# Repeated nicotine vapor inhalation induces behavioral sensitization in male and female C57BL/6 mice

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**Electronic cigarette use has significantly increased over the past decade. However, there is limited preclinical research on the behavioral and abuse-related effects of nicotine vapor inhalation in rodents. The present study evaluates the effects of repeated nicotine vapor inhalation in male and female mice using a nicotine behavioral sensitization model. Male and female C57BL/6 mice were administered vaporized nicotine (0–10.0 mg/ml) or the positive control of intraperitoneally administered nicotine (0.5 mg/kg) once daily for 5 days, and locomotor activity was assessed. Body temperatures were measured before and after nicotine vapor inhalation to assess hypothermia. Nicotine vapor inhalation (1.0–3.0 mg/ml) produced a dose-dependent behavioral sensitization effect and produced hypothermia in male and female mice. Nicotine (0.5 mg/kg) also produced significant behavioral sensitization. No sex differences were found for nicotine behavioral sensitization with either route of administration. Pretreatment with the nonselective nicotinic antagonist mecamylamine blocked the behavioral sensitization produced by 1.0 mg/ml of nicotine vapor inhalation. These results established that nicotine vapor inhalation produces behavioral sensitization in an inverted U-shaped curve that is similar to the effects of injected nicotine across several behavioral models. Additionally, pretreatment with mecamylamine demonstrated that nicotinic receptor activation was responsible for the behavioral sensitization produced by nicotine vapor inhalation and was not a conditioned response to the vapor. The methods used in the present study provide an additional behavioral approach for evaluating the behavioral effects of repeated nicotine vapor inhalation that allows the manipulation of several variables, including e-liquid oil blend, e-liquid flavors, puff duration, etc. *Behavioural Pharmacology* 31: 583–590 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.**

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## Introduction

Historically, nicotine has been consumed via tobacco products (e.g., cigarettes, cigars, pipe or chew tobacco, etc.); however, over the past decade electronic-cigarettes (e-cigs; e.g., vaporizers, vape pens, mods, etc.) have been popularized as a less harmful alternative to traditional tobacco. E-cigs function to administer a flavored aerosolized vapor for inhalation without the use of combustion, which has commonly been perceived by users and non-users to be less harmful than traditional cigarettes (Ambrose *et al.*, 2014; Baeza-Loya *et al.*, 2014; Case *et al.*, 2016). Approximately 3.7% to 4.5% of the USA adult population are regular e-cig users (National Health Interview Survey, 2015; Hu *et al.*, 2016; Mirbolouk *et al.*, 2018). The popularity of e-cigs has substantially increased among youth. For example, e-cigs use rose from 1.5% in 2011 to 20.8% in 2018 among middle and high school students (Cullen *et al.*, 2018). The increased use among the youth is particularly concerning because e-cig use significantly increases the likelihood of later cigarette use (Loukas *et al.*, 2018). E-cig research involving human participants is expanding in many areas including use trends, effectiveness of vaping as a method of smoking cessation, addictive properties of vapor delivered nicotine, and safety of e-cigs (Farsalinos *et al.*, 2013; Etter, 2015; Etter and Eissenberg, 2015; Spindle *et al.*, 2015; Hiler *et al.*, 2017; Liu *et al.*, 2017; Spindle *et al.*, 2017). Several studies of vaping-related lung injury have revealed substantial evidence of health risks related to e-cig use (for a review see, Fonseca *et al.*, 2019). For example, the Centers for Disease Control and Prevention has found, as of 21 January 2020, 2711 patients have been hospitalized for vaping-related lung injuries, and 60 concomitant deaths have been confirmed in the United States (Outbreak of Lung Injury Associated with the Use of E-Cigarette, or Vaping, Products, 2020). As such, e-cig research to evaluate health-related issues of long-term use, lung function, receptor mechanisms, etc. are becoming increasingly more difficult and unethical to measure in humans. Therefore, the development of appropriate rodent models is critical for nicotine vapor inhalation research.To date, there are limited studies on the health risks and behavioral effects of e-cigs in rodents. Several studies have found that chronic nicotine vapor inhalation significantly impairs physiological and lung functions in mice (Sussan *et al.*, 2015;



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Garcia-Arcos *et al*., 2016; Larcombe *et al*., 2017; Laube *et al*., 2017; Chun *et al*., 2018; Lee *et al*., 2018). Behavioral studies have found that acute high-dose nicotine vapor inhalation has been shown to decrease locomotor activity (LMA) and produce hypothermia in rodents (Lefever *et al*., 2017; Javadi-Paydar *et al*., 2019). Additionally, nicotine vapor inhalation produces somatic withdrawal symptoms following subchronic (George *et al*., 2010) and chronic (Ponzoni *et al*., 2015) administration, which is indicative of nicotine dependence in rodents.

The aim of this study was to evaluate nicotine vapor inhalation using a model of behavioral sensitization with intraperitoneal (i.p.) administration of nicotine serving as a positive control. A second aim was to determine if there are sex differences for either route of nicotine administration. Behavioral sensitization is a model in which repeated exposure to a drug of abuse, especially psychostimulants, increases a response (e.g., LMA) caused by neurobiological adaptations that relate to drug-seeking behaviors or development of addiction (Vanderschuren and Kalivas, 2000; Berridge and Robinson, 2003; Robinson and Berridge, 2008). For example, nicotinic α4β2 receptors are highly expressed on dopamine neurons in the ventral tegmental area, and activation of α4β2 receptors stimulate the release of dopamine in the nucleus accumbens. Previous studies have found that repeated nicotine administration alters the dopaminergic system and these neurobiological changes align with abuse-related behaviors like conditioned place preference (CPP) and locomotor sensitization (De Biasi and Dani, 2011; McGranahan *et al*., 2011; Goutier *et al*., 2015a; Goutier *et al*., 2015b).

## Methods

### Subjects

Sensitization experiments were conducted with 124 adult C57BL/6 mice (60 male, 64 female), either purchased from Charles River Laboratories (Raleigh-Durham, North Carolina, USA) or bred at Weber State University. In-house breeder pairs were purchased from Charles River Laboratories to avoid any strain-related differences between purchased and bred mice. No differences were observed between purchased and bred mice. Mice were at least 8 weeks of age at the start of the experiments. Mice were group housed (n = 3–4) in standard plastic cages on a 12-h light/dark cycle (lights on 0600–1800 hours) in a temperature (20–22°C) controlled vivarium. Experiments were performed during the light cycle. Mice had free access to food and water except during experimental sessions. All procedures were approved by the Institutional Animal Care and Use Committee at Weber State University and complied with federal guidelines (Institute of Laboratory Animal Resources, 2011).

### Apparatus

One bench top e-Vape delivery system provided vaporized nicotine administration (La Jolla Alcohol Research, Inc). The system consisted of a vapor generator that allowed for standard Mod tank attachment, a hose connected to the tank delivered vapor into a pressurized transparent Plexiglass chamber (29 cm × 20 cm × 15 cm), and a pump that maintained airflow (1 L/min). Cloud Beast tanks (TFV8) and atomizer (0.15 Ω) were used (SMOK, Nanshan District, Shenzhen, China). Battery wattage output was adjusted to 125 W for optimal performance according to tank specifications. The vapor puff frequency and duration were controlled by a digital interface. For vaporized nicotine administration, a 3-second puff was delivered every 2 minutes for 10 minutes (6 puffs total). Mice were removed from the chamber after the last puff completely cleared the chamber, approximately 90 seconds.

Behavioral sensitization was measured using a standard open-field Plexiglas chamber (29 cm × 29 cm × 20 cm) equipped with three 16-beam IR arrays (Med Associates, Inc., St. Albans, Vermont, USA). Open-field chambers were each enclosed in a cabinet equipped with a house light and a small fan. Distance traveled was measured with Med-Associates Activity Monitor (version 7; Med Associates, Inc.).

### Behavioral sensitization

Mice were handled prior to the start of the behavioral sensitization according to Grabus *et al*., 2006. Behavioral sensitization procedures were adapted from published literature (Biala and Weglinska, 2004; Bernardi and Spanagel, 2013; Carboni *et al*., 2018). Mice received three consecutive habituation sessions separated by 24 hours in which mice were placed in the open field for 60 minutes (no treatments were administered). For each of the subsequent five sensitization sessions (days 1–5), mice received vaporized nicotine (0–10.0 mg/ml) or i.p. nicotine (saline or 0.5 mg/kg), then were immediately placed in the open-field chambers for 30 minutes and LMA was measured. To determine if nicotine vapor inhalation produced a physiological effect in addition to the behavioral changes, body temperatures were measured before and after nicotine vapor inhalation administration (prior to being placed in the open-field chamber) using a RET-3 rectal probe for mice (inserted 24 mm) and digital thermometer (Digi-Sense Type J/K/T Thermocouple Thermometer). Temperatures were not measured in the i.p. nicotine group.

An antagonist study was conducted to confirm that the behavioral sensitization produced by nicotine vapor inhalation was mediated by activation of nicotinic receptors and not a conditioned response in which the vapor served as the conditioned stimulus. For this experiment, the nonselective nicotinic antagonist mecamylamine (1.0 mg/kg, s.c.) or saline was administered 30 mins prior to 1.0 mg/ml nicotine vapor inhalation session, administered as described above. Mice were placed in the open

field immediately after temperatures were measured. Both male and female mice were used in this experiment because sex differences were NS in previous experiments (Fig. 1a).

### Drugs

(–) Nicotine hydrogen tartrate salt, nicotine freebase, and mecamylamine were dissolved with the corresponding vehicle for behavioral studies (Sigma-Aldrich, St. Louis, Missouri, USA). For vaporized nicotine administration, the nicotine freebase was dissolved in e-liquid, which consisted of an unflavored 50/50 oil blend of propylene glycol and vegetable glycerin (vaporvapes.com, Sand City, California, USA). (–) Nicotine and mecamylamine were dissolved in a 0.9% saline solution for i.p. and subcutaneous (s.c.) administration (at a volume of 10 ml/kg), respectively. When necessary, sodium hydroxide was used as a buffer to ensure a pH balance of approximately 7.0 for (–) nicotine solutions. All vaporized nicotine doses are expressed as mg/ml based on the concentration that was used to fill the vaporizer tank; however, it may not represent the actual nicotine concentration inhaled by each mouse. I.p. nicotine (saline or 0.5 mg/kg; immediately) and mecamylamine (saline or 1.0 mg/kg; 30 min pretreatment) doses and pretreatment times were based on published literature (Biala and Weglinska, 2004; Grabus *et al.*, 2006; Bernardi and Spanagel, 2014; Freitas *et al.*, 2016; Lefever *et al.*, 2017). Nicotine vapor inhalation administration times (see below for details) were adapted from Lefever *et al.*, 2017 and vaporized nicotine doses were based on preliminary behavioral studies from our lab.

### Statistical analysis

The primary dependent variable to determine behavioral sensitization was the change in distance traveled on day 5 as compared to day 1. Due to the inherent variability between subjects on unconditioned behavior like LMA (Bernardi and Spanagel, 2014), we normalized the data for each animal. Normalizing these data provide more control over individual differences and expression of behavioral changes. To normalize these raw data, distance traveled for each mouse was converted to a difference score: Δ distance traveled (cm) = day 5 distance traveled – day 1 distance traveled. These normalized data were averaged across mice for statistical analysis using a 2-way between-subjects ANOVA, with nicotine dose and sex as factors. Body temperature changes were used to measure hypothermia after nicotine vapor inhalation (Δ body temperature [°C] = post-nicotine temperature – pre-nicotine temperature). Body temperature changes were analyzed separately for male and female mice using a 2-way mixed factor ANOVA with treatment day as the within-subject factor and nicotine dose as the between-subject factor. All significant ANOVAs were followed by a Newman-Keuls post hoc test (significance set at $P < 0.05$). An independent *t*-test was conducted for the mecamylamine antagonist experiment. Data were analyzed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, California, USA).

## Results

### Sensitization

Nicotine vapor inhalation produced a dose-dependent behavioral sensitization effect in both male and female mice after 5 days of repeated nicotine administration (Fig. 1a; nicotine dose $F(4, 63) = 8.16$, $P < 0.001$; sex $F(1, 63) = 0.17$, NS; interaction $F(4, 63) = 0.57$, NS). The post hoc tests revealed that 1.0 mg/ml and 3.0 mg/ml doses significantly increased LMA as compared to all other doses ($P < 0.05$). Injected nicotine (0.5 mg/kg. i.p.) produced a significant increase in LMA (i.e., behavioral sensitization) in both male and female mice after 5 days of treatment as compared to the saline control group ($P < 0.01$) (Fig. 1b; nicotine dose $F(1, 25) = 11.70$, $P < 0.005$; sex $F(1, 25) = 0.53$, NS; interaction $F(1, 25) = 0.32$, NS).

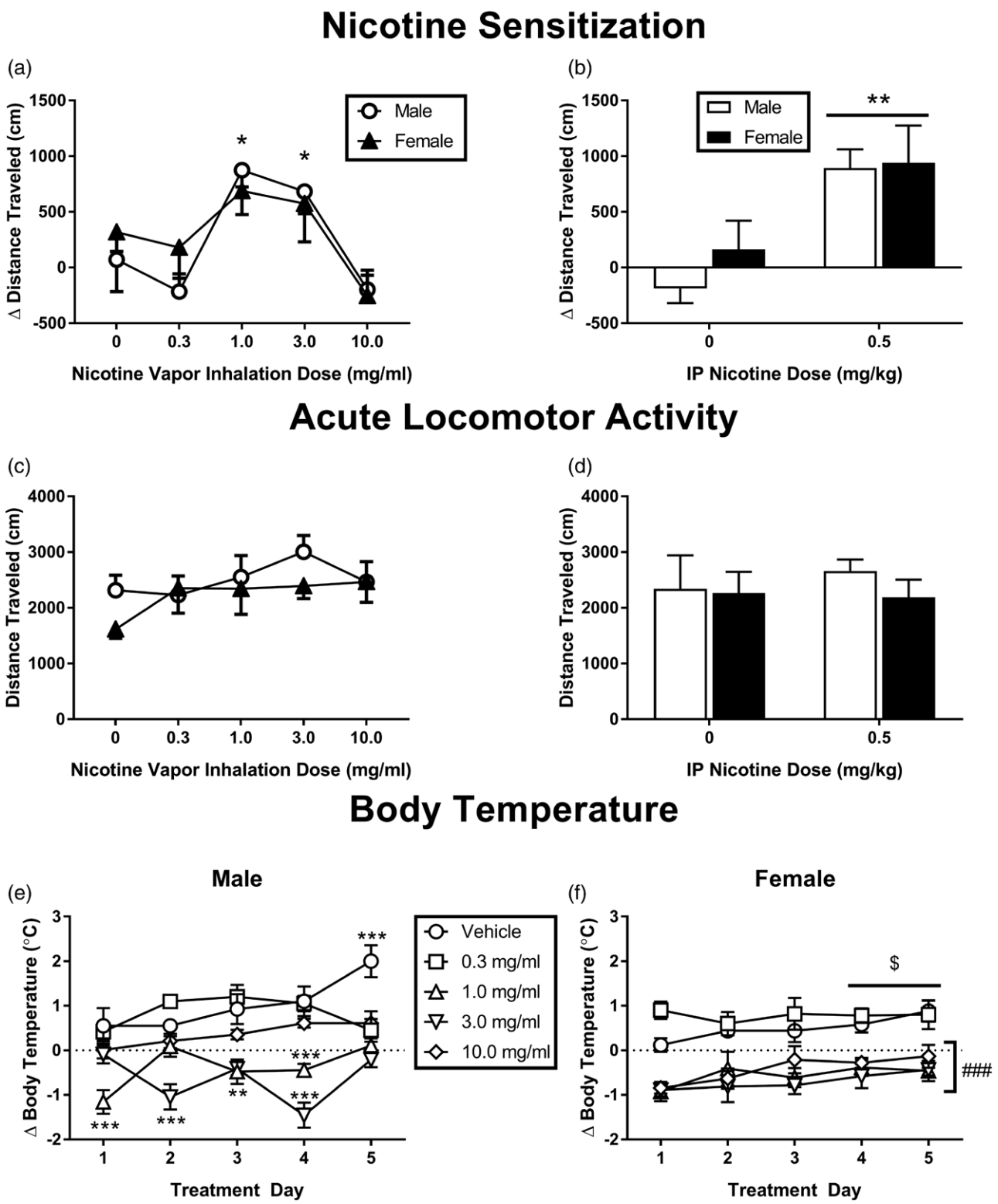
We assessed LMA on day 1 to determine if the 10 mg/ml nicotine vapor inhalation group had significantly higher LMA after the acute treatment, which could mask the expression of behavioral sensitization on day 5. Nicotine vapor inhalation (0–10 mg/ml) did not significantly alter LMA on day 1 of treatment in male or female mice (Fig. 1c; nicotine dose $F(4, 65) = 1.04$, NS; sex $F(1, 65) = 1.48$, NS; interaction $F(4, 65) = 0.48$, NS). Treatment with 0.5 mg/kg (i.p.) nicotine did not significantly change LMA on day 1 for either sex (Fig. 1d; Nicotine dose $F(1, 25) = 0.12$, NS; sex $F(1, 25) = 0.59$, NS; interaction $F(1, 25) = 0.30$, NS).

### Body temperature

Nicotine vapor inhalation significantly decreased body temperature in male mice (Fig. 1e; nicotine dose $F(4, 32) = 26.90$, $P < 0.001$; treatment day $F(4, 125) = 6.22$, $P < 0.001$; interaction $F(16, 128) = 5.49$, $P < 0.001$). Based on the interaction, the post hoc test revealed that 1.0 mg/ml nicotine significantly decreased body temperature on days 1, 3, and 4 as compared to vehicle, 0.3, 10 mg/ml nicotine ($P < 0.01$). Treatment with 3.0 mg/ml nicotine significantly decreased body temperature on days 2, 3, and 4 as compared to vehicle, 0.3, 10 mg/ml nicotine ($P < 0.001$). Lastly, all doses produced significantly lower body temperatures on day 5 as compared to vehicle ($P < 0.001$).

Treatment with vaporized nicotine significantly decreased body temperature in female mice (Fig. 1f; nicotine dose $F(4, 30) = 17.16$, $P < 0.001$; treatment day $F(4, 120) = 3.18$, $P = 0.025$; interaction $F(16, 120) = 0.57$, NS). The post hoc test for the main effect of dose found that vaporized nicotine (1.0–10.0 mg/ml) significantly decreased body temperature as compared to vehicle and 0.3 mg/ml ($P < 0.001$). Based on the main effect for treatment day, the post hoc test revealed that overall temperature

**Fig. 1**




Effect of nicotine vapor inhalation and i.p. administered nicotine and nicotine vapor inhalation behavioral sensitization, acute locomotor activity, and body temperatures. Behavioral sensitization (top panels [a and b]): abscissae: nicotine dose (nicotine vapor = mg/ml; i.p nicotine = mg/kg). Ordinates: Δ distance traveled (cm). Sex indicated in legend. Acute locomotor activity (middle panels [c and d]): abscissae: nicotine dose (nicotine vapor = mg/ml; i.p nicotine = mg/kg). Ordinates: distance traveled (cm). Sex indicated in legend. Body temperature (bottom panels [e and f]): abscissae: treatment day. Ordinates: Δ body temperature (ºC). Nicotine doses (mg/ml) are indicated in legend. Significant 2-way ANOVAs were followed by a Newman-Keuls post hoc test. All data show mean ± SEM. N = 5–9 for each drug dose. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. all doses. +++$P < 0.001$ vs. 10.0 mg/ml; ###$P < 0.001$ versus vehicle and 0.3 mg/ml; \$$P < 0.05$ versus treatment day 1.

(regardless of dose) increased on days 4 and 5 as compared to day 1 ($P < 0.05$).

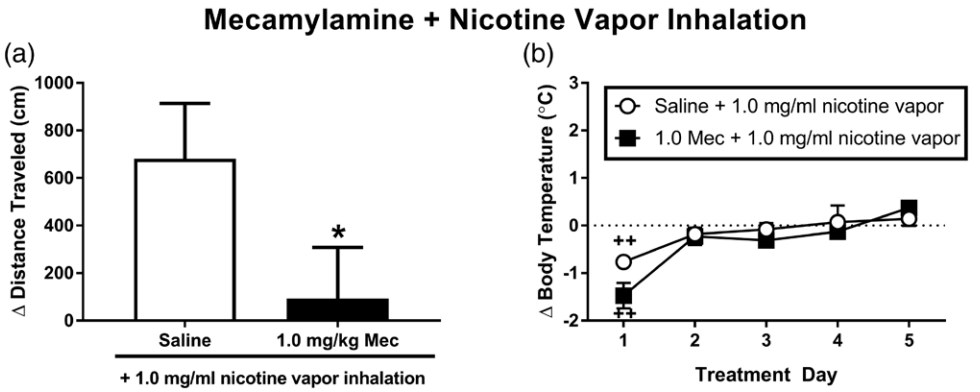
### Mecamylamine antagonist experiment

Mice were pretreated with saline or 1.0 mg/kg mecamylamine to determine if the behavioral sensitization produced by nicotine vapor inhalation was mediated by activation of nicotinic receptors. Under control conditions, mice pretreated with saline exhibited a behavioral sensitization effect consistent with 1.0 mg/ml nicotine vapor inhalation from the first experiment (Figs. 1a and 2a). Pretreatment with 1.0 mg/kg mecamylamine completely blocked the behavioral sensitization effect of 1.0 mg/ml nicotine vapor inhalation (Fig. 2; $t(20) = 1.86$, $P = 0.03$). Both saline and mecamylamine pretreated mice had a significant decrease in body temperature on day 1 as compared to all other days ($P < 0.01$). Additionally, mice pretreated with mecamylamine had a significantly greater decrease in body temperature on day 1 as compared to saline pretreated mice ($P < 0.05$) (Fig. 2b; nicotine dose $F(1, 20) = 1.17$, NS; treatment day $F(4, 80) = 23.42$, $P < 0.001$; interaction $F(4, 80) = 2.56$, $P < 0.05$).

## Discussion

This is the first study to demonstrate that nicotine vapor inhalation produces abuse-related effects in mice using the behavioral sensitization model. There were three main findings. First, nicotine vapor inhalation produced similar behavioral sensitization at 1.0 and 3.0 mg/ml as compared to injected nicotine, which is consistent with published literature (Biala and Weglinska, 2004; Biała and Budzyńska, 2010; Kotagale *et al.*, 2010; Bernardi and Spanagel, 2013; Bernardi and Spanagel, 2014). The incentive sensitization theory of addiction states that an organism becomes hypersensitive to the motivational effects of drug-associated cues, and this hypersensitivity can be attributed to neurobiological changes, associative learning, or a combination of neurobiological changes and associative learning (Segal and Schuckit, 1983; Post *et al.*, 1984; Wolf, 1998; Robinson and Berridge, 2008; Goutier *et al.*, 2015a; Goutier *et al.*, 2015b). Pavlovian conditioning is responsible for converting neutral stimuli into conditioned stimuli (i.e., drug-associated cues) that will elicit a conditioned response (i.e.,wanting or drug seeking). LMA is one way to measure behavioral sensitization as animals are administered drug and placed immediately into an open-field arena (neutral stimulus). After repeated conditioning sessions (drug administration and open-field arena), the open-field arena becomes a conditioned stimulus (drug-associated cue) that elicits a conditioned response (increased LMA). Sensitization is a behavioral assay similar to CPP in that repeated drug conditioning sessions elicits a conditioned response; however, the main difference in CPP is that the animal has the ability to choose between two different environments (saline-paired or drug-paired). In the behavioral sensitization assay, the increased LMA is generally expressed in the open-field arena used for conditioned (drug-associated cue) and does not typically generalize to a neutral environment. Although behavioral sensitization does not directly assess abuse-related or drug-seeking effects, there is a correlation between the drug doses that produce behavioral sensitization and abuse-related effects in other behavioral models such as self-administration, CPP, and intracranial self-stimulation (ICSS) (Segal and Schuckit, 1983; Wolf, 1998; Robinson and Berridge, 2008). Relatively, few studies have evaluated the abuse-related effects of nicotine vapor inhalation. For example, 12-hour/day exposure to nicotine vapor enhanced intravenous nicotine self-administration responding in male rats (Gilpin *et al.*, 2014). Although not administered as a vapor, injected nicotine e-liquid decreased ICSS thresholds in rats (Harris *et al.*,

**Fig. 2**




Effect of 1.0 mg/kg mecamylamine pretreatment on 1.0 mg/ml nicotine vapor inhalation behavioral sensitization and body temperature. (a) Abscissae: drug treatment. Ordinates: Δ distance traveled (cm). (b) Abscissae: treatment day. Ordinates: Δ body temperature (°C). Drug treatment is indicated in legend. All data show mean ± SEM. N = 11 for each treatment condition. *$P < 0.05$ vs. saline + 1.0 mg/ml nicotine vapor inhalation. ++$P < 0.01$ vs. day 2, 3, 4, and 5. Mec, mecamylamine.

2018). Moreover, 7 weeks of vaporized nicotine exposure produces somatic withdrawal symptoms and anxiolytic behaviors in male mice, which is consistent with nicotine dependence in rodents. These withdrawal-like behaviors were accompanied with upregulation of α4β2 receptors in the cortex, hippocampus, nucleus accumbens, and caudate-putamen (Ponzoni *et al.*, 2015).

The second main finding is that nicotine vapor inhalation produced an inverted-U shaped function for behavioral sensitization with 1.0 and 3.0 mg/ml producing sensitization, whereas 10.0 mg/ml failed to produce sensitization. We hypothesize that 10 mg/ml is on the downward slope of the inverted U-shaped curve and that 30.0 mg/ml would likely decrease LMA on day 1. We evaluated acute LMA to determine if increased activity on day 1 was masking the expression of sensitization; however, acute 10 mg/ml dose of nicotine did not significantly influence (increase or decrease) LMA (Fig. 1). This inverted U-shaped curve is not uncommon when assessing the behavioral effects of nicotine. Systemic administration of nicotine has been shown to produce this same inverted U-shaped curve in several behavioral assays including sensitization (Celik *et al.*, 2006), anxiolytic-like behaviors (McGranahan *et al.*, 2011), CPP (Grabus *et al.*, 2006; Walters *et al.*, 2006; Kota *et al.*, 2007; Kota *et al.*, 2008; Brunzell *et al.*, 2009; McGranahan *et al.*, 2011) and ICSS (Freitas *et al.*, 2016; Harris *et al.*, 2018). For example, systemic administration of nicotine will produce CPP in mice at low doses (0.1, 0.3, and 0.5 mg/kg) but not at higher nicotine doses (0.7 or 1.0 mg/kg) (Walters *et al.*, 2006; Kota *et al.*, 2007; Kota *et al.*, 2008). Collectively, these studies suggest there is an optimal dose range for nicotine effects in behavioral assays and that higher doses tend to decrease behavior regardless of administration route.

The third main finding is the sensitization effects produced by nicotine vapor inhalation were a direct result of activation of nicotinic receptors. In the antagonist experiment, 1.0 mg/ml nicotine vapor inhalation produced behavioral sensitization consistent with experiment 1 in mice pretreated with saline; however, behavioral sensitization was completely blocked in animals pretreated with mecamylamine. Additionally, all experiments used an unflavored 50:50 propylene glycol:vegetable glycerin oil blend e-liquid, which does not have an appealing scent or flavor, to reduce the likelihood that the vapor would serve as a conditioned stimulus to produce a conditioned response of increased LMA. Moreover, the vapor e-liquid vehicle and 0.3 mg/ml did not produce behavioral sensitization demonstrating that vapor alone did not elicit the behavioral sensitization effect.

The present study found that acute nicotine vapor inhalation (Fig. 1c and d) did not significantly alter LMA, which is consistent with previous reports that found acute 1.0, 10.0, and 12.0 mg/ml nicotine vapor inhalation did not alter LMA (Lefever *et al.*, 2017; Javadi-Paydar *et al.*, 2019). However, Lefever *et al.* (2017) found that acute 30.0 mg/ml vaporized nicotine decreased LMA, which is consistent with our inverted U-shaped curve hypothesis that 30.0 mg/ml would decrease activity in our dosing regimen. It is important to note that acute treatment with 30.0 mg/ml did not alter LMA in male rats (Javadi-Paydar *et al.*, 2019). Interestingly, we found hypothermia effects at much lower doses (1–10.0 mg/ml) than previously reported. For example, Lefever *et al.* (2017) and Javadi-Paydar *et al.* (2019) found hypothermia at high doses of nicotine (24 and 30 mg/ml) but not at lower doses (1–12.0 mg/ml) similar to doses tested in the present study. Several factors might contribute to the differences in LMA and hypothermia: (1) species/strain (rat vs. mouse; C57LB/6 vs. ICR), (2) methods for measuring body temperature (rectal vs. implant), (3) time of body temperature measurement (immediate vs. 10 vs. ≥30 minutes), and (4) dosing procedures. Finally, the present study or Lefever *et al.* (2017) found no sex differences when evaluating LMA, but both studies found that female mice were more sensitive to the hypothermic effects of nicotine vapor inhalation. In the present study, the hypothermic effect was more variable in male mice and 10 mg/ml failed to produce hypothermia. Unlike the inverted U-shaped curved found in behavior, the hypothermic effect should be a monotonic function in which 10 mg/ml should have produced hypothermia. Here are two possible explanations. First, we used a rectal probe to measure body temperature. It is known that the rectal probe increases stress in rodents and can produce hyperthermia that may persist even after acclimation to probe insertion (Poole and Stephenson, 1977; Bae *et al.*, 2007). The hypothermic effect found in this study was approximately 1°C to 1.5°C, and thus it is possible that probe induced hyperthermia is masking the hypothermic effect. Second, the male mice are larger in size and thus dose to body weight ratio might be difference for male and female mice. It is difficult to control the exact amount of drug being administered when using a vapor or smoke, making it particularly difficult to track any dosing differences between male and female mice in this context. Previous studies evaluating sex differences following nicotine administration have found mixed results dependent upon the route of administration and behavioral assays (Caldarone *et al.*, 2008; Illenberger *et al.*, 2018). The present study has provided a behavioral method that will allow the use of male and female mice without the risk of increased variability due to sex difference.

In conclusion, the results from the present study add to an emerging body of literature focused on the behavioral effects of nicotine vapor inhalation. Specifically, we demonstrated that nicotine vapor inhalation produces abuse-related effects in a model of behavioral sensitization that is mediated by nicotinic receptor activation. The methods used in the present study provide an additional behavioral approach for evaluating the behavioral

effects of repeated nicotine vapor inhalation that allows the manipulation of several variables, including e-liquid oil blend, e-liquid flavors, puff duration, etc.

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### Conflicts of interest

There are no conflicts of interest.

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