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A genetic animal model of differential sensitivity to methamphetamine reinforcement

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Abstract

Sensitivity to reinforcement from methamphetamine (MA) likely influences risk for MA addiction, and genetic differences are one source of individual variation. Generation of two sets of selectively bred mouse lines for high and low MA drinking has shown that genetic factors influence MA intake, and pronounced differences in sensitivity to rewarding and aversive effects of MA play a significant role. Further validation of these lines as a unique genetic model relevant to MA addiction was obtained using operant methods to study MA reinforcement. High and low MA drinking line mice were used to test the hypotheses that: 1) oral and intracerebroventricular (ICV) MA serve as behavioral reinforcers, and 2) MA exhibits greater reinforcing efficacy in high than low MA drinking mice. Operant responses resulted in access to an MA or non-MA drinking tube or intracranial delivery of MA. Behavioral activation consequent to orally consumed MA was determined. MA available for consumption maintained higher levels of reinforced instrumental responding in high than low MA drinking line mice, and MA intake in the oral operant procedure was greater in high than low MA drinking line mice. Behavioral activation was associated with amount of MA consumed during operant sessions. High line mice delivered more MA via ICV infusion than did low line mice across a range of doses. Thus, genetic risk factors play a critical role in the reinforcing efficacy of MA and the oral self-administration procedure is suitable for delineating genetic contributions to MA reinforcement.

1. Introduction

Methamphetamine (MA) is a strong reinforcer with high potential for abuse. Prolonged use of MA results in neurotoxicity, neuropsychiatric disorders, and negative social consequences (Gonzales et al., 2010; Zweben et al., 2004). Several factors contribute to the level of MA use. MA has a long half life (up to 12 h in humans) and easily penetrates neurons because of its lipophilic nature. The reinforcing properties of moderate doses of MA (e.g., 0.1 – 0.4 mg/

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kg in humans; Cruickshank and Dyer, 2009) stem from activation of motivation centers in the brain that manifest in euphoria and behavioral activation. Depending on the dose administered (typically > 1.0 mg/kg), MA can induce anxiety, cause severe headaches, and stimulate the cardiovascular system (Cruickshank and Dyer, 2009; Grilly and Loveland, 2001). Individual differences in sensitivity to rewarding and aversive effects likely influence risk for MA addiction, but few studies have examined the role of genetic factors in these individual differences.

Many animal studies that have explored the influence of genes on amphetamine-related traits have utilized single gene mutants and have examined sensitivity traits like locomotor activation and stereotypic behaviors (for review see Phillips et al., 2008). Fewer (but some) have utilized unbiased gene mapping approaches (e.g., Bryant et al., 2009; Potenza et al., 2008) or have focused on traits with greater face validity for human addiction, such as drug self-administration (Meyer et al., 2010). The relative paucity of these types of studies is understandable, because methods used for measuring amphetamine self-administration in animals have typically involved indwelling intravenous (IV) catheters and extensive training (Roberts et al., 2007), and complex trait mapping typically requires large scale studies involving many individuals. Because the IV route of drug administration is not amenable to the testing of many individuals, and poses particular challenges in mice, a species that has been used extensively to study the genetics associated with addiction-related traits (Crabbe et al., 1999; Fehr et al., 2005; Kirstein et al., 2002; Palmer et al., 2005; Phillips et al., 1998; Phillips et al., 2008), we utilized the oral route of drug administration in the development of a genetic mouse model of high and low MA intake.

MA and amphetamine are commonly taken orally by humans (<http://www.drugabuse.gov/ResearchReports/Methamph/methamph3.html>), are readily absorbed in the digestive system (Sulzer et al., 2005), and can lead to addiction. Nevertheless, the pharmacological onset of an ingested drug is slower than by the IV and some other routes, and can be subject to first-pass metabolism in the liver. Drugs that do not have an appetitive taste *per se*, may not be as readily identified by animals, and thus, not initially imbibed at levels producing reinforcement. Despite these potential complications, operant oral self-administration has been successfully used to measure drug reinforcement in research on alcohol and other psychoactive drugs, including cocaine and amphetamine (George et al., 1991; Meisch, 2001; Samson et al., 2000).

Using a two-bottle choice procedure, two selective breeding projects for MA drinking (MADR) demonstrated that level of MA consumption is heritable, and produced replicate sets of mouse lines that voluntarily consume different amounts of MA, but not solutions with novel tastes (Shabani et al., 2011; Wheeler et al., 2009). Earlier work in rats also demonstrated individual variation in oral consumption of d-amphetamine when offered vs water (Heyne and Wolffgramm, 1998; Galli and Wolffgramm, 2004). Subsequent research in the selected mouse lines established a genetic relationship between MA intake and sensitivity to the rewarding and aversive effects of MA, such that heightened MA drinking was associated with increased sensitivity to reward and reduced sensitivity to aversion (Shabani et al., 2011a and b; Wheeler et al., 2009). Furthermore, the high and low MA drinking selected lines started at the same initial level of intake, but the high MA drinking line (MAHDR) escalated their MA intake within the first 4 days of access to MA, whereas the low MA drinking line (MALDR) reduced their intake across the same time period (Shabani et al., 2011a). The face validity of this genetic animal model of MA addiction would be further strengthened by demonstrating a difference in MA reinforcement using operant methods. Thus, we tested MADR line mice for operant oral self-administration of MA, with methods developed using mice from the founding population of the selected lines. We also tested MADR line mice for acquisition of intracerebroventricular (ICV) self-

administration of MA. Drug is rapidly delivered to the brain via this route, and we chose it over an IV route because of greater success in maintaining ICV cannulae in mice.

2. Materials and Methods

2.1. Animals

All procedures were approved by the Institutional Animal Care and Use Committees of the Portland VA Medical Center or Oregon Health & Science University. Animals were housed 2–4 per cage ($28.5 \times 17.5 \times 12$ cm) on Bed-O-CobTM bedding with same-sex littermates. Mice were provided free access to rodent chow (Purina 5001TM, 4.5% fat content; Animal Specialties Inc., Hubbard, OR, USA) and water, except during water or food restriction periods early in training (as detailed below) and during testing. The animal vivarium was maintained at $21 \pm 1^\circ\text{C}$ on a 12:12 h light:dark schedule.

Selective breeding procedures and response to selection for the two sets of MADR and lines have been published (Shabani et al., 2011; Wheeler et al., 2009). These lines were derived from the F2 cross of the inbred C57BL/6J (B6) and DBA/2J (D2) strains (B6D2F2), corresponding with use of these genotypes in prior gene mapping and expression profiling studies of behavioral traits related to addiction (Fehr et al., 2005; Janowsky et al., 2001; Kirstein et al., 2002; Mulligan et al., 2006; Palmer et al., 2005; Phillips et al., 1998; Tabakoff et al., 2008). A two-bottle choice drinking procedure, with access to MA versus water, was used to identify mice that consumed low and high amounts of MA. Breeding pairs were selected to produce offspring based on level of consumption. By the end of 4 generations of selection (S4), high line mice consumed about 6 mg MA/kg/18-h, compared to almost undetectable intake in low line mice. All mice used in these studies were drug- and experiment-naïve and 89–101 days of age at experiment onset.

2.2. Drugs

(+)-Methamphetamine hydrochloride was provided by the NIDA drug supply program (Research Triangle Institute, Research Triangle Park, NC, USA) or purchased from Sigma (St. Louis, MO). For ICV delivery, MA was dissolved in artificial cerebrospinal fluid (aCSF; in mM, 128 NaCl, 3.9 KCl, 1.2 CaCl_2 , 1.0 MgCl_2 , 25 NaHCO_3 , 0.3 NaH_2PO_4 pH 7.3) for administration into the lateral ventricle. Angiotensin-II (Sigma, St. Louis, MO, USA), for cannulae patency verification, was also dissolved in aCSF. MA and saccharin (sodium salt, Sigma, St. Louis, MO) were dissolved in autoclaved water for drinking solutions.

2.3. Apparatus

Mouse operant chambers ($22 \times 18.75 \times 12.5$ cm; Med-Associates Inc., St Albans, VT) were equipped with two retractable levers (2 cm above the rod floor), a cue light above each lever and a house light. For the oral self-administration procedure, responding on the active lever under the schedules described below resulted in cue light illumination and sipper access, and volume changes were measured to the nearest 0.05 ml using calibrated 6 ml pipettes fitted with ball bearing sipper tubes. For the ICV procedure, during food training, the cue light illuminated and a pellet dispenser delivered food pellets (BioServe) to a hopper located between the two levers. During the ICV MA phase, responding resulted in cue light illumination and 0.1 μl MA was remotely delivered over 2 seconds to the ICV cannula through a prefilled line connected to a single channel fluid swivel.

Locomotor activity was measured using AccuScan automated activity monitors (AccuScan Instruments, Inc., Columbus, OH, USA). Each activity monitor was enclosed in an outer chamber that was designed to provide internal light and ventilation, and attenuate external

sound. Movement in the 40 × 40 × 30 centimeter space was recorded by eight evenly spaced photocell beams and receptors located on an x-y axis 2 cm above the chamber floor. Photocell beam interruption data were translated into distance travelled in centimeters.

2.4. Oral Self-administration Procedure

2.4.1. Lever Training and Saccharin Fading—The procedure and set up were modified from previous work for measuring ethanol reinforcement (Ford et al., 2009). B6D2F2 mice were used to develop oral MA self-administration procedures (see Supplementary Materials), which, to our knowledge, have not been previously used. Using these general methods, female MADR-2 mice from selection generations S2–S3 were subsequently examined for MA seeking and oral MA self-administration behaviors (n=28–32 mice/line). Female mice were used because only females were available, no sex differences were found in the F2 study, and our previous data have demonstrated that high and low MADR line mice of both replicates show large differences in MA intake, regardless of sex (Shabani et al., 2011a). Animals were trained to respond for access to a 0.2% w/v saccharin (0.2S) solution on a FR1 schedule during daily, 60-min trials for up to 16 trials. As in the F2 mice, to expedite lever training, mice were water restricted until they met the training criterion of > 10 reinforcers/trial. The sipper tube was presented for 60 sec for the first 5–16 trials and subsequently presented for 30 sec. Responses on the inactive lever (location counterbalanced across animals) were recorded, but had no scheduled consequences. The first cohort of MADR-2 mice (n=28) reached the lever training criterion in 16 trials, whereas the second cohort (n=32) did so in 8 trials. The discrepancy was due to exposure of the first cohort to a lever retraction contingency upon sipper presentation during the first 10 training trials that was discontinued for the second cohort of mice (due to apparent inhibitory effects of lever retraction noise on behavior). Animals that failed to meet the lever training criterion were excluded from all analyses (MAHDR-2 mice, n=1; MALDR-2 mice, n=6). Following lever training, MA was introduced into the 0.2S solution in a step-wise fashion and the saccharin was faded out according to the schedule and FR requirements shown in Table 1. Sessions were run 5 days/week (Monday-Friday). We increased the number of sessions for each parameter change (change of solution and schedule), compared to the F2 study (see Supplementary Materials Table S1), so that data were collected on the same days of the week, relative to each change. This also allowed for further stabilization of behavior associated with each fluid type. The pattern of appetitive responding was recorded as lever responses, and volume consumed was recorded (volume changes were measured to the nearest 0.05 ml). A control group was included for which the saccharin fading schedule matched that of the MA group, but MA was never added to the solution.

2.4.2. Lever Reversal (Tracking)—*This procedure was used to determine whether animals would track the location of the active lever (please also see data from B6D2F2 mice in Supplementary Materials Figure S2).* During these trials, a 40 mg/l MA solution was presented on a FR2 schedule and the programmed consequences of the active and inactive levers were switched. Data were collected for 4 trials following lever reversal.

2.4.3. Locomotor Activity—Amphetamines elicit locomotor activation and sensitization in mice (Phillips et al., 1994; Pierce and Kalivas, 1997). To determine if amount of locomotor behavior corresponded with amount of MA consumed during operant trials, as it did in F2 mice (see Supplementary Materials Figure S3), locomotor activity was measured every Friday for 15min, beginning 10–15 min after the oral self-administration trial ended on that day. The activity assessments also included an evaluation of baseline activity collected on two days prior to initiation of oral operant self-administration.

2.5. ICV Procedure

2.5.1. Food Training Prior to ICV Self-administration—Male MADR line mice from selection generations S3–S5 were restricted to 2 grams of food daily for the first few days of food training. Mice were placed into individual operant chambers and allowed to lever-press for 20 mg food pellets on a FR1 schedule of reinforcement for two 30-min sessions daily. Lever presentation and house light illumination signaled the beginning of each session. The location of the active lever was counterbalanced across animals. The number of operant responses required to obtain a pellet was increased until the animal responded consistently on an FR10 schedule ($> 10 \pm 3$ pellets for 3 consecutive days, 7:1 active-to-inactive lever responding). Food restriction was lifted as the FR requirement was increased by adding 1 gram of food per day in the home cage over a few days until mice were on an *ad libitum* diet prior to surgery. Sessions were run 5 days/week (Monday-Friday).

2.5.2. Surgery—After food training, mice were anesthetized using isoflurane and placed onto a stereotaxic frame for cannulae placement above the lateral ventricle (AP: -0.46 , ML: ± 0.90 , DV: -1.75 ; Paxinos and Franklin, 2001). The skull surface was exposed and anchor screws (000–120 cap screws; Small Parts, Miami Lakes, FL, USA) were placed anterior to Bregma and posterior to Lambda to secure the dental acrylic head mount to the skull. The 26 gauge guide shaft was surrounded by a threaded Teflon pedestal (3 mm above, 5 mm pedestal, 3 mm below; Plastics One, Roanoke, VA USA) and implanted just dorsal of the Corpus Callosum. A dummy cannulae cap was threaded onto the pedestal when not in use. Mice were singly housed after surgery to prevent damage to head mounts and allowed one week to heal before returning to operant training.

2.5.3. Injector Construction and Cannulae Verification—Injectors were constructed from stainless steel 21 gauge hypodermic tubing. The tubing was cut to a length of 10 mm and soldered onto a 26 gauge, 12 mm section of tubing to act as a depth stop. Fused silica glass tubing ($150 \mu\text{m}$ od $\times$ $75 \mu\text{m}$ id) was inserted into the 26 gauge tubing and cut to protrude 1.0 or 1.5 mm from the end of the 26 gauge guide cannulae. During MA self-administration, the injector was attached to a MA-filled line of PE20 tubing on the swivel assembly, which passed through a plastic cap that threaded onto the pedestal and held the injector in place.

To verify the placement of cannulae and ensure that microinjectors were penetrating the lateral ventricle, human angiotensin-II was administered one week post surgery. Angiotensin-II induces a short-latency robust drinking behavior in mice and rats when administered into the ventricular system (Johnson and Epstein, 1975; Johnson et al., 2003; Johnson et al., 2003). In the present study 25 ng of angiotensin-II was administered in 500 nl at a rate of 10 nl/sec. Mice were habituated to the chamber in which they would be tested overnight to ensure they knew the location and function of the water bottle. The latency to drink, bout length, and weight of the water bottle before and after the test was recorded. Mice that did not drink within 5 minutes of the injection were tested a second time with a longer injector. Mice that failed to drink were removed from the study.

2.5.4. ICV MA Self-administration—Acquisition of MA self-administration began with responding for 1 μg MA/0.1 μl infusion on a FR5 schedule in daily, 3-h trials. Completion of the response requirement resulted in lever retraction (10 sec), stimulus light illumination (2 sec) and infusion over a 2-sec period. During the first day of transfer from food to MA, mice were removed from the chamber if they administered more than 25 μg in the first 900 sec to avoid over-dose or creating an aversion. Once they consistently averaged 4 infusions/h for 3 consecutive days ($\pm 25\%$ of the first day), mice were assigned to receive higher (2.5, then 5.0 μg /infusion) or lower (0.5, then 0.1 μg /infusion) doses. Each subsequent dose was

administered for 4 days. Once the mouse completed its assigned side of the dose curve, it was exposed to the remaining doses. To assess if the two lines administered MA differently, data from the last two days of each dose were averaged. Unlike the MAHDR mice, the MALDR mice inconsistently maintained responding for 1 μ g infusions of MA. In order to re-establish operant responding in the MALDR mice before introducing a new MA dose, it was sometimes necessary to intercalate a few days of food-reinforced training. The highest dose (5.0 μ g/infusion) was not tested in MALDR mice.

2.6. Statistical Analysis

Data were analyzed using Statistica software (StatSoft, Tulsa, OK, USA; version 9). Data from the operant oral self-administration study were analyzed by analysis of variance (ANOVA) with repeated measures when appropriate. Independent variables were lever, group (MA or control), line, and trial or session (session was the average of the last two trials at each condition). Two-way interactions were examined using simple effect analyses, followed by post hoc Newman-Keuls mean comparisons when appropriate. Correlations were determined using Pearson's r . ICV self-administration data were examined using non-parametric statistics.

3. Results

3.1. Operant Oral MA Self-administration in MADR Mice

3.1.1. Responding in MA-reinforced and Control Group Mice—Inclusion of the control group in this study allowed for the evaluation of appetitive behavior (active lever responding) for an artificial sweetener that mimics natural sweet reinforcers, compared to this behavior for MA presented in the sweetener. These groups were also compared for responding on the active lever after saccharin fade-out.

Significant differences in appetitive behavior between the MA-reinforced and control groups for the two lines were observed (Figure 1A). There was a significant line $\times$ group $\times$ session ($F(6,797) = 6.8, p < .001$) interaction, and subsequent analyses revealed changes in behavior across sessions that were significantly different for the MA compared to control groups for both lines (MALDR-2: $F(6,144) = 4.2, p < .001$ and MAHDR-2: $F(6,150) = 3.3, p < .01$ for the group $\times$ session interaction). For MAHDR-2 mice, there were significant differences in responding between the control and MA group during every session. The MAHDR-2 MA group showed an increase in responding during saccharin fade-out, when the schedule was changed to FR2 ($p < .001$ for the effect of session). For the MAHDR-2 control group, there were significant differences in responding across sessions ($p < .05$), but the differences were small and could not be attributed to the increase in FR schedule (Figure 1A). In stark contrast, for MALDR-2 mice, there was a significant difference between the control and MA group during only a single session (Figure 1A). Changes in responding across session were not significant in the MALDR-2 MA group, but there was a significant increase in responding in the control group ($p < .01$ for the effect of session) that was associated with the increase in FR schedule. Inactive lever pressing data are not shown, but inactive lever pressing was significantly lower than active lever pressing for both the MA group ($F(1,29) = 91.7, p < .001$) and control group ($F(1,22) = 49.3, p < .001$), regardless of line. Means across sessions for inactive lever presses ranged from 7.3 ± 1.4 to 13.8 ± 1.8 for the MA group and 6.5 ± 1.0 to 14.1 ± 3.2 for the control group.

When data across sessions for the two lines were directly compared, there was a significant line difference for both the MA group ($F(6,168) = 5.0, p < .001$) and control group ($F(6,126) = 3.0, p < .01$). For the MA group, responding was not significantly different between the lines during the MA fade-in period, when saccharin concentration in the solution was high.

However, during saccharin fade-out, when the FR schedule was increased, lever pressing for MA was significantly higher in MAHDR-2 than MALDR-2 mice in every session. For the control group, MALDR-2 mice generally exhibited higher active lever responding for saccharin or for the saccharin-associated lever, compared to MAHDR-2 mice.

3.1.2. Volume Consumed in MA-reinforced and Control Group Mice—Analysis of volume consumed from the sipper tube (Figure 1B) revealed a significant line $\times$ group $\times$ session interaction ($F(6,294) = 4.5$, $p < .001$). When effects of group were examined within each line, there was a significant group $\times$ session interaction for only MALDR-2 mice ($F(6,144) = 13.3$, $p < .001$). There were changes in volume consumed across sessions in both the MALDR-2 control ($p < .001$) and MA ($p < .01$) groups. Further, volume consumed differed between the MALDR-2 MA and control groups during every session except session 1, when there was no MA in the solution for either group. For MAHDR-2 mice, although there were no significant results involving group, there was a significant decrease in volume consumed across sessions ($F(6,150) = 12.0$, $p < .001$), as saccharin was removed from the solutions.

3.1.3. MA intake (mg/kg)—Consistent with their selection phenotype, MA intake was significantly greater in MAHDR-2, than MALDR-2, mice (Figure 2A). There was a significant line $\times$ session interaction ($F(5,255) = 2.8$, $p < .05$), and the lines differed significantly in intake during every session.

For physiological effects of MA to influence MA intake, a sufficient amount of MA must be initially consumed. To examine this, MA intake was examined for the two selected lines for the initial four trials, when MA was first present in the offered solution (20 mg MA/l in 0.2% Saccharin; Figure 2B). MALDR-2 and MAHDR-2 consumed a similar amount of MA during the first trial and then the lines differed significantly in intake ($F(3,84) = 7.7$, $p < .001$ for the line $\times$ trial interaction). MA intake remained consistent across trials in MAHDR-2 mice, but declined significantly in MALDR-2 mice after the first trial, indicating an avoidance of the solution, even in though MA was offered in saccharin.

3.1.4. Lever Reversal—Data for the lever reversal phase are shown in Figure 3. For the MA group (Figure 3A), both selected lines changed their responding in accordance with the change in lever assignment ($F(4,104) = 8.9$, $p < .001$ for the line $\times$ lever $\times$ trial interaction); however, MAHDR-2 mice directed their behavior more rapidly toward the new MA-reinforced lever. MAHDR-2 mice exhibited significant differences in level of inactive vs active lever responding during all trials ($F(4,56) = 34.9$, $p < .001$ for the lever $\times$ trial interaction). In MALDR-2 MA group mice, differences in responding on the two levers were present only during the baseline trial and the first trial after lever reversal ($F(4,48) = 19.0$, $p < .001$ for the lever $\times$ trial interaction). Thus, responding in the MAHDR-2 mice was higher for the MA-reinforced, compared to inactive lever at the end of the lever reversal phase, whereas levels of responding were similar on the two levers in MALDR-2 line mice. In support of greater motivation for the new reinforced lever in MAHDR-2 mice, the ratio of reinforced to non-reinforced lever responding for trial 4 was 2.8 for MAHDR-2 mice and 1.5 for MALDR-2 mice.

For the control group (Figure 3B), both selected lines also changed their behavior in accordance with the change in lever assignment ($F(4,84) = 5.0$, $p < .01$ for the line $\times$ lever $\times$ trial interaction); however, it was MALDR-2 line mice that more clearly directed their behavior toward what was once the saccharin-reinforced lever. MAHDR-2 mice responded more on the active than inactive lever only during the baseline trial ($F(4,44) = 11.8$, $p < .001$ for the lever $\times$ trial interaction), whereas responses of MALDR-2 mice on the active vs inactive lever differed both at baseline, and during the final trial ($F(4,40) = 13.0$, $p < .001$ for

the lever $\times$ trial interaction). This suggests that MALDR-2 mice showed stronger tracking of the lever that was once associated with access to saccharin.

3.1.5. Locomotor activity—Locomotor activity levels were positively correlated with MA intake in the F2 mice (see Supplementary Materials Figure S3). This factor was also examined in the selected lines and expanded to include all phases of the study. For the two activity measurements obtained prior to initiating the operant self-administration procedures (Figure 4A), there was a significant line $\times$ day interaction ($F(1,51) = 8.0$, $p < .01$). On both days, MALDR-2 mice were more active than MAHDR-2 mice, with the difference being somewhat greater on the second day. This line difference can also be seen in the locomotor activity scores of the control groups on subsequent days (Figure 4B). For activity measures following operant sessions (Figure 4B), there was a significant line $\times$ group $\times$ session interaction ($F(6,288) = 2.4$, $p < .05$). For MAHDR-2 mice, distance traveled during the 15-min test was significantly greater in the MA group than in the control mice for every activity test except the first, when no MA was present in the drinking solution ($F(6,150) = 9.2$, $p < .001$ for the group $\times$ session interaction). In addition, there were significant changes in activity level across sessions within the MAHDR-2 MA group ($p < .001$) that showed a striking parallel to the MA intake data shown in Figure 2A. In MALDR-2 mice ($F(6,138) = 2.7$, $p < .05$ for the group $\times$ session interaction), there were significant changes in activity level across sessions within the MA group ($p < .05$), but not within the control group. MA group mice differed from control group mice only during sessions when MA was present in solutions containing the higher concentration of saccharin. This is also when low line mice consumed somewhat more MA (see Figure 2A). Thus, the amount of activation in MA group mice was consistent with the line difference in MA intake.

3.2. ICV MA Self-administration in MADR Mice

MALDR and MAHDR mice reached the pre-operative food training criterion in 9.7 ± 1.0 and 10.1 ± 0.9 trials, respectively. There was no difference between the lines in the number of food pellets received throughout training. These mice were next allowed to acquire self-administration of 0.1 μ g/infusion MA. Line differences in the number of infusions obtained during the final two, 3-h trials (Figure 5) were evaluated with the Mann-Whitney U test, because the data were not normally distributed and because not all animals were tested at all doses; a number of MALDR mice did not maintain self-administration behavior for doses on the higher end of the curve and a number of MAHDR mice did not maintain responding for the lowest MA dose. All mice largely extinguished their behavior when saline was substituted for MA; however, MAHDR mice self-administered significantly more MA than MALDR mice, consistent with their difference in two-bottle choice oral MA intake (Wheeler et al., 2009; Shabani et al., 2011a) and the differences shown here for operant oral self-administration of MA. Significant line differences were detected at most doses. The total mean doses (in 3 hrs) of MA self-administered were 0.06, 0.29, 0.77, 1.93 and 2.95 mg/kg for MAHDR mice and 0.02, 0.09, 0.26, and 0.56 mg/kg for MALDR mice across the unit doses shown in Figure 5.

4. Discussion

These data are the first to show that MA reinforcing effects can be demonstrated using an operant oral self-administration procedure in rodents. In addition, these data show that levels of MA-reinforced responding and intake in both an ICV and oral self-administration procedure are related to genetic risk for voluntary MA consumption. Compared to low MA drinking line mice, high MA drinking line mice exhibited higher appetitive responding and consumption of MA in an oral self-administration procedure and acquired higher levels of ICV MA self-administration. Further, data from the first several operant sessions after MA

was introduced into the oral solution support the rapid development of avoidance of MA by MALDR-2 mice, and the continued intake in MAHDR-2 mice. MAHDR-2 mice also quickly tracked the MA-reinforced lever and maintained high appetitive responding levels, unlike MALDR-2 mice. On the other hand, MALDR-2 mice exhibited heightened reinforcement by saccharin, compared to MAHDR-2 mice. Finally, mice that consumed above a minimum threshold of MA (~0.2 mg/kg) also displayed evidence of behavioral activation by the drug.

Two bottle choice assessments of consumption and preference for solutions with different tastes, including bitter quinine, suggest that the difference in consumption of MA between the selected lines is not due to a difference in taste sensitivity (Shabani et al., 2011a; Wheeler et al., 2009). Operant ICV MA self-administration bypasses potential orosensory factors completely; thus, the line difference in MA self-administration via this route supports a difference in the effects of MA in these lines. Results from place and taste conditioning studies indicate that MAHDR and MAHDR-2 mice are sensitive to the rewarding effects of MA, but have greatly reduced sensitivity to the aversive effects of MA, whereas MALDR and MALDR-2 mice exhibit the opposite pattern of sensitivities (Shabani et al., 2011a and b; Wheeler et al., 2009). These factors are likely contributors to the line differences in operant MA self-administration. Differential learning abilities between the lines are not likely to account for the difference in MA self-administration, since the lines exhibited largely comparable patterns of learning for operant self-administration of both food and saccharin. Thus, we suggest that these data support differential sensitivity of the MADR lines to the reinforcing effects of MA.

The study design that we used for operant oral MA self-administration testing allowed for the separation of appetitive and consummatory behaviors and showed that they did not necessarily correspond. B6D2F2 mice, mice of the parent genotype to the MADR lines, exhibited higher rates of responding on the active than inactive lever; however, their level of MA intake (consummatory behavior) was influenced by the concentration of saccharin in the offered solution. Although MA intake was higher in the presence of saccharin, the F2 mice continued to respond at high rates on the active lever and to ingest some MA even after saccharin was completely faded out (see Supplementary Materials Figure S1). A similar result was found in the high MA drinking line, however, their appetitive responding and MA intake were considerably higher than for the low MA drinking line. These data suggest that selective breeding for differential MA consumption in a two-bottle choice procedure resulted in a genetically correlated difference in sensitivity to MA reinforcement. Further, that the lines consumed similar amounts of MA during the first trial during which drug was offered, and that intake declined in the following trials only in MALDR-2 mice (Figure 2B), indicates markedly different responses to the initial post-ingestional effects of MA in the two lines. This result is consistent with previous findings for two-bottle choice drinking (Shabani et al., 2011a).

In our study in the MADR-2 lines, responses of an MA and non-MA control group were compared. In high MA drinking line mice, significantly more active lever responses occurred in the MA group than the control group. Number of presses in the MA group largely doubled when the schedule changed from FR1 to FR2, whereas a schedule-specific increase was not seen in the control group. In low MA drinking line mice, responses of the MA and control groups were more similar, although it is interesting to note that the schedule change had a larger impact on the behavior of the control than the MA group in this line. These results suggest that MA had a larger impact on appetitive behavior in the high MA drinking line, and saccharin or the light cue associated with saccharin had a larger impact in the low MA drinking line. Even more striking are the line differences in consummatory behavior. High MA drinking line mice reduced solution consumption as saccharin was

removed, but continued to consume the MA-containing solutions. The amount of MA consumed (~0.3 mg/kg/hr) by the MAHDR-2 mice after saccharin fade-out is similar to that consumed in a two-bottle choice procedure (~6 mg/kg/18-h = 0.33 mg/kg/hr), if one simply divides equally across the longer time span of access (Shabani et al., 2011a; Wheeler et al., 2009), though we acknowledge that we do not have data for pattern of consumption. Similar amounts of 0.1 – 0.5 mg/kg of amphetamines are known to induce rewarding and activating effects in rats, mice, and humans (Cunningham and Noble, 1992; Grilly and Loveland, 2001; Shabani et al., 2011a; Wheeler et al., 2009).

Data for saccharin alone in the current study suggest that voluntary two-bottle choice consumption of saccharin does not predict sensitivity to the reinforcing effects of saccharin, as the MADR lines differed little in saccharin intake or preference in previous two-bottle choice studies (Shabani et al., 2011a; Wheeler et al., 2009). Drugs of abuse are known to have effects on the processing of natural rewards (Anselme, 2009). However, the current data suggest that the high MA drinking line mice have reduced sensitivity to the reinforcing effects of a natural reward, compared to the low MA drinking line, in the absence of prior MA exposure. Since some neural processes that are activated by both natural rewards and drugs show differential levels of activation (Deadwyler, 2010), it would be of interest to examine activation patterns for the low and high MA drinking line mice for each reward type to get at differences in neural processing.

It should be noted that in both lines (and in F2 mice; see Supplementary Materials Figure S1), active lever pressing remained high, even when there was only water in the presented tube (for the control group) and in some cases, when little solution was consumed. That high levels of active lever pressing accompanied significant reductions in consumption suggests that appetitive behavioral responses for a reinforcer were resistant to changes in value of the reinforcer. This phenomenon is especially robust in some mouse strains when cues associated with a previous reward remain present (Malkki et al., 2011; Thomsen and Caine, 2011). It has been argued that initial instrumental learning is mediated by goal-directed action-outcome (A–O) associations, meaning that an animal voluntarily directs its actions to reach a specific outcome (Killcross and Coutureau, 2003). However, goal-directed performance is cognitively demanding and as training continues, instrumental behavior can be driven by stimulus-response (S–R) associations or habits that are insensitive to goal value. Studies in rats show that approach responses, such as magazine approach remain sensitive to motivational shifts and devaluation procedures, but that instrumental responses, such as lever pressing do not (Nelson and Killcross, 2006). Approach and consumption behaviors remain sensitive to changes in goal/reward value because of their closer proximity to goal/reward than instrumental responses such as lever pressing (Simon et al., 2009). In our study, fluid consumption dropped with saccharin fade-out. However, remarkably, even low MA drinking line mice that display a strong aversion for MA (Shabani et al., 2011a and b; Wheeler et al., 2009) and consumed little fluid during operant sessions after MA was introduced maintained high levels of appetitive responding. This is not as surprising as it might seem, since rats and, even more so, mice are resistant to extinction of appetitive responses particularly when reinforcer-associated cues are present (Shelton and Beardsley, 2008; Yan and Nabeshima, 2009). However, it has been suggested that approach and consumption behaviors are more dependent on Pavlovian conditioning, whereas appetitive behavior depends more on instrumental learning (Nelson and Killcross, 2006), and that Pavlovian conditioning cues can interfere with learned instrumental responses (Holmes et al., 2010; Lederle et al., 2011).

The independence of appetitive and consummatory behaviors seen here is similar to results for some ethanol studies in rodents. In the case of ethanol, both genetic and neurochemical substrates have been examined (Samson and Czachowski, 2003). For example, two selected

lines of rats, both bred for high ethanol intake, but from different progenitor populations, consumed similar amounts of ethanol, but exhibited different levels of appetitive responses (Files et al., 1998). Pharmacological manipulations of GABA and glutamatergic systems affected ethanol consumption, but did not affect appetitive behaviors (Czachowski et al., 2006; Ford et al., 2007), whereas manipulation of the D2 receptor affected appetitive behavior for ethanol, but did not affect ethanol consumption (Czachowski et al., 2002). Pharmacological manipulations of other neurochemical substrates suggest similar conclusions, though some manipulations can affect both behaviors (Ford et al., 2009; Freedland et al., 2001; Sharpe and Samson, 2001). To dissociate appetitive and consumption behaviors, some studies have employed a model in which responding and reinforcer access (20 continuous min or more) are procedurally separated (Samson and Czachowski, 2003; Samson et al., 2000). It is possible that using a similar procedure would have resulted in higher levels of intake in the current study

Strong evidence of MA reinforcement in both B6D2F2 mice (see Supplementary Materials Figure S2) and MAHDR-2 mice comes from their behavior during the lever reversal procedure. The more rapid acquisition of responding on the newly active lever in MAHDR-2 mice may suggest heightened sensitivity to MA reinforcement compared to mice from their originating population. The reversal procedure has been used to provide evidence that animals can track the location of the reinforced manipulandum and to demonstrate their motivation to respond on the newly active lever (or other operant requirement; e.g., nosepoke) (Thomsen and Caine, 2006, 2007). MA group MAHDR-2 mice rapidly reversed their lever responding so that it was directed toward the newly active lever and they responded more on the new MA-paired lever than the non-reinforced lever by the second trial of lever reversal. MALDR-2 mice also demonstrated tracking of the active lever; however, at the end of the fourth trial after reversal they exhibited equivalent behavior directed toward the new active and inactive levers, suggesting neutrality. Data for the F2 mice were intermediate to those for the selected lines. It is possible that more complete reversal in the F2 mice would have been seen with additional days of testing. Control group responses during lever reversal were also very interesting and reflected the higher motivation of the MALDR-2 line mice for cues associated with the tube that had contained saccharin without MA.

MA consumed in the operant oral procedure was strongly associated with behavioral activation in both F2 (see Supplementary Materials Figure S3) and MADR-2 mice. Consistent with their greater MA intake, MA group mice of the MAHDR-2 line exhibited a greater difference in locomotor activity when compared to their control mice than did MALDR-2 MA group mice. The lowest effective range of d-amphetamine doses for increased motor and operant behaviors in rats is in a similar dose range (0.1–0.4 mg/kg) (Grilly and Loveland, 2001). In humans, similar amounts of MA or amphetamine have been shown to induce subjective rewarding effects. For example, oral doses of 5–30 mg of MA in humans (which would be a dose of 0.07 – 0.43 mg/kg for a 70 kg person) induce heightened motor activity and euphoric responses, whereas higher doses than 55 mg (~ 0.8 mg/kg) induce anxiety, paranoia and hallucinations (Cruickshank and Dyer, 2009; Grilly and Loveland, 2001).

5. Conclusions

MA has potent reinforcing effects via multiple routes of administration. We have developed a genetic mouse model consisting of lines of mice that consume different amounts of MA and also show differential sensitivity to the reinforcing, rewarding and aversive effects of MA. The operant oral self-administration procedure developed here shows promise for the study of mechanisms associated with MA reinforcement, for example, by examining the

effects of pharmacological agents on self-administration behavior in high MA drinking line mice. Furthermore, because drug seeking is chained with drug taking in this procedure, other behaviors important in MA addiction, such as habitual learning, could be assessed (Zapata et al., 2010).

Highlights

- Breeding for methamphetamine (MA) drinking strongly impacts MA reinforcement.
- Genetic factors influence susceptibility to MA seeking and use.
- An operant oral self-administration method can be used to measure MA seeking and reinforcement.
- MA has potent reinforcing effects via multiple routes of administration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Appetitive (A) and consummatory (B) behaviors in MAHDR-2 and MALDR-2 mice. The saccharin and MA concentrations offered to the MA group mice (closed symbols) during the operant sessions are indicated below the x-axis. For control group mice (open symbols), the indicated saccharin concentrations were offered, but the MA concentration was always zero. The FR schedule applies to both groups and the vertical dotted line separates FR1 from FR2 sessions. Shown are mean $\pm$ SEM values depicted as 2-day averages of data from the two consecutive days when solutions were not changed. * $p < .05$, ** $p < .01$, and *** $p < .001$ for differences between MA and Control groups. $N = 10-15$ /selected line. MAHDR-2: MA high drinking replicate 2; MALDR-2: MA low drinking replicate 2.

A

B

Figure 2.

MA intake in MAHDR-2 and MALDR-2 mice. Data are for the same animals as in Figure 1. Note that session 1 is excluded from Panel A because no MA was present in the offered solution during this session. Shown in A are mean $\pm$ SEM values depicted as 2-day averages as described in the legend for Figure 1. MAHDR-2 mice consumed significantly more MA than MALDR-2 mice. Shown in B are mean $\pm$ SEM active lever response and intake data for each of the first 4 operant trials during which MA was present in the drinking solution. When these data were examined, MALDR-2 and MAHDR-2 mice consumed similar amounts of MA during the first trial and then differed significantly thereafter. ** $p < .01$ and *** $p < .001$ for differences between the MAHDR-2 and MALDR-2 lines.

Figure 3.

Lever presses in MAHDR-2 and MALDR-2 mice during the lever reversal phase of the study. Shown in the top panels (A) are mean $\pm$ SEM responses for the MA group mice for the trial just prior to lever reversal (baseline) and for four trials after lever reversal. Shown in the bottom panels (B) are similar data for control group mice. For all trials, the schedule was FR2 and the solution offered for the MA group was 40 mg/L MA and for the control group was water. * $p < .05$, ** $p < .01$ and *** $p < .001$ for the difference in responding between levers.

Figure 4.

Locomotor activity in MAHDR-2 and MALDR-2 mice measured during 15-min tests as distance in cm. The first two locomotor activity tests (A) were obtained on two consecutive days, one day prior to initiation of operant trials, when mice were naïve to the operant chambers and to MA. Subsequent activity measures (B) were taken 10–15 min after the oral operant data shown in Figure 2 were collected. These measures were corrected for baseline activity from day 2 (A). The saccharin concentration, MA concentration and FR schedule information shown below the x-axis is as described for Figure 1 and indicates the parameters during the operant session prior to activity assessment. Values are mean $\pm$ SEM. * $p < .05$, ** $p < .01$, and *** $p < .001$ for line (A) and group differences (B). MAHDR-2: MA high drinking replicate 2; MALDR-2: MA low drinking replicate 2.

Figure 5. Acquisition of ICV self-administration in MAHDR and MALDR mice. Shown are mean $\pm$ SEM number of MA infusions during 3-hour trials under an FR5 schedule. The numbers of animals contributing to the self-administration data at a given dose are indicated in brackets near each symbol. * $p < .05$ and ** $p < .01$ for differences between the MAHDR and MALDR lines. MAHDR: MA high drinking replicate 1 mice; MALDR: MA low drinking replicate 1 mice.

Table 1

Operant schedule and solutions offered to MA group mice.

Operant phase	Sac (%)	MA (mg/l)	FR	Number of operant trials
MA fade-in	0.2	0	1	5
	0.2	20	1	5
	0.2	40	1	5
Saccharin fade-out	0.2	40	2	5
	0.1	40	2	5
	0.05	40	2	5
	0	40	2	5
Lever Reversal	0	40	2	4

Note: control group mice had the same operant schedule and the solutions offered were the same except that MA was 0 mg/l throughout. Sac: saccharin; MA: methamphetamine.