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The bed nucleus of the stria terminalis regulates ethanol-seeking behavior in mice

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ABSTRACT

Drug-associated stimuli are considered important factors in relapse to drug use. In the absence of drug, these cues can trigger drug craving and drive subsequent drug seeking. One structure that has been implicated in this process is the bed nucleus of the stria terminalis (BNST), a chief component of the extended amygdala. Previous studies have established a role for the BNST in cue-induced cocaine seeking. However, it is unclear if the BNST underlies cue-induced seeking of other abused drugs such as ethanol. In the present set of experiments, BNST involvement in ethanol-seeking behavior was assessed in male DBA/2J mice using the conditioned place preference procedure (CPP). The BNST was inhibited during CPP expression using electrolytic lesions (Experiment 1), co-infusion of $GABA_A$ and $GABA_B$ receptor agonists muscimol and baclofen (M + B; Experiment 2), and activation of inhibitory designer receptors exclusively activated by designer drugs (hM4Di-DREADD) with clozapine-N-oxide (CNO; Experiment 3). The magnitude of ethanol CPP was reduced significantly by each of these techniques. Notably, infusion of M + B (Exp. 2) abolished CPP altogether. Follow-up studies to Exp. 3 showed that ethanol cue-induced c-Fos immunoreactivity in the BNST was reduced by hM4Di activation (Experiment 4) and in the absence of hM4Di, CNO did not affect ethanol CPP (Experiment 5). Combined, these findings demonstrate that the BNST is involved in the modulation of cue-induced ethanol-seeking behavior.

1. Introduction

Drug addiction is a chronic disorder characterized by periods of abstinence and relapse, where relapse to use is often preceded by intense desire for the drug (craving) and the subsequent motivation to obtain the drug (seeking). It is known that environmental contexts and discrete cues therein contribute to relapse by triggering craving (Ehrman et al., 1992; Grant et al., 1996; Sinha and Li, 2007) and driving drug seeking even after sustained periods of abstinence or extinction (Ciccocioppo et al., 2001a, 2001b; Crombag and Shaham, 2002; Krank and Wall, 1990; Weiss et al., 2001; Zironi et al., 2006). These cues become associated with the rewarding and aversive properties of drugs through a Pavlovian learning process. It is the result of this learning, in addition to drug exposure, that leads to alterations in neural structures associated with motivation and reward.

Over the past several decades progress has been made in identifying the neurobiological substrates underlying drug craving and seeking. One neural structure routinely implicated in relapse and drug-seeking processes is the bed nucleus of the stria terminalis (BNST), a chief component of the extended amygdala (Alheid, 2003). Anatomically, the BNST is a complex cluster of nuclei and there is some disagreement regarding the total number of

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subdivisions and their boundaries (Ju and Swanson, 1989; Moga et al., 1989). However, it is clear that the dorsal and ventral subdivisions of the BNST (dBNST and vBNST) send dense projections to the ventral tegmental area (VTA; Dong and Swanson, 2004, 2006a, 2006b; Kudo et al., 2012; Mahler and Aston-Jones, 2012), a region critical for reward seeking (Adamantidis et al., 2011; Bechtholt and Cunningham, 2005; Di Ciano and Everitt, 2004). Moreover, these inputs appear to potently innervate VTA dopamine (DA) neurons (Georges and Aston-Jones, 2001, 2002), leading to their phasic excitation, which is a neural process fundamental to motivated behavior (Adamantidis et al., 2011; Schultz, 1986; Wanat et al., 2009).

Presentation of drug-associated stimuli leads to pronounced activation in dBNST and vBNST, as indicated by increased c-Fos immunoreactivity (Hill et al., 2007; Mahler and Aston-Jones, 2012). In addition, pharmacological inactivation of several BNST subdivisions has been shown to reduce drug-seeking behavior induced by conditioned cue exposure. For example, inactivating the vBNST blocked the expression of cocaine-induced conditioned place preference (CPP; Sartor and Aston-Jones, 2012). Likewise, inactivation across dBNST and vBNST has been shown to block cue-induced reinstatement of cocaine seeking (Buffalari and See, 2011). In other studies, it appears that vBNST inactivation blocks heroin-primed reinstatement while medial posterior BNST inactivation blocks heroin and cue-primed reinstatement (Rogers et al., 2008). These findings support a role for the BNST in cue-induced drug seeking and suggest that the involvement of distinct subdivisions may vary by drug of abuse.

As illustrated by the above studies, a broad range of work has identified the BNST as an important candidate neural structure involved in relapse. However, the majority of this work has examined cue-induced seeking of cocaine and heroin. Therefore, it is not known whether these findings extend to other drugs such as ethanol. Previously, our lab identified the BNST as one of several areas activated by presentation of an ethanol-associated cue (Hill et al., 2007). Beyond this, little evidence exists to indicate that the BNST is involved in cue-induced ethanol-seeking behavior. Hence, our goal was to directly examine this region in the context of cue-induced ethanol seeking using an ethanol-induced CPP procedure that has been well-established by our laboratory (Cunningham et al., 2006).

To evaluate the BNST in ethanol seeking, we used electrolytic lesions, pharmacological inactivation, and chemogenetic inhibition. Given the limitations inherent to each of these intracranial manipulations, we sought to increase the generality of our conclusions by incorporating all three techniques. These manipulations were intended to inhibit BNST activity during ethanol CPP expression. Based on the existing literature, we reasoned that inhibiting the BNST by each of these techniques would disrupt ethanol place preference expression.

2. Materials and methods

2.1. Animals

Adult male DBA/2J mice (n = 214) were purchased from Jackson Laboratory (Sacramento, CA) at 6–7 weeks of age. Mice were housed 2–4 per cage in a colony room maintained at 21 ± 1 °C on a 12:12 light–dark cycle with lights on at 07:00 am. Food and water were available *ad libitum* in home cages throughout the experiment. Surgeries were performed on mice 6–11 weeks of age. All procedures were carried out in accordance 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 the Oregon Health & Science University Institutional Animal Care and Use Committee.

2.2. Drugs

Ethanol (95%) was prepared 20% v/v in a solution of 0.9% saline and administered intraperitoneally (IP) at a dose of 2 g/kg in a 12.5 mL/kg volume.

In exp. 2, the BNST was transiently inactivated using a cocktail of the $GABA_A$ and $GABA_B$ receptor agonists muscimol and baclofen (M + B; Sigma–Aldrich, St. Louis, MO). Muscimol (0.1 mM) and baclofen (1.0 mM) were dissolved in 0.9% saline and administered bilaterally (100 nL/side) into the BNST. Inactivation of the BNST with these concentrations has previously been shown to reduce cue-induced cocaine and heroin seeking (Buffalari and See, 2011; Rogers et al., 2008). Infusions were delivered over 60 s and injectors were left in place for an additional 30 s to allow for complete diffusion of drug from the injectors.

In exps. 3, 4, and 5, clozapine-N-oxide (CNO; Tocris Bioscience, Ellisville, MO) was dissolved in 0.9% saline and administered at 10 or 20 mg/kg (10 mL/kg, IP) 30 min before the CPP test. These doses were selected based on the following considerations. First, compared to Gq-coupled (hM3Dq) DREADDs, which are very effective at eliciting neuronal firing, Gi-coupled (hM4Di) DREADDs are reportedly less effective at inhibiting activity and may therefore require higher CNO doses (Farrell and Roth, 2013). Moreover, these doses were based on previously published studies showing that CNO alone produced no physiological or behavioral response in rodents at doses of 10 mg/kg and above (Li et al., 2013; Mahler et al., 2014; Ray et al., 2011, 2013; Vazey and Aston-Jones, 2014). Finally, a maximum dose of 20 mg/kg was specifically chosen for a control experiment as it was, to our knowledge, the highest reported in the literature (Mahler et al., 2014).

2.3. Stereotaxic surgery

2.3.1. General procedure

In exps. 1–4, mice were anesthetized with isoflurane (1–4% in O_2) and placed in a stereotaxic apparatus (Model No. 1900; Kopf Instruments, Tujunga, CA). Non-steroidal anti-inflammatory drugs, meloxicam (0.2 mg/kg) or carprofen (0.1 mg/kg) were injected subcutaneously (in 10 mL/kg) immediately before and 24 h after surgery to minimize post-operative discomfort. Coordinates targeting the BNST (from bregma: AP +0.14, L ±0.8, DV −4.25) were used in exps. 1A, 1B and 2 based on a standard mouse brain atlas (Paxinos and Franklin, 2001). In exps. 3 and 4, the lateral ventricles were avoided during virus infusions by approaching the BNST at a 20° angle. Burr holes were drilled laterally ±2.3 mm from bregma (AP +0.26) and injectors were lowered 4.33 mm from the top of the skull. These values were derived from the atlas-based coordinates: AP +0.26, ML ±0.8, DV −4.07.

2.3.2. Electrolytic lesions

In exps. 1A and 1B, electrodes (Rhodes Medical Instruments, Woodland Hills, CA) were lowered bilaterally into the BNST to administer electrolytic (0.5 mA for 10 s) or sham (no current) lesions (Model 3500; Ugo Basile, Varese, Italy). Due to reduced body weights in the BNST lesioned group, mice were given 8–13 days of recovery before the start of conditioning (exp. 1A) or the CPP test (exp. 1B). This recovery period ensured weights between lesioned and sham mice were comparable.

2.3.3. Cannulations

In exp. 2, bilateral cannulae (10 mm, 25 ga) were implanted 2.0 mm above the BNST and held in place with carboxylate cement (Durelon™, 3M, St. Paul, MN) anchored to the skull with stainless