Published in final edited form as:

Genes Brain Behav. 2020 June ; 19(5): e12640. doi:10.1111/gbb.12640.

Differential genetic risk for methamphetamine intake confers differential sensitivity to the temperature-altering effects of other addictive drugs

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Abstract

Mice selectively bred for high methamphetamine (MA) drinking (MAHDR), compared to mice bred for low MA drinking (MALDR), exhibit greater sensitivity to MA reward and insensitivity to aversive and hypothermic effects of MA. Previous work identified the trace amine-associated receptor 1 gene (*Taar1*) as a quantitative trait gene for MA intake that also impacts thermal response to MA. All MAHDR mice are homozygous for the mutant *Taar1*^{m1J} allele, whereas all MALDR mice possess at least one copy of the reference *Taar1*$^{+}$ allele. To determine if their differential sensitivity to MA-induced hypothermia extends to drugs of similar and different classes, we examined sensitivity to the hypothermic effect of the stimulant cocaine, the amphetamine-like substance 3,4-methylenedioxymethamphetamine (MDMA), and the opioid morphine in these lines. The lines did not differ in thermal response to cocaine, only MALDR mice exhibited a hypothermic response to MDMA, and MAHDR mice were more sensitive to the hypothermic effect of morphine than MALDR mice. We speculated that the μ-opioid receptor gene (*Oprm1*) impacts morphine response, and genotyped the mice tested for morphine-induced hypothermia. We report genetic linkage between *Taar1* and *Oprm1*; MAHDR mice more often inherit the *Oprm1*^{D2} allele and MALDR mice more often inherit the *Oprm1*^{B6} allele. Data from a family of recombinant inbred mouse strains support the influence of *Oprm1* genotype, but not *Taar1* genotype, on thermal response to morphine. These results nominate *Oprm1* as a genetic risk factor for morphine-induced hypothermia, and provide additional evidence for a connection between drug preference and drug thermal response.

Keywords

addiction; methamphetamine; μ-opioid receptor; opioids; thermal regulation; trace amine-associated receptor 1

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

1 INTRODUCTION

Chronic methamphetamine (MA) use is linked to numerous deleterious health effects and an increased mortality rate.[1,2] Genetic variation impacts risk for MA addiction[3,4] and rodent research supports the contribution of genetic variation to MA sensitivity, which could impact use. For example, in mice, genetic variation impacts locomotor[5,6] and thermal responses,[7] as well as sensitivity to rewarding and aversive effects of MA.[5,7–9] Our lab created the selectively bred MA drinking (MADR) mouse lines, comprised of MA high drinking (MAHDR) and MA low drinking (MALDR) lines, to investigate genetic influences on risk for MA intake and genetic relationships between MA sensitivity traits and MA intake. The MADR lines do not differ in the amounts of several novel tastants voluntarily consumed, including quinine, potassium chloride, and saccharin,[9,10] and they consume similar amounts of MA on the first day it is offered.[11,12] This suggests the difference in MA consumption between the lines on subsequent days is not due to differential sensitivity to the taste of MA, but rather to differences in sensitivity to effects of MA experienced on the initial day of consumption. Further, sensitivities to reinforcing, rewarding, and aversive MA effects are genetically correlated with MA intake in the MADR lines, suggesting that some of the genes impacted by selective breeding have pleiotropic influences on these traits. Accordingly, MAHDR mice operantly self-administer MA and display MA-conditioned reward, but not MA-conditioned aversion, whereas MALDR mice are phenotypically opposite in their MA-related responses.[9–11,13] Notably, following an MA injection MALDR mice become hypothermic, whereas MAHDR mice do not, a result we have consistently obtained across replicated sets of the MADR lines.[7] Although MA is typically characterized for its hyperthermic effects, at normothermic ambient temperatures MA can induce hypothermia,[7] which may be protective against MA-induced neurotoxicity.[14] Hypothermia can also increase the period during which negative associations are conditioned.[15] Thus, hypothermia experienced after MA consumption may enhance the association of MA with subjective aversive effects.

The MADR lines represent an animal model of differential genetic risk for MA use.[16] To identify the genes that may confer high vs. low risk, we performed a quantitative trait locus (QTL) analysis and identified a region on mouse chromosome 10 accounting for 60% of the genetic variance in MA intake between the MADR lines.[17] The trace amine-associated receptor 1 gene (*Taar1*), at 23.9 Mb on chromosome 10, was identified as a major contributor.[7,18] We discovered a spontaneous mutation within the coding region of *Taar1* in one of the founder strains of the MADR lines, the DBA/2J (D2) inbred strain, and found that this mutant allele ($Taar1^{m1J}$) codes for a nonfunctional form of the receptor (TAAR1).[7] The other founder strain, C57BL/6J (B6), contributes the reference allele ($Taar1^{+}$), which codes for a functional TAAR1 and is present in all 28 other mouse strains that have been examined.[19,20] TAAR1 is an intracellular g protein-coupled receptor (GPCR) activated by endogenous trace amines, monoamines and amphetamines,[21–23] and modulates monoamine transmission and reuptake.[23–25] In the 5 replicate sets of MADR lines we have produced, selective breeding for MA intake has resulted in homozygosity for $Taar1^{m1J}$ in all MAHDR mice, with the majority of MALDR mice homozygous for $Taar1^{+}$ and none possessing more than one copy of the mutant allele.[7,20] We also considered the μ-opioid receptor gene

(*Oprm1*), at 6.75 Mb on chromosome 10, for its role in the difference between the MADR lines in MA intake and found that *Oprm1* is not directly associated with risk for MA intake.[26] Rather, it serves as a "hub" for regulation by the top-ranked transcription factor differential gene expression network for MA intake risk.[17]

In addition to impacting MA intake, TAAR1 functionality impacts the hypothermic response to MA. *Taar1* knockout mice do not become hypothermic following MA treatment, whereas their wildtype littermates do.[7,27] Likewise, $Taar1^{+/+}$ mice from recombinant inbred (RI) mouse strains derived from the F2 cross of the B6 and D2 progenitors (the BXD RI strains) become hypothermic, whereas $Taar1^{m1J/m1J}$ BXD RI mice do not[18,20]. Further, D2 mice from The Jackson Laboratory, which are homozygous for the mutant $Taar1^{m1J}$ allele, are insensitive to MA-induced hypothermia, whereas D2 mice from 3 other vendors, which are homozygous for the reference $Taar1^{+}$ allele, become hypothermic after MA treatment.[20] Furthermore, $Taar1^{m1J/m1J}$ D2 mice consume significantly more MA than do $Taar1^{+/+}$ D2 or B6 mice.[20,28]

The MADR lines do not differ in thermal response to ethanol.[7] The effects of other addictive drugs on body temperature have not been examined in these mice, and may provide information about pleiotropic gene actions across drugs. Here, we investigated the effects of the psychostimulant cocaine, the amphetamine-like substance 3,4-methylenedioxy-methamphetamine (MDMA), and the opioid morphine, all of which can induce hypothermia.[29–32] The MADR lines differ in sensitivity to MA-conditioned, but not cocaine-conditioned reward and aversion,[33] suggesting distinct mechanisms contribute to these effects of MA vs. cocaine. We predicted that the MADR lines would display comparable thermal responses to cocaine, and because MDMA and MA are both amphetamine-like drugs and TAAR1 agonists,[34,35] MDMA would induce hypothermia only in MALDR mice. As MAHDR mice consume less morphine than MALDR mice[36] and a greater hypothermic response to MA corresponds with lower MA intake,[7] we predicted that MAHDR mice would exhibit greater morphine-induced hypothermia than MALDR mice. Finally, we examined associations of *Taar1* and *Oprm1* genotype with morphine-induced temperature change, and the results of this study led us to explore the potential independent and interactive influences of *Taar1* and *Oprm1* genotypes on morphine-induced thermal effects in BXD RI mice.

2 METHODS

2.1 Animals

Prior to experimentation, all mice were group-housed in polycarbonate shoebox cages (28.5 × 17.5 × 12 cm) with wire tops and Bed-O'Cobs bedding (The Andersons, Maumee, OH, USA). Mice had free access to rodent food (Purina 5001 or 5LOD PicoLab Rodent Diet; Animal Specialties, Woodburn, OR) and were maintained on a 12:12 h light:dark cycle with lights on at 0600 h. Colony room temperature was 21 ± 1 °C. All procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the Veterans Affairs Portland Health Care System (VAPORHCS) Institutional Animal Care and Use Committee.

Participating in the MADR experiments were 168 male and 167 female MAHDR and MALDR mice, ages 58–89 days. Numbers for each experiment are given below. All mice were experimentally naïve at the time of testing. Details of selective breeding have been previously published.[9,10,37] Briefly, each replicate set of the MADR lines was selectively bred from a founding population of B6D2F2 mice. The choice of breeders for each selection generation was based on voluntary consumption of a 40 mg/l MA solution consumed in a 2-bottle choice drinking procedure, during which they had access to water and 20 mg/l MA for 4 days and then water and 40 mg/l MA for 4 days. Thus, the mice that consumed the highest average amounts of MA were chosen to establish and perpetuate the MAHDR line, whereas the lowest consumers established and perpetuated the MALDR line. Results across replicate for response to selection have been reproducible.[9,10,37] Mice in these studies were from selection generation 5 (S5) of the fourth replicate set of the MADR lines (157 mice) and from S1–S3 of the fifth replicate set (178 mice). Replicate 5 was under development at the time of these studies, and only early selection generation mice were available. We did not see this as a problem, because the greatest divergence in MA intake between the lines occurs in S1,[9,10,37] due to the major impact of *Taar1* on MA intake and the nearly complete fixation of the $Taar1^{m1J}$ allele within the first generation of selection.[20] MADR line differences for multiple traits have been highly reproducible.[7,9,10,37]

Participating in the BXD morphine experiment were 120 female and 120 male BXD RI mice, ages 59–84 days. All mice were experimentally naïve at the time of testing. Breeding pairs of BXD RI strains were obtained from Dr. Robert Williams (University of Tennessee Health Science Center, Memphis, TN), and established within the VAPORHCS. Specific strains were chosen based on their combined *Taar1* and *Oprm1* genotypes and breeding potential. In total, there were 14 BXD RI strains that had four genotypes: $Taar1^{+/+}$/$Oprm1^{B6/B6}$ (n=16 BXD184, n=26 BXD154, n=12 BXD196, and n=6 BXD218); $Taar1^{m1J/m1J}$/$Oprm1^{B6/B6}$ (n=27 BXD161, n=8 BXD199, and n=25 BXD205); $Taar1^{+/+}$/$Oprm1^{D2/D2}$ (n=22 BXD113, n=12 BXD171, and n=26 BXD194); $Taar1^{m1J/m1J}$/$Oprm1^{D2/D2}$ (n=17 BXD178, n=18 BXD186, n=5 BXD210, and n=20 BXD216), evenly distributed by strain across the morphine dose groups.

2.2 Drug-induced core body temperature changes

Core body temperature was assessed using established procedures.[7,20] All experiments were performed during the light phase, between 0900 and 1300 h. Mice were weighed, isolated in acrylic cubicles to prevent huddling-associated body temperature changes, and left undisturbed for 1 h to allow acclimation to the testing environment, also maintained at 21 ± 1 °C. Following acclimation, baseline temperature was taken at time 0 (T0), using a 5 mm rectal probe attached to a Thermalert TH-8 digital thermometer (Sensortek, Clifton, NJ). Mice were then immediately treated with vehicle or the appropriate drug dose, placed back into their holding cubicles, and removed to obtain temperatures at T15, T30, T60, T90, T120, and T150 min post-injection (T30 to T150 for BXD RI).

2.3 Drugs

Cocaine hydrochloride was purchased from Sigma (St. Louis, MO). MDMA and morphine sulfate were obtained from the National Institute on Drug Abuse drug supply program

(Bethesda, MD, USA). All drugs were dissolved in sterile physiological saline (0.9% NaCl, Baxter Healthcare Corporation, Deerfield, IL) and injected intraperitoneally in a 10 ml/kg volume.

2.4 Experiment 1

Thermal responses to the 3 drugs were assessed in a single study, so that a common saline control group could be used, allowing fewer animals to be included. The study was completed in 4 equal passes in S5 mice of the fourth replicate and S1 mice of the fifth replicate (140 mice total; 5/sex/line/drug-specific dose or saline). Doses of cocaine were 15 and 30 mg/kg, MDMA doses were 2.5 and 5 mg/kg, and morphine doses were 15 and 30 mg/kg. These doses were chosen based on a literature review demonstrating behavioral and thermal effects.[28,30,32] Following the final temperature recording, mice were euthanized and tail samples taken for genotyping.

For morphine, initial analyses identified sex and dose effects. Because the group size per sex and dose was small for the initial morphine study, there were concerns about reliability of effects. Therefore, 6 additional passes of 14–42 mice were added, increasing the total sample size to 20–23 mice per sex, line, and saline or morphine dose (95 additional replicate 4 S5 mice and 100 additional replicate 5 S2 and S3 mice). One mouse died during the experiment and its data were excluded.

2.5 Experiment 2

BXD RI mice were used to investigate the respective impacts of *Oprm1* and *Taar1* genotype on thermal response to morphine. Procedures and morphine doses were as in Experiment 1. A total of 240 mice were tested for a final group size of 10 per sex, *Oprm1/Taar1* genotype, and drug dose.

2.6 Genotyping

Genomic DNA from the morphine-treated MADR mice was extracted using QuickExtract DNA extraction solution (Epicenter, Madison, WI). *Oprm1* was amplified using a Hotstart polymerase kit (Qiagen, Valencia, CA) with sequence-specific primers surrounding the region of interest (forward 5′-ggttatgcctctctggattag-3′, reverse 5′-tccatcgcttacatcttacca-3′). To determine *Oprm1* genotype, amplified polymerase chain reaction (PCR) products were run on an agarose gel that was scanned on a Bio-Rad Gel Doc™ XR+ Imaging System to determine band intensity. *Taar1* was amplified (forward 5′-ctttctgctgggctgtctga-3′, reverse 5′-caacagcgctcaacagttctc-3′) and genotype was determined using an rtPCR assay developed in our lab[7,20] and based on standard Taqman procedures.[38]

2.7 Statistical Analysis

Data were analyzed using Statistica Academic software, version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). Body temperature data were analyzed by repeated measures ANOVA, with time as the repeated measure and line, sex, drug treatment, *Oprm1* genotype, and *Taar1* genotype as possible independent variables. Effects were considered significant at $p < 0.05$. Significant interactions were examined with simple main effects analyses, and Neuman–Keuls *post hoc* mean comparisons were performed when appropriate. Correlations between

Taar1 and *Oprm1* genotype and temperature change were determined with Pearson's r. Observed versus expected *Taar1* and *Oprm1* genotype frequencies were assessed with the chi-square test.

3 RESULTS

3.1 Experiment 1: Thermal responses to multiple drugs in MADR mice

3.1.1 Cocaine—Data are presented in Figure 1. In the initial multifactor ANOVA, there were significant time × sex ($F_{6, 288} = 2.1$, $p < 0.05$) and time × dose interactions ($F_{12, 288} = 21.0$, $p < 0.001$, but no significant effects involving line. When the time × sex interaction was examined, there was a significant effect of time within each sex (ps < 0.001), but no significant sex difference at any time point was found. When the time × dose interaction was examined, we found that temperatures were dependent on time for each dose, including saline (ps < 0.05; Figure 1a–c). Mice treated with saline had significantly lower body temperatures compared to T0 at T90-T150. Mice treated with the 15 mg/kg cocaine dose had significantly lower body temperatures at T150 compared to T0, but their temperatures did not differ from T0 at any other time point. The 30 mg/kg cocaine-treated mice were hypothermic at T15, but by T30, their mean temperature was not significantly different from T0.

For the effect of dose at each time, there were no significant differences at T0 or T30, but at T15, the 30 mg/kg cocaine group had a significantly lower mean body temperature than the saline or 15 mg/kg cocaine groups (ps < 0.001). At T60-T150, the 30 mg/kg cocaine group had significantly higher mean body temperatures, compared to the saline group (ps < 0.001 – 0.05) and the mean temperature of this group was also higher than that of the 15 mg/kg cocaine dose group at T150 ($p < 0.05$). These differences are not indicated by symbols in Figure 1, due to representation of the effects of each dose in separate panels for clarity.

3.1.2. MDMA—Data are presented in Figure 2. The initial multifactor ANOVA identified significant time × line × dose ($F_{12, 288} = 2.0$, $p < 0.05$) and time × sex ($F_{6, 288} = 4.5$, $p < 0.001$) interactions. Females had higher T0 and T150 temperatures than males (ps < 0.05), but the sexes did not differ at any other time point. There was a significant effect of time within each sex (ps < 0.001). Next, the significant 3-way interaction was examined by repeated measures time × line ANOVAs for each dose. For the saline group (Figure 2a), only the effect of time was significant ($F_{6, 108} = 20.6$, $p < 0.001$), with decreases in mean temperature at T90-T150, regardless of line. For the 2.5 mg/kg MDMA group (Figure 2b), there was a significant time × line interaction ($F_{6, 108} = 4.7$, $p < 0.001$), with a significant effect of time in both lines (ps < 0.001 – 0.05). Compared to T0, the mean temperature of MAHDR mice was significantly higher at T30 and significantly lower at T120 and T150 (Figure 2b); however, these differences in temperature did not exceed 0.5°C. No significant changes in body temperature from T0 were detected for the MALDR line by post-hoc analysis. However, following treatment with the 2.5 mg/kg dose of MDMA, the mean temperature of MALDR mice was significantly lower than that of MAHDR mice at T15 and T60 (Figure 2b). For the 5 mg/kg MDMA group (Figure 2c), there was a significant time × line interaction ($F_{6, 108} = 6.3$, $p < 0.001$), with a significant effect of time only in the

MALDR line ($p < 0.001$). Compared to T0, the mean temperature of the MALDR line was significantly lower at T15 and T30. The maximal decrease in temperature was approximately 2°C, occurring at T15. In addition, the mean temperature of MALDR mice was significantly lower than that of MAHDR mice at T15 and T30 after treatment with this MDMA dose.

We next examined the effect of dose at each time point for each line. At T0, there was no effect of dose. At T15, temperature was dependent on dose for both lines (ps < 0.001 – 0.05). There were no differences between the saline and 2.5 mg/kg MDMA groups for either line, but mean body temperatures of the 5 mg/kg-treated MALDR and MAHDR groups were lower than those of the other 2 treatment groups (all ps < 0.001 – 0.05), with more profound effects in the MALDR line. At T30, temperatures were dependent on dose only in the MALDR line ($p < 0.001$), with significantly lower mean temperature for the 5 mg/kg group, compared to the other 2 groups (ps < 0.01 – 0.05). There were no significant dose-dependent effects at any other time point. These differences are not indicated by symbols in Figure 2, due to representation of the effects of each dose in separate panels for clarity.

3.1.3 Morphine—Data are presented in Figure 3. In the initial multifactor ANOVA, there were significant time × line × dose ($F_{12, 1452} = 4.5$, $p < 0.001$) and time × sex × dose interactions ($F_{12, 1452} = 2.0$, $p < 0.05$). First, data were examined for time and sex effects within each dose. Temperatures were dependent on time for both sexes at all doses (all ps < 0.001). A significant time × sex interaction was found for the saline ($F_{6, 498} = 4.5$, $p < 0.001$) and 30 mg/kg morphine ($F_{6, 486} = 2.98$, $p < 0.01$) groups. Compared to females, male saline-treated mice had lower temperatures of less than 0.5°C, and only at T150 ($p < 0.01$). Males in the 30 mg/kg morphine group also had lower temperatures, but only at T0 ($p < 0.001$).

Because sex did not influence line-dependent responses to morphine, data for the sexes were combined and a significant time × line × dose interaction was confirmed ($F_{12, 1488} = 4.3$, $p < 0.0001$). For the saline group, there was a significant effect of time ($F_{6, 498} = 100.0$, $p < 0.001$), due largely to a progressive reduction in body temperature (Figure 3a). For each morphine dose, there was a significant time × line interaction ($F_{6, 504} = 8.78$, $p < 0.001$ and $F_{6, 486} = 6.78$, $p < 0.001$ for 15 and 30 mg/kg, respectively). For the 15 mg/kg dose, both the MAHDR and MALDR lines exhibited time-dependent hypothermia (ps < 0.001) and the mean temperature of MAHDR mice was significantly lower than that of MALDR mice at every time point (Figure 3b). The thermal response followed a biphasic pattern, characterized by a rapid decrease in temperature after injection followed by an increase in temperature. The mean difference between the 2 lines of mice was 0.4°C at baseline, compared to a maximal difference of 2.2°C at T60. For the 30 mg/kg dose, both lines displayed hypothermia (ps < 0.001), that persisted across the entire measurement period (Figure 3c). MAHDR mice had significantly lower mean temperatures than MALDR mice, at every time point, except T0. When time and dose effects were examined, there was a significant time × dose interaction for each line ($F_{12, 744} = 45.1$, $p < 0.001$ and $F_{12,744} = 39.6$, ps < 0.001 for MAHDR and MALDR, respectively). For each line, the effect of dose was significant at every time point, except T0 (ps < 0.001). Temperature changed significantly across time for saline and each morphine dose (ps < 0.001), with a linear decrease in the saline group, and biphasic responses in the morphine groups.

3.1.4. Morphine: genotyping results and correlations in MADR mice—The genotyping results for *Taar1* and *Oprm1* in the MADR lines are summarized in Figure 4. MAHDR mice were predominantly $Oprm1^{D2/D2}$ and $Oprm1^{D2/B6}$, whereas MALDR mice were predominantly $Oprm1^{B6/B6}$. For *Taar1*, MAHDR mice were almost entirely $Taar^{m1J/m1J}$; a single animal from S1 replicate 5 was $Taar1^{+/m1J}$. MALDR mice were almost entirely $Taar1^{+/+}$ or $Taar1^{+/m1J}$; a single animal from S1 replicate 5 was $Taar1^{m1J/m1J}$. If there had been no impact of selective breeding on the frequency of *Oprm1* or *Taar1*, the expected ratio of the different possible genotypes for each gene ($Oprm1^{B6/B6}$: $Oprm1^{B6/D2}$: $Oprm1^{D2/D2}$ and $Taar1^{+/+}$: $Taar1^{+/m1J}$: $Taar^{m1J/m1J}$) would be 1 : 2 : 1, within each line or 31.75 : 63.5 : 31.75 for each of the 127 MAHDR and MALDR mice tested in this study. Chi-square tests indicated that the observed ratios differed significantly from the expected ratios for both the MAHDR (chi-squared = 106 and 373 for *Oprm1* and *Taar1*, respectively, ps < 0.001) and MALDR (chi-squared = 284.4 and 197.9 for *Oprm1* and *Taar1*, respectively, ps < 0.001) lines.

Correlations were separately calculated between thermal response to morphine and *Oprm1* or *Taar1* genotype. There were insufficient mice of every possible combined *Oprm1* and *Taar1* genotype to assess potential correlations with allele combinations. In fact, there was a significant correlation between progenitor source of *Oprm1* and *Taar1* (r = .80, p < 0.01), so that *Taar1* genotype predicted *Oprm1* genotype 64% of the time, indicating linkage disequilibrium for these 2 genes. Because the hypothermic response to morphine was greatest at T60, a change value was calculated for each mouse as temperature at T60 minus temperature at T0 (T60-T0), and used to calculate correlations with genotype (D2 or B6 allele, or *m1J* or + allele, homozygous or heterozygous) for each dose. For the saline group, the correlations with *Oprm1* genotype (r = 0.06, p > 0.05) and *Taar1* genotype (r = 0.00, p < 0.05) were not significant. For 15 mg/kg morphine, there were significant correlations with both *Oprm1* (r = 0.50, p < 0.01) and *Taar1* (r = 0.48, p < 0.01) genotype. Similarly, for 30 mg/kg morphine, there were significant correlations with *Oprm1* (r = 0.44, p < 0.01) and *Taar1* (r = 0.43, p < 0.01) genotype.

3.1.5 Experiment 2: Thermal response to morphine in BXD RI mice—Due to linkage disequilibrium, we could not study the individual contributions of *Oprm1* and *Taar1* genotype in the MADR lines. Therefore, BXD RI mice were tested in which the linkage is disrupted. Results are summarized in Figure 5 for *Oprm1* genotype (Figure 5a–c) and *Taar1* genotype (Figure 5d–f). The analysis for which the results are presented did not include strain as a factor, rather the BXD RI mice were considered as a single population, because they were all derived from the D2 and B6 progenitor strains. This afforded a large population size of related individuals for examination of the genetic effects (see same approach in our published paper[20]). There were significant *Oprm1* × *Taar1* × sex ($F_{1, 216}$ = 4.8, p < 0.05), time × sex ($F_{5, 1080}$ = 4.5, p < 0.001), time × *Oprm1* × *Taar1* ($F_{5, 1080}$ = 5.8, p < 0.0001), and time × *Oprm1* × dose ($F_{10, 1080}$ = 11.9, p < 0.0001) interactions. However, there were no interactions of sex and morphine dose nor of *Taar1* genotype and morphine dose, indicating that neither sex nor *Taar1* genotype (Figure 5d–f) impacted the response to morphine. Thus, we examined the significant time × *Oprm1* × dose interaction, because it is relevant to the question of whether genotype impacts response to morphine (Figure 5a–c).

Within the saline group (Figure 5a), there was a significant time × *Oprm1* interaction ($F_{5, 390} = 2.4$, $p < 0.05$). Temperature decreased across time for both genotypes (ps < 0.01), differing from T0 at each time point, but the genotypes did not significantly differ from each other at any time point. For 15 mg/kg morphine (Figure 5b), the time × *Oprm1* interaction was significant ($F_{5, 390} = 15.24$, $p < 0.001$). For both genotypes, the 15 mg/kg morphine dose had time-dependent biphasic effects (ps < 0.001) and mice of both genotypes displayed significant hypothermia at all post-injection measurement times. $Oprm1^{D2/D2}$ mice had significantly lower mean temperatures than $Oprm1^{B6/B6}$ mice at T30-T120 (Figure 5b). For 30 mg/kg morphine (Figure 3c), the time × *Oprm1* interaction was significant ($F_{5, 390} = 23.51$, $p < 0.001$). Both genotypes exhibited time-dependent, biphasic hypothermia (ps < 0.001). $Oprm1^{D2/D2}$ mice had significantly lower temperatures than $Oprm1^{B6/B6}$ mice at T30-T150 (Figure 5c).

When correlations were calculated between temperature change (T60-T0) and *Taar1* or *Oprm1* genotype for each morphine dose group, there were no significant correlations for the saline group or with *Taar1* genotype, regardless of morphine dose. However, the correlation with *Oprm1* genotype was significant for both the 15 mg/kg ($r = 0.49$, $p < 0.01$) and the 30 mg/kg ($r = 0.63$, $p < 0.01$) morphine doses.

4 DISCUSSION

Our findings indicate that genetic risk for MA intake in the MADR mouse lines is tied to thermal response to some other addictive drugs. The lines do not differ in thermal response to cocaine or ethanol,[7] but, similar to previous results for MA,[7] MALDR mice exhibit dose-dependent hypothermia to the amphetamine-like stimulant, MDMA, whereas MAHDR mice are insensitive to MDMA-induced hypothermia. Overall, saline-treated mice had a decrease in body temperature over time, which can be attributed to loss of body heat due to isolate housing.[39] *Taar1* impacts sensitivity to the hypothermic effect of MA[7,18,20] and the current results are consistent with a similar role for *Taar1* in sensitivity to the hypothermic effect of MDMA. The MADR lines differ in thermal response to the opioid, morphine. However, their sensitivity order is reversed, so that MAHDR mice exhibit a larger dose-dependent hypothermic response than MALDR mice. Our analysis in the BXD RI mice indicates that *Oprm1* genotype, rather than *Taar1* genotype, is associated with sensitivity to the hypothermic effect of morphine, and we identified linkage disequilibrium for *Oprm1* and *Taar1* in the MADR lines that likely accounts for their differential sensitivity to morphine-induced hypothermia.

Cocaine, MDMA, and MA all directly affect dopamine (DA) systems. However, MDMA and MA have effects that are distinct from cocaine. Amphetamine-like substances (e.g., MDMA and MA) are DA transporter (DAT) substrates, entering the presynaptic cell via DAT, facilitating the vesicular release of DA in the cytosol, and increasing extracellular DA via reverse transport at DAT.[40–43] Cocaine is a DAT inhibitor, interfering with DA uptake by DAT, causing a buildup of DA in the synapse.[44,45] In all cases, there is more DA available for receptor stimulation. However, MDMA and MA are also TAAR1 agonists, whereas cocaine is not.[21,35] Cocaine elicited a dose-dependent thermal response in both MADR lines, but the lines did not differ in this response. Similarly, the MADR lines do not differ in

sensitivity to cocaine-conditioned reward or aversion, or locomotor stimulation[33], whereas they do differ for these traits in relation to MA.[9–11,13,16] All of these findings suggest distinct mechanisms impact these responses after treatment with cocaine vs. MA in the MADR mouse lines, and one important factor is *Taar1* genotype.

We have determined that *Taar1* is a quantitative trait gene for MA intake, and also impacts sensitivity to the hypothermic effect of MA.[7,18,20] Because the $Taar1^{m1J}$ mutation codes for a nonfunctional receptor, the MAHDR line is a naturally occurring functional knockout. In the presence of functional TAAR1, MA and MDMA induce hypothermia at lower doses and hyperthermia at higher doses, whereas only hyperthermia occurs in response to some doses of these drugs in the absence of functional TAAR1.[7,20,27,29,30,46] This indicates that different biological mechanisms drive these 2 types of thermal response. Because cocaine is not a TAAR1 agonist, yet reduces body temperature similarly in the MADR lines, cocaine must elicit its hypothermic effects via a TAAR1-independent mechanism not impacted by selective breeding for MA intake.

MAHDR mice are more sensitive to the hypothermic effect of morphine, and the MAHDR line also voluntarily consumes less morphine than the MALDR line.[36] Thus, a negative relationship between drug-induced hypothermia and drug intake has been found for both morphine and MA in the MADR lines, suggesting there may be common genetic factors that influence the hypothermia and intake traits for each drug. For both MA and morphine, hypothermia may also increase the period during which negative associations can be conditioned;[15] thus the greater hypothermia experienced by MAHDR mice following morphine exposure may facilitate associations with the negative subjective effects of morphine. *Taar1* has a pleiotropic effect on MA-induced hypothermia and intake,[20] and the current data suggest that *Oprm1* impacts morphine-induced hypothermia. We found that *Oprm1* alleles from the D2 and B6 progenitor strains exist at different frequencies in the MAHDR vs. MALDR lines and this appears to be due to linkage disequilibrium, rather than because *Oprm1* impacts risk for MA intake.[17,26] Thus, the $Oprm1^{D2}$ allele is linked to the $Taar1^{m1J}$ allele and occurs at higher frequency in MAHDR mice and the $Oprm1^{B6}$ allele is linked to $Taar1^{+}$ allele and occurs at higher frequency in MALDR mice. The progenitor D2 strain consumes less morphine and exhibits greater morphine-induced hypothermia, than the progenitor B6 strain,[47–53] consistent with the *Orpm1* genotypes and morphine phenotypes of the MAHDR (largely $Oprm1^{D2}$; less morphine intake; greater morphine-induced hypothermia) and MALDR (largely $Oprm1^{B6}$; more morphine intake; less morphine-induced hypothermia) lines.

It should be acknowledged that morphine intake was measured in an earlier replicate of MADR mice,[36] and has not been evaluated in the replicates used here. However, we believe that it is likely that the same outcome would be obtained in all replicates of the MADR lines for the following reasons. First, the response to selection and differences between the MAHDR and MALDR lines for MA-related and several non-MA-related phenotypes have been highly reproducible across replicates.[7,9,10,16,37] Second, mapping results are consistent across replicates and support *Taar1* as a quantitative trait gene for MA intake in every replicate.[17,20] Third, here we demonstrate linkage disequilibrium between *Taar1* and *Oprm1*, and thus the difference in $Oprm1^{B6}$ *and* $Oprm1^{D2}$ allele frequencies between the lines that

we observed, likely occur in all replicates. Finally, published data from other labs for the B6 and D2 progenitors of the MADR lines are consistent with a negative correlation between morphine drinking and hypothermic response.[47–53]

Initial mapping suggested *Oprm1* as a candidate for our chromosome 10 MA intake QTL. However, fine mapping in existing chromosome 10 congenic strains derived from B6 and D2 mice[49,50] excluded *Oprm1* as a genetic risk factor.[26] Although not involved in risk for high MA intake, evidence from a gene expression microarray experiment using nucleus accumbens tissue from the MADR lines indicates that *Oprm1* is regulated by a gene expression network associated with risk for MA intake,[17] and *Taar1* and *Oprm1* interact to impact both MA intake and MA-induced hypothermia.[18] This led us to consider whether there might be an interaction between *Taar1* and *Oprm1* in their influence on the thermal response to morphine. Particular BXD RI strains were chosen for the current research to allow us to examine the independent and interactive effects of *Taar1* and *Oprm1* genotype on thermal response to morphine. *Oprm1*, but not *Taar1*, genotype was supported as a contributor. Mice with the $Oprm1^{D2/D2}$ genotype displayed greater hypothermia than mice with the $Oprm1^{B6/B6}$ genotype, but there was no correlation with *Taar1* genotype. However, in a recent exploration, *Oprm1* and *Taar1* were found to have interactive effects on MA intake and thermal response to MA.[18] BXD RI mice with the $Taar1^{m1J/m1J}$ genotype consumed significantly more MA, and MA intake was synergistically enhanced in mice with the $Oprm1^{D2/D2}$ genotype. $Taar1^{+/+}$ BXD RI mice exhibited a hypothermic response to MA which was also synergistically enhanced in mice with the $Oprm1^{D2/D2}$ genotype. Correlational data for the BXD RI mice indicate that *Oprm1* genotype accounted for 24% and 40% of the variance in hypothermic response to the 15 and 30 mg/kg morphine doses. However, genes linked to *Oprm1* could impact morphine intake and some of the genes near *Oprm1* are known to be involved in the actions or effects of opioids, such as regulation of μ-opioid receptor signaling.[49] We cannot rule out a potential role for these genes in the current results for morphine-induced hypothermia.

Some evidence indicates a role for human *OPRM1* variants in risk for opioid and MA use.[54–56] These variants may confer differences in receptor function, which may in turn contribute to risk for opioid use. Investigations into a QTL for differences in morphine preference between the B6 and D2 strains identified *Oprm1* as a candidate gene.[49,50,57] Several *Oprm1* polymorphisms exist between these strains that may result in functional differences,[49] which could impact receptor function and may explain the differences in thermal responses presented here. Future studies should examine the binding affinity and kinetics of opioids at these receptor variants.

The BXD RI mice could be enlisted to explore whether *Taar1* plays a role in the differences in morphine intake between the MADR lines and whether *Oprm1* genotype impacts the buprenorphine-induced reduction in MA intake that we previously reported in MAHDR mice.[36] If *Oprm1* genotype, and not *Taar1* genotype, determines morphine intake, then $Oprm1^{B6/B6}$ mice should consume more morphine than $Oprm1^{D2/D2}$ mice, regardless of *Taar1* genotype. Groups of BXD RI mice, all with the $Taar1^{m1J/m1J}$ genotype to induce MA intake, but with either of the 2 *Oprm1* genotypes, could be used to determine if the buprenorphine effect is dependent on $Oprm1^{D2/D2}$ vs. $Oprm1^{B6/B6}$ genotype, or if a

reduction in MA intake occurs regardless of *Oprm1* genotype. The latter result could suggest that the reduction in MA intake involves μ-opioid receptor-independent mechanisms.

ACKNOWLEDGEMENTS

We thank Nicholas Varra and Meley Habtegiorgis for assistance with data collection, and Robert W. Williams for providing BXD RI strain breeding pairs. This research was supported by NIH NIDA grants P50DA018165, R01DA046081, U01DA041579, and T32DA07262; NIH NIAAA grant R24AA020245; the Department of Veterans Affairs I01BX002106; and the VA Research Career Scientist program. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

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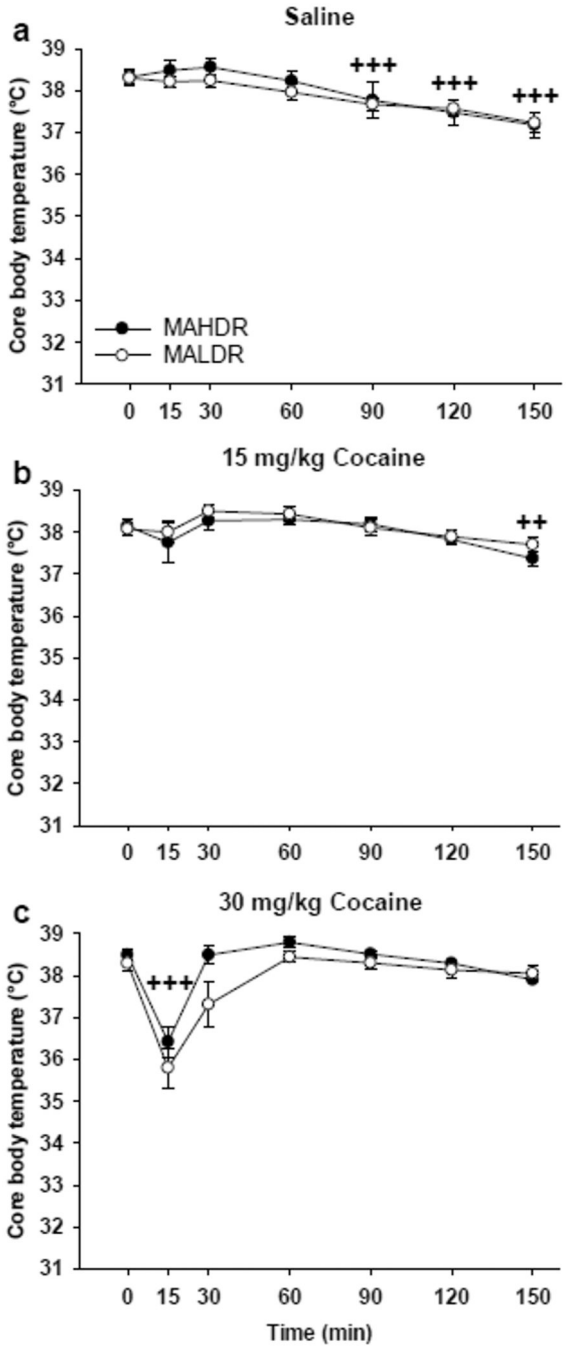

Figure 1.

Mice bred for high and low methamphetamine (MA) intake exhibit similar sensitivity to the hypothermic effects of cocaine. There were no significant effects of sex involving line or cocaine dose, therefore the data are presented for the sexes combined. Shown are the effects of (a) saline, (b) 15 mg/kg cocaine, and (c) 30 mg/kg cocaine on core body temperature in MAHDR and MALDR mice across time in minutes (min). Data are means ± SEM. ++$p < 0.01$, and +++$p < 0.001$ for temperature change from T0 at the indicated time point for the

main effect of time. MAHDR: methamphetamine high drinking; MALDR: methamphetamine low drinking

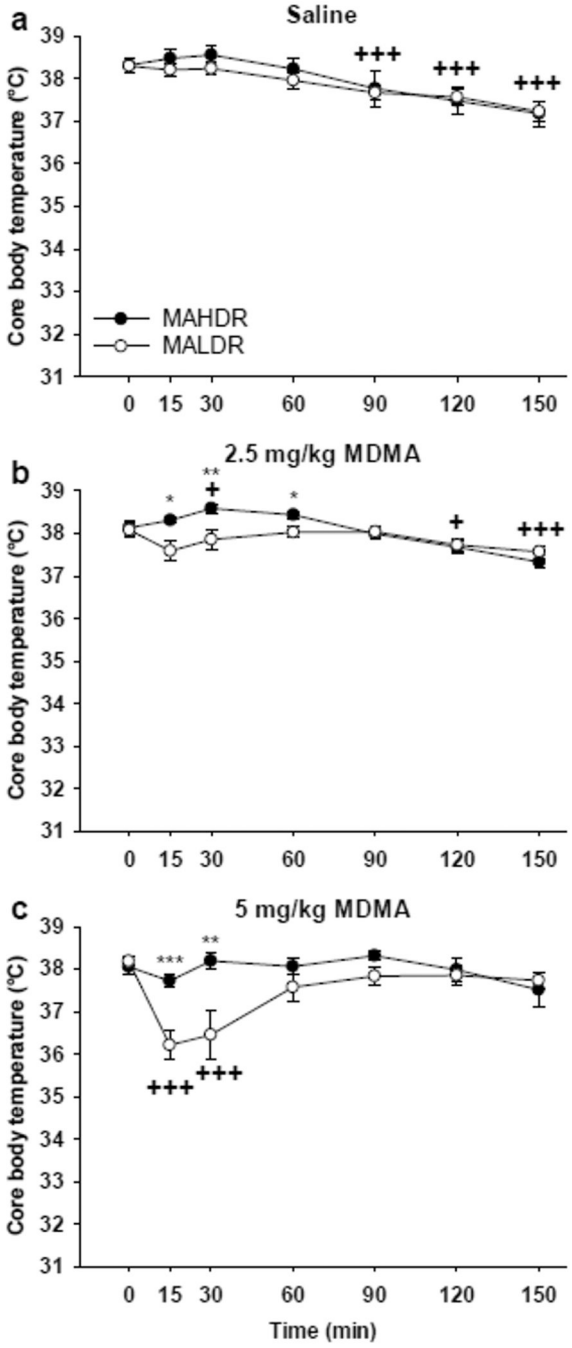

Figure 2.

Mice bred for low methamphetamine (MA) intake are more sensitive to hypothermic effects of MDMA on core body temperature than mice bred for high methamphetamine intake. There were no significant effects of sex involving line or dose, so the data are presented for the sexes combined. Shown are the effects of (a) saline, (b) 2.5 mg/kg MDMA, and (c) 5 mg/kg MDMA on body temperature in MAHDR and MALDR mice across time in minutes (min). Data are means ± SEM. +$p < 0.05$ and +++$p < 0.001$ for temperature change from T0 at the indicated time point for (a) the main effect of time, (b) the MAHDR line, and (c) the

MALDR line; *p < 0.05, **p < 0.01, and ***p < 0.001 for the difference between the lines at the indicated time point. MAHDR: methamphetamine high drinking; MALDR: methamphetamine low drinking; MDMA: 3,4-methylenedioxymethamphetamine

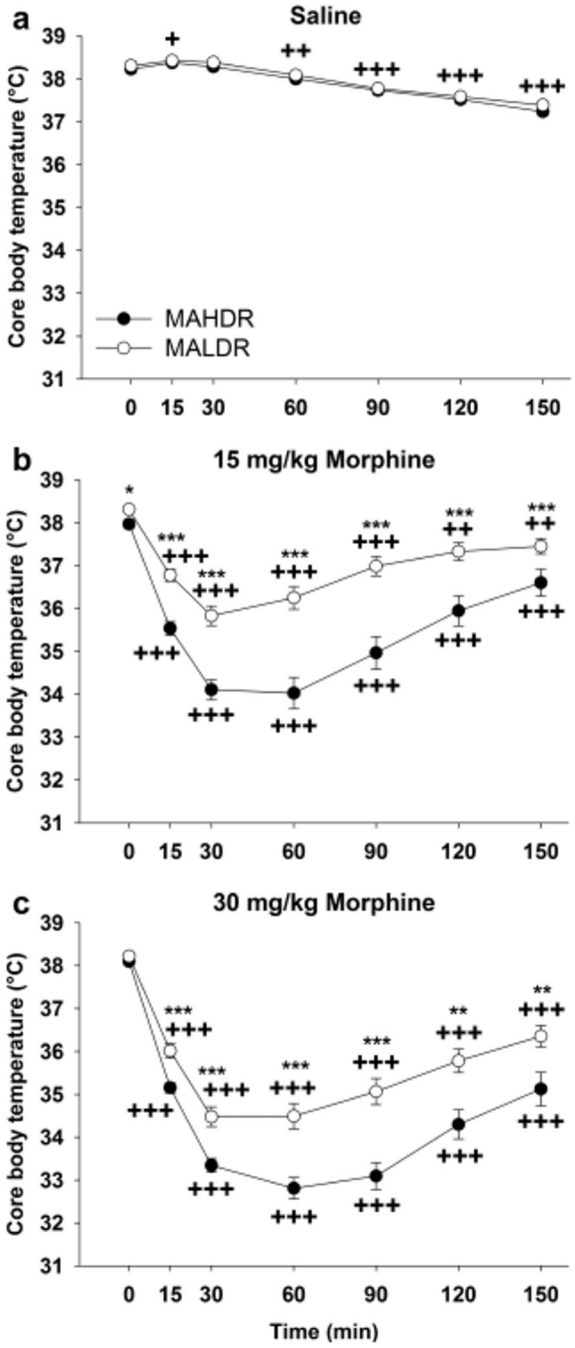

Figure 3.

Mice bred for high methamphetamine (MA) intake are more sensitive to the hypothermic effects of morphine than mice bred for low methamphetamine intake. Because sex did not play a role in line-dependent responses to morphine, the data are presented for the sexes combined. Shown are the effects of (a) saline, (b) 15 mg/kg morphine, and (C) 30 mg/kg morphine on core body temperature in MAHDR and MALDR mice across time in minutes (min). Data are means ± SEM. +$p < 0.05$, ++$p < 0.01$, and +++$p < 0.001$ for temperature change from T0 at the indicated time point for (a) the main effect of time; (b and c) the

MAHDR or MALDR line; *p < 0.05, **p < 0.01, and ***p < 0.001 for the difference between the lines at the indicated time point. MAHDR: methamphetamine high drinking; MALDR: methamphetamine low drinking

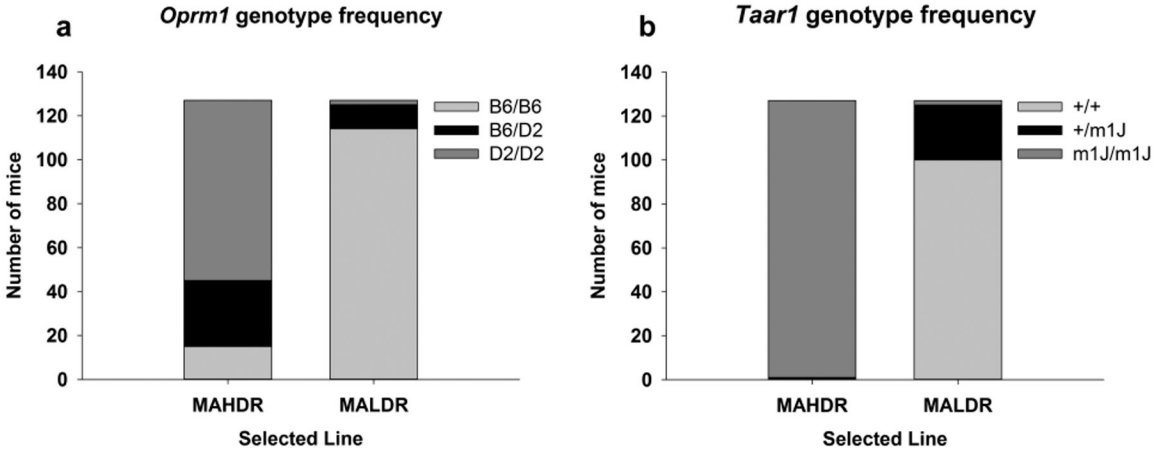

Figure 4.

(a) *Oprm1* and (b) *Taar1* genotype frequencies for methamphetamine high and low drinking mice tested in the study of morphine thermal effects. +: reference *Taar1* allele; B6: C57BL/6J; D2: DBA/2J; *m1J*: mutant *Taar1* allele found only in D2 mice; MAHDR: methamphetamine high drinking; MALDR: methamphetamine low drinking; *Oprm1*: mu-opioid receptor gene; *Taar1*: trace amine-associated receptor 1 gene

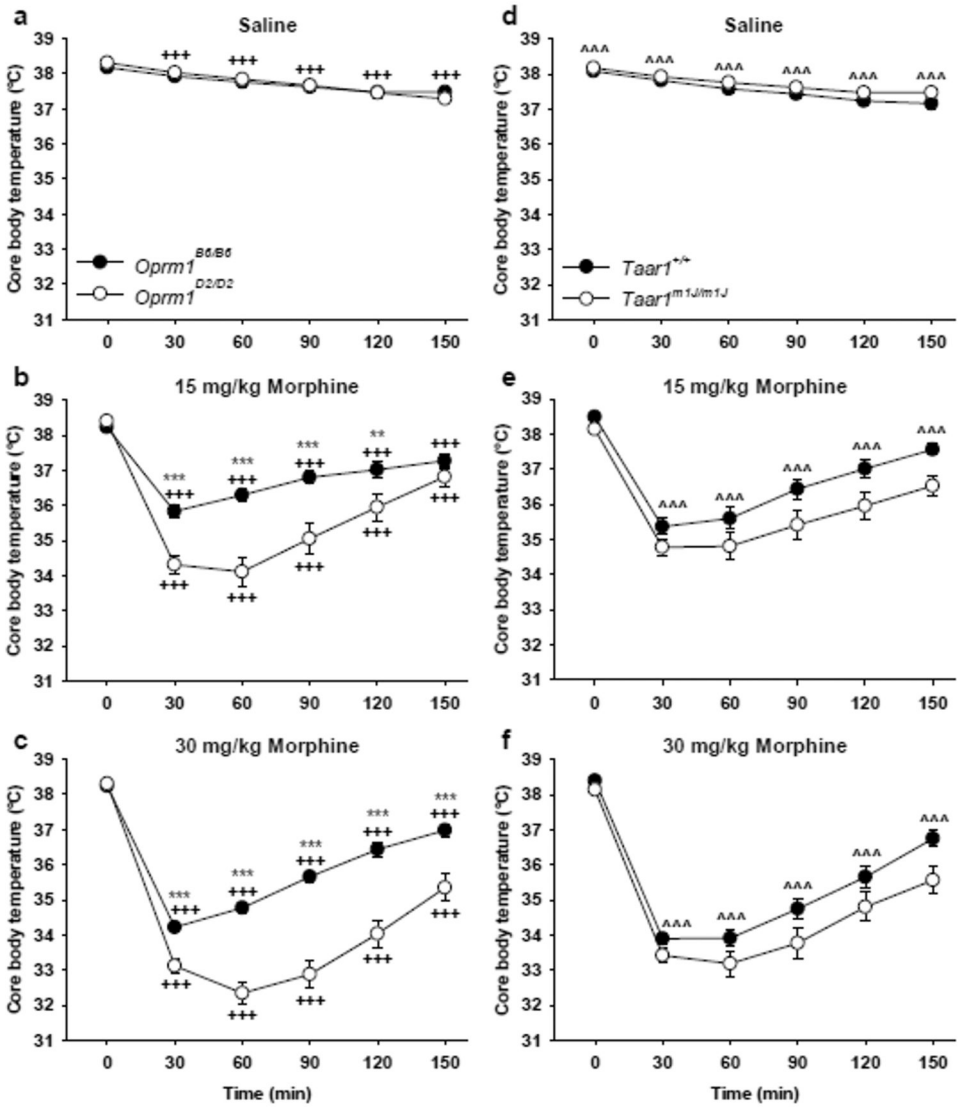

Figure 5.

Mice possessing the *Oprm1*$^{D2/D2}$ genotype are more sensitive to the hypothermic effects of morphine than *Oprm1*$^{B6/B6}$ mice. There were no interactions of sex and morphine dose, therefore data are presented for the sexes combined. Shown are the effects of saline, 15 mg/kg morphine, and 30 mg/kg morphine on core body temperature across time in minutes (min) for BXD RI mice with (a-c) *Oprm1*$^{B6/B6}$ and *Orpm1*$^{D2/D2}$ genotypes and (d-f) *Taar1*$^{+/+}$ and *Taar1*$^{m1J/m1J}$ genotypes. +++p < 0.001 for temperature change from T0 at the indicated time point for the main effect of time (a,d-f) or for the *Oprm1*$^{B6/B6}$ or *Oprm1*$^{D2/D2}$ genotype (b,c); **p < 0.01, ***p < 0.001 for differences between genotypes at the indicated time point. +: reference *Taar1* allele; B6: C57BL/6J; D2: DBA/2J; *m1J*: mutant *Taar1* allele found only in D2 mice; *Oprm1*: mu-opioid receptor gene; *Taar1*: trace amine-associated receptor 1 gene