Pharmacology, Biochemistry and Behavior 93 (2009) 59–66

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of mecamylamine on nicotine-induced conditioned hyperactivity and sensitization in differentially reared rats

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ARTICLE INFO

Article history:
Received 23 December 2008
Received in revised form 12 April 2009
Accepted 13 April 2009
Available online 18 April 2009

Keywords:
Environmental enrichment
Nicotine
Conditioned hyperactivity
Sensitization
Locomotor activity
Mecamylamine

ABSTRACT

Rats reared in an enriched condition (EC) display less sensitization to nicotine than rats reared in an impoverished condition (IC). However, it is unknown what effect differential rearing has on nicotine-induced conditioned hyperactivity. The present study determined whether differential rearing affects nicotine-induced conditioned hyperactivity. This study also examined the effects of mecamylamine on conditioned hyperactivity and sensitization. EC, IC, and social condition (SC) rats were reared from 21 to 51 days of age before receiving repeated nicotine injections (.4 mg/kg) prior to 1-h locomotor sessions. Following the conditioned-hyperactivity test, rats received additional training sessions followed by a drug-free rest period before the sensitization test. Mecamylamine (1.0 mg/kg) was administered prior to the conditioned-hyperactivity test and sensitization test. Nicotine treatment resulted in sensitization and conditioned hyperactivity in all differential rearing groups. EC rats displayed less locomotor activity in response to nicotine than both IC and SC rats. Pretreatment with mecamylamine blocked the expression of conditioned hyperactivity only in EC and SC rats and attenuated sensitization in all three rearing groups. These findings suggest that environmental enrichment may alter nicotinic acetylcholine receptors during development and may be a protective factor in the initiation and relapse of smoking behavior.

1. Introduction

Exposure to novel stimuli during development has been widely used to investigate the effects of rearing environment on the subsequent response to drugs of abuse. For example, while rats raised in an enriched environment (EC) self-administer less amphetamine at low unit doses (Bardo et al., 2001), they are more sensitive to the locomotor effects of acute amphetamine at both moderate (.5 mg/kg) and high (2.0 mg/kg) doses (Bowling and Bardo, 1994), but not at low doses (.1 or .3 mg/kg) (Bardo et al., 1995). In contrast however, rats raised in an impoverished environment (IC) have been found to be more sensitive to the locomotor effects of chronic amphetamine administration at a low unit dose (.3 mg/kg) (Bardo et al., 1995).

Interestingly, very little is understood about how the rearing environment contributes to nicotine addiction, cessation, and relapse. The effects of nicotine on locomotor activity in rats consist of a biphasic effect on activity (Clarke and Kumar, 1983; Stolerman et al., 1973). In non-tolerant rats, acute nicotine initially produces hypoactivity for roughly 15 min followed by a period of hyperactivity. With repeated exposure to nicotine, sensitization develops to the hyperactivity, which is reflected by an increase in locomotor activity (Benwell and Balfour, 1992; Clarke and Kumar, 1983; Walter and Kuschinsky, 1989).

Green et al. (2003) observed that EC rats pretreated with nicotine display less development of sensitization relative to IC and socially reared rats (SC), suggesting that environmental enrichment produces decreased sensitivity to the stimulant effects of nicotine. In this study, rats were pretreated with either a high dose (.8 mg/kg) or low dose (.2 mg/kg) of nicotine for 8 days and challenged immediately after the last training day with only the high dose of nicotine. However, this study did not examine the effects of rearing environment on nicotine-induced conditioned hyperactivity.

Given that previous research has implicated associative learning, specifically Pavlovian conditioning processes, to play a role in the etiology of nicotine dependence (Rose et al., 1993; Koob and Le Moal, 2001), it is important to also examine the effects of differential rearing on conditioned hyperactivity. The procedure of Pavlovian conditioning refers to establishing a relationship between two events or stimuli. One of these stimuli is a relatively neutral stimulus called the conditioned stimulus (CS) while the other is termed the unconditioned stimulus (US) and is more biologically significant. The phenomenon of Pavlovian conditioning occurs when the CS elicits a response it did not before, indicating a learned association (Frieman, 2002; Pavlov, 1927). Nicotine has been found to stimulate contextual conditioning in rats (Reid et al., 1996; Belluzzi et al., 2004). More specifically, conditioned

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0091-3057/$ – see front matter
doi:10.1016/j.pbb.2009.04.007

hyperactivity can be observed in rats that have had repeated nicotine administrations (US) paired with a distinct context (CS) (Walter and Kuschinsky, 1989; Reid et al., 1996; Bevins et al., 2001; Palmatier and Bevins, 2002; Bevins and Palmatier, 2003). After repeated pairings, the context alone can come to produce an increase in activity relative to control rats. This learned association is thought to partially mediate continued tobacco use and relapse by contributing to withdrawal effects and cravings (Rose et al., 1993; Lazev et al., 1999).

While it is clear that Pavlovian conditioning contributes to nicotine dependence, relatively few studies have examined the role of nicotinic acetylcholine receptors (nAChRs) in the expression of conditioned hyperactivity. Research examining the physiological factors that influence nicotine dependence have found that nAChRs play a large role in mediating the effects of nicotine (Matta et al., 1998; Laviolette and van der Kooy, 2004). Additionally, the effects of nicotine on attention, learning, and memory are believed to be mediated through these receptors (Blokland, 1995; Olausson et al., 2004; Levin et al., 2006). Given this role of nAChRs, an abundant amount of research has investigated the effects of nAChR antagonists on the behavioral and physiological effects of nicotine.

Mecamylamine, a nonselective nAChR antagonist, dose dependently decreases self-administration of nicotine in rats (Corrigall and Coen, 1989; Shoaib et al., 1997; Watkins et al., 1999), attenuates cue-induced reinstatement of nicotine-seeking behaviors (Liu et al., 2007), and blocks the rewarding effects of nicotine in conditioned place preference paradigms (Fudala et al., 1985). Although mecamylamine alone has not been found to alter the locomotor activity of rats, pretreatment with a moderate dose (1.0 mg/kg) has been shown to attenuate the acute and chronic effects of nicotine-induced locomotor activity (Clarke and Kumar, 1983; Stolerman et al., 1995; Neugebauer et al., 2006). When a low dose of mecamylamine is administered (.1 mg/kg), nicotine-induced locomotor hypoactivity is blocked.

While it appears that enrichment may be a protective factor against drugs of abuse such as amphetamine, it is not clear if environmental enrichment is also protective against nicotine addiction. It is also unclear what neural mechanisms mediate the effects of differential rearing on the subsequent response to nicotine. The current study examined the effects of repeated nicotine administration on locomotor activity in rats reared in enriched, social, and impoverished conditions. In these conditions, rats are raised in three distinctly different environments. The enriched condition consists of a group of rats (10–12) that are housed in a relatively large cage with novel objects and are handled by the experimenter daily. In the social condition, rats are housed in pairs under standard laboratory conditions and are handled once a week. While the impoverished condition consists of rats housed individually without any novel stimuli and are not handled throughout the rearing period.

In accordance with past research, it is predicted that EC rats will display less sensitization relative to SC and IC rats. Another goal of this study was to assess the effects of mecamylamine on conditioned hyperactivity and sensitization to nicotine in differentially reared rats. Currently, very little is known about the effects of differential rearing on Pavlovian conditioned drug cues to nicotine and the role of nAChRs in mediating these effects. It is hypothesized that the nicotinic antagonist, mecamylamine, will attenuate both the hypoactive and stimulant effects of nicotine and, additionally, will decrease the expression of conditioned hyperactivity. It is expected that this effect will be greatest in EC rats since they have been shown to have increases in ACh relative to IC rats (Degroot et al., 2005).

If it is observed that differential rearing alters the behavioral response to nicotine, it will suggest that the rearing environment is one factor that contributes to the individual differences found in nicotine addiction, cessation, and relapse. The observed effects of mecamylamine on conditioned hyperactivity and sensitization will further our understanding of the neural processes that mediate vulnerability to drug abuse.

2. Method

2.1. Animals

Male Sprague–Dawley rats (Charles River, Portage, MI, USA) were obtained at 21 days of age. Rats had access to food and water throughout the experiment. The colony room was maintained at 24 °C and 45% humidity with a 12 h light:dark cycle. Behavioral testing was conducted during the light portion of the cycle. All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University and were in compliance with the *Guide for the Care and Use of Laboratory Animals* (Council, 1996).

2.2. Drugs

S (−)-Nicotine ditartrate (.4 mg/kg; Sigma, St. Louis, MO) and mecamylamine hydrochloride (1.0 mg/kg; Sigma, St. Louis, MO) were dissolved in .9% saline solution. Nicotine dose was calculated as freebase weight and adjusted to a pH of 7.4. Treatments were administered in a volume of 1 ml/kg subcutaneously. Drug doses were chosen based on previous research (Green et al., 2003; Neugebauer et al., 2006).

2.3. Apparatus

The locomotor chamber measured 40.64×40.64×40.64 cm. The chamber consisted of plexiglass walls and plastic flooring which was covered by pine bedding. The chamber was fitted with a photobeam sensor ring consisting of a 16×16 (*x*-axis) photocell array. These photocells were spaced 2.54 cm apart (Coulbourn Instruments, TruScan 2.01) and linked to a personal computer located outside the chamber. Photobeam interruptions were continuously recorded for all sessions, measuring the total distance traveled by the rat in centimeters. Cumulative photobeam interruptions, in 5-min blocks of time, were also recorded within each session. A white-noise generator (~70 dB) was used to create ambient background noise to mask sounds from outside the chamber.

2.4. Environmental conditions

Upon arrival, rats were randomly assigned to one of three conditions; EC ($n=34$), SC ($n=34$), or IC ($n=33$). Rats were housed in these conditions for the duration of the study. EC rats were housed together (10–14 rats) in a large metal cage (60×120×45 cm) with pulp paper bedding. This environment contained 14 novel objects (i.e., PVC pipe, buckets, children's toys, etc.). Each day, rats were handled and 7 of the objects were replaced with 7 new objects; the remaining items were rearranged into a novel configuration. One to two times a week, all objects were replaced with new items. SC rats were housed in pairs in standard laboratory cages (20×20×42 cm) with paper pulp bedding and a wire rack top. These rats were handled once a week during scheduled bedding changes in compliance with the *Guide for the Care and Use of Laboratory Animals* (1996). IC rats were housed individually in hanging wire cages with a wire mesh floor and front panel (17×24×20 cm), and solid metal sides, back and top. IC rats were not handled during their rearing period (21–51 days of age).

2.5. Behavioral procedures

2.5.1. Acquisition of conditioned hyperactivity

At 51 days of age, rats were randomly assigned to one of 3 groups: Paired ($n=38$), Unpaired ($n=29$), and Control ($n=34$). All rats were brought into the testing room at approximately the same time daily. The Paired group was administered nicotine (.4 mg/kg; s.c.) immediately prior to a 1-h locomotor session. On alternating days, rats received saline injections and remained in their home cage. All

rats in the Unpaired group were injected with saline prior to being placed in the locomotor chambers. To control for repeated nicotine exposure, the Unpaired group was administered nicotine in the home cage on the rest days. The Control group received saline injections in both the locomotor chamber and home cage. Each group received a total of 10 locomotor sessions and 10 home cage injections. Following each 1-h locomotor session, rats were removed and returned to their home cage.

2.5.2. Conditioned-hyperactivity test

On the day following the last acquisition session, all rats received two injections. Rats were administered either mecamylamine (1.0 mg/kg; s.c.) ($n=53$) or saline ($n=48$) in the home cage 15-min prior to a saline injection in the locomotor room. Rats were placed into the locomotor chambers immediately following the second injection for 1-h.

2.5.3. Sensitization training

Following the conditioned-hyperactivity test, all rats received 4 additional training sessions (Paired = 38, Unpaired = 28, Control = 34). During sensitization training, one IC rat became ill and thus, was excluded from the analysis. Procedures were identical to those described for acquisition of conditioned hyperactivity. Following the last day of sensitization training, rats rested in their home cages for 16 days. During this time, no home cage injections were administered.

2.5.4. Sensitization test

Following the 16 day rest period, mecamylamine (1.0 mg/kg) or saline was administered 15-min prior to a nicotine (.4 mg/kg) injection. Mecamylamine ($n=47$) and saline ($n=52$) treatments were counterbalanced between rats from the conditioned-hyperactivity test. For the sensitization test, an additional IC rat became ill and thus, was excluded from the analyses. All rats were placed into the locomotor chambers immediately following the nicotine injection for 1 h.

2.6. Data analysis

The total distance traveled in centimeters during acquisition and sensitization sessions was analyzed using a mixed-factorial analysis of variance (ANOVA) with rearing condition (EC, SC, IC) and nicotine treatment (Paired, Unpaired, Control) as between subjects factors and sessions as within-subjects factors. To examine differences between the rearing conditions in hypoactivity, a mixed-factorial ANOVA was performed with rearing condition and nicotine treatment as between subjects factors and cumulative 5-min photobeam interruptions as within-subjects factors.

The total distance traveled (cm) during the conditioned hyperactivity and sensitization test was analyzed using a between subjects ANOVA with rearing condition, nicotine treatment, and mecamylamine treatment as between subjects factors. To standardize any observed baseline differences in saline-treated control rats, the total distance traveled (cm) during the conditioned-hyperactivity test and sensitization test was also converted into *z*-scores and analyzed using a $3\times3\times2$ (rearing condition, nicotine treatment, mecamylamine treatment) between subjects ANOVA. To examine the effects of mecamylamine on the hypoactive effects of nicotine during the sensitization test, a mixed-factorial ANOVA was performed with rearing condition and mecamylamine treatment as between subjects factors and cumulative 5-min photobeam interruptions as within-subjects factors. The alpha level was set to .05 for all analyses. Bonferroni corrected simple effects were performed to probe the interactions.

3. Results

3.1. Acquisition of conditioned hyperactivity

Repeated nicotine administration increased locomotor activity across sessions for Paired rats and differentially affected rearing groups. During acquisition sessions, there was a significant main effect of session, $F(9, 828)=4.57$, $p<.001$, a main effect of rearing condition, $F(2, 92)=72.11$, $p<.001$, a session × nicotine treatment interaction, $F(18, 828)=31.42$, $p<.001$, a session × rearing condition interaction, $F(18, 828)=4.54$, $p>.001$, and a rearing condition × nicotine treatment × session interaction, $F(36, 828)=1.97$, $p<.001$ (Fig. 1). Nicotine significantly increased locomotor activity in Paired groups. The IC-Paired group significantly differed from IC-Controls on sessions 3–10, Fs$(1, 92)=15.72$–109.61, ps$<.001$. The SC-Paired group also significantly differed from SC-Controls on sessions 3–10, Fs$(1, 92)=26.58$–78.08, ps$<.001$. The EC-Paired group significantly differed from EC-Controls on sessions 2–7 and 9–10, Fs$(1, 92)=13.19$–70.8, ps$<.001$.

In examining differences between rearing groups treated with nicotine, IC-Paired rats displayed significantly greater locomotor activity than EC-Paired rats on sessions 2–10, Fs$(1, 92)=20.03$–97.99, ps$<.001$. SC-Paired rats were observed to display significantly greater locomotor activity than EC-Paired rats on sessions 2–7, 9, and 10, Fs$(1, 92)=12.48$–25.28, ps$<.001$. IC-Paired rats had significantly greater locomotor activity than SC-Paired rats only on session 9, $F(1, 92)=12.42$, $p<.001$ and session 10, $F(1, 92)=22.27$, $p<.001$ (Fig. 2A). Control groups were also found to significantly differ in locomotor activity based on rearing condition, with IC- and SC-Control groups displaying greater activity than EC-Controls. SC- and EC-Controls were found to differ on sessions 1–3, 5, 6, 9, and 10, Fs$(1, 92)=12.83$–23.12, ps$<.001$. IC- and EC-Control groups were also found to significantly differ on sessions 1–7, 9, and 10, Fs$(1, 92)=18.58$–47.00, ps$<.001$ (Fig. 2B).

The biphasic effect of nicotine was examined between rearing groups. There were no significant differences in hypoactivity or hyperactivity during session 1 of acquisition between EC-, SC-, and IC-Paired rats (Fig. 3).

3.2. Conditioned-hyperactivity test

When treated with saline, in substitute for nicotine, rats in the Paired groups displayed conditioned hyperactivity relative to Control groups. Main effects of rearing, $F(2, 83)=53.99$, $p<.001$, and nicotine

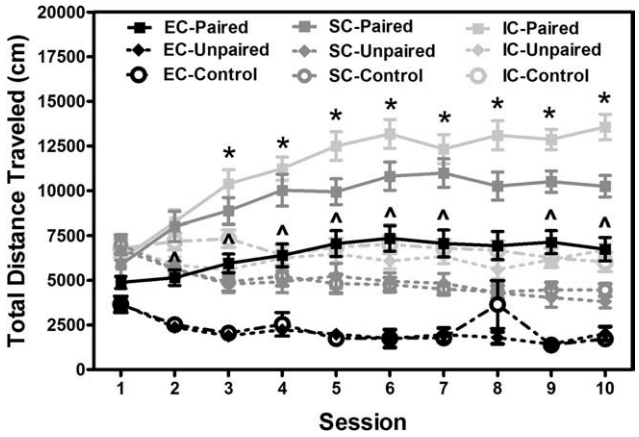

Fig. 1. The mean (±S.E.M.) total distance traveled (cm) for EC, IC, and SC rats in the Paired, Unpaired, and Control groups during sessions 1–10 of acquisition. Asterisks (*) denote a significant difference ($p<.001$) between IC- and SC-Paired from IC- and SC-Control groups. Carrot signs (^) indicate a significant difference between EC-Paired and EC-Control groups ($p<.001$).

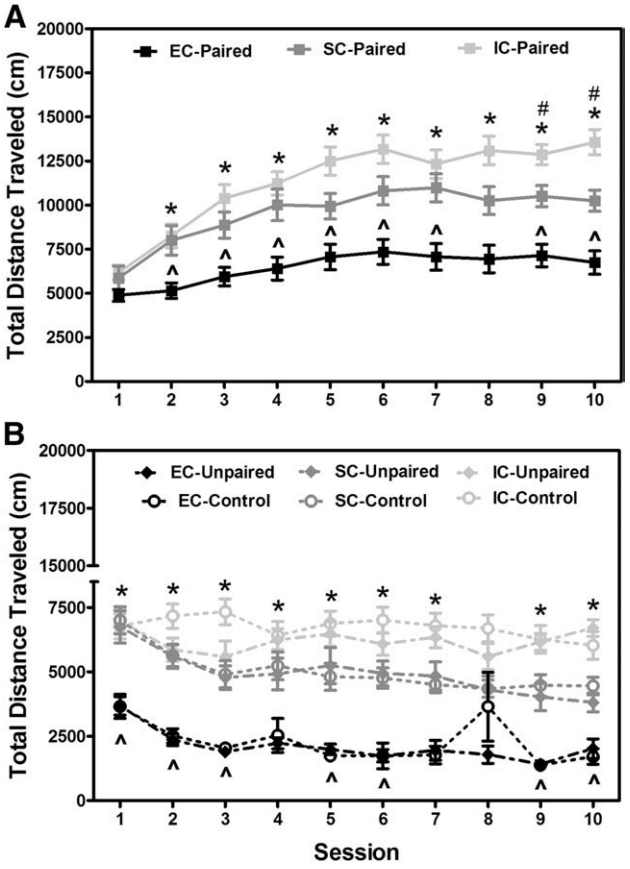

Fig. 2. Panel A shows the mean (±S.E.M.) total distance traveled (cm) for EC, IC, and SC rats in the Paired groups during sessions 1–10 of acquisition. Panel B displays the mean total distance traveled (cm) for EC, IC, and SC rats in the Unpaired and Control groups. Asterisks (*) denote a significant difference between IC and EC rats. Numerical signs (#) denote a significant difference between IC and SC rats. Carrot signs (^) denote a significant difference between EC and SC rats ($p<.001$).

treatment, $F(2, 83)=20.44$, $p<.001$, were found. When rats were pretreated with saline only prior to the session, rats in each rearing condition displayed conditioned hyperactivity, with Paired groups displaying greater locomotor activity than Controls. IC-Paired rats significantly differed from IC-Controls, $F(1, 83)=11.79$, $p<.001$. SC-Paired and SC-Control groups significantly differed, $F(1, 83)=8.99$, $p<.01$ and EC-Paired and EC-Control groups significantly differed, $F(1, 83)=8.61$, $p<.01$. EC rats in the Paired group had significantly less locomotor activity than both SC-Paired, $F(1, 83)=8.62$, $p<.01$ and IC-Paired groups, $F(1, 83)=27.00$, $p<.001$. EC-Unpaired rats were also found to display significantly less locomotor activity than IC-Unpaired rats, $F(1, 83)=18.41$, $p<.001$. In addition, differences in rearing groups between saline-treated Control rats were also found. EC-Controls had significantly less locomotor activity than both IC-Controls, $F(1, 83)=18.65$, $p<.001$, and SC-Controls, $F(1, 83)=8.24$, $p<.01$ (Fig. 4A).

Pretreatment with mecamylamine was found to attenuate conditioned hyperactivity only in EC and SC rats (Fig. 4B). IC-Paired rats still displayed conditioned hyperactivity as they had significantly greater locomotor activity than IC-Unpaired, $F(1, 83)=7.38$, $p<.01$, and IC-Controls, $F(1, 83)=12.90$, $p<.001$.

In order to standardize the observed baseline differences in saline-treated Control rats, data from the conditioned-hyperactivity test were transformed into *z*-scores. A main effect of injection was found, $F(2, 83)=18.71$, $p<.001$. EC, SC, and IC rats pretreated with saline in the Paired groups were still observed to display conditioned hyperactivity relative to Control groups, $F(1, 83)=7.43$, $p<.01$, $F(1, 83)=9.29$, $p<.01$, $F(1, 83)=9.70$, $p<.01$ (Fig. 5A). Although there was no main effect of rearing, IC rats pretreated with mecamylamine were still observed to display conditioned hyperactivity relative to IC-Controls, $F(1, 83)=7.24$, $p<.01$, while EC and SC rats pretreated with mecamylamine did not display conditioned hyperactivity (Fig. 5B).

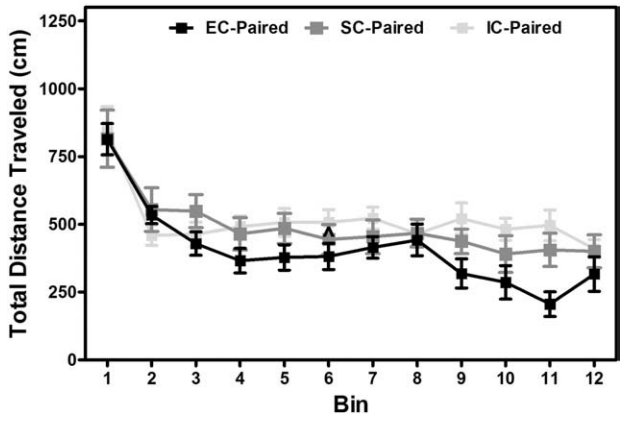

Fig. 3. The mean (±S.E.M.) total distance traveled (cm) for EC, IC, and SC rats in the Paired groups across 5-min bins during session 1 of acquisition.

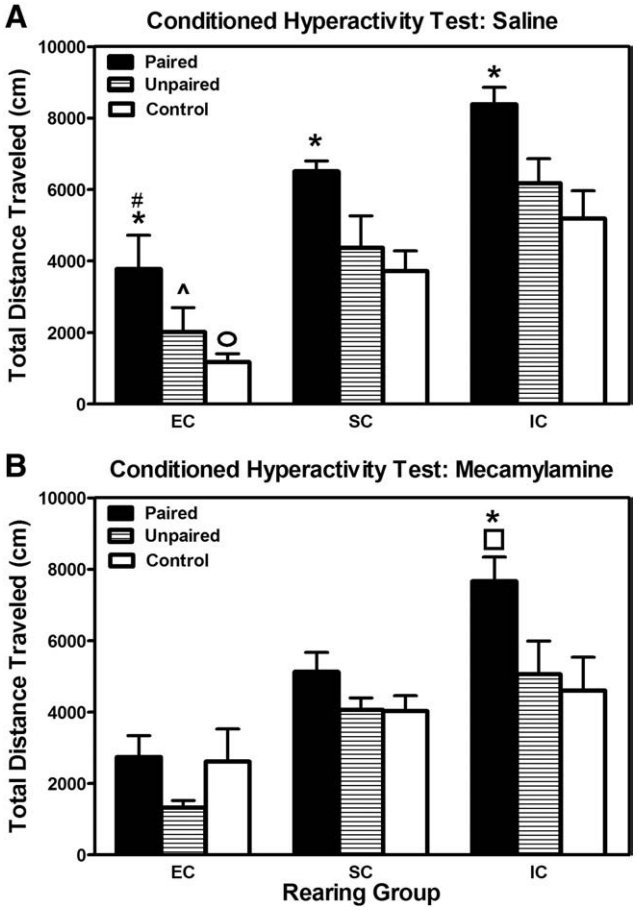

Fig. 4. Panel A shows the mean (±S.E.M.) total distance traveled (cm) for EC, IC, and SC rats pretreated with saline in the Paired, Unpaired, and Control groups during the conditioned-hyperactivity test. Panel B displays the mean total distance traveled (cm) for EC, IC, and SC rats pretreated with mecamylamine (1.0 mg/kg). Asterisks (*) denote a significant difference between Paired and Control groups. Numerical signs (#) denote a significant difference in EC-Paired rats from IC- and SC-Paired rats. Carrot signs (^) indicate a significant difference between EC-Unpaired and IC-Unpaired groups. Open circles (○) indicate a significant difference in EC-Control rats from IC- and SC-Controls ($p<.01$). Open squares (□) indicate a significant difference between IC-Paired and IC-Unpaired rats ($p<.01$).

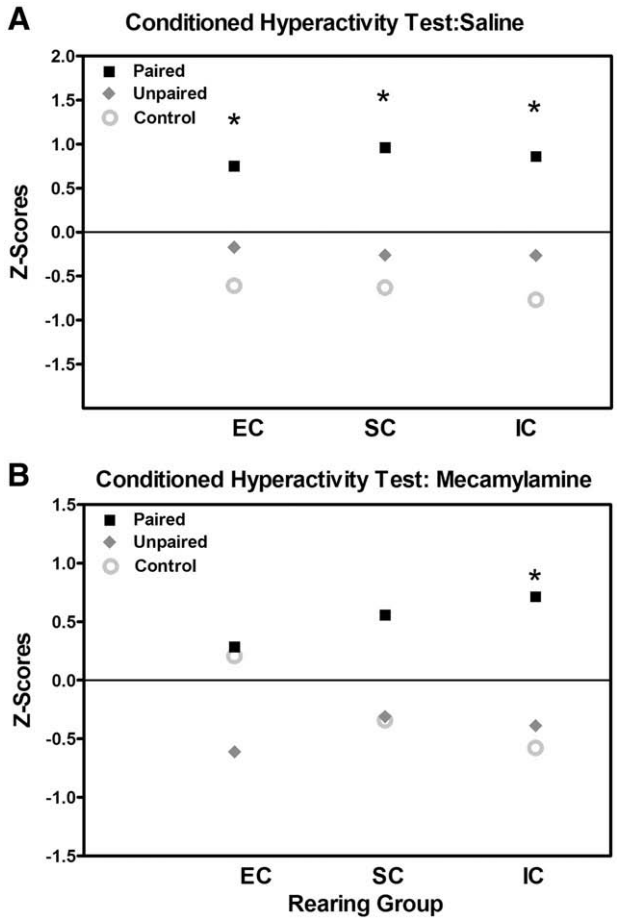

Fig. 5. Panel A displays the mean (1 ± S.E.M.) *z*-score for EC, IC, and SC rats pretreated with saline in the Paired, Unpaired, and Control groups during the conditioned-hyperactivity test. Panel B shows the mean *z*-score for EC, IC, and SC rats pretreated with mecamylamine (1.0 mg/kg). Asterisks (*) denote a significant difference between Paired and Control groups ($p<.01$).

3.3. Sensitization training

Across sensitization training sessions, rats in the Paired groups were observed to display increased locomotor activity. Repeated measures revealed a significant session × nicotine injection interaction, $F(6, 273)=2.43$, $p<.05$, a main effect of nicotine injection, $F(2, 91)=137.34$, $p<.001$, and a main effect of rearing condition, $F(2, 91)=71.22$, $p<.001$. EC, IC, and SC rats in the Paired groups were found to display significantly greater locomotor activity than both Unpaired and Control groups across all 4 sessions of training, F's$(1, 91)=35.22$–94.68, $ps<.001$. IC rats in the Paired group displayed significantly greater locomotor activity than SC-Paired rats only on day 4 of sensitization training, $F(1, 91)=20.05$, $p<.001$. However, EC-Paired rats had less locomotor activity than both SC-Paired, F's$(1, 91)=17.64$–28.56, $ps<.001$, and IC-Paired rats, F's$(1, 91)=55.95$–79.92, $ps<.001$, on all 4 days of training. EC-Controls had significantly less locomotor activity than SC-Controls only on sessions 2, $F(1, 91)=23.32$, $p<.001$, and 3, $F(1, 91)=13.00$, $p<.001$. However, EC-Controls displayed significantly less locomotor activity than IC-Controls across all 4 sensitization training sessions, F's$(1, 91)=24.17$–33.77, $ps<.001$ (Data not shown).

3.4. Sensitization test

During sensitization testing, all rats were treated with nicotine. Rats in the Paired groups were found to display significant sensitization to nicotine. An overall main effect of rearing, $F(2, 810)=24.42$, $p<.001$, and a main effect of nicotine treatment, $F(2, 81)=34.82$, $p<.001$, was found. Also, a two-way interaction of nicotine treatment × mecamylamine treatment was observed, $F(2, 81)=5.28$, $p<.001$. For rats pretreated with saline only prior to the testing session, Paired groups in each rearing condition displayed sensitization. IC-Paired rats significantly differed from IC-Controls, $F(1, 81)=32.98$, $p<.001$. SC-Paired rats significantly differed from SC-Controls, $F(1, 81)=20.32$, $p<.001$, and EC-Paired rats were found to significantly differ from EC-Controls, $F(1, 81)=18.23$, $p<.001$. Additionally, EC-Unpaired rats significantly differed from EC-Controls, $F(1, 81)=8.59$, $p<.01$. Comparisons between rearing conditions revealed that IC rats in the Paired group displayed significantly greater sensitization than EC-Paired rats, $F(1, 81)=7.72$, $p<.01$ (Fig. 6A).

Pretreatment with mecamylamine attenuated sensitization in all three rearing conditions. IC- and SC-Paired rats displayed significant sensitization relative to Controls, $F(1, 81)=4.23$, $p<.05$, $F(1, 81)=5.97$, $p<.05$ while EC-Paired rats only significantly differed from EC-Unpaired rats, $F(1, 81)=4.17$, $p<.05$. Comparisons between rearing conditions in rats pretreated with mecamylamine revealed that EC-Paired rats had less locomotor activity than IC-Paired, $F(1, 81)=8.33$, $p<.01$, and SC-Paired rats, $F(1, 81)=11.67$, $p<.001$. Similarly, EC-

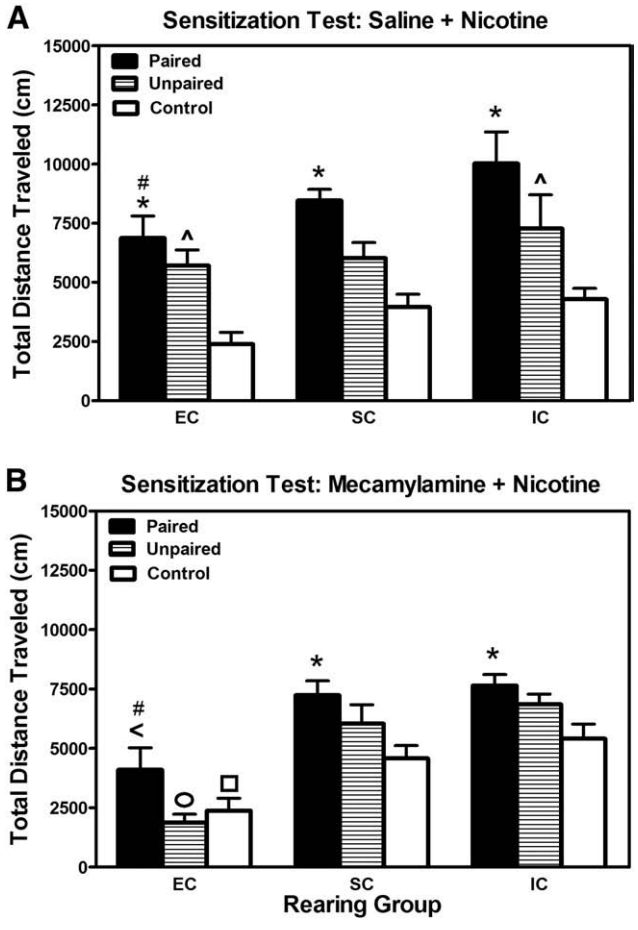

Fig. 6. Panel A shows the mean (± S.E.M.) total distance traveled (cm) for EC, IC, and SC rats pretreated with saline in the Paired, Unpaired, and Control groups during the sensitization test. Panel B displays the mean total distance traveled (cm) for rats pretreated with mecamylamine (1.0 mg/kg) during the sensitization test. Asterisks (*) denote a significant difference between Paired and Control groups ($p<.05$). Carrot signs (^) indicate a significant difference between Unpaired and Control groups ($p<.05$). Numerical signs (#) denote a significant difference in EC-Paired rats from IC-Paired rats (6A) and SC-Paired rats (7B; $p<.01$). Less than signs (<) denote a significant difference between Paired and Unpaired groups ($p<.05$). Open Circles (○) indicate a significant difference in EC-Unpaired rats from IC- and SC- Unpaired rats ($p<.01$). Open squares (□) indicate a significant difference between EC-Control and IC-Control rats ($p<.01$).

Unpaired rats displayed significantly less locomotor activity than both IC-Unpaired, $F(1, 81) = 11.92$, $p < .001$, and SC-Unpaired rats, $F(1, 81) = 7.71$, $p < .01$. EC-Control rats pretreated with mecamylamine were also found to have significantly less locomotor activity than IC-Controls, $F(1, 81) = 10.81$, $p < .01$ (Fig. 6B).

When data from the sensitization test was transformed into *z*-scores, a main effect of injection was found, $F(2, 81) = 36.82$, $p < .001$. However, there was no effect of rearing condition. Rats in the Paired groups pretreated with saline only, were observed to display significant sensitization. EC-Paired rats significantly differed from EC-Controls, $F(1, 81) = 14.06$, $p < .001$. SC-Paired rats were found to significantly differ from SC-Control rats, $F(1, 81) = 25.33$, $p < .001$. IC-Paired rats significantly differed from IC-Controls, $F(1, 81) = 13.39$, $p < .001$ (Fig. 7A).

Mecamylamine was found to attenuate expression of sensitization, however, it did not completely block sensitization. *z*-score analyses revealed that EC-Paired rats had significantly greater locomotor activity than EC-Unpaired rats, $F(1, 81) = 6.91$, $p < .01$, and EC-Controls, $F(1, 81) = 4.54$, $p < .05$. Similarly, IC-Paired rats displayed sensitization relative to IC-Controls, $F(1, 81) = 10.57$, $p < .01$, and SC-Paired rats displayed sensitization relative to SC-Controls, $F(1, 81) = 10.33$, $p < .01$ (Fig. 7B).

In order to examine the effects of mecamylamine on the hypoactive effects of nicotine (.4 mg/kg), only the Control groups were used in the analyses as this was their first experience with nicotine.

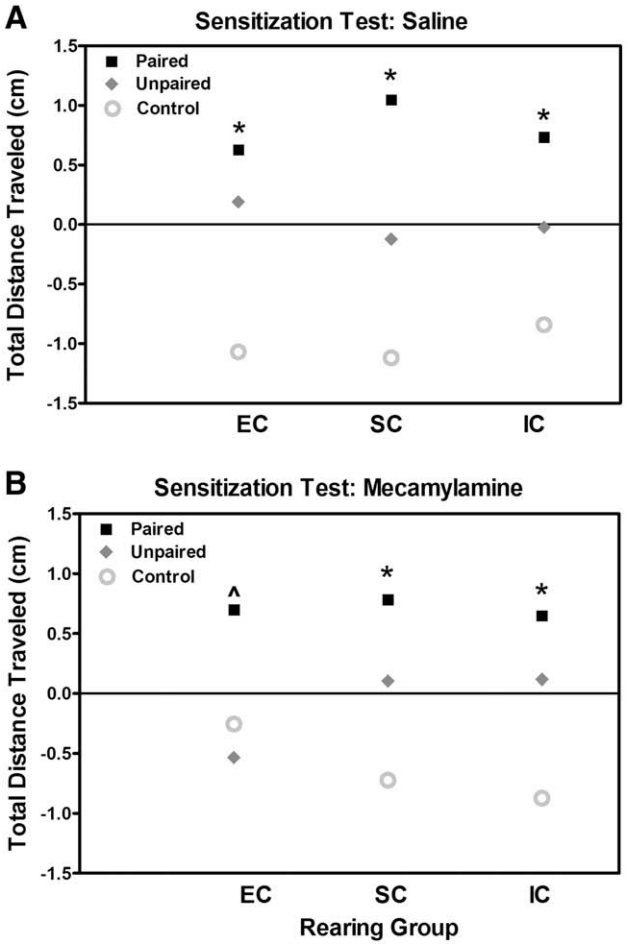

Fig. 7. Panel A displays the mean (1 ± S.E.M.) *z*-score for EC, IC, and SC rats pretreated with saline in the Paired, Unpaired, and Control groups during the sensitization test. Panel B shows the mean *z*-score for EC, IC, and SC rats pretreated with mecamylamine (1.0 mg/kg). Asterisks (*) denote a significant difference between Paired and Control groups. Carrot signs (^) denote a significant difference between Paired and Unpaired groups ($p < .01$).

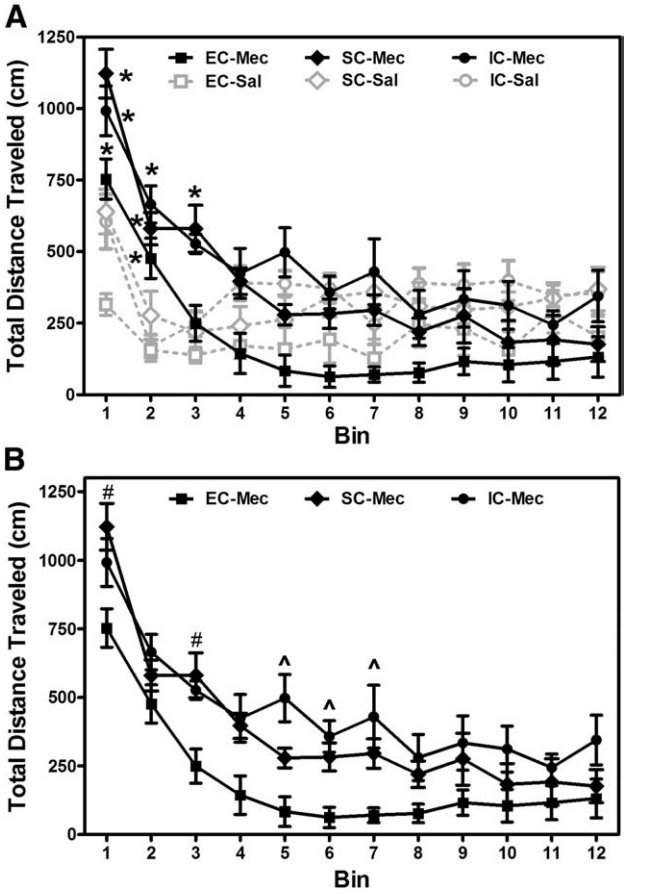

Fig. 8. Panel A shows the mean (± S.E.M.) total distance traveled (cm) for EC, IC, and SC rats in the Control groups pretreated with mecamylamine (1.0 mg/kg) or saline across 5-min bins during the sensitization test. Panel B displays the mean total distance traveled (cm) only for EC, IC, and SC rats in the Control groups pretreated with mecamylamine (1.0 mg/kg). Asterisks (*) denote a significant difference between mecamylamine and saline-treated rats. Numerical signs (#) denote a significant difference between EC and SC rats. Carrot signs (^) denote a significant difference between EC and IC rats ($p < .001$).

Mecamylamine was found to differentially block the hypoactive effects of nicotine (Fig. 8). Repeated measures revealed a significant main effect for 5-min bins, $F(11, 308) = 40.03$, $p < .001$, and a main effect of rearing condition, $F(2, 28) = 11.40$, $p < .001$. An interaction effect was also found between 5-min bins × rearing condition, $F(22, 308) = 1.72$, $p < .05$, and a 5-min bins × mecamylamine treatment interaction, $F(11, 308) = 20.46$, $p < .001$.

Mecamylamine was found to block the hypoactive effects of nicotine during the first 15-min of the testing session (Fig. 8A). IC rats treated with mecamylamine were found to have significantly greater locomotor activity than IC-Saline rats during bin 1, $F(1, 28) = 11.43$, $p < .001$, and bin 2, $F(1, 28) = 30.17$, $p < .001$. SC-Mecamylamine rats were found to have significantly greater locomotor activity than SC-Saline rats during bin 1, $F(1, 28) = 19.55$, $p < .001$, bin 2, $F(1, 28) = 12.42$, $p < .001$, and bin 3, $F(1, 28) = 19.68$, $p < .001$. EC-Mecamylamine rats were observed to display significantly greater locomotor activity than EC-Saline rats during bin 1, $F(1, 28) = 14.67$, $p < .001$, and bin 2, $F(1, 28) = 12.62$, $p < .001$.

Saline-treated rats were not found to significantly differ between rearing conditions, however, EC rats treated with mecamylamine were found to have significantly less locomotor activity than IC and SC rats (Fig. 8B). Early in the session, EC-Mecamylamine rats significantly differed from SC-Mecamylamine rats during bin 1, $F(1, 28) = 11.43$, $p < .001$, and bin 3, $F(1, 28) = 16.55$, $p < .001$. Later in the session, EC-Mecamylamine rats significantly differed from IC-Mecamylamine rats

during bin 5, $F(1, 28) = 19.45$, $p < .001$, bin 6, $F(1, 28) = 12.23$, $p < .001$, and bin 7, $F(1, 28) = 13.99$, $p < .001$.

4. Discussion

This study examined the effects of differential rearing on nicotine-induced conditioned hyperactivity and sensitization. Rats raised in an enriched environment were less sensitive to the hyperactive locomotor effects of nicotine than both rats raised in an impoverished and social environment while IC rats were most sensitive to nicotine-associated contextual cues. This study also examined the effects of mecamylamine on the expression of conditioned hyperactivity and sensitization in differentially reared rats. Although conditioned hyperactivity and sensitization was observed in all three rearing conditions, mecamylamine treatment was found to differentially affect EC, SC, and IC rats. Of particular interest was the finding that mecamylamine blocked conditioned hyperactivity in only EC and SC rats. These results suggest that conditioned hyperactivity is, in part, mediated by neural nAChRs and that environmental enrichment may alter these receptors.

One of the neuropharmacological mechanisms that may contribute to the observed differences between EC, SC, and IC rats in response to repeated nicotine administration is alterations in the mesolimbic dopamine (DA) pathway. Repeated nicotine exposure facilitates clearance of extracellular DA in the terminal regions of the mesolimbic DA pathway (Hart and Ksir, 1996). Environment enrichment regulates DA transporter (DAT) functioning. EC rats display decreased cell surface DAT expression in the medial prefrontal cortex (mPFC) (Zhu et al., 2005). Furthermore, nicotine administration (.4 mg/kg) increases the clearance of extracellular DA in the mPFC in EC rats, but not in IC rats (Zhu et al., 2007). These results suggest that the observed differences in DAT functioning in the mPFC, as a result of enrichment, may contribute to the differences in locomotor activity between EC and IC rats in response to nicotine.

The present study also examined the role that rearing environment may have on the contextual conditioning processes of nicotine. Following repeated pairings of nicotine administration with the locomotor context, rats in the Paired group were found to display conditioned hyperactivity when saline was substituted for nicotine. Interestingly, although environmental enrichment has been shown to enhance learning of contextual cues (Woodcock and Richardson, 2000; Barbelivien et al., 2006), EC rats were found to display significantly less conditioned hyperactivity than IC and SC rats. However, baseline differences were observed in Control groups. In order to standardize these differences, data were transformed into *z*-scores. With the use of *z*-score analyses, rats in the Paired groups were still observed to display conditioned hyperactivity, however, there were no observed differences between rearing groups. Since enrichment has been found to improve learning and memory performance, future studies will need to examine the process of extinction of nicotine-induced hyperactivity in differentially reared rats.

Similar to conditioned-hyperactivity testing, rats in the Paired group were observed to display sensitization relative to Control groups following a 16 day rest period. IC rats showed the greatest sensitization compared to EC rats. Although baseline differences in activity were not observed in the Control groups, data was transformed into *z*-scores in order to make results comparable to the conditioned-hyperactivity test. *z*-score analyses yielded similar results with rats in the Paired group displaying sensitization relative to Control groups. However, there was no effect of rearing condition in the expression of sensitization.

When treated with the nonselective antagonist, mecamylamine, expression of conditioned hyperactivity was blocked only in EC and SC rats. Mecamylamine has previously been found to block cue-induced nicotine-seeking behaviors (Liu et al., 2007) and the rewarding effects of nicotine in conditioned place preference paradigms (Fudala et al., 1985), suggesting that nAChRs contribute to both the conditioning and rewarding effects of nicotine. The results of the current study support this hypothesis. Control groups treated with saline did not significantly differ from groups treated with mecamylamine. Thus, it is likely that this suppression of conditioned hyperactivity in EC and SC rats was due to antagonistic effects at the nAChR sites and not due to a decrease in locomotor activity caused by mecamylamine. Interestingly, only IC-Paired rats were still found to display conditioned hyperactivity relative to IC-Controls. This difference between EC and SC rats in comparison to IC rats suggests that rearing environment may alter nAChR binding. It has been shown that IC rats have less ACh relative to EC rats, thus the number of receptor sites may be influenced by rearing environment (Degroot et al., 2005).

When rats were treated with mecamylamine (1.0 mg/kg) 15-min prior to nicotine administration, expression of sensitization was attenuated when compared to saline-treated controls. However, mecamylamine did not differentially affect the expression of sensitization in EC, IC, and SC rats. Given that the expression of conditioned hyperactivity varied as a function of the rearing condition, the sensitization results suggest that environmental enrichment may alter the neuronal pathways contributing to the primary reinforcing effects and the conditioned effects of nicotine differently. While it has been hypothesized that nAChRs mediate the processes involved in nicotine-induced sensitization, contextual conditioning processes also play a role in this expression (Reid et al., 1996; Miller et al., 2001). Previous research has observed dose dependent effects of mecamylamine on the primary reinforcing effects of nicotine versus the conditioned effects of nicotine, however, the neuronal pathways for these differential effects remain unclear (Liu et al., 2007). Further, there is selectivity for a high versus low dose of mecamylamine in the blockade of hyperactivity following chronic nicotine administration (Ericson et al., 2000). Given that the current study only examined one dose of nicotine and mecamylamine, it still remains to be determined if the enrichment-dependent mecamylamine effects are dose dependent.

Another goal of this study was to determine if rearing condition would affect mecamylamine sensitivity during the hypoactive phase of acute nicotine administration. Mecamylamine (1.0 mg/kg) was found to effectively block hypoactivity following the first 15-min of nicotine administration. Most interestingly, EC rats treated with mecamylamine displayed significantly less locomotor activity than SC rats treated with mecamylamine early in the 1-h session. Conversely, EC rats treated with mecamylamine displayed significantly less locomotor activity than IC rats treated with mecamylamine later in the 1-h session. Due to nicotine's biphasic nature, an increase in locomotor activity can be observed 15-min following nicotine administration (Clarke and Kumar, 1983). Although mecamylamine did significantly attenuate hypoactivity compared to saline-treated Controls within this first 15 min, EC rats appear to be less sensitive to mecamylamine during the hypoactive phase. Furthermore, despite mecamylamine treatment, IC rats appear to remain most sensitive to the hyperactive effects of nicotine. Taken together, these results further suggest that rearing environment alters nAChR binding.

Environmental enrichment alters the behavioral response to a variety of psychostimulants. In response to nicotine, EC rats display lower sensitivity to the hyperactive effects in comparison to IC rats. Furthermore, mecamylamine was found to effectively block conditioned hyperactivity in EC and SC rats, suggesting that environmental enrichment alters nAChR sensitivity. These differences in differentially reared rats in response to nicotine may be due to DA neurotransmission in the mPFC, given that EC and IC rats differ in DAT functioning and DAT clearance (Zhu et al., 2004, 2005, 2007). Regardless of the neurological mechanisms that mediate these responses to nicotine, taken together, the results of the current study indicate that environmental enrichment appears to be a protective factor in repeated nicotine use and relapse.

Acknowledgments

The authors would like to thank Steven Pittenger and Shay Ioerger for their assistance with this project. Research was funded by the Kansas State University. We declare that no financial support or compensation has been received from any individual or corporate entity for and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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