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Orbitofrontal participation in sign- and goal-tracking conditioned responses: Effects of nicotine

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ABSTRACT

Pavlovian conditioned stimuli can acquire incentive motivational properties, and this phenomenon can be measured in animals using Pavlovian conditioned approach behavior. Drugs of abuse can influence the expression of this behavior, and nicotine in particular exhibits incentive amplifying effects. Both conditioned approach behavior and drug abuse rely on overlapping corticolimbic circuitry. We hypothesize that the orbitofrontal cortex (OFC) regulates conditioned approach, and that one site of nicotine action is in the OFC where it reduces cortical output. To test this, we repeatedly exposed rats to 0.4 mg/kg nicotine (s.c.) during training and then pharmacologically inactivated the lateral OFC or performed *in vivo* electrophysiological recordings of lateral OFC neurons in the presence or absence of nicotine. In Experiment 1, animals were trained in a Pavlovian conditioning paradigm and behavior was evaluated after inactivation of the OFC by microinfusion of the GABA agonists baclofen and muscimol. In Experiment 2, we monitored phasic firing of OFC neurons during Pavlovian conditioning sessions. Nicotine reliably enhanced conditioned responding to the conditioned cue, and inactivation of the OFC reduced conditioned responding, especially the sign-tracking response. OFC neurons exhibited phasic excitations to cue presentation and during goal tracking, and nicotine acutely blunted this phasic neuronal firing. When nicotine was withheld, both conditioned responding and phasic firing in the OFC returned to the level of controls. These results suggest that the OFC is recruited for the expression of conditioned responses, and that nicotine acutely influences this behavior by reducing phasic firing in the OFC.

1. Introduction

Environmental stimuli associated with nicotine or other drugs of abuse can acquire incentive motivational properties, becoming salient, attractive, and able to motivate behavior (Robinson and Berridge, 1993). In humans attempting to abstain from drug use, encountering these 'incentives' - stimuli that acquire motivational properties based on associations with drug rewards (Logan, 1964) - can lead to craving and promote relapse (Obrien et al., 1992). Bio-behavioral models of substance dependence implicate long-term changes in the brain circuitry that mediates responses to incentives as central to substance use disorders (Di Chiara et al., 1992; Robinson and Berridge, 1993). Preclinical studies have confirmed that frontolimbic circuitry plays a critical role in the motivational effects of many drugs of abuse (Kalivas and Volkow, 2005). This circuit includes ascending dopaminergic projections from the midbrain, including the ventral tegmental area, and descending glutamatergic projections from the frontal cortex, including the anterior cingulate gyrus and prefrontal cortex (PFC). These projections converge on subcortical circuits that include the ventral striatum, ventral pallidum, and subthalamic nucleus.

The PFC has been implicated in substance dependence because of its role in top-down control of behavior, attention, decision making, and other functions that, when compromised, contribute

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to addiction vulnerability (Perry et al., 2010). Chronic drug use increases the influence of ascending midbrain systems while reducing cognitive control, resulting in an enhanced drive to seek the drug and a decrease in the ability to inhibit drug-seeking (Olausson et al., 2007). The orbitofrontal cortex (OFC), in particular, has been linked to incentive motivation and representations of outcome value or salience in both humans and animals (Gottfried et al., 2003; Ogawa et al., 2013), as well as the expression of behavioral responses and reward-seeking behaviors (Burton et al., 2014; Moorman and Aston-Jones, 2014). While the exact function of the OFC has yet to be precisely defined (see Stalnaker et al., 2015 for review) the OFC has consistently been characterized as involved in behaviors such as impulsivity (Mar et al., 2011; Zeeb et al., 2010) and Pavlovian conditioned approach (Chudasama and Robbins, 2003; Gallagher et al., 1999; Ostlund and Balleine, 2007).

Incentive stimuli that predict both drug and non-drug rewards evoke 'Pavlovian conditioned approach' behavior, which can take one of two forms. Approach behaviors oriented toward the location of reward delivery are traditionally referred to as 'goal tracking,' whereas behaviors oriented toward the location of the incentive, if it is spatially separated from the reward, are referred to as 'sign tracking' (Brown and Jenkins, 1968). Sign tracking has recently come under increasing scrutiny in substance dependence research because of its association with drug abuse vulnerability (Saunders and Robinson, 2013; Tomie et al., 2008). Although both sign and goal tracking rely on the same mesotelencephalic systems implicated in substance dependence (Flagel et al., 2011b; Saunders and Robinson, 2012), individual subjects who display a greater propensity to sign track show increased drug self-administration (Saunders and Robinson, 2011; Versaggi et al., 2016). These individual differences are also linked to variation in stress responses, neurotransmitter release, and neuronal activation in areas including the PFC and the nucleus accumbens (Saunders and Robinson, 2013; Tomie et al., 2008). For example, one study found that c-fos mRNA induction in the OFC was increased only in animals that displayed the sign-tracking response (Flagel et al., 2011a). While it appears that the OFC is involved in Pavlovian conditioned behaviors, there is still much to be learned, including the differential involvement of this region based on specific conditioned responses.

Recent studies from multiple laboratories suggest a special relationship between the effects of nicotine and approach to incentives (Palmatier et al., 2014; Versaggi et al., 2016; Yager and Robinson, 2015). The interaction between nicotine and incentives is especially relevant to tobacco use and dependence because preclinical studies have repeatedly demonstrated that nicotine is a weak primary reinforcer (Foll and Goldberg, 2009; Palmatier et al., 2006). Caggiula, Donny, Chaudhri and others (Caggiula et al., 2001; Donny et al., 2003; Chaudhri et al., 2006) have argued that nicotine self-administration follows from three effects of nicotine on behavior. First, nicotine is a primary reinforcer, albeit a weak one, meaning that nicotine delivery alone supports self-administration. Second, nicotine is a reinforcement enhancer; i.e., nicotine delivery increases responding for non-drug reinforcers (Chaudhri et al., 2007; Donny et al., 2003; Palmatier et al., 2006). Third, serving as a primary reinforcer, nicotine can establish associated non-drug stimuli as 'conditioned reinforcers' (i.e., incentives; Palmatier et al., 2008). More recently, Palmatier and colleagues (Palmatier et al., 2014, 2013a, 2012) have argued that the second effect of nicotine, enhanced responding for non-drug reinforcers, reflects an effect of nicotine on underlying neurobiological substrates that mediate responses to incentives, including conditioned stimuli. Accordingly, they have found that nicotine promotes Pavlovian conditioned approach, including sign-tracking (Palmatier et al., 2013b), and that the increase in approach is abolished by dopaminergic antagonists (Palmatier et al., 2014).

The present study sought to more thoroughly explore the neurobiological underpinnings of the incentive-promoting effects of nicotine by evaluating the role of the OFC in sign-and goal-tracking. We hypothesized that the OFC would be directly involved in both sign- and goal-tracking conditioned responses, and that nicotine exposure would reduce the ability of the OFC to exert top down control over this behavior. We tested this hypothesis with pharmacological inactivation of the OFC and by examining OFC firing patterns *in vivo* during Pavlovian conditioning sessions.

2. Materials and methods

2.1. Animals

Adult, male, Sprague Dawley rats (225–250 g on arrival) were purchased from Harlan/Envigo (Indianapolis, IN), pair housed during initial training, and then individually housed after surgery. Experiment 1 used 16 animals and Experiment 2 used 25 animals. Animals were provided with food and water *ad libitum* during the entire experiment. Rats were housed in a vivarium on a 12:12 h light:dark cycle, and all experiments were conducted during the light cycle. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

2.2. Behavioral training and nicotine regimen

Before training, animals were allowed 1-h access to the 20% sucrose (w/v) solution that would be used as the unconditioned stimulus. Animals were then assigned to either a nicotine exposure group (NIC) or a saline control group (SAL). Nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline and the pH was adjusted to 7.0 ± 0.2. Animals in the NIC group received one injection of 0.4 mg/kg nicotine (s.c., calculated using the freebase form) and animals in the SAL group received an equivalent volume of saline for two days prior to conditioning to habituate them to the injection procedure. This dose was chosen because it is commonly used for repeated subcutaneous injections of nicotine, and we and others have previously shown that this dose influences conditioned responding (e.g., Guy and Fletcher, 2014; Palmatier et al., 2013b). Training sessions were conducted in standard behavioral chambers (MedAssociates, St Albans, VT) assembled with Plexiglas walls. A recessed reward receptacle, stimulus light, and retractable lever directly below the light were located on one wall of the chamber, and a house light was positioned on the opposite wall. A photobeam detector across the reward cup detected head entries into the receptacle. Animals were habituated to the testing chambers during one day of receptacle training, in which they were injected with the assigned drug or control solution, returned to their home cage for 10 min, and then placed in the testing chamber for 5 min before session initiation. During this session, 20% sucrose was dispensed into the receptacle on a variable interval (VI) 120 s schedule. Animals rarely failed to consume the reward, and NIC and SAL groups did not differ in the amount of fluid left in the reward cups at the end of the session (data not shown). Next, 20 (Experiment 1) or 25 (Experiment 2) Pavlovian conditioning sessions were conducted, Monday-Friday, in which the animals were injected with nicotine or saline 15 min before session initiation as described above. The house light was illuminated throughout the session and stimulus-reward pairings occurred on a VI 120 s reinforcement schedule. The conditioned stimulus (cue) consisted of illumination of a cue light and extension of the lever located directly below the light. Cue presentations lasted 30s, and