



## Similar precipitated withdrawal effects on intracranial self-stimulation during chronic infusion of an e-cigarette liquid or nicotine alone



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### ABSTRACT

The FDA recently extended their regulatory authority to electronic cigarettes (ECs). Because the abuse liability of ECs is a leading concern of the FDA, animal models are urgently needed to identify factors that influence the relative abuse liability of these products. The ability of tobacco products to induce nicotine dependence, defined by the emergence of anhedonia and other symptoms of nicotine withdrawal following cessation of their use, contributes to tobacco abuse liability. The present study compared the severity of precipitated withdrawal during chronic infusion of nicotine alone or nicotine-dose equivalent concentrations of three different EC refill liquids in rats, as indicated by elevations in intracranial self-stimulation (ICSS) thresholds (anhedonia-like behavior). Because these EC liquids contain constituents that may enhance their abuse liability (e.g., minor alkaloids), we hypothesized that they would be associated with greater withdrawal effects than nicotine alone. Results indicated that the nicotinic acetylcholine receptor antagonist mecamylamine precipitated elevations in ICSS thresholds in rats receiving a chronic infusion of nicotine alone or EC liquids (3.2 mg/kg/day, via osmotic pump). Magnitude of this effect did not differ between formulations. Our findings indicate that nicotine alone is the primary CNS determinant of the ability of ECs to engender dependence. Combined with our previous findings that nicotine alone and these EC liquids do not differ in other preclinical addiction models, these data suggest that product standards set by the FDA to reduce EC abuse liability should primarily target nicotine, other constituents with peripheral sensory effects (e.g. flavorants), and factors that influence product appeal (e.g., marketing).

The Food and Drug Administration Center for Tobacco Products (FDA CTP) recently extended their regulatory authority to electronic cigarettes (ECs) (Food and Drug Administration, 2016), which have become increasingly popular despite their unknown health consequences (Brandon et al., 2015; Glasser et al., 2017; Walton et al., 2015). Development of appropriate preclinical methodology for evaluating the abuse liability of ECs is needed to inform FDA CTP regulation of these products.

Animal models of tobacco addiction typically involve administration of nicotine alone or other isolated tobacco constituents (e.g., minor alkaloids). Models using EC liquids would provide insights into interactions between nicotine and other behaviorally relevant non-nicotine constituents present in EC liquids (e.g., minor alkaloids, acetaldehyde, and propylene glycol, see Etter et al., 2013; Goniewicz et al., 2014; Han et al., 2016), similar to how study of extracts of smokeless tobacco or cigarette smoke has been useful for understanding product abuse

liability (e.g., Brennan et al., 2014; Costello et al., 2014; Harris et al., 2015). While the systemic (i.v., s.c., i.p.) routes used in these models do not simulate the route of administration for tobacco products, they have the major advantage of controlling for sensorimotor stimuli associated with tobacco use (e.g., taste, smell). This provides critical information for interpreting human studies that do not control these variables.

We found that acute injection of low to moderate doses of nicotine alone and nicotine dose-equivalent concentrations of three EC liquids (Aroma E-Juice, Janty, and NicVape) similarly enhanced the reinforcing effects of non-drug stimuli as measured by reductions in intracranial self-stimulation (ICSS) thresholds, and Aroma E-Juice also had similar primary reinforcing effects in an i.v. self-administration model (LeSage et al., 2016; Harris et al., submitted). In contrast, all three EC liquids produced less ICSS threshold-elevating effects than nicotine alone at a high acute nicotine dose, which may reflect a reduction in nicotine's aversive/anhedonic effects (Fowler et al., 2011;

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Spiller et al., 2009). Extending preclinical evaluation of EC liquids to additional aspects of abuse liability is needed to better understand the role of non-nicotine constituents in EC use.

The ability of ECs and other tobacco products to induce nicotine dependence, defined by the emergence of anhedonia and other symptoms of nicotine withdrawal following cessation of use, contributes to their abuse liability (Etter and Eissenberg, 2015; Foulds et al., 2015; Markou, 2008; Paolini and De Biasi, 2011). The current study compared the ability of chronic infusion of nicotine alone and Aroma E-Juice, Janty, and NicVape EC liquids to induce nicotine dependence in rats, measured as elevations in ICSS thresholds (anhedonia-like behavior) following administration of the nicotinic acetylcholine receptor antagonist mecamylamine (precipitated withdrawal) and abrupt cessation of the infusion (spontaneous withdrawal). Precipitated withdrawal was the primary outcome because it is typically more robust than spontaneous nicotine withdrawal (Skjei and Markou, 2003; Watkins et al., 2000), and because prior studies showing an enhancement of nicotine dependence by isolated non-nicotine tobacco constituents used precipitated withdrawal assays (Alsharari et al., 2015; Guillem et al., 2008). We hypothesized that EC liquids would elicit greater withdrawal effects than nicotine alone due to the presence of behaviorally active non-nicotine constituents in the EC liquids.

## 1. Materials and methods

### 1.1. Animals

Male Holtzman Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 250–300 g upon arrival were housed individually under a reversed 12-h light/dark cycle and allowed unlimited access to water. Rats were food restricted to 18 g/day to facilitate operant performance and avoid detrimental health effects of long-term ad libitum feeding (Keenan et al., 1997; Keenan et al., 1999). Protocols were approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation in accordance with the 2011 NIH Guide for the Care and Use of Laboratory Animals and the 2003 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

### 1.2. Drugs

Nicotine bitartrate and mecamylamine hydrochloride (MEC) were obtained from Sigma Chemical Co. (St. Louis, MO). Aroma E-Juice Whole Tobacco Alkaloid (WTA) EC refill liquid (Dark Honey Tobacco flavor), Janty EC refill liquid (DK Port flavor), and NicVape EC refill liquid (Fruit Stripe Gum/Fruit Twist flavor) were obtained from Aroma E-Juice (<http://www.aromaejuice.com>, Scottsdale, AZ), Janty USA (<http://www.usa.janty.com>, Blasdel, NY), and NicVape (<http://www.nicvape.com>, Spartanburg, SC), respectively. These EC liquids were used because we have previously studied them in other preclinical addiction models (LeSage et al., 2016; Harris et al., submitted). In addition, Aroma E-Juice is advertised as containing higher levels of minor alkaloids than other ECs, and Janty and NicVape EC liquid contained relatively high (Janty) and low (NicVape) levels of minor alkaloids relative to nicotine in our analysis of 20 different EC liquids (Harris et al., submitted). According to the labels, Aroma E-Juice refill liquid contained 80% vegetable glycerine (VG) and 20% propylene glycol (PG), while Janty refill liquid contained 66.1% PG, 15.0% vanillin tincture, 1.0% peach aldehyde, and 2.0% 2,5-dimethylpyrazine. The remaining 15.9% of ingredients for Janty were unaccounted for. NicVape refill liquid was advertised as containing 50% PG and 50% VG. Labeled nicotine content of all EC liquids was 24.0 mg/ml. Actual nicotine concentrations were determined as described previously (Hieda et al., 1999), and the EC liquids were diluted in saline to the concentrations required for the current studies. The average measured nicotine concentration across vials for all 3 EC liquids was

$22.88 \pm 0.48$  SEM mg/ml (range 21.5–25.9 mg/ml). When expressed as a percentage of nicotine, levels of total minor alkaloids (normicotine + anabasine + anatabine) in Aroma E-Juice, Janty, and NicVape EC liquids were 1.15%, 2.28%, and 0.11%, respectively (LeSage et al., 2016; Harris et al., submitted). These labeled levels of nicotine, VG, and PG, and actual levels of nicotine and minor alkaloids, are within the typical range of those reported for EC liquids (Etter et al., 2013; Goniewicz et al., 2014; Han et al., 2016). The pH of all nicotine-containing solutions was adjusted to 7.4 with NaOH. Nicotine alone and EC liquids were administered s.c. via osmotic minipump (see below), while MEC injections were administered s.c. in a volume of 1.0 ml/kg. Nicotine and MEC doses are expressed as the base and salt, respectively.

### 1.3. Intracranial self-stimulation

Surgery, apparatus, and training procedure used here are described in detail elsewhere (Harris et al., 2010; Harris et al., 2011). Briefly, animals were anesthetized with i.m. ketamine (75 mg/kg)/dexmedetomidine (0.025 mg) and implanted with a stimulating electrode in the medial forebrain bundle. Rats were trained to respond for electrical brain stimulation using a modified version of the Kornetsky and Esposito (1979) discrete-trial current-threshold procedure (Harris et al., 2010; Harris et al., 2011; Markou and Koob, 1992).

### 1.4. Behavioral protocol

Rats were tested in daily ICSS sessions conducted Mon–Fri until thresholds were stable (< 10% variability over a 5-day period and no trend). Rats were then implanted s.c. with an osmotic pump delivering either saline ( $n = 7$ ), nicotine alone ( $n = 10$ ), Aroma E-Juice EC liquid ( $n = 8$ ), Janty EC Liquid ( $n = 13$ ), or NicVape EC liquid ( $n = 9$ ) as described previously (Manbeck et al., 2013). Osmotic pumps were always implanted on Mondays after that day's ICSS test. Nicotine-containing solutions were administered at a rate (3.2 mg/kg/day) that reliably induces nicotine dependence as indicated by elevations in ICSS thresholds (e.g., Epping-Jordan et al., 1998; Manbeck et al., 2013; Roiko et al., 2009). This infusion rate also produces nicotine serum levels ( $\approx 40$  ng/ml) within the range of those observed in experienced EC users (Farsalinos et al., 2015; LeSage et al., 2002; Spindle et al., 2017). Rats continued to be tested for ICSS Mon–Fri throughout the duration of the 4-week infusion. MEC-precipitated withdrawal testing commenced during the 2nd week of the infusion. On test days, ICSS sessions were preceded by 10 min pretreatment with MEC (0, 0.3, 0.6, 1.0, 2.0, or 3.0 mg/kg). These MEC doses precipitate elevations in ICSS thresholds in nicotine-dependent rats, but do not affect ICSS in nicotine-naïve rats (Bruijnzeel et al., 2007; Watkins et al., 2000). MEC-precipitated withdrawal tests were conducted on Tuesdays and Fridays (half-life of MEC is  $\approx 1$  h, Debruyne et al., 2003), and MEC doses were administered in a counterbalanced order. Seventy-two hours after the final test day, osmotic pumps were removed to elicit spontaneous withdrawal as previously described (Manbeck et al., 2013) and ICSS thresholds were tested 22, 46, 70, 94, and 166 h later (time points based on Cryan et al., 2003; Harris et al., 2010; Skjei and Markou, 2003). Drug delivery was confirmed by measuring the residual volume of formulations in osmotic pumps following their removal. Data for any animals with osmotic pump failure (> 10% difference between actual and predicted flow rate) were excluded.

### 1.5. Statistical analyses

Intracranial self-stimulation thresholds (a measure of the function of brain reinforcement pathways, in  $\mu$ A) and response latencies (a measure of non-specific (e.g., motor) effects, in sec) during each session were expressed as percentage of baseline (mean during the last 5 sessions prior to osmotic pump implantation). Within each test phase, ICSS threshold and latency data were analyzed using separate two-factor

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