

Article history:
Received 2 September 2016
Accepted 16 November 2016
Available online 17 November 2016

Keywords:
BNST
VTA
CPP
Ethanol
DREADD
HSV

ABSTRACT

Abstinent alcohol-dependent individuals experience an enduring sensitivity to cue-induced craving and relapse to drinking. There is considerable evidence indicating that structures within the midbrain and extended amygdala are involved in this process. Individually, the ventral tegmental area (VTA) and the bed nucleus of the stria terminalis (BNST) have been shown to modulate cue-induced ethanol-seeking behavior. It is hypothesized that cue-induced seeking is communicated through a direct projection from the BNST to VTA. In the current experiments, an intersectional viral strategy was used in DBA/2J mice to selectively target and inhibit BNST projections to the VTA during a test of ethanol conditioned place preference (CPP). Inhibitory designer receptors exclusively activated by designer drugs (hM4Di DREADDs) were expressed in VTA-projecting BNST (BNST-VTA) cells by infusing a retrograde herpes-simplex virus encoding cre recombinase (HSV-Cre) into VTA and a cre-inducible adeno-associated virus encoding hM4Di (AAV-DIO-hM4Di) into BNST. Before testing the expression of preference, clozapine-N-oxide (CNO) was peripherally administered to activate hM4Di receptors and selectively inhibit these cells. Ethanol CPP expression was blocked by CNO-mediated inhibition of BNST-VTA cells. A follow-up study revealed this effect was specific to CNO activation of hM4Di as saline- and CNO-treated mice infused with a control vector (HSV-GFP) in place of HSV-Cre showed significant CPP. These findings establish a role for a direct BNST input to VTA in cue-induced ethanol-seeking behavior.

1. Introduction

It is thought that mesocorticolimbic dopamine (DA) transmission directs reward-related behaviors and is a prime neural signal driving drug seeking and relapse. Originating predominantly from within the ventral tegmental area (VTA), this DA signal has been the focus of many studies. The bulk of these studies have centered on neural circuits originating within the VTA and projecting to outside targets such as the nucleus accumbens (reviewed in Ikemoto, 2007). However, attention to VTA afferents and their influence in generating diverse motivational states is growing.

Evidence is emerging that one source of input to VTA, the extended amygdala, is highly involved in regulating VTA-mediated states of reward and aversion. Specifically, the bed nucleus of the stria terminalis (BNST) of the extended amygdala sends strong projections to VTA (Dong & Swanson, 2004; 2006a; 2006b; Kudo et al., 2012) that potently innervate DA cells (Georges & Aston-Jones, 2001, 2002) and inhibit gamma-aminobutyric acid (GABA) cells (Jennings et al., 2013; Kudo et al., 2014). Behavioral evidence further implicates the BNST in drug seeking induced by exposure to stress- and drug-associated cues. Inactivation of this region has been shown to impair drug- and cue-primed reinstatement of heroin seeking (Rogers, Ghee, & See, 2008) as well as stress- and cue-induced reinstatement of cocaine seeking (Buffalari & See, 2011), and cocaine conditioned place preference (CPP) expression (Sartor & Aston-Jones, 2012). The BNST appears to be also involved in ethanol seeking. Not only is this structure activated by ethanol-associated cues (Dayas, Liu, Simms, & Weiss, 2007; Hill, Ryabinin, & Cunningham, 2007; Zhao et al., 2006), but its direct inhibition impairs the expression of an ethanol place preference (Pina, Young, Ryabinin, & Cunningham, 2015).

It is unclear however whether the BNST's involvement in cue-induced drug seeking is driven by its input to the VTA. Previous studies attempted to address this question using a combination of tract tracing and c-Fos staining or intracranial pharmacological manipulations. For example, retrogradely labeled BNST projections to the VTA showed enhanced c-Fos immunoreactivity following cue-induced cocaine seeking and cocaine CPP expression (Mahler

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http://dx.doi.org/10.1016/j.nlm.2016.11.013
1074-7427/

& Aston-Jones, 2012; Sartor & Aston-Jones, 2012). Pharmacological disconnection procedures have also shown that a BNST-VTA projection is involved in cocaine CPP expression (Sartor & Aston-Jones, 2012) and stress-induced cocaine seeking (Vranjkovic, Gasser, Gerndt, Baker, & Mantsch, 2014).

Though informative, these studies involved methodologies that do not allow direct circuit-selective manipulation during seeking behavior. Recent advances in viral-mediated gene transfer have provided an unprecedented opportunity for not just cell-type selective targeting but also circuit-selective targeting. Most notably, an intersectional strategy involving infusion of two viruses – a retrograde vector encoding cre recombinase and a cre-dependent adeno-associated virus (AAV) vector – into two distinct yet directly connected nuclei has provided a means for discrete circuit modulation. This approach has been successfully employed in rats using a cre-encoding canine adenovirus-2 (CAV-2) to express designer receptors exclusively activated by designer drugs (DREADD) in projection neurons to and from the VTA (Boender et al., 2014; Nair, Strand, & Neumaier, 2013). This approach enables the direct manipulation of VTA circuit activity during the performance of behavioral tasks. A similar strategy has also been applied in mice using an HSV vector to express channelrhodopsin-2 in distinct populations of VTA efferents (Fenno et al., 2014; Stamatakis et al., 2013).

In the present experiments, we investigated the role of a direct neuronal projection from the BNST to VTA in seeking behavior induced by an ethanol-associated cue. We employed a convergent dual-virus strategy to selectively manipulate BNST-VTA cells in mice during ethanol-seeking behavior. An HSV encoding cre recombinase (HSV-Cre) was combined with a cre-inducible AAV encoding the inhibitory hM4Di DREADD (AAV-DIO-hM4Di). In this manner, hM4Di expression was restricted to BNST-VTA projection neurons, enabling us to inhibit their activity during ethanol seeking modeled in a CPP procedure. We hypothesized that inactivation of BNST-VTA cells during the CPP test would disrupt ethanol place preference expression, thus suggesting that ethanol seeking is conveyed through a direct BNST projection to VTA.

2. Materials and methods

2.1. Animals

Male DBA/2J mice (n = 116; The Jackson Laboratory, Sacramento, CA) were 7 weeks old at arrival. Mice were housed 4/cage in a colony room maintained at 21 ± 1 °C set to a 12:12 light-dark cycle (lights on at 07:00 am). Home cage access to food and water was provided *ad libitum* throughout all experiments. All procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 2011) and were approved by Oregon Health & Science University's Institutional Animal Care and Use Committee and Institutional Biosafety Committee.

2.2. Drugs

Ethanol (20% v/v in 0.9% saline) was administered intraperitoneally (IP) at a dose of 2 g/kg (12.5 mL/kg).

To stimulate hM4Di receptors, clozapine-N-oxide (CNO; Tocris Bioscience, Ellisville, MO) dissolved in 0.9% saline was administered at a dose of 10 mg/kg (10 mL/kg, IP) 30 min before the CPP test. In the absence of DREADD expression, this dose of CNO produces no physiological or behavioral response in rodents (Mahler et al., 2014; Ray et al., 2011, 2013; Vazey & Aston-Jones, 2014). In addition, we have shown that in the absence of hM4Di, CNO (10 or 20 mg/kg) does not affect ethanol CPP expression or locomotor activity in DBA/2J mice (Pina et al., 2015).

2.3. Stereotaxic surgery

Mice were anesthetized with isoflurane (4% in O_2 for induction) and secured in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Anesthesia was maintained (1–3% isoflurane in O_2) for the duration of the procedure. To minimize post-operative discomfort, meloxicam (0.2 mg/kg; 10 mL/kg) was subcutaneously delivered immediately before and 24 h after surgery. Stereotaxic coordinates for VTA and BNST were derived from a standard atlas of the mouse brain (Paxinos & Franklin, 2001) and both regions were targeted during the same surgery. For VTA, injectors were aimed at the more medial aspect of this region (from bregma: posterior (AP) −3.2, lateral (ML) ± 0.5, ventral (DV) −4.69). To avoid the lateral ventricles, the BNST was approached at a 20° coronal angle. Starting coordinates for BNST were as follows: AP + 0.26, ML ± 0.8, DV − 4.07 from bregma. Entry holes were drilled in the skull ±2.3 mm lateral and +0.26 mm rostral to bregma. The head was then tilted 20° left or right on a coronal axis and an injector was lowered 4.33 mm from the top of the skull on each side. Vectors were delivered using 32-ga stainless steel injectors (26-ga encasing) attached via polyethylene tubing (PE-20) to 1 μl Hamilton syringes. Infusions were delivered by syringe pump (Harvard Apparatus, Plymouth Meeting, PA) at a rate of 20 nL/min and injectors were left in place for 5 min after infusions. A post-surgical delay of 8 weeks was used. This delay was based on the following: (1) VTA to BNST retrograde transport and full transgene expression were achieved within 2 weeks of HSV-GFP delivery (Supplementary Fig. 1); (2) Axonal transport of most AAV serotypes occurs within 4–9 weeks (Castle, Gershenson, Giles, Holzbaur, & Wolfe, 2014; Salegio et al., 2012; Smith, Bucci, Luikart, & Mahler, 2016); and (3) our previous work has demonstrated robust AAV-mediated hM4Di expression in soma and axons of BNST neurons within 6 weeks (Pina et al., 2015).

2.4. Viral-mediated gene transfer

To inhibit the BNST-VTA circuit, hM4Di DREADDs (Armbruster, Li, Pausch, Herlitze, & Roth, 2007) were selectively expressed in BNST-VTA cells. Transgene expression in this discrete subset of BNST neurons was achieved using a retrograde intersectional approach that combines two viral vectors (Fig. 1). First, a retrograde HSV-Cre vector was infused into the VTA to drive long-term expression of cre-recombinase in VTA-projecting cells. Next, cre-inducible AAV-DIO-hM4Di was delivered into the BNST to selectively express hM4Di in VTA-projecting BNST cells.

2.4.1. Herpes simplex virus (HSV) vector

HSV vectors were purchased from Massachusetts Institute of Technology's Viral Gene Transfer Core (Cambridge, MA) and infused bilaterally into VTA (200 nL/side). HSV encoding for cre recombinase (HSV-Cre; hEF1α-EGFP-IRES-Cre, >3 × 10e8 TU/mL) was used in Exp. 1, whereas a control HSV carrying an enhanced green fluorescence protein-encoding gene (HSV-GFP; hEF1α-EGFP, >3 × 10e8 TU/mL) was used in Exp. 2. The control HSV-GFP vector served as a (1) retrograde tracer to determine the post-infusion delay required for maximal transgene expression in BNST (Supplementary Fig. 1), and a (2) control vector to test for non-specific effects of surgery and transgene expression on behavior (Exp. 2).

2.4.2. Adeno-associated virus (AAV) vector

In all experiments, AAV8-hSyn-DIO-hM4D(Gi)-mCherry (AAV-DIO-hM4Di; 5–7 × 10e12 vg/mL, University of North Carolina