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GENETICS OF NOVELTY SEEKING, AMPHETAMINE SELF-ADMINISTRATION AND REINSTATEMENT USING INBRED RATS

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Abstract

Previous research using outbred rats indicates that individual differences in activity in a novel environment predict sensitivity to the reinforcing effect of psychostimulant drugs. The current study examined if the link between responses related to novelty and amphetamine self-administration is heritable. Twelve inbred rat strains were assessed for locomotor activity in a novel environment, preference for a novel environment, and intravenous amphetamine self-administration (acquisition, extinction and amphetamine-induced reinstatement). Strain differences were observed in activity in a novel environment, novelty preference and amphetamine self-administration, indicating a genetic influence for each of these behaviors. While there was no relation between activity in an inescapable novel environment and amphetamine self-administration, strain-dependent differences in novelty preference were positively correlated with the amount of amphetamine self-administered. There was also a positive correlation between the dose-dependent rate of amphetamine self-administration and magnitude of reinstatement. These results demonstrate that activity in an inescapable novel environment and preference for a novel environment are different genetically, and thus likely to reflect different behavioral constructs. Moreover, these results implicate a genetic influence on the relation between novelty seeking and stimulant self-administration, as well as on the relation between stimulant reward and reinstatement.

Keywords

amphetamine; novelty seeking; self-administration; genetics; rat; addiction

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Introduction

A heritable trait linked reliably to drug use is sensation seeking or novelty seeking (Cloninger, 1987; Zuckerman, 1994). To understand the fundamental neural mechanisms underlying novelty seeking, investigators have used laboratory animal models. Piazza *et al.* (1989) classified rats as high responders (HR) or low responders (LR) based on a median split of their level of locomotor activity in a novel environment. HR rats subsequently self-administer more amphetamine (AMPH), cocaine, or nicotine than LR rats (Mantsch *et al.*, 2001; Piazza *et al.*, 1989; Suto *et al.*, 2001). While the HR/LR model has been used to predict individual differences in drug self-administration, interpretation of data from this model may be complicated for two reasons. First, differences in activity may have nothing to do with the response to the novelty of the apparatus, making the relationship to human sensation- or novelty-seeking tenuous. Second, even if some aspect of situational reactivity plays a role, the activity differences may be due to the influence of stress (Kosten & Ambrosio, 2002).

Free-choice preference for novelty may be a useful alternate model (Bardo *et al.*, 1996). In this test, rats are exposed repeatedly to one environment over several days and then are allowed to choose between a novel and now-familiar environment. Rats are classified as either high or low novelty seekers based on the degree of preference for the novel environment over the familiar environment (Cain *et al.*, 2004; 2005). Using novelty preference, individual differences in novelty seeking have been found to predict AMPH conditioned place preference (Robinet *et al.*, 1998). When a regression analysis is used with a large sample size, similar to studies with humans, novelty seeking is also related to AMPH self-administration in rats (Cain *et al.*, 2005).

The majority of preclinical work examining individual differences in activity or novelty seeking has used outbred rats. Yet, the rationale for studying these behaviors in rodents seems often to imply that they represent heritable traits. However, these studies have not sought to determine whether novelty seeking represents a heritable trait. The purpose of the present experiment was to determine whether genetics plays a role in the relationship among activity, novelty seeking and drug self-administration. Rats from 12 inbred rat strains were screened initially for activity and their response to free-choice novelty, and then were assessed for acquisition, extinction and reinstatement of AMPH self-administration. The various strains chosen offered a diversity of characterized phenotypes (traits) that were useful in analyzing the critical determinants of novelty seeking. Within any inbred strain, all same-sex individuals are nearly identical, and allelic genetic differences across strains have been fixed randomly during their development. By comparing multiple strains in a carefully controlled environment, individual differences within each strain are presumably due to environmental influences, while differences among strain means estimate genetic influences on the behaviors involved (Hegmann & Possidente, 1981). Covariance among traits may also be partitioned into genetic vs non-genetic influences, which allowed us to assess whether genetic differences in novelty-related behaviors were predictive of genetic differences in AMPH-related behaviors.

Methods

Animals

Male ACI (N = 6); Brown Norway (BN; N = 6); Buffalo (BUF; N = 14); Fischer (F344; N = 8); Lewis (LEW; N = 8); Spontaneous Hypertensive Rat (SHR; N = 12); and Wistar Kyoto (WKY; N = 6) inbred rats between the ages of 70-75 days were obtained from Harlan Industries (Indianapolis, IN, USA). Male BDIX (BD9; N = 11); Dahl Salt Sensitive (DSS; N = 11); Fawn Hooded (FH; N = 9); Wistar Albino Glaxo (WAG; N = 8); and Wistar Furth

(WF; N = 6) inbred rats between the ages of 70-75 days were obtained from Charles Rivers Laboratories (Wilmington, MA, USA). For each replicate experiment, 3-8 rats from each strain were tested simultaneously to control for potential cohort effects; however, due to the variable availability of strains from vendors, full counterbalancing of strains within each replicate experiment was not possible. Nonetheless, each strain was represented in 2-3 cohorts, except for WAG, which arrived as a single shipment from an international source (Charles Rivers Laboratories, Germany). Rats were housed individually with *ad libitum* access to food and water (except where noted) and were maintained on a 12:12-hr light:dark cycle; testing was conducted during the light. Procedures were approved by IACUC at the University of Kentucky.

Drugs

d-AMPH sulfate was purchased from Sigma (St. Louis, MO) and was prepared in 0.9% NaCl (saline).

Apparatus

Activity in a novel environment was recorded using a monitoring system (AccuScan Instruments Inc., Columbus, OH). Rats were placed in a chamber (42 × 42 × 30 cm) made of clear acrylic that incorporated a 16 × 16 grid of photo beam sensors. Activity was expressed as total horizontal distance traveled.

Free-choice novelty place preference was measured using an automated 3-compartment conditioned place preference apparatus (ENV-013, Med Associates, St. Albans, VT), measuring 21 × 21 × 68 cm and consisting of two 28-cm long end compartments separated by a 12-cm long central compartment. One end compartment had black walls and metal rod floor; the other end compartment had white walls and mesh floor; and the center compartment had gray walls and smooth PVC floor.

AMPH self-administration was conducted in a 2-lever operant conditioning chamber (ENV-001; Med Associates, St Albans, VT) housed in a sound-attenuated outer chamber. The end walls of the operant conditioning chamber were aluminum, the front and back walls were clear Plexiglas and the floor was 18 stainless steel rods. At the bottom center of one of the end walls was an opening (5 × 4.2 cm) to a recessed food tray. Located on each side of the food tray was a response lever. A 28-V white cue light was located 6 cm above each response lever. A 28-V white house light was centered 20 cm above the floor on the wall opposite the response levers. An infusion pump delivered drug via a silastic tube attached to a swivel mounted on the outside of the back wall.

Procedures

Activity and Novelty-Seeking Tests—To assess activity, rats were placed individually in the center of the activity monitor for 30 min and total distance traveled was recorded. On the next day, rats were assessed for free-choice novelty place preference. Rats were habituated to either the black or white compartment (counterbalanced within strain) for 30 min on two consecutive days. On the following day, animals were placed in the center compartment with access to both the familiar and novel end compartments. Time spent in each compartment was monitored for 15 min. A novelty preference ratio was calculated as the duration in the novel compartment divided by the total duration in both the novel and familiar compartments. Some rats did not enter each compartment; individual rats with preference ratios (PR) less than 0.10 or greater than 0.90 were excluded from the statistical analysis, which eliminated 5 rats (2 BUF; 1 F344; and 2 WKY).

AMPH Self-Administration—Within five days of completing novelty place preference, rats were anesthetized (80 mg/kg ketamine, 5 mg/kg diazepam, i.p.) and implanted with an indwelling jugular catheter. The silastic catheter was threaded subcutaneously to exit from a dental acrylic head mount secured to the top of the skull with screws and an infusion pump was attached to the head mount via a silastic leash during self-administration sessions. Rats were given 7 days to recover from surgery before AMPH self-administration began.

Phase 1: Acquisition: Acquisition of AMPH self-administration was initiated using an autoshaping procedure similar to that described previously (Carroll & Lac, 1993). Three days prior to autoshaping, animals were restricted to 20 g food per day to enhance motivation for drug (Sparber *et al.*, 1991). For 5 consecutive days, rats were given a 60-min autoshaping session, followed 30 min later by a 60-min contingent AMPH self-administration session. During the autoshaping session, the house light was illuminated and an inactive lever (no programmed consequence, counterbalanced for position across rats within each strain) was extended throughout the session. For the first 15 min of each autoshaping session, an active lever was also extended 10 times at random intervals and remained extended for 10 sec. If the active lever was pressed, AMPH (0.1 mg/kg/infusion, 5.9 sec duration, 0.1 ml volume) was delivered immediately; if the active lever was not pressed during the 10-sec extension, a non-contingent infusion of AMPH was delivered when the lever retracted. For the remaining 45 min of the session, only the inactive lever was extended.

For the subsequent contingent AMPH self-administration session, both levers (active and inactive) were extended and infusions of AMPH were contingent on pressing the active lever using a continuous reinforcement schedule (fixed ratio 1; FR1); each reinforced response was followed immediately by a 20-sec time out interval during which both cue lights above the levers were illuminated and responding on neither lever was reinforced. Throughout the session, responding on the inactive lever was recorded but there was no programmed consequence. Following 5 days of autoshaping and contingent AMPH self-administration, rats underwent 3 days of only contingent 60-min self-administration sessions (no autoshaping) under food restriction, followed by 3 self-administration sessions with no food restriction. Rats remained on free feed for the remainder of the experiment.

Phase 2: Incremental FR Schedule: Daily 60-min self-administration sessions continued and the schedule of reinforcement was incremented from FR1 to FR5, with rats spending 3 consecutive sessions on each FR value. The unit dose of AMPH during this phase remained at 0.1 mg/kg/infusion, with a 20-sec signaled time out.

Phase 3: Dose Response: Rats spent 3 consecutive sessions (60 min) on the FR5/20-sec signaled time out schedule at each of the following unit doses: 0.056, 0.03, 0.01 and 0.001 mg/kg/infusion; doses were given in descending order. Catheter patency was checked at the conclusion of this phase of the study by administering 0.2 ml of 15 mg/ml morphine sulfate i.v. to induce rapid catalepsy; all rats tested at this point of the experiment showed patent catheters.

Phase 4: Extinction: Rats spent 10 consecutive sessions (60 min) on the FR5/20-sec signaled time out schedule, but received infusions of saline only.

Phase 5: Reinstatement: Rats received 2 consecutive sessions (60 min) on the FR5/20-sec signaled time out with saline self-infusions. Immediately prior to the first session, rats received saline (s.c); immediately prior to the second session, rats received AMPH (0.3 mg/kg, s.c.).

Data Analysis

The two activity and novelty-seeking tests were analyzed separately using a one-way ANOVA with strain as a between-subjects factor. Operant response data were analyzed by ANOVA for each phase of the experiment (acquisition, incremental FR, dose response, extinction and reinstatement), with strain as a between-subjects factor. For the incremental FR and dose response phases, ANOVA was performed using the average number of infusions earned on the last two sessions at each FR requirement or at each dose. The effect of varying the unit dose of AMPH was also assessed by one-way ANOVA using data derived as an area under the curve (AUC) from the lowest dose (saline extinction) to the highest dose (0.1 mg/kg/infusion) using the trapezoidal rule (GraphPad Prism Software Inc., San Diego, CA, USA). For the reinstatement phase, paired sample t-tests were performed for each strain to compare the last day of extinction to the challenge test with saline, as well as to compare the saline challenge to the AMPH challenge. In all cases, *P* values were 0.05.

Genetic Correlations

Genetic correlational analyses were conducted among the following variables: (1) activity in the novel environment; (2) novelty preference ratio; (3) number of AMPH self-infusions at each FR level; (4) number of AMPH self-infusions at each unit dose; (5) dose response AUC; and (6) the difference between saline self-infusions reinstated by AMPH (0.3 mg/kg) compared to saline challenge. In these correlational analyses, a single value for each strain was derived by determining the mean value for all rats tested from the same strain. Hegmann and Possidente (1981) have shown that if strains have been maintained and tested under similar conditions, then the influence of a common set of genes on two traits can be estimated from the correlation of inbred strain means. For 12 inbred strains (*df* = 10) a correlation of $|r| \geq 0.58$ would be necessary for statistical significance ($p < 0.05$). *P* values were not adjusted for multiple testing, as we were interested in the overall pattern of relationships among the variables explored as described previously (Crabbe *et al.*, 1994; 2005). Based on *a priori* predictions, the following correlations are presented in the results: (1) activity in the novel environment and average number of AMPH self-infusions on the terminal FR5 schedule; (2) novelty preference ratio and average number of AMPH self-infusions on the terminal FR5 schedule; and (3) dose response AUC and the difference between saline self-infusions reinstated by AMPH (0.3 mg/kg) compared to saline challenge. Genetic effect size was estimated from each strain mean ANOVA for activity in inescapable novelty, novelty preference, AMPH self-administration, dose response AUC, and reinstatement by dividing sum of squares (SS) among strains by total SS as described previously (Falconer & Mackay, 1996). This method for estimating genetic effect size has been previously used by Belknap *et al.* (1996).

Results

Genetic Correlations

Correlations across all of the variables measured can be found in S1.

Activity and Novelty-Seeking Tests

Results from activity in the novel environment and novelty place preference are presented in Table 1. One-way ANOVA revealed a significant effect of strain on total distance traveled [$F(11,104) = 18.27, p < 0.001$], with strain means differing more than 3-fold. For novelty place preference ratios, a t-test across all animals showed $PR = 0.55 \pm 0.02$, which differed significantly from the null hypothesis of a $PR = 0.50$ [$t(99) = 3.31, p < 0.01$]. However, the range of average scores among strains was relatively narrow (mean preference ratios ranged from 0.47-0.63) and strains did not differ significantly [$F(11,99) = 0.92, NS$;

genetic effect size = 0.10]. Reasoning that the restricted variability across strains limited power to detect strain differences, we explored the individual strains' PRs. Of the 12 strains tested, only ACI, LEW and WAG showed novelty preference that differed significantly from 0.50 [$t(5) = 5.04, p < 0.01$; $t(7) = 3.35, p < 0.05$; $t(7) = 4.44, p < 0.01$; respectively], and no strain showed significant novelty avoidance. There was no significant genetic correlation between activity in inescapable novelty and novelty place preference among the strain means [$r(12) = 0.01$].

AMPH Self-Administration

Phase 1: Acquisition—Results from the acquisition phase for AMPH self-administration are shown in Figure 1. Strains differed significantly in performance across acquisition sessions as shown by significant main effects of strain [$F(11,65) = 8.03, p < 0.001$] and session [$F(10,650) = 40.42, p < 0.001$], as well as a significant interaction between the two factors [$F(110,650) = 2.11, p < 0.001$]. Separate ANOVAs for each strain revealed significant increases in infusions across sessions in all but SHR and BD9 [BUF: $F(10,70) = 4.42, p < 0.001$; F344: $F(10,50) = 5.39, p < 0.001$; WKY: $F(10,40) = 13.65, p < 0.001$; ACI: $F(10,40) = 7.04, p < 0.001$; BN: $F(10,50) = 6.40, p < 0.001$; LEW: $F(10,60) = 7.30, p < 0.001$; DSS: $F(10,80) = 11.63, p < 0.001$; FH: $F(10,50) = 4.86, p < 0.001$; WF: $F(10,40) = 3.73, p < 0.001$; WAG: $F(10,50) = 3.95, p < 0.001$; respectively]. SHR showed a steady, moderate infusion rate (~10 infusions) across all sessions, including session 1, which may reflect a rapid rate of acquisition during the initial session. In contrast, BD9 earned essentially no infusions on any session, indicating that acquisition was not obtained in that strain.

Phase 2: Incremental FR—Strains performed differently across the incremental FR sessions in which the response requirement increased from FR1 to FR5 (see Figure 2). There were significant main effects of strain [$F(11,51) = 12.35, p < 0.001$] and FR value [$F(4,204) = 3.68, p < 0.05$], as well as a significant interaction of strain and FR value [$F(44,204) = 1.88, p < 0.05$]. Separate ANOVAs for each strain revealed significant main effects of FR value in FH and WAG only [$F(4,16) = 3.10, p < 0.05$; $F(4,16) = 3.11, p < 0.05$; respectively]. For FH and WAG, the number of infusions earned declined across the incremented ratio requirement. For all other strains, number of infusions earned did not change across the incremented ratio requirement, thus indicating that these strains adjusted their response rate to maintain a relatively constant intake of AMPH. However, similar to the acquisition phase, BD9 continued to show no responding for AMPH during this phase.

Due to the failure of BD9 to show any responding for AMPH across the first two phases of the study, a follow-up experiment using BD9, LEW and F344 ($n=3$ per strain) was conducted as described previously, except that food pellets (45 mg, Noyes) were used instead of AMPH infusions. While acquisition of food-reinforced responding was slowest in BD9 during the autoshaping phase, the number of pellets earned did not differ significantly among BD9, LEW or F344 during the incremental FR phase (mean number of pellets earned per FR2-5 session ranged from 15-30 for BD9, LEW and F344; results not shown). Thus, BD9 successfully acquired the task when food reward was substituted for AMPH.

As shown in Figure 3, a correlational analysis for activity in inescapable novelty and average number of AMPH infusions (training dose) on the terminal FR5 schedule failed to reach significance [$r(12) = -0.05$]. However, there was a significant genetic correlation between novelty place preference and average number of AMPH infusions [$r(12) = 0.59, p < 0.05$]. While the correlations in Figure 3 were based on mean values derived from all rats at each phase of testing, the results were similar when the mean values for only those subjects that completed the AMPH self-administration portion of the study were used in the

correlational analyses; i.e., the correlation between activity and AMPH infusions was not significant, while the correlation between novelty preference and AMPH infusions was significant [$r(12) = 0.62, p < 0.05$]. Thus, attrition across the study did not influence the outcome.

Since SHR and BD9 failed to acquire AMPH self-administration, correlational analyses for activity in inescapable novelty, novelty place preference and the average number of AMPH infusions earned were also recomputed with these two strains removed. The overall pattern of correlations did not change (Activity vs AMPH self-administration [$r(10) = 0.32$]; Novelty Place Preference vs AMPH self-administration [$r(10) = 0.56$]; results not shown).

Phase 3: Dose Response—Results from the AMPH dose response phase showed that strains differed significantly in their sensitivity to changes in unit dose (see Figure 4). An overall ANOVA revealed a significant main effect of strain [$F(11,41) = 11.55, p < 0.001$] and dose [$F(5,205) = 33.72, p < 0.001$], as well as a significant strain $\times$ dose interaction [$F(55,205) = 2.92, p < 0.001$]. Only BUF, WAG, FH, WKY, SHR and DSS showed significant alterations in responding with different unit doses [$F(5,15) = 5.45, p < 0.05$; $F(5,20) = 4.14, p < 0.05$; $F(5,15) = 4.31, p < 0.05$; $F(5,15) = 11.32, p < 0.001$; $F(5,10) = 4.72, p < 0.05$; $F(5,20) = 4.40, p < 0.05$; respectively]. Trend analyses revealed significant linear trends for dose in BUF, WKY, SHR, and DSS [$F(1,3) = 15.35, p < 0.05$; $F(1,4) = 9.89, p < 0.05$; $F(1,3) = 11.49, p < 0.05$; $F(1,3) = 63.75, p < 0.01$; respectively], as well as significant quadratic trends for dose in WAG and DSS [$F(1,3) = 21.34, p < 0.05$; $F(1,4) = 27.14, p < 0.01$; respectively]. Expressing these results as an area under the curve (AUC) revealed that BD9 were least sensitive and DSS were most sensitive to changes in unit dose [$F(11,53) = 10.75, p < 0.001$; see Figure 5].

Phase 4: Extinction—An overall ANOVA revealed significant main effects of strain [$F(11,42) = 5.38, p < 0.001$] and session [$F(9,378) = 2.66, p < 0.05$]; there was no significant interaction (results not shown). Collapsed across strain, the mean ($\pm$ SEM) number of responses on the first extinction session to the last extinction session declined significantly from 5.7 (± 0.66) to 3.9 (± 0.46) [$t(53) = 3.95, p < 0.001$]. The relatively low response rate on the first extinction session was likely due to the descending dose procedure used in the previous dose response phase, thus establishing some loss of responding prior to saline substitution.

Phase 5: Reinstatement—Results from the reinstatement session are shown in Figure 6. There was no significant difference between the last day of extinction and the test day with saline for any strain (results not shown); however, amphetamine-induced reinstatement expressed as a change in number of infusions from the saline session was evident in WAG, WKY and DSS [$t(4) = 3.59, p < 0.05$; $t(4) = 3.67, p < 0.05$; $t(4) = 3.04, p < 0.05$; respectively]. As shown in Figure 7, a correlation was found for AUC in the dose response curve and the magnitude of AMPH-induced reinstatement [$r(12) = 0.83, p < 0.05$]. The correlational analysis was also recomputed with the SHR and BD9 strains removed, but this did not alter the overall pattern of the correlation [$r(10) = 0.83$; results not shown].

Estimated Genetic Effect Size

The estimated genetic effect size across the main measures were as follows: activity in inescapable novelty, $h^2 = 0.68$; novelty preference, $h^2 = 0.10$; AMPH self-administration, $h^2 = 0.74$; AMPH dose response AUC, $h^2 = 0.74$; and AMPH reinstatement, $h^2 = 0.53$.

Discussion

The present study determined whether a heritable link between novelty related responses and amphetamine self-administration exists. Among twelve inbred rat strains, no relationship was found between activity in a novel environment and AMPH self-infusions. However, a relationship was found between novelty preference ratio and AMPH self-infusions, with higher novelty seeking strains self-administering more AMPH. A positive strain-dependent relation was also found between dose response AUC and strength of reinstatement.

Previous work using outbred rats shows that AMPH self-administration is predicted by individual differences in activity in a novel environment (Piazza *et al.*, 1989). The current study found no significant genetic correlation between activity in a novel environment and AMPH self-administration across strain means, suggesting that HRs examined in previous studies may self-administer more AMPH than LR rats due to environmental influences. Another possible explanation is that distance traveled may not be the most relevant measure for indexing behavior in a novel environment. Previous work by Kosten *et al.* (2007) found time spent in the center of the novel environment, but not total distance traveled, predicted cocaine self-administration. Exner and Clark (1993) found that the “escape” responses in a novel environment predicted behavior responses to AMPH. Predictive individual differences may also depend on test duration, drug dose, and measures of self-administration (acquisition vs. drug intake; Marinelli, 2005; Mitchell *et al.*, 2005). Our study extends these findings by showing a genetic relationship between novelty place preference and AMPH self-administration, but not between activity in a novel environment and AMPH self-administration.

Davis *et al.* (2008) tested rats selectively bred based on a variant of the HR/LR phenotype and found that HR rats acquired cocaine self-administration more rapidly than LR rats of the 14th selected generation. The overall rate of cocaine self-administration was greater in female HR rats than in female LR, male HR and male LR rats, although the difference between HR and LR male rats was relatively small. However, regardless of sex, and consistent with our results, the selected lines used in Davis *et al.* (2008) did not differ reliably in cocaine self-administration when responding stabilized after approximately 7 sessions, suggesting that genes influencing HR/LR are not involved in the rate of stimulant self-administration at the plateau of responding. Some caution is needed in comparing results across these studies, however, as Davis *et al.* (2008) used cocaine and selectively bred lines, whereas our study used AMPH and commercially available inbred strains.

The lack of significant correlation between activity in a novel environment and preference for a novel environment across strain means suggests that these behaviors are genetically distinct. We have previously reported the lack of a phenotypic correlation between these traits in Sprague Dawley rats (Cain *et al.*, 2005; 2008). This genetic finding corroborates recent findings in inbred mouse strains compared for five different novelty-related responses: activity in a novel environment, novel environment preference, head dipping on a hole-board, novel object preference, and a two-trial version of a spontaneous alternation task (Kliethermes & Crabbe, 2006). Differences among strains were observed for all tasks, but strain mean performance in any given task was generally not predictive of performance in any other. Also, Kliethermes *et al.* (2007) selectively bred mice for high exploratory behavior (HEB) and low exploratory behavior (LEB), one measure of novelty seeking based on the number of head dips in a hole-board apparatus. When HEB and LEB mice were tested for sensitivity to develop AMPH conditioned place preference, no difference between lines was seen. Thus, any common genetic basis for novelty preference and AMPH-related behaviors is likely to depend upon the specific measures used to assess each trait.

In the current study, strain-dependent differences in acquisition of AMPH self-administration were obtained, although BD9 failed to show reliable acquisition under the procedures used. The differences in acquisition among strains may be attributed, at least in part, to heritable differences in sensitivity to the reinforcing effect of AMPH. While other factors may be involved, such as sensory or learning differences, BD9 acquired a food-reinforced response as readily as LEW and F344, indicating that the results obtained do not reflect merely a difference in learning ability. As AMPH self-administration continued and the FR value was increased, the majority of strains maintained a relatively constant number of infusions throughout the incremental FR phase of the experiment by increasing their responding on the active lever. This finding is generally consistent with previous work showing that rats regulate drug intake to some optimal level of reward or satiation by adjusting their response rate (Lynch & Carroll, 2001; Tsibulsky & Norman, 1999).

Results from the dose response phase showed that several strains (BUF, WAG, FH, WKY, SHR and DSS) adjusted their responding when the unit dose of AMPH was altered. These inbred rat strains displayed an inverted U-shaped dose response curve, which is typical for drugs self-administered on a FR schedule of reinforcement (Dworkin & Stairs, 2003). For all strains showing a dose-dependent change in responding, peak responding occurred at an AMPH unit dose of either 0.03 or 0.056 mg/kg/infusion. While there was little difference among strains in the dose that produced peak responding, the height of the peak differed substantially among strains. These differences in peak responding may reflect genetic differences in sensitivity to the reinforcing effect of AMPH; the estimated genetic effect size for peak responding was substantial ($h^2 = .74$). Unexpectedly, during the dose response phase of the study, LEW and F344 did not significantly adjust their responding to alterations in the unit dose of AMPH. These results contrast with Kosten *et al.*, (2007), who found that F344 earn more cocaine than LEW on a progressive ratio (PR) schedule. F344 also earn more cocaine than either ACI and BN under a PR schedule (Ward *et al.*, 1996). These discrepant results may reflect differences in the schedules of reinforcement (FR5 vs PR) and/or the stimulant tested (AMPH vs cocaine).

During the extinction phase, substitution of saline for an AMPH unit dose of 0.001 mg/kg/infusion resulted in a decrease in responding across strains. Since a descending dose order was used to establish the dose response curve, the extinction obtained was relatively small in magnitude (decline from 5.7 to 3.9 mean responds per session), likely because the lowest unit dose of AMPH (0.001 mg/kg/infusion) was not sufficient to maintain responding. More importantly, following the extinction phase, inbred strains were subsequently tested for reinstatement following AMPH (0.3 mg/kg, s.c.). Only WAG, WKY and DSS showed a significant reinstatement of AMPH seeking. Interestingly, these three strains also displayed the greatest adjustments in responding during the dose response phase of the experiment as defined by the AUC. Dose response AUC was also correlated with magnitude of reinstatement, suggesting a common genetic influence (shared variance in strain means = 69%) on sensitivity to both the reinforcing and reinstating effects of AMPH. These results add to previous work showing that Roman high-avoidance (RHA) rats maintain cocaine self-administration at greater rates and reinstate cocaine seeking behavior at lower doses than Roman low-avoidance (RLA) rats (Fattore *et al.*, 2009). To the extent that the reinstatement procedure models drug relapse in humans, a view generating some controversy (Epstein *et al.*, 2006; Katz & Higgins, 2003), the current results implicate a heritable link between sensitivity to drug reward and propensity to relapse following a period of abstinence. Future research may determine whether treatment interventions that specifically target individuals with a high reward sensitivity phenotype would enhance the efficacy of relapse prevention strategies.

Genetic effect size for activity in a novel environment was high, consistent with other published data (e.g., Ramos *et al.*, 1997). AMPH self-administration and dose-response AUC showed high genetic effect sizes ($h^2 = 0.74$ each) for a behavioral trait, suggesting that 55% of the variance in each trait was due to genetic causes. While genetic effect sizes for reinstatement and novelty preference were lower ($h^2 = 0.53$ or 0.10 respectively), they are similar to those reported for many behavioral and drug-related traits. For example, the estimated genetic effect size in mice selectively bred for loss of righting reflex after ethanol was less than 0.18 , but the selected lines differ by more than 30-fold in their response (McClearn & Kakihana, 1981).

Finally, while the neural mechanisms involved in the strain-dependent behavioral effects observed here are unknown, AMPH is known to reverse the dopamine transporter (DAT; Goodwin *et al.*, 2009). Previous research found that LEW have reduced DAT protein and slower basal dopamine clearance compared to F344, whereas F344 have greater AMPH-induced dopamine clearance via DAT (Gulley *et al.*, 2007). Further research is necessary to determine to what extent, if any, differences in DAT function underlie the heritable behavioral associations observed here in response to AMPH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Mean ($\pm$ SEM) number of AMPH self-infusions earned during 60-min contingent self-administration sessions during autoshaping sessions 1-5, food restricted (20 g per day) sessions 6-8, and free feed sessions 9-11 on a FR1 schedule of reinforcement. Individual strain main effects of session were obtained for the following strains: BUF, FH, WAG, WKY, WF, DSS, ACI, BN, F344, and LEW. Strains are assigned to panels arbitrarily. No significant main effect of session was obtained for either SHR or BD9.

Figure 2.

Mean ($\pm$ SEM) number of AMPH self-infusions earned, averaged across sessions 2 and 3 at each FR value, for each strain. Individual strain main effects of FR value were obtained for the following strains: FH and WAG. No significant main effect of FR value was obtained for BUF, WKY, SHR, WF, DSS, ACI, BN, F344, LEW or BD9.

Figure 3.

Left Panel: Genetic correlation between activity in a novel environment and AMPH self-administration. Points for each strain are based on group average for distance traveled and number of AMPH self-infusions. Right Panel: Genetic correlation between novelty place preference and AMPH self-administration. Points for each strain are based on average novelty place preference ratio and number of AMPH self-infusions. Note that a preference ratio of 0.5 indicates no preference, whereas a number above 0.5 indicates a preference for the novel compartment over the familiar compartment.

Figure 4.

Mean ($\pm$ SEM) number of AMPH self-infusions earned across varying unit doses of AMPH, averaged over sessions 2 and 3 at each dose, for each strain. Individual strain main effects of dose were obtained for the following strains: BUF, WAG, FH, WKY, SHR and DSS. No significant main effect of dose was obtained for WF, ACI, BN, F344, LEW or BD9.

Figure 5.
Mean ($\pm$ SEM) area under the curve (AUC) for responding across varying unit doses of AMPH for each strain.

Phase 5: Reinstatement

Figure 6.

Mean ($\pm$ SEM) change in saline self-infusions from the first reinstatement session following saline challenge (s.c.), and the second reinstatement session following AMPH (0.3 mg/kg, s. c.) for each strain. AMPH-induced reinstatement was obtained for WKY, DSS, and WAG; the asterisk (*) represents a significant difference between AMPH and saline challenge sessions within strain, $p < 0.05$.

Figure 7. Genetic correlation between AUC during the dose response phase and the magnitude of AMPH-induced reinstatement. Points for each strain are based on average dose response AUC and change in number of saline self-infusions reinstated by AMPH challenge (0.3 mg/kg, s.c.) compared to saline challenge.

Table 1

Activity and Novelty Place Preference Ratio

Strain	n	Total Distance ($\pm$ SEM)	n	Preference Ratio ($\pm$ SEM)
BUF	14	1455 ($\pm$ 150)	12	0.55 ($\pm$ 0.05)
F344	8	4677 ($\pm$ 681)	7	0.50 ($\pm$ 0.06)
WKY	6	1323 ($\pm$ 409)	4	0.60 ($\pm$ 0.07)
ACI	6	3937 ($\pm$ 498)	6	0.63 ($\pm$ 0.03)
BN	6	3136 ($\pm$ 446)	6	0.58 ($\pm$ 0.08)
SHR	12	3958 ($\pm$ 198)	12	0.53 ($\pm$ 0.05)
LEW	8	4681 ($\pm$ 435)	8	0.60 ($\pm$ 0.03)
DSS	11	3950 ($\pm$ 196)	11	0.55 ($\pm$ 0.04)
BD9	11	1395 ($\pm$ 188)	11	0.49 ($\pm$ 0.05)
FH	9	5251 ($\pm$ 295)	9	0.55 ($\pm$ 0.06)
WF	6	2969 ($\pm$ 475)	6	0.47 ($\pm$ 0.04)
WAG	8	2398 ($\pm$ 276)	8	0.63 ($\pm$ 0.03)

Total distance is expressed as cm traveled during 30-min test. Preference ratio is expressed as duration in novel compartment divided by duration in both novel and familiar end compartments; note that preference ratios above 0.50 indicate a preference for novelty.