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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 than influence product appeal (e.g., marketing).

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All authors declare that they have no conflict of interest.

Keywords

Nicotine; mecamylamine; withdrawal; electronic cigarettes; intracranial self-stimulation

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 Dark Honey, Janty, and NicVape) similarly enhanced the reinforcing effects of non-drug stimuli as measured by reductions in intracranial self-stimulation (ICSS) thresholds, and 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; 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.

2. Materials and Methods

2.1. Animals

Male Holtzman Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 250–300 g upon arrival were housed individually under a reversed 12-hr 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 Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

2.2. 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>, Blasdell, 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 ± 0.48 SEM mg/ml (range 21.5–25.9 mg/ml). When expressed as a percentage of nicotine, levels of total minor alkaloids (nornicotine + 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.

2.3. 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).

2.4. 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). 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 (≈ 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 ≈ 1 hour, 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 hours 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.

2.5. Statistical analyses

Intracranial self-stimulation thresholds (a measure of the function of brain reinforcement pathways, in μ 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 ANOVAs with group (formulation) as a between-subjects factor and either session or MEC dose as a within-subjects factor.

Degrees of freedom for ANOVAs were adjusted using the Greenhouse-Geisser correction to account for possible violations of sphericity. Independent sample Welch's t-tests with a Bonferroni correction were used for between-formulation comparisons at each session or MEC dose.

3. Results

3.1 Attrition and baseline measures

Data for 4 animals from the Janty EC liquid group were removed due to osmotic pump failure. There were no significant differences in baseline ICSS thresholds or response latencies between groups (Table).

3.2. ICSS thresholds

Although ICSS thresholds appeared lower for the nicotine alone and EC liquid groups compared to the saline group during the first week of chronic infusion (Fig 1A), the effect of group was only marginally significant ($F(4,36)=2.4$, $p=0.07$). There was a significant effect of session ($F(2.4,87.2)=4.2$, $p<0.05$), but no significant group $\times$ session interaction (Fig 1A).

There were significant effects of group ($F(4,36)=3.8$, $p<0.05$) and MEC dose ($F(3.4,124)=15.0$, $p<0.00001$) on ICSS thresholds during MEC-precipitated withdrawal, but no group $\times$ MEC dose interaction (Fig 1B). Comparison of marginal means indicate that ICSS thresholds were significantly elevated in the nicotine alone group ($t(11.0)=3.7$, $p<0.05$), the Aroma E-Juice group ($t(8.5)=3.4$, $p<0.05$), the Janty group ($t(8.2)=4.8$, $p<0.01$), and the NicVape group ($t(13.0)=5.7$, $p<0.01$) compared to the saline group. There were no other significant differences between groups.

There was no effect of group on ICSS thresholds during spontaneous withdrawal, but there was a significant effect of session ($F(3.5,121.3)=7.4$, $p<0.0001$) and a significant group $\times$ session interaction ($F(13.9,121.3)=2.2$, $p<0.05$). ICSS thresholds were modestly increased in the nicotine alone and EC liquid groups compared to the saline group on days 1, 2, and/or 3 of spontaneous withdrawal (Fig 1C), although none of these differences were significant.

3.3 ICSS latencies

There were no significant differences in response latencies between groups during any test phase (Fig 2A–2C).

Discussion

The primary finding of this study was that magnitude of ICSS threshold elevations during MEC-precipitated withdrawal did not differ in rats receiving a chronic infusion of nicotine alone versus three EC liquids. None of these formulations produced a significant spontaneous withdrawal effect. Although previous studies have demonstrated significant increases in ICSS thresholds during spontaneous nicotine withdrawal (Cryan et al., 2003; Harris et al., 2010; Skjei and Markou, 2003), this effect is often modest and typically not studied in animals with a history of MEC injections during the chronic infusion, as in the current study. There was also a non-significant trend for chronic infusion of nicotine alone

and the EC liquids to reduce ICSS thresholds during the induction of dependence, an effect that is typically modest and variable in our lab and others (e.g., Epping-Jordan et al., 1998; Harris et al., 2010; Harrison et al., 2001; Lindblom et al., 2005). This study is the first to examine severity of withdrawal from EC liquid exposure in a preclinical model and provides novel insights into the role of non-nicotine constituents in this aspect of EC abuse liability.

The similar effects of nicotine alone and EC liquids in this study contrast with findings indicating that these 3 EC liquids produced less ICSS threshold-elevating (aversive / anhedonic) effects than nicotine alone at a high acute nicotine dose (LeSage et al., 2016; Harris et al., submitted), and suggest different mechanisms underlying ICSS threshold elevations induced by acute versus chronic nicotine exposure (see also Fowler et al., 2013). However, our data are consistent with findings indicating that acute injections of low to moderate doses of these EC liquids and nicotine alone produced similar ICSS threshold-lowering (reinforcement-enhancing) effects (LeSage et al. 2016, Harris et al submitted), and that Aroma E-Juice EC liquid and nicotine alone had similar reinforcing effects in an i.v. self-administration model (LeSage et al., 2016). Complementing our findings, degree of nicotine addiction and withdrawal in humans was no greater for ECs than for nicotine gum (Etter and Eissenberg, 2015).

The use of only a single nicotine dose for the induction of dependence (3.2 mg/kg/day) represents a limitation of this study. This dose was chosen because it reliably induces dependence, produces clinically relevant nicotine serum levels, and is used in nearly all studies examining withdrawal from a chronic nicotine infusion in rats. While use of this dose represents a logical starting point for comparing withdrawal effects from nicotine alone versus EC liquids, future studies should examine other nicotine doses, which would also deliver different levels of non-nicotine constituents in the EC liquid conditions. Use of a higher nicotine dose may also produce a significant spontaneous withdrawal effect, which was not observed under the current dosing conditions.

The use of systemic administration of EC liquids allowed us to isolate the direct CNS effects of nicotine and non-nicotine constituents from sensory factors (e.g., taste, smell), and was appropriate for the purposes of the current study. However, sensory aspects of ECs and other tobacco products can contribute to their abuse potential (Litt et al., 2016; Rose, 2006; Rose et al., 2000) and should be incorporated into future studies. For example, preclinical models involving oral ingestion of EC liquid or inhalation of EC aerosol would provide insights into whether constituents with sensory effects (e.g., flavorants) contribute to EC withdrawal.

Overall, these findings suggest that nicotine is the primary CNS determinant of EC abuse liability. The main regulatory implication of these findings is that FDA product standards to alter the abuse liability of ECs should primarily target other factors including nicotine, other constituents with peripheral sensory effects (e.g. flavorants, odorants, airway irritants or anesthetics), and factors than influence product appeal such as design and marketing/messaging. For example, to the extent that the FDA is interested in enhancing the abuse liability of ECs in order to provide a better substitute for cigarettes, the impact of increasing nicotine content in ECs could be evaluated. Alternatively, product standards requiring a reduction in EC nicotine content could be considered in order to reduce EC abuse potential.

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Highlights

1. We compared withdrawal from nicotine alone vs. electronic cigarette (EC) refill liquids.
2. All formulations produced similar anhedonia-like behavior during withdrawal.
3. Nicotine alone mediates EC withdrawal effects.
4. FDA product standards for ECs should target CNS effects of nicotine.

Figure 1.

ICSS thresholds (expressed as percent of baseline, mean $\pm$ SEM) during the first week of chronic infusion of saline, nicotine alone, or EC liquids (A). ICSS thresholds during MEC-precipitated and spontaneous withdrawal testing are shown in (B) and (C), respectively. ICSS was not tested during days 5 and 6 during either the chronic infusion or during spontaneous withdrawal due to the weekend break in testing. *, ** Significantly different from saline group (marginal means), $p < 0.05$ or 0.01 , respectively.

Figure 2.

ICSS response latencies (expressed as percent of baseline, mean $\pm$ SEM) during the first week of chronic infusion of saline, nicotine alone, or EC liquids (A), as well as during MEC - precipitated (B) and spontaneous (C) withdrawal testing.

Table

Mean ($\pm$ SEM) ICSS thresholds (in μ A) and response latencies (in sec) in experimental groups during baseline sessions.

	Thresholds	Latencies
Nicotine Alone	89.0 ± 5.9	2.4 ± 0.1
NicVape EC Liquid	94.4 ± 11.8	2.7 ± 0.2
Janty EC Liquid	115.0 ± 23.11	2.8 ± 0.2
Aroma E-Juice EC Liquid	92.4 ± 9.9	2.4 ± 0.1
Saline	117.2 ± 19.8	2.7 ± 0.2