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Exposure to passive nicotine vapor in male adolescent rats produces a withdrawal-like state and facilitates nicotine self-administration during adulthood

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Abstract

Electronic cigarette use is particularly prevalent in adolescents, but the effects of secondhand exposure to nicotine vapor in adolescents on the propensity to develop nicotine dependence and increase nicotine self-administration in adulthood are poorly known. The present study explored the effects of nicotine vapor exposure on withdrawal-like states (hyperalgesia, spontaneous withdrawal signs, and locomotor activity) in adolescent rats and the vulnerability to acquire intravenous nicotine self-administration in adulthood. Adolescent (postnatal day 38) rats were exposed to intermittent nicotine vapor (14 h/day) for 7 consecutive days in a range of doses (0, 0.4, and 7 mg/m^3). The rats were tested for somatic, emotional, and motivational withdrawal symptoms. When the animals reached adulthood, they were allowed to self-administer nicotine (0.03 mg/kg/0.1 ml) intravenously in operant chambers for 1 h/day for 12 consecutive days. Rats that were exposed to nicotine vapor presented moderate to severe signs of spontaneous withdrawal after the cessation of nicotine vapor. No effect on anxiety-like behavior was observed. Rats that were exposed to high levels of nicotine vapor in adolescence had lower pain thresholds and exhibited faster and higher acquisition of nicotine self-administration in adulthood. Chronic exposure to nicotine vapor in adolescent rats produced a withdrawal-like state and facilitated the acquisition of intravenous nicotine self-administration in adulthood. These

results suggest that exposure of adolescents to nicotine vapor may confer higher risk of developing nicotine dependence when they become adults.

Introduction

Electronic cigarettes (e-cigarettes) are battery-operated devices that are designed to deliver nicotine through vapor instead of smoke. Epidemiological population-based studies indicate that the rate of e-cigarette use across countries is increasing among both current and former smokers and dramatically increasing among nonsmokers, particularly among adolescents (https://www.drugabuse.gov/publications/drugfacts/electronic-cigarettes-e-cigarettes; accessed July 7, 2019). Recent clinical data suggest that e-cigarette use is most appealing and prevalent among adolescents. Findings from the 2014 National Youth Tobacco Survey showed that current e-cigarette use among high school students increased from 4.5% in 2013 to 13.4% in 2014, increasing from approximately 660,000 to 2 million students. In fact, e-cigarette use has surpassed the use of every other tobacco product overall, including conventional cigarettes, since 2011 to date. Nicotine dependence is often characterized by the emergence of a nicotine abstinence syndrome after the cessation of chronic nicotine exposure. Such an abstinence syndrome has been characterized in both humans and rats and is associated with both somatic and motivational components (Shiffman & Jarvik, 1976; Hughes *et al.*, 1991; Malin *et al.*, 1992; Malin *et al.*, 1993; Malin *et al.*, 1994; Hildebrand *et al.*, 1997; Epping-Jordan *et al.*, 1998; Watkins *et al.*, 2000). In rats, the somatic signs of nicotine withdrawal (both spontaneous withdrawal and withdrawal that is precipitated by the nicotinic acetylcholine receptor [nAChR] antagonist mecamylamine) include

abdominal constrictions, facial fasciculation, ptosis, and hyperalgesia. The motivational components include anxiety-like behavior and higher motivation (craving) for nicotine (Bruijnzeel, 2016). However, unclear are the ways in which adolescent exposure to nicotine vapor affects the somatic and motivational aspects of nicotine dependence.

The first goal of the present study was to measure the effect of chronic exposure to nicotine vapor on key behaviors that are related to nicotine withdrawal (somatic signs, hyperalgesia, and anxiety-like behavior) in adolescent rats. The second goal was to test whether chronic exposure to nicotine vapor during adolescence produces long-lasting changes that confer greater vulnerability to self-administer intravenous nicotine in adulthood. Human studies have shown that the air concentrations of nicotine that are emitted by various brands of e-cigarettes range from 0.82 to 6.23 μg/m^3 (Czogala *et al.*, 2014). We tested the effects of two concentrations of nicotine vapor (0.4 and 7 mg/m^3) over 7 days of exposure (14 h/day). Intermittent nicotine vapor exposure, with 14 h vapor ON and 10 h OFF, was used based on previous studies (Baiamonte *et al.*, 2014; Gilpin *et al.*, 2014; McGinn *et al.*, 2016; Xue *et al.*, 2018) that showed that this protocol produces nicotine dependence with somatic signs of withdrawal and hyperalgesia when exposed to high levels of nicotine (7 mg/m^3). This protocol is highly relevant to adolescent e-cigarette users, who often vape intermittently because of either parental or societal restrictions. Withdrawal in rats peaks 16 h into withdrawal but can be observed 10 h into withdrawal (Grieder *et al.*, 2010).

Our overall hypothesis was that repeated mild withdrawal (10 h) is an important factor in the transition to addiction in both rats and humans, thus this parameter is particularly important in the present study.

Materials and Methods

Animals

Male Wistar rats (*n* = 18) were obtained from Charles River (Wilmington, MA, USA) 1 week after their weaning period (postnatal day [PND] 28). The rats weighed 120-150 g upon arrival in the laboratory. The rats were tested during adolescence (PND 38-46; 200-220 g). Nicotine self-administration was performed during adulthood (PND 66-78; 300-330 g). The animals were group housed (3 rats/cage) in standard cages in a room with artificial lighting (12 h/12 h light/dark cycle, lights off at 8:00 AM) at a constant temperature (20-22°C) and humidity (45-55%) with food (2018 Teklad global 18% protein rodent diet; Teklad, Madison, WI, USA) and water available *ad libitum* except during testing. The rats were handled twice for 5 min after arrival in the animal facility. The animal procedures met the guidelines of the National Institutes of Health and were approved by The Scripps Research Institute Institutional Animal Care and Use Committee (protocol no. 08-0015). All of the surgical procedures were performed under isoflurane anesthesia, and all necessary efforts were made to minimize suffering of the animals.

Nicotine vapor exposure

The rats (*n* = 6/group) were group housed in standard rat cages (3 rats/cage) and exposed daily from 9:00 AM to 11:00 PM to intermittent nicotine vapor (14 h ON/10 h OFF). Every cage had an air-tight transparent Plexiglas lid. (-)Nicotine (≥ 99% [GS], 1.010 g/ml liquid, Sigma-Aldrich, St. Louis, MO, USA) was used at 20°C. For the entire duration of nicotine vapor exposure (14 h) and air exposure (10 h) for 7 consecutive days, the rats were left undisturbed in the nicotine vapor chamber with food and water available *ad libitum*. Nicotine vapor was produced as previously described (George *et al.*, 2010) to reach nicotine vapor concentrations of 0.4 and 7 mg/m^3. The nicotine:air ratio was 0:20 L/min for the air control group, 1:19 L/min for the 0.4 mg/m^3 nicotine group, and 10:10 L/min for the 7.0 mg/m^3 nicotine group. Gas chromatography was used to analyze the level of nicotine in the test chamber. A glass tube (70 mm length, 7 mm outer diameter) that contained two sections of XAD-4 (front = 80 mg, back = 40 mg) that were separated by silicate glass wool was used. The concentration (*C*) of nicotine was calculated as the following: $C = Wf \times Wb \times Bf \times Bb / V$ (mg/m^3 = μg/L). Mass (μg) was corrected for desorption efficiency (DE). *Wf* is the sample front of the sorbent sections, and *Wb* is the sample back of the sorbent sections, and in the average media blank front (*Bf*) and back (*Bb*) sorbent sections. Air control rats were treated similarly to nicotine-exposed rats, with the exception that the air that entered the chambers did not contain nicotine.

Withdrawal signs

After 7 days of intermittent nicotine vapor exposure (14 h ON/10 h OFF), the rats were maintained nicotine-free for 16-24 h and experienced spontaneous withdrawal in their home cages. On the test day, after 16 h of no exposure to nicotine vapor, the rats were placed inside a transparent cylinder (50 cm height × 30 cm diameter) and observed for 30 min. The number of wet dog shakes, front paw tremors, teeth chattering, genital licks, and abdominal constrictions was counted. The sum of the observation scores was used as a quantitative measure of withdrawal severity. Three experimenters who were blind to the treatment group scored the withdrawal signs. The average of the three measures was used for the analyses.

Open field test

The open field test was performed at 16-24 h of nicotine abstinence after 7 days of nicotine vapor exposure. Locomotor activity and anxiety-like behavior were evaluated in an opaque open field apparatus (100 cm × 100 cm × 40 cm) that was divided into 20 cm × 20 cm squares. On the experimental day, the animals were placed in the center of the open field, and locomotor activity (number of lines crossed), time in the center, entries into the center, and total distance traveled (in centimeters) were scored for 10 min. Behavior was videotaped, and AnyMaze software was used to analyze the recordings.

Mechanical nociception in the von Frey test

Mechanical nociception was evaluated using the von Frey test. The test was first performed 1 day before placing the animals into the nicotine exposure chamber to determine the baseline pain threshold of each rat. The second test was conducted after 7 days of nicotine vapor exposure at 16-24 h of nicotine withdrawal (immediately after withdrawal signs were scored and the locomotor activity test was performed). The hindpaw withdrawal threshold was determined using von Frey filaments, ranging from 3.63 to 125.89 g (Edwards *et al.*, 2012; Xue *et al.*, 2018). A test session began after 10 min of habituation to the testing environment. A series of von Frey filaments were applied in ascending order from below a wire mesh to the central region of the plantar surface of the left hindpaw, beginning with the lowest filament (3.63 g). The filament was applied until buckling of the hair occurred, and it was maintained for approximately 2 s. A withdrawal response was considered valid only if the animal completely removed its hindpaw from the mesh platform. The stimulus was incrementally increased until a positive response was observed and then decreased until a negative response was observed to determine a pattern of responses to apply to the statistical method of Dixon (Dixon, 1980). The 50% paw withdrawal threshold was calculated as the following: $Xf + k\delta$, where Xf is the last von Frey filament employed, k is the Dixon value that corresponds to the response pattern, and δ is the mean difference between stimuli. Once the threshold was determined for the left hindpaw, the same testing procedure was

applied to the right hindpaw after 5 min.

Intravenous catheterization

Chronic intravenous jugular catheters were implanted in all of the rats on PND 58 as described previously (Caine & Koob, 1993). The rats were anesthetized with 1-3% isoflurane in an oxygen mixture. Incisions were made to expose the right jugular vein. A catheter that was made from Micro-Renathane tubing (0.020 inches inner diameter; 0.037 inches outer diameter; Braintree Scientific, Braintree, MA, USA) was subcutaneously implanted. After insertion into the vein, the proximal end of the catheter was anchored with surgical silk to the muscles under the vein. The distal end of the catheter was attached to a stainless-steel cannula that was bent at a 90° angle. The cannula was anchored in dental cement on the back of the rats. For 1 week after surgery, the rats were treated daily with 0.2 ml of the antibiotic Cefazolin (262 mg/ml). For the duration of the experiments, the catheters were flushed daily with 0.2-0.3 ml of heparinized saline solution. Body weight was monitored every other day. Catheter patency was confirmed at the end of the experiment with an injection of 0.2-0.3 ml of Brevital sodium solution (10 mg/ml). Catheter patency was assumed if there was an immediate loss of reflexes after the injection. The rats were given 1 week to recover from surgery before the self-administration experiments.

Operant training

The self-administration chambers consisted of operant conditioning chambers (Med Associates, St. Albans, VT, USA) that were enclosed in sound-attenuating, ventilated environmental cubicles. Each chamber was equipped with two retractable levers that were located in the front panel. A plastic tube that was connected to the catheter before beginning the session delivered nicotine. An infusion pump was activated by responses on the right ("active") lever, and responses on the left ("inactive)" lever were recorded but did not result in any programmed consequences. Activation of the pump resulted in the delivery of 0.1 ml of nicotine solution (0.03 mg/kg/0.1 ml) on a fixed-ratio 1 (FR1) schedule of reinforcement for 1 h, paired with illumination of the chamber by the cue light. The delivery of nicotine solution was followed by a 20-s timeout (TO) period, during which further lever presses did not result in any consequences. Nicotine solution was prepared fresh every other day from (-)nicotine hydrogen tartrate salt (Sigma-Aldrich) based on the animals' body weight, and the pH was adjusted to 7.3 with 1 M NaOH.

Experimental design

The experimental design is summarized in Fig. 1. Adolescent rats (PND 37) were subjected to the von Frey test prior to the behavioral tests so that we could perform within-subjects analyses, based on the known between-subjects variability in pain thresholds in rats. The animals were equally divided into three groups (n = 6/group). They were placed in the nicotine

vapor chambers and exposed daily to intermittent nicotine vapor (14 h ON/10 h OFF). Body weight was recorded every other day. After 7 days of intermittent nicotine exposure, the animals were returned to their home cages in the animal facility. Withdrawal scores, including teeth chattering, paw tremors, genital licking, wet-dog shakes, and abdominal constrictions, were recorded after 16 h of spontaneous withdrawal for 30 min starting at 3:00 PM the next day. After the assessment of somatic withdrawal signs, locomotor activity was assessed in a 10 min test at 16-24 h of nicotine withdrawal. The total distance traveled, number of entries into the central zone of the arena, and time spent in the central zone were recorded. Immediately after scoring spontaneous withdrawal signs and evaluating locomotor activity, the von Frey test was performed. On PND 58, the rats underwent intravenous catheter implantation surgery. They were allowed to recover for 1 week after surgery. Starting on PND 66, the rats were given daily 1-h access to nicotine self-administration (0.03 mg/kg/0.1ml) for 12 consecutive days.

Statistical analysis

The data were analyzed using mixed-factorial or one-way analysis of variance (ANOVA), followed by the Newman-Keuls *post hoc* test when appropriate. For individual means comparisons, Student's paired or unpaired *t*-test was used. Data from the mechanical nociception test during spontaneous withdrawal were analyzed using Student's *t*-test by comparing

the withdrawal results with the rats' baselines during the naive state. Values of $p < 0.05$ were considered statistically significant.

Results

Vapor exposure induced nicotine dependence in adolescent rats

The animals that were exposed to the highest concentration of nicotine vapor exhibited significant body weight loss compared with rats that were exposed to air and the low concentration of nicotine vapor (Fig. 2a). The mixed-factorial ANOVA, with group (passive nicotine dose) as the between-subjects factor and time as the within-subjects factor, revealed main effects of group ($F_{2,15} = 34.2$, $p < 0.001$) and time ($F_{3,15} = 17.73$, $p < 0.001$) and a significant group $\times$ time interaction ($F_{6,45} = 17.434$; $p < 0.001$). The Newman-Keuls *post hoc* test showed that rats that were exposed to the high concentration of nicotine vapor (7 mg/m^3) exhibited a significant loss of body weight on day 3 ($p < 0.05$), day 5 ($p < 0.01$), and day 7 ($p < 0$ .01) compared with the air-exposed group.

Rats that were exposed to the highest concentration of nicotine vapor exhibited a decrease in mechanical thresholds during spontaneous withdrawal ($t_5 = 3.8$, $p < 0.01$) relative to their own baseline pain threshold that was established prior to nicotine vapor exposure (from 46.7 ± 7.1 g to 21.5 ± 4.3 g; Fig. 2b). The one-way ANOVA revealed that 7-day exposure to intermittent nicotine vapor significantly increased pain sensitivity ($F_{2,15} = 5.73$, $p < 0.05$) during nicotine withdrawal. Rats that were exposed to the

highest concentration of nicotine vapor (7 mg/m^3) were more sensitive to pain ($p < 0.05$) compared with air-exposed rats. The number of spontaneous withdrawal signs, measured 16-24 h after the cessation of nicotine vapor, is shown in Fig. 2c. The one-way ANOVA revealed that 7-day exposure to nicotine vapor induced significant spontaneous withdrawal signs after the discontinuation of intermittent nicotine vapor ($F_{2,15} = 10$, $p < 0.0013$). The Newman-Keuls *post hoc* test showed that both concentrations of nicotine vapor (0.04 and 7 mg/m^3) significantly increased withdrawal scores ($p < 0.05$ and $p < 0.01$, respectively) compared with air-exposed rats.

Effect of nicotine vapor withdrawal on anxiety-like behavior

Immediately after the assessment of somatic withdrawal signs, locomotor activity was recorded over a 10-min period (Fig. 3). The ANOVA revealed a nonsignificant trend toward an overall difference in the time spent in the center ($F_{2,15} = 0.36$, $p = 0.7$; Fig. 3a), number of entries into the center ($F_{2,15} = 2.52$, $p = 0.11$; Fig. 3b), and total distance traveled ($F_{2,15} = 3.43$, $p = 0.059$; Fig. 3c) between the air- and nicotine-exposed groups. A trend toward a decrease in the number of entries into the center and total distance traveled was observed in nicotine-withdrawn animals compared with air-exposed controls.

Effect of nicotine vapor exposure during adolescence on intravenous nicotine self-administration during adulthood

After completing the first set of experiments, the rats were left undisturbed in their home cages in the animal facility. On PND 58, the animals were implanted with intravenous catheters to assess nicotine self-administration. After 1 week of recovery from surgery, the animals were placed in operant chambers and allowed to self-administer nicotine (0.03 mg/kg/0.1 ml) in 1 h daily sessions. Animals that were previously exposed to the highest concentration of nicotine (7 mg/m^3) during adolescence exhibited higher nicotine intake during adulthood compared with rats that were exposed to air or low-concentration nicotine vapor during adolescence (Fig. 4a). The mixed-factorial ANOVA, with group (passive nicotine dose) as the between-subjects factor and time as the within-subjects factor, revealed main effects of group ($F_{2,15} = 3.98$, $p < 0.05$) and time ($F_{10,15} = 3.88$, $p < 0.001$) but no group $\times$ time interaction ($F_{20,150} = 0.32$, $p > 0.05$). The one-way ANOVA showed that total nicotine intake over the 12 sessions of intravenous nicotine self-administration was higher in animals that were previously exposed to 7 mg/m^3 nicotine vapor during adolescence ($F_{2,15} = 15.49$, $p < 0.0001$; Fig. 4b**)**. The Newman-Keuls *post hoc* test revealed that rats that were exposed to 7 mg/m^3 nicotine vapor during adolescence exhibited higher intravenous nicotine intake in adulthood compared with the air-exposed group ($p < 0.001$).

Discussion

The present study evaluated the effects of nicotine vapor exposure on withdrawal-like states in adolescent rats and the propensity to initiate intravenous nicotine self-administration in adulthood. Chronic exposure to 0.4 mg/m^3 nicotine vapor during adolescence increased somatic signs of nicotine withdrawal but was not associated with hyperalgesia, anxiety-like behavior, or the vulnerability to self-administer nicotine in adulthood. Chronic exposure to 7 mg/m^3 nicotine vapor during adolescence increased somatic signs of nicotine withdrawal and increased hyperalgesia and the vulnerability to intravenously self-administer nicotine in adulthood.

We first tested whether intermittent exposure to low or high levels of nicotine vapor for 7 days in adolescent rats produces nicotine dependence. Withdrawal from nicotine induces somatic signs of withdrawal and negative affect in humans, leading to bradycardia, insomnia, gastrointestinal discomfort, increased appetite, weight gain, irritability, depressed mood, restlessness, anxiety, increased stress, and craving for tobacco (Hughes *et al.*, 1991; Kallupi & George, 2017). In rodents, the cessation of nicotine administration or administration of nAChR antagonists in rats that are chronically exposed to nicotine results in the emergence of somatic signs of nicotine withdrawal, including abdominal constrictions, facial fasciculation, increased eye blinks, and ptosis (Malin *et al.*, 1992; Malin *et al.*, 1994; Hildebrand *et al.*, 1997; Epping-Jordan *et al.*, 1998). Our results showed that nicotine vapor exposure at levels that are sometimes observed in environments that allow prolonged and high secondhand exposure to

tobacco or e-cigarettes (Pellegrino *et al.*, 2012; Evans & Hoffman, 2014; Schroeder & Hoffman, 2014) produced nicotine dependence, reflected by the emergence of spontaneous somatic signs of withdrawal. Notwithstanding the slight possibility that a fraction of nicotine was absorbed through the skin, toxicological studies have demonstrated that skin absorption represents less than 10% of the total dose that is received from combined skin and inhalation exposure (Hayes & Bakand, 2010). These results are consistent with our previous findings (George *et al.*, 2010) and further demonstrate that 1 week of exposure to nicotine vapor (0.4 mg/m^3) is sufficient to produce mild signs of nicotine dependence in adolescent rats. We also observed a significant reduction of body weight and pronounced hyperalgesia in rats that were exposed to a high level of nicotine compared with rats that were exposed only to air or a low level of nicotine, suggesting that the high nicotine concentration resulted in a greater level of dependence, similar to observations in heavy smokers (Shiffman & Paty, 2006; Scheuermann *et al.*, 2015). This finding is consistent with other studies that showed that high doses of nicotine reduce appetite and alter feeding patterns, typically resulting in a decrease in body weight (Grunberg, 1986; Grunberg *et al.*, 1986; Blaha *et al.*, 1998; Miyata *et al.*, 1999; Bray, 2000; Zhang *et al.*, 2001).

No significant alterations of locomotor activity were observed in the open field test. One possible explanation is that withdrawal symptoms may have interfered with measures of locomotor activity, such as entries in the center zone and total distance traveled. Compared with nicotine-naive rats,

nicotine-tolerant rats develop greater mechanical hyperalgesia. Clinically, long-term smokers have a higher risk of suffering chronic pain (Ditre *et al.*, 2011; Fishbain *et al.*, 2013; Baiamonte *et al.*, 2014; Cohen *et al.*, 2015). Our data are consistent with our previous studies that reported the development of hyperalgesia in nicotine-dependent rats with extended access to nicotine self-administration (George *et al.*, 2010; Cohen & George, 2013; Cohen *et al.*, 2015). A few clinical studies have provided preliminary evidence that e-cigarettes may serve as a smoking cessation aid. These studies reported the alleviation of craving and a decrease in nicotine withdrawal symptoms over time when these devices are used as substitutes for regular tobacco cigarettes (Dawkins *et al.*, 2015; Lechner *et al.*, 2015; Malas *et al.*, 2016). However, more studies are needed to establish the long-term effects of the cessation of e-cigarette use. Unclear is whether e-cigarette use in adolescence produces dependence in humans. Several studies have investigated potential differences in nicotine intake and relapse during adolescence and adulthood using established rat models of nicotine self-administration (Shram *et al.*, 2008). Other studies have examined the rewarding properties of nicotine in adolescents compared with adults using conditioned place preference and conditioned taste aversion procedures (Vastola *et al.*, 2002; Belluzzi *et al.*, 2004; Torrella *et al.*, 2004; Shram *et al.*, 2006). Several studies have reported that adolescent rats may acquire nicotine self-administration faster than adult rats (Belluzzi *et al.*, 2005; Levin *et al.*, 2007) but one caveat of these studies is that they used

different routes of administration. The present results demonstrate that 1 week of exposure to a wide range of concentrations of nicotine vapor (0.4-7 mg/m^3) is sufficient to produce nicotine dependence. Such behavioral changes may contribute to the maintenance and progression of e-cigarette use through a mechanism of negative reinforcement.

One limitation of the present study was that the blood nicotine levels was not measured. However, we previously reported that this level of nicotine exposure leads to blood nicotine levels in the 10-100 ng/ml range (George *et al.*, 2010; Gilpin *et al.*, 2014). Air nicotine concentrations in the 1-10 mg/m^3 range closely mimic conditions of human smoking. Moreover, other groups (Baiamonte *et al.*, 2014; McGinn *et al.*, 2016; Xue *et al.*, 2018) have replicated our original findings, showing that the delivery of 7 mg/m^3 nicotine at 10 L/min produced nicotine dependence with somatic signs of withdrawal and hyperalgesia, similar to other animal models (e.g., minipumps and intravenous self-administration), and blood nicotine levels in the 10-100 ng/ml range. Another limitation of the present study was that we included male adolescent rats only. Further studies in females and human studies are needed to clarify the effect of e-cigarette use on nicotine dependence in adolescence.

In a previous study, we found that chronic intermittent nicotine vapor inhalation produced the escalation of nicotine self-administration and physical dependence in adult rats (Gilpin *et al.*, 2014). However, the long-term consequences of adolescent exposure to nicotine vapor on intravenous

self-administration in adulthood has not been previously investigated. The present results showed that exposure to high levels of nicotine (7 mg/m^3) during adolescence facilitated the acquisition of intravenous nicotine self-administration in adulthood compared with rats that were exposed only to air or low nicotine levels. These results suggest that adolescent exposure to high-dose nicotine can either increase the positive reinforcing effects of nicotine or increase the negative reinforcing effects of withdrawal.

In summary, the present study demonstrated that chronic exposure to nicotine vapor in adolescence produced a withdrawal-like state and facilitated the acquisition of intravenous nicotine self-administration in adulthood. Secondhand exposure to e-cigarettes that contain high levels of nicotine may have long-term consequences on the vulnerability to develop nicotine dependence and the motivation for nicotine. Future animal studies will elucidate the neurobiological changes that are associated with secondhand exposure to e-cigarettes.

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Figure Legends

Figure 1. Timeline of the experiments and representative schematic diagram of the nicotine vapor machine.

Figure 2. (**a**) Body weight of rats that were exposed to air (0.0 mg/m^3) and nicotine vapor (0.4 and 7 mg/m^3). *$p < 0.05$, ***$p < 0.001$, compared with day 1; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, difference between air-exposed rats and nicotine (7 mg/m^3)-exposed rats on days 3, 5, and 7. (**b**) Pain thresholds. The data are expressed as a percent difference from baseline. **$p < 0.01$, significant decrease in pain thresholds in nicotine (7 mg/m^3)-exposed rats compared with their own baseline; $^{\#}p < 0.05$, difference between nicotine (7 mg/m^3)-exposed rats and air-exposed rats. (**c**) Spontaneous withdrawal symptoms. *$p < 0.05$, **$p < 0.01$, compared with air-exposed rats.

Figure 3. Open field test. (**a**) Time in center. (**b**) Entries into center. (**c**) Total distance traveled. No significant differences were observed between groups.

Figure 4. Intravenous nicotine self-administration in adulthood. (**a**) Number of nicotine rewards in 1 h sessions over 12 consecutive days. (**b**) Average number of nicotine rewards in air-exposed and nicotine-exposed rats. ***$p < 0.001$, compared with air-exposed rats. (**c**) Inactive lever responses. No differences were observed between groups.

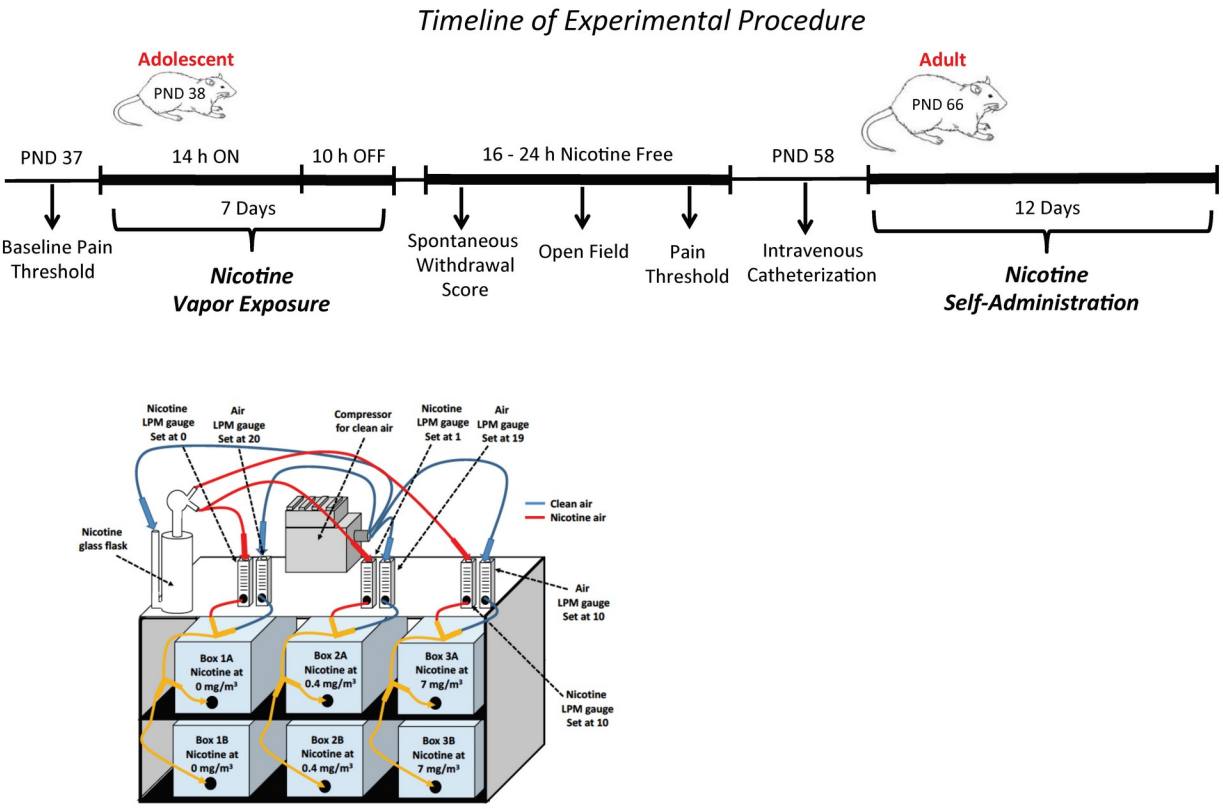
Timeline of Experimental Procedure
Adolescent
PND 38
Adult
PND 66
PND 37
14 h ON
10 h OFF
16 - 24 h Nicotine Free
PND 58
7 Days
12 Days
Baseline Pain Threshold
Nicotine Vapor Exposure
Spontaneous Withdrawal Score
Open Field
Pain Threshold
Intravenous Catheterization
Nicotine Self-Administration
Nicotine LPM gauge Set at 0
Air LPM gauge Set at 20
Compressor for clean air
Nicotine LPM gauge Set at 1
Air LPM gauge Set at 19
Clean air
Nicotine air
Nicotine glass flask
Air LPM gauge Set at 10
Nicotine LPM gauge Set at 10
Box 1A Nicotine at 0 mg/m³
Box 2A Nicotine at 0.4 mg/m³
Box 3A Nicotine at 7 mg/m³
Box 1B Nicotine at 0 mg/m³
Box 2B Nicotine at 0.4 mg/m³
Box 3B Nicotine at 7 mg/m³

Figure 1

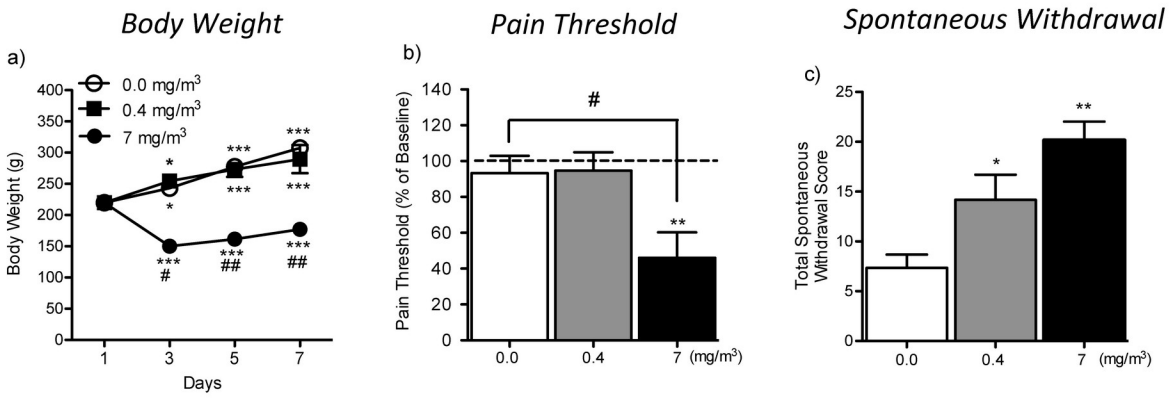
Body Weight
a)
0.0 mg/m3
0.4 mg/m3
7 mg/m3
Body Weight (g)
Days
Pain Threshold
b)
Pain Threshold (% of Baseline)
(mg/m3)
Spontaneous Withdrawal
c)
Total Spontaneous Withdrawal Score
(mg/m3)

Figure 2

Open Field

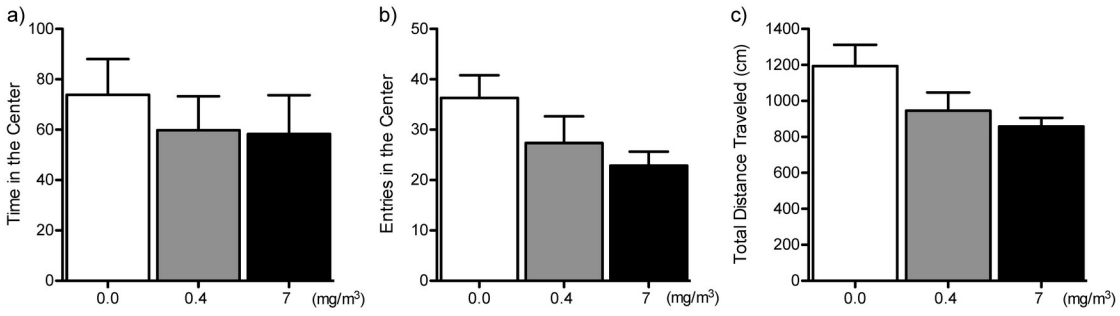

Figure 3

Nicotine Self-Administration

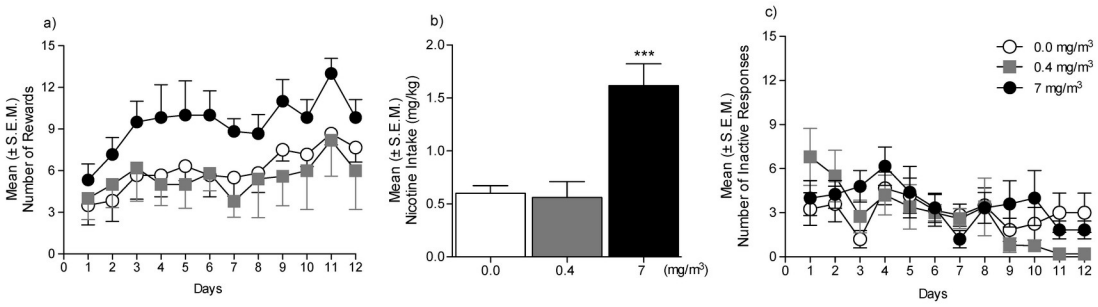

Figure 4