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Black agouti (ACI) rats show greater drug- and cue-induced reinstatement of methamphetamine-seeking behavior than Fischer 344 and Lewis rats

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Abstract

Fischer 344 (F344) and Lewis (LEW) rats differ in methamphetamine self-administration (SA) and methamphetamine-induced reinstatement of previously extinguished behavior. We sought to determine whether genetic background also influences methamphetamine reinforcement efficacy, conditioned reinstatement, and methamphetamine-primed reinstatement of responding in F344, LEW, and Black Agouti (ACI) rats. We implanted rats with jugular catheters and trained them to self-administer methamphetamine (0.06 mg/kg/infusion) under a progressive ratio (PR) schedule of reinforcement during daily 2-h SA sessions. A compound stimulus (light+tone; LT) was paired with each infusion. Dose-dependent intake was determined for each rat. Rats then entered the extinction phase of the experiment where responding resulted in no programmed consequences. Following extinction sessions, rats underwent conditioned reinstatement testing. For conditioned reinstatement, rats received response-contingent presentations of the LT and no methamphetamine. Last, methamphetamine-primed reinstatement test sessions where conducted where subjects received experimenter delivered infusions of methamphetamine (0.06, 0.12, or 0.24 mg/kg). The strains did not differ in PR responding across the doses tested. The ACI rats demonstrated the highest behavioral output during extinction training, conditioned- and methamphetamine-primed reinstatement of previously extinguished behavior compared to the other strains. These data suggest that genetic background differentially influences extinction, conditioned reinstatement and methamphetamine-primed reinstatement in rats.

Keywords: Rat; Self-administration; Methamphetamine; Genetics; Relapse

1. Introduction

The prevalence of methamphetamine abuse and subsequent addiction is rapidly increasing in North America (SAMSHA, 2004). While environment undoubtedly contributes to access and prevalence of methamphetamine abuse, the contribution of genetic background may also influence methamphetamine addiction. In humans, genetics strongly influences the transition from drug use to dependence (Tsuang et al., 1999). Additionally, animal models of methamphetamine abuse demonstrated that inbred strains of rats (Camp et al., 1994; Kruzich and Xi, 2006b) differ in behavioral responses to methamphetamine. Specifically, LEW rats demonstrate a heightened sensitivity to methamphetamine-induced stereotypy than F344 rats (Camp et al., 1994). Moreover, F344 rats show increased locomotor activity compared to LEW rats when placed in a novel and inescapable environment, and show increased locomotor activation following administration of a dose amphetamine not associated with inducing stereotypy (Miserendino et al., 2003).

We previously reported strain differences in the acute reinforcing characteristics, patterns of extinction responding, and methamphetamine-primed reinstatement of previously extinguished behavior between Fischer 344 (F344) and Lewis (LEW) rats (Kruzich and Xi, 2006b). LEW rats more readily self-administer methamphetamine along a continuous schedule of reinforcement than F344 rats (Kruzich and Xi, 2006b). LEW rats also show a greater propensity to reinstate previously extinguished responding following methamphetamine primes (Kruzich and Xi, 2006b). However, this study did not determine whether strain differences in responding for methamphetamine are influenced by reinforcement efficacy—how much work the

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particular strain is willing to perform to receive methamphetamine (Hodos, 1961). Also, we did not assess the ability of visual and auditory stimuli to acquire secondary reinforcing characteristics by pairing them with methamphetamine infusions. This is important to determine because humans frequently relapse following periods of abstinence when confronted with cues associated with drug self-administration (Childress et al., 1999; Shalev et al., 2002). Last, only 2 strains were compared. Use of extinction, conditioned reinstatement, and drug-primed reinstatement methods provide useful information regarding the persistence of drug-seeking behavior in the absence of drug, learned associations with drugs, and susceptibility to relapse following ingestion of small doses of drug relative to what was typically self-administered, respectively. While the validity of extinction/reinstatement procedures in rats is currently experiencing significant debate (see Epstein et al., 2006 and Katz and Higgins, 2003 for review), all are useful for determining the effects of genetics and environment on drug-seeking behaviors.

The purpose of the present study was to determine the role of genetic background in: methamphetamine self-administration under a progressive ratio schedule of reinforcement, extinction responding, conditioned reinstatement, and methamphetamine-primed reinstatement in F344, LEW, and ACI rats. We chose F344 and LEW rats based on previously published reports with these strains showing different responses to methamphetamine (Camp et al., 1994), and our previous report demonstrating that LEW rats show heightened self-administration and methamphetamine primed reinstatement behavior compared to F344 rats (Kruzich and Xi, 2006b). The ACI rats were chosen especially due to the polymorphism found on the CYP2D1 gene in this strain, which contributes to a "poor metabolizers of methamphetamine" phenotype for these rats (Vorhees et al., 1998). This P450 enzyme is responsible for the metabolism of methamphetamine and a number of other psychotropic drugs (Vorhees et al., 1998). We therefore sought to test the hypothesis that genetic background differentially influences several methamphetamine-seeking behaviors in inbred rats.

2. Methods

2.1. Subjects

Eleven Fischer 344 (F344), 14 Lewis (LEW), and 8 Black Agouti (ACI) rats (Harlan, Indianapolis, IN) weighing 250–300 g upon arrival were used in this study. Rats were individually housed and maintained in a 12/12 h light/dark cycle (lights on 0700 h). Rats received ad libitum access to tap water and standard rodent chow. All of our protocols were approved by the Institutional Animal Care and Use Committee at the Medical College of Georgia, and complied 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 saline and filtered (0.2 μm). The infusion bolus was 0.05 ml. Pentobarbital sodium (50 mg/ml; Ovation Pharmaceuticals, Deerfield, IL) was intravenously infused in a bolus of 0.1 ml (5-mg total dose) to determine catheter patency in animals demonstrating irregular self-administration or drug-primed reinstatement behavior. Intravenous (iv) administration of this concentration of pentobarbital induces loss of righting reflex that recovers after approximately 10 min.

2.3. Apparatus

All experiments were conducted in 16 identical operant conditioning chambers (Coulbourn Instruments, Allentown, PA). The chambers were housed in sound-attenuated cubicles. The chambers contained two retractable levers, a pellet hopper, and a house light located outside the chamber. Intravenous methamphetamine was delivered through 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 by a PC clone computer (Colbalt, Allentown, PA) that ran Graphic State Notation 3.0 software (Coulbourn Instruments, Allentown, PA).

2.4. Procedures

2.4.1. Lever training

Rats were food 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. Lever presses on the right lever were reinforced along a continuous reinforcement schedule. Responding on the left lever resulted in no programmed consequences. Successful lever training was defined as earning ≥100 food pellets in a single 1-h session. Following lever training, the metal food hoppers were removed from the chambers and replaced with a metal plate in order to remove as many food-associated cues as possible. Rats were returned to ad libitum access to food in their home cages for the remainder of the study.

2.4.2. Surgery

Rats were implanted with silastic tubing jugular catheters according to previously described methods (Kruzich and Xi, 2006a). Rats were anesthetized with 90-mg/kg ketamine and 2.0-g/kg xylazine (F344, ACI, rats) or 90-mg/kg ketamine and 1.6-mg/kg xylazine (LEW rats). Different anesthesia regimens were used because LEW rats are more sensitive to the cardiovascular depressing effects of xylazine in our laboratory. Animals received 7 days to recover from surgery. Catheters were flushed daily by administering 0.1 ml of 100-U/ml heparinized saline.

2.4.3. Methamphetamine self-administration

Following surgical recovery, rats received daily limited access to methamphetamine during 2-hour self-administration sessions 7 days a week. Initially, a lever press on the right lever was reinforced according to a fixed ratio-1 (FR-1) schedule of reinforcement. Reinforced responses resulted in 5-s methamphetamine infusions (0.06 mg/kg/iv in a volume of 0.05 ml)

plus 5 s of timeout. All infusions were signaled by a 3-light cue (red, yellow, and green bulbs, located over the active lever) and concurrent presentation of a 5-s 4-kHz 80-dB tone (LT compound stimulus, LT), which is 15 dB louder than ambient noise levels. The duration of the LT presentation was always 5 s. Responses emitted during the infusions, stimulus presentations, or timeouts resulted in no programmed consequences, but were recorded. The first reinforced infusion lasted 9 s (total volume ~0.09 ml) plus a 1-s timeout, which accounts for the dead volume of the catheter. The LT stimulus was presented for the initial 5 s of the first infusion.

After rats acquired stable methamphetamine self-administration behavior that did not vary by 20% in daily intake over 3 days, animals self-administered methamphetamine according to a progressive ratio (PR) schedule of reinforcement. This schedule measures reinforcement efficacy (Hodos, 1961; Richardson and Roberts, 1996). The response requirement for each subsequently earned methamphetamine infusion was increased according to Richardson and Roberts (1996). The number of responses required for infusions was: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, and 603. Rats could receive a total of 24 infusions during a daily session (603 responses were required to receive the 24th infusion). The total number of infusions earned in a 2-h session served as our index of reinforcement efficacy. Catheters were flushed immediately after the self-administration session with 0.1 ml of 100-U/ml heparinized saline. Rats were then returned to their home cages where they had unrestricted access to food and water.

Once stable responding for 0.06-mg/kg/iv methamphetamine was established for each subject (less than 20% variability in earned infusions), a dose–response curve was generated from all rats. We chose to investigate reinforcement efficacy of ascending limb doses of methamphetamine in order to determine possible strain differences. Individual differences in intake are best detected along the ascending limb of the dose–response curve for psychostimulants (e.g. Roth and Carroll, 2004). The doses available for self-administration during this phase of the experiment were: 0.01, 0.03, and 0.06-mg/kg/infusion. Rats were required to self-administer each dose for a minimum of 3 days. Once intake did not vary by over 20% at the dose tested, the animal was provided access to a different dose. The dosing order was counterbalanced for each subject to avoid order effects (Keppel, 1991). Following generation of the dose–response curve, subjects were once again allowed to self-administer 0.06-mg/kg methamphetamine for 3 days under the PR schedule and then underwent the extinction phase of the study.

2.4.4. Extinction

Following methamphetamine self-administration testing, rats underwent extinction sessions. During daily 2-h extinction sessions, responding on either lever resulted in no programmed consequences, but was recorded. All rats underwent 5 days of extinction training prior to starting reinstatement testing.

2.4.5. Conditioned reinstatement

Once an animal underwent 5 days of extinction training, a conditioned reinstatement test was performed the next day. During the conditioned reinstatement test session, responding on the formerly active lever according to an FR-1 10-s timeout schedule of reinforcement resulted in a 5-s presentation of the LT. A 5-s timeout followed each response-contingent presentation of the LT. No methamphetamine was infused during this test. This test assesses the secondary reinforcing characteristics of the LT (e.g. Kruzich et al., 2001). Following the conditioned reinstatement test, rats underwent additional extinction sessions. Once a rat's number of responses during a 2-h extinction session was once again ≤ 10 responses, methamphetamine-primed reinstatement was investigated.

2.4.6. Methamphetamine-primed reinstatement

Rats received experimenter-delivered methamphetamine infusions (0.0. 0.06, 0.12, or 0.24 mg/kg/iv) 1 min into separate 2-h test sessions. The infusions were delivered in a single 0.09-ml bolus. Responding on either lever resulted in no programmed consequences. The primes were not signaled. The test sessions were separated by at least 1-extinction session. Responding during an intervening extinction session had to be at 10 or fewer emitted responses prior to testing methamphetamine-primed reinstatement the next day. The doses were tested once a dosing order was determined according to a counterbalanced Latin squares design. This methamphetamine priming procedure is based on our previous studies with inbred strains of rats (Kruzich and Xi, 2006a,b).

All catheters were tested for patency prior to beginning methamphetamine-primed reinstatement testing and/or if behavior became erratic at any point in the study (responding that varied from the rat's usual pattern of behavior). Catheter patency was verified by drawing blood from the catheter. If no blood was drawn, 5 mg of pentobarbital sodium was intravenously administered to the rat. If pentobarbital failed to produce immediate somnolence, the catheter was surgically reinserted into the left jugular vein of the animal. This measure was taken on one F344 rat. This rat received 5 days of recovery from the second surgery.

2.5. Statistics

Methamphetamine infusions (strain × session), number of responses emitted during the conditioned reinstatement test (strain), and responding during methamphetamine-priming tests (strain × dose) were analyzed with separate repeated measures (RM) analyses of variance (ANOVA) tests. If a significant RM-ANOVA was determined, post-hoc comparisons utilizing the Fisher LSD test were performed. Significance was set at $p<0.05$.

3. Results

3.1. Methamphetamine self-administration under an FR-1 schedule of reinforcement

There was not a significant effect of "strain" on active lever presses when responding was reinforced according to an FR-1 10-s timeout schedule ($F(2,112)=0.006$; $p=0.94$), nor was there a significant effect of self-administration session on active

lever pressing ($F(4,112)=0.654$; $p=0.63$). There was a statistically significant strain × session interaction ($F(8,112)=2.92$; $p<0.01$). The F344 rats emitted significantly more responses during session #1 compared to the LEW and ACI rats ($p<0.05$ for all comparisons; Fig. 1, *Top*). The strains did not differ in the number of infusions earned when responding according to an FR-1 10-s timeout schedule of reinforcement ($F(2,112)=0.04$; $p=0.97$; Fig. 1, *Middle*). There was not a significant effect of "session" ($F(4,112)=0.20$; $p=0.94$) nor was there a significant strain × session interaction for number of earned infusions ($F(8,112)=1.41$; $p=0.20$).

The strains did differ in the number of responses emitted on the inactive lever ($F(2,112)=8.78$; $p<0.001$; Fig. 1, *Bottom*). The LEW rats emitted significantly more responses on the inactive lever than the F344 and ACI rats ($p<0.05$ for all comparisons). Responding on the inactive lever did not statistically vary by session ($F(4,112)=1.77$; $p=0.14$). There was not a significant strain × session interaction for inactive lever responses ($F(8,112)=0.39$; $p=0.93$).

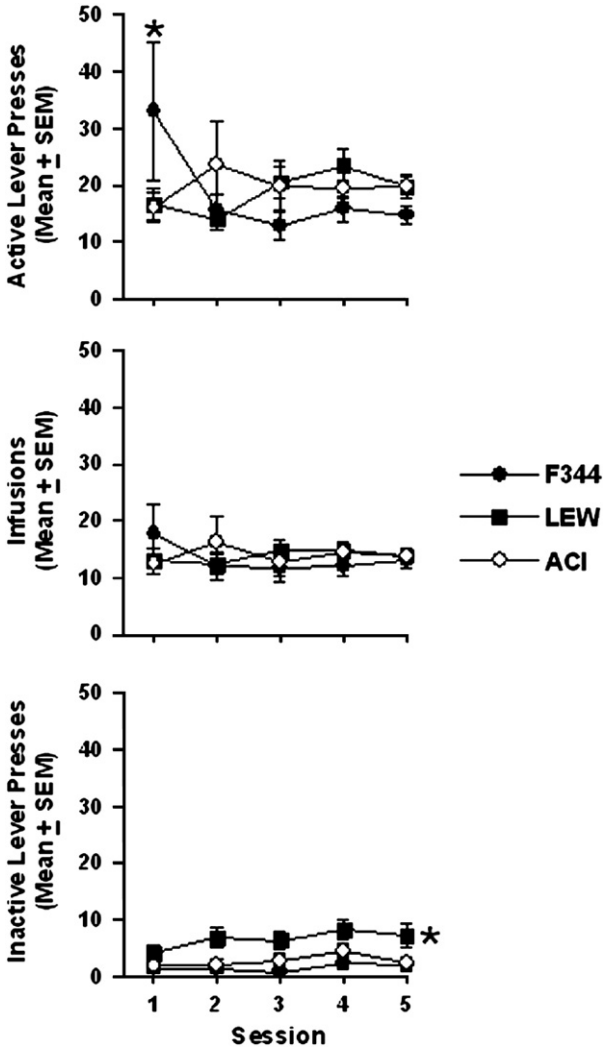

Fig. 1. Methamphetamine self-administration under an FR-1 schedule of reinforcement. *Top*: The strains did not differ in active lever presses nor was there a significant effect of session on active lever pressing. There was a significant strain × session interaction; F344 rats emitted significantly more responses during session #1 compared to the LEW and ACI rats (*$p<0.05$ for all comparisons). *Middle*: The strains did not differ in the number of infusions earned. There was not a significant effect of "session" or a significant strain × session interaction for number of earned infusions. *Bottom*: The LEW rats emitted significantly more responses on the inactive lever than the F344 and ACI rats (*$p<0.05$ for all comparisons).

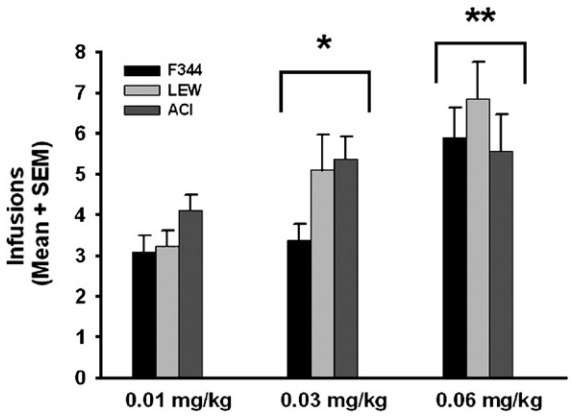

Fig. 2. Methamphetamine self-administration under a PR schedule of reinforcement. There were no significant strain differences in intake across any of the doses tested ($p=0.35$). However, the strains did demonstrate robust increases in responding for 0.03 and 0.06 mg/kg/iv/infusion compared to 0.01 mg/kg/iv/infusion (*$p<0.05$). Last, the strains exerted more work to receive 0.06 mg/kg/iv/infusion compared to 0.03 mg/kg/iv/infusion (**$p=0.05$).

3.2. Methamphetamine self-administration under a PR schedule of reinforcement

The strains did not differ in the number of earned methamphetamine infusions while responding under a PR schedule of reinforcement ($F(2,30)=0.97$; $p=0.39$; Fig. 2). There was a significant difference in number of infusions earned across the various doses ($F(2,60)=27.21$; $p<0.001$). Post-hoc comparisons revealed that rats earned more infusions at the 0.03 and 0.06 mg/kg/injection doses of methamphetamine compared to 0.01 mg/kg methamphetamine ($p<0.05$ for both comparisons) and emitted more responses for 0.06 mg/kg than 0.03 mg/

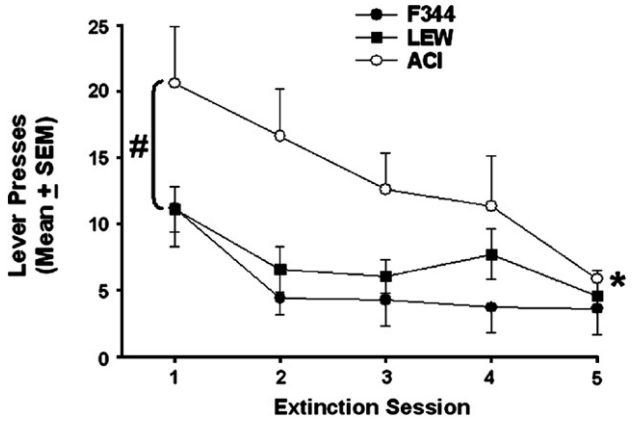

Fig. 3. Extinction responding. The ACI rats emitted more responses during extinction training than the F344 and LEW rats (*$p<0.05$ for all comparisons). All 3 strains emitted significantly more responses during the first extinction session compared to sessions 2–5 ($^{\#}p<0.05$ for all comparisons).

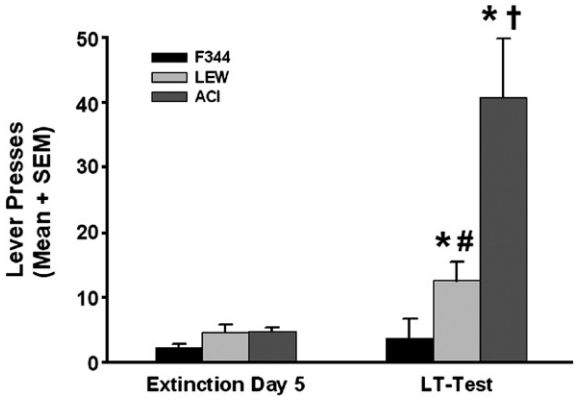

Fig. 4. Conditioned reinstatement. Response-contingent presentation of the LT compound stimulus significantly reinstated responding relative to extinction Day 5 output for the LEW and ACI rats (*$p<0.05$). The F344 rats did not demonstrate significant conditioned reinstatement ($p>0.7$). The LEW rats responded significantly more during the conditioned reinstatement test than the F344 rats ($^{\#}p<0.05$). The ACI rats emitted significantly more responses during the conditioned reinstatement test compared to the F344 and LEW rats ($^{\dagger}p<0.05$ for all comparisons).

kg ($p<0.05$). The interaction was not statistically significant ($F(4,98)=1.4$; $p=0.25$). There were no differences in inactive lever responses (data not shown).

3.3. Extinction

There was a significant effect of strain on extinction responding ($F(2,112)=6.55$; $p<0.01$; Fig. 3). The ACI rats emitted more responses during extinction training than the F344 and LEW rats ($p<0.05$ for all comparisons). There was a significant effect of session on extinction responding ($F(4,112)=10.49$; $p<0.001$). Rats from all 3 strains emitted significantly more responses during the first extinction session compared to sessions 2–5 ($p<0.05$ for all comparisons). The interaction was not statistically significant ($F(8,112)=1.56$; $p=0.14$).

3.4. Conditioned reinstatement

The strains did not differ in response output for extinction Day 5. Despite the finding that all rats from all 3 strains responded for at least one presentation of the LT, the ability of the LT to reinstate previously extinguished responding relative to extinction Day 5 response levels was significantly influenced by strain ($F(2,30)=15.13$; $p<0.001$; Fig. 4). The response-contingent presentations of the LT reinstated the highest response levels from the ACI rats compared to the F344 and LEW rats ($p<0.05$ for all comparisons). The LEW rats responded for more presentations of the LT than the F344 rats ($p<0.05$). There was a significant difference in responding by test day ($F(1,30)=28.86$; $p<0.001$). Significantly more responding occurred during the conditioned reinstatement test session compared to extinction Day 5 ($p<0.05$). Last, there was a significant strain × test day interaction ($F(2,30)=15.77$; $p<0.001$). While the LEW and ACI rats reinstated significant responding during the conditioned reinstatement test compared to extinction Day 5 ($p<0.05$ for all comparisons), the amount of responses emitted by the F344 rats did not vary by session ($p=0.73$). The strains did not differ in inactive lever responses and responses on the inactive lever did not vary by session day (data not shown). The average number of LT presentations earned for each strain was: F344=3, LEW=7.14, ACI=22.

3.5. Methamphetamine-primed reinstatement

There was a significant effect of strain for methamphetamine-primed reinstatement of previously extinguished responding ($F(2,90)=4.7$; $p<0.05$; Fig. 5). The ACI and LEW rats emitted significantly more responses during methamphetamine priming compared to the F344 rats ($p<0.05$ for all comparisons). There were statistically significant differences in responding across doses ($F(3,90)=9.7$; $p<0.001$). The most responding was evoked by administration of 0.24 mg/kg methamphetamine compared to all other doses administered ($p<0.05$ for all comparisons). Response levels following 0.12 mg/kg methamphetamine were greater than saline priming ($p<0.05$). A significant strain × dose interaction was found ($F(6,90)=2.17$; $p=0.05$).

Methamphetamine failed to reinstate robust responding relative to saline within the F344 rats following 0.06 and 0.12 mg/kg ($p>0.10$ for all comparisons). The 0.24 mg/kg prime did significantly reinstate responding compared to saline in the F344 rats ($p=0.05$). Within the LEW rats, 0.12 mg/kg/iv methamphetamine was capable of reinstating significant levels of responding compared to saline ($p<0.05$ for all comparisons). The response output following 0.12 mg/kg was almost statistically greater for the LEW rats compared to levels they emitted following 0.06 mg/kg methamphetamine ($p=0.55$). Interestingly, a single bolus infusion of 0.24 mg/kg/iv

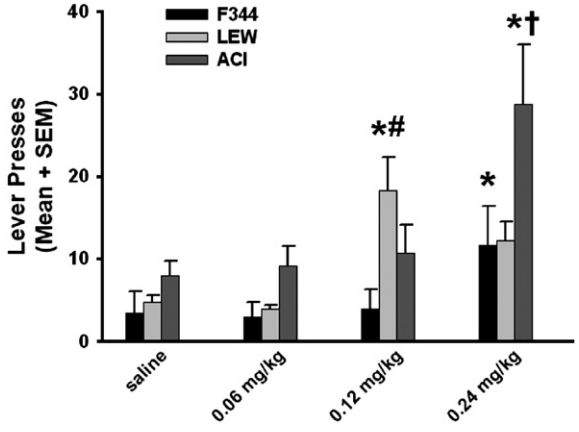

Fig. 5. Methamphetamine-primed reinstatement. Experimenter-delivered methamphetamine dose-dependently reinstated previously extinguished responding in the strains relative to saline pretreatment. The F344 rats emitted significantly more responses following 0.24 mg/kg methamphetamine compared to saline (*$p<0.05$). The LEW rats reinstated significant responding compared to saline following 0.12 mg/kg methamphetamine (*$p<0.05$). The ACI rats emitted significantly more responses following the 0.24 mg/kg prime compared to saline (*$p<0.05$). The response output demonstrated by the LEW rats following 0.12 mg/kg methamphetamine was significantly greater than the F344 rats' output at this dose ($^{\#}p<0.05$). The ACI rats responded significantly more following the 0.24 mg/kg prime compared to the F344 and LEW rats ($^{\dagger}p<0.05$ for all comparisons).

methamphetamine did not reinstate significant responding compared to saline priming in the LEW rats. Although we did not formally test for the incidence of stereotypy, we anecdotally did notice head weaving and increased locomotion in the LEW rats following injection of 0.24 mg/kg/iv. The ACI rats demonstrated significant reinstatement of responding following 0.24 mg/kg/iv compared to any other dose tested within this strain ($p<0.05$ for all comparisons).

None of the strains differed in response output from one another following saline or 0.06 mg/kg/iv methamphetamine ($p>0.30$ for all comparisons). The LEW rats did emit more responses following the 0.12-mg/kg/iv prime compared to the F344 rats ($p<0.05$) but not the ACI rats ($p=0.35$). The ACI rats emitted significantly more responses compared to the F344 and LEW rats following 0.24 mg/kg/iv ($p<0.05$ for all comparisons). No differences in inactive lever responding were found, and inactive lever responses did not vary as a function of dose (data not shown).

4. Discussion

The present study sought to determine if genetic background influences several methamphetamine-seeking behaviors: methamphetamine self-administration under a PR schedule of reinforcement, extinction, conditioned reinstatement, and methamphetamine-primed reinstatement in rats. Strain differences were found in extinction and both reinstatement paradigms.

The strains did not differ in number of infusions earned during self-administration under FR or PR schedules of reinforcement. We previously demonstrated that LEW rats self-administer more methamphetamine than F344 rats when responding under a fixed ratio-1 schedule of reinforcement (Kruzich and Xi, 2006b). A possible factor influencing differences between the present study and our previous report is food restriction. During the present study, animals were allowed to free-feed during methamphetamine self-administration. In our previous study, rats were maintained at approximately 90% of their free-feeding weights (Kruzich and Xi, 2006b). Food restriction influences the acute reinforcing effects of psychostimulants (Comer et al., 1995; Oei, 1983). Potentially, if the strains were food deprived in the present study, our results would have been different—suggesting a possible strain × environmental manipulation interaction. However, the characteristic pattern of increased inactive lever responding compared to F344 rats under an FR-1 schedule was demonstrated by LEW rats and supports our earlier study (Kruzich and Xi, 2006b).

We were somewhat surprised the ACI rats did not differ in intake compared to the other rats (please see Fig. 1)—because they poorly metabolize methamphetamine (Vorhees et al., 1998). Surprisingly little is known about genetic influences on methamphetamine self-administration and the reinforcement efficacy of methamphetamine in rats. Evaluation and identification of significant genetic influences on other methamphetamine induced behaviors (e.g. locomotion and stereotypy) have been reported in inbred rats (Camp et al., 1994). LEW rats show an increased release of dopamine following methamphetamine compared to F344 rats, and LEW rats also show greater and sustained plasma levels of methamphetamine compared to F344 rats (Camp et al., 1994). Perhaps the "poor metabolizer" phenotype in the ACI rats contributed to their heightened sensitivity to methamphetamine in our measures. To our knowledge, no prior study has evaluated the ACI's neurochemical response to methamphetamine. We are reluctant to conclude definitively that genetic background does not influence the reinforcement efficacy of methamphetamine. Nevertheless, under our testing conditions and parameters, with the strains we chose, genetic background contributed very little to methamphetamine intake.

That we did not find significant strain differences in methamphetamine intake definitely simplified our interpretation of the extinction, conditioned reinstatement, and pharmacological reinstatement experiments. We previously demonstrated that F344 rats show markedly different patterns of extinction responding following methamphetamine self-administration under a FR-1 schedule of reinforcement compared to LEW rats (Kruzich and Xi, 2006b). F344 rats tend to show greater response output compared to LEW rats during the first extinction session encountered (Kruzich and Xi, 2006a,b). The lack of difference between the F344 and LEW rats in extinction responding in the current study may again reflect the schedule of reinforcement used during maintenance of self-administration (e.g. FR versus PR schedules). We are unaware of any other published reports investigating extinction responding in ACI rats.

The literature with outbred rats would suggest that high levels of extinction responding would predict significant conditioned reinstatement (Kruzich et al., 2001) and cocaine-seeking behavior (Homberg et al., 2004). Extinction responding was a good predictor of conditioned reinstatement in the present study. Moreover, the number of earned infusions also represents the number of LT pairings with methamphetamine. All strains received statistically equal presentations of the LT with methamphetamine. Conditioned reinstatement is a valid assessment of the secondary reinforcing characteristics of stimuli when they are paired with primary reinforcers such as drugs of abuse (Di Ciano and Everitt, 2005; See, 2005). Despite showing equivalent intake and therefore receiving equal numbers of LT presentations with methamphetamine, the ACI rats demonstrated the highest level of response output during the conditioned reinstatement test.

There are no previously published reports investigating genetic background on conditioned reinstatement of previously extinguished psychostimulant-seeking behavior in rats, although both C57BL/6J and 129X1/SvJ mouse strains demonstrate high levels of conditioned reinstatement following cocaine self-administration (Fuchs et al., 2003; Highfield et al., 2002). While the neural substrates of conditioned reinstatement of psychostimulant-seeking behavior are becoming better understood (Di Ciano and Everitt, 2005; See, 2005; Weiss, 2005), genetic influences are largely unknown. Earlier studies have shown that conditioned reinstatement is influenced by: sex and estrous status (Fuchs et al. 2005; Kippin et al., 2005), duration of withdrawal (Tran-Nguyen

et al., 1998; Grimm et al., 2001), response-contingent presentation of cues during testing (Fuchs et al., 1998; Grimm et al., 2000) and presentation of neutral stimuli as a compound stimulus (See et al., 1999).

We do not interpret the reduced conditioned reinstatement demonstrated by the F344 rats to be indicative of a learning deficit. All strains readily acquired the operant tasks at similar rates. Potentially, differences in attaching salience to conditioned cues underlie the differences. One potential factor could be eyesight—the F344 and LEW rats are albino whereas ACI rats have pigmented eyes (e.g. http://www.harlan.com/models/usmodels.asp). However, a commonly used outbred strain, the albino Sprague–Dawley rat, shows exceptional conditioned reinstatement, and is a parental strain of F344 and LEW rats (e.g. Kruzich et al., 2001). We are unaware of any hearing deficits reported in any of these strains. Nevertheless, genetic background should be added to the growing list of factors influencing conditioned reinstatement of psychostimulant-seeking behavior.

We are forced to temper our discussion of the methamphetamine-induced reinstatement of responding in inbred strains of rats because only one published study investigating methamphetamine-primed reinstatement in inbred strains of rats at the time of writing this report exists (Kruzich and Xi, 2006b). Previously, we reported that LEW rats show a greater propensity to reinstate previously extinguished methamphetamine-seeking behavior following experimenter delivered iv infusions of methamphetamine compared to F344 rats (Kruzich and Xi, 2006b).

The LEW rats showed good reinstatement of responding following the 0.12-mg/kg pretreatment. The level of reinstatement demonstrated by the LEW rats in the present study following the 0.12-mg/kg challenge was in the same general range as our previous report (Kruzich and Xi, 2006b). There are a number of procedural differences between these studies that may have influenced the differences in findings at the other doses tested. First, in the previous study, experimenter delivered methamphetamine infusions were injected in bolus concentrations of 0.06 mg/0.05 ml (Kruzich and Xi, 2006b). A 0.12-mg/kg priming injection in the previous report required 2 infusions spaced by 1 min. In the present study, infusions were delivered in a single 0.09-ml bolus. The concentration of methamphetamine in the syringe was adjusted for each subject's weight and delivered all at once in this study. Perhaps number and spacing of infusions exerts an effect on response output. It is known that LEW rats display a heightened sensitivity to the catalepsy inducing effects of methamphetamine compared to F344 rats (Camp et al., 1994). The LEW rats' sensitivity to methamphetamine-induced stereotypy potentially appears to have affected their performance in methamphetamine-primed reinstatement. To our best knowledge, this is the first report seeking to characterize methamphetamine-seeking behavior in ACI rats.

The second major difference between the two studies is the schedule of reinforcement used during training (PR versus FR-1). Potentially, the differences in response requirements influenced responding during methamphetamine priming. Leaner schedules of reinforcement during maintenance are associated with robust drug-primed reinstatement (e.g. Anderson et al., 2003). However, the use of a PR schedule may have prevented an augmentation of responding following extinction compared to a continuous schedule of reinforcement.

In conclusion, 3 inbred strains of rats displayed different patterns of methamphetamine-seeking behaviors across a number of measures. Genetic background does not appear to influence significantly the reinforcement efficacy of methamphetamine (Fig. 2). Extinction responding was a good predictor of methamphetamine reinstatement behavior (Fig. 3). Conditioned reinstatement does appear to be influenced by genetic background (Fig. 4). Lastly, all strains demonstrated at least partial methamphetamine-induced reinstatement of responding compared to saline (Fig. 5). ACI rats demonstrated the highest level of methamphetamine-seeking behavior compared to the other strains tested with our reinstatement procedure.

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