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Effects of active anti-methamphetamine vaccination on intravenous self-administration in rats

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Abstract

BACKGROUND—*D*-methamphetamine (METH) addiction is a serious public health concern for which successful treatment remains elusive. Immunopharmacotherapy has been shown to attenuate locomotor and thermoregulatory effects of METH. The current study investigated whether active vaccination against METH could alter intravenous METH self-administration in rats.

METHODS—Male Sprague-Dawley rats (Experiment 1: N=24; Experiment 2: N=18) were vaccinated with either a control keyhole-limpet hemocyanin conjugate vaccine (KLH) or a candidate anti-METH vaccine (MH6-KLH) or. Effects of vaccination on the acquisition of METH self-administration under two dose conditions (0.05, 0.1 mg/kg/inf) and post-acquisition dose-substitution (0, 0.01, 0.05, 0.20 mg/kg/inf, Experiment 1; 0.01, 0.05, 0.10, 0.15 mg/kg/inf, Experiment 2) during steady-state responding were investigated. Plasma METH concentrations were determined 30 min after an acute challenge dose of 3.2 mg/kg METH.

RESULTS—Active vaccination inhibited the acquisition of METH self-administration under the 0.1 mg/kg/inf dose condition, with 66% of the MH6-KLH-vaccinated rats compared to 100% of the controls reaching criteria, and produced transient and dose-dependent effects on self-administration during the maintenance phase. Under the 0.05 mg/kg/inf dose condition, MH6-KLH-vaccinated rats initially self-administered more METH than controls, but then self-administration decreased across the acquisition phase relative to controls; a subsequent dose-

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Contributors MAT designed the study with significant input from MLM and SMA. KMC, SMA and MLM collected and organized the in vivo data, and completed initial data analyses. AYM and KDJ designed and created the MH6 conjugate vaccine and AYM performed antibody titer assessments. MLM and SMA conducted literature searches and provided summaries of previous related work. MAT undertook the statistical analysis and MAT, SMA and MLM created figures and drafted the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest. The authors have no conflicts of interest to report for this study.

response assessment confirmed that MH6-KLH-vaccinated rats failed to acquire METH self-administration. Finally, plasma METH concentrations were higher in MH6-KLH-vaccinated rats compared to controls after an acute METH challenge, and these were positively correlated with antibody titers.

CONCLUSIONS—These data demonstrate that active immunopharmacotherapy for METH attenuates the acquisition of METH self-administration.

Keywords

Drug addiction; immunopharmacotherapy; active vaccination; methamphetamine; self-administration

1. INTRODUCTION

D-methamphetamine (METH) abuse and addiction continues to be a serious public health concern for which successful treatment remains elusive (SAMHSA, 2010). Behavioral therapy shows moderate success (Roll et al., 2006), but maintaining long-term abstinence is a challenge for recovering addicts. Pharmacotherapies also have limited efficacy for treating METH addiction (Karila et al., 2010; Vocci and Appel, 2007), and additional approaches are needed. Immunopharmacotherapy has shown promise as a treatment for drug addiction in recent years (Gentry et al., 2009; Janda and Treweek, 2012; LeSage et al., 2006b; Moreno and Janda, 2009; Shen et al., 2013). In outline, conjugate vaccines stimulate drug-specific antibodies that sequester drug molecules in the blood stream, thereby reducing distribution to the brain.

Clinical trials for vaccines against cocaine (Haney et al., 2010; Kosten et al., 2002; Martell et al., 2009) and nicotine (Cornuz et al., 2008; Hatsukami et al., 2011) were advanced due to promising pharmacokinetic and behavioral results from preclinical studies. Anti-nicotine vaccines generate nicotine-specific antibodies (Cerny et al., 2002; de Villiers et al., 2004; Pentel et al., 2000), reduce brain nicotine concentrations (de Villiers et al., 2004; Pentel et al., 2000), and delay nicotine elimination (Keyler et al., 1999, 2005). Similar pharmacokinetic results exist for anti-cocaine vaccines (Carrera et al., 1995). Behavioral effects of drugs such as cocaine (Carrera et al., 1995, 2001, 2000; Kantak et al., 2001, 2000; Wee et al., 2012) and nicotine (Carrera et al., 2004; LeSage et al., 2006a; Lindblom et al., 2002; Roiko et al., 2008) are likewise attenuated by vaccination. Kantak et al (2001) reported that vaccination decreased cocaine intravenous self-administration (IVSA) in rats by about 30%. Wee et al (2011) showed that vaccination reduced cocaine IVSA in rats across several phases of the self-administration procedure, including a dose-response assessment, progressive ratio schedule, extinction and reinstatement. LeSage et al (2006) reported that 70% of controls while only 36% of vaccinated rats acquired nicotine IVSA, and that by the end of the study vaccination reduced the amount of nicotine self-administered by 38%. Collectively, these successes prompted the development of vaccines capable of opposing the actions of METH.

An initial study found no effect of an active anti-METH vaccine on METH-induced locomotor activity (Byrnes-Blake et al., 2001) but recent findings show that active

vaccination with the MH6-KLH conjugate vaccine (Moreno et al., 2011) blocks METH-induced locomotor and thermoregulatory disruptions in rats (Miller et al., 2013), and another vaccine alters METH-induced locomotion in mice (Shen et al., 2013). Lastly, active vaccination altered METH IVSA in rats; that is, vaccinated rats initially self-administered *more* METH than controls, but then self-administration decreased to a level indistinguishable from controls as the response requirement progressively increased across sessions (Duryee et al., 2009).

The current study investigated the effects of an anti-METH vaccine on METH IVSA, with a primary focus on the *acquisition* of self-administration. Since drug dependence is a minority outcome for most humans who sample a given drug (Anthony et al., 1994; Schramm-Sapyta et al., 2009), prevention of the establishment of a compulsive use pattern is important to model pre-clinically. Although anti-drug vaccine investigators frequently assume that broad spectrum vaccination of, e.g., adolescents is unimaginable, the approval and acceptance of a vaccine against human papilloma virus (Constantine et al., 2007; Shi et al., 2007) shows such views are unduly pessimistic. Preclinical investigators should determine what is biologically possible rather than fail to do so based on suppositions about what might be approved as an eventual treatment. We have previously shown that the MH6-KLH conjugate vaccine is capable of sequestering METH in the blood compartment of the rat while decreasing brain levels and that actively vaccinated rats are protected from thermoregulatory and locomotor effects of METH (Miller et al., 2013). Consequently, rats were not lever trained prior to self-administration sessions, the response requirement remained constant throughout the study, and two different training doses were used (unlike the Duryee et al. study). Effect of vaccination across a range of METH doses during the maintenance phase of self-administration was investigated, along with an assessment of antibody titers and plasma METH concentrations at the end of the study.

2. METHODS

2.1 Animals

Male Sprague-Dawley rats (Experiment 1: N=24; Experiment 2: N=18; Charles River, NY, USA) weighing ~250 grams on arrival were group housed in clear shoebox cages in a vivarium with a 12:12 reverse light-dark cycle. Food pellets and water were available ad libitium in the vivarium. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Clark et al. 1996) and under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of The Scripps Research Institute.

2.2 Drug and Hapten

D-methamphetamine HCl (provided by RTI under contract to the National Institute on Drug Abuse) was dissolved in sterile saline and administered intravenously in a volume of 0.1 ml per infusion. Doses are expressed as the salt.

Methamphetamine hapten (MH6) was coupled with the KLH (control) carrier protein and administered (100 micrograms per innoculation) in formulation with the Sigma Adjuvant System® as previously reported (Miller et al., 2013).

2.3 Equipment

Standard self-administration chambers (MED Associates, St. Albans, VT, USA; Model ENV-007) equipped with 2 response levers and cue lights, pellet magazine, and drug infusion pump (Med Associates Model ENV-045) were used. Each chamber was enclosed in a sound-attenuating box and all equipment was controlled by MED-PC IV software.

2.4 Vaccination Procedure

For vaccination, either MH6-KLH or KLH (control) were added to adjuvant to create 100 ug/0.5 ml vaccine for each rat, which was administered across 3 sites (0.2 ml s.c. in the nape; 0.2 ml s.c. in the left hind quadricep/flank; 0.1 ml i.p.). Rats were vaccinated during weeks 0, 2, and 5 (Experiment 1) and weeks 0, 2, 5, 9, and 13 (Experiment 2). The vaccination schedule was designed to match that used in a prior report from our laboratory (Miller et al., 2012). As such, a vaccination is typically administered during week 9. In Experiment 1 of the current study, however, the week 9 vaccination was not administered because it coincided with the dose-response assessment. However, an additional vaccination was administered (during week 13) in Experiment 2 because the self-administration conditions ran 6 weeks longer than in Experiment 1; vaccine administration occurred between the acquisition and maintenance phases for that reason. Vaccinations administered during the acquisition were administered after self-administration sessions. A summary of experimental conditions is shown in Table 1.

2.5 Surgery

Chronic intravenous catheters were surgically implanted into all rats as described in (Aarde et al., 2015a; Creehan et al., 2015; Miller et al., 2012). There were 4 days of surgical recovery prior to starting self-administration sessions; for the first 3 days, cephazolin (0.4 g/ml; 2.0 ml/kg s.c.; once daily) and flunixin (2.5 mg/ml; 2.0 ml/kg s.c.; once daily) were administered. Catheters were flushed with sterile physiological saline containing either timentin (before sessions; 0.1 g/ml; 0.2–0.3 ml/rat) or heparin (after sessions; 10 USP units/ml; 0.2–0.3 ml/rat). Catheter patency was checked weekly after the session by administering 0.2–0.3 ml of the ultra-short-acting barbiturate anesthetic Brevital sodium (1% methohexital sodium, Eli Lilly, Indianapolis, IN) through the catheter. Rats with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 sec of the Brevital injection. Failure to produce loss of muscle tone was considered a sign of a faulty catheter. In those cases, the faulty catheter was surgically removed and a new catheter was implanted into the left jugular vein; after recovery, the rats resumed self-administration sessions.

2.6 Immunologic Assays

Blood samples were collected from the tail vein during weeks 18 and 20, and from the heart during exsanguination on week 22 (Experiment 2 only). Samples were placed on ice to prevent clotting and then centrifuged at 10,000 g for 15 min; the plasma was extracted and then stored at −80°C until further processing.

Antibody titers were assessed by enzyme-linked immunosorbent assay (ELISA) as previously described (Moreno et al., 2011) using MH6-BSA conjugates as coating antigens.

Titers were calculated as the dilution corresponding to an absorbance reading 50% of the maximal value from the plot of absorbance versus log dilution.

METH concentrations in terminal blood samples were assessed using high-throughput liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) at the Scripps Center for Metabolomics and Mass Spectrometry. For this assessment, blood samples were obtained 30 min after a 3.2 mg/kg (s.c.) METH challenge during week 22 (Experiment 2 only), and were prepared for LC-MS/MS using a trichloroacetic acid-based extraction described in (Hendrickson et al., 2006) to dissociate drug from antibody.

2.7 Experiments

2.7.1 Experiment 1: METH Self-Administration (0.10 mg/kg/inf)—In both experiments, rats were trained to self-administer METH according to a fixed ratio (FR1) schedule of reinforcement. That is, every response on the left lever activated the drug infusion pump for 4 seconds, and delivered either 0.10 (Experiment 1) or 0.05 (Experiment 2) mg/kg/inf METH. After the lever press, a 20-s time-out ensued with the illumination of the stimulus light above the lever. Responses on either lever had no programmed consequences during the time-out. Sessions were conducted within the first 3 hrs of the dark phase, at ambient temperature ~23 °C.

In Experiment 1, rats self-administered METH (0.10 mg/kg/inf) in 2-hr daily sessions for 13 days. A priming dose of METH (equal to one infusion) was administered via activation of the drug-infusion pump if there was no responding for 2 consecutive sessions (i.e., no lever press in the previous session and no lever press within 30 min in the current session). METH priming was necessary for 2 and 3 rats in the KLH and MH6-KLH groups, respectively (i.e., one KLH rat received 2 and the other 5 primes; two MH6-KLH rats received 1 and the other 10 primes). Acquisition of self-administration was defined as obtaining 11 or more infusions on 2 consecutive sessions, with the first session denoting the date of acquisition. If a rat reached criterion on the last day of the acquisition period (i.e., session 13), it received one additional session. If it reached criterion during this additional session, it was considered to have met acquisition on session 13.

Dose-Substitution: The dose-substitution condition and subsequent dose-response assessments were conducted to ascertain vaccine efficacy across multiple doses of METH. Therefore, immediately following the acquisition period in Experiment 1, there were 2 sessions in which either 0.05 or 0.10 mg/kg/inf was available; order of presentation was balanced within each group. Thereafter, the training dose (0.10 mg/kg/inf) was reinstated for 3 sessions followed by a subsequent dose-response assessment.

The effect of a range of doses of METH (0.0, 0.01, 0.05, 0.20 mg/kg/inf) on self-administration was next investigated. These doses were selected based on our prior work with METH (Aarde et al., 2013a; Aarde et al., 2013b). For this assessment, each dose was presented once, in a randomized order, within a sequential 4-session block (i.e., cycle), with 3 repetitions of each cycle in order to detect systematic differences across cycles.

2.7.2 Experiment 2: METH Self-Administration (0.05 mg/kg/inf)—Experiment 2 was conducted to investigate the acquisition of self-administration under threshold conditions less likely to establish responding in all rats. Consequently, additional groups (N=9 per group, one KLH animal failed to complete the study) of MH6-KLH-vaccinated and KLH-control rats were allowed access to a lower per infusion dose (0.05 mg/kg/inf) and the session duration was reduced to 1 hour. Furthermore, no priming doses were administered, regardless of performance, during the entire 21-day acquisition period for these rats. The self-administered infusion data were analyzed in terms of individual sessions and summated across blocks of 7 sessions.

Dose-Substitution: Following the acquisition period, the effects of a range of doses of METH (0.01, 0.05, 0.10, 0.15 mg/kg/inf) on self-administration was investigated; this condition was the same as described above for Experiment 1.

2.8 Data Analysis

Analysis of the self-administration data generally employed repeated-measures analyses of variance (ANOVA) with a between-subjects factor of vaccine Group and within-subjects factors of Sessions, per-infusion dose and dose block (i.e., cycle), where relevant. *Posthoc* analyses of signi cant effects in the ANOVA were conducted using the Neuman-Keuls test including all pairwise comparisons. The dose-substitution condition (Experiment 1 only) included pre-planned comparison between groups at each dose and between doses within group. Analysis of acquisition data used a Wilcoxon Survival analysis for the percent of each group reaching criterion. For the days-to-acquisition analysis, any rats that had not reached criterion were assigned the maximum, 13 sessions. The criterion for signi cance for all analyses was $p < 0.05$. Analyses were conducted with GB-STAT v7.0; Dynamic Microsystems, Silver Spring MD or Prism 6 for Windows (v. 6.02; GraphPad Software, Inc, San Diego CA). Graphs were generated with Excel (Microsoft, Redmond WA) and figures were created in Canvas (v.12; ACD Systems of America, Inc, Seattle, WA).

3. RESULTS

3.1 Experiment 1: METH Self-Administration (0.10 mg/kg/inf)

3.1.1 Acquisition—The percentage of rats reaching self-administration criterion by session 13 was lower for the MH6-KLH group (66%) than the KLH group (100%; Figure 1A). A Wilcoxon Survival analysis confirmed a significant group difference ($p<0.05$). Similarly, there was a main effect of group on the number of days until acquisition criteria were met (t (22) = −2.216; $p<0.05$; Figure 1B) with KLH-control rats reaching acquisition a mean of 3 days earlier than MH6-KLH-vaccinated rats.

The number of METH infusions increased significantly across the 13-day acquisition period (main effect of session: $F_{12,12} = 33.55$; $p<0.001$; Figure 1C) but there was no main effect of group or interaction confirmed. The cumulative METH dose across the acquisition period was approximately 25% less in MH6-KLH-vaccinated rats compared to KLH-control rats, but the analysis did not confirm this as a statistically significant difference (Figure 1D).

3.1.2 Initial Dose-Substitution—The mean number of infusions differed with per-infusion dose (Figure 2), as was confirmed with a significant main effect of dose in the ANOVA ($F_{1,20}$ = 23.26; p<0.001). Pre-planned comparisons confirmed significant between-group differences at each per-infusion dose and within-group differences between the per-infusion doses.

3.1.3 Full Dose-Response Assessment—Figure 3 shows the effects of a range of doses of METH (0, 0.01, 0.05, 0.20 mg/kg/inf) on self-administration in KLH-control and MH6-KLH-vaccinated groups across 3 iterations of the dose-response assessment (i.e., cycles 1, 2, and 3). The number of infusions at the 0.01 mg/kg/inf dose during cycles 1 and 2 were statistically different between groups. The omnibus analysis confirmed a main effect of dose ($F_{3,66}$ = 10.13; p<0.001), of the interaction of dose with cycle ($F_{6,132}$ = 3.16; p<0.01), and the three-way interaction with treatment group ($F_{6,132}$ = 2.83; p<0.05). When collapsed across cycles, the *post-hoc* test confirmed significant differences in the number of responses for vehicle and 0.01 mg/kg/inf, as well as between 0.01 and 0.2 mg/kg/inf within each group. This same pattern was also confirmed for each group across all 3 cycles, except for MH6-KLH-vaccinated rats during the 3rd cycle where no differences were found. Significant differences in responding between groups were confirmed at the 0.01 mg/kg/inf dose in cycles 1 and 2, with the KLH-control rats self-administering more METH than MH6-KLH-vaccinated rats in the 1st cycle and less in the 2nd cycle. Across cycles, the KLH-control rats self-administered more METH at the 0.01 mg/kg/inf dose in cycle 1 compared to the subsequent cycles; MH6-KLH-vaccinated rats self-administered more METH at the 0.01 mg/kg/inf dose in cycle 2 compared to cycle 3.

3.2 Experiment 2: METH Self-Administration (0.05 mg/kg/inf)

3.2.1 Acquisition—Figure 4A shows the number of METH infusions for MH6-KLH-vaccinated and KLH-control rats in 7-session averages during the acquisition period; individual session data are shown in Figure 4B. The MH6-KLH-vaccinated rats self-administered more METH than KLH-control rats during the first 7 sessions; however, this effect was transient and the number of infusions for the MH6-vaccinated rats decreased across the 21-day acquisition period. The ANOVA confirmed a significant interaction between treatment group and the blocks of sessions ($F_{2,32}$ = 10.21; p<0.0005), but no main effect of either factor. *Post-hoc* tests further confirmed significant differences between groups during the first and last 7 sessions of the acquisition period. The *post hoc* also confirmed that within the MH6-KLH group, fewer infusions were *obtained in the last two 7-session blocks relative to the first 7-session block.*

3.2.2 Dose Response Assessment—Figure 4C shows that when a range of METH doses was evaluated (0.01, 0.05, 0.10, 0.15 mg/kg/inf), there was a slight increase in the number of infusions in the KLH group at the lower per-infusion doses (i.e., 0.01 and 0.05 mg/kg/inf), but no change in the MH6-KLH group at any dose. The statistical analysis did not confirm any significant differences between or within groups.

3.2.3 Blood METH concentration—Figure 5A shows the effects of vaccination on plasma METH concentration obtained 30 min after a 3.2 mg/kg (s.c.) METH challenge; this

approach was selected so as to be able to compare with our prior finding (Miller et al., 2013). MH6-KLH-vaccinated rats had a significantly higher concentration of METH in peripheral blood than KLH-control rats, as confirmed by main effect of group ($F_{2,17.61}$ = −6.67; p<0.0001). Figure 5B shows the results of a correlation analysis between plasma METH concentration and antibody levels following the 3.2 mg/kg (s.c) METH challenge (week 22) as well as two prior observations (weeks 18, 20). Antibody titers from week 18 were correlated with those from weeks 20 (r^2=0.87, p<0.005) and 22 (r^2=0.95, p<0.0005) and those from week 20 were correlated with those from week 22 (r^2=0.90, p<0.005). Plasma METH concentration was significantly positively correlated (Pearson r; 2-tailed) with antibody titer at the time of this challenge (week 22; r^2=0.63, p<0.05) and also with the antibody titer from blood samples obtained during weeks 18 (r^2=0.58, p<0.05) and 20 (r^2=0.90, p<0.0005).

4. DISCUSSION

This study is the first to show that active immunopharmacotherapy attenuated the rate of the *acquisition* of *d*-methamphetamine (METH) intravenous self-administration (IVSA) in rats by using the MH6-KLH conjugate vaccine previously shown to attenuate locomotor and thermoregulatory effects of METH, as well as to reduce METH entry to the brain (Miller et al., 2013; Moreno et al., 2011). The delay in acquisition was substantial, with less than 17% of the vaccinated group reaching acquisition criteria after 7 sessions compared with 75% of controls. Furthermore, only 66% of the MH6-KLH-vaccinated rats compared with 100% of the controls reached acquisition criteria by 13 sessions of training. When additional groups were trained under less robust conditions (i.e., lower per-infusion dose), the MH6-KLH-vaccinated rats initially self-administered *more* METH than controls, as if insensitive to the effects of the drug, and IVSA was reduced over the course of 21 sessions to a level below that of the control group, which exhibited stable METH intake across the acquisition period. Together, these results show that vaccination with the MH6-KLH conjugate decreases the reinforcing efficacy of METH.

Although this study is the first to report the effects of active vaccination on the *acquisition* of METH IVSA, a prior study found effects similar to the results of Experiment 2 (Duryee et al., 2009). In that study, rats were trained to lever press (using an autoshaping procedure) prior to the start of self-administration sessions. Vaccinated rats initially self-administered *more* METH than controls, but with increasing response requirements, METH IVSA decreased across 10–15 sessions, which is similar to the present data. In both the Duryee et al. (2009) and the current study, METH IVSA did not vary substantially across the sessions for control rats. The one difference was that in the Duryee study, IVSA in vaccinated rats decreased to approximately the same level as controls, whereas in this study vaccinated rats eventually self-administered half as much as controls (i.e., over the final 7 sessions of acquisition). This suggests that although the response requirements in the Duryee et al (2009) study were not physically strenuous to the rats (i.e., scores of responses per infusion are common under progressive-ratio schedules) and the response requirement in Experiment 2 (i.e., FR1) was not altered over the course of the experiment, MH6-KLH-vaccinated rats did not sustain compensatory responding for the presumably lower METH concentrations entering the brain. Therefore, the overall pattern across the acquisition interval is consistent

with prior studies that showed passive vaccination reduces cocaine IVSA in a manner consistent with the extinction of drug effects (Fox et al., 1996). The observed increase in METH IVSA during the initial part of the acquisition phase (under threshold conditions) may be functionally similar to reducing the drug dose under simple fixed-ratio schedules (Pickens and Thompson, 1968; Wilson et al., 1971) and/or treatment with dopamine antagonists (Caine and Koob, 1994; Koob et al., 1987; Wilson and Schuster, 1972). This is consistent with the conclusion that the reinforcing properties of METH are blunted by an antibody-mediated reduction in drug concentration reaching the brain targets.

This conclusion is also supported by the finding of substantially delayed acquisition when the per-infusion dose was higher (i.e., 0.1 mg/kg/inf rather than 0.05 mg/kg/inf), the session was longer (2 h vs. 1 h), and priming procedures were used to ensure some minimal drug exposure in all rats. In Experiment 1, the rats self-administered a mean of 15–20 infusions at the end of the acquisition period and increased their responding when the per-infusion dose was systematically lowered from the training dose. Thus, the intent to examine robust acquisition parameters was satisfied. As outlined above, the MH6-KLH-vaccinated rats in Experiment 1 required more sessions and were less likely to reach acquisition criteria; furthermore, the cumulative METH dose across the acquisition period was approximately 25% less in MH6-KLH-vaccinated rats compared to controls. (The attempt to facilitate the acquisition of self-administration by delivering priming doses of METH throughout the acquisition period was largely ineffective in the MH6-KLH-vaccinated group, as 2 of the 3 vaccinated rats that received METH primes never satisfied acquisition criteria, even though 1 of those rats received 10 primes.) The finding that vaccination slows the acquisition of IVSA is similar to the findings from a prior anti-nicotine study (LeSage et al., 2006a). In that study, 36% of vaccinated and 70% of control rats acquired nicotine IVSA.

Self-administration in MH6-KLH-vaccinated rats was also reduced by 27% compared to KLH-control rats during the initial dose-substitution condition (i.e., 0.05 mg/kg/inf), and was reduced by 38% during the 1st cycle of a subsequent dose-response assessment at the smallest dose tested (i.e., 0.01 mg/kg/inf). The magnitude of the initial reduction is comparable to prior demonstrations showing that active vaccination reduces IVSA of nicotine and cocaine. For example, nicotine intake was reduced by 38% during acquisition and 57% during maintenance (LeSage et al., 2006a), and cocaine intake by 30% (Kantak et al., 2000), in prior studies. These results are also consistent with findings from reinstatement paradigms in which the protection need only last for a single drug challenge. Lindblom and colleagues found that vaccination reduced the reinstatement of nicotine seeking by 69% and 38% after priming doses of 0.001 and 0.03 mg/kg nicotine (Lindblom et al., 2002). It has also been shown that active vaccination reduces the percentage of rats that reinstate responding for cocaine by 32–62% (Carrera et al., 2000), reduces the number of self-administered cocaine infusions by 37% (Kantak et al., 2000), and reduces response rates during reinstatement by 50% and 44% after priming doses of 10 and 15 mg/kg cocaine, respectively (Wee et al., 2012).

One major caveat to the study is common to prior, related studies of drug vaccine efficacy and lies in the fact that protection was partial and surmountable. Acquisition increased in the MH6-KLH groups after eight exposure sessions. Changes were observed in the relative

protection of vaccination in the 0.01 mg/kg/inf condition across three dose substitution cycles. Duryee and colleagues (2009) reported that METH specific antibodies were undetectable in blood one day after the final IVSA session, but these antibodies rebounded a month later. The kinetics of this process may differ across vaccines, drug intake levels and the duration of exposure in behavioral testing but it illustrates that protection in the MH6-KLH rats may have been dynamically reduced by ongoing METH self-administration. Further studies on these dynamics are of significant interest to understanding the manner in which vaccines might be applied within the entire drug addiction cycle.

This study also confirmed that vaccination increased METH levels in peripheral blood in the MH6-KLH-vaccinated rats compared with KLH control rats following an acute METH challenge. In addition, METH serum levels were positively correlated with the titer on the final day, as well as with titers from the 2 prior blood sample collections during weeks 18 and 20, which is similar to our prior report (Miller et al., 2013). This is consistent with findings from previous studies that investigated anti-METH (Byrnes-Blake et al., 2001), anti-nicotine (Pentel et al., 2000; Pravetoni et al., 2011), and anti-cocaine (Wee et al., 2012) vaccines, and further enhances confidence in the specificity of the effects of MH6-KLH vaccination. Titers were not correlated with individual IVSA in Experiment 2, however it has been previously shown that the *in vivo* protection of anti-drug antibodies is not necessarily a simple function of drug binding sites and available drug molecules (Pitas et al., 2006; Ruedi-Bettschen et al., 2013).

A limitation of this study is that the motivation to consume METH beyond the initial acquisition period was not investigated. It is likewise unknown if the specific timing of vaccination (and related antibody titer peaks) would affect the acquisition of METH self-administration or the maintenance post-acquisition, but clearly such extensive investigations are now warranted, following this initial demonstration of efficacy. Interestingly, overnight bouts of exercise on an activity wheel produce effects of similar magnitude (Aarde et al., 2015b; Smith and Witte, 2012) but it is unknown at this time if combined therapeutic approaches of such disparate natures would summate to further reduce METH intake.

To conclude, this is the first study to show that immunopharmacotherapy can delay, and perhaps even prevent, the *acquisition* of METH IVSA. This is a graded phenomenon which affects the probability of establishing stable drug use given the amount experienced per “hit” and the number of times the drug is experienced. This outcome has important translational implications. Many human individuals sample addictive psychoactive drugs a few times and decide that the experience is just not attractive to repeat- a large percentage of vaccinated rats experienced eight exposure sessions without acquiring stable METH intakes where three-quarters of the unvaccinated rats had already done so. Inevitably, the potential therapeutic application for anti-drug vaccines has an influence on which models are used to assess efficacy. The popularity of reinstatement models (Carrera et al., 2000; Kantak et al., 2001; Lindblom et al., 2002; Wee et al., 2012) is in part due to the assumption that these relatively invasive, novel procedures will be initially used in treatment-seeking addicts. Indeed, Young and colleagues have raised social, legal, and ethical issues for an immunotherapeutic approach for the treatment of drug (cocaine) addiction and suggested that preventative vaccination of, e.g., adolescents, is unlikely (Young et al., 2012). Concern

on this front should be moderated by the approval and adoption of a vaccine against the human papilloma virus (Constantine et al., 2007; Shi et al., 2007), which is similar in the sense of being wide scale prophylactic vaccination against the consequences of voluntary behavior. Such discussions were moot in the absence of demonstrations that vaccines are capable of affecting initial acquisition of habitual drug taking; the present data supply such evidence. Similarly, it may be the case that "failures" of clinical trials for anti-cocaine (Martell et al., 2009) and anti-nicotine (Hoogsteder et al., 2014) vaccines which examined potential reductions in *ongoing* drug use may not mean these vaccines would fail if used prophylactically.

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Highlights (for review)

- Methamphetamine addiction has no currently approved treatment medications
- Vaccination produces antibodies which can sequester drug in the blood
- Active vaccination attenuated the acquisition of self-administration in rats
- Prophylactic vaccination against drug use is possible to achieve

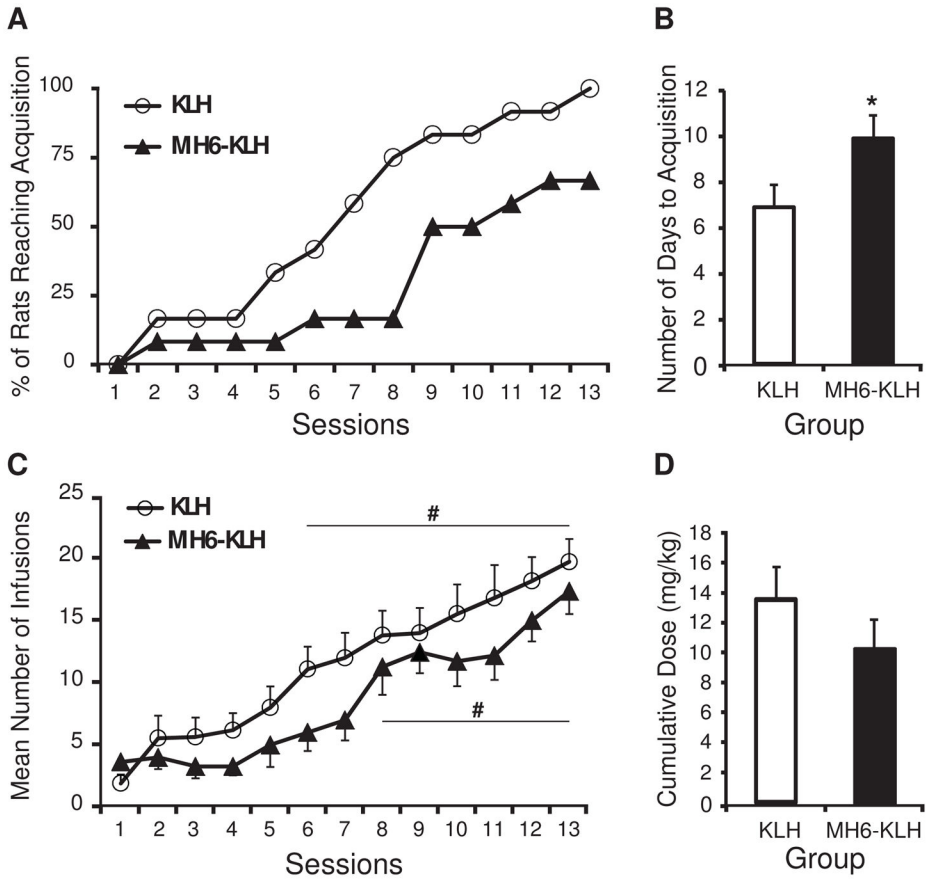

Figure 1.

A. Cumulative percent of MH6-KLH-vaccinated and KLH-control rats (N=12 per group) that acquired intravenous METH self-administration. B. Mean number of days to acquire self-administration for each group. C. Mean number of infusions for each group across the 13-day acquisition period. D. Cumulative METH dose (mg/kg) during the acquisition period for both groups. Significant difference between groups are depicted with * and differences relative to the first session within group by #. Error bars are ±SEM.

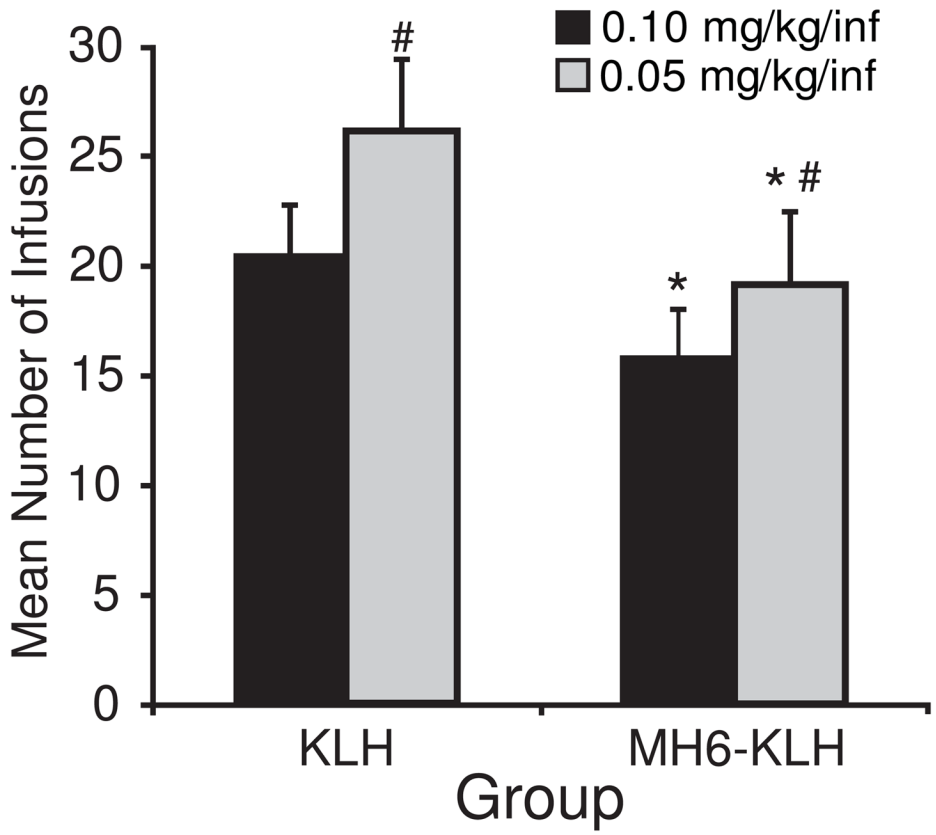

Figure 2.

Mean (N=12 per group) number of infusions after a dose substitution of 0.05 mg/kg/inf and a return to the training dose (0.10 mg/kg/inf) for MH6-KLH-vaccinated and KLH-control rats. Significant difference between and within groups are shown by * and #, respectively. Error bars are ±SEM.

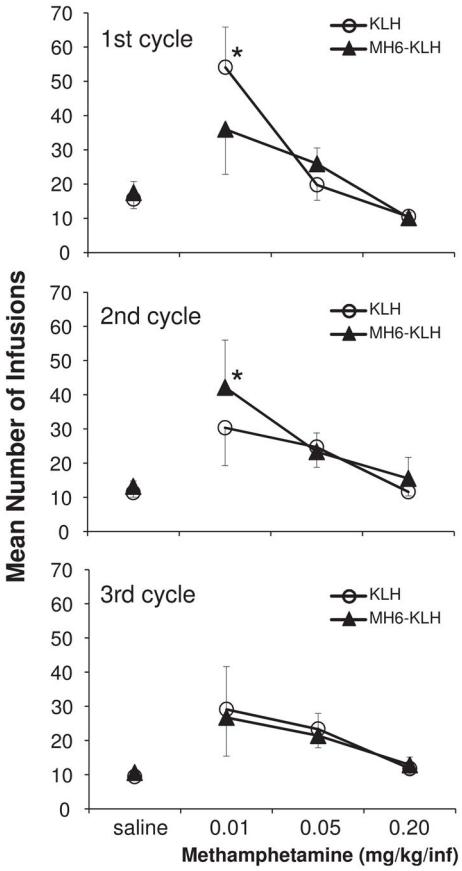

Figure 3.

Mean (N=12 per group) number of infusions across a range of METH doses (saline, 0.01, 0.05, 0.20 mg/kg/inf) shown for each 4-session block of the dose-response assessment (i.e., 1st, 2nd, and 3rd cycle) for KLH-control and MH6-KLH-vaccinated rats. Significant differences between groups within a cycle are depicted with *; see text for additional differences. Error bars are ±SEM.

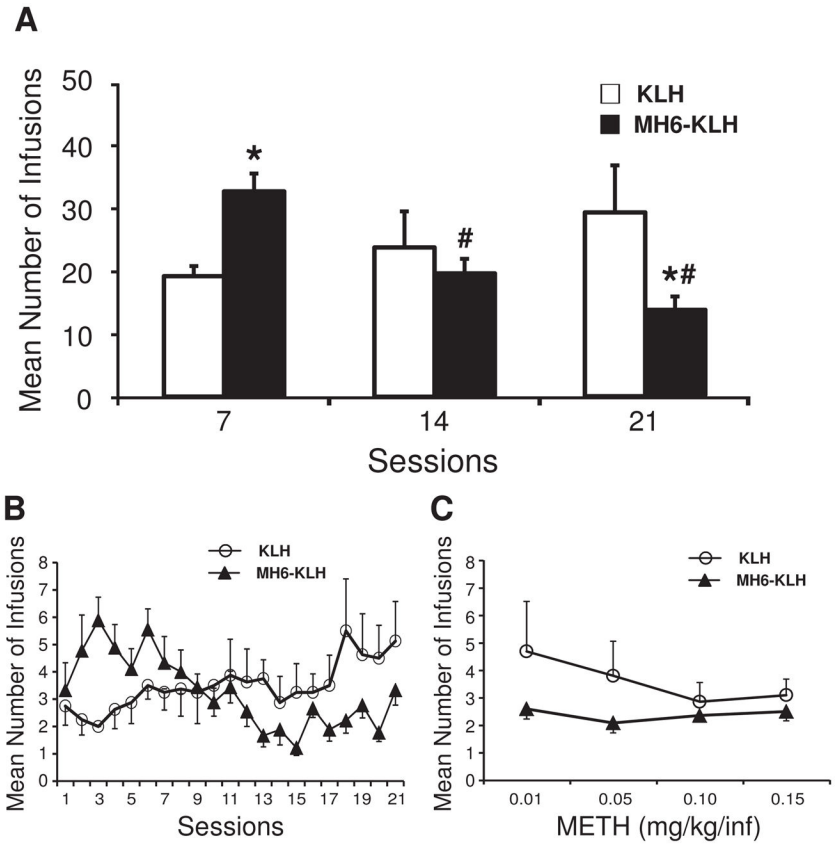

Figure 4.

A. Mean number of infusions during acquisition of METH self-administration for MH6-KLH-vaccinated (N=9) and KLH-control (N=8) rats summated across 7-session bins. B. Mean number of infusions in the individual 21-session acquisition period is depicted for both groups. C. Mean number of infusions across a range of doses of METH (0.01, 0.05, 0.10, 0.15 mg/kg/inf) for both groups. Significant differences between groups are depicted with * and within groups (relative to the first 7 sessions) are depicted with #. Error bars are ±SEM.

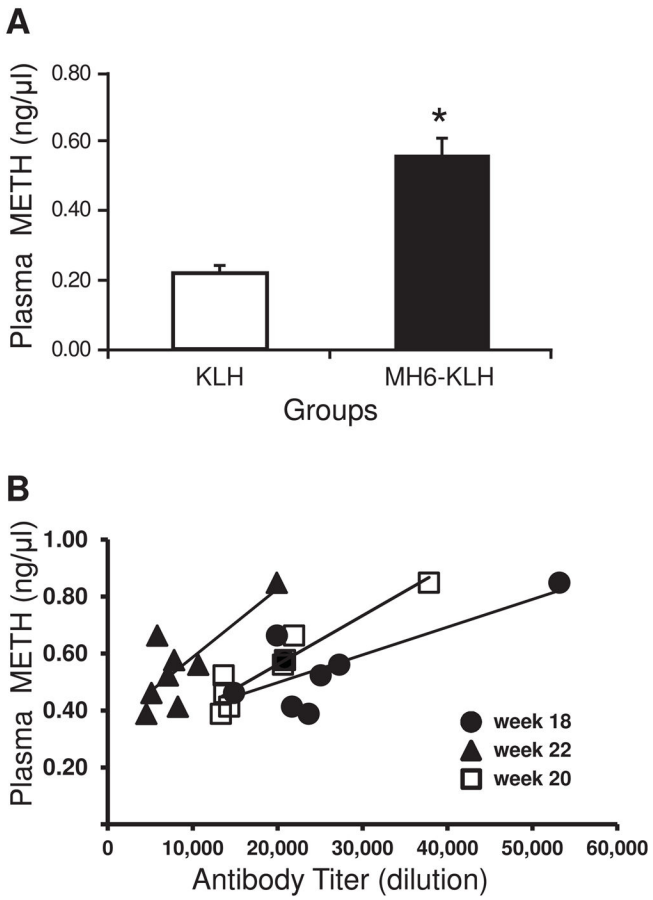

Figure 5.

A. Mean (N=8 per group) plasma METH concentrations (ng/ul) for KLH-control and MH6-KLH-vaccinated rats. B. 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 (s.c.) METH challenge given during week 22 of the study. 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), self-administration condition (Phase), methamphetamine doses, surgeries, and blood collection (B). Both experiments investigated effects of active vaccination on METH self-administration in rats.

Experiment 1				Experiment 2			
Week	**Vaccine**	**Phase**	**Dose(s)**	**Week**	**Vaccine**	**Phase**	**Dose(s)**
0	V			0	V		
1				1			
2	V			2	V		
3	surgery			3	surgery		
4		Acquisition	.10 mg/kg/inf	4			
5	V	Acquisition	.10 mg/kg/inf	5	V		
6		Acquisition	.10 mg/kg/inf	6			
7		Maintenance	.05, .10 mg/kg/inf	7			
8		Maintenance	0, .01, .05, .20 mg/kg/inf	8			
9		Maintenance	0, .01, .05, .20 mg/kg/inf	9	V		
10		Maintenance	0, .01, .05, .20 mg/kg/inf	10		Acquisition	.05 mg/kg/inf
				11		Acquisition	.05 mg/kg/inf
				12		Acquisition	.05 mg/kg/inf
				13	V	Acquisition	.05 mg/kg/inf
				14		Maintenance	.01, .05, .10, .15 mg/kg/inf
				15		Maintenance	.01, .05, .10, .15 mg/kg/inf
				16		Maintenance	.01, .05, .10, .15 mg/kg/inf
				17			
				18		B	
				19			
				20		B	
				21			
				22		B	