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# Monoclonal IgG affinity and treatment time alters antagonism of (+)-methamphetamine effects in rats

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**Abstract**

The roles of monoclonal antibody affinity and treatment time of (+)-methamphetamine-induced pharmacological effects in rats were studied using two anti-(+)-methamphetamine monoclonal antibodies. These studies tested the preclinical protective effects of monoclonal antibody antagonists in (+)-methamphetamine overdose and pretreatment scenarios. The higher affinity antibody (mAb6H4; $K_D$=11 nM for (+)-methamphetamine) more effectively antagonized (+)-methamphetamine-induced behavioral effects (distance and rearing) than the low affinity antibody (designated mAb6H8; $K_D$=250 nM) and had a longer duration of action. Both antibodies more effectively reduced (+)-methamphetamine effects in the overdose model than in the pretreatment model. (+)-Methamphetamine pharmacokinetic studies showed the mAb6H4 significantly reduced brain concentrations over time in both models. However, while mAb6H4 immediately reduced brain concentrations in the overdose model, it did not prevent the initial distribution of (+)-methamphetamine into the brain in the pretreatment model. Thus, anti-(+)-methamphetamine monoclonal antibody affinity and administration time (relative to (+)-methamphetamine dosing) are critical determinants of therapeutic success.


*Keywords:* (+)-Methamphetamine; (+)-Amphetamine; Monoclonal antibody; (Rat)

## 1. Introduction

(+)-Methamphetamine is a widely abused drug that can cause long-lasting effects. Because tolerance develops to the desired euphoric effects, (+)-methamphetamine is often taken repeatedly at high doses, which increases the incidence and severity of (+)-methamphetamine's adverse effects and addiction liability (Cho, 1990). Clinically significant central effects range from acute psychosis to chronic schizophrenia-like symptoms and depression (Cho, 1990; Seiden et al., 1993). There are also reports of long-term neurotoxicity caused by (+)-methamphetamine use (McCann et al., 1998; Ernst et al., 2000; Volkow et al., 2001). These effects can be severe, debilitating and even lethal at high doses. Thus, users often require treatment for overdose and for long-term addiction.

Unfortunately for (+)-methamphetamine users, there are no specific medications for use in an overdose situation or for reducing the potential for relapse to drug use (NIDA Research Report Series, 2002). Patients with a (+)-methamphetamine overdose can receive supportive care for their most serious symptoms, but full recovery requires the patient to wait for elimination of the drug from the body. These supportive and passive clinical treatments can be life saving, but since they do not remove (+)-methamphetamine agent from its sites of action in the brain, the potential for neurotoxicity still exists

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even if patients appear to be stabilized. For preventive therapy and rehabilitation, behavioral modification treatments are helpful for some addicts, and antidepressants can be used to combat the depression often found during (+)-methamphetamine abstinence; however, the relapse rate is still very high (Murray, 1998; NIDA Research Report Series, 2002).

Due in part to (+)-methamphetamine's multiple sites (e.g., central nervous system, cardiovascular system) and diverse mechanisms of action (Cho, 1990; Seiden et al., 1993; Sulzer et al., 1995), development of a traditional receptor antagonist has not been proven successful. An alternative approach is antibody-based therapy. A purposely designed monoclonal antibody with the suitable characteristics (e.g., affinity and specificity) could be used as a drug specific antagonist of drug effects. Used this way the monoclonal antibody would serve as a pharmacokinetic antagonist, which limits the drug to serum and intracellular fluid compartments through high affinity binding.

Previous studies in our laboratory have shown that a single dose of a high-affinity anti-phencyclidine monoclonal Fab ($K_D$=1.8 nM; the antigen binding fragment of IgG) effectively reverses phencyclidine overdose in rats (Valentine et al., 1996), and that the intact monoclonal antibody ($K_D$=1.3 nM; McClurkan et al., 1993) can produce immediate and long-term, reductions in brain phencyclidine concentrations (Proksch et al., 2000a; Laurenzana et al., 2003b). Furthermore, only a single dose of this anti-phencyclidine monoclonal antibody significantly protects against phencyclidine-induced locomotor effects in rats after repeated i.v. challenges for up to 2 weeks (Hardin et al., 2002). The pharmacokinetic and protective effects of anti-phencyclidine monoclonal antibody persist even when the binding capacity of the antibody was far exceeded and apparently saturated.

One of the reasons the anti-phencyclidine monoclonal antibody is so effective is that it has a very high affinity for phencyclidine. Indeed, the affinity of this antibody for phencyclidine is about 80 times higher than the affinity of the highest PCP binding sites in the central nervous system (Proksch et al., 2000b). Previous studies have evaluated the role of antibody affinity in treating overdose of other drugs. In studies of the effects of monoclonal and polyclonal anti-imipramine antibodies, Ragusi et al. (1998) demonstrate that higher affinity antibodies are more effective in redistributing the drug from the brain. While it is intuitive that a high affinity antibody for a drug would provide a significant clinical advantage in vivo, the optimal balance between in vivo drug association with the antibody (to block effects) and drug dissociation from the antibody (to allow regeneration of the binding capacity) is inadequately elucidated.

The purpose of the current experiments was to study the role of monoclonal antibody affinity in antibody-induced reductions of (+)-methamphetamine-induced pharmacological effects (behavioral and pharmacokinetic), in two preclinical models of (+)-methamphetamine abuse. This was accomplished by conducting new studies and integrating these data with a single data set from the previously published study of Byrnes-Blake et al. (2003). Two different anti-(+)-methamphetamine monoclonal antibodies, differing by about 25-fold in their affinities for (+)-methamphetamine ($K_D$=11 nM and 250 nM), were tested in each of the preclinical models. Because the higher affinity antibodies were more effective in both models, and because both antibodies were more effective in the overdose model, these studies showed that monoclonal antibody affinity and time of administration (relative to (+)-methamphetamine dosing) are critical determinants of therapeutic success.

## 2. Materials and methods

### 2.1. Drugs

$^3$H-(+)-methamphetamine ((+)-[2′,6′-$^3$H($n$)]methamphetamine; 23.5 Ci/mmol), (+)-methamphetamine, and (+)-amphetamine were obtained from the National Institute on Drug Abuse (Bethesda, MD). Other reagents were obtained from Sigma Chemical Corporation (St. Louis, MO), unless otherwise noted. The synthesis of the (+)-P6-METH hapten (*S*-(+)-4-(5-carboxypentyl) methamphetamine HCl) is described by Byrnes-Blake et al. (2003). The (+)-P4-METH hapten (*S*-(+)-4-(3-carboxypropyl) methamphetamine HCl) was synthesized in a similar fashion. Both haptens were conjugated (Davis and Preston, 1981) to bovine serum albumin (BSA) for immunization, and to ovalbumin for antibody screening by enzyme-linked immunoassay.

### 2.2. Generation and characterization of anti-(+)-methamphetamine monoclonal antibodies

Female BALB/c mice (Charles River Laboratories, Wilmington, MA) were subcutaneously immunized with 100 μg of (+)-P4-METH-BSA or (+)-P6-METH-BSA antigen in TiterMax (CytRx Corporation, Norcross, GA) near each hindquarter per the adjuvant manufacturer's instructions. This initial immunization was followed by monthly boosts of 50 μg antigen in saline. A hybridoma cell line developed from each antigen was produced by the methods described by Valentine et al. (1994). The relative affinities of mAb6H8 and mAb6H4 for (+)-methamphetamine and related compounds were determined by radioimmunoassay similar to the method of Owens et al. (1988).

### 2.3. Large-scale monoclonal antibody production and purification

Antibodies were produced in hollow-fiber bioreactors by the method of Valentine et al. (1996) and purified by the method of Hardin et al. (1998). The monoclonal antibodies were formulated in a buffer containing 15 mM sodium phosphate and 150 mM sodium chloride (pH 6.5). The monoclonal antibody formulations were checked to assure that the concentration of endotoxin were well below physiologically significant levels. The monoclonal antibody medications were then ultra-centrifuged to eliminate large molecular-weight antibody

complexes, which can be highly antigenic (Spiegelberg and Weigle, 1967).

### 2.4. Animals

Male Sprague–Dawley rats (Hilltop Laboratories, Scottsdale, PA) with an indwelling silastic jugular venous catheter were maintained between 270 and 300 g body weights. All animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health, USA.

### 2.5. Overdose studies

Rats were habituated to testing chambers after being assigned to one of two anti-(+)-methamphetamine antibody dosing groups (mAb6H4 or mAb6H8; $n=6$ per group). On each (+)-methamphetamine (or saline) dosing day, rats were placed in the monitoring chambers at 08:30 h (1 h prior to drug administration) to obtain stable baseline locomotor activity for that day. On study day 1, both groups received physiologic saline (1.0 ml/kg as a 15 s i.v. infusion) followed at 30 min by antibody buffer to obtain saline-induced baseline behavior. On study days 4, 7 and 10, they received a single dose of 1.0 mg/kg (+)-methamphetamine (as a 15 s i.v. infusion). On study days 4 and 7, the (+)-methamphetamine dose was followed 30 min later by antibody buffer. These (+)-methamphetamine doses were designated as the control doses. On study day 10, each group of rats received a single i.v. injection of mAb6H4 or mAb6H8 (8.0 mL as a 2.0 mL/min infusion) 30 min after the 1.0 mg/kg (+)-methamphetamine dose. The 30 min time point was chosen because this is the time point of near maximal locomotor activity and the time point at which the (+)-amphetamine (the principle pharmacological active metabolite of (+)-methamphetamine) concentrations in tissues are near maximum (Rivière et al., 1999, 2000). No further (+)-methamphetamine doses were given after the monoclonal antibody dose in the overdose studies.

The (+)-methamphetamine-induced locomotor activity was recorded for 6 h on each study day, beginning 30 min before saline or (+)-methamphetamine dosing. The dose of mAb6H4 (367 mg/kg) was calculated to be equimolar in binding sites to the body burden of what remains of a 1.0 mg/kg (+)-methamphetamine dose at 30 min. This determination was made based on pharmacokinetic calculations using previously determined pharmacokinetic values for (+)-methamphetamine determined in our laboratory (Rivière et al., 1999). This calculation showed that approximately 70% of the original (+)-methamphetamine dose still remained at 30 min after the 1.0 mg/kg (+)-methamphetamine injection. The dose of mAb6H8 (1000 mg/kg) was chosen to improve and maximize chances for this low affinity antibody to successfully antagonize (+)-methamphetamine's locomotor effects. Therefore, it should be noted that the dose of mAb6H8 was 2.7 times greater than the dose of mAb6H4. We have safely administered similar large doses of monoclonal antibodies to rats in numerous experiments without adverse effects (e.g., Proksch et al., 2000a; Hardin et al., 2002, Laurenzana et al., 2003b).

### 2.6. Pretreatment studies

For the pretreatment studies, rats were randomly assigned to one of two anti-(+)-methamphetamine antibody treatment groups (mAb6H4 or mAb6H8; $n=7$ per group) and habituated to the behavioral monitoring chambers. Similar to the overdose study, saline and (+)-methamphetamine-induced locomotor activity (control data) were determined before beginning the antibody treatment phase. On each (+)-methamphetamine (or saline) dosing day, rats were placed in the monitoring chambers at 08:30 h (1 h prior to drug administration) to obtain stable baseline locomotor activity. The rats were then administered physiologic saline (1.0 ml/kg; study day 1) or (+)-methamphetamine (1.0 mg/kg in 1 ml of physiological saline per kg of body weight, study days 2 and 5) as a 15 s i.v. infusion. Then, on study day 7 at 1630 h, one group of rats received a 503 mg/kg dose of the high affinity mAb6H4 via the jugular venous catheter. The other group of rats received a 1000 mg/kg dose of the low affinity mAb6H8. These doses were chosen to be consistent with the overdose studies (i.e., equal to the (+)-methamphetamine body burden at the time of antibody administration for mAb6H4 and higher doses of mAb6H8 in an attempt to improve the chances for therapeutic success).

In these studies, additional (+)-methamphetamine challenges (1.0 mg/kg) were then administered on the following day (study day 8 or monoclonal antibody day 1) and on study days 11 and 14 (monoclonal antibody days 4 and 7) to determine if the single dose of the antibody would continue to be effective over time. The resultant locomotor activity after each (+)-methamphetamine i.v. challenge was recorded for 6 h starting 30 min before the saline or (+)-methamphetamine dose. A total of three (+)-methamphetamine challenges were administered after the single antibody dose in the pretreatment studies.

### 2.7. Quantification of locomotor activity

Locomotor activity was quantified for each animal by video imaging technology (Noldus Information Technology, Sterling, VA); which has been extensively validated in our lab for these types of studies (Hardin et al., 1998; Rivière et al., 1999). The distance traveled and number of rearing events were quantified in each consecutive 2 min period and totaled from the time of antibody administration (overdose study) or (+)-methamphetamine administration (pretreatment study) to over 4 h after the pharmacological effects returned to baseline values. The duration of drug action was calculated based on the locomotor activity (either distance traveled or rearing) beginning at the same time points, until the time at which the locomotor activity returned to baseline levels. Locomotor activity was considered to have returned to baseline values when the first of two consecutive 4 min time intervals were equal to or below the mean + 1 S.D. of the 30 min baseline behavior prior to drug administration.

### 2.8. Integration of previous data into the current analysis

The data in the (+)-methamphetamine overdose group treated with mAb6H4 were previously published by this laboratory (Byrnes-Blake et al., 2003). This previous study examined the ability of a single dose of mAb6H4 to reverse the effects of one of three (+)-methamphetamine doses (0.3, 1.0 or 3.0 mg/kg, i.v.). The experiments in the current study were performed for the three new groups (overdose treated by mAb6H8; pretreatment with mAb6H4; pretreatment with mAb6H8) exactly as in the Byrnes-Blake et al. (2003) paper to facilitate comparison of monoclonal antibody affinity and administration time, relative to (+)-methamphetamine administration. The (+)-methamphetamine overdose followed by treatment with mAb6H4 experiments were not repeated to minimize animal and antibody consumption.

### 2.9. Statistical analysis

Raw (i.e., untransformed to percentage) data were used for all analyses. For each rat and type of locomotor activity (distance traveled or rearing events), we calculated two response ratios: (1) (+)-methamphetamine-induced locomotor activity in the presence of monoclonal antibody to control (+)-methamphetamine-induced locomotor activity and (2) (+)-methamphetamine-induced locomotor activity in the presence of monoclonal antibody to saline activity. The natural logarithms of these ratios were used for the analyses. We used a significance level of 0.05. All analyses were conducted with the MIXED procedure in SAS/STAT® Version 9.1.

To compare between the two affinities of antibody and between the two models through the first dose of (+)-methamphetamine in the presence of antibody, we analyzed the (+)-methamphetamine log ratios (for each type of locomotor activity) with analysis of variance (ANOVA) controlling for monoclonal antibody affinity, model, and their interaction. We examined the residuals from these analyses for violations of assumptions, and found none. If the interaction effect was significant, we examined only the conditional effects of antibody affinity given a model (or vice versa); otherwise, we also examined the marginal effects of antibody affinity and model. In the same analyses, we evaluated the therapeutic efficacy for each antibody affinity by model combination. This was done by computing the upper 95% confidence limit (95% UCL) for the mean (+)-methamphetamine log ratio.

To determine if monoclonal antibody administration brought (+)-methamphetamine-induced behavior back to saline levels, we analyzed the saline log ratios in the same way as the (+)-methamphetamine log ratios. For each monoclonal antibody affinity by model combination, we computed the lower 95% confidence limit (95% LCL) for the mean saline log ratio and report the exponentiated 95% LCL, which is a 95% LCL for the median saline response ratio.

To assess the prophylactic effect of a single pretreatment monoclonal antibody dose on each type of locomotor activity over repeated (+)-methamphetamine challenges, we used the log ratios from days 1, 4, and 7. These were fitted with a linear mixed model having fixed effects for antibody affinity, day of challenge, and their interaction, and a random effect for rat. We modeled the correlation of observations within a rat with a first order autoregressive structure. For a given affinity of antibody, we assessed efficacy at days 1, 4, and 7 with a Bonferroni-corrected 95% UCL. We used a two-sided $t$-test to compare between low and high affinity monoclonal antibody. Residuals showed no evidence of violations of ANOVA assumptions.

## 3. Results

### 3.1. Anti-(+)-methamphetamine monoclonal antibodies

The low affinity monoclonal antibody (mAb6H8; $K_D$=250 nM) was generated from immunization with the (+)-P4-METH-BSA antigen, and the high affinity monoclonal antibody (mAb6H4; $K_D$=11 nM) was generated from immunization with the (+)-P6-METH-BSA antigen. The monoclonal antibodies (IgG1, κ light chain) were highly specific for (+)-methamphetamine. All of the structurally unrelated drugs and neurotransmitters that were tested had no significant cross-reactivity (Byrnes-Blake et al., 2003). (+)-Amphetamine and (−)-amphetamine had <0.1% cross reactivity. The one exception

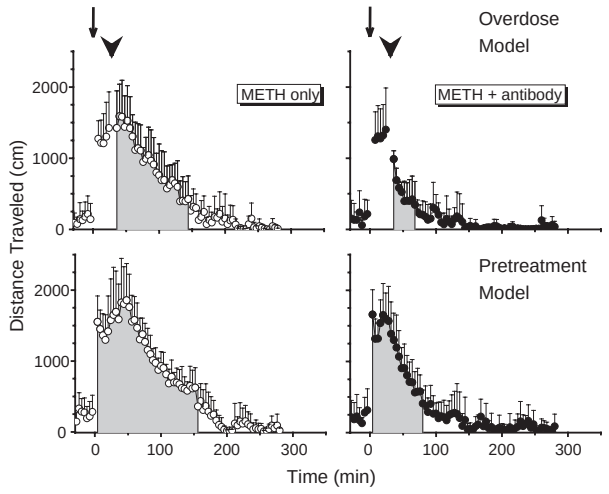


Fig. 1. Time course of (+)-methamphetamine-induced distance traveled in rats. In the overdose model (upper panels), rats ($n$=6) received (+)-methamphetamine followed 30 min later by antibody buffer on study day 7 ('METH only'; open circles ○). Three days later (study day 10) the same animals received (+)-methamphetamine followed 30 min later by the mAb6H4 (367 mg/kg monoclonal antibody; METH+antibody; closed circles ●). In the pretreatment model (lower panels), rats ($n$=7) received (+)-methamphetamine alone on study day 5 (open circles ○). On study day 7 at approximately 1630, the same animals received the mAb6H4 (503 mg/kg). On study day 8, they were given (+)-methamphetamine (closed circles ●). The antibody was thus administered 17 h before the (+)-methamphetamine (not shown on graphs). The average distance traveled for all rats in each antibody group during each 2 min interval (+1SD) is shown. The arrow on the overdose model graphs indicates the time (0 min) of (+)-methamphetamine administration, and the arrowhead indicates the time of mAb6H4 administration (30 min, overdose rats only). Shading indicates the time period of the duration of drug action above saline-induced locomotor activity following buffer or antibody treatments. The time needed to return to baseline activity was determined by statistical comparison of the behavior starting at $t$=30 min (time of treatment) with each animal's predosing behavior from −30 min to $t$=0.

was the drug of abuse, (+)-3,4-methylenedioxymethamphetamine (MDMA or "ecstasy"), which mAb6H4 bound with a slightly higher relative affinity than (+)-methamphetamine ($K_D$=9 nM vs. 11 nM, respectively). Both antibodies were stereospecific, having an approximately 50–200 times higher relative affinity for (+)-methamphetamine than (−)-methamphetamine (Byrnes-Blake et al., 2003).

### 3.2. Representative data from control and mAb6H4-treated animals

Fig. 1 shows the time course of the distance traveled after (+)-methamphetamine administration in rats (without and with mAb6H4 treatment) in both (+)-methamphetamine abuse models. These data are used to illustrate how the experiments were conducted and the quality of the data used for the analysis. The data used for analytical comparisons are shaded in Fig. 1. Although data are only shown for mAb6H4, experiments with mAb6H8 were conducted in an identical manner, with similar variance for individual data points.

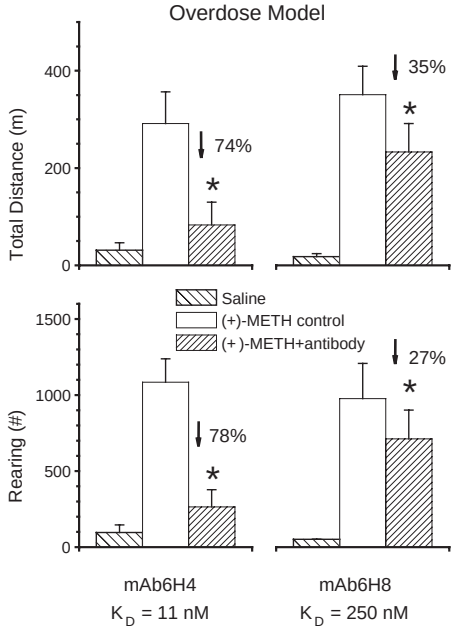


Fig. 2. Comparison of locomotor activity in the overdose model after administration of 367 mg/kg of a high affinity monoclonal antibody (mAb6H4; $K_D$=11 nM) or 1000 mg/kg of a low affinity monoclonal antibody (mAb6H8; $K_D$=250 nM). Distance traveled (top panel) and the number of rearing events (bottom panel) over the period of analysis in each experiment were measured. An example of the data sets from which the bar graphs were derived is shown in Fig. 1 for mAb6H4. The animals received saline followed 30 min later by buffer (saline data—open bars). Three days later, they received a 1.0 mg/kg i.v. dose of (+)-methamphetamine followed 30 min later by buffer (data not shown). This was followed 3 days later by a second 1.0 mg/kg i.v. (+)-methamphetamine dose with buffer at $t$=30 min ((+)-methamphetamine control data—closed bars). Three days later, they received a final 1.0 mg/kg i.v. (+)-methamphetamine dose followed at $t$=30 min by the high-affinity mAb6H4 or the low-affinity mAb6H8 ((+)-methamphetamine+mAb—hatched bars). The data are shown as the mean+1 S.D. ($n$=6 per group). The * indicates a statistical difference from the control (+)-methamphetamine and saline data ($P$<0.05).

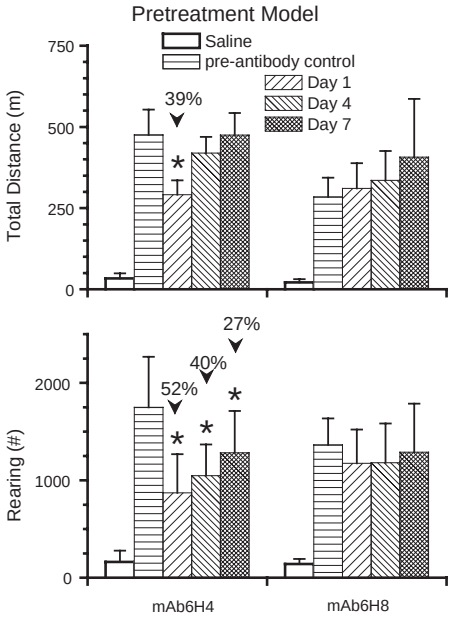


Fig. 3. Comparison of locomotor activity after administration of 503 mg/kg of high affinity mAb6H4 or 1000 mg/kg of low affinity mAb6H8. Distance traveled (top panel) and the number of rearing events (bottom panel) were recorded. The animals received saline on study day 1, and then a single dose of 1.0 mg/kg i.v. (+)-methamphetamine every 3 days. The monoclonal antibodies were administered on study day 7, prior to the (+)-methamphetamine doses on study days 8, 11, and 14 (monoclonal antibody days 1, 4 and 7; hatched bars). The arrowhead and associated values indicate the antibody-induced percentage reductions in locomotor activity. The data are shown as the mean+1 S.D. ($n$=7 per group). The * indicates a statistical difference from the control (+)-methamphetamine and saline data ($P$<0.05).

### 3.3. Effect of anti-(+)-methamphetamine monoclonal antibodies on methamphetamine-induced locomotor activity and comparison of low and high affinity antibodies

In the overdose model (Fig. 2), both antibodies significantly reduced (+)-methamphetamine-induced locomotor activity from control (+)-methamphetamine induced locomotor activity; however, activity did not decrease to saline levels. mAb6H4 reduced distance traveled to 26.2% (95% UCL=31.8%) of control and rearing events to 22.1% (95% UCL=28.4%) of control. Though reductions were not as great in magnitude as mAb6H4, mAb6H8 reduced distance traveled to 65.3% (95% UCL=79.3%) of baseline and rearing events to 72.4% (95% UCL=93.1%) of control. Statistical comparison of the effect of affinity on antibody function revealed that the high affinity mAb6H4 was significantly more effective at antagonizing both of the measured (+)-methamphetamine induced behaviors in the overdose model ($P$<0.001).

In the pretreatment model (Fig. 3), effectiveness of the antibodies in reducing (+)-methamphetamine induced behavior from control was determined for 1, 4 and 7 days after antibody treatment. In animals that received mAb6H4, (+)-methamphetamine-induced locomotor activity was reduced to 61.4% (95% UCL=73.5%) and 47.6% (95% UCL=60.1%) of control distance traveled and rearing, respectively, on the first day after mAb treatment (mAb day 1), but activity was also

significantly different from saline levels. (+)Methamphetamine-induced distance traveled was back to pre-antibody levels on monoclonal antibody days 4 and 7. However, the rearing behavior was still significantly reduced on monoclonal antibody days 4 and 7 (Fig. 3). A similar analysis showed that the lower affinity mAb6H8 was not effective at antagonizing (+)-methamphetamine induced effects on any of the antibody post-treatment days (Fig. 3).

Statistical comparison of the effect of affinity on antibody function revealed that the high affinity mAb6H4 was significantly more effective at antagonizing both of the measured (+)-methamphetamine induced behaviors in the pretreatment model. Distance traveled (Day 1 only) was 61.4% of control for mAb6H4 compared to 110.1% for mAb6H8 ($P<0.001$), and for rearing events 47.6% for mAb6H4 compared to 88.2% for mAb6H8 ($P=0.002$).

### *3.4. Comparison of anti-(+)-methamphetamine monoclonal antibody behavioral effects in overdose and pretreatment models*

Fig. 4 shows a comparison of the antibody effects on distance traveled and rearing events between the two different preclinical abuse models on the first day of behavioral testing. For both monoclonal antibodies, the reductions in (+)-methamphetamine response ratios for distance traveled were significantly greater in the overdose model than in the pretreatment model ($P<0.001$ for mAb6H4 and $P=0.001$ for mAb6H8). For rearing behavior with mAb6H4, antagonism of (+)-methamphetamine effects was greater in the overdose model than in the pretreatment model ($P<0.001$). For the low affinity antibody, mAb6H8, the difference in antagonism of rearing effects between the overdose model and the pretreatment model was not significant ($P=0.300$).

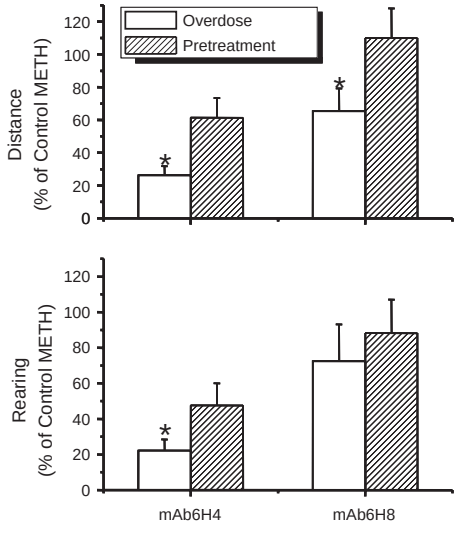


Fig. 4. Effect of time of monoclonal antibody administration (model) on therapeutic efficacy. For the two preclinical models of (+)-methamphetamine abuse, the reductions in the response ratio for (+)-methamphetamine-induced distance traveled (top panel) and rearing (bottom panel) in each model on day 1 are shown. Values represent medians (expressed as % of baseline (+)-methamphetamine activity) and the error bars represent the 95% UCL. The * indicates a significant difference between monoclonal antibody models ($P<0.05$).

## 4. Discussion

The overall goal of these studies was to determine the role of antibody affinity and time of monoclonal antibody dosing (relative to (+)-methamphetamine dosing) in antibody antagonism of (+)-methamphetamine-induced pharmacological effects. Both monoclonal antibodies were of the same isotype and light chain, and they were highly specific for (+)-methamphetamine, with no significant cross-reactivity with (+)-amphetamine. They differed in only one important aspect: an approximate 25-fold difference in $K_D$ value (11 nM vs. 250 nM).

The high affinity mAb6H4 was significantly more effective than the lower affinity mAb6H8 at antagonizing the effects of (+)-methamphetamine in both preclinical models. Other investigators also show superior effectiveness of a high affinity anti-imipramine antibody compared with a lower affinity antibody (Ragusi et al., 1998). Unlike the current studies, effectiveness was only defined by the ability of the antibody to redistribute imipramine from tissues into the blood. Their high affinity sheep polyclonal anti-imipramine antibody ($K_a=1.3\times10^{10}$ $M^{-1}$ or $K_D=0.77$ nM) had a greater impact on imipramine tissue redistribution than a lower affinity monoclonal mouse $IgG_1$ antibody ($K_a=3.8\times10^{7}$ $M^{-1}$ or $K_D=27$ nM). The investigators used a rat model with imipramine dosing prior to antibody administration, similar to the overdose model in the current studies.

Interestingly, in the Ragusi et al. (1998) studies and the current studies, the dose of the lower affinity antibody was much higher relative to the amount of target drug (e.g., imipramine or (+)-methamphetamine, respectively) than in the studies with the higher affinity antibody. This resulted in a significant excess of antibody binding sites (on a molar basis) over the amount of drug in the body in the lower affinity antibody groups. In the current studies, the ratio of antibody binding sites to the (+)-methamphetamine molar dose was 2.7:1 for mAb6H8. Nevertheless, the higher affinity antibodies were still significantly more efficacious.

Other studies have demonstrated that anti-drug antibodies can be effective even in the presence of extreme molar drug excess; that is, when there is a substantial shortage of antibody binding sites compared to the amount of drug in the body. Laurenzana et al. (2003b) demonstrated that a high-affinity anti-phencyclidine monoclonal antibody reverses phencyclidine-induced locomotor activity, prevents weight loss, and reduces chromodacryorrhea (an indicator of animal stress due to high drug doses) in rats given a continuous high dose infusion of phencyclidine for 2 weeks. This antibody prevented adverse health effects even when administered on day 1 as a single dose equivalent to only 1/100th the amount of phencyclidine in the body, and phencyclidine continued to be replaced at a rate of 15% of the body burden per hour. These data emphasize that antibody affinity is a more important determinant of efficacy than binding capacity.

The efficacy of the monoclonal antibody also appeared to be dependent on the preclinical model in which it was tested. Both the high affinity and low affinity antibodies were significantly better at reversing (+)-methamphetamine effects in the overdose model than preventing (+)-methamphetamine effects in the pretreatment model.

Pharmacokinetic data collected in previous (separate) studies from our laboratory for mAb6H4 (Byrnes-Blake et al., 2003; Laurenzana et al., 2003a; Fig. 5) provide significant insight into the mechanism for the differing effectiveness of the antibodies in the two preclinical models in the current studies. For the pharmacokinetic studies, 1.0 mg/kg i.v. (+)-methamphetamine doses were given 30 min before (Byrnes-Blake et al., 2003) or 17 h after (Laurenzana et al., 2003a) a 1 mol-eq mAb6H4 antibody dose. In the pretreatment model (Laurenzana et al., 2003a), brain concentrations were not significantly reduced in animals treated with mAb6H4 for the first 5 min after the (+)-methamphetamine dose (Fig. 5). Conversely, in the overdose model (Byrnes-Blake et al., 2003) (+)-methamphetamine brain concentrations were significantly reduced immediately after mAb6H4 administration. Starting at 15 min, the (+)-methamphetamine brain concentrations in the two studies were decreased and virtually superimposable, except for the 4.5 h time point.

We think a mechanism for this difference is as follows. First, (+)-methamphetamine has a very high volume of distribution, with very little of the dose in the blood. In the overdose model, the anti-drug antibody was administered at 30 min, after the (+)-methamphetamine was distributed to highly perfused tissues like the brain. Thus, there was a very large dose of mAb6H4 in the serum relative to the dose of (+)-methamphetamine in the brain. The presence of the monoclonal antibody in the blood stream caused rapid redistribution of (+)-methamphetamine out of highly perfused tissues, which can quickly re-equilibrate. In the antibody pretreatment model, a different scenario occurred. At the time point of (+)-methamphetamine administration, the antibody was already fully distributed into extracellular fluid (Bazin-Redureau et al., 1997). When (+)-methamphetamine was administered via a rapid i.v. bolus dose, the dose of drug entering the bloodstream temporarily overwhelmed the capacity and/or the rate of association of the monoclonal antibody, such that free drug (not bound to antibody) was available to penetrate into tissues. This was why the brain concentrations up to 5 min were not significantly lower in the brain, but they were significantly lower by 15 min. The trend of the later data concentration-time points in the overdose model is consistent with this interpretation. At the last concentration-time point (4.5 h) in the overdose model (collected well after the behavioral effects are over), the capacity of the antibody starts to become more limited due to repartitioning from slower equilibrating tissues, and the drug begins to slowly reenter the brain. We think an even higher affinity monoclonal antibody would lead to lower brain concentrations at earlier time points and improve the protective effects in the overdose scenario. In support of our hypothesis, our very high affinity anti-phencyclidine monoclonal antibody ($K_D$=1.3 nM) offers protection against brain phencyclidine penetration that is far greater than predicted by the antibody binding capacity for phencyclidine (see Proksch et al., 2000a; Hardin et al., 2002).

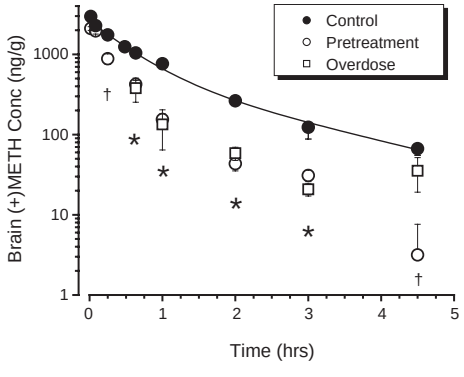


Fig. 5. Average concentration vs. time profiles for (+)-methamphetamine in the brain without mAb6H4 (●), in the overdose model (□), and in the pretreatment model (○). The (+)-methamphetamine only (control), overdose data, and pretreatment data are combined from the original data sets of Byrnes-Blake et al. (2003) and Laurenzana et al. (2003a). All values are represented as the mean±1 S.D., $n$=3 animals per time point. The * indicates that both the overdose and pretreatment concentration-time points are statistically different from the control (+)-methamphetamine only concentration-time points ($P$<0.05). The † indicates that only the pretreatment concentration-time points are statistically different from the control (+)-methamphetamine only concentration-time points ($P$<0.05).

The design of the experiments for the pretreatment model also allowed us to characterize reductions in (+)-methamphetamine-induced locomotor effects over time (Fig. 3). Thus, we tested the efficacy of the antibody during repeated (+)-methamphetamine challenges over a week long period in the rats. Because both sensitization (e.g., Nishikawa et al., 1983; Fujiwara et al., 1987; Ujike et al., 1989) and tolerance (e.g., Schmidt et al., 1985a,b; Gygi et al., 1996) to (+)-methamphetamine occurs with very frequent dosing regimens, the (+)-methamphetamine dosing used in the overdose and pretreatment studies was designed to minimize the influence of tolerance and sensitization. Since (+)-methamphetamine was administered every 3 days, this regimen allowed for complete clearance of the previous (+)-methamphetamine dose ($t_{1/2\lambda Z}$=approximately 1 h; Rivière et al., 1999, 2000), when the antibody was not present. In a preliminary study conducted to determine if sensitization or tolerance occurs with this dosing schedule, (+)-methamphetamine-induced distance traveled was determined to be stable and not statistically different for 19 days of repeated dosing (results not shown). Rearing behavior showed a downward trend with multiple dosing, indicating mild tolerance. However, this time-dependent decrease in rearing was not statistically significant (data not shown).

When (+)-methamphetamine was given on the day after the high affinity mAb6H4 dose, (+)-methamphetamine-induced locomotor activity was significantly decreased. However, the antibody did not provide significant protection on days 4 and 7 after antibody administration. While sensitization to (+)-methamphetamine effects could have contributed to the apparent lack of antibody effect on days 4 and 7, this lack of longer-term protection was unexpected because mouse monoclonal antibodies have been reported to have an

elimination half-life of ~8 days in rats (Bazin-Redureau et al., 1997). In addition, previous studies from our laboratory have shown that pretreatment with an anti-phencyclidine antibody significantly reduces phencyclidine-induced locomotor activity for up to 2 weeks (Hardin et al., 2002). However, there are important differences between our studies with the anti-phencyclidine antibody and the current study. First, the anti-phencyclidine antibody has about a 10-fold higher affinity for phencyclidine than the high affinity mAb6H4 has for (+)-methamphetamine. Second, the (+)-methamphetamine studies are complicated by the formation of a pharmacologically active metabolite (+)-amphetamine, which does not bind to the antibody. In addition, we have previously shown that the anti-phencyclidine antibody has a "functional" elimination half-life of ~15 days (Proksch et al., 2000a), as measured by its ability to produce prolonged high serum concentrations of phencyclidine and prolonged low brain concentrations. Although the anti-phencyclidine antibody and anti-(+)-methamphetamine antibodies are all $IgG_1$ (κ light chains), there could be significant differences in the in vivo stability and thus the functional half-life. We are currently studying this hypothesis.

In conclusion, the current studies showed that the therapeutic efficacy of anti-drug monoclonal antibodies was highly dependent on monoclonal antibody affinity. In addition, the time of antibody administration, relative to the time of drug administration, was also an important factor. Anti-drug monoclonal antibodies provide an attractive alternative to traditional drug abuse treatment medications due to their specificity, relatively long half-lives (~8 days in rats and 21 days in humans; Bazin-Redureau et al., 1997), and lack of interaction with drug receptor sites of pharmacological action in tissues. Monoclonal antibodies with significantly improved affinity for (+)-methamphetamine, and possibly increased cross-reactivity with (+)-amphetamine, could offer improvements in the effectiveness of the therapy. These second generation monoclonal antibody medications are currently under development.

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