Published in final edited form as:
Biol Psychiatry. 2013 April 15; 73(8): 721–728. doi:10.1016/j.biopsych.2012.09.010.

A methamphetamine vaccine attenuates methamphetamine-induced disruptions in thermoregulation and activity in rats

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Abstract

Background—There are no approved pharmacotherapies for *d*-methamphetamine (METH) addiction and existing therapies have limited efficacy. Advances in using immunotherapeutic approaches for cocaine and nicotine addiction have stimulated interest in creating a similar approach for METH addiction. This study investigated whether active vaccination against METH could potentially attenuate responses to METH, *in vivo*.

Methods—Male Sprague Dawley rats (N=32) received a 4-boost series with 1 of 3 candidate anti-METH vaccines (MH2(R), MH6, and MH7) or a control keyhole limpet hemocyanin conjugate vaccine (KLH). Effects of METH on rectal temperature and wheel activity at 27°C ambient temperature were determined. The most efficacious vaccine, MH6, was then contrasted with KLH in a subsequent experiment (N=16), wherein radiotelemetry determined home cage locomotor activity and body temperature at 23°C ambient temperature.

Results—The MH6 vaccine produced high antibody titers with nanomolar affinity for METH and sequestered METH in the periphery of rats. In Experiment 1, the thermoregulatory and psychomotor responses produced by METH at 27°C were blocked in the MH6 group. In Experiment 2, METH-induced decreases in body temperature and locomotor activity at 23°C were also attenuated in the MH6 group. A pharmacokinetic study in Experiment 2 showed that MH6-vaccinated rats had higher METH serum concentrations, yet lower brain METH concentrations than controls, and METH concentrations correlated with individual antibody titer.

Conclusions—These data demonstrate that active immunopharmacotherapy provides functional protection against physiological and behavioral disruptions induced by METH.

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Financial Disclosures
The authors reported no biomedical financial interests or potential conflicts of interest.

Keywords

Drug addiction; immunopharmacotherapy; *d*-methamphetamine; thermoregulation; activity; stimulants

INTRODUCTION

D-methamphetamine (METH) addiction is a growing public health concern, but effective treatments are lacking. Pharmacotherapies have limited success for treating drug addiction, and often produce adverse side effects (1). Immunopharmacotherapy is a promising alternative (2–6). In the active immunization approach, vaccination stimulates the immune system to produce antibodies against the drug of abuse. The drug-recognizing antibodies sequester drug molecules in the blood stream, which reduces distribution to the brain, thereby reducing drug effects. To date vaccines have been shown to effectively attenuate effects of drugs such as cocaine (7–12), nicotine (13–21), morphine and heroin (22, 23), tetrahydrocannabinol (24) and phencyclidine (25, 26). Clinical studies of anti-cocaine and anti-nicotine vaccines are ongoing (27–29) and have shown titer-dependent efficacy during abstinence (28–31) as well as reduced subjective ratings of pleasurable drug effects (28). This translational success has encouraged the development of anti-METH vaccines.

Efficacy of both passive and active anti-METH vaccines has been investigated in preclinical studies. Passive administration of monoclonal antibodies can reduce METH self-administration (32), reduce METH-induced locomotor activity (33–35), and impair METH discrimination in a drug discrimination paradigm (36). Although passive immunotherapy has the advantage of producing immediate and dose-dependent antagonist effects, it may be limited as a therapeutic approach. Monoclonal antibodies are expensive to manufacture and effects are transient, which complicates patient compliance.

Active vaccination offers an improved alternative because the immune system provides antibody protection across a long period of time. Although this protection can last for years to decades for microbe vaccination, at present efficacy for only weeks to months (after each boost) has been shown for drug vaccines. Active vaccination is more cost effective and requires minimal patient compliance; however, immune responses can vary and the resulting vaccine efficacy might differ among individuals. Prior preclinical investigations using *active* anti-METH vaccines are limited and show mixed results. Byrnes-Blake et al. (37) found no change in METH-induced locomotor activity in vaccinated rats even though antibody titers reached significant levels. More recently, however, active vaccination was shown to transiently increase METH self-administration in a manner that might be interpreted as consistent with reduced brain penetrance of drug (38); unfortunately no data on METH distribution were presented. This limited evidence, along with preclinical evaluations of cocaine and nicotine vaccines, suggests that a diversity of METH vaccines may be necessary to further basic understanding of anti-drug vaccination biology and, ultimately, to ensure efficacy in a variant population of addicts.

Novel strategies for creating an active vaccination for METH have been explored in several laboratories (38–40). Most relevant to the current study, Moreno et al. (2011) systematically generated a series of chemical structures to target the most stable conformation of METH using GIX+ mice. Following vaccination 3 of 6 candidates (MH2(R), MH6, and MH7) generated elevated antibody titers and nanomolar (+)-METH affinity. The present study sought to determine whether any of the three anti-METH candidates alter METH-induced disruptions in the thermoregulatory and locomotor behavior of rats.

METHODS AND MATERIALS

Experimental Design

There were two experiments in this investigation. Experiment 1 was an initial screen to determine which of 3 most promising candidate anti-METH vaccines from a previous study in mice (40) would confer effects consistent with the attenuation of METH's impact *in vivo* in rats. Experiment 1 therefore assessed rectal temperature values under a high ambient temperature condition (T_A = 27±1°C) to determine effects on METH-induced hyperthermia and locomotor activity as previously described (41, 42). Experiment 2 focused on the vaccine to emerge from the first experiment as the most promising (MH6) in order to determine effects on METH-induced hypothermia under a typical laboratory ambient temperature condition (T_A = 23±1°C). This experiment used radiotelemetry devices for precise assessment of body temperature and locomotion under freely-moving conditions in the standard shoe-box style cages (43). Table 1 shows experimental conditions for both experiments.

Animals

Forty eight male Sprague Dawley rats (Experiment 1: N=32, Experiment 2: N=16; Harlan, Livermore, CA) weighing ~320 grams on arrival were group housed in clear shoebox cages (2–3 per cage) in a vivarium with a 12:12 light-dark cycle. Food pellets and water were available ad libitium. Rats were 11 weeks old at the start of both experiments. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and under protocols approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Drugs and Haptens

D-methamphetamine was dissolved in 0.9% sterile saline and administered subcutaneously (s.c.) for acute challenges. A constant injection volume of 1 ml/kg was used. *D*-methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), and 4-methylmethcathinone (4-MMC) were provided by RTI under contract to NIDA; amphetamine (AMPH) was purchased from Sigma-Aldrich.

Methamphetamine haptens (MH2(R), MH6, and MH7 were coupled with a keyhole limpet hemocyanin (KLH) carrier protein and in formulation with the Sigma Adjuvant System® as previously reported (40).

Equipment

Standard activity wheels attached to clear shoebox cages were used (Med Associates Model ENV-046), and the number of wheel quarter rotations in each session was collected by MED-PC IV software (Experiment 1 only). Radiotelemetry transmitters (CTA-F40; Data Sciences International, DSI) and corresponding telemetry plates were used in conjunction with DSI Dataquest A.R.T. system™ software to collect locomotor activity and body temperature data (Experiment 2 only). Ambient temperature was controlled by a 1000/1500-watt utility heater (Patton PUH680-U) in both experiments.

Surgery

Radiotelemetry transmitters were implanted into the abdominal cavities of all rats in Experiment 2. An incision was made along the abdominal midline posterior to the xyphoid space, large enough to pass the miniature transmitter into the abdominal cavity. Absorbable sutures closed the abdominal muscle incision and the skin incision was closed with a liquid tissue adhesive (3M™ Vetbond™ Tissue Adhesive). There were at least 6 days of recovery

prior to drug challenges. For the first 3 days of recovery, cephazolan (0.4 g/ml; 2.0 ml/kg s.c.) and flunixin (2.5 mg/ml; 2.0 ml/kg s.c.) were administered once per day to prevent bacterial infection.

Immunologic Assays

Blood was collected from the tail vein (weekly in Experiment 1; every two weeks in Experiment 2), immediately placed on ice to prevent clotting, centrifuged at 10,000 g for 15 min, plasma extracted and then stored at −80°C until further use.

Antibody titers were assessed by enzyme-linked immunosorbent assay (ELISA) as previously described (40) using MH6- and MH7-BSA conjugates as coating antigens (i.e., MH2(R) sera was overlaid on MH6-BSA plates). Titers were calculated from the plot of absorbance versus log dilution, as the dilution corresponding to an absorbance reading 50% of the maximal value.

Antibody affinities and concentrations for the MH2(R), MH6, and MH7 haptens and the specificity of MH6 antibodies for METH and AMPH were determined by equilibrium dialysis using a solution-based radioimmunoassay as described in (40) (Experiment 1 only).

Drug concentrations in terminal blood samples and brains were assessed using high-throughput liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) at the Scripps Center for Metabolomics and Mass Spectrometry (Experiment 2 only). For this assessment, blood samples and brains were collected 30 min after a 3.2 mg/kg METH challenge for all rats, except for 2 KLH rats that were inadvertently euthanized by animal care staff prior to this assessment. Rats were anesthetized under isoflurane, exsanguinated via cardiac puncture, and then decapitated; brains were quickly removed, weighed and homogenized in saline (1:4 ratio). All samples were centrifuged at 8000 rpm for 10 min and the extracted plasma was stored at −80°C. Subsequently, these plasma samples were prepared using a trichloroacetic acid-based extraction described in (44) to dissociate drug from antibody.

Vaccination Procedure

All rats were vaccinated during weeks 0, 2, 5, and 9. For each vaccination, haptens (MH2(R), MH6, MH7, and KLH) were added to adjuvant to create a 0.5 ml vaccine for each rat, which was administered across 3 sites (0.2 ml subcutaneous in the nape; 0.2 ml subcutaneous in the hind quadriceps; 0.1 ml intraperitoneal). Rats in Experiment 1 received MH2(R), MH6, MH7, or KLH (control) haptens; rats in experiment 2 rats received either MH6 or KLH (control) haptens.

Drug Challenges

Experiment 1—After completion of the vaccination procedure, the effects of METH (0.0, 1.0, 5.6 mg/kg, s.c.) on rectal temperature and wheel activity were determined in vaccinated (N=8 per hapten; MH2(R), MH6, and MH7 haptens) and non-vaccinated control rats (N=8; KLH) at T_A = 27±1°C. METH challenges were administered in a balanced order, with a 3–4 day interval between each challenge. In each experimental session, rectal temperature values were collected 10 min prior to and then 30, 60, 90 and 120 min after the METH challenge and wheel activity was recorded throughout the entire 2-hr post-injection interval.

Experiment 2—Throughout the vaccination procedure, effects of METH (0.0, 0.5, 1.0, 3.2, 5.6 mg/kg, s.c.) on body temperature and locomotor activity were determined in MH6-vaccinated (N=8) and KLH-control rats (N=8) at T_A = 23±1°C. Body temperature and

locomotor activity data were collected in 5-min bins, starting 1-hr prior to and continuing for 3-hrs after METH challenge during each 4-hr session (2 sessions per week).

Data Analysis

Analysis of the majority of the physiological and behavioral data was conducted using analysis of variance (ANOVA) with a between-subjects factor of treatment group and a within-subjects factor of session time (3, 60, 90, 120 min post-injection). Two-sample t-tests were used to assess between-group differences in plasma METH levels in Experiment 2. In the first experiment, two sets of analyses were conducted: the first included all 4 groups and then the second set of analyses compared KLH to the candidate anti-METH groups individually. Drug-treatment condition (vehicle, METH doses) was included as a third repeated factor in Experiment 2. Post-hoc analyses of significant main effects in the ANOVA were conducted using the Fisher's LSD 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. Detailed summaries of the statistical analyses are described in Supplementary Materials.

RESULTS

Experiment 1

Antibody Titers—An equilibrium dialysis assay was performed in order to normalize the results among MH2(R), MH6, and MH7 haptens. The MH6 and MH7 haptens produced similar binding constants (K_d =~25–30 nM; Table 2) but the MH6 hapten produced 2–3 times more antibody (Figure 1). Although MH6 and MH2(R) produced similar levels of antibody, MH2(R) had much lower affinity than MH6 (K_d = ~2.24 μM). Specificity of MH6 antibodies for METH, AMPH, MDMA, and 4-MMC was assessed by equilibrium dialysis, and the results are shown in Table 2. Binding affinity for METH and AMPH was in the nanomolar range whereas that for MDMA and 4-MMC was in the low micromolar range.

Antibody titers obtained following MH6 vaccination for both experiments are shown in Figure 1. Similar to the previously reported findings in mice (40), titer production for MH6 was 1:70,000–1:80,000, and was maintained for 3 weeks following the final boost. A similar pattern of titer production was observed up to week 12 in Experiment 2.

Thermoregulation and Activity—The KLH-control rats had significantly higher rectal temperature values following 5.6 mg/kg METH compared with the MH6-vaccinated rats (Figure 2), as was confirmed by a main effect of time post-injection ($F_{4,112} = 19.46$; $p<0.0001$) and an interaction of treatment group with time post-injection ($F_{12,112} = 2.38$; $p<0.01$) in the four group analysis. The peak mean rectal temperature values following administration of 5.6 mg/kg METH for the KLH group was 39.40 °C (SEM 0.40; 60 min post-injection) compared to 39.53 °C (SEM 0.18; 30 min post-injection) for the MH2(R) group, 39.53 °C (SEM 0.49; 30 min post-injection) for the MH7 group, and 38.85 °C (SEM 0.29; 60–90 min post-injection) for the MH6 group. In addition, the KLH-control rats had significantly more wheel activity (quarter rotations) compared with MH6-vaccinated rats, as confirmed by a main effect of time post-injection ($F_{11,154} = 6.21$; $p<0.0001$) and an interaction of treatment group with time post-injection ($F_{11,154} = 3.43$; $p<0.0005$). The peak mean numbers of wheel rotations following administration of 5.6 mg/kg METH for the KLH group was 78.5 (SEM 21.10; 15–20 min post-injection) compared to 105.13 (SEM 35.25; 15–20 min post-injection) for the MH2(R) group, 98.88 (SEM 43.96; 10–15 min post-injection) for the MH7 group, and 38.63 (SEM 9.68; 0–5 min post-injection) for the MH6 group. Data from the 1.0 mg/kg METH challenge are not shown because there were no

significant differences between the KLH-control and vaccinated (i.e., MH2(R), MH6, MH7) groups.

Experiment 2

Thermoregulation and Activity—Figure 3 shows effects of MH6 on body temperature and locomotor activity, as measured by radiotelemetry, 60 min prior to and up to 120 min after METH challenges (0.0, 0.5, 1.0, 3.2, 5.6 mg/kg) at T_A=23°C (Experiment 2). KLH-control rats had significantly lower body temperature values compared with MH6-vaccinated rats, as confirmed by main effects of drug dose ($F_{4,56}$ = 3.11; $p<0.05$) and time post-injection ($F_{5,70}$ = 19.95; $p<0.0001$), and interactions of drug dose with time post-injection ($F_{20,280}$ = 6.08; $p<0.0001$) and treatment group with time post-injection ($F_{5,70}$ = 3.19; $p<0.05$).

METH decreased locomotor activity in KLH-control rats relative to MH6-vaccinated rats at the 5.6 mg/kg dose, as confirmed by a main effect of time post-injection ($F_{4,28}$ = 3.04; $p<0.05$), with an interaction of dose and time post injection ($F_{20,140}$ = 3.10; $p<0.0001$) and a 3-way interaction of treatment group, dose, and time post-injection ($F_{20,140}$ = 1.74; $p<0.05$).

Blood METH Concentration—Figure 4 shows the effects of vaccination on METH concentration in both peripheral blood and brain tissue 30 min after a 3.2 mg/kg METH challenge. MH6-vaccinated rats had a significantly higher concentration of METH in peripheral blood than KLH-control rats, as confirmed by main effect of group ($F_{2,27.42}$ = −6.28; $p<0.0001$). In addition, MH6-vaccinated rats had a significantly lower concentration of METH in brain tissue than KLH-control rats, as confirmed by main effect of group ($F_{2,11.27}$ = −2.50; $p<0.05$). Plasma METH concentration was significantly positively correlated (see Supplementary Materials) with antibody titer at the time of this challenge (r^2=0.53, depicted) and also with the antibody titer from blood samples obtained during weeks 14 (r^2=0.75), 16 (r^2=0.50) and 18 (r^2=0.86).

DISCUSSION

This study provides unequivocal evidence that active vaccination is capable of attenuating physiological and behavioral effects of *d*-methamphetamine (METH), unlike prior attempts at active immunotherapy for METH (37, 38). It was shown that active vaccination attenuated METH-induced disruptions in thermoregulation (i.e., hyper- and hypothermia at 27°C and 23°C, respectively), wheel activity (Experiment 1) and stereotypy (Experiment 2) after the highest METH dose administered. The progression of psychomotor effects across successive METH challenges (Supplementary Materials, Figures S1, S2) was also attenuated by vaccination, which is consistent with an alteration in the trajectory of stimulant sensitization.

The efficacy of an immunologic approach depends on the magnitude of the antibody concentration response (i.e., titer) in conjunction with the affinity and specificity of the antibodies towards METH (Moreno and Janda, 2009). We report here that vaccination with MH6 satisfies these criteria. That is, MH6 produced moderate and sustained antibody titers relative to the other haptens tested (i.e., MH2(R), MH7), with good affinity and specificity for METH and AMPH. The binding properties of AMPH are an important consideration for the development of an anti-METH vaccine because AMPH is a metabolite of METH that produces psychoactive effects independent of METH's effects. An immunologic response that prevents both METH and AMPH from activating the central nervous system is especially germane to clinical application.Titer production generated by MH2(R) and binding constants generated by MH7 were similar to those produced by MH6, yet both had no effect on METH-induced disruptions in thermoregulation and wheel activity (Experiment

1). However, given the variability of individual titer production in human clinical populations (29) it may be worthwhile to investigate the efficacy of a multivalent vaccine to address this diversity. Overall, the pharmacokinetic findings reported here are consistent with observations from preclinical studies of anti-METH (33, 45), anti-cocaine (46), and anti-nicotine (14, 17, 47) vaccinations.

The development of the titer response in MH6-vaccinated rats followed a consistent, replicable pattern in both experiments. That is, antibody titers increased across a 6-week period and then stabilized thereafter. In Experiment 2, when titers were assessed 1–3 months after the final vaccine boost, a gradual decline in titers was observed and the final assessment of titers (i.e., week 20) showed titer values that were similar to those found after the priming vaccine dose. Although the decline in titers may be a function of time since last boost, it may also be due to the concomitant presence of METH in the blood due to the intermittent challenges. When antibody loads were assessed 30-min after a 3.2 mg/kg challenge dose of METH, titers were approximately $1/10^{th}$ of previous titers possibly because binding of the antibodies to the ELISA plate competed with the presence of METH. A similar finding was reported by Duryee and colleagues (38) since when METH-specific antibodies were assessed upon completion of the METH self-administration study, marginal titers were found in vaccinated rats; however, after a 34-day drug-free period, titers in vaccinated rats returned to previous levels. We show here that individual METH levels were nevertheless positively correlated with the titer on the final day as well as with titers from the three prior blood sample collections during weeks 14–18. This finding further enhances confidence in the specificity of the effects of MH6 vaccination.

This study showed that vaccination increased METH levels in serum and decreased METH levels in the brain following an acute injection of METH (Experiment 2). This is consistent with findings from previous studies that investigated anti-METH (48), anti-nicotine (4, 17, 47), and anti-cocaine (9, 10, 46) vaccines, and suggests that the anti-METH vaccine engenders neuroprotection against centrally-mediated effects of METH.

The present study is also the first to report effects of anti-METH vaccination on thermoregulatory responses. These findings support the *in vivo* specificity of the protection, and are an important consideration for clinical application because physiological effects of drugs are often of highest concern for acute mortality and morbidity (49–51). Methamphetamine *increased* rectal temperatures at 27±1°C (Experiment 1) and *decreased* body temperatures at 23±1°C (Experiment 2). These were expected outcomes consistent with prior reports that show METH-induced hypo- and hyperthermia depends on low and high ambient temperatures, respectively (52). Each of the effects (hypo- and hyperthermic) were attenuated in the MH6-vaccinated groups, again indicating an effect specific to the drug.

The overt behavioral effects of METH were likewise attenuated by vaccination in both experiments at the highest dose administered (5.6 mg/kg, s.c.). In the first experiment, vaccination attenuated METH-induced increases in wheel activity, which is consistent with prior vaccine studies that show reductions in cocaine- and nicotine-induced locomotor activity (9, 10, 13, 34, 35). Similar beneficial locomotor effects were not reported in an initial study of active vaccination (37). In that study, METH was administered daily and the anti-METH vaccine was administered during weeks 0, 3, and 6. Total distance travelled prior to, during, and following active vaccination was ascertained (using a within-group comparison) and vaccination had no effect on total distance traveled. Because there was no control group it is not possible to determine whether METH produced either sensitization or habituation due repeated administration (53). An alternative consideration is that home-cage locomotor activity and voluntary wheel activity are affected differentially by psychomotor

stimulants (42, 54, 55). This latter possibility is reinforced by the contrasting psychomotor effects observed in the current study between wheel activity (Experiment 1) and locomotor activity (Experiment 2).

Vaccinated rats showed *increased* locomotor activity after the 5.6 mg/kg METH dose relative to KLH-control rats in Experiment 2. Similarly, Byrnes-Blake and colleagues showed that *passive* immunotherapy with an anti-METH monoclonal antibody increased locomotor activity at the 3.0 mg/kg dose but reduced locomotor activity at the 0.3 and 1.0 mg/kg doses of METH (33). The increased locomotor activity in MH6-vaccinated rats in the present study is most plausibly interpreted as protection from the induction of stereotypy that is associated with high-dose psychomotor stimulant administration, and the protection against stereotypy was progressive across time (Figure S2). The suppression of locomotor activity after the 5.6 mg/kg dose of METH became increasingly complete in the KLH-control group across 3 successive challenges, while the MH6-vaccinated group became progressively sensitized to increased locomotor activity. A prior study found decreased stereotypy in anti-cocaine vaccinated rats, although there was no change in locomotor activity following the highest cocaine dose (10). Gentry et al. found that distance traveled was inversely related to stereotypy rating during the first 100 min following 3.0 mg/kg (s.c.) METH (56). In the current study, diminished locomotor activity in KLH-control rats suggests that stereotyped behaviors interfered with the rats' ability to ambulate; increased locomotor activity in the MH6-vaccine group suggests that MH6 blocked METH-induced stereotypy.

These results underline the need for relatively complete animal models for *in vivo* assessment since there were conditions that showed no difference between groups. It was not simply a matter of the right METH dose, because thermoregulatory and locomotor distinctions between the MH6 and KLH groups were observed at different METH doses (1.0 vs. 5.6 mg/kg) in Experiment 2. In tactical sense, this cautions developers of anti-drug vaccines to assay a wide range of preclinical models lest a vaccine with efficacy be missed because of selecting a single (insensitive) assay. In the broader perspective, it further emphasizes that eventual human clinical application of vaccines may be specific to different aspects of the addiction cycle and/or drug-induced physiological morbidities.

A limitation of the current study is that the reported effects are quantitative and selective, yet these are consistent with previous preclinical reports for anti-cocaine vaccination. For example, Carrera et al. (2000) found differential effects of an anti-cocaine vaccine on self-administered cocaine in rats depending on cocaine dose: low doses (10 and 20 mg/kg) *increased* the number of infusions whereas high doses (30 and 40 mg/kg) *decreased* cocaine infusions by 50 and 70%, respectively. A study by Kantak et al. (2000) showed only a 30% reduction in cocaine self-administration in vaccinated rats. In addition, Duryee et al. (2009) found that vaccinated rats actually self-administered almost *twice* as much cocaine as non-vaccinated control rats under FR1 and FR3 schedules, whereas negligible differences were found between groups under FR5 and FR10 schedules. Although those prior effects were similarly quantitative rather than a complete blockade, they supported translational investigation for immunopharmacotherapy for cocaine addiction, and indeed clinical trials are ongoing. Recent evidence from clinical trials suggests that anti-cocaine vaccination is efficacious in individuals that have high antibody reactivity to the vaccine (57), whereas minimal protection is engendered in those individuals with low antibody reactivity. This is an inherent limitation with the immunotherapeutic approach at present and is an active target to be addressed. Nevertheless, at present it is valuable to consider immunopharmacotherapy as one component of a treatment package that could include, for example, a contingency management program (57–60) to promote abstinence in recovering addicts.

In summary, this study shows that active vaccination attenuates physiological and behavioral effects of METH. Protection was observed across multiple models and/or METH-related endpoints. The data were consistent across environmental conditions under which the effects of METH in control animals differed in quality, such as hyper- vs. hypothermia and increased vs. suppressed locomotor activity. The plasma and brain METH levels in Experiment 2 confirm that the antibodies sequestered METH in the periphery, thereby reducing METH distribution to the brain. Together these studies confirm the generality of the anti-METH effects of the MH6 vaccine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIDA grant #DA024705 (to M.A.T.). The NIH/NIDA had no further role in the design of the study, the analysis of results or in the decision to publish. This is manuscript #21720 from The Scripps Research Institute.

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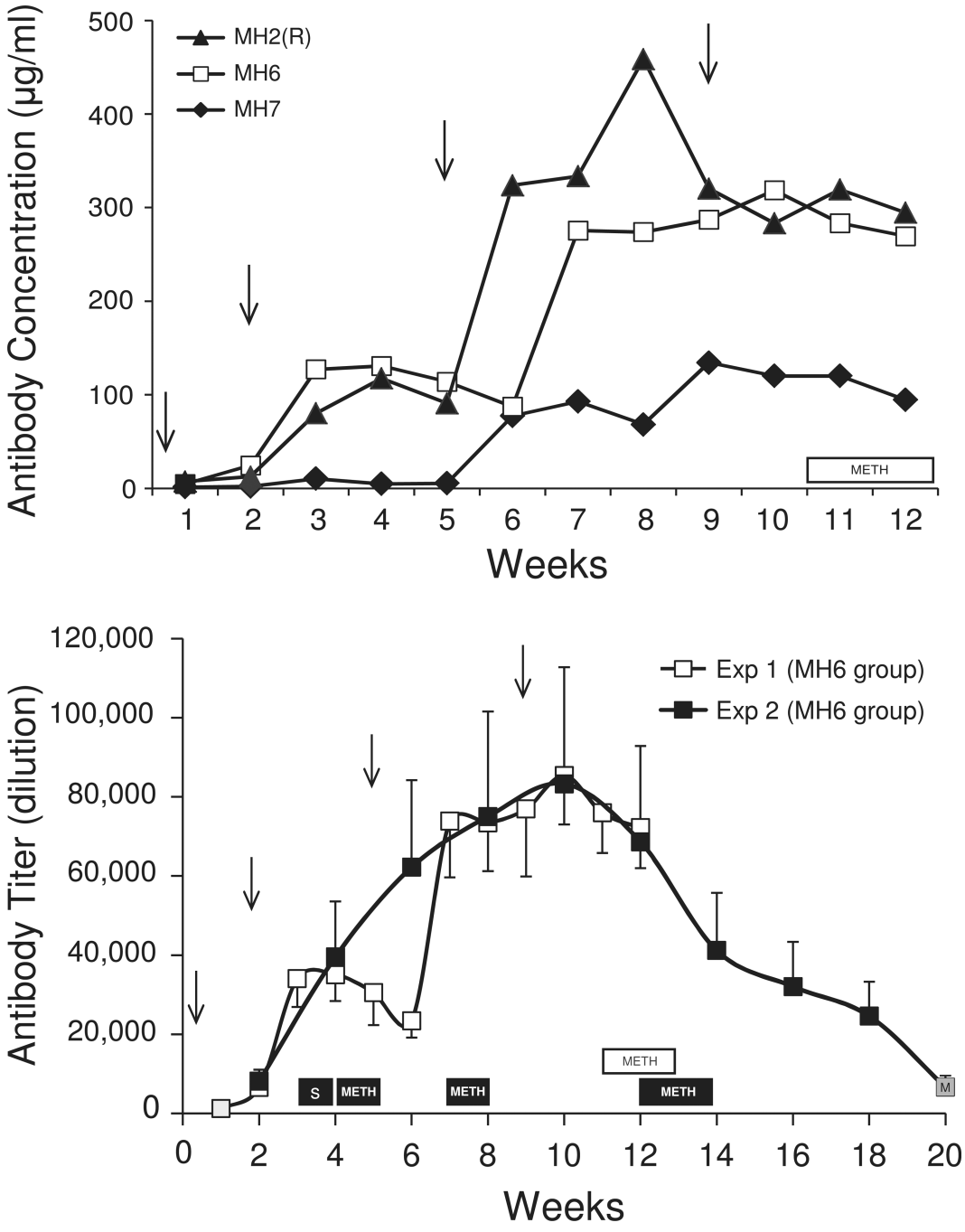

Figure 1.

Top panel: Normalized mean antibody titer concentrations (µg/ml) for MH2(R), MH6, and MH7 across 12 weeks. Arrows depict vaccinations (weeks 0, 2, 5, and 9). Acute METH challenges are shown by the box labeled 'METH.' Bottom panel: Mean antibody titer (dilution) across 12 (Experiment 1) and 20 weeks (Experiment 2) for MH6-vaccinated rats (N=8 per group). Acute METH challenges for Experiments 1 and 2 are shown by the boxes labeled 'METH' or 'M' in the top and bottom row, respectively; surgery is depicted by "s" for rats in Experiment 2. Error bars are ±SEM.

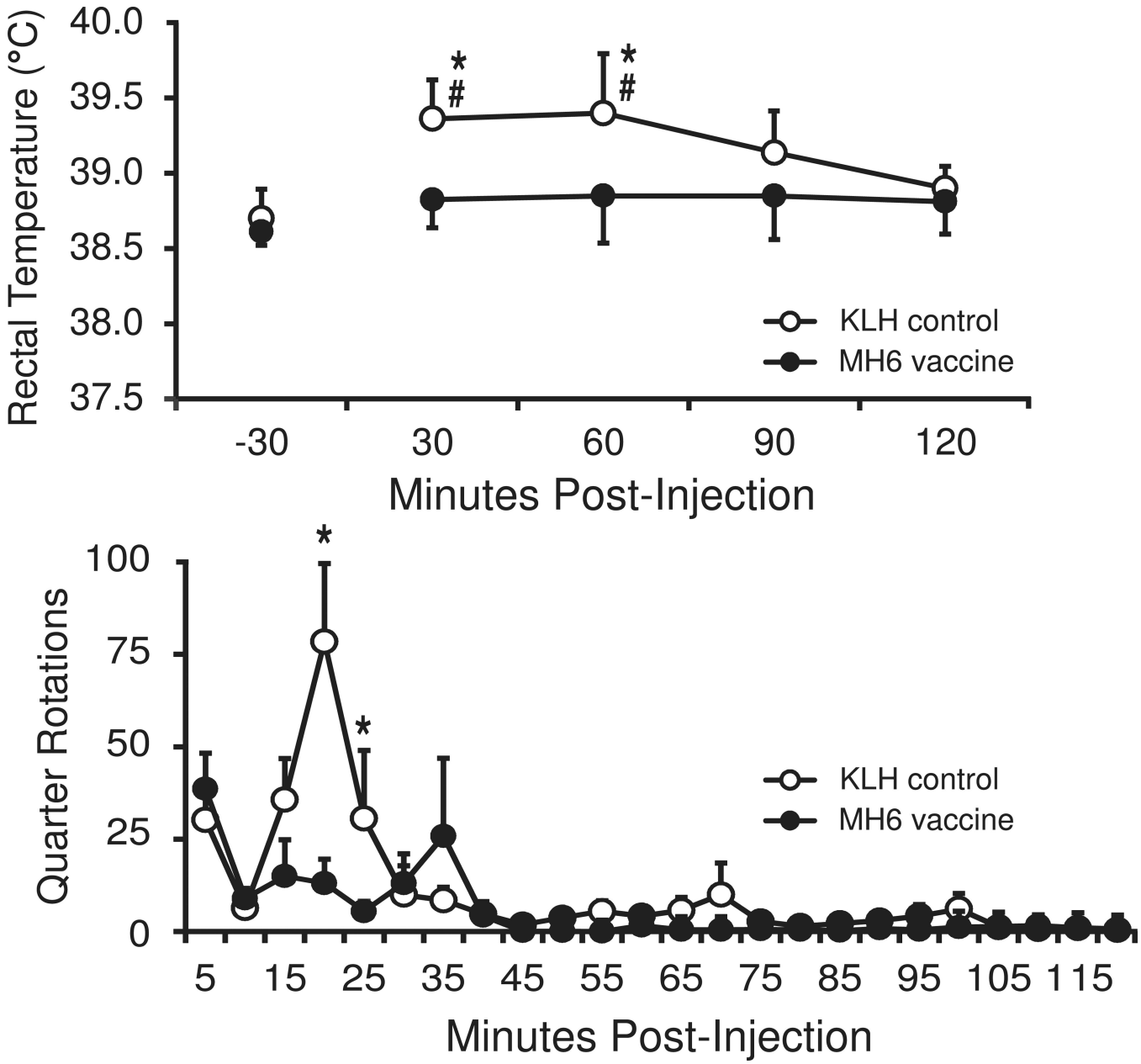

Figure 2.

Mean rectal temperature values (°C) across successive 30-min intervals (top panel) and number of quarter wheel rotations across successive 5-min intervals (bottom panel) following a challenge dose of 5.6 mg/kg METH in MH6-vaccinated and KLH-control groups at T_A = 27°C (Experiment 1). Significant differences between and within groups are shown by * and #, respectively. Error bars are ±SEM.

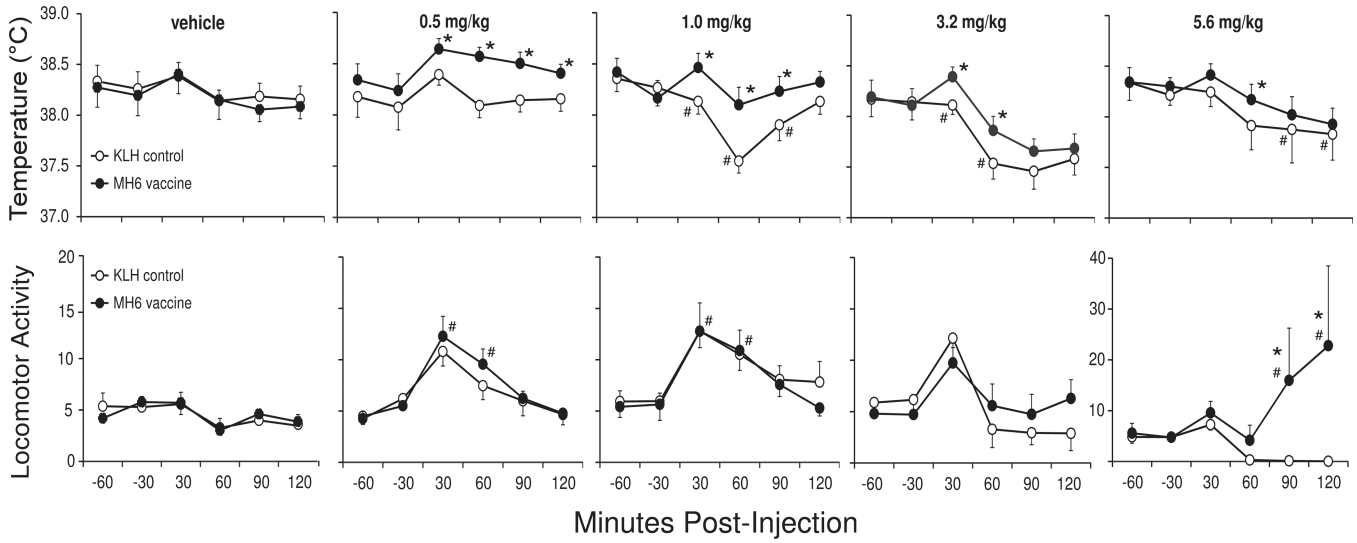

Figure 3.

Mean body temperature values (°C; top panel) and locomotor activity (bottom panel) across successive 30-min intervals following challenge doses of METH (0.0, 0.5, 1.0, 3.2, 5.6 mg/kg) in MH6-vaccinated and KLH-control rats at T_A = 23°C (Experiment 2). Significant differences between and within groups are shown by * and #, respectively. Note that the y-axis values differ for locomotor activity at the 5.6 mg/kg dose. Error bars are ±SEM.

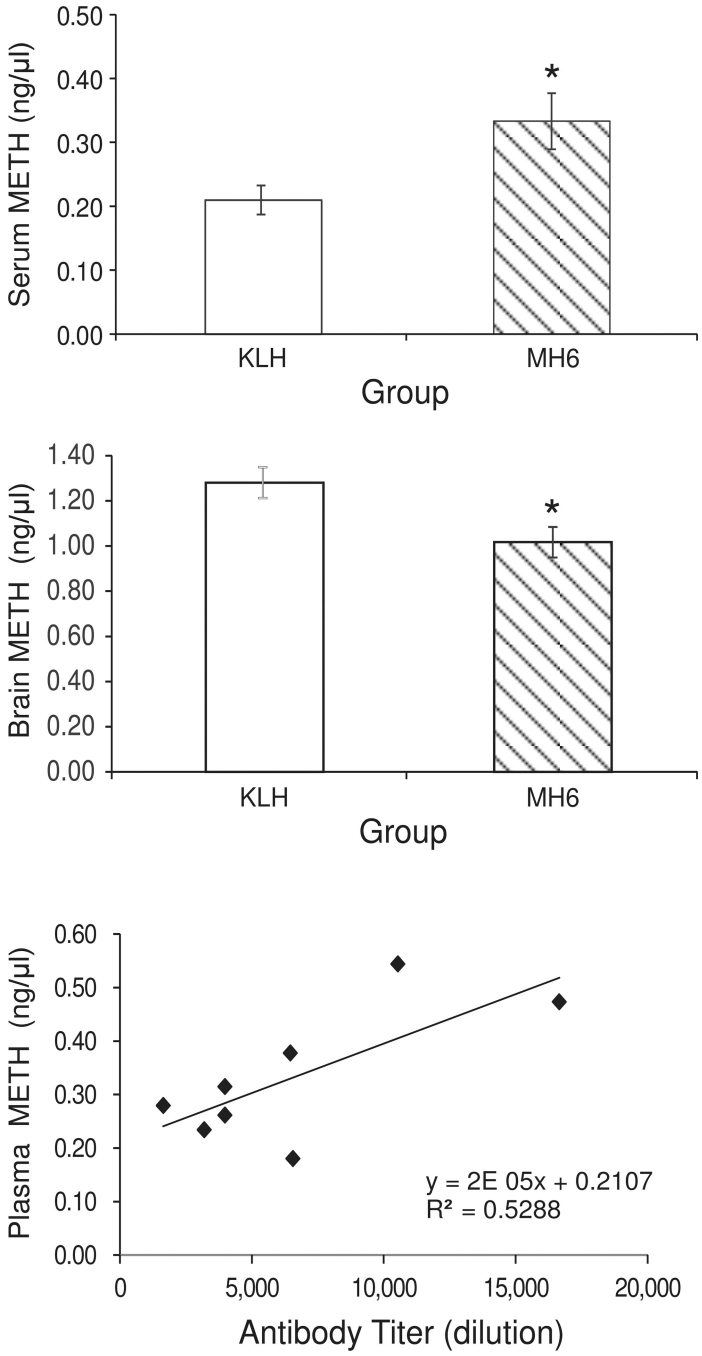

Figure 4.

Top & middle panels: Serum and brain METH concentrations (ng/ul) for KLH-control and MH6-vaccinated rats. Bottom panel: Individual plasma METH concentrations (ng/ul) as a function of individual antibody titer (dilution). All samples were obtained 30 min after a 3.2 mg/kg METH challenge.

Significant differences between groups are shown by * and error bars are ±SEM.

Table 1

Chronological summaries of the experimental procedures are shown: vaccine administration (V), blood collection (B), acute methamphetamine challenges (doses), and surgery (Experiment 2 only). Experiment 1 investigated effects of vaccination with MH2(R), MH6, MH7, and KLH (control) on rectal temperature and wheel activity in rats at T_A = 27±1°C. Experiment 2 investigated effects of vaccination with MH6 and KLH (control) on body temperature and locomotor activity in rats at T_A = 23±1°C.

Experiment 1				**Experiment 2**			
Week	**Vaccine (V)**	**Blood (B)**	**METH (doses)**	**Week**	**Vaccine (V)**	**Blood (B)**	**METH (dose)**
0	V (Prime)			0	V (Prime)		
1		B		1			
2	V (Boost 1)	B		2	V (Boost 1)	B	
3		B		3	Surgery		
4		B		4		B	0.0, 1.0, 5.6 mg/kg
5	V (Boost 2)	B		5	V (Boost 2)		
6		B		6		B	
7		B		7			0.0, 1.0, 5.6 mg/kg
8		B		8		B	
9	V (Boost 3)	B		9	V (Boost 3)		
10		B		10		B	
11		B	1.0, 5.6 mg/kg	11			
12		B	1.0, 5.6 mg/kg	12		B	0.0, 0.5, 1.0, 3.2, 5.6 mg/kg
				13			0.0, 0.5, 1.0, 3.2, 5.6 mg/kg
				14		B	
				15			
				16		B	
				17			
				18		B	
				19			
				20		B	3.2 mg/kg

Table 2

Binding affinities for anti-METH vaccines (MH2(R), MH6, MH7) and amphetamines (d-methamphetamine, METH; amphetamine, AMPH; 3,4-methylenedioxymethamphetamine, MDMA; 4-methylmethcathinone, 4-MMC).

Anti-METH vaccines	**Binding Affinities**
MH2(R)	2.24 ± 1.89 μM
MH6	30.4 ± 5.79 nM
MH7	25.91 ± 0.83 nM
Amphetamines	Binding Affinities
(+)-METH K_d	0.030 ± 0.005 μM
(+)-AMPH K_I	0.194 ± 0.071 μM
(±)- MDMA K_I	0.964 ± 0.454 μM
(±) 4-MMC K_I	15.13 ± 3.7 μM