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OPTOGENETIC ACTIVATION OF AMYGDALA PROJECTIONS TO NUCLEUS ACCUMBENS CAN ARREST CONDITIONED AND UNCONDITIONED ALCOHOL CONSUMMATORY BEHAVIOR

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Abstract—Following a Pavlovian-pairing procedure, alcohol-paired cues come to elicit behavioral responses that lead to alcohol consumption. Here we used an optogenetic approach to activate basolateral amygdala (BLA) axonal terminals targeting the shell of nucleus accumbens (AcbSh) and investigated a possible influence over cue-conditioned alcohol seeking and alcohol drinking, based on the demonstrated roles of these areas in behavioral responding to Pavlovian cues and in feeding behavior. Rats were trained to anticipate alcohol or sucrose following the onset of a discrete conditioned stimulus (CS). Channelrhodopsin-mediated activation of the BLA-to-AcbSh pathway concurrent with each CS disrupted cued alcohol seeking. Activation of the same pathway caused rapid cessation of alcohol drinking from a sipper tube. Neither effect accompanied an overall change in locomotion. Finally, the suppressive effect of photoactivation on cue-triggered seeking was also evidenced in animals trained with sucrose. Together these findings suggest that photoactivation of BLA terminals in the AcbSh can override the conditioned motivational properties of reward-predictive cues as well as unconditioned consummatory responses necessary for alcohol drinking. The findings provide evidence for a limbic-striatal influence over motivated behavior for orally consumed rewards, including alcohol.

Key words: ethanol, Pavlovian, conditioning, reward, learning, appetitive, consummatory.

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Abbreviations: BLA, basolateral amygdala; ChR2, channelrhodopsin; CS, conditioned stimulus; D1R, dopamine D1-receptor; MSN, medium spiny neurons; PBS, phosphate-buffered solution; VI, variable interval.

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INTRODUCTION

The motivational properties of alcohol-associated cues contribute critically to the relapsing nature of alcohol use disorders (Brandon et al., 2007; Heinz et al., 2009; Schacht et al., 2013; Courtney et al., 2016). In humans, alcohol cues can increase self-report of craving, induce approach and attentional bias, impair behavioral control, and increase alcohol consumption (Field and Duka, 2002; Field and Cox, 2008; Jones et al., 2013; Kreusch et al., 2013; Jasinska et al., 2014; Wiers et al., 2014). Similarly, in animal models, alcohol-related cues can invigorate and reinstate instrumental seeking (Chaudhri et al., 2008a; Corbit and Janak, 2007, 2016; Dayas et al., 2008; Krank, 2003; Krank et al., 2008; Nie and Janak, 2003), elicit cue approach (Krank, 2003; Krank et al., 2008; Srey et al., 2015), and also trigger reward approach and consumption (Chaudhri et al., 2008b, 2010; Hauser et al., 2016; Knight et al., 2016; Krank, 2003; Krank et al., 2008; Millan et al., 2015; Remedios et al., 2014; Srey et al., 2015). In many cases, these multiple behavioral effects of alcohol-paired cues may be observed within the same behavioral session and within the same subjects (Krank et al., 2008; Srey et al., 2015). Recently we sought to isolate the impact of alcohol cues on alcohol approach and consumption using a Pavlovian design in which auditory cue presentations are followed by alcohol delivery; using this procedure, alcohol-paired cues increase entries into a recessed reward port where alcohol is delivered for oral consumption (Chaudhri et al., 2008b, 2010; Remedios et al., 2014; Sparks et al., 2014; Millan et al., 2015), allowing study of the neural mechanisms underlying cue-elicited alcohol approach and consumption.

We previously found that pharmacological inactivation of the basolateral amygdala (BLA) or the nucleus accumbens reduces these Pavlovian cue-elicited port entries for alcohol (Chaudhri et al., 2010, 2013; Millan et al., 2015). Multiple lines of evidence suggest that reward-paired cues impact reward seeking in part though activation of excitatory projections from the BLA to the nucleus accumbens (Ambroggi et al., 2008; Beyeler et al., 2016; Everitt et al., 1989, 1999; Gremel and Cunningham, 2008; Namburi et al., 2015). In this conception, excitation of BLA neurons by reward-paired cues would excite relevant populations of accumbens neurons

that in turn activate appetitive behavior, i.e., reward-seeking actions. In agreement with this, the onset of cue-evoked excitation in the BLA, measured as increases in spike rates of single units, generally precedes the onset of cue excitations in the accumbens, and pharmacological inactivation of the BLA reduces excitatory cue responses in the accumbens (Ambroggi et al., 2008). These findings might suggest that direct activation of BLA-to-accumbens projections would facilitate cue-elicited port entries for alcohol.

On the other hand, a substantial body of work implicates decreases, rather than increases, in nucleus accumbens neuronal firing, especially within the shell subregion (AcbSh), in the actual reward consummatory behavior itself (Kelley and Swanson, 1997; Krause et al., 2010; Maldonado-Irizarry et al., 1995; Nicola et al., 2004; O'Connor et al., 2015; Roitman et al., 2005; Stratford and Kelley, 1997; Taha and Fields, 2005, 2006). For example, in single-unit *in vivo* recording studies of accumbens neurons, considerably more inhibitions, measured as decreases in spike activity, than excitations are typically observed during oral consumption of sucrose (Day et al., 2006; Janak et al., 2004; Nicola et al., 2004; Wan and Peoples, 2008). These decreases in neural firing are observed time-locked to consummatory responses during opportunities for free-feeding and in the setting of conditioned behavior. For example, O'Conner and colleagues (2015) recently showed that spiking activity in dopamine D1-receptor (D1R)-expressing AcbSh medium spiny neurons (MSN) decreased upon initiation and during maintenance of liquid fat consumption from a sipper tube. In addition, when recording in a Pavlovian port-approach procedure, in which a light cue was paired with sucrose delivery into a recessed port, Wan and Peoples (2006) found that $>50\%$ of recorded accumbens neurons showed a long-lasting inhibition that paralleled the time in the reward port, with half of these inhibitions beginning after cue onset and before port entry. Since the subjects approached and entered the port during the cue prior to reward delivery, the inhibitions were proposed to potentially regulate the approach and port entry behavior as well as the subsequent consumption (Wan and Peoples, 2006). Similar long-lasting inhibitions in the nucleus accumbens during cue approach have been reported by Day and colleagues (2006) recording in a Pavlovian-autoshaping procedure. Importantly, observation of the onset of accumbal inhibitions and reward port entry and exit, as well as sipper tube contact, suggest that for the most part the inhibitions are permissive for consummatory behavior rather than directly correlated with motoric actions of licking (Nicola et al., 2004; Taha and Fields, 2005).

In agreement with the notion that accumbal inhibitions permit, or gate, consummatory behavior, classic studies demonstrated that pharmacological inhibition of the AcbSh with GABAergic agonists or AMPA glutamate receptor antagonists induces eating even in sated subjects (Maldonado-Irizarry et al., 1995; Kelley and Swanson, 1997; Stratford and Kelley, 1997; Kelley, 2004), and recent studies show that optogenetic inhibition of D1R MSNs does as well (O'Connor et al., 2015). Conversely, when targeted directly through electrical, optogenetic, or pharmacological means, activation of the AcbSh can diminish reward consumption (Stratford et al., 1998; Krause et al., 2010; O'Connor et al., 2015). A recent study reported that photoactivation of glutamatergic terminals in the AcbSh in mice decreased licking behavior for sucrose (Prado et al., 2016).

Because alcohol is consumed orally, one might expect similar inhibitory neural signals to accompany alcohol intake. Indeed, spike activity decreases during port entries when alcohol is consumed have been recorded in the AcbSh (Janak et al., 1999). Together, these findings support a suppressive influence for AcbSh neural activity over approach and consumption of rewards. The AcbSh receives excitatory glutamatergic inputs from multiple corticolimbic and thalamic sites, including the BLA (Kelley et al., 1982; McDonald, 1991; Brog et al., 1993; Thompson and Swanson, 2010), which are candidates for regulating AcbSh activity relevant to consumption. Taken with the experimental evidence provided above, the hypothesis that activation of excitatory BLA-to-AcbSh projections could decrease, not increase, both cue-elicited approach and consumption of oral alcohol emerges. Here we tested this idea.

We show that channelrhodopsin (ChR2)-mediated optogenetic activation of BLA–AcbSh innervation during the onset of an alcohol-predictive cue significantly disrupts Pavlovian cue-triggered alcohol approach under non-reinforced and reinforced test conditions. Second, this disruption is not specific for conditioned, cue-elicited behavior, as activation of this pathway can interrupt alcohol drinking itself. Finally, we show that the suppressive effect of BLA–AcbSh stimulation on conditioned behavior is generalizable to non-drug consummatory rewards. Taken together, these findings support the notion that distinct patterns of neural activity in the accumbens regulate appetitive and consummatory behaviors, with excitation biasing away from consummatory behavior, and provide new information on the neural regulation of cue-elicited alcohol intake.

EXPERIMENTAL PROCEDURES

Subjects

Experimentally naïve male Long-Evans rats (Harlan, IN; 200–215 g) were individually housed in ventilated polycarbonate cages in a temperature (21 °C) and light-regulated vivarium (lights on 7 am, 12-h light/dark cycle) with partial enrichment. Food and water were freely available throughout the duration of these studies. All procedures were approved by the institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized with isoflurane and infused bilaterally in BLA with adeno-associated viruses (AAV; $\sim 10^{12}$ infectious units ml^{-1}; UNC Viral Vector Core, Chapel Hill, NC, USA) expressing channelrhodopsin

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