EFFECTS OF MECAMYLAMINE ON NICOTINE-INDUCED CONDITIONED HYPERACTIVITY AND SENSITIZATION IN DIFFERENTIALLY REARED RATS

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Abstract

Rats reared in an enriched condition (EC) with novel stimuli and social contact with cohorts display less sensitization to nicotine than rats reared under impoverished conditions (IC). However, it is currently unknown what effect differential rearing has on nicotine-induced conditioned hyperactivity. The present study determined whether differential rearing affects conditioning to a nicotine-associated context. In addition, this study also examined the effects of mecamylamine, an antagonist to nicotinic acetylcholine receptors, on conditioned hyperactivity and sensitization. This antagonistic drug has been shown to attenuate the locomotor effects of nicotine. In the current study, EC, IC, and social condition (SC) rats were reared from 21 to 51 days of age before training for the acquisition of conditioned hyperactivity and sensitization. Nicotine (0.4 mg/kg) was administered prior to 1-h locomotor sessions. Conditioned hyperactivity testing followed. Rats then received 5 sessions of sensitization training followed by a 16-day drug-free rest period before being tested for sensitization. Mecamylamine (1.0 mg/kg) was administered to rats 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 in EC and SC rats and attenuated sensitization in all three rearing groups. These findings suggest that environmental enrichment may alter nAChR binding during development and may be a protective factor in the initiation and relapse of smoking behavior.

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Dedication

To my Mom and Dad, for inspiring me to go for what I dream.

And to Joe, for always being there to hold my hand.

Effects of Mecamylamine on Nicotine-Induced Conditioned Hyperactivity and Sensitization in Differentially Reared Rats

Substance abuse is a large problem in today's society. This challenging issue affects many facets of life including economic, relational, and individual well-being. Currently, many researchers are trying to identify the behavioral, genetic, and neural processes that contribute to drug addiction. An increasing body of research focuses on individual differences that may predict vulnerability to drug abuse. These findings are being used to develop treatment programs that may facilitate cessation of drug use. Although there has been an abundant amount of attention centered on genetic factors responsible for individual differences to drug addiction, genetics only partially contributes to substance abuse. Environmental factors also play a large role in drug use and dependence, resulting in a genotype-environment interaction (McGue, Lykken, & Iocono, 1996).

One environmental factor that may influence an individual's vulnerability to drug abuse is the amount of novel stimuli experienced during development. A method used to study the effects of this environmental factor is the environmental enrichment paradigm (Renner & Rosenzweig, 1987). In this paradigm, rats are reared in two distinctly different environmental contexts. The enriched condition (EC) 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. Daily, half of the objects are replaced with new objects while the remaining items are rearranged into a novel configuration. All objects are replaced 1-2 times a week. The impoverished condition (IC) consists of rats housed individually without any novel stimuli and are not handled throughout the rearing period.

Differential rearing has been shown to produce many behavioral and neurobiological changes in rats in as little as 4 days of rearing (Ferchmin, Eterovic, & Caputto, 1970). Although past research has shown robust differences between rats raised in enrichment and impoverishment after 90 days of rearing, significant differences can still be found following 30 days (Cain, Green, & Bardo, 2006; Green, Cain, Thompson & Bardo, 2003; Renner & Rosenzweig, 1987). Similarly, many researchers have examined the effects of age at which the differential rearing experience starts. Neuronal changes have been observed in rats given differential housing starting from weaning age to 600 days of age (Renner & Rosenzweig, 1987).

Rats reared in enriched environments have been found to have greater brain weight (Huntley & Newton, 1972) and increased dendritic branching relative to impoverished rats (Greenough, Volkmar, & Juraska, 1973). While a number of neurochemical changes also occur, of particular interest to drug abuse are the changes that occur in the dopamine (DA) and acetylcholine (ACh) neurotransmitter systems. The mesolimbic DA reward system is critical for the reinforcing effects of nicotine (Laviolette & van der Kooy, 2004). It has been suggested that exposure to novelty activates this system (Bardo, Donohew, & Harrington, 1996). Although research to date has been inconclusive if EC and IC rats differ in baseline levels of DA in the nucleus accumbens (NAcc), EC rats have been shown to have increased DA release in the NAcc in response to amphetamine (Bowling & Bardo, 1994). Given that nicotine induces DA release in the NAcc (Laviolette & van der Kooy, 2004), it is reasonable to suggest that EC and IC rats would differ in DA release in response to nicotine administration. In addition to alterations in the DA system, increases in ACh have also been observed in rats

reared in enriched environments (Degroot, Wolff, & Nomikos, 2005). This neurotransmitter has been found to be critical for the consolidation of long-term memory (Blokland, 1995).

In conjunction with these neurobiological changes are the observed behavioral differences between rats reared in enriched and impoverished environments. For example, it has been shown that EC rats perform better than IC rats on spatial learning tasks. In one study, EC rats were observed to make the correct choice in a 17-radial arm maze more accurately and efficiently relative to their IC counterparts (Juraska, Henderson, & Muller, 1984). EC rats have also been found to be more successful than IC rats in passive avoidance tasks (Freeman & Ray, 1972) and display increased contextual conditioning in signaled Pavlovian fear conditioning paradigms (Barbelivien et al., 2006; Woodcock & Richardson, 2000). It is believed that this effect is due in part to improved memory and learning. Environmental enrichment has also been shown to accelerate habituation to novel stimuli (Schrijiver, Bahr, Weiss & Wurbel, 2002; Zimmermann, Stauffacher, Langhans, & Wurbel, 2001) and decrease activity in an inescapable novel environment relative to IC rats (Bowling, Rowlett & Bardo, 1993; Green, et al., 2003).

Since the development of this paradigm, it has been argued that social stimulation may solely be the cause of the observed differences found between EC and IC rats. Thus, many researchers have included a third context (social condition; SC) in which rats are housed in pairs under standard laboratory conditions and are handled once a week during weekly bedding changes. SC rats have been found to be between EC and IC rats in multiple brain measures, indicating that social stimulation alone is not responsible for the

observed differences between EC and IC rats (Rosenzweig, Bennett, Hebert, & Morimoto, 1978).

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 such as amphetamine. While EC rats self-administer less amphetamine at low unit doses (Bardo, Klebaur, Valone, & Deaton, 2001), they are more sensitive to the locomotor effects of acute amphetamine at both moderate (0.5 mg/kg) and high (2.0 mg/kg) doses (Bowling & Bardo, 1993), but not at low doses (0.1 or 0.3 mg/kg) (Bardo et al., 1995). In contrast however, IC rats have been found to be more sensitive to the locomotor effects of chronic amphetamine administration at a low unit dose (0.3 mg/kg) (Bardo et al., 1995). Additionally, enrichment appears to increase the rewarding effects of amphetamine in conditioned place preference paradigms (CPP) (Bardo et al., 1995). It is important to note the apparent contradiction that, although EC rats are less sensitive to repeated amphetamine administrations, they are more sensitive to amphetamine-induced CPP relative to IC rats. It has been suggested that since EC rats are more sensitive to acute amphetamine, this initial sensitivity may be reflected in one-trial CPP (Bardo et al., 1995).

Alternatively, the enhanced CPP may be due to an enhanced ability to process contextual cues. EC rats display greater contextual conditioning and are better at context discrimination relative to IC rats (Barbelivien et al., 2006; Woodcock & Richardson, 2000). This would suggest that EC rats are also more sensitive to Pavlovian-conditioned drug cues. Although much research has investigated the effects of differential rearing on

addictive drugs such as amphetamine and cocaine, one of the most commonly abused and addictive drugs, nicotine, has been sparsely investigated within this paradigm.

Nicotine addiction is one of the most visible public health concerns today. It is estimated that 20.9 % (45.1 million) adults in the United States currently smoke, contributing to an estimated 438,000 preventable deaths (Centers for Disease Control & Prevention, 2005a, 2005b). Although nicotine is generally not considered to be one of the 'harder' addictive substances, with repeated exposure, it is often just as difficult to discontinue its use (Laviolette & van der Kooy, 2004).

Interestingly, very little is understood about how the rearing environment contributes to nicotine addiction, cessation, and relapse. For example, why is it that some individuals can smoke a cigarette on occasion and never become addicted while others readily seek tobacco after their first encounter, leading to addiction? Or, why do some successfully quit smoking while others struggle to kick the habit? Understanding how the rearing environment contributes to these individual differences in drug abuse will aid in the development of prevention and cessation programs.

Previous research has implicated associative learning, specifically Pavlovian conditioning processes, to play a role in the etiology of nicotine dependence (Koob & Le Moal, 2001; Rose, Behm, & Levin, 1993). 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 (Belluzzi, Lee, Oliff, Leslie, 2004; Reid, Ho, Berger, 1996). More specifically, conditioned-hyperactivity can be observed in rats that have had repeated nicotine administrations (US) paired with a distinct context (CS) (Bevins, Besheer, & Pickett, 2001; Bevins & Palmatier, 2003; Palmatier & Bevins, 2002; Reid et al. 1996; Walter & Kuschinsky, 1989). 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 (Lazev, Herzog, & Brandon, 1999; Rose et al., 1993).

The effects of nicotine on locomotor activity in rats are quite complex and consists of a biphasic effect on activity (Clarke & Kumar, 1983; Stolerman, Fink, & Jarvik, 1973). In non-tolerant rats, acute nicotine initially produces hypoactivity for roughly 15 min followed by a period of hyperactivity depending upon the dose and habituation to the testing apparatus. Rats that have habituated to the testing apparatus display less sensitivity to the depressant effects of nicotine. With repeated exposure to nicotine, sensitization develops to the hyperactivity. Behavioral sensitization in response to nicotine can be observed following 5 days of nicotine treatment when tested on the sixth day and is reflected by an increase in locomotor activity (Benwell & Balfour, 1992; Clarke & Kumar, 1983; Walter & Kuschinsky, 1989). In one previous study (Miller, Wilkins, Bardo, Crooks, & Dwoskin, 2001), rats were administered nicotine once a week for 6 weeks followed by a 21 day no drug period. Interestingly, when given a nicotine challenge, rats still expressed behavioral sensitization. This suggests that even occasional nicotine exposure may initiate neuroadaptive processes that contribute to addiction.

As mentioned previously, relatively few studies have investigated the effects of rearing environment on nicotine addiction. Green et al., (2003) observed that EC rats pretreated with nicotine display less development of sensitization relative to IC and SC rats, suggesting that environmental enrichment produces decreased sensitivity to the stimulant effects of nicotine. In this study, rats were pretreated with either a high dose (0.8 mg/kg) or low dose (0.2 mg/kg) of nicotine for 8 days and challenged with only the high dose of nicotine. However, this study did not look at the effects of rearing environment on nicotine-induced conditioned hyperactivity. In another study (Faraday, Scheufele, Rahman, & Grunberg, 1999), the effects of social rearing on nicotine-induced locomotor activity were examined. The results of this study showed that rats housed individually displayed decreased locomotor activity in response to nicotine while rats housed in groups increased in locomotor activity, contradicting the results found by Green et al. (2003). However, rats were not housed in their experimental condition until the start of drug administration and nicotine was administered continuously through osmotic mini-pumps whereas Green et al. (2003) administered nicotine subcutaneously.

Research examining the physiological factors that influence nicotine dependence have found that nicotinic acetylcholine receptors (nAChRs) play a large role in mediating the effects of nicotine (Laviolette & van der Kooy, 2004; Matta, Fu, Valentine, & Sharp, 1998). Activation of nAChRs has been shown to contribute to the reinforcing effects of nicotine, acutely enhancing the release of dopamine in the NAcc (Benwell & Balfour, 1992; Corrigall, Franklin, Coen, & Clarke, 1992). Additionally, the effects of nicotine on attention, learning, and memory are believed to be partially mediated through these receptors (Blokland, 1995; Levin, McClernon, & Rezvani, 2006; Olausson, Jentsch, &

Taylor, 2004). 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 is a nonselective nAChR antagonist that readily crosses the blood-brain barrier (Clarke & Kumar, 1983). Its therapeutic potential in smoking cessation has been widely investigated using various paradigms. Previous studies have shown that mecamylamine dose dependently decreases self-administration of nicotine in rats (Corrigall & Coen, 1989; Shoaib, Schindler, & Goldberg, 1997; Watkins, Epping-Jordan, Koob, & Markou, 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, Teoh, & Iwamoto, 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 & Kumar, 1983; Neugebauer et al., 2006; Stolerman, Garcha, & Mirza, 1995). When a low dose of mecamylamine is administered (0.1 mg/kg) nicotine-induced locomotor hypoactivity is blocked. However, mecamylamine does not seem to attenuate the stimulant effects of chronic nicotine administration (Bevins & Besheer, 2000).

Interestingly, few studies have examined how differential rearing may predict sensitivity to the antagonistic effects of drugs, such as mecamylamine, on nicotine-induced locomotor activity. Nicotine has been found to disrupt individual differences in locomotor activity in high and low responding rats (HR, LR) (Bevins & Besheer, 2001). HR rats display high amounts of activity when placed in an inescapable novel

environment while LR rats display low amounts of activity. Mecamylamine was found to restore these individual differences, thus suggesting that the disruptive affects of nicotine on HR and LR rats is mediated by nAChRs. Investigating these differences in sensitivity to receptor antagonism could potentially lead to identifying processes that mediate vulnerability to drug abuse.

While it appears that enrichment may be a protective factor against drugs of abuse such as amphetamine, it is not clear if this environmental factor is also protective against nicotine addiction. It is also unclear what neural mechanisms mediate the affects 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 accordance with past research, it is hypothesized 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 nACh receptors 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 affect will be greatest in EC rats since they have been shown to have increases in ACh relative to IC rats (Degroot, Wolff, & Nomikos, 2005).

If it is observed that differential rearing alters the behavioral response to nicotine, it will suggest that the rearing environment 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.

Method

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 are in compliance with the *Guide for the Care and Use of Laboratory Animals* (1996).

Drugs

S (–) -Nicotine ditartrate (0.4 mg/kg; Sigma, St. Louis, MO) and mecamylamine hydrochloride (1.0 mg/kg; Sigma, St. Louis, MO) was dissolved in 0.9% 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).

Apparatus

The locomotor chamber measures 40.64 x 40.64 x 40.64 cm. The chamber consists of plexiglass walls and plastic flooring which was covered by pine bedding. The photobeam sensor ring consists of a 16 x 16 (x-axis) photocell array. These photocells

are spaced 2.54 cm apart (Coulbourn Instruments, TruScan 2.01) and linked to a personal computer. The total distance traveled in centimeters was recorded. Cumulative photobeam interruptions, in 5-min blocks of time, were also recorded within each session. A white-noise generator (~70dB) was used to create ambient background noise to mask sounds from outside the chamber.

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 x 120 x 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 x 20 x 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 x 24 x 20 cm), and solid metal sides, back and top. IC rats were not handled during their rearing period (21-51 days of age).

Behavioral Procedures

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 in

the Paired group received repeated pairings of the locomotor chamber (context CS) with the nicotine US. Thus, once every other day, rats in the Paired group were injected with nicotine subcutaneously immediately prior to a 1-h 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 also administered nicotine in the home cage on the rest days. The Control group received saline injects in both the locomotor environment 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.

Conditioned hyperactivity test. Following 10 acquisition sessions, 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-m prior to a saline injection in the locomotor room. Rats were placed into the locomotor chambers immediately following the second injection for 1-h.

Sensitization training. Following the conditioned hyperactivity test, all rats received 4 additional sessions of conditioning (38 rats in the Paired condition, 28 in the Unpaired condition, and 34 rats in the Control condition). 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 underwent a 16 day resting period. During this time, no home cage injections were administered.

Sensitization test. Mecamylamine or saline was administered 15-m prior to a nicotine (0.4 mg/kg) challenge 16 days after nicotine pretreatment. 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.

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. The total distance traveled (cm) during the conditioned hyperactivity test and sensitization test was also converted into z-scores and analyzed using a 3 x 3 x 2 (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 treatement 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.

Results

Acquisition of conditioned hyperactivity

Repeated nicotine administration increased locomotor activity across sessions and differentially affected rearing groups. For 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 X nicotine treatment interaction, $F(18, 828) = 31.42$, $p<.001$, a session X rearing condition interaction, $F(18, 828) = 4.54$, $p<.001$, and a rearing condition X nicotine treatment X session interaction , $F(36, 828) = 1.97$, $p<.001$ (Figure 1). Nicotine significantly increased locomotor activity in Paired groups. The IC-Paired group significantly differed from IC-Controls on session 3-10, F's(1, 92) = 15.72 – 109.61, p's<.001. The SC-Paired group also significantly differed from SC-Controls on sessions 3-10, F's(1, 92) = 26.58 – 78.08, p's<.001. The EC-Paired group significantly differed from EC-Controls on sessions 2-8 and 9-10, F's(1, 92) = 13.19 – 70.8, p's<.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, F's(1, 92) = 20.03 – 97.99, p's<.001. SC-Paired rats were observed to display significantly greater locomotor activity than EC-Paired rats on sessions 2-7, 9, and 10, F's(1, 92) = 12.48 – 25.28, p's<.001. IC-Paired rats had significantly greater locomotor activity than SC-Paired rats only on day 9, $F(1, 92) = 12.42$, $p<.001$ and day 10, $F(1, 92) = 22.27$, $p<.001$ (Figure 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, F's(1, 92) = 12.83 – 23.12, p's<.001. IC- and EC-Control groups were also found to significantly differ on days 1-7, 9, and 10, F's(1, 92) = 18.58 – 47.00, p's<.001 (Figure 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 (Data no shown).

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 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 and 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, $F(1, 83) = 8.24$, $p<.01$ (Figure 3A).

Pretreatment of mecamylamine was found to attenuate conditioned hyperactivity only in EC and SC rats (Figure 3B). 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, $F(1, 83) = 7.43$, $p<.01$, $F(1, 83) = 9.29$, $p<.01$, $F(1, 83) = 9.70$, $p<.01$ (Figure 4A). Although there was no main effect of rearing, IC rats pretreated with mecamylamine were still observed to displayed 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 (Figure 4B).

Sensitization Training

Across sensitization training sessions, rats in the Paired groups were observed to display increased locomotor activity (Figure 5). Repeated measures revealed a significant session X 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$(1, 91) = 35.22 – 94.68$, p's$<.001$. 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$(1, 91) = 17.64 – 28.56$, p's$<.001$, and IC-Paired rats, F's$(1, 91) = 55.95 – 79.92$, p's$<.001$, on all 4 days of training (Figure 6A). 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$(1, 91) = 24.17 – 33.77$, p's$<.001$ (Figure 6B).

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 X 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, $F(1, 81) = 7.72$, $p<.01$ (Figure 7A).

When comparing mecamylamine pretreatment to saline pretreatment, rats in the Paired group treated with mecamylamine were found to display significantly less locomotor activity than rats treated with saline in the Paired group, regardless of rearing condition, $F(1, 81) = 13.82$, $p<.001$ (Figure 7B). 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 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-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, $F(1, 81) = 10.81$, $p<.01$ (Figure 7C)

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-Paired rats, $F(1, 81) = 25.33$, $p<.001$. IC-Paired rats significantly differed from IC-Controls, $F(1, 81) = 13.39$, $p<.001$ (Figure 8A).

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, $F(1, 81) = 10.33$, $p<.01$ (Figure 8B).

In order to examine the effects of mecamylamine on the hypoactive effects of nicotine, only the Control groups were used in the analyses as this was their first experience with nicotine. Mecamylamine was found to differentially block the hypoactive effects of nicotine (Figure 9). 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 X rearing condition, $F(22, 308) = 1.72$, $p<.05$, and a 5-min bins X 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. 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 from IC and SC rats (Figure 10). Early in the

session, EC rats significantly differed from SC 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 rats significantly differed from IC rats during bin 5, $F(1, 28) = 19.45$, $p<.001$, bin 6, $F(1, 28) = 12.23$, $p<.001$, and bin 7, $F(1, 28) = 13.99$, $p<.001$ (Figure 9B).

Discussion

This study examined the effects of differential rearing on nicotine-induced conditioned hyperactivity and sensitization. Rats raised in an enriched environment appear to be less sensitive to the locomotor effects of nicotine than both rats raised in an impoverished and social environment while IC rats appear to be most sensitive to nicotine-associated contextual cues. In addition, this study also examined the effects of mecamylamine, a nonselective nAChR antagonist, on conditioned hyperactivity and sensitization in rats differentially reared. 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 conditioned hyperactivity is, in part, mediated by neural nACh receptors and that environmental enrichment may alter these receptors.

In the current study, repeated nicotine administration was found to induce a period of hypoactivity followed by hyperactivity. Across repeated nicotine injections, sensitization was found to develop to the hyperactivity. These results are consistent with previous research examining the locomotor effects of nicotine administration (Benwell & Balfour, 1992; Clarke & Kumar, 1983; Stolerman, Fink, & Jarvik, 1973). Furthermore, the present results support previous findings that IC rats develop a greater locomotor

response to nicotine relative to EC and SC rats (Green et al., 2003). Rats reared in an impoverished environment appeared to be most sensitive to the hyperactive effects of nicotine in comparison to rats reared in an enriched or social environment.

During acquisition of conditioned hyperactivity and sensitization training sessions, differences in baseline activity levels in saline-treated control rats were observed. These baseline differences are an inherent feature of the environmental manipulation (Bowling & Bardo, 1995). Despite these baseline differences, EC, SC, and IC rats in the Paired group did not significantly differ during session 1 of acquisition. Thus, the baseline difference did not prevent observations between EC, SC, and IC rats in the hypoactive or hyperactive effects of nicotine.

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 the DA neurotransmitter system. The mesolimbic DA reward system is a critical component for the reinforcing effects of nicotine. Nicotine is thought to release DA by impulse-regulated vesicular exocytosis (Vizi & Lendavai, 1999). Extracellular DA is transported into the presynaptic terminal by DA transporters (DAT) (Zhu, Apparsundaram, Bardo, & Dwoskin, 2005). Repeated nicotine exposure facilitates clearance of extracellular DA in the terminal regions of the mesolimbic DA pathways (Hart & Ksir, 1996). It has been shown that environment enrichment also regulates DAT functioning. EC rats display decreased cell surface DAT expression in the medial prefrontal cortex (mPFC) (Zhu et al., 2005). Furthermore, nicotine administration (0.4 mg/kg) has been shown to increase clearance of extracellular DA in the mPFC in EC rats, but not in IC rats (Zhu, Bardo, Green, Wedlund, & Dwoskin, 2007). These results

suggest that the observed differences in DAT functioning in the mPFC, as 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 were found to display conditioned hyperactivity when saline was substituted for nicotine. Interestingly, although environmental enrichment has been shown to enhance learning regarding contextual cues (Barbelivien et al., 2006; Woodcock & Richardson, 2000), 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 examine the process of extinction of nicotine-induced hyperactivity in differentially reared rats.

Similarly 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 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 mediate the conditioning and rewarding effects of nicotine. The results of the current study support the hypothesis that nAChRs may mediate conditioned responses to nicotine-associated cues. 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 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 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, Wolff, & Nomikos, 2005).

Administration of mecamylamine during the sensitization test resulted in attenuation of sensitization. Mecamylamine (1.2 mg/kg) has been reported to attenuate nicotine sensitization (Miller et al., 2001). The results in the present study support this finding. 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. It has been hypothesized that nAChRs mediate the processes involved in sensitization, however, contextual conditioning processes also play a role in this expression (Miller et al., 2001; Reid et al., 1996). In a study conducted by Miller et al. (2001) rats were injected once a week with nicotine (0.35 mg/kg) for six weeks prior to sensitization testing. In the current study, rats underwent a total of 14 conditioning trials prior to testing for sensitization. Thus, it is possible that the residual activity levels displayed by the Paired groups above saline-treated Controls reflect conditioned hyperactivity to the testing chamber.

Alternatively, in another study (Ericson, Olausson, Engel, & Soderpalm, 2000) it was found that following 15 daily injections of nicotine (0.35 mg/kg) mecamylamine (4.0 mg/kg) attenuated sensitization. These results suggest there is selectivity for a high versus low dose of mecamylamine in the blockade of hyperactivity following chronic nicotine administration. This relationship between mecamylamine dose and the number of nicotine pre-exposure trials will require further investigation.

Another goal of this study was to determine if rearing condition would affect mecamylamine sensitivity during the hypoactive phase of acute nicotine administration. Previous research has shown that mecamylamine (1.0 mg/kg) blocks hypoactivity to nicotine in rats (Bevins & Besheer, 2001). The results to the current study support this finding. 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-hr session. Conversely, EC rats treated with mecamylamine displayed significantly less locomotor activity than IC rats treated with

mecamylamine later in the 1-hr session. Due to nicotine's biphasic nature, an increase in locomotor activity can be observed 15-min following nicotine administration (Clarke & Kumar, 1983). Although mecamylamine did significantly attenuate hypoactivity compared to saline-treated Controls within this first 15-mins, 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.

It is important to note that mecamylamine is nonselective in nAChR binding. Further research investigating more selective nAChR antagonists will be important for understanding which receptor subtypes are involved in nicotine sensitization and the learning of nicotine-associated cues. For example, examination of dihydro-β-erythroidine (DHβE), an antagonist for nicotinic receptors with the $\alpha_4\beta_2$ receptor subtype (Williams & Robinson, 1984), and methyllycaconitine (MLA), an α_7-selective antagonist (Alkondon, Pereira, Wonnacott, & Albuquereque, 1992), will allow for further understanding of how nAChRs mediate both physiological and conditioning effects of nicotine.

It has been hypothesized that nAChRs, in part, mediate the locomotor effects of nicotine by enhancing DA release in the NAcc (Nisell, Nomikos, & Stevenson, 1994). It has been suggested that this increase in DA contributes to the conditioned locomotor effects of nicotine (Reid, Ho, & Berger, 1998). Furthermore, the mPFC appears to contribute to conditioning associated with environmental cues paired with the drug (Berridge & Robinson, 1998). Given that EC and IC rats differ in DAT expression and

DA clearance in response to nicotine in the mPFC (Zhu et al., 2004, 2005, 2007), future studies will also investigate DA antagonists in differentially reared rats. For example, SCH-23390, a dopamine D_1 receptor antagonist has been shown to block expression of conditioned locomotor activity to nicotine (Bevins, Besheer, & Pickett, 2001), however, no studies to date have examined differential effects of SCH-23390 in rats reared in enriched, impoverished, and social conditions.

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. However, given past research indicating that EC and IC rats differ in DAT functioning and DAT clearance (Zhu et al., 2004, 2005, 2007), it is possible that these differences in differentially reared rats in response to nicotine are due to DA neurotransmission in the mPFC. 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.

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Figure Captions

Figure 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 ($p<.001$).

Figure 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 ($p<.001$).

Figure 3: 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) in the Paired, Unpaired, and Control groups. 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 ($p<.01$)

Figure 4: 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) in the Paired, Unpaired, and Control groups. Asterisks (*) denote a significant difference between Paired and Control groups ($p<.01$).

Figure 5: The mean (± S.E.M.) total distance traveled (cm) for EC, IC, and SC rats in the Paired, Unpaired, and Control groups during sessions 1-4 of sensitization training. Asterisks (*) denote a significant difference ($p<.001$) between Paired and Control groups.

Figure 6: Panel A shows the mean (± S.E.M.) total distance traveled (cm) for EC, IC, and SC rats in the Paired groups during sessions 1-4 of sensitization training. 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 EC and IC rats ($p<.001$). Carrot signs (^) denote a significant difference between EC and SC rats ($p<.001$). Numerical signs (#) denote a significant difference between IC and SC rats ($p<.01$).

Figure 7: 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) or saline during the sensitization test. Panel C displays the mean total distance traveled (cm) for EC, IC, and SC rats pretreated with mecamylamine (0.1 mg/kg) in the Paired, Unpaired, and Control groups. 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 (7A) and SC-Paired rats (7C; $p<.01$). Greater than signs (>) denote a significant difference between mecamylamine treatment and saline treatment for Paired groups ($p<.001$). 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 ($p<.01$).

Figure 8: 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) in the Paired, Unpaired, and Control groups. Asterisks (*) denote a significant difference between Paired and Control groups. Carrot signs (^) denote a significant difference between Paired and Unpaired groups ($p<.01$).

Figure 9: The mean (± S.E.M.) total distance traveled (cm) for EC, IC, and SC rats pretreated with mecamylamine (1.0 mg/kg) or saline in the Control groups across 5-min

bins during the sensitization test. Asterisks (*) indicate a significant difference between mecamylamine treated rats from saline treated rats ($p<.001$).

Figure 10: The mean (± S.E.M.) total distance traveled (cm) for EC, IC, and SC rats pretreated with mecamylamine (1.0 mg/kg) in the Control groups across 5-min bins during the sensitization test. Asterisks (*) indicate a significant difference between IC and EC rats. Numerical signs (#) indicate a significant difference between SC and EC rats ($p<.001$).

Figure 1

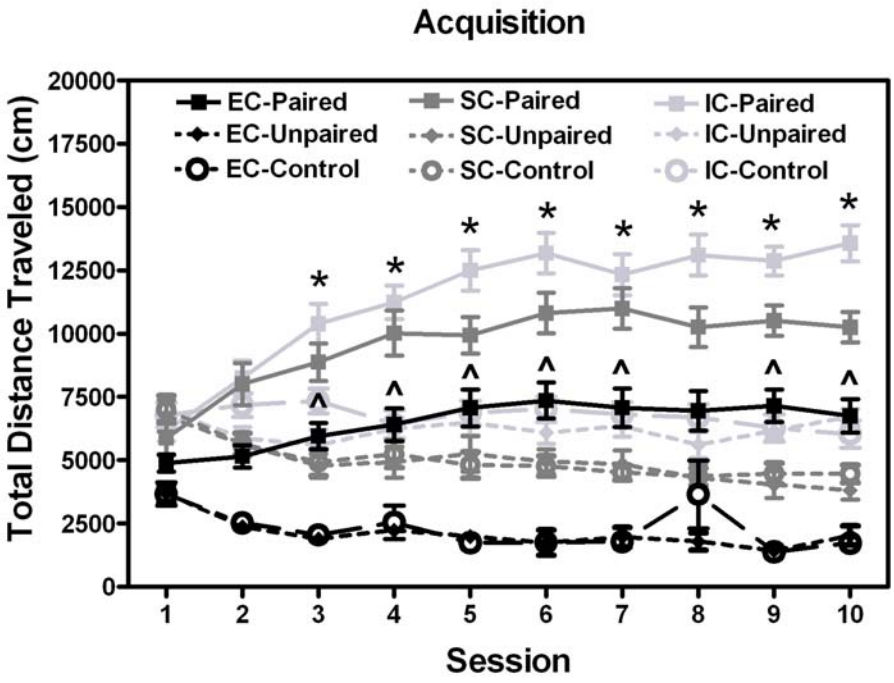

Acquisition
EC-Paired
EC-Unpaired
EC-Control
SC-Paired
SC-Unpaired
SC-Control
IC-Paired
IC-Unpaired
IC-Control
Total Distance Traveled (cm)
20000
17500
15000
12500
10000
7500
5000
2500
0
1
2
3
4
5
6
7
8
9
10
Session

Figure 2

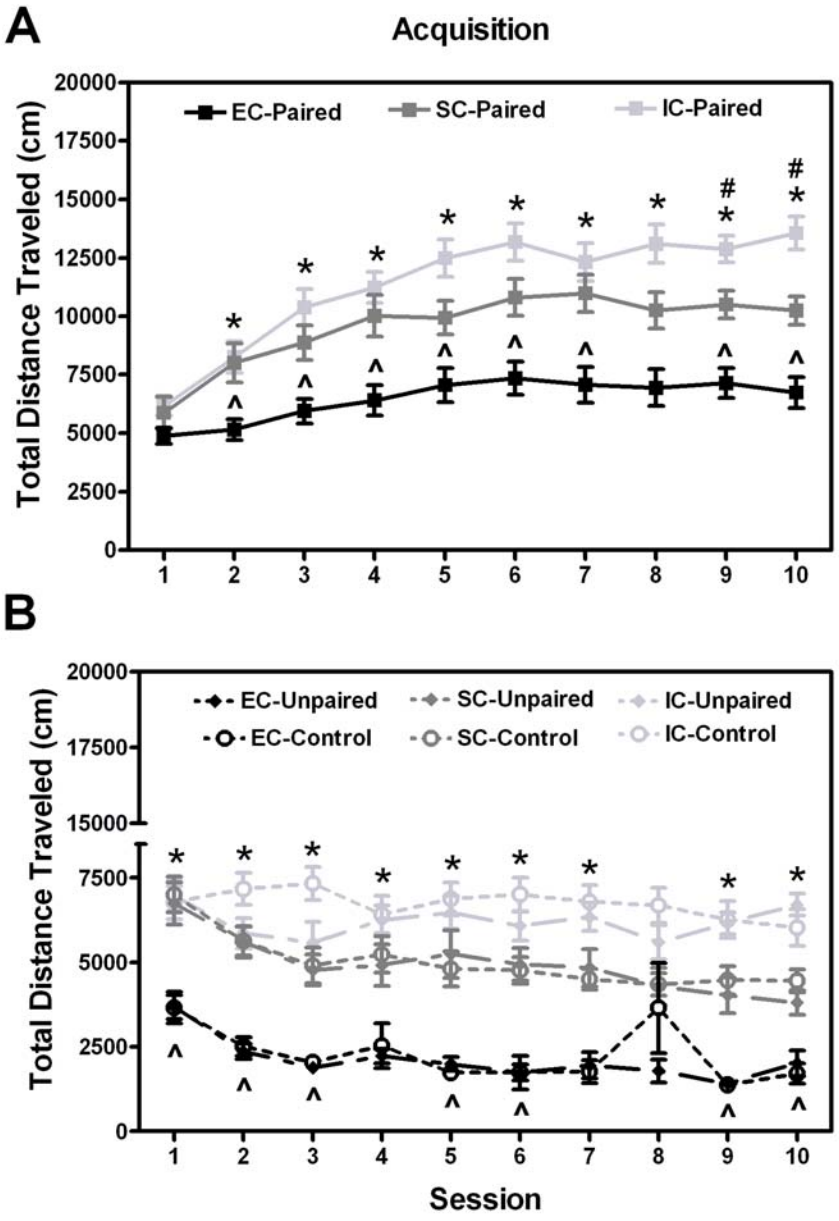

A
Acquisition
EC-Paired
SC-Paired
IC-Paired
Total Distance Traveled (cm)
20000
17500
15000
12500
10000
7500
5000
2500
0
1
2
3
4
5
6
7
8
9
10
B
EC-Unpaired
SC-Unpaired
IC-Unpaired
EC-Control
SC-Control
IC-Control
Total Distance Traveled (cm)
Session

Figure 3

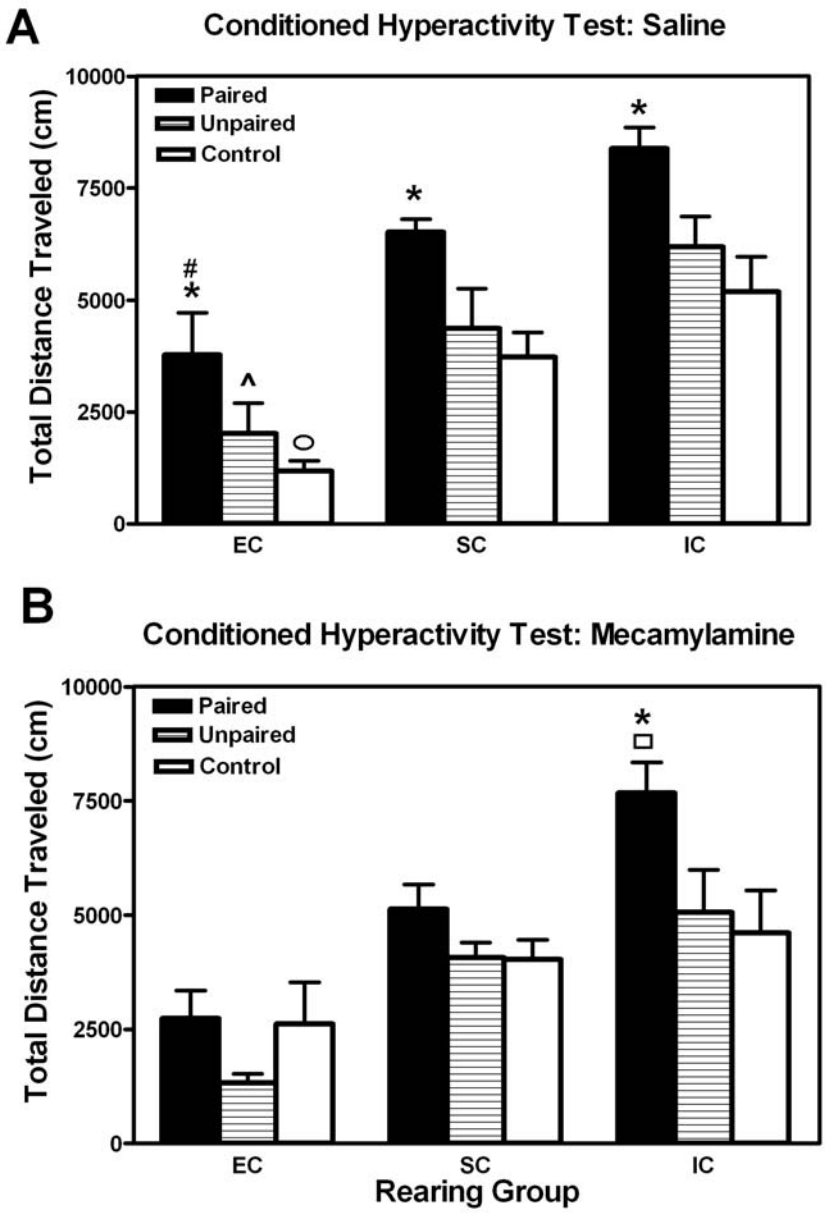

A
Conditioned Hyperactivity Test: Saline
Total Distance Traveled (cm)
10000
7500
5000
2500
0
Paired
Unpaired
Control
#
*
^
o
*
*
EC
SC
IC
B
Conditioned Hyperactivity Test: Mecamylamine
Total Distance Traveled (cm)
10000
7500
5000
2500
0
Paired
Unpaired
Control
*
□
EC
SC
IC
Rearing Group

Figure 4

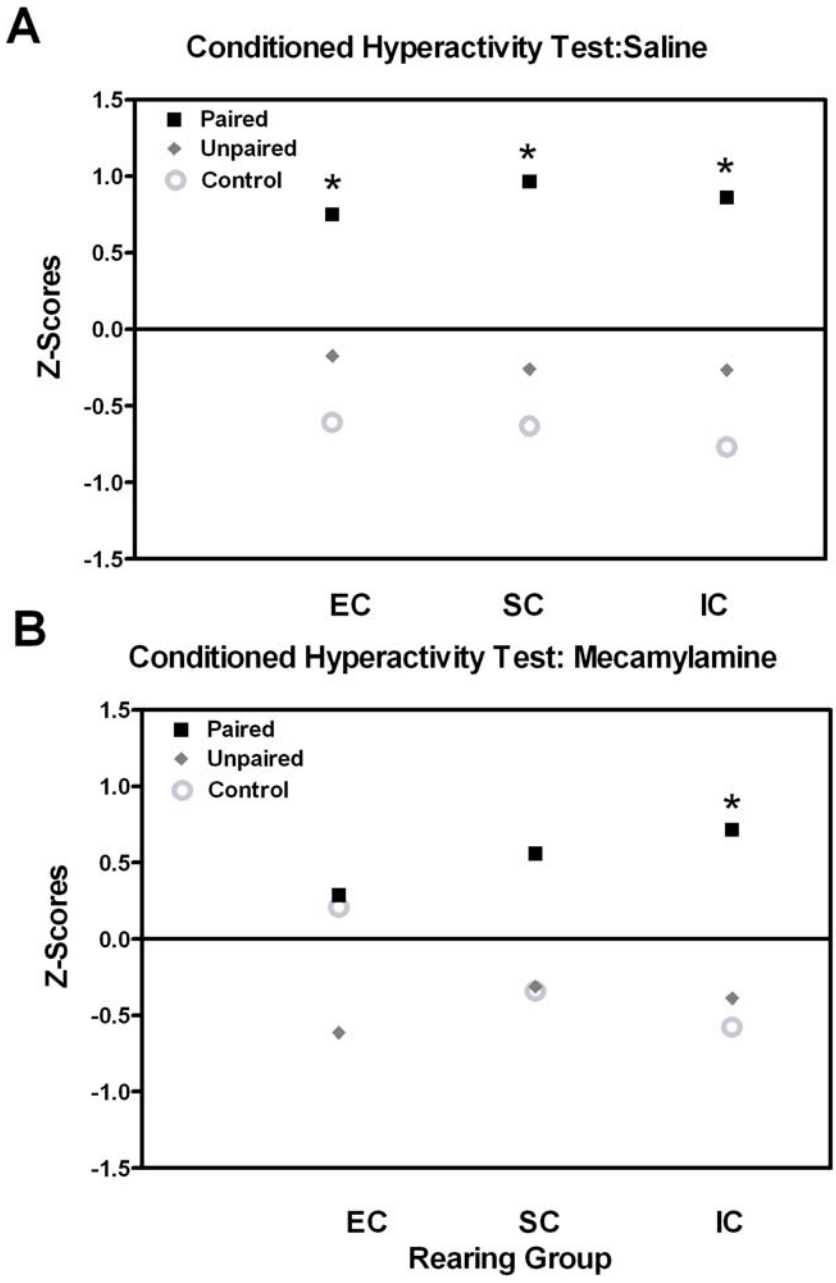

A
Conditioned Hyperactivity Test:Saline
Paired
Unpaired
Control
Z-Scores
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
*
*
*
EC
SC
IC
B
Conditioned Hyperactivity Test: Mecamylamine
Paired
Unpaired
Control
Z-Scores
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
*
EC
SC
IC
Rearing Group

Figure 5

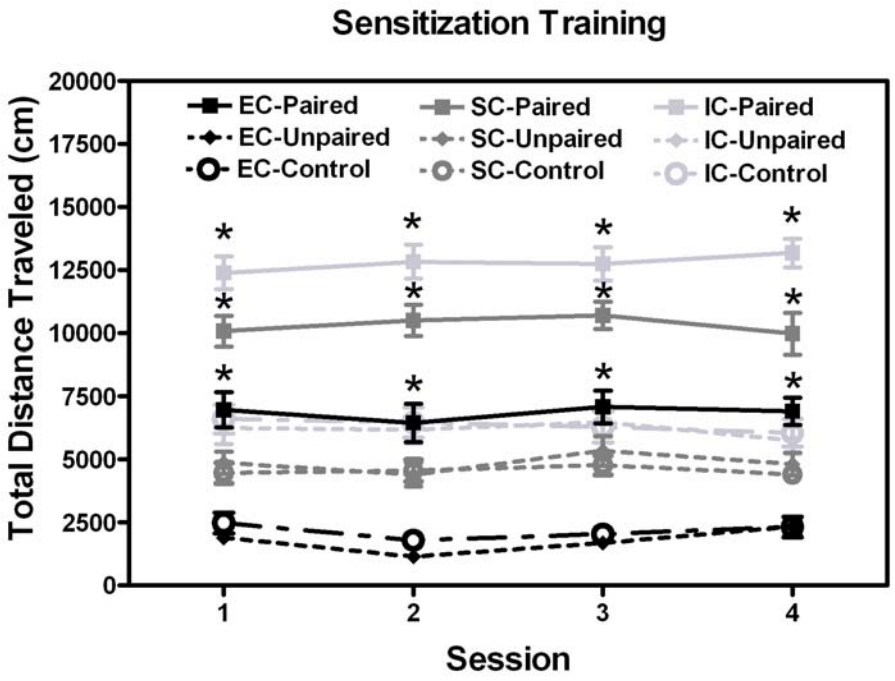

Sensitization Training
Total Distance Traveled (cm)
20000
17500
15000
12500
10000
7500
5000
2500
0
EC-Paired
EC-Unpaired
EC-Control
SC-Paired
SC-Unpaired
SC-Control
IC-Paired
IC-Unpaired
IC-Control
1
2
3
4
Session

Figure 6

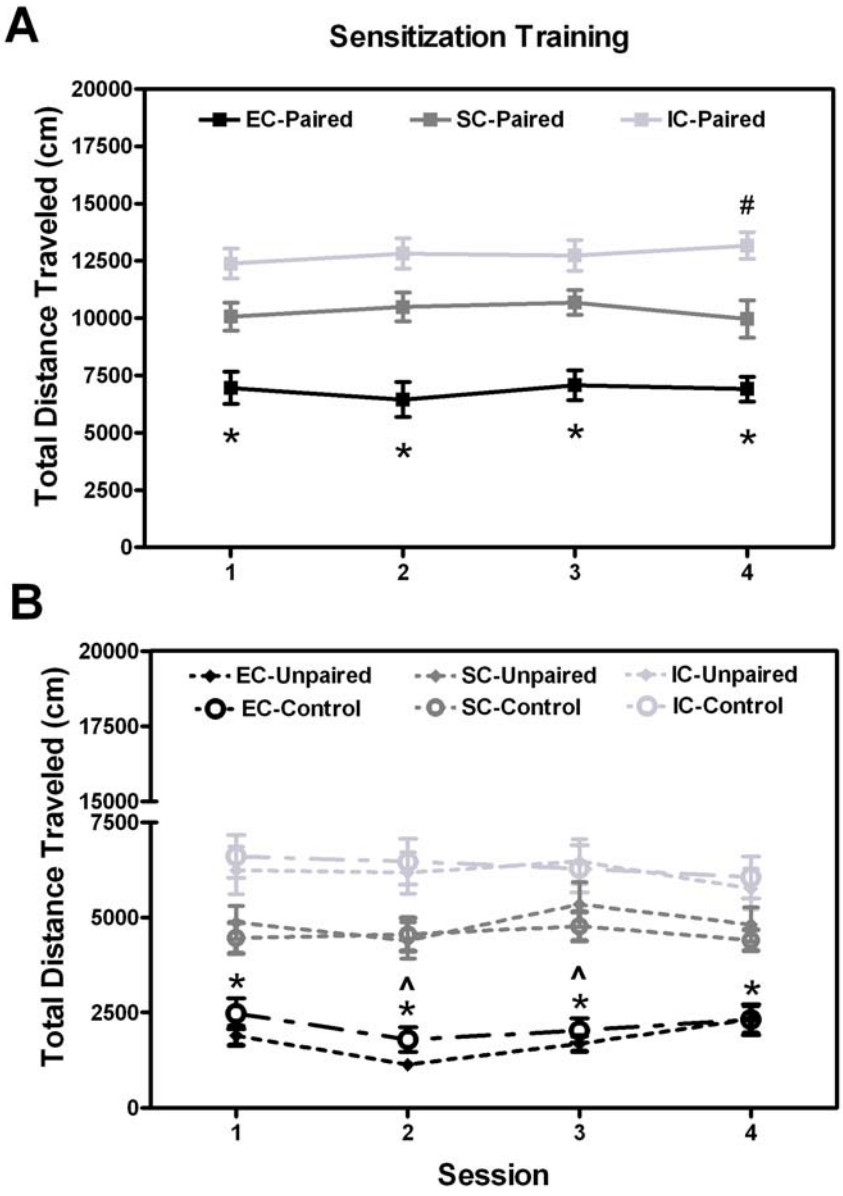

A
Sensitization Training
EC-Paired
SC-Paired
IC-Paired
Total Distance Traveled (cm)
20000
17500
15000
12500
10000
7500
5000
2500
0
1
2
3
4
#
*
B
EC-Unpaired
SC-Unpaired
IC-Unpaired
EC-Control
SC-Control
IC-Control
Total Distance Traveled (cm)
20000
17500
15000
7500
5000
2500
0
1
2
3
4
Session
*
^
*

Figure 7

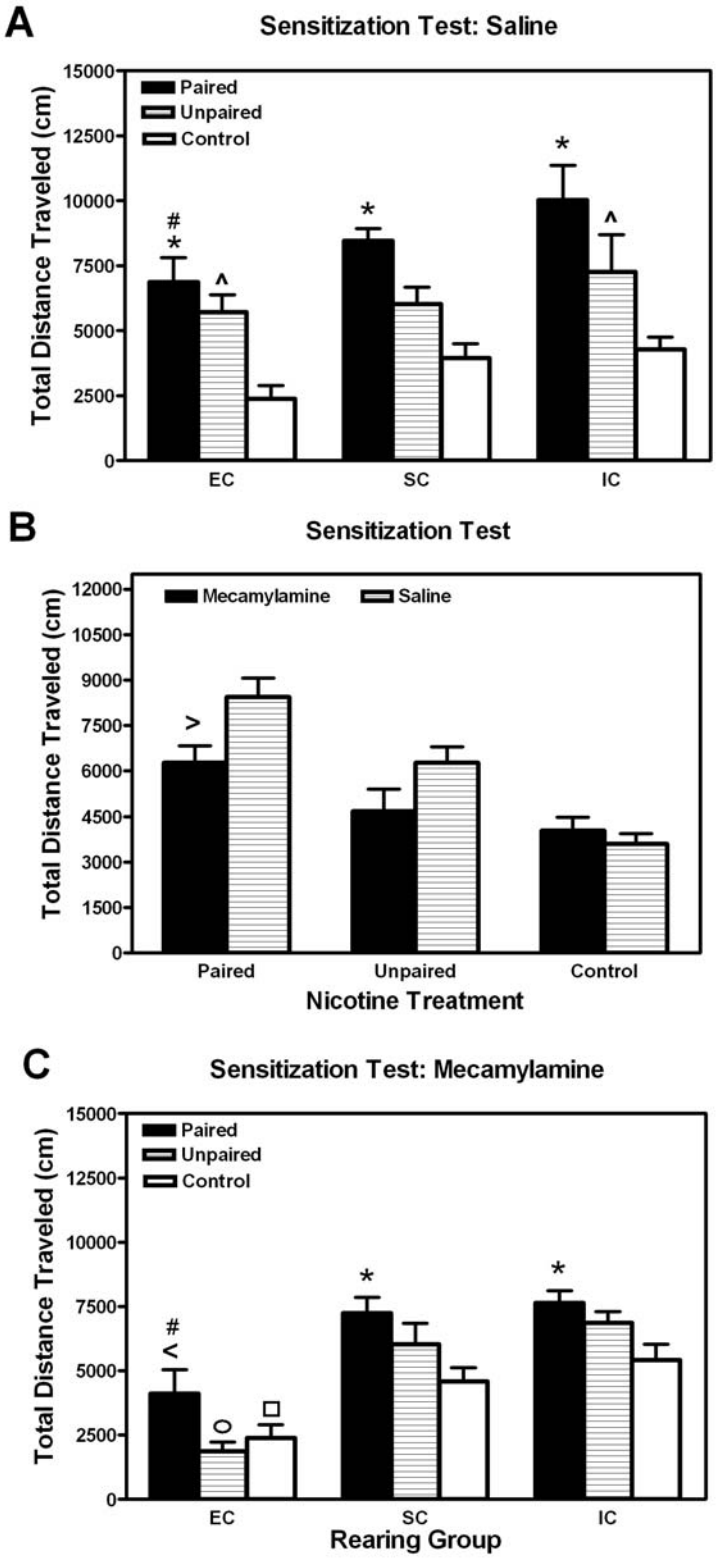

A
Sensitization Test: Saline
Total Distance Traveled (cm)
15000
12500
10000
7500
5000
2500
0
Paired
Unpaired
Control
#
*
^
*
*
^
EC
SC
IC
B
Sensitization Test
Total Distance Traveled (cm)
12000
10500
9000
7500
6000
4500
3000
1500
0
Mecamylamine
Saline
>
Paired
Unpaired
Control
Nicotine Treatment
C
Sensitization Test: Mecamylamine
Total Distance Traveled (cm)
15000
12500
10000
7500
5000
2500
0
Paired
Unpaired
Control
#
<
o
□
*
*
EC
SC
IC
Rearing Group

Figure 8

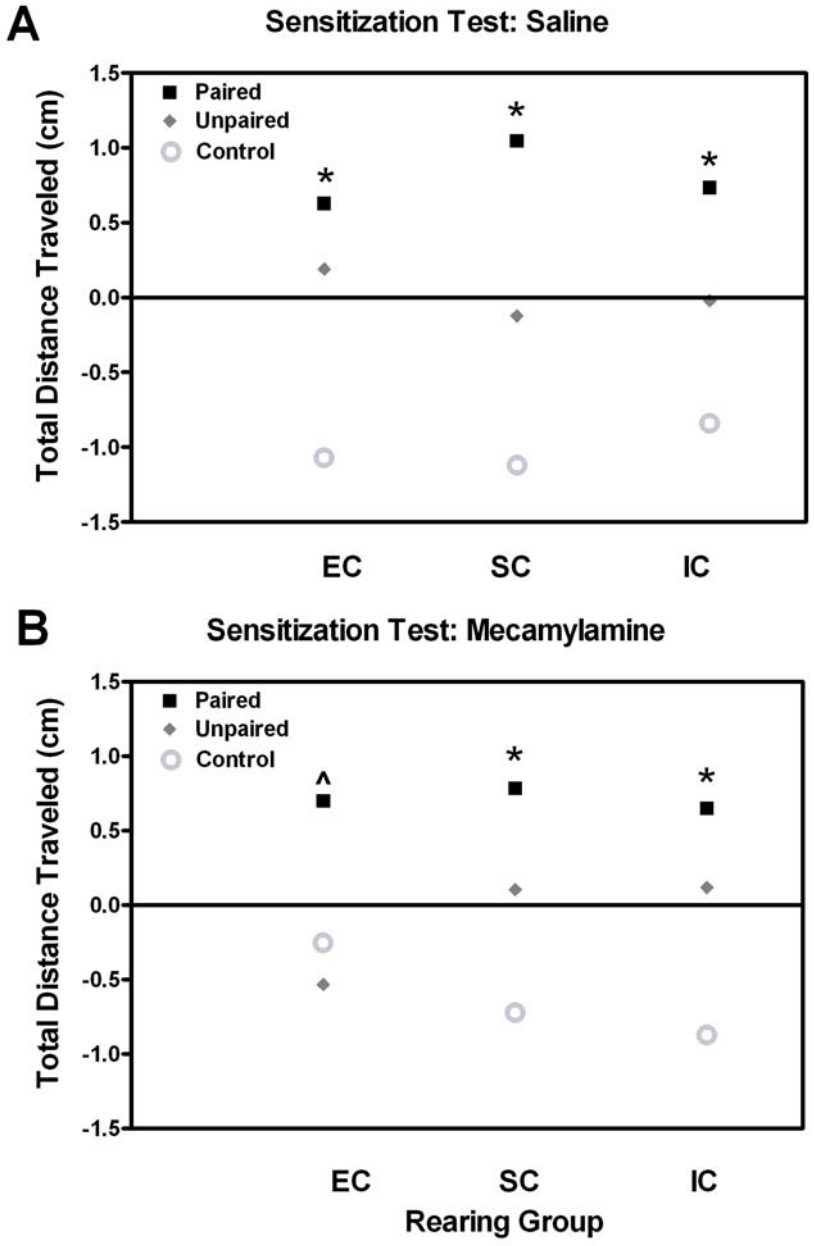

A
Sensitization Test: Saline
Paired
Unpaired
Control
Total Distance Traveled (cm)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
*
*
*
EC
SC
IC
B
Sensitization Test: Mecamylamine
Paired
Unpaired
Control
Total Distance Traveled (cm)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
^
*
*
EC
SC
IC
Rearing Group

Figure 9

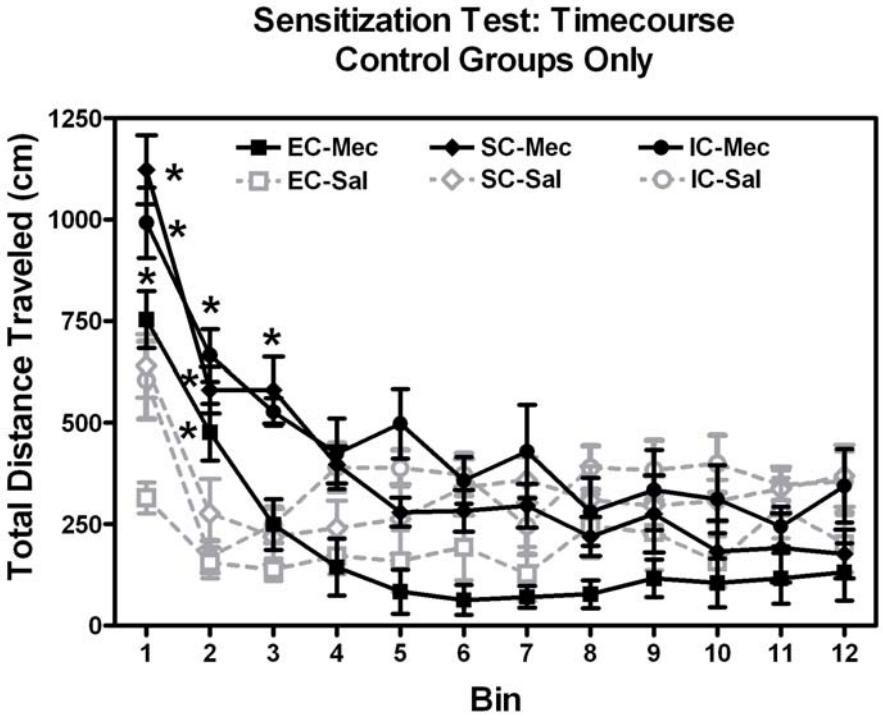
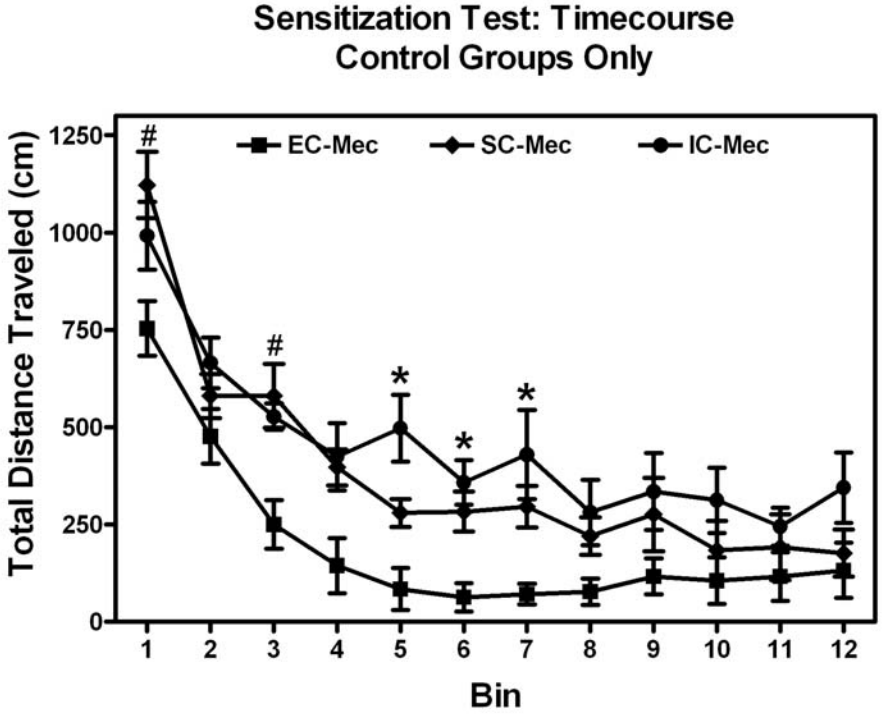

Sensitization Test: Timecourse
Control Groups Only
EC-Mec
SC-Mec
IC-Mec
EC-Sal
SC-Sal
IC-Sal
Total Distance Traveled (cm)
1250
1000
750
500
250
0
1
2
3
4
5
6
7
8
9
10
11
12
Bin

Figure 10


Sensitization Test: Timecourse
Control Groups Only
EC-Mec
SC-Mec
IC-Mec
Total Distance Traveled (cm)
1250
1000
750
500
250
0
#
#
*
*
*
1
2
3
4
5
6
7
8
9
10
11
12
Bin