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The effects of acute and repeated nicotine doses on spontaneous activity in male and female Sprague Dawley rats: Analysis of brain area epibatidine binding and cotinine levels

Alan L. Pehrson [b,*], Scott D. Philibin [b], Daniel Gross [b], Susan E. Robinson [b], Robert E. Vann [b], John A. Rosecrans [b], John R. James [a]

[a] Department of Pharmaceutics, Virginia Commonwealth University, Richmond, VA, 23298 USA
[b] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, 23298 USA

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Abstract

Previous research in this laboratory has shown that nicotine's effects on spontaneous activity are contingent on individual differences, attenuating activity in high active rats and increasing it in low active rats. This study was designed to further evaluate this phenomenon, and to compare it with nicotine's effects on nicotinic acetylcholine receptor (nAChR) expression in several brain regions. Male and female Sprague–Dawley rats selected for differences in baseline activity were administered nicotine twice daily for 14 days, and its effects on spontaneous activity were evaluated following 1, 13 and 27 doses. Furthermore, [^{3}H] epibatidine binding and plasma cotinine levels were evaluated 24 h after the 28th dose. Contrary to previous findings, the effects of repeated nicotine on spontaneous activity were minimally contingent on baseline activity levels. Following an initial attenuation, males, but not females, exhibited sensitization to nicotine's effects on spontaneous activity. [^{3}H] epibatidine was significantly increased in several brain regions in both male and female nicotine-treated animals, and in females selected for high activity at baseline. However, a clear relationship between these effects and spontaneous activity was not found, due to the lack of consistent effects of nicotine administration and baseline activity on spontaneous activity. Interestingly, significant correlations suggest that rats exhibiting higher spontaneous activity on the final test day were differentially marked by higher [^{3}H] epibatidine. Cotinine levels were higher in low activity males than in high activity males, but no differences were observed between high and low activity females. Thus, no clear relationship between this variable and spontaneous activity could be discerned. Based on these data, no simple relationships between the effects of nicotine administration or baseline activity on [^{3}H] epibatidine binding, nicotine metabolism, or spontaneous activity were observed. However, a relationship between [^{3}H] epibatidine and spontaneous activity on the final test day is suggested.

Keywords: Nicotine; Baseline activity; Brain area nAChR binding; Behavioral sensitization

1. Introduction

The evaluation of nicotine's effects on behavior in both humans (via its presence in tobacco) and experimental animals has been both engaging and enigmatic. Nicotine's effects in humans, for example, appear to be 'paradoxical' in nature (Robinson and Pritchard, 1992); some individuals appear to be aroused by nicotine (via smoking) while others appear to be sedated by equivalent levels of nicotine. Animal research has presented a similar picture, in which nicotine's effects on simple behaviors such as spontaneous activity appear contingent on the initial baseline of the experimental subject (Hendry and Rosecrans, 1982; Rosecrans, 1995). For example, the activity of Sprague–Dawley rats pre-selected for high activity was suppressed by nicotine (0.4 mg/kg, subcutaneous, SC), while that of rats selected for low activity was elevated by the same dose. Thus, nicotine appears to normalize behavior in this unconditioned paradigm.

* Corresponding author. University of Pittsburgh, Department of Neuroscience, A210 Langley Hall, 5th Ave. and Ruskin Ave. Pittsburgh, PA, 15260, USA. Tel.: +1 412 624 4537; fax: +1 412 624 9198.
E-mail address: pehrson@pitt.edu (A.L. Pehrson).

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The basic question remaining in this research relates to the potential relationships between nicotine's differential effects on behavior and whether these effects were contingent on nicotine's ability to alter expression of the nAChR (Rosecrans et al., 1995).

The overall objective of the present study was to further evaluate the chronic as well as the acute effects of nicotine on behavior in both male and female rats selected for different baseline arousal levels. It was hypothesized that rats selected for differences in baseline activity would differ in terms of nAChRs in one or more of several brain regions. In order to test these relationships, this research sought to determine whether there is a relationship between [^{3}H] epibatidine binding in several brain regions and the behavior of rats selected for differences in baseline arousal levels. To determine the potential role that nicotine metabolism might have in nicotine's differential effects on behavior, plasma cotinine was also evaluated 24 h after the 28th dose in male and female rats selected for high and low levels of baseline activity. This research included the female Sprague–Dawley rat as previous research has suggested that gender may also influence the effects of nicotine on select behaviors (Rosecrans, 1972).

2. Methods

2.1. Subjects

Forty male and 40 female SD rats (Harlan Laboratories, Indianapolis, IN), were housed in same-sex pairs in a temperature (22±1 °C) and humidity (40–70%) controlled environment, on a 12 h light/dark schedule (0600–1800). Food and water were available ad libitum in the vivarium. Upon arrival in the vivarium, all subjects were allowed one week to habituate to their environment.

All methods were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee prior to the start of the study, and are in compliance with the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* (publication no. 85-23, revised 1985).

2.2. Drugs

(−)-Nicotine hydrogen tartrate (Sigma Chemical Co., St. Louis, MO) was dissolved in a 0.01 M phosphate buffer (pH= 7.4) vehicle at a concentration of 0.8 mg/ml. All injections were administered subcutaneously (sc) at a volume of 1 ml/kg. All concentrations are calculated as free-base.

The 0.8 mg/kg dose of nicotine was chosen based on previous research, which suggested that maximal nAChR upregulation occurred at this dose. Previous attempts to assess nAChR upregulation using [^{3}H] epibatidine binding after a lower dose of nicotine (0.3 mg/kg twice per day) produced very little upregulation. However, higher doses (0.6 mg/kg twice per day and higher) produced maximal nAChR upregulation (Rowell and Li, 1997). Rowell and Li (1997) also demonstrated that administration of nicotine 2× per day was more effective for eliciting nAChR upregulation than higher injection frequencies, including constant infusion via indwelling catheters.

2.3. Activity

Activity levels were assessed in 4 open field test chambers (ENV 515, Med-Associates, St. Albans, VT). Data were collected on a PC using Med-Associates software. After an initial habituation period of one week, activity levels for each subject were assessed in the activity chambers during three-minute sessions on five consecutive days. These three-minute sessions were used in order to minimize the effects of habituation while evaluating baseline activity levels. Male and female rats were ranked based on activity level differences using the average activity score of each rat on days 4 and 5. Then, 32 rats displaying the most extreme activity in each sex (i.e., the 16 rats with the highest and 16 rats with the lowest activity scores) were selected for further evaluation; medium activity rats (8 males and 8 females) were discarded from the study. In this way, subjects of each sex were separated into high and low activity groups.

Rats in the high and low activity groups were further subdivided into two groups of equal size (nicotine and vehicle groups) matched for average activity on days 4 and 5. High activity (N=8) and Low activity (N=8) nicotine rats of each sex received injections (sc) of 0.8 mg/kg nicotine twice per day for 14 days. Similarly, subjects in the high and low activity control groups for each sex received injections (sc) of vehicle twice per day for 14 days. All injections occurred at approximately 9 am and 5 pm daily. With the exception of doses 1, 13 and 27, all injections occurred in the vivarium.

Doses 1, 13, and 27 were administered in the behavioral laboratory 5 min prior to each behavioral test session; each subject's spontaneous activity was recorded for a period of 20 min. The same behavioral apparatus was utilized during the determination of differences in baseline activity and spontaneous activity. On day 15, (i.e., 24 h after the last behavioral test session and approximately 15 h after the 28th nicotine dose) subjects were killed by rapid decapitation. Whole brains were immediately removed, frozen on dry ice and stored at −70 °C. Additionally, blood was collected and stored as frozen plasma until assay. At this time after dosing, no nicotine is present in the plasma, but the metabolite cotinine does remain (Ghosheh et al., 1999).

2.4. [^{3}H] epibatidine receptor binding

Evaluation of nAChR binding using [^{3}H] epibatidine binding was performed in the laboratory of Dr. Peter Rowell at the University of Louisville. Each whole brain was thawed, and the following areas were dissected: frontal cortex, hippocampus, striatum and thalamus. Brain areas were weighed and placed in Beckman Ultraclear centrifuge tubes in a 10-fold volume of ice-cold 0.32 M sucrose containing 1 mM EDTA. The maximal number of α4β2 and similar subtype nAChR sites was determined using a modification of the procedure of Houghtling et al. (1995) with 2 nM (+/−)[^{3}H] epibatidine (56 Ci/mmol, Perkin Elmer; Boston, MA). The homogenates were incubated for 2 h at 4 °C in a 400 μl volume containing the radioligand either with (nonspecific binding in triplicate) or without (total binding in triplicate) 500 μM nicotine. The tissue was rapidly vacuum-filtered through glass fiber filters (Whatman GF/C) using a Brandel tissue harvester,

followed by three rapid washes with ice-cold PBS. Radioactivity on the filters was determined in Bio-Safe NS (Research Products International, Mount Prospect, NY) using a Packard 2300 scintillation counter. Maximum specific binding (Bmax) was determined by subtracting the average nonspecific from total binding and expressed as fmol/mg protein determined by the Bradford protein assay (Bio-Rad, Hercules, CA; Bradford, 1976).

2.5. Determination of cotinine levels

Cotinine samples were analyzed by a liquid chromatography/tandem mass spectrometric (LC/MS/MS) method validated for selectivity, calibration model fit, sensitivity, accuracy and precision. The instrument used was a Micromass Quatro II LC mass spectrometer equipped with dual Shimadzu LC-10 ADVP pumps, a Shimadzu model SCL-10ADVP system controller, a Leap PAL HTS auto sampler and MassLynx version 3.5 software. A Varian Polaris SI- A column was used with a mobile phase consisting of formic acid, ammonium formate, acetonitrile and water. The composition of the mobile phase was varied through a 3-step gradient employed over a 3.5 min period. Plasma samples (500 μl) were transferred into separate 13 × 100 glass tubes with Teflon-lined caps. Twenty-five microliters of internal standard solution (cotinine-methyl-d_3) were added to each tube followed by 250 μl of 0.6% ammonium hydroxide and then 5 ml of 90:10 methyl, tert-butyl,ether:tetrahydrofuran. All tubes were transferred to a rotator for 10 min followed by centrifugation for 5 min at 3000 rpm. Each tube was then frozen in an acetone/dry ice bath and the upper organic layer was poured off. Formic acid (100 μl of 1% formic acid in acetonitrile) was then added to each tube and the contents of the tubes were evaporated to dryness. The samples were then reconstituted in 100 μl of 0.05% trifluoroacetic acid in acetonitrile and stored refrigerated until analyzed. Linearity for cotinine was 1.0 ng/ml to 600 ng/ml. The inter-run precision for cotinine was 7.15%–13.60% across the linear range. Inter-run accuracy for cotinine was −13.80 to 4.04% across the same linear range. Intra-run precision for cotinine was 4.30%. Blanks were less than 20% of the response for the limit of quantitation (LOQ) of 1.0 ng/ml for cotinine.

2.6. Data analysis

Two factor repeated measures ANOVAs were used to evaluate the effects of nicotine and day on spontaneous activity within each baseline activity group (i.e. high activity males, low activity males, etc.). Additionally, two factor ANOVAs were used to analyze the effects of nicotine administration and activity group on [^{3}H] epibatidine binding, and were used to assess the effects of nicotine administration and baseline activity group on spontaneous activity on the final behavioral test day (i.e. after 27 doses of nicotine or saline). Newman–Keuls post hoc analyses were used where appropriate. Two-tailed *t*-tests were conducted to examine differences in cotinine levels as a function of group. Finally, a stepwise multiple correlation was used for each sex to examine whether there was a significant relationship between spontaneous activity on the last behavioral test day and [^{3}H] epibatidine binding.

3. Results

3.1. Baseline activity levels

Male rats selected for high activity exhibited a baseline rate of 272 + 26 counts/3 min while male rats selected for low activity exhibited a baseline rate of 109 ± 9 counts/3 min. On the other hand, female rats selected for high activity averaged 561 ± 87 counts/3 min and those selected for low activity exhibited a rate of 178 ± 59 counts/3 min. Baseline activity levels appeared higher in females than in male subjects, which has been a consistent finding in this laboratory using this behavioral protocol in Sprague–Dawley rats (for reference see Rosecrans, 1972). Additionally, we have recently observed the same phenomenon while using this protocol in Lewis rats (unpublished observation).

3.2. Behavioral effects of nicotine in males

The effects of nicotine administration on spontaneous activity in the male rats are shown in Fig. 1 (mean activity counts ±

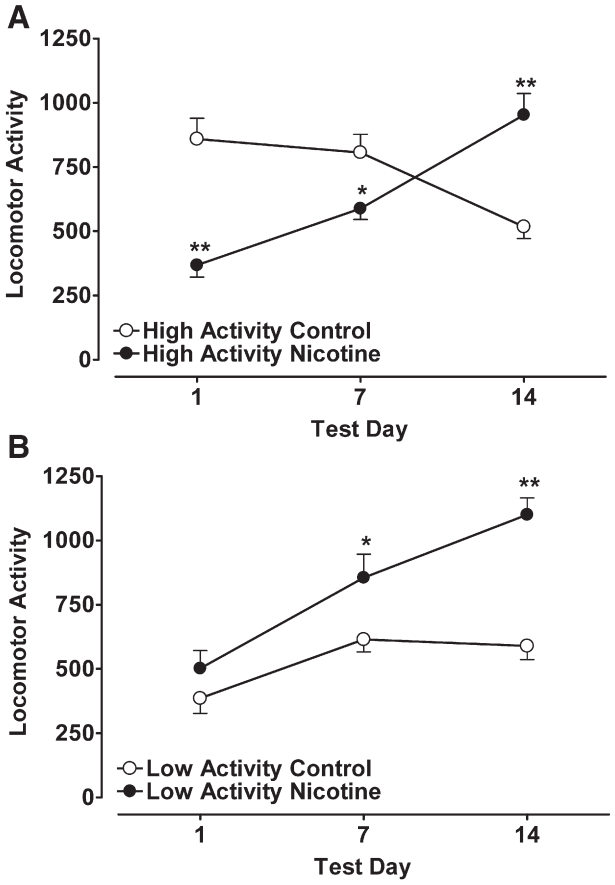

Fig. 1. Spontaneous activity in high activity (top panel) and low activity males (bottom panel) on days 1, 7, and 14 of repeated nicotine administration. Data are presented as the mean ± S.E.M. Filled circles represent the nicotine group, while open circles represent the saline control group. Asterisks represent significant differences between nicotine- and saline-treated animals on a given test day (*$p<0.05$; ** $p<0.01$). In the high activity males, nicotine-treated rats were significantly less active than saline rats on days 1 and 7, but were significantly more active than saline rats on day 14. However, in the low activity males, nicotine-treated rats were not significantly different from saline-treated rats on day 1, but were significantly more active than saline rats on days 7 and 14.

SEM). In the high activity group (top panel), no significant main effects were detected for either the drug group or day factors. However, a significant drug group × day interaction was detected ($F(2,28)=29.04$, $p<0.0001$). A Newman–Keuls post hoc test revealed that nicotine-treated rats were significantly less active than saline-treated controls on test days 1 and 3 (i.e. after 1 and 13 nicotine doses, respectively), but were significantly more active on test day 3 (i.e. after 27 nicotine doses).

In the low activity group (bottom panel, Fig. 1), a significant main effect was observed for the drug group factor ($F(1,14)=33.94$, $p<0.0001$), with nicotine-treated rats being significantly more active than saline-treated rats. Additionally, a significant main effect was observed for the day factor ($F(2,28)=18.21$, $p<0.0001$), with significant increases in activity as compared to test day 1 on days 2 and 3. A significant drug group × day interaction was also detected ($F(2,28)=4.32$, $p<0.05$), and a Newman–Keuls post hoc test revealed that nicotine-treated rats in the low activity group were significantly more active than saline-treated rats test days 2 and 3 (i.e. after, 13 and 27 doses of nicotine, respectively).

For test day 3, a two factor ANOVA was used to compare activity in nicotine- and saline-treated rats from both the high and low activity groups. No significant main effect for activity group was noted on this test day, nor was there a significant activity group × drug group interaction effect. These results suggest that the total activity displayed by rats in the high and low activity groups was similar on the final test day, and that nicotine affected rats from these groups similarly. However, a significant main effect was observed for the treatment group factor ($F(1,28)=55.87$, $p<0.0001$), reflecting the fact that nicotine-treated rats were more active than saline-treated rats.

3.3. Neurochemical effects of nicotine in males

Two factor ANOVAs were also used to compare [^{3}H] epibatidine binding in nicotine- and saline-treated rats from both the high and low activity groups for the cortex, hippocampus, thalamus, and striatum (Table 1). In the cortex, no significant main effect was detected for the activity group factor, nor was there a significant activity group × drug group interaction. However, a significant main effect for the drug group factor revealed that nicotine-treated rats had higher [^{3}H] epibatidine binding than did saline-treated rats ($F(1,27)=6.93$, $p<0.05$).

A similar pattern was observed in the striatum. No significant activity group main effect was observed, nor was there a significant activity group × drug group interaction. However, the drug group main effect was significant ($F(1,28)=5.11$, $p<0.05$), reflecting the fact that nicotine-treated rats expressed more [^{3}H] epibatidine binding than saline-treated rats.

Neither activity group nor drug group appeared to have an effect on [^{3}H] epibatidine binding in the thalamus or hippocampus, as no significant main effects or interaction effects were observed for either brain area.

A stepwise multiple correlation examining the relationships between spontaneous activity on the final behavioral test day (i.e. after 27 doses of nicotine or saline) and [^{3}H] epibatidine binding in the cortex, striatum, thalamus, and hippocampus in the males revealed a significant positive correlation between spontaneous activity and [^{3}H] epibatidine in the cortex ($R=0.405$; $R^2=0.164$; $F(1,29)=5.7$, $p<0.05$). Thus, male rats that were more active on the final test day were differentially marked by higher levels of nAChR expression in the cortex. However, correlations between spontaneous activity and [^{3}H] epibatidine in the striatum, thalamus, and hippocampus were not significant.

Table 1
The effects of repeated nicotine administration (left two columns) or baseline activity levels (right two columns) on nAChR binding in male Sprague–Dawley rats in the cortex, hippocampus, striatum, and thalamus

Brain area	Nicotine	Saline	High activity	Low activity
	$N=16$	$N=16$	$N=16$	$N=16$
Cortex	137±5*	119±4	122±4	134±6
Hippocampus	52±6	47±3	43±2	56±7
Striatum	130±7*	111±4	116±5	125±7
Thalamus	142±6	132±3	136±5	138±5

* significant difference compared to saline; $p<0.05$.
Values represent mean fmol/mg protein [^{3}H] epibatidine binding concentrations ± S.E.M. [^{3}H] epibatidine binding was significantly increased vs. saline in the cortex and striatum of nicotine-treated rats. However, no significant differences were observed between rats selected for differences in baseline activity level.

3.4. Behavioral effects of nicotine in females

The effects of nicotine administration on spontaneous activity in female rats are shown in Fig. 2 (mean activity counts ± SEM). In the high activity females, no significant main effect for the drug group factor was observed. However, a significant main effect for the day factor ($F(2,28)=14.4$, $p<0.0001$), as well as a significant drug group × day interaction ($F(2,28)=5.89$, $p<0.01$) were observed. A Newman–Keuls post hoc test revealed that nicotine-treated animals were significantly less active than saline-treated controls on test day 1 (i.e. after 1 dose of nicotine), however no significant differences in activity were observed on test days 2 or 3 (after 13, and 27 doses of nicotine, respectively).

Analysis of the low activity females' activity revealed very similar results. No significant main effect was observed for the drug group main effect. However, the day main effect ($F(2,28)=17.4$, $p<0.0001$), and the drug group × day interaction effects were both significant ($F(2,28)=7.49$, $p<0.01$). As in the high activity rats, a Newman–Keuls post hoc test revealed that nicotine-treated animals displayed significantly reduced activity compared to saline-treated controls on test day 1, but not on test days 2 or 3.

A two factor ANOVA was used to analyze the effects of activity group and treatment group on total activity scores for test day 3. As one might expect, a significant activity group main effect revealed that rats in the low activity group were significantly less active than those in the high activity group ($F(1,28)=7.13$, $p<0.05$). However, no significant main effect was observed for the drug group factor, nor was a significant treatment group × drug group interaction.

As seen in the baseline measurements of activity, female rats were much more active than were male rats. Again, this has

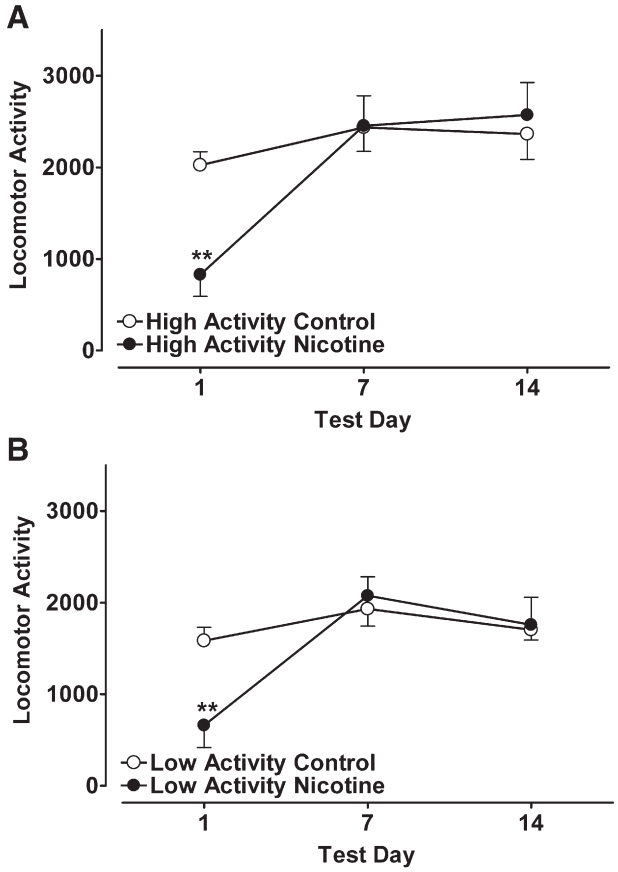

Fig. 2. Spontaneous activity in high activity (top panel) and low activity females (bottom panel) on days 1, 7, and 14 of repeated nicotine administration. Data are presented as the mean ± S.E.M. Filled circles represent the nicotine group, while open circles represent the saline control group. Asterisks represent significant differences between nicotine- and saline-treated animals on a given test day (*$p<0.05$; ** $p<0.01$). In both high and low active females, nicotine-treated rats were significantly less active than saline-treated rats on day 1. However, no significant differences between these groups were noted on days 7 or 14.

been a consistent phenomenon across several studies using this behavioral protocol Sprague–Dawley rats (Rosecrans, 1972), and has also been observed recently in Lewis rats (unpublished observation).

3.5. Neurochemical effects of nicotine in females

Two factor ANOVAs were also used to assess the effects of activity and treatment groups on [^{3}H] epibatidine binding in the cortex, thalamus, hippocampus, and striatum (Table 2). In the cortex, rats in the high activity group displayed significantly higher [^{3}H] epibatidine binding than did those in the low activity group (activity group main effect; $F(1,28)=6.89$, $p<0.05$). Additionally, nicotine-treated rats displayed significantly more [^{3}H] epibatidine binding than did saline-treated rats (drug group main effect; ($F(1,28)=5.35$, $p<0.05$). However, no significant treatment group × drug group interaction was observed.

Analysis of [^{3}H] epibatidine binding in the hippocampus yielded similar results. High activity rats displayed significantly greater [^{3}H] epibatidine binding in the hippocampus than did rats in the low activity group (activity group main effect; $F(1,28)=23.11$, $p<0.0001$), and nicotine-treated rats had significantly more [^{3}H] epibatidine binding than those treated with saline ($F(1,28)=4.84$, $p<0.05$). No significant treatment group × drug group interaction was observed.

Table 2
The effects of repeated nicotine administration (left two columns) or baseline activity levels (right two columns) on nAChR binding in female Sprague–Dawley rats in the cortex, hippocampus, striatum, and thalamus

Brain area	Nicotine	Saline	High activity	Low activity
	$N=16$	$N=16$	$N=16$	$N=16$
Cortex	205±14*	167±22	209±19+	163±16
Hippocampus	149±19*	109±14	172±17+	86±9
Striatum	265±18**	199±11	236±18	230±17
Thalamus	349±27	305±34	323±30	332±32

* significant difference compared to saline; $p<0.05$.
** significant difference compared to saline; $p<0.01$.
+ significant difference compared to LA; $p<0.05$.
Values represent mean fmol/mg protein [^{3}H] epibatidine binding concentrations ± S.E.M. [^{3}H] epibatidine binding was significantly increased vs. saline in the cortex, hippocampus and striatum of nicotine-treated rats. Additionally, significant increases in [^{3}H] epibatidine binding in the cortex and hippocampus were noted in rats selected for high baseline activity, compared to rats selected for low baseline activity.

In the striatum, nicotine-treated rats displayed significantly increased [^{3}H] epibatidine binding compared to saline controls (drug group main effect; $F(1,28)=9.94$, $p<0.01$). However, no significant main effect was observed for the activity group factor, nor was the activity group × drug group interaction significant. In the thalamus, no significant main effects were observed for either the activity group main effect or the drug group main effect. Furthermore, the activity group × drug group interaction was not significant.

A stepwise multiple correlation examining the relationships between spontaneous activity on the final behavioral test day (i.e. after 27 doses of nicotine or saline) and [^{3}H] epibatidine binding in the cortex, striatum, thalamus, and hippocampus in the females revealed a significant positive correlation between spontaneous activity and [^{3}H] epibatidine in the hippocampus ($R=0.492$; $R^2=0.242$; $F(1,29)=8.932$, $p<0.01$). Thus, female rats exhibiting higher activity were differentially marked by increased nAChR expression in the hippocampus. Correlations between spontaneous activity and [^{3}H] epibatidine in the cortex, striatum, and thalamus were not significant.

3.6. Plasma cotinine levels

High and low activity males had mean cotinine levels of 11.3 ng/mL ± 2.0 SEM (range 4.2–16.9 ng/ml) and 36.1 ng/ml ± 3.1 SEM (range 27.5–53.6 ng/mL), respectively (Fig. 3). A t-test revealed significant differences in cotinine levels between high and low activity male rats ($t(12)=6.2$, $p<0.05$). Cotinine levels for control animals were below the limit of quantitation (less than 1 ng/mL).

High and low activity females had mean cotinine levels of 49.3 ng/mL ± 3.9 SEM (range 34.1–66.5 ng/mL) and 57.0 ng/mL ± 4.5 SEM (range 43.0–83.8 ng/mL), respectively (Fig. 3). A *t*-test revealed no significant differences in cotinine levels between high and low activity female rats. Cotinine levels for

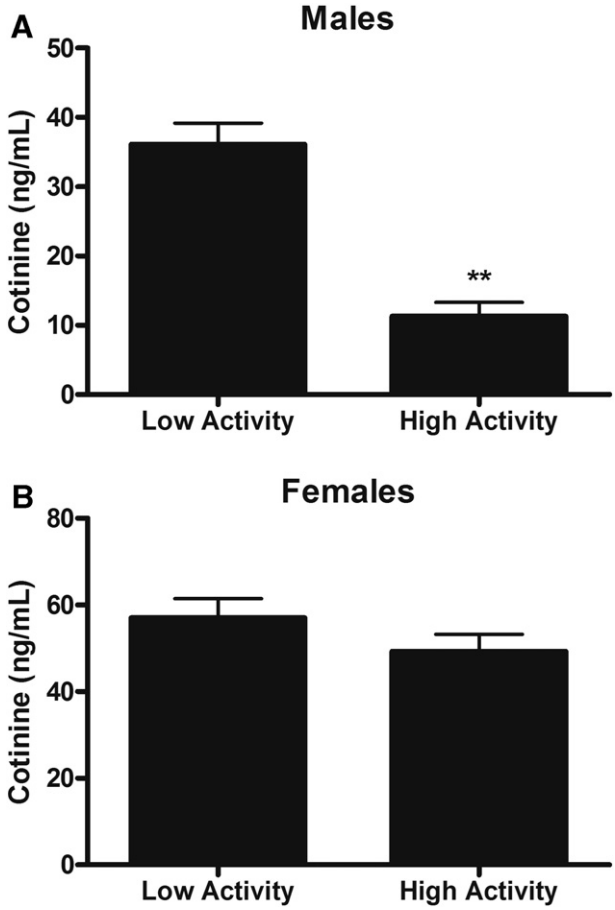

Fig. 3. Cotinine levels (in ng/mL) in low and high activity males (top panel) or females (bottom panel) 15 h after the final dose of nicotine (24 h after the final behavioral test point). Data are presented as the mean±S.E.M. Asterisks represent significant differences between the low and high activity groups (*$p<0.05$; **$p<0.01$). Male rats selected for high baseline activity had significantly lower cotinine levels than male rats selected for low baseline activity. However, no significant differences were observed between female rats selected for differences in baseline activity.

control animals were below the limit of quantitation (less than 1 ng/mL).

4. Discussion

As described in the Introduction, research conducted in this laboratory has previously shown that nicotine's effects on unconditioned behaviors such as spontaneous activity are contingent on the baseline arousal level of each rat studied. While these findings have proven interesting and complement analogous findings in humans (Robinson and Pritchard, 1992), an understanding of potential nAChR mechanisms that might be involved in nicotine's propensity to elicit differential behavioral effects in the rat has not been attained. A clue as to what might be occurring evolved from a study that suggested that nAChR desensitization might be responsible for the inability of a select population of Sprague–Dawley rats to exhibit tolerance to nicotine's discriminative stimulus effects in a two-lever operant procedure (James et al., 1994; Robinson et al., 2006, 2007). That study, while not involving rats pre-selected for differences in behavioral arousal, provided the concept that nicotine's differential effects on unconditioned behaviors might likewise be contingent on the ability of a rat to exhibit nAChR desensitization following nicotine administration. It has been suggested that desensitization may be related to the upregulation of nAChRs (Marks and Collins, 1985; Schwartz and Kellar, 1985; Rowell et al., 1987), although this relationship may not be a direct one (Gentry et al., 2003). Thus, our operational hypothesis suggested that nicotine administration in the high and low activity rat might lead to different levels of nAChR expression, and thus, to differing effects of repeated nicotine on spontaneous activity. However, few significant relationships were found between nicotine-induced increases in nAChR expression and behavior.

4.1. Effects of nicotine on behavior

The pattern of the behavioral effects of multiple injections of nicotine varied between male and female rats. Evidence of a differential effect on behavior based on basal activity level was observed in the male rat following nicotine administration; there was a significant decrease in spontaneous activity in the high activity rat after a single dose of nicotine and a lesser, yet still significant decrease in spontaneous activity after 13 doses. On the other hand, a minimal increase in spontaneous activity in the low activity rat occurred after 13 doses. Both groups of male rats exhibited a significant increase in spontaneous activity after 27 injections of nicotine.

Given the fact that nicotine administration significantly reduced locomotor activity on days 1 and 7, but not on day 14 in the high activity males, one might argue that these rats are developing tolerance to the behavioral effects of this drug. However, the significant increase in locomotor activity observed on day 14 in this group is not consistent with the behavioral profile one might expect in a nicotine-tolerant animal, and may be better explained by sensitization to nicotine's effects. Similarly, the gradual increase in locomotor activity observed in low activity males treated with nicotine is consistent with sensitization to nicotine, a phenomenon that has been well documented in the literature (Booze et al., 1999).

An interesting consideration that may explain some individual differences in locomotor activity and differing rates of sensitization is thyroid function. Recent evidence has demonstrated that mice congenitally expressing low levels of thyroid function also display low levels of locomotor activity (Wilcoxon et al., 2007). Additionally, it has been demonstrated that altered thyroid function causes changes in long term potentiation and depression (Alzoubi et al., 2007a,b). Hence, although it was not measured in this study, individual variations in thyroid function may be an explanation for the differential response to nicotine noted between low and high activity males. Future research should examine this variable and its relationship to nicotine sensitization and nAChR upregulation.

While the male rats' response to nicotine appeared to be somewhat contingent on baseline activity levels, the female rats exhibited a significant decrease in spontaneous activity in both the high and low activity groups following single doses of

nicotine. Repeated nicotine injection (either 13 or 27 doses) produced similar effects on spontaneous activity in both low and high activity female rats. Male and female rats exhibited clearly different behavioral profiles in response to repeated nicotine injection.

An important aspect of the approach we used to study nicotine's ability to induce pharmacological sensitization was our attempt to reduce possible daily conditioning effects by not evaluating activity after every dose of nicotine. In fact, all injections except when behavior was evaluated were conducted in the home cage in the animal quarters to further eliminate potential environmental conditioning. Given the importance of environmental factors in the development of sensitization (Robinson and Berridge, 2001), possible explanations for the lack of behavioral sensitization in female rats include a reduced susceptibility to context-dependent conditioning in the behavioral testing apparatus, or context-insensitive conditioning in the home cage. In other words, female and male rats may not react to nicotine similarly when it is administered in the same environment. The mechanism is not known, but it is unlikely that receptor binding is directly related to the differences observed, as no clear relationships between nicotine's effects on [^{3}H] epibatidine binding and spontaneous activity were observed in this study.

Another explanation may be related to the level of activity of the female rat at the time of nicotine administration. The female rats in this study were extremely aroused as evidenced by the high level of activity observed. Thus, the female rat may have been near a behavioral ceiling that would tend to attenuate the expression of behavioral sensitization. These potential explanations need to be studied experimentally before we can conclude which explanation is correct.

4.2. Receptor binding relationships

In general, administration of nicotine caused increased binding in both high activity groups of each sex, as assessed by [^{3}H] epibatidine binding. Repeated administration of nicotine in male rats elicited increases in nAChR binding compared to control animals in the cortex and striatum. It is tempting to suggest that these increases in [^{3}H] epibatidine binding are related to increases in spontaneous activity, given that male nicotine-treated rats from both the high and low activity groups were significantly more active than saline-treated controls on the last behavioral test day. However, repeated nicotine administration also caused significant increases in [^{3}H] epibatidine binding in the cortex, striatum, and hippocampus of female rats, with no significant differences in spontaneous activity between nicotine- and saline-treated rats on the last test day. Thus, when both sexes are considered, there appears to be no clear relationships between the effects of repeated nicotine administration on [^{3}H] epibatidine binding and spontaneous activity.

The relationship between individual differences on baseline activity levels and [^{3}H] epibatidine binding is also somewhat difficult to discern. In males, no significant difference was observed in [^{3}H] epibatidine binding between animals selected for high or low baseline activity levels in any brain region. However, it should also be noted that significant differences were not observed in spontaneous activity levels between male high and low activity rats on the final day of behavioral testing. On the other hand, high activity female rats displayed significantly increased [^{3}H] epibatidine binding in the cortex and hippocampus, as well as significantly increased spontaneous activity on the final behavioral test day, compared to low activity female rats. Again, based on these data it appears that there is no obvious relationship between baseline activity levels and [^{3}H] epibatidine binding. However, it should be noted that [^{3}H] epibatidine measures are only sensitive to $\alpha4\beta2$* and $\alpha4\beta2$-like nAChR binding. It is possible that other types of nAChRs may correlate to spontaneous activity.

It is also possible that the observed differences in activity were related to changes in other neurotransmitter systems such as the dopamine system, which has been correlated with different levels of activity (Harrod et al., 2004). Research conducted in this laboratory (Johnson et al., 2000) may provide some insight into this as we have observed nicotine (0.4 mg/kg, sc) to induce differential effects on nucleus accumbens dopamine release in male SD rats. Those studies showed that nicotine enhanced dopamine release when baseline levels were low while it attenuated dopamine release when baseline levels were high. In the study of Johnson et al. (2000), a second nicotine injection 90 min later again elicited a "state-dependent" effect; levels that were lowered by nicotine initially were then enhanced by a second injection while the opposite was observed in the group of rats in which dopamine levels were reduced by the first injection of nicotine. These findings are supported by neuropharmacological studies suggesting that the differential effects of nicotine on dopamine levels were induced by nicotine's ability to desensitize select nAChRs in the nucleus accumbens (Harrod et al., 2004).

Although no clear relationships could be discerned between the effects of nicotine administration and baseline activity on [^{3}H] epibatidine binding and spontaneous activity in these animals, significant correlations were observed between spontaneous activity on the final behavioral test day and [^{3}H] epibatidine binding in the cortex (in males), and in the hippocampus (in females). While no causal relationship should be inferred from these data, it does suggest that increases in $\alpha4\beta2$ and $\alpha4\beta2$-like nAChR expression are related to spontaneous activity, at least over short periods of time (i.e. less than 24 h).

4.3. Plasma cotinine levels

While plasma cotinine levels taken at a single time point should not be considered a definitive indicator of nicotine metabolism, what can be ascertained from the data is that at 24 h after the last nicotine injection significant increases in cotinine were found in male low activity rats compared to male high activity rats. However, no differences were observed between the high and low activity female groups. Given the fact that significant differences in spontaneous activity on the final behavioral test day were not observed between high and low activity males, but were observed between the high and low activity females, there appears to be no clear relationship between spontaneous activity and cotinine levels 24 h after the

last nicotine injection. The lack of significant differences between the two groups of females may be due to the effects of the estrous cycle, for which this study had no controls. Gonadal hormones have been reported to affect nicotine-induced activity (Booze et al., 1999), though the opposite finding of no effect has also been reported (Kuo et al., 1999).

Given the fact that female rats in this study demonstrated higher levels of cotinine than males, it is interesting to consider that differences in p450 enzymes known to metabolize nicotine, or some other genetic or epigenetic variable related to metabolism or elimination of nicotine, may exist between the two sexes in this strain of rats. Unfortunately, the lack of data on these variables from this study does not provide an opportunity to speculate meaningfully on these variables, or whether they are related to differences in spontaneous activity.

5. Conclusions

Overall, this study suggests that there are no clear or simple relationships between the effects of repeated nicotine administration or baseline activity levels on [^{3}H] epibatidine binding, nicotine metabolism, and spontaneous activity. However, a relationship between spontaneous activity on the final test day and [^{3}H] epibatidine is suggested. Additionally, it is possible that clear relationships could be found between the effects of repeated nicotine administration on expression of non-$\alpha4\beta2$-like nAChRs and spontaneous activity.

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