Pharmacology, Biochemistry and Behavior 179 (2019) 80–88

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Enrichment-induced differences in methamphetamine drug discrimination in male rats

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ARTICLE INFO

Keywords:
Methamphetamine
Rats
Environmental enrichment
D1 dopamine receptor
Nicotine
Drug discrimination

ABSTRACT

Rats raised in an enriched environment show a decrease in sensitivity to the subjective effects of the psychostimulant d-amphetamine. The purpose of the present study was to determine if environmental enrichment during development alters the subjective effects of the more commonly abused drug methamphetamine. Male Sprague-Dawley rats were raised in either an enriched (EC) or an isolated condition (IC). EC and IC rats were trained on a two-lever operant procedure to discriminate 1.0 mg/kg (i.p.) methamphetamine from saline. Following acquisition of the discrimination a methamphetamine generalization curve (0.1–1.0 mg/kg) was determined. The antagonistic effects of dopamine D_1 receptor antagonist SCH23390 (0.0075–0.06 mg/kg) and the dopamine D_2 receptor antagonist eticlopride (0.01–0.3 mg/kg) were also tested. Finally, the ability of nicotine (0.05–0.5 mg/kg) to generalize and the ability of the nicotinic receptor antagonist mecamylamine (0.125–0.5 mg/kg) to antagonize the discriminative stimulus effects of methamphetamine were determined. EC rats were less sensitive to discriminative stimulus effects of methamphetamine compared to IC rats at a low 0.3 mg/kg dose and showed full antagonism of methamphetamine discrimination following SCH23390 compared to IC rats. There were no environmentally-induced differences in the effects of eticlopride. Nicotine only partially generalized to the effects of methamphetamine in both EC and IC rats. While mecamylamine failed to antagonize the effects of methamphetamine in either EC or IC rats. These results suggest that environmental enrichment decreases sensitivity to the discriminative effects of methamphetamine and the differences may be mediated through changes in the D_1 dopamine receptor.

1. Introduction

While rates of methamphetamine use have declined over the past few years, there are still approximately 402,000 lifetime users of methamphetamine and ~71,000 current users (Center for Behavioral Health Statistics and Quality, 2018). One of the risk factors that has been found to correlate with increased likelihood of methamphetamine use is sensation-seeking personality trait (Herman-Stahl et al., 2007; Mahoney 3rd et al., 2015). There is considerable research showing that the sensation-seeking personality trait is associated with increased vulnerability to drugs of abuse (Wills et al., 1994; M. Zuckerman, 1994; Marvin Zuckerman, 2007). The work by Mahoney 3rd et al. (2015) used the Impulsive Sensation Seeking Scale (ImpSS) to measure impulsive sensation seeking and found that individuals who had a methamphetamine use disorder scored significantly higher on the ImpSS compared to healthy controls. There is increasing evidence that a greater vulnerability to psychomotor stimulants seen in high sensation seekers may be due to changes in dopaminergic neurotransmission (Norbury and Husain, 2015).

There are various preclinical animal models that show correlated behaviors to sensation seeking (Zuckerman and Kuhlman, 2000), which can be useful in studying the underlying biological mechanism for how sensation seeking influences vulnerability to drug use. The rodent environmental enrichment model allows researchers to experimentally control the exposure to novelty and sensory stimulation in rats during development, and this model has been shown to produce a consistent altered sensitivity to drugs of abuse similar to other animal models of sensation seeking (Stairs and Bardo, 2009). The rodent environmental enrichment model can be done in varying ways, which can make cross-laboratory comparisons difficult (Simpson and Kelly, 2011). Studies using an enrichment model like those used by Bardo and colleagues, typically house rats for several weeks, post weaning (≈ 21 days of age), in either an enriched condition (EC) with novel objects and social cohorts or in an isolated condition (IC) without novel objects or social

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https://doi.org/10.1016/j.pbb.2019.02.008
Received 7 September 2018; Received in revised form 15 February 2019; Accepted 18 February 2019
Available online 20 February 2019
0091-3057/

cohorts. Environmental enrichment alters the behavioral effects of both d-amphetamine and cocaine in that EC rats show less sensitization following repeated injections of d-amphetamine and cocaine (Bardo et al., 1995; Smith et al., 1997). Also, EC rats self-administer less d-amphetamine and cocaine compared to IC rats at low unit doses under both Fixed-Ratio (FR) and Progressive-Ratio (PR) schedules of reinforcement (Howes et al., 2000; Smith et al., 2009). Using a cocaine drug discrimination task, IC rats had significantly lower ED_{50} values following a cocaine generalization curve compared to EC rats (Fowler et al., 1993). Moreover, this study found that IC rats also had significantly lower ED_{50} values compared to EC rats in the ability of amphetamine to produce cocaine like effects (Fowler et al., 1993).

While environmental enrichment appears to have consistent protective effects on some of the behavioral effects of d-amphetamine and cocaine, the effects of environmental enrichment on the behavioral effects of the psychostimulant methamphetamine have been less consistent. For instance, Gehrke et al. (2006) found that compared to IC rats, EC animals had a decreased locomotor sensitivity to a low acute dose of methamphetamine and showed a decreased sensitized locomotor response to repeated methamphetamine injections. While there was an effect of enrichment on the locomotor effects of methamphetamine, the study found there were no differences in EC and IC rats on levels of methamphetamine-induced conditioned place preference (CPP) (Gehrke et al., 2006). The lack of an effect of enrichment on CPP was replicated by Thiriet et al. (2011) which found no differences between enriched rats and controls on methamphetamine-induced locomotor behavior. Finally, Hofford et al. (2014) found there were no differences in EC and IC rats in both levels of methamphetamine-induced CPP or in levels of methamphetamine self-administration across a range of doses.

While environmental enrichment has inconsistent to no effects on methamphetamine-induced locomotor behavior, CPP, or methamphetamine self-administration, no research has looked at the effects of enrichment on the discriminative stimulus effects of methamphetamine. Two important testing advantages using the drug discrimination procedure is: First, it models the subjective effects of a drug (Schuster and Johanson, 1988) which is an important aspect of the abuse liability of a compound (Ator and Griffiths, 2003; Stolerman, 1992). Second, the procedure can also be used as an in vivo model to uncover the neural mechanisms or receptors that underlie the discriminative stimulus effects of a drug (Young, 2009).

In standard caged rats methamphetamine has been shown to maintain reliable discrimination versus saline across a range of methamphetamine doses (Desai and Bergman, 2010; Gatch et al., 2005; Munzar and Goldberg, 1999, 2000; Munzar et al., 2002; Suzuki et al., 2004). Discriminative stimulus effects of methamphetamine are primarily modulated via D_1 and D_2 like dopamine receptors (Desai and Bergman, 2010; Munzar and Goldberg, 2000; Tidey and Bergman, 1998) although there is a modulatory effect of serotonin and norepinephrine on methamphetamine drug discrimination (Munzar and Goldberg, 1999; Munzar et al., 1999b). Also, Gatch et al. (2008) found nicotine fully substituted in methamphetamine-trained rats indicating the nicotine and methamphetamine share similar discriminative stimulus effects. Dopamine antagonists, such as haloperidol, have been found effective in partially attenuating discriminative effects while nicotinic antagonists, such as mecamylamine, have little to no effect (Gatch et al., 2008).

The current study was conducted to determine if environmental enrichment can alter the sensitivity to the discriminative stimulus effects of methamphetamine. A second aim was to determine if environmental enrichment altered the ability of dopamine antagonists and the nicotinic antagonist mecamylamine to block the discriminative stimulus effect of methamphetamine. Finally, we also assessed if nicotine would differentially substitute for methamphetamine in methamphetamine-trained enriched and impoverished rats.

2. Methods

2.1. Subjects

Twenty-four 21-day old male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were used as subjects. Only male rats were used in the current study as previous research using this environmental enrichment design showed no differences between male and female rats in drug sensitivity (Bardo et al., 2001). Animals had unlimited access to food and water in their home cage unless otherwise noted. Rats were housed in a colony room maintained on a 12 h light/dark cycle with lights on from 06:00 to 18:00 h. Procedures were approved by the Creighton University Institutional Animal Care and Use Committee, and conformed to the 2011 NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources (U.S.), 2011).

2.2. Environmental conditions

Upon arrival to the colony, rats were placed randomly into one of two conditions; EC (n = 12), IC (n = 12) conditions. EC rats were always housed together in a stainless-steel cage (62 × 62 × 42 cm) with social cohorts (12 per cage) and 14 hard plastic objects (commercial toys, plastic containers, etc.) placed randomly throughout the cage. Seven objects were replaced daily with new objects and all objects were rearranged to create daily novel arrangements. EC rats were removed and handled briefly during the daily object change. IC rats were housed in individual hanging stainless steel cages with wire mesh floor and front panel, and solid metal side walls and top (17 × 24 × 20 cm). IC rats were handled minimally from 21 to 51 days of age. All animals remained in these conditions during postnatal days 21–51 and throughout the duration of the experiment.

2.3. Apparatus

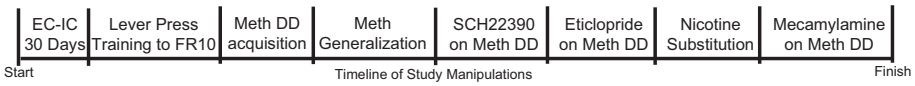
Standard operant conditioning chambers (28 × 21 × 21 cm; ENV-001; MED Associates, St. Albans, VT) that had alternating aluminum and Plexiglas walls and a metal rod floor were located inside sound-attenuating chambers (ENV-018 M; MED Associates, St. Albans, VT). A recessed food tray (5 × 4.2 cm) was located 2 cm above the floor in the center of one of the aluminum walls, and a response lever was located 6 cm above the floor on each side of the food tray. A white stimulus light (28 v; 3 cm diameter) was mounted 6 cm above each lever. Responses were recorded and programmed consequences were controlled by a computer in an adjacent room equipped with Med-PC software (Med Associates, St. Albans, VT).

3. Procedure

3.1. Lever-press training

Starting on PND 52, food access was restricted over a period of 4 days to decrease body weights to approximately 85% of free feeding weights. Following food deprivation, rats were exposed to 5 g. of food pellets (45 mg pellets; BioServ, Frenchtown, NJ) to alleviate neophobia during the training phase. On the day following exposure to the food pellets, rats were placed into the operant conditioning chamber with one lever removed from the chamber. Presses on the available lever resulted in the delivery of a food pellet under a Fixed-ratio 1 (FR) schedule of reinforcement. On the next day of training the previously removed lever was made available with the other lever removed; presses on the available lever again resulted in the delivery of a food pellet under an FR1 schedule of reinforcement. Each individual lever was available on alternating daily sessions; while the FR requirement was increased over successive 15-min daily sessions to a terminal FR10 schedule of reinforcement. Once animals respond on both levers under the FR10 schedule for two available sessions, methamphetamine

Table 1
Timeline of experimental manipulations.



discrimination training commenced. See Table 1 for the timeline of the various study manipulations.

3.2. Methamphetamine drug discrimination

In the training phase of methamphetamine discrimination, methamphetamine (1.0 mg/kg, intraperitoneal; i.p.) or saline injections were administered 15 min prior to the experimental session. Following the pretreatment, rats were placed into the operant conditioning chambers and the onset of the cue lights above both levers signaled the start of the 15-min experimental session. The assignment of the methamphetamine or saline-appropriate lever was counterbalanced across animals. Also, the daily pretreatment of methamphetamine or saline was counterbalanced across animals so that on each daily session half of the animals received methamphetamine and half received saline.

To facilitate training, the first eight sessions of drug discrimination training consisted of single lever training. For example, following a methamphetamine or saline pretreatment, only the methamphetamine- or saline-appropriate lever was extended into the chamber, completion of an FR10 on that lever resulted in the delivery of a food pellet. Following the eight sessions of single lever training both levers were extended into the chamber and responding on the injection-appropriate lever resulted in the delivery of a food pellet, on an FR10 schedule. Responses on the incorrect lever were recorded but had no programmed consequences. Methamphetamine (M) and saline (S) pretreatments were administered five days a week in the following two-week manner: MMSSM MSSMM. Drug discrimination training continued until the animals meet the following criterion on six consecutive sessions: (1) > 80% of the total session responses occur on the injection-appropriate lever for four consecutive sessions, and (2) no more than four responses on the incorrect lever before the completion of the first FR10 on the correct lever. Once animals met the previous criterion, methamphetamine generalization testing began.

3.3. Methamphetamine generalization testing

Following acquisition of methamphetamine discrimination, a methamphetamine generalization curve was determined in both EC and IC rats. The generalization test sessions were identical to the training sessions, with the exception that they were 3 min in duration and completion of an FR10 on either lever was reinforced. The generalization curve for methamphetamine was determined by substituting varying doses of methamphetamine (0–1.0 mg/kg; i.p.) for the training dose of methamphetamine. The doses of methamphetamine were administered in a Latin Square design 15 min prior to an experimental session. Generalization testing occurred twice a week in the following two-week pattern: MTSMT MTMST (M-methamphetamine training dose; S- saline; *t*-test dose of methamphetamine). This pattern was repeated unit all doses were tested twice in each animal. Following completion of the generalization curves, animals began antagonist testing.

3.4. Dopamine antagonist testing

Following completion of the methamphetamine generalization curves, both the dopamine D_1 receptor antagonist SCH23390 and the dopamine D_2 receptor antagonist eticlopride were tested in their ability to block the discriminative stimulus effects of the training dose of methamphetamine.

3.4.1. SCH23390 pretreatments

Various dose of SCH22390 were administered in a Latin Square design (0–0.06 mg/kg; i.p.) 10 min prior to the administration of the training dose of methamphetamine. Following the methamphetamine pretreatment, the animals were then returned to their transfer cage and 15 min later the animals were placed into the operant conditioning chambers to complete an experimental session. The experimental sessions following SCH23390 pretreatments were identical to the substitution test sessions used in the completion of the methamphetamine generalization curve. Antagonist pretreatment sessions occurred twice a week in the following two-week pattern: MASMA SAMSA (M-Methamphetamine training dose alone; S- Saline; A- Antagonist pretreatment + Methamphetamine training dose). This pattern was repeated unit all doses of SCH23390 were tested twice in each animal.

3.4.2. Eticlopride pretreatments

Following completion of the SCH23390 dose-effect curves, dose-effect curves of the D_2 antagonist eticlopride were completed in which various dose of eticlopride were administered in a Latin Square design (0–0.3 mg/kg; i.p.; 30 min pretreatment). The eticlopride pretreatment sessions were carried out identically to the SCH23390 pretreatment sessions with the only difference being training dose of methamphetamine was not administered until 15 min after the eticlopride injection. All eticlopride doses were tested twice in each animal.

3.5. Nicotine substitution and mecamylamine antagonism

Following completion of the dopamine antagonist pretreatments, the ability of nicotine to substitution for methamphetamine in the EC and IC methamphetamine-trained rats was determined. Finally, we investigated whether mecamylamine could antagonize the discriminative stimulus effects of the training dose of methamphetamine.

3.5.1. Nicotine substitution testing

The nicotine substitution test sessions were conducted the same way as the methamphetamine generalization test sessions with the exception that various doses of nicotine (0, 0.05, 0.1, 0.25, 0.5 mg/kg, s.c) and the 1.0 mg/kg training dose of methamphetamine were administered 15 min prior to a testing session. Nicotine substitution test sessions occurred twice a week in the following two- week pattern: MNSMN SNMSN (M-Methamphetamine training dose alone; S- Saline; N- Nicotine dose). This pattern was repeated unit all doses of nicotine were tested twice in each animal.

3.5.2. Mecamylamine pretreatments

Following completion of the nicotine substitution curve, various dose of mecamylamine were administered in a Latin Square design (0, 0.125, 0.25, 0.5 mg/kg; s.c; 30 min pretreatment). The mecamylamine pretreatment session were conducted identically to the SCH23390 pretreatment except for the antagonist pretreatment. Mecamylamine pretreatments were done twice a week until all doses were tested twice in each animal.

4. Data analysis

As stated above each pretreatment was tested twice, the data from the two sessions were averaged, the averages were then expressed as the percentage of total responses made on the methamphetamine-appropriate lever. Response-rate data were expressed as response per

second averaged over the session. All data are presented as group means (± SEM). Dose-effect curves were analyzed using a mixed factor analysis of variance (ANOVA), with dose of pretreatment drug (methamphetamine or nicotine dose or antagonist pretreatment) being the within-subject factor and environmental condition (EC vs IC) being the between-subject factor. Post hoc comparisons were conducted using Tukey's post hoc test. Effective and inhibition concentration (EC and IC) 50 values were determined from dose-effect curves analyzed using a nonlinear least-squares curve-fitting program (GraphPad Prism; GraphPad Software, San Diego, CA) and compared differences between the enriched and isolated curves using an F-test. For all tests of significance, an alpha of $p \leq 0.05$ was used.

5. Drugs

Methamphetamine hydrochloride, mecamylamine, SCH23390, eticlopride and S-(−)-nicotine bitartrate were purchased from Sigma/RBI (St. Louis, MO) and dissolved in 0.9% *w/v* NaCl (saline) at a volume of 1 ml/kg. The nicotine solutions were adjusted to pH 7.4 using 1 M NaOH. Nicotine doses are expressed as free-base weight, while all other drugs are in salt weight.

6. Results

6.1. Methamphetamine generalization

Given the use of the eight sessions of forced single lever trials and the more moderate training dose of methamphetamine, both EC and IC rats met acquisition criterion by 16 sessions. A two way ANOVA found only significant main effects of session, $F(15, 330) = 9.35$, $p < 0.0001$ and environment, $F(1, 22) = 7.45$, $p < 0.05$. Post hoc analysis revealed that the effect of enrichment seems mainly to be driven by EC rats having a significant lower level of performance on session 3, there were no other differences between EC and IC rats during the other training session (See Fig. 1).

When looking at the effects of environmental enrichment on sensitivity to the discriminative stimulus effects of methamphetamine, a mixed factor ANOVA on the percentage of methamphetamine appropriate responding revealed there was a significant main effect of the methamphetamine dose, $F(4, 88) = 39.87$, $p < 0.001$ and a significant interaction of methamphetamine dose by environment, $F(4, 88) = 2.687$, $p < 0.05$. Post hoc comparisons indicated that at the 0.3 mg/kg dose of methamphetamine EC rats allocated significantly less responding on the methamphetamine-paired lever compared to IC rats

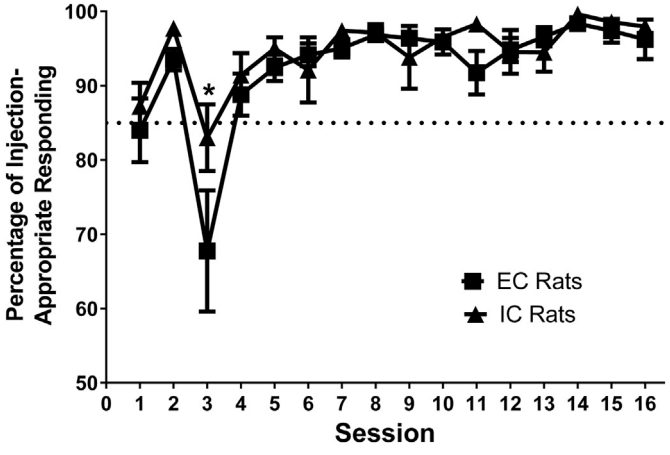


Fig. 1. The mean (± SEM) percentage of injection-appropriate responding in EC and IC rats trained to discriminate 1.0 mg/kg methamphetamine from saline across the first 16 session in which both levers were available. * indicates a significant difference compared to EC rats.

(Fig. 2A). The EC_{50} values for IC rats were 0.1995 (± 0.06) with EC rats being 0.4148 (± 0.01). An F test comparing the EC_{50} values and Hillslope indicated the EC and IC rats had significantly different models for their generalization curves. A mixed factor ANOVA on the overall rate of responding revealed a main effect of methamphetamine dose and environment, $F(4, 88) = 15.63$, $p < 0.001$ and $F(1, 22) = 6.49$, $p < 0.05$, respectively. There was also a significant interaction of methamphetamine dose by environment $F(4, 88) = 3.17$, $p < 0.05$. Post hoc test revealed that IC rats had a higher overall response rate when saline was administered and following the administration of 0.1 mg/kg dose of methamphetamine. Also, following the administration of 0.56 and 1.0 mg/kg doses of methamphetamine, overall response rates were significantly decreased in IC rats compared to the saline condition. Only the 1.0 mg/kg dose of methamphetamine significantly decreased the overall response rate in EC rats compared to saline administration (see Fig. 2B).

6.2. SCH23390 pretreatments

When testing the ability of the D_1 antagonist SCH23390 to block the discriminative stimulus effects of methamphetamine, a mixed factor ANOVA revealed there was a main effect of dose, $F(4, 88) = 16.50$, $p < 0.001$ and environment, $F(1, 22) = 11.29$, $p < 0.01$ on the percentage of methamphetamine responding. There was also a significant interaction of SCH23390 dose and environment, $F(4, 88) = 2.47$, $p \leq 0.05$. Post hoc comparisons revealed that EC rats had a greater antagonism of the discriminative stimulus effects of the training dose of methamphetamine following the two highest doses of SCH23390 compared to IC rats. While SCH23390 significantly decreased methamphetamine appropriate responding in EC rats following administration of the 0.015, 0.03 and 0.06 mg/kg doses compared to saline control injections only the 0.06 mg/kg dose significantly decreased responding in IC rats compared to saline control injections (Fig. 3A). The calculated IC_{50} values for SCH23390 in IC rats were 0.028 (± 0.01) with EC rats being 0.017 (± 0.004). An F test comparing the IC_{50} values and Hillslope indicated the EC and IC rats had significantly different models for their inhibition curves. A mixed factor ANOVA revealed there were no significant effects of SCH23390 on the overall response rates (Fig. 3B).

6.3. Eticlopride pretreatments

When looking at the antagonistic effects of the antagonist eticlopride, a mixed factor ANOVA revealed there was a main effect of eticlopride dose, $F(4, 88) = 11.70$, $p < 0.001$ on level of methamphetamine-appropriate responding. There were no significant main effects of environment or an interaction of dose and environment. Although there was a trend in IC rats showing a stronger antagonistic effect on methamphetamine-appropriate responding following administration of the 0.01 and 0.03 mg/kg doses of eticlopride (Fig. 4A). An F test comparing the IC_{50} values and Hillslope indicated the inhibition curves for EC and IC rats were not significantly different from one another. The estimated IC_{50} values for eticlopride in IC and EC rats were 0.321 (± 0.0014). A mixed factor ANOVA on the effects of eticlopride on overall response rates revealed only main effects of dose and environment, $F(4, 88) = 7.57$, $p < 0.001$ and $F(1, 21) = 5.38$, $p < 0.05$, respectively. Post hoc analysis indicated that EC rats had a lower overall response rate compared to IC rats following the administration of the 0.03 mg/kg dose of eticlopride. Also, compared to saline EC rats had a significant decreasing effect of the 0.03, 0.1 and 0.3 mg/kg doses of eticlopride, where only the 0.3 mg/kg dose of eticlopride significantly decreased responding in IC rats (Fig. 4B).

6.4. Nicotine substitution

When investigating the ability of nicotine to substitute for

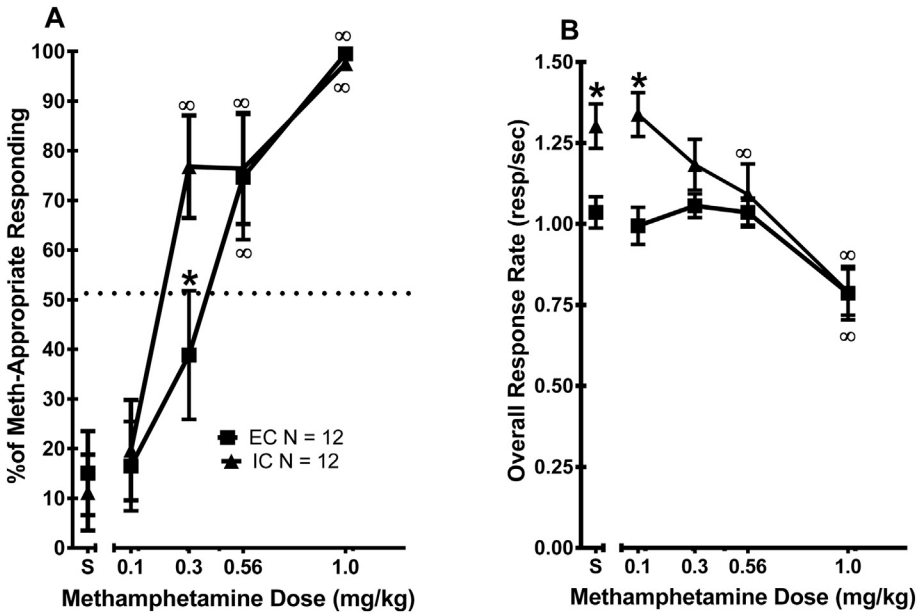


Fig. 2. A. The mean (± SEM) percentage of methamphetamine-appropriate responding in EC and IC rats trained to discriminate 1.0 mg/kg methamphetamine from saline across various doses of methamphetamine and saline (S). * indicates a significant difference compared to EC rats. ∞ indicates behavior is significantly different than saline.
B. The mean (± SEM) overall rate of responding (resp/sec) in EC and IC rats trained to discriminate 1.0 mg/kg methamphetamine from saline across various doses of methamphetamine and saline (S). * indicates a significant difference compared to EC rats. ∞ indicates behavior is significantly different than saline.

methamphetamine drug discrimination, a mixed factor ANOVA revealed there was a main effect of nicotine dose, $F(5, 110) = 19.30$, $p < 0.001$ on level of methamphetamine-appropriate responding. There were no significant main effects of environment or an interaction of nicotine dose and environment. Post hoc test revealed that nicotine resulted in partial substitution for methamphetamine in both EC and IC rats with no significant differences in between the groups. The 0.25 mg/kg dose of nicotine resulted in ~42% methamphetamine responding in EC and IC rats while higher doses significantly disrupted overall response rates (Fig. 5A). An F test comparing the EC_{50} values and Hill-slope indicated the nicotine substitution curves for EC and IC rats were not significantly different from one another. The estimated EC_{50} values for nicotine in both IC and EC rats were 0.21 (± 0.045). A mixed factor ANOVA on the effects of nicotine on overall response rates revealed only main effects of dose and environment, $F(5, 110) = 51.70$, $p < 0.001$ and $F(1, 21) = 5.077$, $p < 0.01$, respectively. Post hoc analysis indicated that IC rats tended to maintain higher rates of responding compared to EC rats (see Fig. 5B). Post hoc analysis on the effects of nicotine dose indicated that the 0.25 and 0.5 mg/kg doses of nicotine decreased responding compared to saline control injections, but only the 0.5 mg/kg dose of nicotine suppressed rates of responding below the rates maintained by the training dose of methamphetamine.

6.5. Mecamylamine pretreatment

When testing to determine if mecamylamine would antagonize the discriminative stimulus effects of methamphetamine, a mixed factor ANOVA found there were no significant effects of mecamylamine on the level of methamphetamine-appropriate responding (Fig. 6A). Given the lack of inhibition of mecamylamine, IC_{50} curves were not calculated. A mixed factor ANOVA on the effects of mecamylamine on overall response rates revealed only a main effect of mecamylamine dose, $F(3, 66) = 05.97$, $p < 0.01$. Post hoc analysis revealed that all active doses of mecamylamine decreased response rates compared to saline control injections (see Fig. 6B).

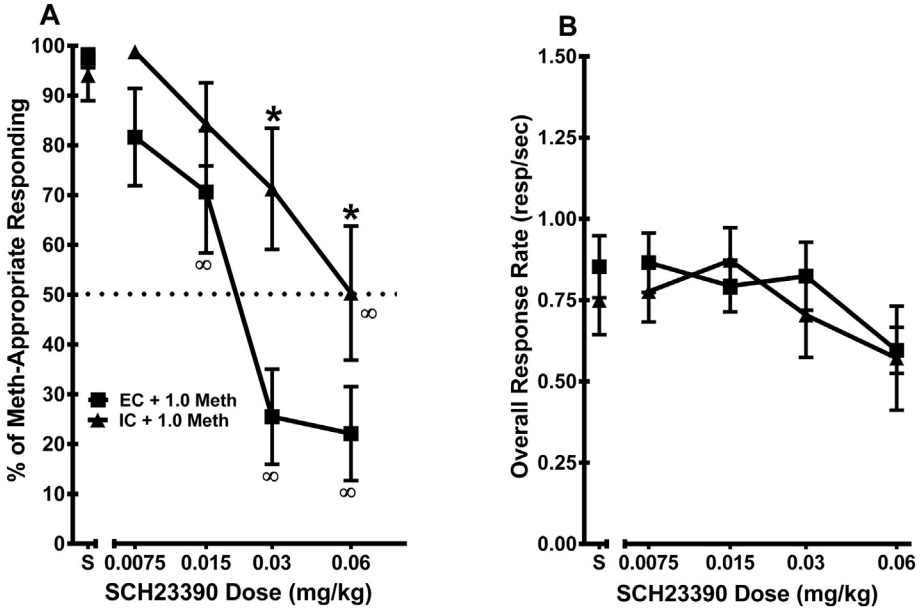


Fig. 3. A. The mean (± SEM; $N = 12$) percentage of methamphetamine-appropriate responding in EC and IC rats following pretreatment with various doses of SCH23390 and 1.0 mg/kg methamphetamine. * indicates a significant difference compared to EC rats. ∞ indicates behavior is significantly different than saline (S).
B. The mean (± SEM; N = 12) rate of responding (resp/sec) in EC and IC rats following pretreatment with various doses of SCH23390 and 1.0 mg/kg methamphetamine.

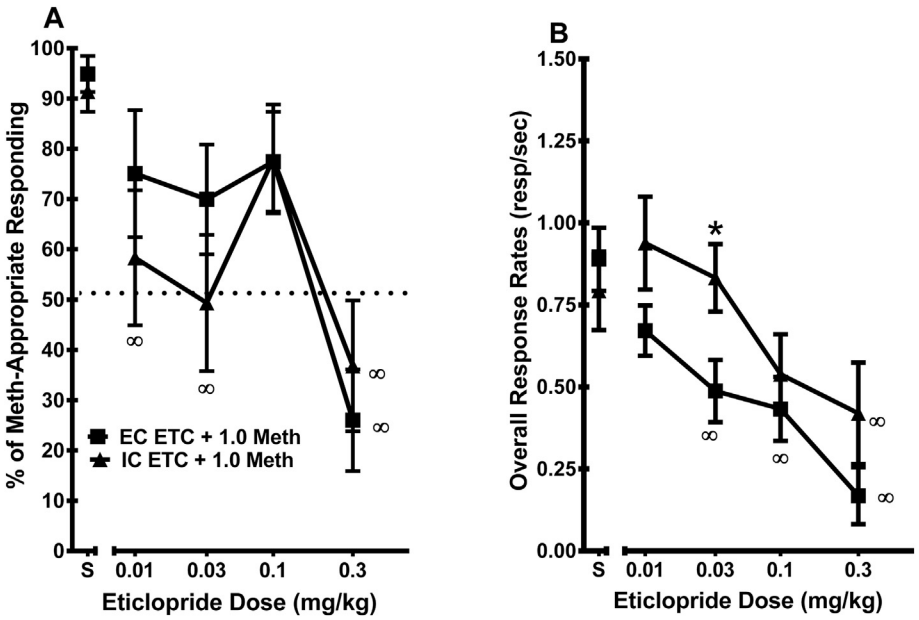


Fig. 4. A. The mean (± SEM; N = 12) Percentage of methamphetamine-appropriate responding in EC and IC rats following pretreatment with various doses of eticlopride and 1.0 mg/kg methamphetamine. ∞ indicates behavior is significantly different than saline (S).
B. The mean (± SEM; N = 12) rate of responding (resp/sec) in EC and IC rats following pretreatment with various doses of eticlopride and 1.0 mg/kg methamphetamine. * indicates a significant difference compared to EC rats. ∞ indicates behavior is significantly different than saline.

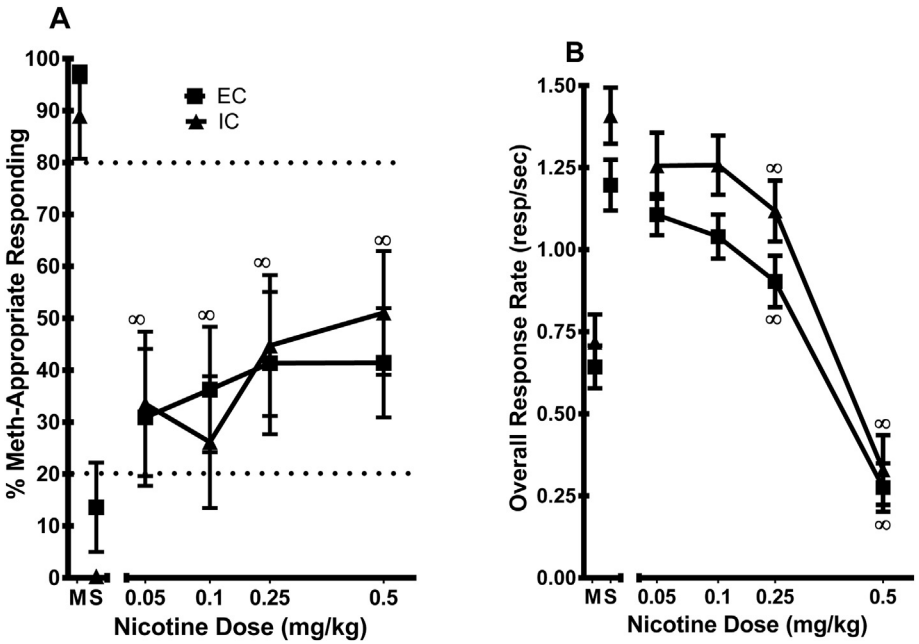


Fig. 5. A. The mean (± SEM; N = 12) Percentage of methamphetamine-appropriate responding in EC and IC rats following substitution with various doses of nicotine the 1.0 mg/kg training dose of methamphetamine (M) and saline (S). ∞ indicates behavior is significantly different than saline.
B. The mean (± SEM; N = 12) rate of responding (resp/sec) in EC and IC rats following substitution with various doses of nicotine. ∞ indicates behavior is significantly different than saline.

7. Discussion

In the current study, we found that environmental enrichment alters the discriminative stimulus effects of methamphetamine using a two-lever operant drug discrimination procedure. This was indicated by the generalization curves being different between EC and IC rats. This study also found that IC rats had a decreased sensitivity to the D_1 antagonist SCH-23390 in its ability to antagonize the discriminative stimulus effects of methamphetamine. There were no enrichment differences in the D_2 antagonist eticlopride, which had, at best, moderate antagonistic effects on the discriminative stimulus effects of methamphetamine. While EC and IC rats did not differ in acquisition of methamphetamine at the 1.0 mg/kg dose, EC rats appeared less sensitive to discriminative stimulus effects of methamphetamine compared to IC rats at the 0.3 mg/kg dose of methamphetamine. The D_1 antagonist SCH23390 showed full antagonism of methamphetamine discrimination in EC rats, but not in IC rats which only showed a ~50% reduction in methamphetamine-like responding following the highest dose of SCH-23390 tested. Both EC and IC rats showed partial generalization of nicotine to methamphetamine drug discrimination. The highest dose of nicotine tested resulted in ~ 50% methamphetamine-appropriate responding, although this dose began to have disruptive effects on overall response rate in the animals decreasing behavior below saline and methamphetamine levels. Finally, there was no significant antagonistic effects of the nicotinic antagonist mecamylamine on the training dose of methamphetamine in nether EC or IC rats.

These results are congruent with a study done by Fowler et al. (1993) which showed IC rats are more sensitive to the discriminative stimulus of cocaine. Results are also congruent with previous literature stating IC rats are more sensitive to low doses of amphetamine and cocaine (Bardo et al., 2001; Green et al., 2002; Howes et al., 2000) compared to EC rats in self-administration paradigms. The effects of enrichment of the on the methamphetamine generalization curve are similar but less pronounced than a previous study from our lab looking

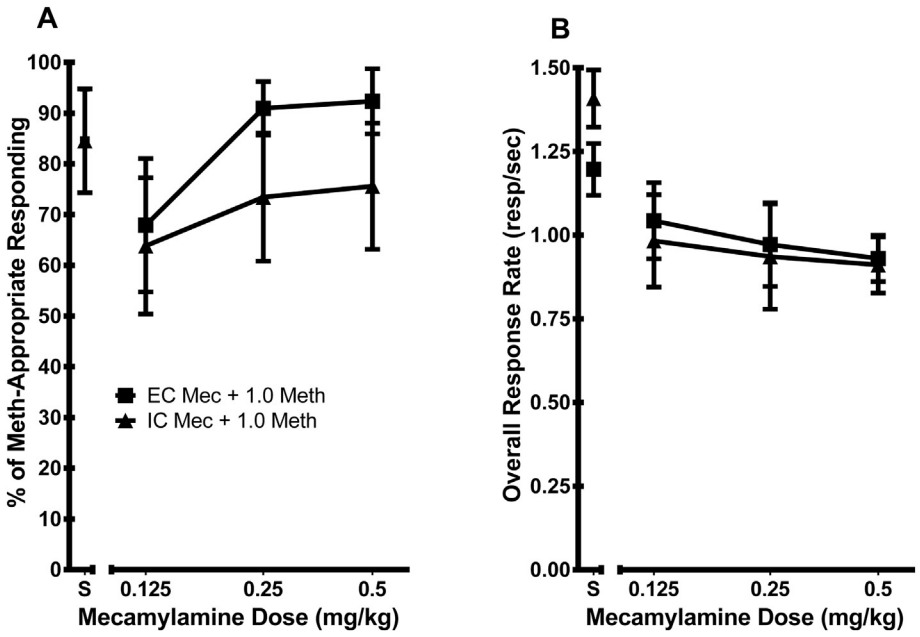


Fig. 6. A. The mean (± SEM; N = 12) Percentage of methamphetamine-appropriate responding in EC and IC rats following pretreatment with various doses of mecamylamine or saline (S) and 1.0 mg/kg methamphetamine.
B. The mean (± SEM; N = 12) rate of responding (resp/sec) in EC and IC rats following pretreatment with various doses of mecamylamine and 1.0 mg/kg methamphetamine.

at nicotine drug discrimination (Bockman et al., 2018). The significant effects of enrichment on the methamphetamine generalization curve is inconsistent with the absence of an effect of enrichment on both the methamphetamine CPP response (Gehrke et al., 2006; Hofford et al., 2014; Thiriet et al., 2011) and methamphetamine self-administration (Hofford et al., 2014).

The effects of the dopamine antagonists on methamphetamine drug discrimination in the current study are supported by previous data investigating the neural mechanisms of methamphetamine drug discrimination. Previous research has shown that dopamine function at the dopamine uptake protein and the D_1 and D_2 receptors mediate the majority of the discriminative stimulus effects of methamphetamine (Munzar et al., 1999a; Munzar and Goldberg, 2000; Sasaki et al., 1995) there is also modulatory roles of 5HT, NE, GABA and adenosine (Gatch et al., 2005; Munzar, Baumann, et al., 1999; Munzar and Goldberg, 1999; Munzar et al., 2002). The effects of the D_1 antagonist SCH23390 showed complete antagonist effects of methamphetamine particularly in EC rats, although there was only a ~50% antagonism in IC rats with the highest dose tested. The full antagonism seen in EC rats is congruent with what has been shown previously in rats (Munzar and Goldberg, 2000). While the D_2 antagonist eticlopride showed only partial antagonism at doses that did not significantly disrupt overall response rates in both EC and IC rats. Previous research with the D_2 antagonist spiperone found a complete blockade of the 1.0 mg/kg training dose of methamphetamine (Martelle and Nader, 2008). The difference in effects may be due to either differences in housing of the animals in the previous study or in differences in selectivity of eticlopride for the D_2 receptor vs spiperone having activity at the adrenergic receptors (Laurila et al., 2011) and serotonergic receptors (Geerts et al., 1999; Vhora and Chiba, 1994). Spiperone may be having greater antagonistic effects on methamphetamine drug discrimination by having activity at NE and 5HT receptor which have been shown to have modulatory effects on methamphetamine drug discrimination.

There was also no effect of environmental enrichment on the ability of nicotine to substitute for the discriminative stimulus effects of methamphetamine. Both EC and IC rats showed only a partial substitution of nicotine for methamphetamine drug discrimination. The ability for nicotine to partially substituted for methamphetamine in the current study is congruent with a previous study by Gatch et al. (2008). Gatch et al. (2008) found that doses of nicotine that did not disrupt response rates had a maximum substitution in methamphetamine of ~60% methamphetamine responding, while higher doses increased methamphetamine-lever responding but significantly decreased response rates. The partial substitution effects of nicotine in EC and IC rats is somewhat incongruent with Desai and Bergman (2010) which found full substitution of nicotine in rats that were trained to discriminate 0.3 mg/kg *d*-methamphetamine from saline. The differences between the two studies are most likely due to different training doses of methamphetamine and procedural differences, for instance Desai and Bergman administered the nicotine doses i.p. and used a within session cumulative dosing procedure compared to our s.c. nicotine injections and between session nicotine dose effect curve.

Finally, mecamylamine failed to antagonize the training dose of methamphetamine at any dose tested in both EC and IC rats. This is consistent with Gatch et al., (2008) which found mecamylamine did not antagonize the effects of a 1.0 mg/kg training dose of methamphetamine. Although again Desai and Bergman (2010) found with a lower training dose of methamphetamine and a within session cumulative dosing regimen of mecamylamine a significant antagonism of the discriminative stimulus effects of methamphetamine. These inconsistent effects of nicotinic compounds to fully substitute or antagonize the discriminative effects of methamphetamine appear most likely to be due to differences in training dose. Perhaps role of the nicotinic receptor is more pronounced with a lower training dose of methamphetamine, and when higher training doses are used the nicotinic receptor has less of a moderating effect on methamphetamine drug discrimination.

Given the greater EC_{50} value in the generalization curve of methamphetamine in IC rats compared to EC rats and greater IC_{50} value of the D_1 dopamine receptor antagonist SCH23390 in IC rats compared to EC rats, it seems plausible that IC rats show a greater sensitivity to the discriminative stimulus effects of methamphetamine because of changes in the D_1 receptor. While an earlier study using a similar enrichment model to the one used in the current study found no differences in D_1 or D_2 receptors (Bardo and Hammer Jr., 1991), a more recent study has found a significant difference in the density of D_1 receptors between EC and IC rats (Gill et al., 2013). Gill et al. (2013) found that rats raised in social isolation have increases densities in D_1 receptors in various striatal areas compared to enriched counterparts, while they found no reported differences in densities of the D_2 receptors between enriched and isolated rats. This same effect of isolation has also been found in mice (Gariepy et al., 1995). These data could in part

explain the increased sensitivity to the discriminative stimulus effects of methamphetamine and the decreased sensitivity to the antagonistic effects of SCH23390 in IC rats. That is, if IC rats have a greater density of D_1 receptors in critical limbic structures they could show stronger discriminative effects of methamphetamine compared to EC rats as well as a weaker antagonist effect of an equivalent dose of SCH23390. This explanation is congruent with findings from our laboratory that looked at nicotine drug discrimination in EC and IC rats in which the antagonist mecamylamine and higher densities of nicotinic receptors in the ventral tegmental area in IC rats produced similar effects (Bockman et al., 2018). While differences in D_1 densities is one potential neural mechanism, there are numerous functional differences in the dopaminergic systems between EC and IC rats (see (Stairs and Bardo, 2009) for a review) that could also account for the differences seen between EC and IC rats in the current study.

While the current study found that EC rats have a decreased sensitivity to methamphetamine and an increased sensitivity of D_1 antagonist to block the discriminative stimulus effects of methamphetamine, there are some limitations in the interpretation of our results given the study design. One limitation of the current study is that the methamphetamine generalization curve was not re-determined at the completion of the various pretreatments. This decreases our confidence in the consistency of enrichment differences seen between EC and IC rats in methamphetamine generalization. A second limitation to the current study is that only a single more moderate training dose of methamphetamine was used in the current study. With the use of a moderate training dose of methamphetamine we were not able to see clear differences in the sensitivity to the acquisition of methamphetamine drug discrimination in EC and IC rats. In future studies we could strengthen the argument that IC rats are more sensitive to the discriminative stimulus effects of methamphetamine by looking at rates of acquisition using lower methamphetamine training doses in both EC and IC rats.

Despite these limitations, the results from the current study suggests that there are differences in high and low sensation seekers and their sensitivity to psychostimulant drugs. To the degree that the IC rats are a rodent model of high sensation seeking, the current data may indicate that the increased vulnerability to methamphetamine use disorder in individuals with high sensation seeking personality trait may be mediated through differential sensitivity to the subjective effects of methamphetamine due to differences in D_1 receptors. While much of the research looking a dopaminergic explanation for sensation seeking has focused on the D_2 receptor (Norbury and Husain, 2015), little research in humans has looked at D_1-like receptors. A previous study investigating the subjective effects of d-amphetamine in high and low sensation seekers, found that high sensations seekers reported higher levels of subjective effects of “liking the drug” and “high” compared to low sensation seekers, although no neural explanation was explored (Kelly et al., 2006). One study looking at alcohol dependent humans did find an association between the D_1 receptor gene *Dde*I polymorphism and sensation-seeking scores in alcohol-dependent males (Limosin et al., 2003). Further investigating the role of the D_1 receptor in sensation-seeking in humans and in the rodent environmental enrichment model could hopefully lead to better individualized pharmacological treatments for psychostimulant addiction.

Acknowledgments

The authors would like to thank Brittany BaDour, Tyson Hickle, and Bard Hovenga for assisting in the collection of the data.

Funding

The authors would like to thank generous support from the Creighton College of Arts and Science. Finally, the project described was also supported by Grant Number G20RR024001 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

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