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Opioid sensitivity in mice selectively bred to consume or not consume methamphetamine

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Abstract

There has been little investigation of genetic factors and associated mechanisms that influence risk for development of methamphetamine (MA) dependence. Selectively bred mouse lines that exhibit high (MAHDR) or low (MALDR) levels of MA intake in a two-bottle choice MA drinking (MADR) procedure provide a genetic tool for this purpose. These lines were used to determine whether opioid sensitivity and MA intake are genetically associated, since opioid mediated pathways influence some effects of MA. . Sensitivity to the analgesic effects of the μ -opioid receptor (MOP-r) agonist fentanyl (0.05, 0.1, 0.2, 0.4 mg/kg) was examined using two acute thermal tests (hot plate and tail flick) and one chronic pain test (magnesium sulfate abdominal constriction). Locomotor stimulant responses to fentanyl (0.05, 0.1, 0.2, 0.4 mg/kg) and morphine (10, 20, 30 mg/kg) were also examined. In addition, MADR was measured in the progenitor strains (C57BL/6J (B6), DBA/2J (D2)) of the F2 population from which the selected lines were generated. The MADR lines did not differ in sensitivity to the analgesic effects of fentanyl; however, MALDR mice exhibited greater locomotor activation than MAHDR mice to both fentanyl and morphine. D2 mice consumed more MA than B6 mice. The line differences for MA consumption and morphine activation recapitulated B6 and D2 strain differences for these two traits, but not strain differences previously found for opioid analgesic responses. These results support a negative genetic correlation between MA consumption and sensitivity to the stimulant effects of opioids and suggest the involvement of MOP-r regulated systems in MA intake.

Keywords

amphetamine; μ -opioid receptor; selective breeding

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Authors' contributions

ECE developed hypotheses, designed the studies, analyzed the data, and wrote the manuscript. TJP assisted with hypothesis development, study design, reviewed data analysis, and edited the manuscript.

INTRODUCTION

Not all initial users of methamphetamine (MA) develop patterns of chronic use. It is possible that genetic factors influence predisposition to escalating MA use and addiction. The MA drinking (MADR) mouse lines were developed to address the question of whether genetic factors influence MA consumption, and subsequently to identify mechanisms that influence MA intake. Two replicate sets of high (MAHDR) and low (MALDR) short-term MA drinking selected lines were serially created from the F2 cross of the DBA/2J (D2) and C57BL/6J (B6) inbred strains, with virtually identical results. After only 4 generations of selection, the MAHDR lines consumed ~ 6 mg/kg MA/18 h, whereas the MALDR lines consumed virtually no MA in a two-bottle choice drinking procedure (Wheeler et al., 2009; Shabani et al., 2011). In addition, MAHDR mice were found to be more sensitive to the rewarding and reinforcing effects of MA and less sensitive to the aversive effects of MA, compared to MALDR mice (Wheeler et al., 2009; Shabani et al., 2011; Shabani et al., 2012a; Shabani et al., 2012b). Other traits that differ between the lines are called genetically correlated traits and share genetic influence with the selection trait (Crabbe et al., 1990).

The effects of MA are mediated through interactions with several monoamine transporters. Dopamine (DA) levels in the nucleus accumbens (NAcc)/ventral striatum, a component of the ventral reward pathway, influence drug-induced reinforcement (Di Chiara et al., 2004; Taber et al., 2012). DA in the NAcc can be increased indirectly through μ -opioid receptors (MOP-r) residing on γ -aminobutyric acid (GABA) interneurons in the ventral tegmental area (VTA). MOP-r activation results in hyperpolarization of these interneurons, thereby reducing inhibitory input to VTA DA neurons and enhancing DA transmission in the NAcc (Johnson & North, 1992). In support of opioid involvement in MA reward, human studies have identified an association between a polymorphism in the MOP-r gene, *OPRM1*, and MA-induced psychosis and dependence (Ide et al., 2004; Ferraro et al., 2005; Ide et al., 2005; Doyle et al., 2006; Doyle et al., 2007). Furthermore, attenuation of MA-induced behavioral sensitization and locomotor activation was found in *Oprm1* knockout mice or after pretreatment with MOP-r antagonists (Jones & Holtzman, 1994; Chiu et al., 2005; Shen et al., 2010). However, the involvement of MOP-r in MA intake has not been examined in animals, and positive results could be relevant to the development of pharmacological treatments to reduce MA consumption.

Because research in both humans and animals has implicated MOP-r mediated systems in MA-related traits, we explored opioid drug sensitivity in the MADR lines. Such differences would suggest that opioid systems were changed in concert with selective breeding for MA consumption, and thus, might play a role in MA intake. We used nociception and locomotor traits as sensitive measures of opioid response to examine opioid drug sensitivity in the MADR lines. More than one measure of sensitivity to analgesic drug effects was used, based on different characteristics of the tests and possible differences in the underlying mechanisms associated with analgesic responses in the tests. The abdominal writhing test provides a model of more chronic pain, compared to the hot plate and tail flick tests, and has been suggested to involve DA pathways (Barton et al., 1980; Basbaum & Fields, 1984; Altier & Stewart, 1999). Because the MADR lines differ in sensitivity to the rewarding effects of MA, we hypothesized that DAergic mechanisms are involved and that the MADR

lines would be more likely to differ in opioid sensitivity in the writhing test. We also considered that similar differences between the selected lines and the inbred strains from which they were derived would provide additional evidence of genetic relationships between MA consumption and opioid drug sensitivity. Existing data show that D2 mice have greater sensitivity to the analgesic effects and reduced sensitivity to the activating effects of morphine (Belknap et al., 1989; Phillips et al., 1994; Bergeson et al., 2001). We examined MA consumption in the B6 and D2 strains, which had not been previously characterized.

MATERIALS AND METHODS

Animals

Adult B6 and D2 mice used in the MA two-bottle choice drinking study were obtained from our breeding facility (Veterans Administration Medical Center, Portland, OR) and were 70–91 days of age at the initiation of testing. Short-term selected lines are serially created to reduce fixation of trait-irrelevant genes due to inbreeding (Belknap et al., 1997). At the time these studies were completed, the replicate 1 lines had been retired and thus, replicate 2 MADR line mice (MAHDR-2 and MALDR-2) were used in the current studies. These mice were 62–110 days of age at the initiation of testing and were the MA- and experiment-naïve offspring of fifth generation parents. Selective breeding procedures for these lines have been described (Wheeler et al., 2009). Each experiment included male and female mice unless otherwise indicated. Mice were housed with dam and sire until they were weaned at 21–23 days of age and then housed in groups of 2–5 with same sex littermates (or sometimes with mice of the same strain or line and similar age to avoid single housing). Mice were housed in shoebox cages (28.5 × 17.5 × 12 cm) with Bed-O-Cob™ bedding and wire cage tops, and maintained at 21 ± 1°C on a 12:12 h light:dark schedule. Mice had free access to water and standard rodent diet (Purina 5001™, Animal Specialties Inc., Hubbard, OR) at all times, except during nociception and locomotor activity testing. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the Portland Veterans Affairs Medical Center.

Drugs

(+) Methamphetamine hydrochloride (MA) and fentanyl citrate were purchased from Sigma (St. Louis, MO). Morphine sulfate was obtained from the NIDA drug supply program (Bethesda, MD). For drinking solutions, MA was dissolved in tap water to the appropriate concentrations. For injections, morphine and fentanyl were dissolved in sterile physiological saline (0.9% NaCl, Baxter Healthcare Corporation, Deerfield, IL). All injections were given intraperitoneally (IP) at a volume of 10 ml/kg body weight.

Hot plate test

The hot plate assay examines a coordinated muscle response to a heated stimulus, during which a mouse licks its hind foot to terminate a trial (Mogil et al., 1996). Each mouse was weighed and placed individually into the cell (8×19×8 cm) of an acrylic plastic isolation chamber that had holes for ventilation. These chambers provide quick access to each animal, and help to equate body temperature prior to conducting the thermal test by preventing

huddling. Mice were housed in these chambers for 10 min and then injected with saline and returned to the chambers for 10 minutes. For hot plate testing, each mouse was placed onto a 10×10 cm aluminum hot plate that was maintained at 53°C. A bottomless box with 15 cm high acrylic plastic walls was used to prohibit escape from the hot plate. Latency to first rear paw lick (in seconds) was used as the nociceptive response (Bryant et al., 2009b). Immediately after the mouse displayed this response, it was removed from the hot plate and returned to its assigned cell in the holding chamber. If no response occurred within a cut-off time of 90 seconds, the mouse was removed from the hot plate to prevent tissue damage and the maximum score of 90 seconds was recorded. Selection of temperature and cut-off latency was based on previous work (Belknap et al., 1995; Bryant et al., 2009b). Two minutes after saline hot plate testing, each mouse was injected IP with fentanyl (0.05, 0.1, 0.2 or 0.4 mg/kg), returned to the holding chamber for 10 minutes, and then tested on the hot plate exactly as described after saline treatment. Doses were selected from previous work (Bryant et al., 2009a; Romero et al., 2010). The measure of drug response was percent of maximum possible effect (% MPE), which was generated using the following formula: (fentanyl latency – saline latency)/(cut off latency – saline latency). This data transformation corrects for differences in saline latency and approximates a more normal distribution (Belknap et al., 1989; Belknap et al., 1995). Mice were tested in 4 passes (n = 6–12 per line and sex per pass).

Tail flick test

A protracted period of 7 days was allowed post hot plate testing to ensure complete drug elimination and to reduce the possibility of a prior exposure effect. The same mice were then examined for sensitivity to the analgesic effects of the same dose of fentanyl that they had received before, using the tail flick test. This assay measures a simple nociceptive reflex, involving reflexive movement of the tail to avoid a targeted heat source (Mogil et al., 1996). Mice were weighed and placed in isolation chambers for 10 min, then injected with saline and returned to the chambers for 10 minutes. Each mouse was then gently restrained, and the distal half of its tail was dipped into a water bath (12 L capacity; 32.7×30×15 cm; VMR Lab Shop, Radnor, PA) maintained at 49°C. Latency to withdraw the tail from the water (in seconds) was recorded and if the mouse did not withdraw its tail within 15 seconds, the test was terminated to prevent tissue damage. In this case, the mouse was assigned the maximum score of 15 seconds. This test was repeated three times, with an inter-trial interval of 20 seconds, during which time the tail was dried off using a paper towel. Three trials were used based on research showing that three averaged trials improve response accuracy (Mogil et al., 1995). After completing the three trials, mice were returned to the isolation chambers. Two min after saline tail flick testing, each mouse was injected with 0.05, 0.1, 0.2, or 0.4 mg/kg fentanyl, returned to the isolation chamber for 10 min, and then tested again for tail flick latency. The measure of drug response was % MPE, as described for the hot plate test.

Magnesium sulfate abdominal constriction test

Drug- and experiment-naïve mice were used. The abdominal constriction test involves administration of a noxious substance that produces a writhing response (Hendershot & Forsaith, 1959). Isolation chambers were used as described above. Mice received injections of either saline or fentanyl (0.05, 0.1, 0.2, 0.4 mg/kg), were returned to holding chambers for

10 minutes, and then given an injection of magnesium sulfate (120 mg/kg), which is known to induce abdominal constrictions or “writhes” (Luo et al., 2010), defined as a lengthwise stretch of the torso with a concomitant concave arching of the back. The number of abdominal writhes was counted during a 5-minute observation period. . Mice were tested in 3 passes (n = 7–15 per line and sex per pass).

Locomotor response to fentanyl

Locomotor activity was measured using eight automated Accuscan activity monitors (Accuscan Instruments, Columbus, OH). Eight pairs of infrared beams and detectors mounted 2 cm above the clear acrylic plastic test chamber (40×40×30 cm) floor detected locomotion. Photocell beam breaks were automatically recorded and translated by Accuscan software to distance traveled in centimeters. The monitors were enclosed in illuminated (3.3 Watt incandescent bulb) and ventilated environmental control chambers that provided sound attenuation (AccuScan Instruments).

The first cohort of animals tested was that used in the thermal nociception assays, and was tested 7 days after nociception testing. Because of possible effects of prior testing and exposure to fentanyl, this study was repeated in a separate group of mice, which were drug- and experiment-naïve. Only female mice were available for the second study; however, there was no significant effect of sex in the initial study (n=7 per line and dose). On each day of testing, animals were moved to the procedure room for a 45–60 minute acclimation period before testing began. On each day, mice were injected and placed individually into the center of the activity monitors, where distance traveled was measured for 30 minutes, with data collected in 5-minute time bins. On days 1 and 2, mice were treated with saline, and on day 3, they were treated with 0.05, 0.1, 0.2 or 0.4 mg/kg fentanyl. Day 1 allowed habituation to the novel environment and experimenter handling, data collected on day 2 provided baseline activity data, and data collected on day 3 provided a measure of drug response. Day 2 baseline data were subtracted from day 3 drug data for each individual animal to obtain a measure of change in baseline behavior induced by drug treatment, a measure that has been used in several of our previous studies (Phillips et al., 1992a; Phillips et al., 1995).

Locomotor response to morphine

Drug- and experiment-naïve mice were used to examine sensitivity to the activating effects of morphine (10, 20 and 30 mg/kg). All experimental details were identical to those described for fentanyl. Doses were chosen from previous work (Phillips et al., 1992b). Mice were run in 4 passes (n=5–9 per sex and line per pass).

Two-bottle choice MA drinking

The identical procedure used for selection of the MADR lines (Wheeler et al., 2009; Shabani et al., 2011) was used. B6 and D2 mice were isolate housed and given 24-h access for two days to two water-filled 25-ml drinking cylinders, fitted with sipper tubes, to familiarize them with the drinking apparatus. Over the following 8 days, one tube was filled with tap water and one with MA in tap water (20 mg/l for 4 days, then 40 mg/l for 4 days). Mice had 24-h access to water throughout the study, but 18-h access to MA (MA tube removed during

hours 3 through 9 of the light cycle). The positions of the tubes were alternated every 2 days to control for side preferences. Body weight was measured every 4 days and used to calculate amount of MA consumed in mg/kg. Volume changes in the tube to the nearest 0.2 ml were used to calculate consumption and preference ratio (ml MA: total ml from both tubes during the same 18-h period) by averaging the second and fourth days for each concentration of MA.

Data analysis

Data from the two-bottle choice drinking procedure, abdominal constriction, and locomotor studies were analyzed by factorial Analysis of Variance (ANOVA) with repeated measures when appropriate, using Statistica software (Statsoft Version 9, Tulsa, OK). Possible independent variables were line, sex and drug dose or concentration, and in the case of locomotor activity, both baseline scores and drug response scores were analyzed. Two-way interactions were resolved using simple main effects analysis and the Newman Keuls test was used for post-hoc mean comparisons. Latency to lift or shake the hind paw in the hot plate test or to withdraw the tail in the tail flick test were transformed to % MPE to correct for differences in saline baseline latencies and were analyzed by Kruskal Wallis non-parametric ANOVA, because the dependent variables included cut-off latencies. Independent variables were line, sex and dose, and both latencies after saline and drug response scores were analyzed. Figures were created using Sigmaplot 2002 for Windows Version 8.0 (SPSS, Chicago, IL). All values are expressed as mean $\pm$ standard error of the mean (SEM). The criterion for significance was set at $p < 0.05$.

RESULTS

Sensitivity to the analgesic effects of fentanyl on thermal nociception in MADR mice

Figure 1 illustrates analgesic responses to fentanyl for the hot plate (Figure 1A) and tail flick (Figure 1B) tests. Data are combined for the two sexes because there were no significant effects of sex. There were no significant line differences in either baseline (saline) nociceptive responses or sensitivity to fentanyl (hot plate saline latencies; mean $\pm$ SEM: MAHDR-2: 17.7 ± 0.9 sec; MALDR-2: 17.8 ± 1.0 sec; tail flick saline latencies; mean $\pm$ SEM: MAHDR-2: 3.3 ± 0.2 sec; MALDR-2: 2.9 ± 0.1 sec). There was a significant main effect of fentanyl dose for both the hot plate test ($H(3, N=167)=91.6$; $p < 0.001$) and tail flick test ($H(3, N=166)=98.4$; $p < 0.001$). In addition, a similar percentage of each of the MADR lines was assigned the hot plate cut-off latency of 90 seconds (MAHDR-2: 45.7%; MALDR-2: 45.8%) and the tail flick cut-off latency of 15 seconds (MAHDR-2: 26.5%; MALDR-2: 28.2%). Although the same animals were tested in these two assays, only 51% of the ones that reached the cut-off latency in the hot plate test were the same ones to reach the cut-off latency in the subsequent tail flick test. Although hyperalgesia has been reported with repeated fentanyl administration, this effect was reported to occur with greater exposure and to fully dissipate within a shorter time frame (within 5 days; (Celerier et al., 2000) than the 7 days between treatments used here.

Sensitivity to fentanyl in the abdominal constriction test in MADR mice

Figure 2 shows the effects of fentanyl on magnesium sulfate-induced abdominal writhing. Female mice were more sensitive than male mice to the analgesic effects of fentanyl in this test ($F(1,93) = 5.51$; $p < 0.05$ for the main effect of sex), but there were no interactions of sex with line or dose; thus, data are shown collapsed on sex. No significant line difference was detected for the number of abdominal writhes. There was a main effect of dose ($F(4,93) = 25.18$; $p < 0.01$), indicating that fentanyl dose-dependently reduced the number of magnesium sulfate-induced abdominal writhes.

Sensitivity to the locomotor activating effects of fentanyl

Analysis of baseline locomotor activity data collected on day 2, demonstrated that the low line had greater baseline activity compared to the high line ($F(1,151) = 11.65$; $p < 0.05$; mean $\pm$ SEM: 5405 ± 204 cm for MALDR-2 and 4271 ± 175 cm for MAHDR-2). The drug response scores (day 3 minus day 2) shown in Figure 3 corrected for this difference. Data for locomotor response to fentanyl were initially analyzed with time as a repeated measure; however, the results with regard to line, sex and dose were comparable to those when data were analyzed for the total 30-minute session. There were no main or interaction effects involving sex, so data are shown for the total session with the sexes combined. Analysis of data from the cohort of mice used in the nociception assays (Figure 3A), demonstrated a greater stimulant response to fentanyl in MALDR-2 than MAHDR-2 mice. There were significant main effects of line ($F(1,51) = 8.1$; $p < 0.01$) and dose ($F(3,151) = 33.4$; $p < 0.001$). Analysis of data from mice that were fentanyl- and experiment-naïve (Figure 3B), revealed largely comparable differences, although in this case, the selected lines did not differ significantly in baseline activity level (mean $\pm$ SEM: 6144 ± 362 cm for MALDR-2 and 5417 ± 577 cm for MAHDR-2). There were significant main effects of line ($F(1,38) = 18.4$; $p < 0.001$) and dose ($F(2,38) = 30.4$; $p < 0.001$), as well as a significant line $\times$ dose interaction ($F(2,38) = 4.5$; $p < 0.05$). Simple effect analysis indicated that MALDR-2 mice were more stimulated than MAHDR-2 mice by both doses of fentanyl.

Sensitivity to the locomotor activating effects of morphine

After obtaining significant results for fentanyl, this study was performed to determine whether the selected line results would recapitulate existing results for morphine in the B6 and D2 strains. Analysis of day 2 baseline locomotor activity demonstrated that MALDR-2 mice had greater baseline activity compared to MAHDR-2 mice ($F(1,80) = 7.01$; $p < 0.05$; mean $\pm$ SEM: 5828 ± 273 cm for MALDR-2 and 4823 ± 275 cm for MAHDR-2). The drug response scores shown in Figure 4 corrected for this difference. Data were initially analyzed including time as a repeated measure; however, the results were comparable when data were analyzed for the total 30-minute session. There were no main or interaction effects involving sex, so data are shown for the total session with the sexes combined. MALDR-2 mice were more sensitive than MAHDR-2 mice to the locomotor stimulant effects of morphine. There were significant main effects of line ($F(1,88) = 29.8$; $p < 0.001$) and dose ($F(3,88) = 6.5$; $p < 0.001$) and a significant line $\times$ dose interaction ($F(3,88) = 3.7$; $p < 0.05$). Simple effect analysis indicated that MALDR-2 mice were more stimulated than MAHDR-2 mice by all doses of morphine.

MA drinking in B6 and D2 mice using a two-bottle choice test

As shown in Figure 5A, D2 mice consumed more MA than B6 mice. Repeated measures ANOVA revealed significant effects of strain ($F(1,31)=84.2$; $p<0.001$) and concentration ($F(1,31)=19.4$; $p<0.001$), and a significant strain $\times$ concentration interaction ($F(1,31)=18.7$; $p<0.001$). Simple effects analyses indicated significantly greater MA consumption in D2 compared to B6 mice for both concentrations. MA consumption in D2 mice was increased with increasing concentration. A similar strain difference was found for MA preference ratio (Figure 5B). There were significant main effects of strain ($F(1,31)=81.7$; $p<0.001$) and concentration ($F(1,31)=15.5$; $p<0.001$), with D2 mice having larger preference ratios compared to B6 mice, and B6 mice showing considerable avoidance of the MA solutions. For total volume (ml/kg) during the 18-h period when both MA and water tubes were offered (Figure 5C), significant main effects of strain ($F(1,31)=7.6$; $p<0.05$), sex ($F(1,31)=10.8$; $p<0.05$) and concentration ($F(1,31)=25.5$; $p<0.001$) were found. D2 mice consumed greater total volume compared to B6 mice (234.2 ± 10.6 ml/kg for D2 and 203.8 ± 8.8 ml/kg for B6) and female mice consumed more total volume compared to male mice (235.5 ± 11.6 ml/kg for female and 199.2 ± 6.4 ml/kg for male). More total volume was consumed when the higher concentration of MA was available compared to when the lower concentration was available (227.7 ± 7.2 ml/kg for 40 mg/l and 205.9 ± 7.1 ml/kg for 20 mg/l).

DISCUSSION

Selective breeding for high and low MA consumption has altered locomotor sensitivity, but not analgesic sensitivity, to MOP-r agonist drugs. MA consumption levels in the B6 and D2 mice indicate that the MAHDR lines are more D2-like for MA intake, while the MALDR lines are more B6-like. The MADR line difference also recapitulated this strain difference for locomotor stimulation by opioid agonist drugs; D2 and MALDR-2 mice were more stimulated than their respective B6 and MAHDR-2 counterparts (data shown here and in Belknap et al., 1989). Overall, we show a negative genetic correlation between sensitivity to the locomotor activating effects of MOP-r agonist drugs and MA consumption, providing some evidence for a change in MOP-r mediated systems as a result of selective breeding for high vs. low MA intake.

Differences in behavioral stimulation between the high and low MADR lines were found for both fentanyl and morphine. We initiated our studies using fentanyl because it has been shown to be more selective for the MOP-r subtype, while morphine acts on both μ - and κ -, but not δ - opioid receptor subtypes. Although morphine is less selective, it has higher affinity for the μ than for the κ receptor (Raynor et al., 1994; Kalvass et al., 2007). Fentanyl and morphine have similar affinity for the MOP-r, however fentanyl is 100 times more potent, compared to morphine, and crosses the blood brain barrier with greater ease (Volpe et al., 2011). Though these opioid receptor drugs have different potencies and specificity for the opioid receptor subtypes, they both produced locomotor stimulation and similar differences in response were seen between the selected lines. This supports a role for differences in MOP-r-mediated effects between the two lines. Concerns about drug carryover effects influencing the line difference results seen for fentanyl-induced locomotion led to evaluation in experimentally naïve animals. Similar results were observed,

with MALDR-2 line mice showing greater locomotor activation to fentanyl compared to MAHDR-2 mice. Completed studies (see Supplementary Data) indicate that the MADR lines do not differ in peak levels or clearance of morphine or fentanyl, suggesting that differences in sensitivity, rather than pharmacokinetic factors, are responsible for the observed behavioral differences.

Significant baseline locomotor activity differences were found for two of the three locomotor activity studies, with higher levels of baseline activity in MALDR-2 than MAHDR-2 mice. There was a tendency toward a similar difference in the third study. Clearly this difference does not account for the line difference in opioid response because (1) the drug response data were corrected for baseline activity level and (2) one might expect the line with the lower activity level (the MAHDR-2 line) to be able to show greater activation, yet the opposite result was obtained.

No differences were found between the MADR lines in sensitivity to the analgesic effects of fentanyl. Pain has been placed into two categories; phasic and tonic pain. The first refers to pain spanning minutes or less, such as that induced in the hot plate and tail flick tests, which are terminated with a simple reflexive response. Tonic pain, refers to pain of greater duration, such as that associated with the acetic acid and magnesium sulfate abdominal constriction tests (Barton et al., 1980). Analgesic drug effects in phasic pain tests have been suggested to act through direct spinal pathways that activate opioid receptors in the periaqueductal grey (PAG) brainstem region, whereas analgesic drug effects in tonic pain tests are thought to involve changes in mesolimbic DA activity in the VTA via opiate actions (Barton et al., 1980; Basbaum & Fields, 1984; Altier & Stewart, 1999). We hypothesized that the tonic pain assay would be more likely to detect a line difference in opioid sensitivity, if MA consumption were being mediated through the indirect action of opioids on DA pathways. However, the current results suggest that sensitivity to the analgesic effects of fentanyl in both pain assays does not share a common genetic mechanism with MA consumption.

Data for MA preference drinking have not been previously published for the D2 and B6 inbred mouse strains. We predicted that each of the selected lines might resemble one of the inbred strains for MA consumption, based on rapid response to selection, suggesting the possibility of a major gene effect on MA intake (Shabani et al., 2011). The D2 strain consumed more MA, making them more similar to the MAHDR lines, while the B6 strain consumed low amounts of MA, making them more similar to the MALDR lines. The D2 mice also consumed more total fluid volume; however, this difference was small compared to the large magnitude difference in MA consumption between the inbred strains. A similar strain-line association was found for sensitivity to opioid-induced locomotor activation. B6 mice have been found to show greater locomotor activation to morphine compared to D2 mice (Belknap et al., 1989; Phillips et al., 1994), and MALDR-2 mice had greater locomotor activation to both MOP-r agonist drugs compared to MAHDR-2 mice. However, these similarities did not extend to sensitivity to the analgesic effects of MOP-r drugs. It is not surprising that the selected lines did not resemble the inbred strains for all traits examined, since the lines were created to differ for a specific response and for those that share some genetic codetermination with that response. Genetic differences between inbred strains arise

by chance, rather than by selective breeding; thus, not all phenotypic differences, even if under genetic control, would be expected to share common genetic regulation in a pair of inbred strains. Overall, these results suggest that some of the same D2-like genes that have a role in greater MA consumption also have a role in reduced sensitivity to the locomotor activating effects of opioids, but not in sensitivity to opioid analgesic effects.

It is possible that the MADR lines differ in level of opioid consumption. If MA and opioid consumption are genetically related and predicted from B6 and D2 strain differences, then opioid consumption should be greater in the low than high MADR line mice. We make this directional prediction because B6 mice have been shown to consume more morphine compared to D2 mice in two-bottle choice drinking procedures (Belknap et al., 1993; Berrettini et al., 1994b). Although the possibility that there is no genetic relationship between MA and morphine intake must be considered, it is interesting to note that quantitative trait locus mapping results for MADR mice have identified a locus on proximal mouse chromosome 10 (unpublished) in the vicinity of the MOP-r gene, *Oprm1*, where morphine drinking has also been mapped (Berrettini et al., 1994a; Berrettini et al., 1994b; Ferraro et al., 2005).

The current data support additional consideration of the MOP-r system for its role in differences in MA consumption between the high and low MADR line mice. To more directly investigate this, our future studies will examine MOP-r receptor levels and distribution and the effects of MOP-r agonists and antagonists on MA drinking. The greater sensitivity of MALDR-2 mice to some effects of MOP-r agonist drugs suggests that they may possess more of these receptors in brain regions relevant to these effects. Our findings suggest that greater MOP-r activity could result in lower MA intake and that MOP-r agonists could reduce MA intake.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Sensitivity to the analgesic effects of fentanyl in MADR mice using the hot plate and tail flick tests

Shown are means ($\pm$ SEM) for the analgesic response to several doses of IP fentanyl for the (A) hot plate and (B) tail flick tests. Means are shown as % maximum possible effect (MPE) [(fentanyl latency – saline latency)/(cut off latency – saline latency)], where 90 and 15 seconds were the cut off latencies for hot plate and tail flick, respectively. MAHDR, methamphetamine high drinking line; MALDR, methamphetamine low drinking line

Figure 2. Sensitivity to the analgesic effects of fentanyl in MADR mice using the magnesium sulfate-induced abdominal writhing test.

Shown are mean ($\pm$ SEM) number of abdominal writhes (constrictions) during a five-minute observation period after IP injection of 120 mg/kg magnesium sulfate. Mice received pretreatment with IP saline or fentanyl, 10 minutes before magnesium sulfate treatment. MAHDR, methamphetamine high drinking line; MALDR, methamphetamine low drinking line

Figure 3. Sensitivity of MADR mice to the locomotor stimulant effects of fentanyl

Shown are mean ($\pm$ SEM) difference scores for intraperitoneal saline and several doses of fentanyl, created by subtracting horizontal distance traveled on saline day 2 from distance traveled on drug day 3 during 30-min locomotor activity sessions. Data shown in (A) are from mice that had been used to obtain the nociception data shown in Figure 1. Data shown in (B) are from experiment- and fentanyl-naïve mice. + $p < 0.05$ for the main effect of line. * $p < 0.05$ and *** $p < 0.001$ for the line difference at the indicated dose. MAHDR, methamphetamine high drinking line; MALDR, methamphetamine low drinking line

Figure 4. Sensitivity of MADR mice to the locomotor stimulant effects of morphine

Shown are mean ($\pm$ SEM) difference scores for IP saline and several doses of morphine, created by subtracting horizontal distance traveled on saline day 2 from distance traveled on drug day 3 during 30-min locomotor activity sessions. *** $p < 0.001$ for the line difference at the indicated dose. MAHDR, methamphetamine high drinking line; MALDR, methamphetamine low drinking line

Figure 5. Consumption of MA in D2 and B6 mice in a two-bottle choice drinking procedure
Data shown are (A) average MA consumed (mg/kg) on the second and fourth days of access to the 20 mg/l and 40 mg/l MA solutions, (B) average drug preference using data from the same days, calculated as the ratio of the volume of drug solution to the total volume of fluid consumed, and (C) average total volume consumed on the same days, corrected for body weight (ml/kg). All values are expressed as mean $\pm$ SEM. *** p <0.001 for the strain difference at each concentration. + p <0.05 and +++ p <0.001 for the main effect of strain. D2:

DBA/2J; B6: C57BL/6J. MAHDR, methamphetamine high drinking line; MALDR, methamphetamine low drinking line