A Methamphetamine Vaccine Attenuates Methamphetamine-Induced Disruptions in Thermoregulation and Activity in Rats

Supplemental Information

Supplementary Methods and Materials

Post-hoc analyses of significant main effects in the analysis of variance were conducted using the Fisher's least significant difference test including all pairwise comparisons; the criterion for significance was $p < 0.05$. Analyses were conducted with GB-STATv7.0; Dynamic Microsystems, Silver Spring MD.

All rats were weighed upon arrival to the vivarium and then every week throughout the course of the study using a SOEHNLE 66111 Olympia Plus Digital scale.

Supplementary Results

Post-hoc Analyses

Figure 2: The KLH-control rats had significantly higher rectal temperature values following 5.6 mg/kg METH compared with the MH6-vaccinated rats in Experiment 1. The post hoc test confirmed that body temperature was unchanged from the baseline in the MH6 group but elevated in the KLH group (30-60 min post-injection). Elevated body temperature in the MH2(R) (30-120 min post-injection) and MH7 (30-120 min post-injection) groups was comparable to the KLH group (data not shown). Post-hoc tests further confirmed significant differences between the KLH and MH6 groups at 30 and 60 min after the METH challenge.

In addition, the KLH-control rats had significantly more wheel activity (quarter rotations) compared with MH6-vaccinated rats in Experiment 1. The post hoc test further confirmed significant differences between MH6 and KLH groups at 20 and 25 min post-injection and within groups at 10, 20 and 40-60 min compared with the first 5

min post-injection (KLH group) and at 10-30 and 40-60 min compared with the first 5 min post-injection (MH6 group).

Figure 3: KLH-control rats had significantly lower temperature values compared with MH6-vaccinated rats in Experiment 2. Post-hoc tests confirmed significant differences between groups at 0.5, 1.0, 3.2, and 5.6 mg/kg METH, and within-group compared to corresponding vehicle values at 1.0, 3.2, and 5.6 mg/kg METH.

In addition, METH decreased locomotor activity in KLH-control rats relative to MH6-vaccinated rats at the 5.6 mg/kg dose in Experiment 2. Post-hoc tests confirmed significant differences within groups compared to corresponding vehicle values at 0.5, 1.0, and 5.6 mg/kg METH, and within groups compared to pre-injection values (i.e., -30 and -60 min) at 0.5 and 1.0 mg/kg METH for the KLH group and at 0.5, 1.0, and 5.6 mg/kg METH for the MH6 group.

Figure 4: A correlation analysis was performed including the antibody levels on Weeks 14, 16 and 18, as well as at the terminal collection. Both METH and AMPH levels in plasma were included. Results of the *t*-test identified significant correlations between METH and all other variables and between all of the antibody time-points. AMPH was correlated with METH but not with any of the antibody time-points. A Bonferroni correction for multiple correlations sustained the correlations between all antibody levels and between METH and the antibody levels at Weeks 14 and 18.

Time-Course Effects

To determine whether time-course effects of the vaccination procedure were present in Experiment 2, the effects of successive vaccine injections on body temperature (Figure S1) and locomotor activity (Figure S2) following drug challenges (0.0, 1.0, 5.6 mg/kg METH) were examined. Figure S1 shows that temperature values in MH6-vaccinated and KLH-control groups were significantly different, as confirmed by main effect of drug treatment condition ($F_{8,112} = 6.96$; $p < 0.0001$) and time post-injection ($F_{5,70} = 49.95$; $p < 0.0001$), with interactions between group and time post-injection ($F_{5,70} = 4.07$; $p < 0.005$), and treatment condition and time post-injection ($F_{40,560} = 3.31$; $p < 0.0001$). Post-hoc tests further confirmed significant differences between groups at the 1.0 mg/kg METH dose across the vaccination procedure. Significant differences within

groups were confirmed relative to baseline (i.e., 30 and 60 min prior to the drug challenge) and relative to the 2nd vaccination across all doses, including a baseline shift in saline vehicle relative to the 2nd vaccination.

Figure S2 shows that locomotor activity in the MH6-vaccinated and KLH-control groups was significantly different, as confirmed by main effect of treatment condition (drug dose) ($F_{8,112}$ = 2.36; $p < 0.05$) and time post-injection ($F_{5,70}$ = 10.62; $p < 0.0001$), with an interaction between treatment condition (drug dose) and time post-injection ($F_{40,560}$ = 2.93; $p < 0.0001$). Post-hoc tests further confirmed significant differences between groups at 5.6 mg/kg METH across successive vaccinations. Significant differences within groups were confirmed relative to baseline and relative to the effects following the 2nd vaccination at 1.0 and 5.6 mg/kg METH but not after administration of saline vehicle. See Figure S1 for complete details of the post-hoc comparisons.

Blood and Brain Tissue AMPH Concentrations

Figure S3 shows that vaccination produced marginal differences in serum and brain AMPH concentrations obtained 30 min after a 3.2 mg/kg METH challenge. A correlation analysis was performed including the antibody levels on Weeks 14, 16 and 18, as well as at the terminal collection (depicted). Both METH and AMPH levels in serum were included. Results of the *t*-test identified significant correlations between AMPH and METH (r^2 = 0.24), but not with any of the antibody time-points.

Body Weight

MH6-vaccinated rats gained significantly more weight than KLH-control rats in Experiment 2 (Figure S4), as confirmed by a main effect of weeks ($F_{22,308}$ = 473.62; $p < 0.0001$), and an interaction of treatment group and weeks ($F_{22,308}$ = 3.00; $p < 0.0001$). Significant differences in weights between KLH-control and MH6-vaccinated rats were observed starting with week 7 until the end of the study (week 20). During this time period, KLH-control rats weighed on average 18.71 grams less than MH6-vaccinated rats. No significant differences in weight were observed between MH6-vaccinated and KLH-control rats in Experiment 1 (data not shown).

Supplementary Discussion

The effects of successive vaccine boosts across the 9-week period show that METH-induced hypothermia was attenuated in MH6-vaccinated rats relative to KLH-control rats at both doses tested (1.0 and 5.6 mg/kg, subcutaneous (s.c.)); this protection was evident after the first boost and was maintained across subsequent boosts. The hypothermic effects of 1.0 mg/kg METH in KLH-control rats were greater than those seen at the 5.6 mg/kg dose, thus the vaccine appears to have provided better protection at the smaller dose. This pattern of METH-induced hypothermia is similar to prior reports that show amphetamine's effects on body temperature response (under different ambient temperatures) are characterized by nonlinear functions (MDMA: (1); METH: (2)). That is, moderate doses may produce a maximum decrease of body temperature and any further increase in dose produces effects that are neutral or even hyperthermic.

Although it appears that there was a difference in thermoregulatory responses in the MH6-vaccinated and KLH-control rats following a vehicle administration after the first boost, this difference may be at least partially attributed to a procedural difference between rats. Experimental sessions were conducted either 2 or 5.5 hours into the rats' light cycle due to space and equipment limitations for this study, and this produced modestly different effects on body temperature regardless of whether the rats were vaccinated or not. Temperature values were slightly lower when sessions were conducted 5.5 hrs into the light cycle and tended to decrease more across the experimental session. For the first set of challenges, 5/8 KLH rats were run later and 5/8 MH6 rats run earlier; this was corrected to 4/4 for the subsequent challenges. Nevertheless it is still possible that some minor, unanticipated differences in physiological effects were produced by the two vaccines; if so these were most likely relevant only earlier in the study since baseline differences disappeared by the second set of METH challenges.

METH-induced effects on locomotor activity were observed only at the highest dose administered (5.6 mg/kg, s.c.), and protection engendered by vaccination followed a similar pattern. The disruptive effects of METH on locomotor activity increased across

successive METH challenges in a manner consistent with sensitization to psychomotor stimulant effects. That is, KLH-control rats showed progressive decreases in locomotor activity, which is presumably due to stereotypy that interfered with their ability to ambulate. MH6-vaccinated rats showed increased activity across the vaccination procedure. These findings are highly consistent with the interpretation that the effects of MH6 vaccination were to reduce brain exposure of a given injected METH dose.

METH produced biphasic effects on locomotor activity, as well as thermoregulatory responses, that were dependent on dose and ambient temperature (i.e., hypo- and hyperthermia at low and high ambient temperatures, respectively), which is consistent with prior reports (3, 4). In addition, METH produced differential effects on locomotor activity and body temperature; that is, hyper- and hypothermia occurred independent of wheel or locomotor activity. A study by Phelps *et al.* (3) reported similar findings; they found that METH produced hyperthermia at 24°C in a manner uncorrelated with locomotor activity.

An unexpected finding of this study was the difference in weights between the KLH-control and MH6-vaccine rats. A prior study found that an anti-PCP antibody dose-dependently attenuated PCP-induced weight loss but that study used chronic drug exposure (5). Although the administration of METH was limited in the present study it is still possible that anorexic effects of METH were greater in the KLH-control group compared with the MH6-vaccinated rats. This would again be consistent with an interpretation of MH6 vaccination lowering the effective brain exposure after any given dose of METH. The bodyweight effect wasn't large, but given body composition issues in some METH users, it would be worthwhile to further investigate the relationship between anti-drug vaccination and its efficacy to protect against drug-induced weight loss.

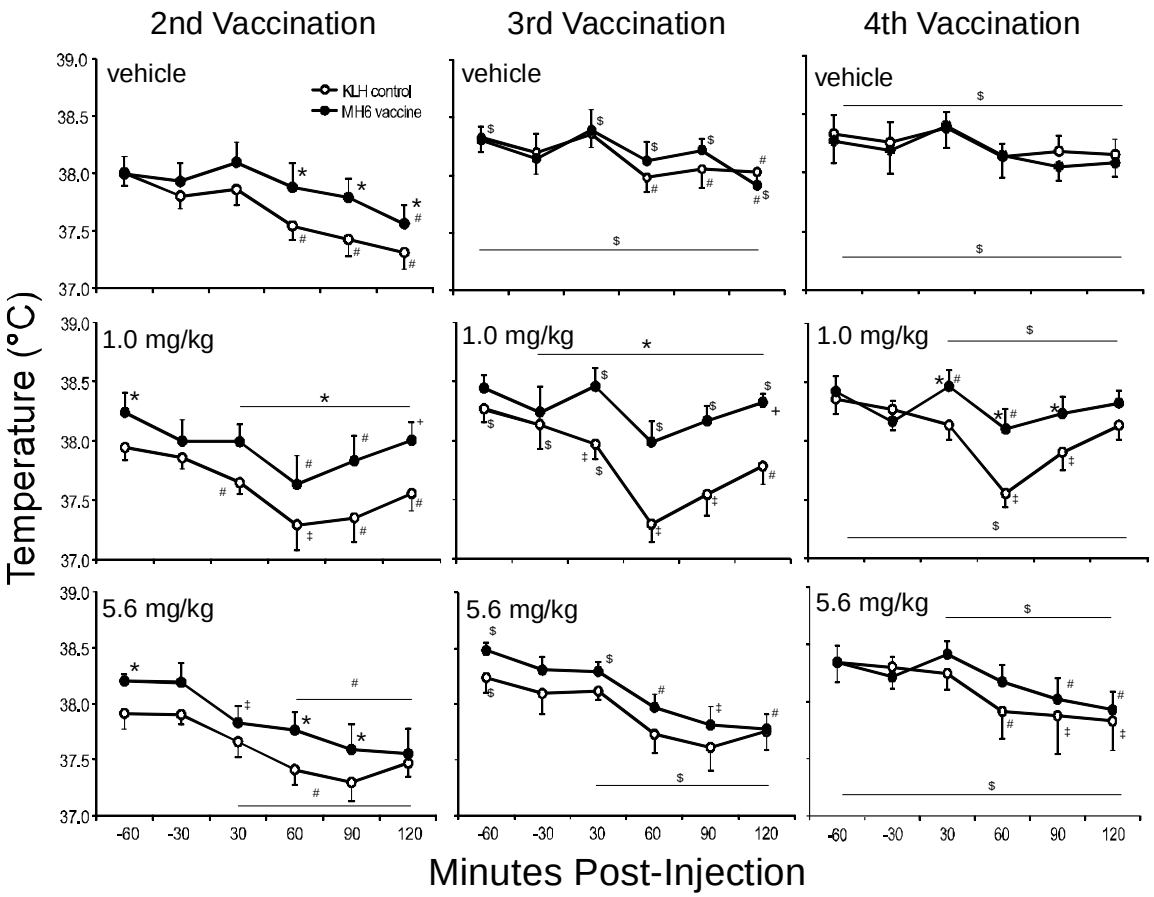

Figure S1. Mean body temperature values (°C) during successive 30-min intervals across a range of doses of METH (0.0, 1.0, 5.6 mg/kg, s.c.) in MH6-vaccinated and KLH-control rats (Experiment 2). Graphs in columns (left-to-right) show data obtained after the 2nd, 3rd, and 4th vaccinations. Significant differences between groups (within each graph) are shown by *. Significant differences (a) within group compared to baseline (i.e., 30 and 60 min prior to drug challenge) are shown by #; (b) within group, across vaccinations, and across doses are shown by $; (c) within group, within vaccination, and across doses compared to vehicle are shown by +; and, (d) within group, within vaccination, and across doses compared to vehicle *and* within group compared to baseline are shown by ‡. Error bars are SEMs. These data partially overlap with data shown in Figure 3. KLH, keyhole limpet hemocyanin; METH, d-methamphetamine; s.c., subcutaneous.

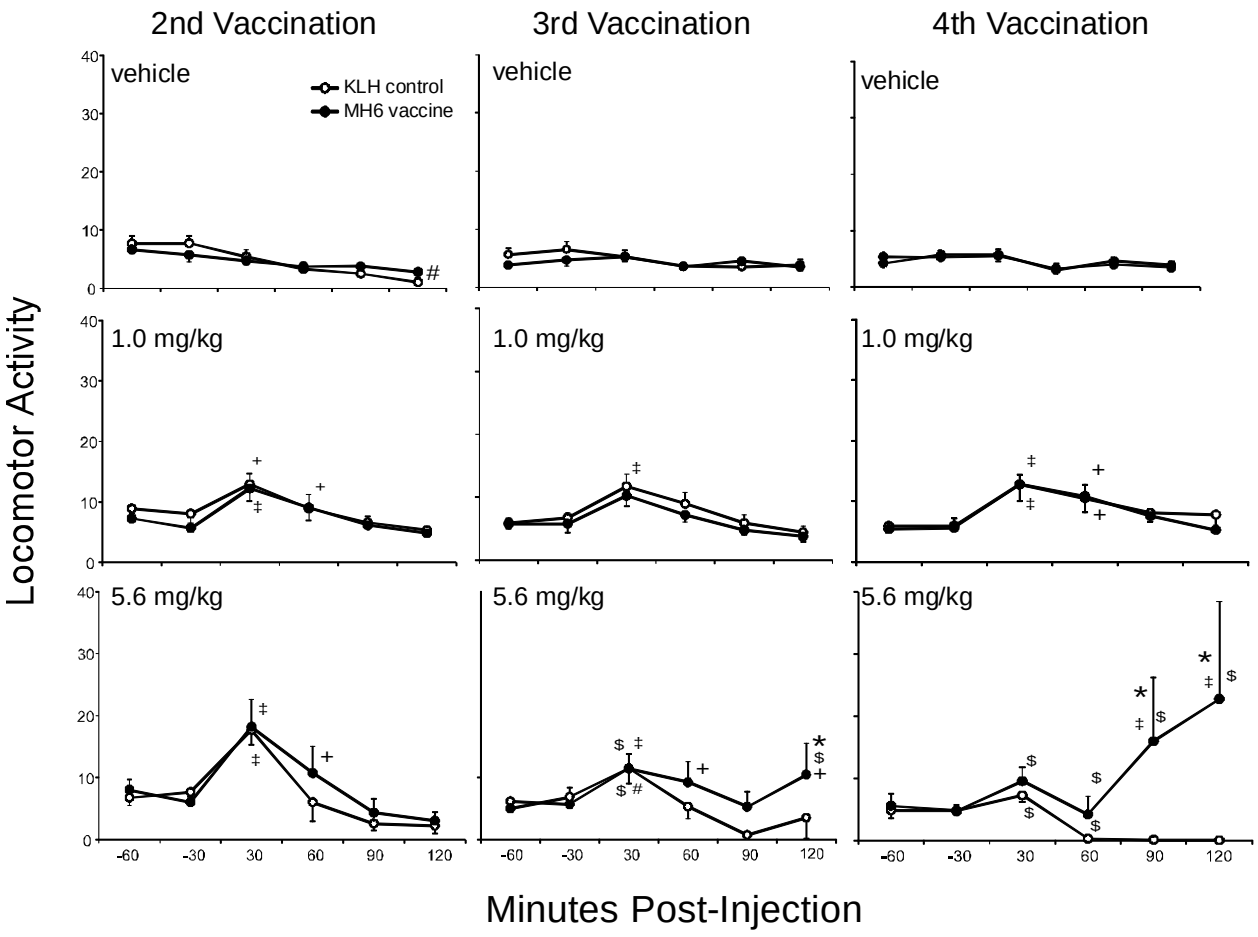

Figure S2. Mean locomotor activity during successive 30-min intervals across a range of doses of METH (0.0, 1.0, 5.6 mg/kg, s.c.) in MH6-vaccinated and KLH-control rats (Experiment 2). Graphs in columns (left-to-right) show data obtained after the 2nd, 3rd, and 4th vaccinations. Symbols for significant differences between and within groups are the same as described in Figure 4. Error bars are SEMs. These data partially overlap with data shown in Figure 3. KLH, keyhole limpet hemocyanin; METH, d-methamphetamine; s.c., subcutaneous.

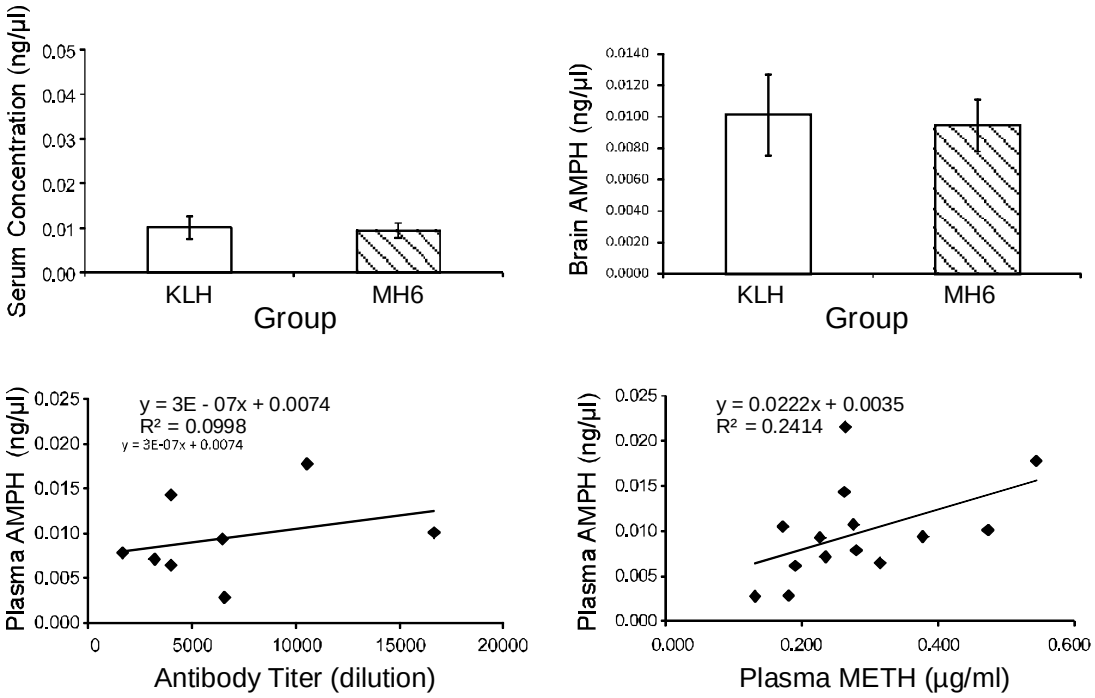

Figure S3. Top panel: AMPH concentrations (ng/ul) in blood serum (left) and brain serum (right) for KLH-control and MH6-vaccinated rats. Bottom panel: Plasma AMPH concentrations (ng/ul) across a range of antibody titer (dilution; left) and plasma AMPH concentrations (ng/ul) as a function of plasma METH concentrations (ng/ul; right). AMPH, amphetamine; KLH, keyhole limpet hemocyanin; METH, d-methamphetamine; s.c., subcutaneous.

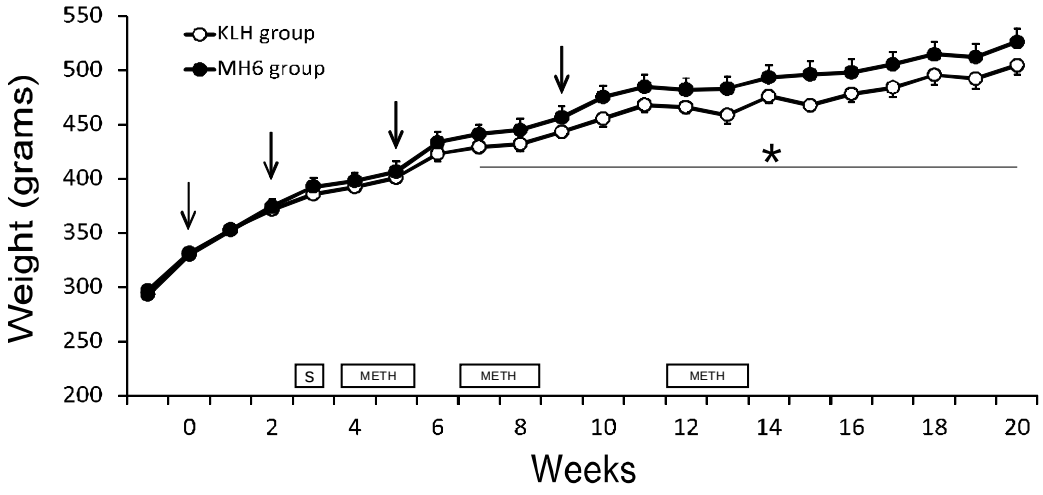

Figure S4. Mean body weights (grams) for KLH-control and MH6-vaccinated rats across successive weeks during Experiment 2. Arrows depict vaccinations (weeks 0, 2, 5, and 9). Acute METH challenges are shown by boxes labeled 'METH' and surgery is depicted by "s." Significant differences between groups are shown by *. Error bars are ± SEM. KLH, keyhole limpet hemocyanin; METH, d-methamphetamine.

Supplementary References

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