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Ablation of μ opioid receptor-expressing GABA neurons in rostromedial tegmental nucleus increases ethanol consumption and regulates ethanol-related behaviors

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ABSTRACT

There has been increasing interest in the rostromedial tegmental nucleus (RMTg), given its potential regulatory role in many aversion-related behaviors. The RMTg contains mostly GABAergic neurons, sends a dense inhibitory projection to dopamine neurons in the midbrain, and is rich with μ-opioid receptors (MOR). Like most addictive drugs, ethanol has both aversive and rewarding properties. However, the cellular mechanisms underlying the effects of ethanol, particularly the aversive effect that limits its intake are not well understood. Recent studies have linked aversion with synaptic inhibition of dopamine neurons in the ventral tegmental area. To determine a potential role that the RMTg plays in the effect of ethanol, in this study, we employed a neurotoxin, dermorphin-saporin (DS), to lesion RMTg neurons prior to assessing ethanol-related behaviors. Rats were infused with DS bilaterally into the RMTg. This manipulation substantially increased the intake and preference for ethanol but not sucrose. It also reduced the number of neurons with MOR and glutamic acid decarboxylase 67 immunoreactivity within the RMTg. These changes did not occur after intra-RMTg infusion of blank saporin or vehicle. Importantly, intra-RMTg DS infusion significantly enhanced expression of conditioned place preference induced by ethanol (2 g/kg, i.p.), and slowed the extinction process. These results suggest that MOR-expressing GABAergic neurons in the RMTg contribute significantly to the regulation of ethanol consumption and related behaviors.

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1. Introduction

Alcohol use disorders are a serious economic and health problem in our society, but the underlying neurobiological mechanisms are not completely understood. Ethanol has both rewarding and aversive properties, and a balance between them may influence ethanol consumption. Ethanol's rewarding property has been linked to its ability to increase the activity of dopamine neurons in the ventral tegmental area (VTA) and the release of dopamine in the target projection regions (Di Chiara, 1998; Nicola and Malenka, 1997; Thomas et al., 2001), however, significantly less is known about its aversive property.

There has been increasing interest in the rostromedial tegmental nucleus (RMTg), because it encodes aversive signals, is excited by various aversive stimuli (Jhou et al., 2009a; Lecca et al., 2012), and its activation produces conditioned aversion (Jhou et al., 2013). The RMTg exhibits distinct neuroanatomical, physiological, and behavioral properties. It consists of GABAergic neurons that project intensively to midbrain dopamine neurons, and exerts a major inhibitory drive on the dopamine system (Lecca et al.,

Abbreviations: aCSF, artificial cerebrospinal fluid; AUDs, alcohol use disorders; BS, blank saporin; CLi, caudal linear nucleus of the raphe; CPP, conditioned place preference; DOR, delta opioid receptor; DS, dermorphin saporin; GAD67, glutamic acid decarboxylase 67; i.p., intraperitoneal; IPN, interpeduncular nucleus; IR, immunoreactive; LHb, lateral habenula; MnR, median raphe nucleus; MOR, mu opioid receptor; pVTA, posterior VTA; RMTg, rostromedial tegmental nucleus; s.c, subcutaneously; SEM, standard error of the mean; tth, trigeminothalamic tract; VTA, ventral tegmental area.

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2011). The RMTg could act as a hub that converges and integrates widespread multimodal signals towards the dopamine system (Lecca et al., 2011). Several lines of evidence have demonstrated that mu opioid receptors (MOR), but not delta opioid receptors (DOR), are the main opioid receptors in the RMTg. Histologically, high levels of MORs are one of the important markers that distinguish the RMTg from surrounding regions (Jhou et al., 2009a). Electrophysiological data demonstrated that the MOR agonist DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin) hyperpolarizes RMTg neurons that project to the VTA; but the delta-opioid agonist DPDPE (D-Ala2, D-Leu5 enkephalin) did not have a significant effect. Furthermore, only DAMGO, but not DPDPE, decreased the amplitude of the $GABA_A$ IPSCs evoked by RMTg stimulation in dopamine neurons (Matsui and Williams, 2011), suggesting that GABAergic transmission from the RMTg to VTA dopamine neurons is inhibited by mu, but not by delta opioid receptors.

The RMTg receives strikingly focused afferents from the lateral habenula (LHb), a key structure in the brain that regulates aversion-related behavior. LHb neurons are activated by a variety of aversive stimuli, such as stress, fear, disappointment, or negative prediction errors (Hikosaka, 2010; Lecourtier and Kelly, 2007; Matsumoto and Hikosaka, 2009; Stamatakis and Stuber, 2012). Stimulation of the LHb *in vivo* inhibits dopamine neurons, an action mediated by $GABA_A$ receptors (Ji and Shepard, 2007), probably via the activation of RMTg neurons. In the current study, we measured changes in MOR expression and drinking-related behaviors induced by intra-RMTg infusion of dermorphin-saporin (DS). Dermorphin has a much higher affinity for MORs than DORs, as reflected by the very different K_D values: 1.24 vs 78 nM (Krumins, 1987). DS could induce lesions in MOR-expressing neurons, including the GABA neurons in the VTA of rats (Reynolds et al., 2011; Shank et al., 2007). Here, we provide the evidence that ablation of RMTg neurons robustly increases ethanol intake and preference, as well as enhances ethanol-induced conditioned place preference. Our findings suggest that the RMTg plays a significant role in ethanol-related behaviors.

2. Materials and methods

2.1. Animals and housing

All procedures were approved by the Animal Care and Utilization Committee of Rutgers, the State University of New Jersey, in accordance with National Institutes of Health guidelines, minimizing the number of animals used and their suffering. All experiments were conducted on adult female Sprague–Dawley rats (250–350 g at the start of the experiments). The rats were individually housed. Food and water were available ad libitum unless otherwise indicated. We conducted the current study in female rats to take advantage of their stable head size, their higher alcohol drinking levels, and the request for more studies in females by the NIH (see Discussion for more).

2.2. Intermittent-access to 20% ethanol two-bottle free choice drinking procedure

We used the intermittent-access to 20% ethanol two-bottle free choice drinking procedure (I2BC) described previously (Li et al., 2011). Briefly, after acclimating to the homecage environment, all animals had 24 h concurrent access to two bottles, one with 20% ethanol (v/v) and another with water only, starting on Monday afternoon. After 24 h, the ethanol bottle was replaced with a second water bottle that was available for the next 24 h. This pattern was repeated on Wednesdays and Fridays. On all other days the rats had unlimited access to two bottles of water. In each ethanol drinking session, the placement of the ethanol bottle was alternated to control for side preferences.

The amount of fluid intake was determined by weighing the bottles before and after 24 h of access. Ethanol consumption was determined by calculating grams of ethanol consumed per kilogram of body weight. Two bottles, one containing water and one containing 20% ethanol, were placed in a cage without rats to evaluate the spillage due to the experimental manipulations during the test sessions. The spillage was always <1.0 ml (<2.5% of the total fluid intake) during 24 h. Animals in the control group were allowed unlimited access to water and food. Body weights of all rats were recorded weekly, and no significant difference between the control and the ethanol-drinking rats was found at the end of the experiments. Rats under the I2BC paradigm escalated their ethanol intake and preference, in keeping with previous report (Li et al., 2011). Animals in the control group were allowed access to water and food without limitation.

2.3. Intra-RMTg infusion

After a stable baseline drinking level had been reached (about 6–8 weeks in the I2BC paradigm), rats received bilateral intra-RMTg infusion (1.5 pmol/100 nl/side, 10 nl/min) of DS Advanced Targeting Systems, Inc. San Diego, CA, USA), or the equivalent amount of blank saporin (BS, Advanced Targeting Systems, Inc.) or artificial cerebrospinal fluid (aCSF) under isoflurane anesthesia at the following stereotaxic coordinates (in mm: AP:-6.8; ML:±0.8; DV:-7.9 from the skull's surface) according to the rat brain atlas (Paxinos and Watson, 2007). Following the infusion, the pipette remained in place for 10 min before being withdrawn. Thereafter the burr hole was sealed with sterile bone wax; and the scalp was sutured. Before recovery from anesthesia, rats were given the pain-killer meloxicam (1.0 mg/kg, s.c.), and returned to their home cage. Rats were allowed to recover for ≥7 days before resuming ethanol drinking, during which time they were handled daily. The same investigator executed all procedures.

2.4. Sucrose self-administration

A separate group (n = 6) of rats was trained to drink 2% sucrose (wt/vol) solution under an I2BC procedure, similar to that for ethanol described above. We selected 2% sucrose according to previous studies (Li et al., 2012; Nie et al., 2011). These rats had reached a stable baseline drinking level after 6 drinking sessions, and then received intra-RMTg infusion of DS, BS, or vehicle. Seven days after intra-RMTg infusion, these rats resumed sucrose drinking for an additional 12 sessions.

2.5. Ambulation measurement

We measured the non-habituated locomotor activity, as described (Li et al., 2012), under low-light conditions utilizing standard locomotor testing chambers (TruScan Photobeam Activity Monitors, 40 L × 40 W × 40H cm; Coulbourn Instruments, Whitehall, PA, USA). We quantified ambulation as the total distance (cm) traveled in the horizontal plane. The interruption of two consecutive photobeams resulted in a count of "ambulation" by the control software. On each testing day, the rats rested for 10 min after being transported into the testing room and then were placed in the assessment chamber where locomotion in the X–Y plane was assessed during a 60 min session.

2.6. Quantization of MOR and glutamic acid decarboxylase 67 immunoreactivity

The RMTg expresses high levels of the GABA synthesizing

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