Supplementary Materials

Results

40 mg/l MA preference ratio

Preference ratio for the 40 mg/l solution (Figure 1B of the accompanying manuscript) in MAHDR-2 and MALDR-2 mice showed a similar pattern of differences across generations as seen for the selection trait. This result was similar to that seen in the first set of MADR lines (Wheeler et al. 2009). Analysis of MA preference data for S1-S4 offspring, identified significant effects of line ($F(1,507)=918.8$, $p<.001$) and generation ($F(1,507)=8.8$, $p<.001$), and a significant generation x line interaction ($F(3,507)=5.6$, $p<.001$). The interaction is explained by the increasing divergence in MA consumption between the lines across generations; the line difference was significant in every generation (p values<.001).

A bidirectional change in preference during the course of selection for MA consumption was statistically supported by results of analyses that included data from the F2 (S0 generation) mice in two separate ANOVAs that also included either MALDR-2 S1-S4 data or MAHDR-2 S1-S4 data. There was a significant main effect of generation in both cases ($F(4,386)=42.3$, $p<.001$ for the MAHDR-2; $F(4,375)=17.0$, $p<.001$ for the MALDR-2). Post-hoc mean comparisons indicated significant differences between the F2 (S0) and S1-S4 mice for both lines.

20 mg/l MA consumption

There were statistically significant generation x line ($F(3,509)=4.6$, $p<0.005$) and sex x line ($F(1,509)= 6.8$, $p<0.01$) interactions for consumption of 20 mg/l MA (Figure 1C of the accompanying manuscript) .One-way ANOVA for data from F2 and MAHDR-2 (S1-S4 generation) mice revealed a significant main effect of generation ($F(1,387)=32.0$, $p<.001$). Post-hoc mean comparisons revealed

that F2 mice consumed significantly less MA than MAHDR-2 mice of every generation. Similarly, one-way ANOVA for the F2 and MALDR-2 (S1-S4 generation) mice revealed a significant main effect of generation ($F(1,387)=32.0$, $p<.001$), and post-hoc mean comparisons revealed that F2 mice consumed significantly more MA than MALDR-2 mice of generations S1, S3 and S4. To examine the sex x line interaction, data were collapsed on generation, and a significant sex effect ($p<.001$) was found only in the MAHDR-2 mice, though a similar trend ($p=.09$) was observed for MALDR-2 mice. In both lines, females consumed more than males with an average difference of 0.6 mg/kg in MAHDR-2 mice and 0.2 mg/kg in MALDR-2 mice.

Tastant consumption

For saccharin and quinine, there were no significant interactions involving line; however, there were significant sex x concentration interactions for saccharin ($F(1,44)=9.3$, $p<.01$) and quinine ($F(1,44)=9.4$, $p<.01$) consumption. These interactions were associated with females consuming greater amounts of these tastants especially at high tastant concentrations. For saccharin preference, the only significant effect was concentration ($F(1,44)=4.2$, $p<.05$). For the quinine preference ratio, there was a significant sex x concentration interaction ($F(1,44)=4.5$, $p<.05$), which was associated with similar differences to those described for quinine consumption.

For KCl consumption, there was a significant of line x sex x concentration interaction ($F(1,44)=5.1$, $p<.05$). However, when the data were examined for each concentration, only main effects of sex ($F(1,44)=5.1$, $p<.05$ and $F(1,44)=10.9$, $p<.01$ for the low and high KCl concentrations, respectively) were found; females consumed more KCl than males. Likewise, data examined within each sex yielded no significant line differences. For KCl preference, the only significant results were effects of concentration ($F(1,44)=37.2$, $p<.001$) and sex ($F(1,44)= 4.5$, $p<.05$); there was higher preference for the lower concentration and females had higher preference ratios than males.

To demonstrate discrimination between water and tastant, we compared water and tastant consumption (ml) for each tastant concentration. Animals consumed significantly higher amounts of 0.033% and 0.066% saccharin solution than water (mean ± SEM: 6.1 ± .3 and 7.2 ± .3 ml 0.033% and 0.066% saccharin vs. 0.9 ± .1 and 0.8 ± .2 ml water; p values<.001). Tastant and water consumption did not differ for low concentrations of quinine and KCl but did differ significantly for the higher concentrations of each tastant, with less tastant solution consumed than water [quinine vs. water (mean ± SEM; 2.3 ± .2 ml vs. 4.0 ± .2 ml; p<.001), and KCl vs. water (mean ± SEM; 2.2 ± .2 ml vs. 4.6 ± .2 ml; p values<.001)].

Locomotor activity during place preference tests

B6D2F2 mice

Locomotor activity data collected during the drug-free place preference test for the F2 mice were analyzed by ANOVA grouped on sex, dose and conditioning floor. No significant effects of these factors found. Data are shown in Figure S1.

MALDR-2 and MAHDR-2 mice

Locomotor activity data from the drug-free and drug-present preference tests were examined by ANOVA grouped on line, sex, dose and conditioning floor. For the drug-free test, no significant effects were found (Figure S2A). For the drug-present test, significant effects are described in the accompanying manuscript and the data are summarized in Figure S2B.

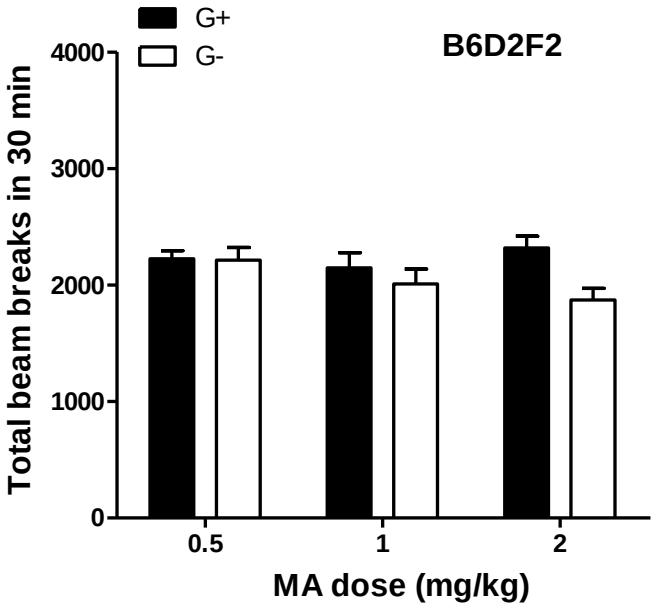

Figure S1. Locomtor activity levels during the drug-free preference test in B6D2F2 mice, measured as photocell beam breaks. n = 15-17 per group and dose. Bars and error bars are means ± SEM.

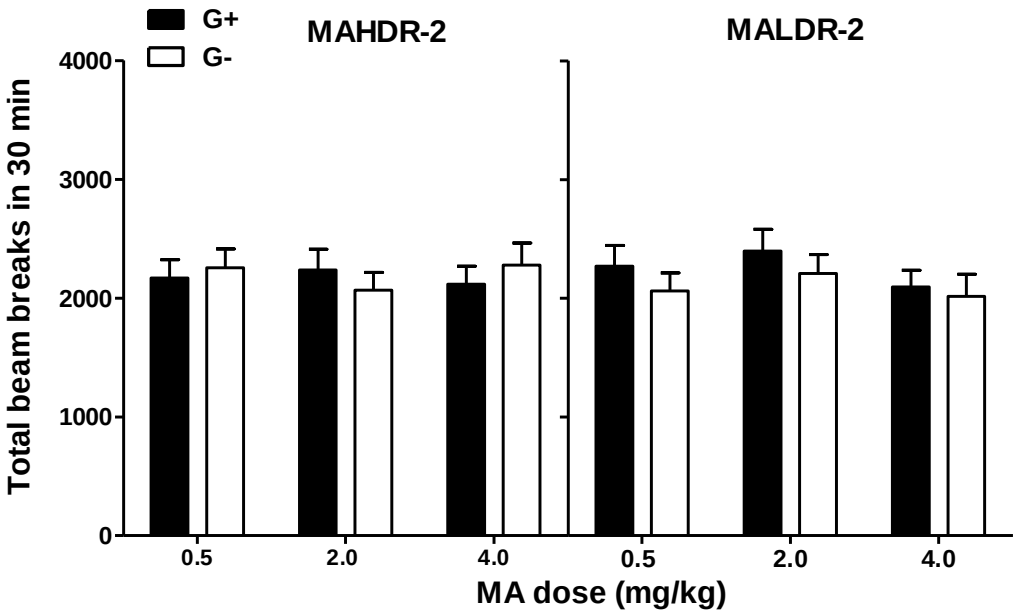

Figure S2. Locomotor activity levels during the drug-free preference test (A), n = 9-15 per line, group and dose, in MADR-2 mice, measured as photocell beam breaks. Bars and error bars are means ± SEM.

References

Wheeler, J.M., Reed, C., Burkhart-Kasch, S., Li, N., Cunningham, C.L., Janowsky, A., Franken, F.H., Wiren, K.M., Hashimoto, J.G., Scibelli, A.C. & Phillips, T.J. (2009) Genetically correlated effects of selective breeding for high and low methamphetamine consumption. *Genes Brain Behav* **8**, 758-771.