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Inactivation of the lateral orbitofrontal cortex increases drinking in ethanol-dependent but not non-dependent mice

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ABSTRACT

Long-term consumption of ethanol affects cortical areas that are important for learning and memory, cognition, and decision-making. Deficits in cortical function may contribute to alcohol-abuse disorders by impeding an individual's ability to control drinking. Previous studies from this laboratory show that acute ethanol reduces activity of lateral orbitofrontal cortex (LOFC) neurons while chronic exposure impairs LOFC-dependent reversal learning and induces changes in LOFC excitability. Despite these findings, the role of LOFC neurons in ethanol consumption is unknown. To address this issue, we examined ethanol drinking in adult C57Bl/6J mice that received an excitotoxic lesion or viral injection of the inhibitory DREADD (designer receptor exclusively activated by designer drug) into the LOFC. No differences in ethanol consumption were observed between sham and lesioned mice during access to increasing concentrations of ethanol (3–40%) every other day for 7 weeks. Adulterating the ethanol solution with saccharin (0.2%) or quinine (0.06 mM) enhanced or inhibited, respectively, consumption of the 40% ethanol solution similarly in both groups. Using a chronic intermittent ethanol (CIE) vapor exposure model that produces dependence, we found no difference in baseline drinking between sham and lesioned mice prior to vapor treatments. CIE enhanced drinking in both groups as compared to air-treated animals and CIE treated lesioned mice showed an additional increase in ethanol drinking as compared to CIE sham controls. This effect persisted during the first week when quinine was added to the ethanol solution but consumption decreased to control levels in CIE lesioned mice in the following 2 weeks. In viral injected mice, baseline drinking was not altered by expression of the inhibitory DREADD receptor and repeated cycles of CIE exposure enhanced drinking in DREADD and virus control groups. Consistent with the lesion study, treatment with clozapine-N-oxide (CNO) further enhanced consumption only in CIE exposed DREADD mice with no change in air-treated mice. These results suggest that the LOFC is not critical for the initiation and maintenance of ethanol drinking in non-dependent mice, but may regulate the escalated drinking observed during dependence.

1. Introduction

Alcohol is regularly consumed by a large (~70%) portion of the U.S. population and mortality due to alcohol-related causes is the third leading preventable cause of death in the United States (NSDUH, 2013). A large number of factors contribute to the risk for alcohol related problems including genetic factors, duration and intensity of drinking and co-occurrence of psychiatric disease and other illnesses. Elucidating how these and other factors contribute to an individual's risk for developing an alcohol use disorder is important for developing more effective treatments for alcohol

Abbreviations: LOFC, lateral orbitofrontal cortex; CIE, chronic intermittent ethanol; CNO, clozapine-N-oxide; DREADD, designer receptor exclusively activated by designer drug; GFP, green fluorescent protein; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; NMDA, N-methyl-D-aspartate; VTA, ventral tegmental area.

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abuse. Alcohol, like other addictive substances, activates midbrain reward-sensitive dopamine neurons that are designed to reinforce and guide responses to natural rewards such as food and social behavior (Hyman and Malenka, 2001). However, as drinking escalates, these reward pathways may become less important in driving alcohol intake as brain areas implicated in habitual and/or compulsive behaviors are engaged. These include striatal regions and those within prefrontal (PFC) and orbitofrontal (OFC) cortex that send and receive extensive projections to limbic and motor areas and that are critical for cognition, learning, and control over adverse and risky behaviors (Fuster, 2008). Although cortical regions including the OFC have long been suspected as being particularly impacted by alcohol in humans, until recently, few preclinical studies had examined the effects of acute and chronic ethanol exposure on this region.

In humans, the OFC is subdivided into several areas including Brodmann areas 10/11/13 and 47 (Fuster, 2008). In rodents, analogous regions have been described and include medial, ventrolateral and lateral subdivisions (Heidbreder and Groenewegan, 2003). The OFC receives inputs from all major sensory areas with especially robust inputs from olfactory and gustatory centers as well as those that signal emotional valence (e.g. amygdala). OFC neurons project widely to sub-cortical structures involved in reward evaluation and motor planning including ventral (nucleus accumbens, NAc) and dorsal striatum (DS) and ventral tegmental area (VTA) (Watabe-Uchida et al., 2012). Inactivating the OFC in mice disrupts goal-directed responding for food while leaving habit-based responses in the same animal intact (Gremel and Costa, 2013). Similarly, contralateral lesions of the lateral OFC and ventral dorsal lateral striatum prevented satiety-induced devaluation for food reward in mice (Gourley et al., 2013) and a similar effect was reported in monkeys following pharmacological inactivation of the lateral OFC (BA13) (West et al., 2011). These findings suggest that lateral OFC neurons are important in mediating reward-based behaviors through interactions with striatal and midbrain circuits. At the cellular level, results from ex vivo slice electrophysiology studies show that action potential spiking of LOFC neurons is inhibited by relevant concentrations of ethanol (U.S legal limit for intoxication is 0.08% or ~17 mM) via a glycine receptor dependent mechanism (Badanich et al., 2013). Following repeated cycles of chronic intermittent ethanol (CIE) exposure that induces dependence (Lopez and Becker, 2005; Griffin et al., 2009), LOFC neurons are hyperexcitable, show changes in markers of glutamatergic synaptic plasticity and are less sensitive to inhibition by acute ethanol (Nimitvilai et al., 2016). CIE exposed mice have also been shown to be impaired during the reversal phase of a naturalistic food foraging task shown to require the LOFC (Badanich et al., 2011; Bissonette et al., 2008).

While these findings clearly indicate that LOFC neurons are an important target for acute and chronic ethanol, little is known regarding whether LOFC neurons regulate voluntary ethanol consumption. In this study, we used two different mouse models of ethanol consumption and excitotoxic lesions and expression of inhibitory DREADD receptors to test if inactivation of the LOFC alters ethanol consumption. The results suggest that LOFC is not involved in initiating or maintaining baseline drinking in non-dependent mice but may serve to limit drinking during the development of dependence.

2. Materials and methods

2.1. Experimental subjects

C57BL/6 J male mice were purchased from the Jackson Laboratory (Bar Harbor, ME) at 6–8 weeks of age and given at least one week to acclimate to the colony room before being used. Colony and testing rooms were maintained on a reverse light-dark cycle (lights off 9:00 a.m.). All procedures were carried out under protocols approved by the MUSC Institutional Animal Care and Use Committee and conform to NIH guidelines.

2.2. Surgeries

Neurotoxic lesions of the lateral OFC were generated using injections of N-methyl-D-aspartate (Sigma Aldrich, St Louis, MO) dissolved in 0.9% saline (Sigma Aldrich) at a concentration of 20 mg/ml (Bissonette et al., 2008). For sham lesions, an equivalent volume of 0.9% saline was injected. Coordinates for bilateral OFC lesions were: anterior-posterior, +2.6 mm from bregma; medial-lateral, ±1.2 mm; dorsal-ventral, 2.2 from dura (Fig. 1). At the injection site, 0.35 μL of N-methyl-D-aspartate or saline was injected at a rate of 0.1 μL/min, and the needle was left in place for 3 min. For viral expression studies, animals were injected at a rate of 0.1 μL/min with 0.3 μl of an AAV virus (UNC Viral Core) encoding the inhibitory (Syn-hM4Di-mCherry) DREADD receptor. Sham animals received an injection of a control AAV virus expressing green fluorescent protein (GFP). For both lesion and viral studies, mice were single housed after surgery and allowed 2 weeks to recover before beginning the drinking studies.

2.3. Histological verification

At the end of the experiments mice that had received NMDA infusions were euthanized with a lethal dose of pentobarbital and perfused transcardially with 0.9% saline solution followed by 4% formaldehyde. The brain was removed and fixed overnight in 4% formaldehyde followed by 30% sucrose-0.1 M PBS for 48 h. The brains were frozen and cut coronally in 30 μm slices using a Thermo Scientific Microm HM550 cryostat. To identify the lesion site, sections were processed for glial fibrillary acidic protein (GFAP; 1:500 dilution) and images of GFAP staining were collected using an Evos epifluorescent microscope (ThermoFisher, Rochester, NY). For viral injected animals, mice were euthanized by isoflurane anesthesia followed by rapid decapitation and brain slices (250 μm) were prepared using a Leica VT-1000S vibratome as previously described (den Hartog et al., 2013). Images of the fluorescent marker (e.g. mcherry, GFP) were collected using an Evos epilfluorescent microscope and used to assess viral expression.

2.4. Ethanol drinking

Mice were individually housed for at least 2 weeks prior to initiating drinking and food was provided *ad libitum* at all times. Where appropriate, the placement of the drinking bottles was alternated for each session to control for side preferences and mice were weighed weekly. Sham cages had drinking tubes but no mice to account for accidental spillage or loss of fluid. Two drinking paradigms were used to assess the effects of OFC lesions on ethanol consumption.

2.4.1. Intermittent access drinking

OFC- and sham-lesioned mice were given intermittent access to one bottle of ethanol and one bottle of tap water during 24-h sessions. Mice received two water bottles on intervening days. Mice were serially offered 3%, 6%, 10%, 15% ethanol (v/v in water) on successive sessions and then 20% ethanol for 13 sessions (26 d). Ethanol was then increased to 40% (v/v) for 8 sessions (16 d). To test how tastants affect drinking, mice were then offered 40% ethanol sweetened with 0.02% saccharin for 8 sessions (16 d) followed by 40% ethanol mixed with 0.06 mM quinine for 5 sessions (10 d).