Neuropharmacology 128 (2018) 33–42

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Evaluating oral flavorant effects on nicotine self-administration behavior and phasic dopamine signaling

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ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form
31 August 2017
Accepted 19 September 2017
Available online 21 September 2017

Keywords:
Nucleus accumbens
Voltammetry
Menthol
Saccharin
Sucrose
Progressive ratio

ABSTRACT

Understanding how tobacco product flavor additives, such as flavorants in electronic cigarettes, influence smoking behavior and addiction is critical for informing public health policy decisions regarding tobacco product regulation. Here, we developed a combined intraoral (i.o.) and intravenous (i.v.) self-administration paradigm in rats to determine how flavorants influence self-administration behavior. By combining i.o. flavorant delivery with fast scan cyclic voltammetry (FSCV) or i.v. nicotine self-administration in adult, male rats, we examined whether flavors alter phasic dopamine (DA) signaling and nicotine self-administration. Oral administration of 10% sucrose or 0.32% saccharin, but not 0.005% menthol, increased phasic DA release in the nucleus accumbens (NAc). Oral sucrose or saccharin, when combined with i.v. nicotine delivery, also led to increased self-administration behavior. Specifically, combined i.o. sucrose and i.v. nicotine decreased responding compared to sucrose alone, and increased responding compared to nicotine alone. In contrast, i.o. flavorants did not alter motivational breakpoint in a progressive ratio task. Oral menthol, which did not alter i.v. nicotine administration, reversed oral nicotine aversion (50 and 100 mg/L) in a two-bottle choice test. Here, we demonstrate that i.o. appetitive flavorants that increase phasic DA signaling also increase self-administration behavior when combined with i.v. nicotine delivery. Additionally, oral menthol effects were specific to oral nicotine, and were not observed with i.v. nicotine-mediated reinforcement. Together, these preclinical findings have important implications regarding menthol and sweet flavorant additive effects on tobacco product use and can be used to inform policy decisions on tobacco product flavorant regulation.

1. Introduction

The 2009 Family Smoking Prevention and Tobacco Control Act gave the Federal Drug Administration (FDA) power to regulate flavor additives, with the exemption of menthol, in tobacco cigarettes. A primary goal was to reduce smoking in adolescents, primary consumers of flavored cigarettes. However, the advent of flavored electronic cigarettes (e-cigarettes) have reinvigorated these discussions. Recent data suggest that flavored e-cigarettes are the most popular form of smokable tobacco products initiated by high school students (Krishnan-Sarin et al., 2015). Moreover, the availability of appetitive flavors is one of the primary reasons why high school students use e-cigarettes (Kong et al., 2015; Villanti et al., 2013). Currently, there is limited data on oral menthol and appetitive flavorant effects on nicotine self-administration behavior and tobacco product use, although a recent finding in young adult smokers found enhanced reward for flavored versus non-flavored e-cigarettes (Audrain-McGovern et al., 2016).

Individuals who smoke menthol cigarettes have lower quit rates (Delnevo et al., 2011; Gandhi et al., 2009) and a faster time to smoke the day's first cigarette, compared to non-menthol smokers (Ahijevych and Parsley, 1999). Menthol's mint-like flavor, cooling, antitussive, and anti-irritant properties are thought to mask the bitter flavor and the irritation of the mouth, lungs, and throat

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https://doi.org/10.1016/j.neuropharm.2017.09.029
0028-3908/

induced by cigarettes (Kreslake et al., 2008; Lee and Glantz, 2011; Strasser et al., 2013; Wickham, 2015; Willis et al., 2011). Menthol also influences the subunit composition, expression, and function of nicotine acetylcholine receptors (nAChRs) in the mesolimbic dopamine (DA) system (Ashoor et al., 2013; Brody et al., 2013; Hans et al., 2012; Henderson et al., 2016). Burst firing of ventral tegmental area (VTA) DA neurons and the subsequent phasic DA release in the nucleus accumbens (NAc) is associated with exposure to rewards and can drive drug-taking and drug-seeking behavior (Day et al., 2007; Phillips et al., 2003; Solecki et al., 2013), while DA release in the NAc core sub-region, in particular, plays a critical role in cue-mediated drug-taking and drug-seeking (Phillips et al., 2003; Solecki et al., 2013). Indeed, based on their recent findings, Wang et al. have proposed that oral menthol may act as a conditioned cue when paired with nicotine (Wang et al., 2014). Due to the complex sensory profiles of menthol and tobacco product flavorants, however, it is unclear whether the tastants and flavorants themselves influence phasic DA signaling and nicotine intake.

In their recent work, Wang et al. specifically sought to examine oral menthol effects on i.v. nicotine taking in rats trained to lick a lickometer that produced simultaneous oral menthol and intravenous (i.v.) nicotine (Wang et al., 2014). However, their model produced low rates of nicotine self-administration and a lack of discrimination between active and inactive operandi. Here, we sought to develop a combined intraoral (i.o.) flavorant and i.v. drug operant paradigm that produced robust rates of nicotine self-administration, a strong dissociation between active and inactive operant responses, and compatibility with *in vivo* electrochemical techniques. We first combined fast scan cyclic voltammetry (FSCV) with i.o. flavorant delivery to determine whether flavorants alter phasic DA signaling in the NAc. Secondly, we combined i.o. flavorant delivery with i.v. self-administration to demonstrate that oral flavorants which increased phasic DA release also increased self-administration behavior when combined with i.v. nicotine under fixed ratio schedules. In contrast, these i.o. flavorants did not alter the motivation for nicotine taking in a progressive ratio task. These experiments demonstrate the ability of oral flavorants to alter nicotine self-administration and provide a foundation for future investigations of the neurochemical and behavioral effects of other tobacco product flavorants.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (250–350 g, Charles River Laboratories, Wilmington, MA, USA) were placed on ad libitum food and water and housed 2–3 per cage on a 12-h light/dark cycle (lights on at 7 a.m.). One week after combined i.o. and i.v. catheter surgery, rats were maintained at 85–90% body weight throughout behavioral training. After surgery, each animal was housed individually to prevent cage mates from damaging each other's catheters. 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.

2.2. Drugs

Nicotine hydrogen tartrate salt (Sigma, St. Louis MO) was dissolved in 0.9% saline solution (pH = 7.0) and filtered using a 0.22 μm filter. Nicotine was delivered at 30 μg/kg/infusion (free base) and infused for 6 s at 17.7 μL/s (106 μL total). For i.o. delivery, 10% sucrose, 0.32% saccharin, 0.005% menthol (all w/v) were prepared in deionized (DI) water and infused at a rate of 33 μL/s for 6 s (362 μL). Flavorants and doses were selected to allow for examination of caloric and non-caloric appetitive flavorants (sucrose and saccharin, respectively) and menthol, encompassing multiple types of tobacco product flavorants. The reinforcing 10% sucrose served as a positive control for our newly established behavioral methodology. The appetitive saccharin dose was selected from two-bottle choice tests showing that 0.32% saccharin was most preferred compared to water. For menthol, 0.001%, 0.005% and 0.01% all showed equal preference to water (two bottle choice data not shown). Here, we chose to use the 0.005% concentration to maintain consistency with recently published work in mice showing the ability of 0.005% menthol to decrease oral nicotine aversion, even with demonstrated equal preference between water and 0.005% menthol (Fan et al., 2016). For two-bottle choice experiments, nicotine hydrogen tartrate salt (Sigma, St. Louis MO) was dissolved in DI water and 0.1 M NaOH was used to set the pH to ~7.4.

2.3. Surgical procedures

Rats were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p. Sigma Aldrich, USA). First, we implanted a silastic catheter into the external jugular vein, as described previously (Solecki et al., 2013). Second, we implanted an ethylene oxide sterilized polyethylene i.o. catheter that was anchored to the first molar and protruded dorsally through the skin between the ears. For additional, detailed i.o. surgical methodology, see (Wickham et al., 2015). Carprofen (5 mg/kg, s.c.) was administered prior to any surgical incision and was administered for three days post-surgery. In preparation for voltammetry experiments, a guide cannula (Bioanalytical Systems, West Lafayette, IL) was positioned above the NAc (AP +1.2 mm, ML -1.4 mm) and an Ag/AgCl reference electrode (previously baked at 120 °C for 1 h) was implanted in the contralateral hemisphere (Wickham et al., 2015). Subsequently, a bipolar stimulating electrode was implanted in the VTA/substantia nigra (SN) (AP −5.2 mm, ML -0.5 to −1.5 mm, DV −8.0 to −9.0 mm). Dental cement (Dentsply, Milford, DE) and screws (Gexpro, High Point, NC) were used to secure the cannula and reference electrode to the skull.

2.4. Measurement of phasic DA combined with intraoral infusion

A micromanipulator containing a carbon fiber microelectrode was lowered to the NAc core and a low-pass filtered (2 kHz), a triangular potential waveform (−0.4 V to +1.3 V and back to −0.4 V, at a rate of 400 V/s) was applied at 60 Hz for 15 min, and then applied at 10 Hz for recordings. An initial training set of DA and pH was collected by stimulating the VTA at varying frequencies and pulses (10–20 Hz, 10–40 pulses, all at 150 μA). Then, a single flavor was administered (Fig. 1A) at pseudorandom intervals of 60, 120, and 180 s, for a total of 25 infusions, similar to previously published methodology (Roitman et al., 2008; Wickham et al., 2015).

After the experiment, the carbon fiber microelectrode was removed and calibrated in vitro to determine DA concentrations. Using principal component regression and the DA training set, we identified and quantified phasic DA responses to flavorants, consistent with published methodology (Keithley et al., 2009, 2010). In a subset of rats, the carbon fiber was used to lesion the recording site for histological verification (Fig. 1D) as previously described (Addy et al., 2010). Since the lesioned electrode could not be used for calibration, in rats used for histology, we used our obtained average calibration factor (7 nA/μM DA), consistent with our published methodology (Addy et al., 2010).