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Differences in extinction responding and reinstatement of methamphetamine-seeking behavior between Fischer 344 and Lewis rats

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Abstract

Fischer 344 (F344) and Lewis (LEW) rats differ in a number of self-administration behaviors. Whether or not these strains differ in methamphetamine-primed reinstatement of extinguished responding is unknown. F344 and LEW rats were trained to self-administer intravenous (i.v.) methamphetamine (0.06 mg/kg) during daily 2-h limited access sessions for 14 days. Following methamphetamine self-administration, subjects underwent a minimum of 6 extinction sessions where responding on the previously active lever resulted in no programmed consequences. Following extinction sessions, we evaluated strain and dose dependency of methamphetamine-primed (0.06, 0.12, or 0.24 mg/kg/i.v.) reinstatement of responding. All subjects received each dose once. Dosing order was determined by utilizing a within-subjects Latin square design. We found partial strain differences in daily methamphetamine self-administration. In addition, F344 rats responded significantly more during the first extinction session compared LEW rats. Last, the LEW rats demonstrated a heightened propensity to reinstate responding following methamphetamine priming injections compared to F344 rats. Our results suggest that genetic background influences differences in methamphetamine-seeking behaviors in rats.

Keywords: Methamphetamine; Self-administration; Inbred rats; Fischer 344; Lewis; Rat; Extinction; Reinstatement

1. Introduction

A popular rodent model of psychostimulant relapse is the drug-primed reinstatement procedure (de Wit and Stewart, 1981; Kalivas and McFarland, 2003; Shaham et al., 2003). The increased use of drug-primed reinstatement procedures has greatly enhanced the understanding of mechanisms underlying drug-induced neuroplasticity and drug-seeking behavior following extinction/withdrawal (Kalivas et al., 2005). Genetic/individual differences have also been found to influence the transition from drug use to drug addiction and subsequently relapse (Tsuang et al., 1999).

Investigation of individual differences in the susceptibility to reinstate cocaine-seeking behaviors in preclinical models is experiencing increased interest (Homberg et al., 2004; Kruzich et al., 1999). These previous studies used outbred strains of rats. Another approach for understanding individual differences that influence susceptibility to reinstate extinguished drug-seeking behaviors is the utilization of inbred strains or genetic models (Crabbe and Phillips, 2004). Inbred strains are useful for determining genetic influences on behavior because their genes are homozygous at every allele. Identified strain differences in a particular behavior can be attributed to genetic influences when the experimental manipulations (e.g. dosing) are equal. Also, any differences seen within an inbred strain can be attributed to environmental/pharmacological manipulations (Crabbe and Phillips, 2004).

Previous studies have used inbred strains to evaluate the role of genetics in the acquisition and maintenance of cocaine and morphine self-administration by rats (reviewed by Kosten and Ambrosio, 2002). However, only recently have inbred strains of rats been utilized to investigate drug-primed reinstatement (Kruzich and Xi, 2006). We recently demonstrated that Lewis (LEW) rats show a heightened sensitivity to cocaine-primed reinstatement of cocaine-seeking behavior, and a blunted response to AMPA-stimulated cocaine-seeking behavior following extinction compared to Fischer 344 (F344) rats (Kruzich

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and Xi, 2006). Other related studies have shown that LEW rats demonstrate an augmented response to methamphetamine-induced stereotypy compared to F344 rats (Camp et al., 1994) and this increased sensitivity is not predicted by their responses to cocaine (Haile et al., 2001; Sircar and Kim, 1999). Possible differences in sensitivity and behavioral output between these two strains in extinction/reinstatement models of methamphetamine-seeking behavior are unknown. The purpose of the present study was to determine if F344 and LEW rats differ in methamphetamine self-administration behaviors (maintenance and extinction) and methamphetamine-primed reinstatement of previously extinguished responding.

2. Methods

2.1. Subjects

Twelve F344 and fourteen LEW rats (Harlan, Indianapolis, IN) weighing 250–300 g upon arrival were used. Rats were singly housed and maintained in a humidity and temperature controlled vivarium on a 12/12 h light/dark cycle (lights on at 0700 h) with free access to food and water, except during food-reinforced lever training, where they were maintained at ~90% of their free-feeding weights. All rats were habituated to the vivarium and handled daily by the experimenters for 7 days before initiating the self-administration studies. All protocols were approved by the Institutional Animal Care and Use Committee at the Medical College of Georgia, and were in compliance with "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2003).

2.2. Drugs

Methamphetamine HCl (methamphetamine; Sigma, St. Louis, MO) was dissolved in sterile isotonic saline and 0.2-μm filtered. The infusion bolus for methamphetamine was 0.05 ml. Pentobarbital sodium (50.0 mg/ml; Ovation Pharmaceuticals, Deerfield, IL) was infused in an intravenous (iv.). bolus of 0.1 ml to determine catheter patency in animals demonstrating erratic self-administration or drug-primed reinstatement behavior. Administration of this concentration results in immediate loss of righting reflex that is restored after approximately 10 min.

2.3. Apparatus

Lever training and methamphetamine self-administration experiments were conducted in 8 standard operant conditioning chambers (Coulbourn Instruments, Allentown, PA). Each chamber was housed within a sound-attenuated cubicle. The chambers were affixed with two retractable levers, a pellet hopper, and a house light located outside the chamber. Intravenous methamphetamine was delivered through one channel of 2-channel liquid swivels (Instech, Plymouth Meeting, PA) by selectable speed infusion pumps (model A73-02-SEL, Razel Scientific Instruments, St. Albans, VT). The behavioral programs, pumps, and data collection were controlled and collected by a PC clone computer (Colbalt, Allentown, PA) that ran Graphic State Notation 3.0 software (Coulbourn Instruments, Allentown, PA).

2.4. Lever training

Rats were diet restricted to ~90% of their free-feeding weights and trained to lever press for 45 mg food pellets (Formula A/I, Research Diets, New Brunswick, NJ) during daily 1-h sessions for 5 days. Each lever press on the right lever was reinforced along a continuous reinforcement schedule. Responding on the left lever resulted in no programmed consequences, but was recorded. Following lever training, the metal food hoppers/magazines in the experimental chambers were removed and replaced with a metal plate in order to remove as many food-associated cues as possible.

2.5. Surgery

Rats were implanted in the jugular vein with chronic indwelling Silastic® catheters according to methods described in full elsewhere (Kruzich and Xi, 2006). Briefly, animals were anesthetized with a combination of 90.0 mg/kg ketamine and 2.0 mg/kg xylazine (F344 rats) or 72.0 mg/kg ketamine and 1.6 mg/kg xylazine (LEW rats). Different anesthesia regimens were used because LEW rats experience significant cardiovascular depression following the dose used for F344 rats. Following catheter implantation, animals were allowed 7 days for recovery. Catheters were flushed daily during surgical recovery by administering 0.1 ml of 100 U/ml heparinized saline.

2.6. Methamphetamine self-administration, extinction, and reinstatement

2.6.1. Methamphetamine self-administration

After recovery from surgery, rats received daily limited access to methamphetamine during 2-h self-administration sessions 7 days a week. Lever pressing on the right lever was reinforced according to a fixed ratio-1 (FR-1) schedule of reinforcement followed by a 10-s timeout period. A reinforced response resulted in a 5-s infusion of methamphetamine (0.06 mg/kg/i.v. in a volume of 0.05 ml) plus an additional 5 s of timeout. An infusion was signaled by illumination of a 3-light cue over the active lever and delivery of a 4 kHz 80 dB tone. Responding during the infusion or timeout resulted in no programmed consequences, but was recorded. The first reinforced infusion lasted 9-s (total volume ~0.1 ml) plus a 1-s time out, in order to account for the dead volume of the catheter and to avoid passively infusing drugs to load catheters at the beginning of the self-administration sessions. All rats self-administered methamphetamine for a minimum of 14 sessions. Our criteria for successful self-administration were: 14 completed sessions, at least 5 self-administered infusions/day in the final 10 of the 14 sessions, and the number of self-administered infusions could not vary by over 20% in the final 3 self-

administration sessions. The catheters were flushed immediately after the self-administration session with 0.1 ml of the 100 U/ml heparinized saline solution. Rats were returned to their home cages following the 2-h sessions where they received free-access to food and water.

2.6.2. Extinction

Following 14 days of methamphetamine self-administration, rats underwent extinction sessions. During a daily 2-h extinction session, responses on either lever resulted in no programmed consequences, but were recorded. Subsequent to a minimum of 6 extinction sessions (responding had to be at or below 50% of self-administration levels), animals began the methamphetamine-primed reinstatement phase of the study.

2.6.3. Methamphetamine-induced reinstatement of previously extinguished responding

Rats received passively infused methamphetamine (0.0, 0.06, 0.12, or 0.24 mg/kg/i.v.) at the beginning of separate 2-h test sessions. For the remainder of the session, responding on either lever resulted in no programmed consequences. The primes were not signaled by any programmed cues. The test sessions were separated by at least 1-extinction session (response levels were typically ≤ 10% of maintenance response levels during the intervening extinction sessions). Each dose was delivered once and dosing order was determined according to a counterbalanced Latin squares design. Methamphetamine was delivered in a 0.05-ml i.v. bolus 1 min after subjects were placed into the chambers. The infusions were separated by 1 min for the 0.12 (2 infusions) and 0.24 (4 infusions) mg/kg/i.v. tests. The methamphetamine prime was delivered intravenously to avoid introducing additional extraneous variables such as route of administration (e.g. i.p. injections) and because we have previously validated this method for reinstating extinguished responding in F344 and LEW rats (Kruzich and Xi, 2006). Catheter patency was verified after the test sessions by drawing blood from the catheter. If no blood was drawn, 5.0 mg of pentobarbital sodium was administered to the rat.

2.7. Data analysis

Self-administered methamphetamine infusions (strain × session), responding during extinction (strain × session) and responding during methamphetamine-priming tests (strain × dose) were analyzed with separate 2-way repeated measures analysis of variance (RM-ANOVA) tests. If a significant RM-ANOVA was determined, post-hoc comparisons utilizing the Student–Newman–Keuls test were performed. Significance was set at $p<0.05$.

3. Results

3.1. Methamphetamine self-administration

There was a significant effect of strain on methamphetamine self-administration ($F(1,24)=4.3$; $p=0.043$; Fig. 1, top). There was a significant trend of "session" ($F(13,312)=1.7$; $p=0.053$).

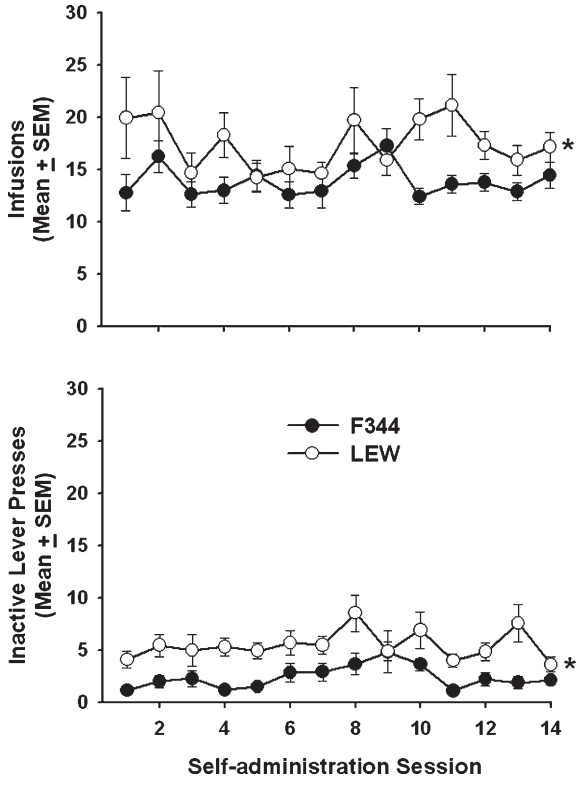

Fig. 1. Methamphetamine self-administration in F344 and LEW rats. Top: Reinforced responses by F344 and LEW rats. The LEW rats earned significantly more methamphetamine infusions than F344 rats (*$p<0.05$), and this level of intake was not significantly influenced by session ($p>0.05$). Bottom: Responses on the inactive lever. The LEW rats demonstrated significantly elevated responding on the inactive (left) lever compared to F344 rats during methamphetamine self-administration sessions (*$p<0.05$).

There was not a significant strain × session interaction ($F(13,312)=1.4$; $p>0.17$). The strains demonstrated significant differences in responding on the inactive lever ($F(1,24)=17.46$; $p<0.001$; Fig. 1, bottom). There was a significant effect of "session" on inactive lever pressing ($F(13,312)=2.52$; $p<0.01$). Post-hoc comparisons revealed that inactive lever responding on self-administration day 8 was significantly greater than responding days 1, 11, and 14 ($p<0.05$ for all comparisons). There was not a significant strain × session interaction ($F(13,312)=1.03$; $p=0.4$).

3.2. Extinction

While there was not a significant strain difference in extinction responding on the formerly active (right) lever ($F(1,24)=1.1$; $p=0.31$; Fig. 2, top), there was a significant effect of session ($F(5,120)=36.39$; $p<0.001$) and a significant strain × session interaction ($F(5,120)=7.53$; $p<0.001$; Fig. 2). The F344 rats emitted significantly more responses during the first extinction session compared to the LEW rats ($p<0.05$). Both strains demonstrated significant decreases in responding during sessions 2–6 versus the first extinction session ($p<0.05$ for all comparisons). There was not a significant effect of "strain" on inactive (left) lever responding during extinction sessions ($F(1,24)=2.26$; $p=0.15$; Fig. 2, bottom). There were

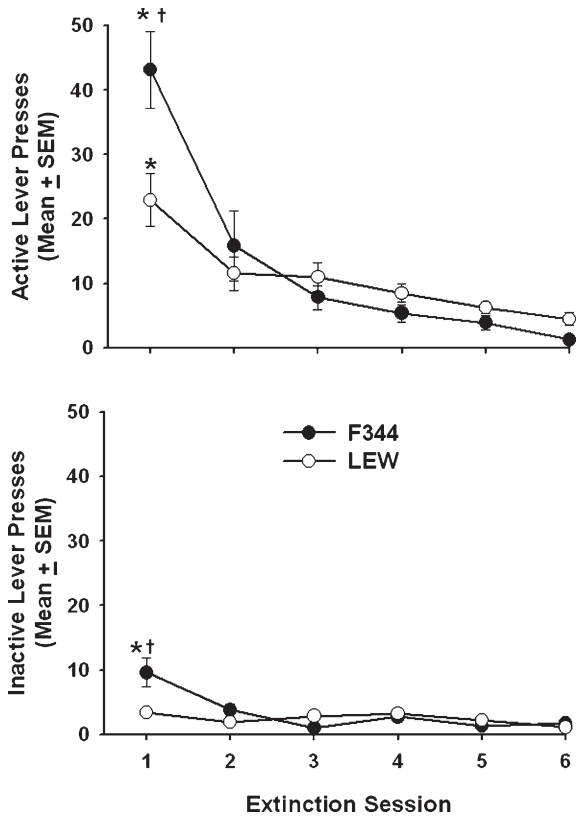

Fig. 2. Extinction responding in F344 and LEW rats. Top: Both strains significantly reduced their response output on the formerly active lever during sessions 2–6 of extinction vs. session 1 (*$p<0.05$). F344 rats engaged in significantly greater methamphetamine-seeking behavior than LEW rats during the first extinction session ($^{\dagger}p<0.05$). Bottom: Responses on the inactive (left) lever during extinction sessions. Responding significantly decreased after session 1 of extinction (*$p<0.05$). The number of responses emitted by the F344 rats during extinction session 1 was significantly greater than responses emitted by LEW rats ($^{\dagger}p<0.05$). However, the LEW rats maintained the same pattern of responding on the inactive lever during extinction which did not vary as a function of session.

significant differences in responding across the 6 extinction sessions ($F(5,120)=13.12$; $p<0.001$). Post-hoc comparisons demonstrated that responding on the left lever significantly declined following session 1 for the F344 rats. Responding on the left lever during extinction was not influenced by session in the LEW rats. There was a significant strain × session interaction ($F(5,120)=7.97$; $p<0.001$). The F344 rats emitted significantly more responses on the left lever than the LEW rats during extinction session 1 ($p<0.05$).

3.3. Methamphetamine-induced reinstatement

Two of the original LEW rats were excluded from analysis of methamphetamine-induced reinstatement due to catheter failure during the methamphetamine-primed reinstatement phase. All 12 of the F344 rats' data were used for the methamphetamine-primed reinstatement tests. There was a significant effect of strain for responding on the previously active (right) lever ($F(1,22)=9.0$; $p<0.01$) on methamphetamine-primed reinstatement (Fig. 3, top). A significant effect of dose was also determined ($F(3,66)=19.3$; $p<0.001$). Post-hoc tests revealed that the 0.06, 0.12, and 0.24 mg/kg/i.v. doses significantly reinstated lever pressing relative to vehicle ($p<0.05$ for all comparisons). The 0.24 mg/kg/i.v. prime evoked significantly more responding in both strains compared to all other doses tested ($p<0.05$ for all comparisons). There was not a significant strain × dose interaction ($F(3,66)=1.4$; $p>0.2$). Responding on the inactive (left) lever differed by strain during the methamphetamine reinstatement sessions ($F(1,22)=8.46$; $p<0.01$; Fig. 3, bottom). However, there was not a significant effect of dose on methamphetamine induced responding ($F(3,66)=1.40$; $p=0.25$) nor a significant dose × session interaction ($F(3,66)=1.84$; $p=0.15$) for left lever responding.

4. Discussion

These results are the first demonstration that F344 and LEW rats differ in: 1) extinction behavior following methamphetamine self-administration and 2) methamphetamine-primed reinstatement of methamphetamine-seeking behavior. While the F344 rats demonstrated significantly higher levels of responding during the first extinction session, the LEW rats

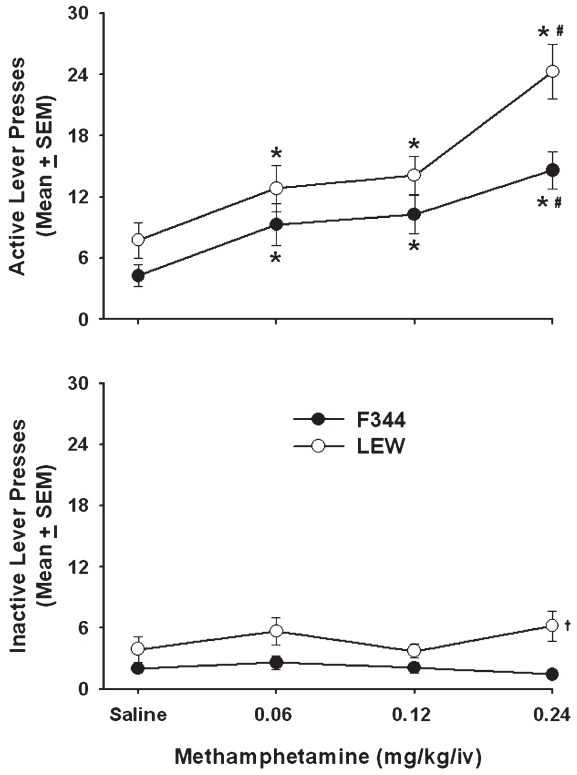

Fig. 3. Methamphetamine-primed reinstatement of previously extinguished methamphetamine-seeking behavior in F344 and LEW rats. Top: Methamphetamine priming reinstated significant unreinforced responding on the formerly active lever in both strains compared to saline (*$p<0.05$). The 0.24 mg/kg/i.v. dose evoked the highest level of responding in both strains ($^{\#}p<0.05$ for all comparisons). Bottom: Effects of methamphetamine priming on the inactive (left) lever. The LEW rats emitted more responses on the left lever during the methamphetamine reinstatement sessions compared to the F344 rats ($^{\dagger}p<0.05$). Response levels on the left lever did not vary as a function of dose in the F344 rats ($p>0.05$).

showed the highest behavioral output during the methamphetamine-primed reinstatement tests.

No previously published reports investigating methamphetamine self-administration in F344 and LEW rats exist. A number of studies have investigated strain differences in behavioral sensitization to methamphetamine (Camp et al., 1994; Yoshida et al., 1998). The prevailing findings in those reports were: LEW rats are more sensitive to methamphetamine-induced stereotypy and locomotion (Camp et al., 1994; Yoshida et al., 1998), and F344 rats develop methamphetamine-altered cliff avoidance behaviors, whereas LEW rats do not (Yoshida et al., 1998). Both strains readily self-administered methamphetamine in the current study (Fig. 1, top). The strain-dependent differences in intake did not appear to be systematic or robust. Therefore, we are hesitant to conclude that the consumption differences found in the present study are significantly influenced by strain. Future studies examining potential strain differences in the reinforcement efficacy of methamphetamine with progressive ratio schedules of reinforcement should be performed. A comprehensive investigation of different maintenance doses was beyond the scope of this study (extinction and reinstatement).

The extinction response patterns found here compliment our earlier studies where F344 and LEW rats self-administered cocaine and then underwent extinction (Kruzich and Xi, 2006). As was found in our earlier study, F344 rats displayed significantly greater behavioral output during the first extinction session compared to LEW rats. A previous study where F344 and LEW rats underwent two extinction sessions following cocaine self-administration reported no strain differences in extinction responding (Kosten et al., 1997). However, in that study, a passive infusion of cocaine was intravenously delivered at the beginning of both extinction sessions. The dose of the passive infusion was comparable to the amount delivered during a single reinforced lever press. In the present study, subjects did not receive infusions of drugs during extinction sessions, and we measured extinction responding over 6 days (Fig. 2). Possibly, these differences in extinction procedures account at least in part for disparate results between the previous Kosten et al. (1997) report and the present study.

To our knowledge, this is the first report investigating differences in methamphetamine-induced reinstatement of responding in LEW and F344 rats. The LEW rats demonstrated increased lever-pressing behavior compared to F344 rats following intravenous priming infusions with methamphetamine (Fig. 3, top). The response output between the strains increased as a function of dose following the methamphetamine challenges. Our results compliment methamphetamine behavioral sensitization studies demonstrating that LEW rats display greater methamphetamine-induced alterations of locomotor behavior compared to F344 rats (Camp et al., 1994; Yoshida et al., 1998). Our findings provide further evidence that genetic background influences several methamphetamine-mediated behaviors. These differences in sensitivity to methamphetamine displayed by F344 and LEW rats were not predicted by their previously established responses to cocaine (Camp et al., 1994; Kruzich and Xi, 2006).

In conclusion, significant differences in extinction/reinstatement behaviors between F344 and LEW rats were found in the present study. The use of F344 and LEW rats to understand methamphetamine-primed reinstatement may serve as a unique and powerful tool for investigating the mechanisms underlying individual differences influencing susceptibility for reinstatement following periods of withdrawal.

Acknowledgements

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