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Research report

Involvement of ventral tegmental area ionotropic glutamate receptors in the expression of ethanol-induced conditioned place preference

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HIGHLIGHTS

- Ethanol seeking was modeled using a conditioned place preference (CPP) procedure.
- Intra-VTA infusion of an iGluR antagonist cocktail blocked ethanol CPP expression.
- At a high dose, intra-VTA iGluR antagonism increased basal locomotor activity.
- Findings show iGluR activity in VTA is necessary for ethanol CPP expression.

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ABSTRACT

The ventral tegmental area (VTA) is a well-established neural substrate of reward-related processes. Activity within this structure is increased by the primary and conditioned rewarding effects of abused drugs and its engagement is heavily reliant on excitatory input from structures upstream. In the case of drug seeking, it is thought that exposure to drug-associated cues engages glutamatergic VTA afferents that signal directly to dopamine cells, thereby triggering this behavior. It is unclear, however, whether glutamate input to VTA is directly involved in ethanol-associated cue seeking. Here, the role of intra-VTA ionotropic glutamate receptor (iGluR) signaling in ethanol-cue seeking was evaluated in DBA/2J mice using an ethanol conditioned place preference (CPP) procedure. Intra-VTA iGluRs α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR)/kainate and *N*-methyl-D-aspartate (NMDAR) were blocked during ethanol CPP expression by co-infusion of antagonist drugs 6,7-dinitroquinoxaline-2,3-dione (DNQX; AMPA/kainate) and D-(−)-2-Amino-5-phosphonopentanoic acid (AP5; NMDA). Compared to aCSF, bilateral infusion of low (1 DNQX + 100 AP5 ng/side) and high (5 DNQX + 500 AP5 ng/side) doses of the AMPAR and NMDAR antagonist cocktail into VTA blocked ethanol CPP expression. This effect was site specific, as DNQX/AP5 infusion proximal to VTA did not significantly impact CPP expression. An increase in activity was found at the high but not low dose of DNQX/AP5. These findings demonstrate that activation of iGluRs within the VTA is necessary for ethanol-associated cue seeking, as measured by CPP.

1. Introduction

Dopaminergic (DA) transmission within the mesocorticolimbic system is thought to play a key role in motivated behavior. The predominant source of central DA, the midbrain [1], has been the focus of considerable research aimed at understanding the neural events that promote reward seeking. Much of this work supports the idea that reward-related signals are predominantly generated by DA cells that originate in the substantia nigra (SN) and ventral tegmental area (VTA) [2].

For example, early work has established that midbrain DA neurons are phasically activated by primary rewards [3–5]. Remarkably, reward-predicting stimuli also appear to elicit similar levels of phasic DA cell firing. In fact, after training and formation of stimulus-reward associations, the activity of midbrain DA neurons is increased almost exclusively by conditioned stimuli and not the primary rewarding stimulus [3,6]. The idea that midbrain DA activity mediates reward and cue-induced motivated behavior is also supported by behavioral studies using animal models. For example, conditioned DA release in the nucleus accumbens (NAc) core

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has been observed following cocaine-associated cue presentation [7]. Accordingly, antagonism of DA D1-like receptors within the NAc reduces context-induced reinstatement of ethanol seeking [8]. Similarly, blockade of amygdala D1- and D2-like receptors inhibits cue-induced ethanol-seeking behavior as suggested by its disruption of ethanol conditioned place preference (CPP) expression [9].

Additional studies identify the VTA, as a region fundamental to primary reward and cue-induced reward seeking. For instance, VTA inactivation reduced the acquisition and expression of morphine-induced CPP [10]. Activating $GABA_B$ receptors in the VTA, which putatively inhibits DA activation, also reduced morphine-induced CPP acquisition [11] and ethanol-induced CPP expression [12]. Moreover, exposure to an ethanol-associated cue activated the VTA resulting in increased c-Fos immunoreactivity [13]. These studies illustrate the VTA's importance in the acute rewarding effects of morphine and conditioned rewarding effects of morphine and ethanol.

Although a role for VTA activity in cue-induced seeking behavior has been established, less is known about what neurochemical inputs are responsible for the excitation of VTA DA cells during drug-associated cue exposure. Considering that activity of VTA dopamine cells is regulated in part by several glutamatergic afferents [14], it is highly likely that glutamate may be involved. It has been suggested that glutamate input to the VTA may serve as a principal source of DA activation that is required for behaviorally relevant burst firing [15]. Some direct evidence does indeed indicate that glutamate input to the VTA plays a critical role in the motivational effects of abused drugs and drug-associated cues. For example, intra-VTA glutamate receptor antagonism blocked the development of place preference for environmental stimuli paired with cocaine and morphine [16,17]. Moreover, conditioned glutamate release in anticipation of cocaine delivery has been observed in VTA [18]. Taken together, this literature suggests that glutamate may serve as an important source of VTA DA innervation and is a likely signal driving cue-induced drug seeking. Despite this, few studies have assessed glutamatergic involvement in conditioned reward using ethanol as a primary reinforcer.

In the present experiment, we assessed whether glutamatergic input to the VTA was involved in the expression of ethanol-induced CPP. A well-characterized ethanol-induced CPP procedure [19] was used to establish an ethanol-cue association (acquisition) in order to evaluate the impact of iGluR antagonism on ethanol CPP expression. *N*-Methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors were blocked in the VTA during the ethanol-induced CPP expression test. Based on the existing literature, we hypothesized that blocking the action of this excitatory input to the VTA would reduce ethanol place preference expression.

2. Materials and methods

2.1. Animals

Male DBA/2J mice (n = 123; The Jackson Laboratory, Sacramento, CA) 6–7 weeks of age upon arrival were received in 4 separate shipments (n = 32–36/shipment). This inbred strain was chosen based on evidence from our laboratory showing that DBA/2J mice consistently develop robust ethanol-induced CPP [19]. Mice were housed in polycarbonate cages (2–4 per cage) lined with cob bedding in a colony room maintained at 21+/−1C on a 12:12 h light-dark cycle with lights on at 7:00 a.m. All procedures were conducted during the light phase (7:00 a.m.–7:00 p.m.). Mice were given approximately 1 week to acclimate to the colony before surgery. During this time, mice were housed in groups of four. After surgeries, mice were housed 2 per cage to reduce headmount damage and cannula loss from allogrooming. Home cage access to lab chow (5L0D PicoLab® Rodent Diet, St. Louis, MO) and water was provided ad libitum. Procedures were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee and carried out in compliance with the National Institutes of Health Guide For the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 2011).

2.2. Apparatus

Place conditioning was conducted using an unbiased two-compartment apparatus. Conditioning chambers (30 × 15 × 15 cm) composed of acrylic and aluminum were enclosed in ventilated boxes (Coulbourn Instruments Model E10-20) that attenuated light- and sound. Within each apparatus, six infrared phototransistors were used to detect locomotor activity and time spent on each side of the chamber. Infrared light emitting diodes were mounted opposite these detectors at 5-cm intervals, 2.2 cm above the floor on the front and rear sides of each inner chamber. During each session, locomotor activity and chamber position were continuously recorded by computer. Two distinct interchangeable floors placed inside the conditioning chamber served as tactile cues. Floors were characterized by a grid (2.3-mm stainless steel rods mounted 6.4 mm apart in an acrylic frame) or hole (16-ga. stainless steel sheets perforated with 6.4 mm diameter holes on 9.5-mm staggered centers) pattern. These floor cues are equally preferred by groups of experimentally naïve DBA/2J mice [20], demonstrating the unbiased nature of the apparatus. A removable clear acrylic divider was used to separate floor cues and partition the apparatus into two compartments. To disperse olfactory cues, floors and chambers were wiped clean with a damp sponge between animals. Additional details about the apparatus and procedure can be found elsewhere [19].

2.3. Drugs

Ethanol (95%; Decon Labs, King of Prussia, PA) was prepared in a 20% v/v solution of 0.9% saline and administered intraperitoneally (IP) at a dose of 2 g/kg in a 12.5 mL/kg volume. Vehicle injections of saline were also administered IP (12.5 mL/kg).

Stock solutions of the AMPA/kainate antagonist 6,7-Dinitroquinoxaline-2,3-dione disodium salt (DNQX; 1 mg/mL; Tocris, Minneapolis, MN) and NMDA antagonist D-(−)-2-Amino-5-phosphonopentanoic acid (AP5; 10 mg/mL; Tocris) were prepared in artificial cerebrospinal fluid (aCSF; Tocris). Aliquots were stored at −80 °C and diluted to final concentrations in aCSF then combined on the day of use. Drugs were administered as a cocktail in final doses (in ng/100 nL/side) of 1 DNQX + 100 AP5 (DNQX/AP5 1 group) and 5 DNQX + 500 AP5 (DNQX/AP5 5 group). Doses were chosen based on previously published intracranial work in mice [9,21,22].

2.4. Surgical procedure

Anesthesia was induced with 4% isoflurane (Terrell™, Piramal Critical Care Inc., Orchard Park, NY) and maintained with 1–3% in oxygen with a flow rate of 1 L/min. Mice were secured in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and guide cannulae (10 mm, 25 ga) were implanted 2.0 mm above the VTA (AP −3.2, ML ±0.5, DV −4.69 mm, from bregma) and held in place with permanent glass ionomer luting cement (Ketac-Cem Maxicap; 3 M ESPE, St. Paul, MN). Coordinates were derived from a standard mouse brain atlas [23] and selected based on literature suggesting that more medial aspects of the VTA are involved in approach behavior [24].