Neuroscience 301 (2015) 384–394

DISTINCT EFFECTS OF VENTRAL TEGMENTAL AREA NMDA AND ACETYLCHOLINE RECEPTOR BLOCKADE ON CONDITIONED REINFORCEMENT PRODUCED BY FOOD-ASSOCIATED CUES

R. J. WICKHAM,[a] W. B. SOLECKI,[a] E. J. NUNES[a] AND N. A. ADDY[a,b,c*]

[a] *Interdepartmental Neuroscience Program, Yale Graduate School of Arts and Sciences, New Haven, CT 06520, USA*

[b] *Department of Psychiatry, Yale School of Medicine, New Haven, CT 06511, USA*

[c] *Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT 06511, USA*

Abstract—Stimuli paired with rewards acquire reinforcing properties to promote reward-seeking behavior. Previous work supports the role of ventral tegmental area (VTA) nicotinic acetylcholine receptors (nAChRs) in mediating conditioned reinforcement elicited by drug-associated cues. However, it is not known whether these cholinergic mechanisms are specific to drug-associated cues or whether VTA cholinergic mechanisms also underlie the ability of cues paired with natural rewards to act as conditioned reinforcers. Burst firing of VTA dopamine (DA) neurons and the subsequent phasic DA release in the nucleus accumbens (NAc) plays an important role in cue-mediated behavior and in the ability of cues to acquire reinforcing properties. In the VTA, both AChRs and N-methyl-D-aspartate receptors (NMDARs) regulate DA burst firing and phasic DA release. Here, we tested the role of VTA nAChRs, muscarinic AChRs (mAChRs), and NMDARs in the conditioned reinforcement elicited by a food-associated, natural reward cue. Subjects received 10 consecutive days of Pavlovian conditioning training where lever extension served as a predictive cue for food availability. On day 11, rats received bilateral VTA infusion of saline, AP-5 (0.1 or 1 μg), mecamylamine (MEC: 3 or 30 μg) or scopolamine (SCOP: 3 or 66.7 μg) immediately prior to the conditioned reinforcement test. During the test, nosepoking into the active (conditioned reinforced, CR) noseport produced a lever cue while nosepoking on the inactive (non-conditioned reinforced, NCR) noseport had no consequence. AP-5 robustly attenuated conditioned reinforcement and blocked discrimination between CR and NCR noseports at the 1-μg dose. MEC infusion decreased responding for both CR and NCR while 66.7-μg SCOP disrupted the subject's ability to discriminate between CR and NCR. Together, our data suggest that VTA NMDARs and mAChRs, but not nAChRs, play a role in the ability of natural reward-associated cues to act as conditioned reinforcers.

Key words: conditioned reinforcement, ventral tegmental area, nicotinic receptor, muscarinic receptor, NMDA receptor, incentive salience.

INTRODUCTION

The ability to make informed decisions about food availability is critical for an animal's survival and ultimate reproductive fitness. When an environmental stimulus is paired with food availability, animals learn to utilize this conditioned stimulus (CS) to guide their ongoing behavior. Conditioned stimuli have been demonstrated to powerfully promote both food-craving and food-seeking (Nicola et al., 2005; Ball et al., 2011; Grimm et al., 2011; Guy et al., 2011; Jastreboff et al., 2013) and drug-craving and drug-seeking (Sinha et al., 2000; Caggiula et al., 2001; Fuchs et al., 2006; Bossert et al., 2007; Liechti et al., 2007; Chaudhri et al., 2010) in both humans and in preclinical rat models. During conditioned reinforcement, subjects readily learn to perform a new operant action in order to receive a CS, which serves as a conditioned reinforcer (CR), in the absence of primary reward (Wolterink et al., 1993; Parkinson et al., 1999). It has also been proposed that reward-associated stimuli can acquire ''incentive salience,'' where the cues themselves become wanted or desired (Robinson and Berridge, 1993), and these incentive properties may further facilitate the ability of these cues to act as reinforcers. Thus, understanding the neurobiological mechanisms of conditioned reinforcement can provide important insights into the underlying processes that allow cues to powerfully guide motivated behavior.

Dopaminergic activity in the ventral tegmental area (VTA) to nucleus accumbens (NAc) pathway has been shown to play an important role in several cue-related behaviors (Wolterink et al., 1993; Schultz, 1998; Nicola et al., 2005; Bossert et al., 2007; Saunders and Robinson, 2012). The presentation of reward-associated cues leads to increased burst firing of dopamine (DA)

*Correspondence to: N. A. Addy, 34 Park Street, 3[rd] Floor Research, New Haven, CT 06520, USA. Tel: +1-203-737-5646; fax: +1-203-737-2043.
E-mail address: nii.addy@yale.edu (N. A. Addy).

Abbreviations: AP, anteroposterior; CR, conditioned reinforced; CS, conditioned stimulus; DA, dopamine; DV, dorsoventral; mAChRs, muscarinic AChRs; MEC, mecamylamine; ML, mediolateral; NAc, nucleus accumbens; nAChRs, nicotinic acetylcholine receptors; NCR, non-conditioned reinforced; NMDARs, N-methyl-D-aspartate receptors; PCA, Pavlovian Conditioning Approach; PPTg, pedunculopontine tegmentum; SCOP, scopolamine; VTA, ventral tegmental area.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.021
0306-4522/

neurons in the VTA (Schultz, 1998; Pan et al., 2005) and increased phasic DA release in the NAc (Phillips et al., 2003; Day et al., 2007; Sombers et al., 2009). Previous work has also demonstrated that DA release in the NAc is both necessary and sufficient for cue-mediated reward-seeking behavior (Nicola et al., 2005). Specifically, the concentration of cue-evoked phasic DA release is proportional to the ability of these cues to predict reward (Sugam et al., 2012) and also proportional to the magnitude of the predicted reward (Beyene et al., 2010). Dopaminergic activity in the NAc is also a critical mechanism underlying the ability of drugs of abuse to enhance conditioned reinforcement for natural reward-associated cues (Taylor and Robbins, 1984; Wolterink et al., 1993; Parkinson et al., 1999). Given the evidence that cue presentation is encoded by burst firing activity of VTA DA neurons and downstream phasic DA release in the NAc (Schultz, 1998; Phillips et al., 2003; Pan et al., 2005; Day et al., 2007; Sombers et al., 2009), it is likely that VTA mechanisms are also important for regulating the ability of cues to act as reinforcers. Indeed, recent studies have revealed VTA mechanisms involved in cue-induced drug-seeking behavior (Zhou et al., 2007; Lu et al., 2009; Solecki et al., 2013). However, there is limited understanding of the VTA mechanisms that underlie the ability of reward-associated cues to act as conditioned reinforcers.

In the VTA to NAc circuit, DA burst firing and phasic DA release is mediated by N-methyl-D-aspartate receptor (NMDAR) and acetylcholine receptor (AChR) mechanisms (Forster and Blaha, 2000, 2003; Grace et al., 2007; Sombers et al., 2009; Wickham et al., 2013) and recent evidence points toward a role for VTA NMDARs and AChRs in cue-dependent behavior. In particular, the mesopontine tegmentum (MPT), which contains the laterodorsal tegmentum (LDTg) and pedunculopontine tegmentum (PPTg), sends both cholinergic and glutamatergic projections to the VTA (Clements et al., 1991; Honda and Semba, 1995; Oakman et al., 1995; Takakusaki et al., 1996). PPTg inactivation has been shown to impair stimulus-reward learning, conditioned reinforcement (Inglis et al., 2000) and also impairs the ability of VTA DA neurons to burst fire in the presence of reward-predictive cues (Pan et al., 2005). Within the VTA, NMDARs have been shown to regulate the magnitude of phasic DA release to reward-associated cues (Sombers et al., 2009; Zweifel et al., 2009), and to alter cue-dependent reward learning (Zweifel et al., 2009). However, it is unclear whether VTA NMDARs play a role in the ability of cues to act as conditioned reinforcers. Importantly, a previous study demonstrated an important role for VTA AChRs in conditioned reinforcement for drug cues, as antagonism of VTA nAChRs decreased conditioned reinforcement for ethanol-associated cues (Lof et al., 2007). However, no studies have determined whether similar mechanisms also underlie the ability of natural reward-associated cues to act as conditioned reinforcers or whether AChR mechanisms are specific to drug-associated cues. Here, we tested the hypothesis that VTA AChR and NMDAR mechanisms mediate the ability of a natural reward-associated cue to act as a reinforcer.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague Dawley rats (250–350 g) were acquired from Charles River Laboratories (Wilmington, MA, USA) and were placed on ad libitum food and water. Animals were housed 2–3 per cage on a 12-h light/dark cycle (lights on at 7 am) and were allowed 1 week to acclimate to the facility prior to any surgical procedures. Animals were handled every other day during acclimation and every day after surgery. After surgery, all animals were fed *ad libitum* and were allowed to recover for 1 week prior to Pavlovian conditioning training. During training and subsequent testing for conditioned reinforcement, animals' body weights were maintained at 100% of their pre-surgical weight (usually between 300 and 320 g). After each session, rats were immediately given food (~15 g), which was consumed within 1 hour. Thus, animals were trained and tested in a non-sated state, consistent with previously published methodology (Robinson and Flagel, 2009). All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee.

Drugs

Mecamylamine (MEC) (3 or 30 μg, MP Biomedicals, Solon, OH, USA), AP-5 (0.1 or 1 μg, Sigma Aldrich, St. Louis, MO, USA), or scopolamine (SCOP) (3 or 66.7 μg, Sigma Aldrich, St. Louis, MO, USA) were dissolved into 0.9% saline and infused into the VTA in a 0.5-μL volume at a rate of 0.5 μL/min using a Hamilton 25 gauge syringe. After infusion, the internal cannula was left in place for one additional minute to allow adequate absorption of the drug. The doses for all experiments were calculated based on previous work from our laboratory and others' demonstrating the ability of VTA administration of these doses to modulate reward-related behavior and to modulate phasic DA release in the NAc (Yeomans and Baptista, 1997; Sombers et al., 2009; Solecki et al., 2013; Wickham et al., 2013). Importantly, we have previously determined that VTA administration of these drugs at these doses does not alter locomotor activity (Solecki et al., 2013). Animals were tested for conditioned reinforcement immediately after local drug micro-infusion. The 0.5-μL infusion volume was selected based on our previous histological verification, where we observed that infusion of 0.5 μL Chicago Blue Dye led to staining that was restricted to the VTA (Solecki et al., 2013).

Surgery

Rats were anesthetized with ketamine HCl (100 mg/kg, i.m., Sigma Aldrich, USA) and xylazine (10 mg/kg, i.m., Sigma Aldrich, USA) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) for cranial implantation of cannula. All coordinates were obtained from the rat brain atlas (Paxinos and Watson, 2007) with anteroposterior (AP), mediolateral (ML) and dorsoventral