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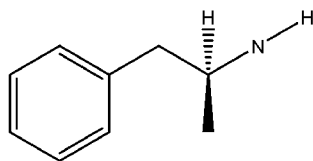
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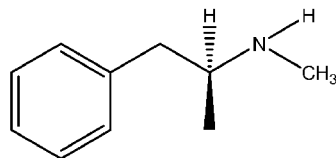
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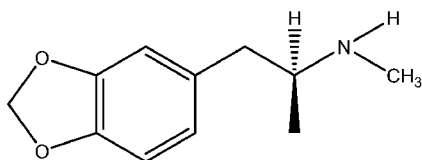
(54) Title: MONOCLONAL ANTIBODIES THAT SELECTIVELY RECOGNIZE METHAMPHETAMINE AND METHAMPHETAMINE LIKE COMPOUNDS



(+) Amphetamine



(+)Methamphetamine



(+)-3,4-Methylenedioxyamphetamine

(57) Abstract: The invention generally relates to monoclonal antibodies that recognize at least one compound from the group consisting of (+) methamphetamine, (+) amphetamine, and (+) 3,4-methylenedioxyamphetamine ((+) MDMA). Generally speaking, the monoclonal antibodies do not recognize (-) methamphetamine, (-) amphetamine, or (-) MDMA.

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MONOCLONAL ANTIBODIES THAT SELECTIVELY RECOGNIZE METHAMPHETAMINE AND METHAMPHETAMINE LIKE COMPOUNDS

GOVERNMENTAL RIGHTS

[0001] This invention was made with government support under the National Institute on Drug Abuse grant Nos. DA11560, DA14361, and DA05477. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] The invention provides monoclonal antibodies that recognize (+) methamphetamine, (+)amphetamine, and/or (+)3,4-methylenedioxymethamphetamine ((+)MDMA), but typically do not recognize (-)methamphetamine, (-)amphetamine, or (-)MDMA.

BACKGROUND OF THE INVENTION

[0003] (+)Methamphetamine ((+)METH) abuse has become America's number one drug threat (NACO, 2005). Known strategies for combating (+)methamphetamine drug use all have drawbacks. Current pharmacotherapies for managing the acute cardiovascular system, central nervous system and toxic effects are mostly supportive (Sato, 1992; Albertson et al., 1999; Richards et al., 1999); and do nothing to remove the drug from its sites of action in the brain. Also lacking are medications that can reduce or treat the medically crippling effects of (+)METH addiction. Antibodies provide an attractive potential medication that can target the drug instead of the site of action (Kosten and Owens, 2005). These high affinity protein-based medications act as so-called pharmacokinetic antagonists, sequestering the drug in the bloodstream away from medically vulnerable tissues like the brain and heart.

[0004] Unlike nicotine and cocaine where the effects are caused by a single, specific compound, drugs like opiates (e.g., morphine), arylcyclohexylamines (e.g., phencyclidine) and amphetamines (e.g., (+)METH) are starting structures from which many pharmacologically similar compounds can be synthesized. These so-called "designer drugs" can be chemically modified to alter their effects. Thus, for an antibody

to have the broadest medical applicability, it should have high affinity and specificity for more than one medically important member of this drug class (i.e., (+)METH, (+)amphetamine ((+)AMP) and (+)MDMA).

[0005] There are other medication design issues that further complicate the development of effective treatments for (+)METH-like stimulants. First, (+)METH is one of several stimulant drugs of abuse with similar or overlapping effects. In particular, (+)AMP is both a pharmacologically active metabolite of (+)METH and a frequently used drug of abuse that could be substituted for (+)METH. Next, (+/-)3,4-methylenedioxymethamphetamine is the racemic mixture commonly referred to as MDMA or ecstasy. The plus isomer ((+)MDMA) has predominately dopaminergic, stimulant-like activity with overlapping effects with (+)METH, while (-)MDMA has predominately serotonergic effects (Cho and Segal, 1994). (+)METH, (+)AMP, and (+)MDMA can produce life threatening effects at high doses (Cho and Segal, 1994; Farre et al., 2004). Additionally, all of these drugs are plus stereoisomers, with the minus isomers having a significantly different pharmacological profile of effects, some of which may be beneficial. For example, (-)methamphetamine is commonly used as a bronchodilator in over the counter medications. The minus isomers of these drugs could potentially be purposely taken by drug abusers to neutralize mAb medications with high affinity binding for both plus and minus stereoisomers. In a related way, there are many structurally similar compounds like ephedrine and pseudoephedrine that could be used to lessen the efficacy of antibodies if the antibody is not highly specific for (+)METH-like structures.

[0006] There is a need in the art for specific antibodies that recognize at least one or more of (+)methamphetamine, (+)amphetamine, or (+)MDMA, and that do not substantially cross-react with (-)methamphetamine, (-)amphetamine, or (-)MDMA. In particular, there is a need for specific monoclonal antibodies that recognize each compound of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, and that do not substantially cross-react with (-)methamphetamine, (-)amphetamine, or (-)MDMA.

REFERENCE TO COLOR FIGURES

[0007] The application file contains at least one photograph executed in color. Copies of this patent application publication with color photographs will be provided by the Office upon request and payment of the necessary fee.

BRIEF DESCRIPTION OF THE FIGURES

[0008] **Fig. 1** depicts the chemical structures of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine ((+)MDMA).

[0009] **Fig. 2** presents amino acid sequence alignments of the variable regions of five moderate to high affinity anti-(+)METH and anti-(+)METH/(+)AMP, and anti-(+)METH/(+)AMP/(+)MDMA mAb (herein, mAb refers to monoclonal antibodies, both singular and plural). **Panel A** presents the amino acid sequences of the heavy chains. **Panel B** presents the amino acid sequences of the light chains. The sequences are presented in single letter amino acid notation and numbered according to Kabat and Wu (1991 J Immunol 147:1709-1719). Location of the framework (FR) and complementarity determining region (CDR) residues are indicated for the heavy chains and light chains.

[0010] **Fig. 3** presents molecular models of three anti-(+)METH mAb. **Upper panel:** Stereo view of superimposed molecular models of anti-(+)METH mAb. The variable regions of the three mAb were modeled, structurally aligned and represented in cartoon format. The framework residues are represented in blue. The CDRs are colored according to mAb: mAb6H4, blue; mAb6H8, red; mAb4G9, green. The heavy chain, light chain, and CDRs are labeled. **Lower panel:** Root mean square deviations (RMSD) (Å) of CDRs from the main chain conformation of mAb6H4.

[0011] **Fig. 4** illustrates modeled structures of the anti-(+)METH mAb variable chains. In this model, METH (magenta) has been computationally docked into a pocket at the interface of the VH and VL chains with FlexX software. Left panels: Surface rendering of deep pocket in mAb6H4 and mAb4G9. The VL chain domain is on the left side in blue and the VH chain domain is on the right in green. Right panels: Stick representation of mAb6H4 and mAb4G9. Only side chains within 8 angstroms of the

METH molecule are shown for clarity. The view is oriented in a “top view” with the same color scheme as in left panel. The side chains are labeled and numbered in the Kabat scheme as in Figure 2.

[0012] **Fig. 5** presents a graph depicting serum concentration of (+)METH over time. (+)METH concentrations before (□ symbol with “M” inside) and after treatment (open symbols) with five different anti-(+)METH mAb (n=3 rats/time point) and (+)AMP concentrations before (□ symbol with “A” inside) and after treatment (filled circles) with mAb4G9. (+)AMP concentrations (filled circles) are shown only for mAb4G9 because the other four mAb did not produce long-term increases in (+)AMP concentrations. The best-fit line was determined using a weighted two-compartment pharmacokinetic model.

[0013] **Fig. 6** presents graphs depicting the effect of mAb4G9 on METH discrimination (Panel A) and AMP self-administration (Panel B) in rats. (A) Rats were trained to discriminate cocaine and METH and then administered mAb4G9. Drug discrimination dose response curves were then determined for METH. (B) (+)AMP self-administration before and after treatment with mAb4G9 (3-4 rats/time point). Dashed lines are used to show the groupings of responding for (+)AMP before and after mAb. *P<0.05 compared to control group before antibody (open circles).

[0014] **Fig. 7** depicts two graphs showing the effect of mAb6H4 (Panel A) and mAb4G9 (Panel B) on METH (closed circles) and AMP (open circles) concentrations in rat serum. METH was infused using a subcutaneous osmotic minipump and once steady state concentrations were reached, anti-METH mAb was administered. Rats were sacrificed at various time points for determination of serum METH and AMP concentrations. One way ANOVA followed by a Dunnett’s test was used to compare control and post-mAb METH and AMP values. mAb6H4 significantly increased METH serum concentrations at the 1, 5, 30, 75, and 120 min, but not at 24 hr, compared to the pre-mAb levels. AMP concentrations were increased briefly at the 1 and 5 min time points. MAb4G9 significantly increased METH and AMP serum concentrations from 1 min to 24 hrs.

[0015] **Fig. 8** depicts two graphs showing the effect of mAb6H4 (Panel A) and mAb4G9 (Panel B) on METH (closed circles) and AMP (open circles) concentrations in the rat brain. METH was infused using a subcutaneous osmotic minipump and once steady state concentrations were reached, anti-METH mAb was administered. Rats were sacrificed at various time points for determination of brain METH and AMP concentrations. One way ANOVA followed by a Dunnett's test was used to compare control and post-mAb METH and AMP values. METH brain levels significantly different from the no mAb group are indicated with *, while # indicates METH and AMP levels significantly different from the no mAb group. METH brain concentrations were decreased at all times except 24 hr after mAb6H4 treatment. mAb6H4 had no effect on AMP brain concentrations. mAb4G9 significantly decreased METH brain concentrations at all time points, while AMP brain concentrations were significantly decreased at the 5 and 30 min time points.

SUMMARY OF THE INVENTION

[0016] One aspect of the invention encompasses a monoclonal antibody that recognizes each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine.

[0017] Another aspect of the invention encompasses a method of treating drug use in a subject. The method comprises administering to the subject a monoclonal antibody that recognizes each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine. The monoclonal antibody decreases the concentration of at least one compound selected from the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine in the brain of the subject.

[0018] Yet another aspect of the invention encompasses an assay for detecting the presence of at least one compound in a sample. The compound is selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine. The assay comprises contacting the sample with a monoclonal antibody that recognizes each compound of

the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine and detecting the association of the monoclonal antibody in the sample with at least one compound selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine.

[0019] A further aspect of the invention encompasses a kit. The kit comprises a monoclonal antibody that recognizes each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine.

[0020] Other aspects and iterations of the invention are described more thoroughly below.

DETAILED DESCRIPTION OF THE INVENTION

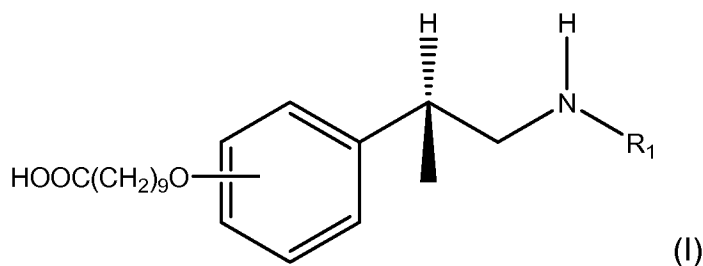
[0021] The present invention provides monoclonal antibodies that have specificity and affinity for (+)methamphetamine and (+)methamphetamine-like compounds. These antibodies recognize at least one compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)MDMA, and do not substantially recognize (-)amphetamine, (-)methamphetamine, and (-)MDMA. Because of their specificity and affinity, the monoclonal antibodies may be used to treat drug use in a subject.

I. Monoclonal Antibodies of the Invention

[0022] In one embodiment the monoclonal antibodies may recognize at least one compound of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA. In another embodiment, the monoclonal antibodies may recognize at least two compounds from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA. In an exemplary embodiment, the monoclonal antibodies may recognize all three compounds of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA. In another exemplary embodiment, the monoclonal antibodies may recognize all three compounds of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, and not substantially

recognize over the counter medications. In yet another exemplary embodiment, the monoclonal antibodies may recognize all three compounds of the group consisting of (+) methamphetamine, (+)amphetamine, and (+)MDMA, and not substantially recognize non-(+)methamphetamine like prescription medications. In each of the above embodiments, the monoclonal antibodies do not substantially recognize (-) methamphetamine, (-)amphetamine, and (-)MDMA.

[0023] An antibody of the invention “recognizes” a compound if the IC_{50} ratio for that antibody is greater than about 20%. The IC_{50} ratio may be calculated in reference to either (+)methamphetamine or (+)amphetamine. Typically, if the antibody is generated with a hapten derived from (+)methamphetamine, then the IC_{50} ratio should be determined in reference to (+)methamphetamine. Similarly, if the antibody is generated with a hapten derived from (+)amphetamine, then the IC_{50} ratio should be determined in reference to (+)amphetamine. For instance, if the hapten was derived from a compound of formula (I),



wherein R_1 is hydrogen (i.e. forming (+)amphetamine), then the IC_{50} ratio should be determined in reference to (+)amphetamine. Alternatively, if R_1 of formula (I) is a methyl group (i.e. forming (+)methamphetamine), then the IC_{50} ratio should be determined in reference to (+)methamphetamine.

[0024] In reference to (+)methamphetamine, the IC_{50} ratio refers to the ratio of the IC_{50} for (+)methamphetamine in the presence of labeled (+)methamphetamine to the IC_{50} for a test ligand in the presence of labeled (+)methamphetamine. The IC_{50} value is the concentration of test ligand required to inhibit 50% of the labeled (+)methamphetamine binding. For instance, see Table 2 in the Examples.

[0025] In reference to (+)amphetamine, the IC₅₀ ratio refers to the ratio of the IC₅₀ for (+)amphetamine in the presence of labeled (+)amphetamine to the IC₅₀ for a test ligand in the presence of labeled (+)amphetamine. The IC₅₀ value is the concentration of test ligand required to inhibit 50% of the labeled (+)amphetamine binding.

[0026] Irrespective of whether the IC₅₀ ratio is determined in reference to (+)methamphetamine or (+)amphetamine, an antibody of the invention "recognizes" a compound if the IC₅₀ ratio is greater than about 20%. In one embodiment, a monoclonal antibody of the invention has an IC₅₀ ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about 43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater than about 95%, greater than about 97%, or greater than about 100% for (+)methamphetamine (e.g. an anti-(+)METH monoclonal antibody).

[0027] In another embodiment, a monoclonal antibody of the invention has an IC₅₀ ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about 43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater than about

95%, greater than about 97%, or greater than about 100% for (+)amphetamine (e.g. an anti-(+)AMP monoclonal antibody).

[0028] In yet another embodiment, a monoclonal antibody of the invention has an IC₅₀ ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about 43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater than about 95%, greater than about 97%, or greater than about 100% for (+)MDMA (e.g. an anti-(+)MDMA monoclonal antibody).

[0029] In still another embodiment, a monoclonal antibody of the invention has an IC₅₀ ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about 43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater than about 95%, greater than about 97%, or greater than about 100% for (+)methamphetamine and (+)amphetamine (e.g. an anti-(+)METH/(+)AMP monoclonal antibody).

[0030] In a further embodiment, a monoclonal antibody of the invention has an IC₅₀ ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about

43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater than about 95%, greater than about 97%, or greater than about 100% for (+)methamphetamine and (+)MDMA (e.g. an anti-(+)METH/(+)MDMA monoclonal antibody).

[0031] In a further embodiment, a monoclonal antibody of the invention has an IC_{50} ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about 43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater than about 95%, greater than about 97%, or greater than about 100% for (+)amphetamine and (+)MDMA (e.g. an anti-(+)AMP/(+)MDMA monoclonal antibody).

[0032] In an exemplary embodiment, a monoclonal antibody of the invention has an IC_{50} ratio of greater than about 20%, greater than about 23%, greater than about 25%, greater than about 27%, greater than about 30%, greater than about 33%, greater than about 35%, greater than about 37%, greater than about 40%, greater than about 43%, greater than about 45%, greater than about 47%, greater than about 50%, greater than about 53%, greater than about 55%, greater than about 57%, greater than about 60%, greater than about 63%, greater than about 65%, greater than about 67%, greater than about 70%, greater than about 73%, greater than about 75%, greater than about 77%, greater than about 80%, greater than about 83%, greater than about 85%, greater than about 87%, greater than about 90%, greater than about 93%, greater

than about 95%, greater than about 97%, or greater than about 100% for (+)methamphetamine, (+)amphetamine, and (+)MDMA (e.g. an anti-(+)METH/(+)AMP/(+)MDMA monoclonal antibody).

[0033] An antibody of the invention “substantially does not recognize” a compound if the IC₅₀ ratio is less than about 15%. In one embodiment, a monoclonal antibody of the invention has an IC₅₀ ratio of less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% for (-)methamphetamine, (-)amphetamine, and (-)MDMA. In another embodiment, a monoclonal antibody of the invention may have an IC₅₀ ratio of less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% for over the counter medications. In yet another embodiment, a monoclonal antibody of the invention may have an IC₅₀ ratio of less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% for non-(+)methamphetamine like prescription medications.

[0034] The invention also encompasses the amino acid and nucleic acid sequences of monoclonal antibodies of the invention. For instance, in one embodiment, the heavy chain of a monoclonal antibody of the invention has the amino acid sequence of SEQ ID NO:25 or SEQ ID NO:19. In another embodiment, heavy chain of a monoclonal antibody of the invention has the nucleic acid sequence of SEQ ID NO:23 or SEQ ID NO:20. In yet another embodiment, the light chain of a monoclonal antibody of the invention has the amino acid sequence of SEQ ID NO:26 or SEQ ID NO:21. In still another embodiment, the light chain of a monoclonal antibody of the invention has the nucleic acid sequence of SEQ ID NO:24 or SEQ ID NO:22.

[0035] An exemplary embodiment of a monoclonal antibody that recognizes each compound of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, but does not substantially recognize (-)methamphetamine, (-)amphetamine, and (-)MDMA is a monoclonal antibody comprising the heavy chain amino acid sequence of SEQ ID NO:25 and the light chain amino acid sequence of SEQ ID NO:26 [i.e. the monoclonal antibody referred to herein as mAb4G9]. Another exemplary embodiment of an antibody that recognizes each compound of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, but does not substantially recognize (-)methamphetamine, (-)amphetamine, and (-)MDMA is a monoclonal antibody comprising the heavy chain amino acid sequence of SEQ ID NO:19 and the light chain amino acid sequence of SEQ ID NO:21 [i.e. the monoclonal antibody referred to herein as mAb2F11].

[0036] The monoclonal antibodies may be murine monoclonal antibodies, human monoclonal antibodies, humanized monoclonal antibodies, chimeric monoclonal antibodies, recombinant monoclonal antibodies, or monoclonal antibody fragments without departing from the scope of the invention. In one embodiment, the invention encompasses murine monoclonal antibodies. Non-limiting examples of murine monoclonal antibodies include mouse and rat monoclonal antibodies. In another embodiment, the invention encompasses human monoclonal antibodies. In yet another embodiment, the invention encompasses humanized monoclonal antibodies. In still yet another embodiment, the invention encompasses chimeric monoclonal antibodies. In an alternative embodiment, the invention encompasses recombinant monoclonal antibodies. Recombinant monoclonal antibodies include antibodies that have been engineered so as to reduce the antibodies' immunogenicity when used as a medication. In another alternative, the invention encompasses monoclonal antibody fragments. Non-limiting examples of such fragments include Fab fragments, Fab' fragments, F(ab')₂ fragments, single chain antigen binding fragments (scFv), disulfide stabilized Fv (dsFv) fragments, single domain antigen binding fragments, and other antibody fragments that maintain the binding specificity of the whole monoclonal antibody but that are less

immunogenic, more cost-effective to produce, or more pharmaceutically effective than the whole monoclonal antibody.

[0037] Monoclonal antibodies may have lambda, kappa, or a recombinant light chain. Additionally, monoclonal antibodies are typically, but not necessarily, IgG antibodies. In certain embodiments, the IgG antibodies may include antibodies from the IgG1, IgG2, IgG3, and IgG4 human antibody classes. In other embodiments, the IgG antibodies may include antibodies from the IgG1, IgG2a, IgG2b, and IgG3 mouse antibody classes.

II. Methods of Making Monoclonal Antibodies

a. hapten compounds for making monoclonal antibodies

[0038] Hapten compounds may be used to elicit monoclonal antibodies that recognize at least one of the compounds selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, but not substantially recognize (-)methamphetamine, (-)amphetamine, and (-)MDMA. In certain embodiments, the hapten compound is designed to generate monoclonal antibodies that recognize at least two compounds from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA. In an exemplary embodiment, the hapten compound is designed to generate monoclonal antibodies that recognize all three compounds of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA. Typically, such a hapten compound will comprise either (+)methamphetamine or (+)amphetamine conjugated to a linker (L).

[0039] In general, L is comprised of atoms and is of a sufficient length so that L is flexible enough to facilitate an orientation of the (+)methamphetamine or (+)amphetamine sufficient to generate desired antibodies. In this context, "desired" antibodies include antibodies that recognize at least one compound selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA. L is also typically not strongly immunogenic. In other words, L may be designed so that antibodies generated against a compound of the invention recognize the compound

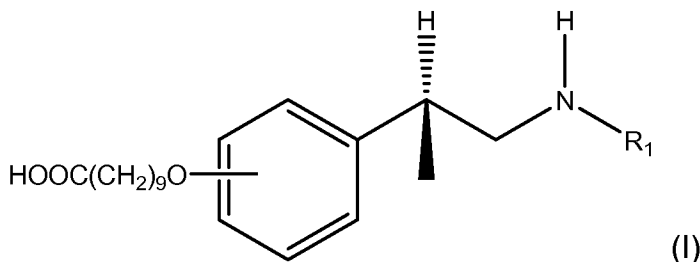
without the need for L to be linked to the compound or present in a subject during treatment.

[0040] The exact length of L can and will vary. Typically, L is at least 9 angstroms long. In one embodiment, L may be from about 9 angstroms to about 27 or more angstroms long. In another embodiment, L is at least 11 angstroms, at least 12 angstroms, at least 13 angstroms, at least 14 angstroms, at least 15 angstroms, at least 16 angstroms, at least 17 angstroms, at least 18 angstroms, at least 19 angstroms, at least 20 angstroms, at least 21 angstroms, at least 22 angstroms, at least 23 angstroms, at least 24 angstroms, at least 25 angstroms, at least 26 angstroms, or at least 27 angstroms. The length of the linker when expressed in angstroms may be determined by performing a modeling study, using, for instance, the MM94 force field. Stated another way, the length of L may be expressed as the number of contiguous atoms forming the shortest path from one substructure that L connects to the other substructure. In one embodiment, L is at least 6 contiguous atoms in length. In another embodiment, L may be from about 8 to about 100 or more atoms in length. In an additional embodiment, L is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more contiguous atoms in length.

[0041] As will be appreciated by a skilled artisan, the atoms comprising L may vary widely. Typically, the atoms impart the appropriate degree of flexibility, as detailed above. Suitable atoms forming L may be selected from the group comprising hydrocarbyl, substituted hydrocarbyls, and heteroatoms. In some embodiments, L may be comprised of amino acids, such as glycine or proline. For instance, L may be a peptide. In other embodiments, L may be comprised of nucleotides. In further embodiments, L may be linear, branched, or may comprise ring structures.

[0042] It is also envisioned that L may be attached to the benzene ring of (+)methamphetamine or (+)amphetamine at a variety of positions without departing from the scope of the invention. For example, in one embodiment, L may be attached at the meta position of the benzene ring. In another embodiment, L may be attached at the ortho position. In yet another embodiment, L may be attached at the para position.

[0043] In an exemplary embodiment, the hapten compound may comprise a compound of formula (I):



wherein:

R_1 is selected from the group consisting of hydrogen and methyl. In some embodiments, R_1 is hydrogen (i.e., forming (+) amphetamine). In other embodiments, R_1 is a methyl group (i.e., forming (+) methamphetamine). In formula (I), L is represented by the $-O(CH_2)_9COOH$ group. Therefore, in one embodiment, the $-O(CH_2)_9COOH$ group may be in the ortho position. In another embodiment, the $-O(CH_2)_9COOH$ group may be in the meta position. In yet another embodiment, the $-O(CH_2)_9COOH$ group may be in the para position. In other exemplary embodiments, the hapten compound may comprise a compound listed in Table 1 or Example 1 herein.

b. immunizing agents comprising hapten compounds

[0044] To elicit an antibody response, immunizing agents comprising hapten compounds may be used. In certain embodiments, an immunizing agent may comprise a hapten compound and an adjuvant. Generally speaking, an adjuvant may be used to increase the immune response to a hapten compound. For instance, an adjuvant may be used to increase antibody affinity, antibody titer, and the duration of the immune response. Non-limiting examples of adjuvants include alum, TiterMax Gold, Ribi, ASO4, Freund's complete adjuvant, and Freund's incomplete adjuvant. In one embodiment, the adjuvant may be alum. In another embodiment, the adjuvant may be TiterMax Gold. In yet another embodiment, the adjuvant may be Ribi. In still another embodiment, the adjuvant may be ASO4. In still yet another embodiment, the adjuvant may be Freund's complete adjuvant. In an additional embodiment, the adjuvant may be Freund's incomplete adjuvant.

[0045] In some embodiments, an immunizing agent may further comprise a pharmaceutically acceptable carrier. Briefly, a pharmaceutically acceptable carrier safely elicits an antibody response in a subject. In this context, safely means that the carrier does not substantially elicit an immune response that cross-reacts with a self-protein, or a regularly ingested protein. Typically, the carrier may be a protein, lipid, carbohydrate, or any combination thereof that is capable of eliciting an immune response. In some embodiments, the carrier is a protein. In a particular embodiment, the carrier may be selected from the group of proteins comprising keyhole limpet hemocyanin (KLH), ovalbumin, bovine serum albumin (BSA), sheep albumin, thyroglobulin, and any modifications, derivatives, or analogues thereof. For instance, in one embodiment, the carrier may be BSA or cationized BSA. In another embodiment, the carrier may be KLH. In yet another embodiment, the carrier may be thyroglobulin.

[0046] In another particular embodiment, the carrier may be a bacterial toxin or toxoid. Non-limiting examples of suitable bacterial toxins or toxoids may include tetanus toxoid, diphtheria toxoid, non-toxic mutant diphtheria toxoid CRM₁₉₇, outer membrane protein complex (OMPC) from *Neisseria meningitidis*, the B subunit of heat-labile *Escherichia coli*, recombinant exoprotein A from *Pseudomonas aeruginosa* (rEPA), cholera toxin B-(CTB), pertussis toxin and filamentous hemagglutinin, shiga toxin, and the LTB family of bacterial toxins.

[0047] In an alternative embodiment, an immunizing agent comprising a hapten compound may further comprise an excipient. Non-limiting examples of excipients include sterile water, salt solutions such as saline, sodium phosphate, sodium chloride, alcohol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycol, gelatin, mannitol, carbohydrates, magnesium stearate, viscous paraffin, fatty acid esters, hydroxy methyl cellulose, and buffer. Other suitable excipients may be used by those skilled in that art.

c. hybridoma methods

[0048] Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal is typically immunized with

an immunizing agent, as described above, to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent.

Alternatively, the lymphocytes may be immunized *in vitro*.

[0049] The immunizing agent will typically comprise a hapten compound capable of eliciting antibodies that recognize at least one of the compounds selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, but not substantially recognize (-)methamphetamine, (-)amphetamine, and (-)MDMA, as described above and as detailed in the examples.

[0050] Generally, the lymphocytes from the host animal immunized with a hapten compound are collected and fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59 103]. Peripheral blood lymphocytes ("PBLs") may be used if cells of human origin are desired, or spleen cells or lymph node cells may be used if non-human mammalian sources are desired. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

[0051] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies

[Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

[0052] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies that recognize at least one compound selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA, but do not substantially recognize (-)methamphetamine, (-)amphetamine, and (-)MDMA. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). In an exemplary embodiment, the binding specificity is determined using the RIA method detailed in the Examples.

[0053] After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

[0054] The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, protein G-Sepharose, hydroxyapatite chromatography, ion exchange chromatography, gel electrophoresis, dialysis, or affinity chromatography.

d. recombinant antibody methods

[0055] The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567. DNA (including cDNA derived from reverse transcription of RNA) encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be

placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Pat. No. 4,816,567] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric antibody. Methods of creating recombinant or chimeric antibodies are well known in the art. See, for instance, Harlow and Lane, *Antibodies: a Laboratory Manual*, Cold Spring Harbor Laboratory (1988).

[0056] The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

[0057] In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

[0058] Monoclonal antibodies and antibody fragments may also be produced in plant expression systems. For more details, see Peterson et al., *The AAPS Journal* 2006; 8(2): E383.

e. human and humanized antibody methods

[0059] The monoclonal antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments

thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin [Jones et al., Nature, 321:522 525 (1986); Riechmann et al., Nature, 332:323 329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593 596 (1992)].

[0060] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522 525 (1986); Riechmann et al., Nature, 332:323 327 (1988); Verhoeyen et al., Science, 239:1534 1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which

some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0061] Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al, *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild et al, *Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995).

[0062] The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

III. *Methods of Using the Monoclonal Antibodies*

[0063] A further aspect of the invention encompasses both therapeutic and non-therapeutic uses for the monoclonal antibodies generated using the methods of section II above.

a. non-therapeutic uses

[0064] In certain embodiments, the monoclonal antibodies may be used in non-therapeutic assays, such as immunostaining, immunoprecipitation, immunoblotting, immunoaffinity purification, immunochromatographic assays, lateral-flow assays and ELISAs. In one embodiment, the monoclonal antibodies may be used for immunostaining. In another embodiment, the monoclonal antibodies may be used for immunoprecipitation. In yet another embodiment, the monoclonal antibodies may be used for immunoblotting. In still another embodiment, the monoclonal antibodies may be used for immunoaffinity purification. In still yet another embodiment, the monoclonal antibodies may be used for ELISAs. In an alternative embodiment, the monoclonal antibodies may be used for immunochromatographic assays. In another alternative embodiment, the monoclonal antibodies may be used for lateral-flow assays. Protocols for each of the above non-therapeutic uses are well known in the art, and may be found, for instance, in Harlow and Lane, Antibodies, Cold Spring Harbor, 1988, Chapters 9-14, U.S. Patent: 5,160,701, U.S. Patent: 5,141,850, U.S. Patent: 5,451,504, U.S. Patent: 5,415,994, and U.S. Patent: 5,559,041, hereby incorporated by reference.

[0065] Additionally, one or more monoclonal antibodies of the invention may be used for detecting (+)methamphetamine, (+)amphetamine, and (+)MDMA in a sample using a non-therapeutic assay described above. For example, one embodiment of the invention is an assay for detecting the presence of at least one compound in a sample selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine. The assay comprises contacting the sample with a monoclonal antibody of the invention and detecting the association of the monoclonal antibody in the sample with at least one compound selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine. In some embodiments, the assay may further comprise quantifying the amount of a compound in the sample selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine.

[0066] A monoclonal antibody may be used to detect and/or quantify at least one compound from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA in a sample. In another embodiment, a monoclonal antibody may be used to detect and/or quantify at least two compounds from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA in a sample. In yet another embodiment a monoclonal antibody may be used to detect and/or quantify each compound of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA in a sample. In an alternative embodiment, more than one monoclonal antibody may be used to detect and/or quantify each compound of the group consisting of (+)methamphetamine, (+)amphetamine, and (+)MDMA in a sample. Methods and devices for using antibodies to detect and/or quantify substances in samples are well known in the art. For instance, see US Patent No. 5,328,828.

[0067] As used herein, "sample" may refer to a biological sample from a subject or a sample of a chemical substance. Non-limiting examples of biological samples from a subject may include fluid samples or tissue samples. Fluid samples may include blood (including serum or plasma), urine, saliva, or other biological fluids that could comprise (+)methamphetamine, (+)amphetamine, or (+)MDMA. Tissue samples may include hair or skin samples, or other biological tissues that could comprise (+)methamphetamine, (+)amphetamine, or (+)MDMA. Methods of collecting biological samples are well known in the art. Non-limiting examples of chemical substances may include powders, pills, and liquids. For instance, a monoclonal antibody of the invention may be used to detect (+)methamphetamine, (+)amphetamine, or (+)MDMA in a powder of unknown composition.

b. therapeutic uses

[0068] Additionally, the monoclonal antibodies may be used for therapeutic purposes. Generally speaking, the monoclonal antibodies may be used to antagonize the effects of (+)methamphetamine, (+)amphetamine, and/or (+)MDMA in a subject. In certain embodiments, the subjects may be using (+) methamphetamine, (+)amphetamine, and/or (+)MDMA. For instance, in one embodiment, the monoclonal

antibodies may antagonize the effects of (+)methamphetamine, (+)amphetamine, and/or (+)MDMA in a subject by decreasing the concentration of (+)methamphetamine, (+)amphetamine, or (+)MDMA in the brain of a subject. In another embodiment, the monoclonal antibodies may be used to decrease drug-seeking behavior in a subject. In yet another embodiment, the monoclonal antibodies may be used to decrease self-dosing behavior in a subject. Methods for each of the above embodiments may be found in the examples.

[0069] In each of the above embodiments, the monoclonal antibodies may be administered passively. Passive administration typically refers to administering at least one monoclonal antibody, generated by a subject, to a second subject, or alternatively, administering at least one monoclonal antibody produced via ex vivo methods to a subject.

[0070] In one embodiment, the invention may encompass a method of treating drug use. The method may comprise administering a monoclonal antibody to a subject, wherein the passive administration decreases the concentration of (+)methamphetamine, (+)amphetamine, or (+)MDMA in the brain of the subject. In another embodiment, the method may comprise administering a monoclonal antibody to a subject, wherein the monoclonal antibody decreases the concentration of two or more of the group consisting of (+)methamphetamine, (+)amphetamine, or (+)MDMA in the brain of the subject. In yet another embodiment, the method may comprise administering a monoclonal antibody to a subject, wherein the monoclonal antibody decreases the concentration of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine in the brain of the subject.

[0071] Alternatively, the method of treating drug use may comprise administering more than one monoclonal antibody to a subject to decrease the concentration of (+)methamphetamine, (+)amphetamine, or (+)MDMA in the brain of the subject. If more than one antibody is administered to a subject, the antibodies may be administered simultaneously or sequentially. In one embodiment, each monoclonal antibody administered to a subject has a different pharmacokinetic profile. For instance, a monoclonal antibody that has a short half-life may be administered together with a

monoclonal antibody that has a longer half-life. In another embodiment, each monoclonal antibody administered to a subject has a different affinity for a particular target compound. For instance, a monoclonal antibody that recognizes (+)methamphetamine may be administered together with a monoclonal antibody that recognizes (+)amphetamine. Alternatively, a monoclonal antibody that recognizes at least one compound selected from the group comprising (+)methamphetamine, (+)amphetamine, and (+)MDMA may be administered with a monoclonal antibody that recognizes another drug of abuse, such as cocaine, phencyclidine, opioids, or nicotine.

c. pharmaceutical compositions

[0072] The monoclonal antibodies of the present invention can be formulated into pharmaceutical compositions and administered by a number of different means that will deliver a therapeutically effective dose. Such compositions may be administered orally, parenterally, by inhalation spray, rectally, intradermally, or transdermally, in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection, or infusion techniques. Formulation of pharmaceutical compositions is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (1975), and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y. (1980).

[0073] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic

mono- or diglycerides. In addition, fatty acids such as oleic acid are useful in the preparation of injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, and polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

[0074] For therapeutic purposes, formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions can be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The compounds can be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

[0075] Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

[0076] The amount of the compound of the invention that can be combined with the carrier materials to produce a single dosage of the composition will vary depending upon the patient and the particular mode of administration. Those skilled in the art will appreciate that dosages may also be determined with guidance from Goodman & Goldman's *The Pharmacological Basis of Therapeutics*, Ninth Edition (1996), Appendix II, pp. 1707-1711 and from Goodman & Goldman's *The Pharmacological Basis of Therapeutics*, Tenth Edition (2001), Appendix II, pp. 475-493. Generally speaking, the dosage of monoclonal antibody may be from about 1 ng/kg subject body weight per month to about 1 g/kg subject body weight per month. In one embodiment, the dosage of monoclonal antibody may be from about 1 mg/kg subject body weight per month to about 125 mg/kg subject body weight per month. In another embodiment, the dosage of monoclonal antibody may be from about 10 mg/kg subject body weight per month to about 150 mg/kg subject body weight per month. In yet

another embodiment, the dosage of monoclonal antibody may be from about 25 mg/kg subject body weight per month to about 100 mg/kg subject body weight per month. In a preferred embodiment, the dosage of monoclonal antibody may be from about 25 mg/kg subject body weight per month to about 40 mg/kg subject body weight per month.

d. kits

[0077] A further aspect of the invention encompasses kits. The kits typically comprise an antibody of the invention described above. The kits may further comprise instructions for detecting and/or quantifying the presence of at least one compound in a sample selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine using the methods described above and in the Examples. The kits may also comprise means for collecting a sample. In some embodiments, the sample may be biological. In other embodiments, the sample may be chemical. For instance, in certain embodiments the kits may be used for detecting and/or quantifying the presence of at least one compound in a hair or blood sample selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine.

DEFINITIONS

[0078] As used herein “(d)” stands for dextrorotatory and “(ℓ)” stands for levorotatory, and refers to the direction in which an enantiomer rotates the plane of polarized light. Herein, (d) is used interchangeably with (+), and (ℓ) is used interchangeably with (-).

[0079] Unless otherwise indicated, the alkyl groups described herein are preferably lower alkyl containing from one to eight carbon atoms in the principal chain and up to 30 carbon atoms or more. They may be straight or branched chain or cyclic and include methyl, ethyl, propyl, isopropyl, butyl, hexyl and the like. Unless otherwise indicated, the alkenyl groups described herein are preferably lower alkenyl containing from two to eight carbon atoms in the principal chain and up to 30 carbon atoms or

more. They may be straight or branched chain or cyclic and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, hexenyl, and the like.

[0080] Unless otherwise indicated, the alkynyl groups described herein are preferably lower alkynyl containing from two to eight carbon atoms in the principal chain and up to 30 carbon atoms or more. They may be straight or branched chain and include ethynyl, propynyl, butynyl, isobutynyl, hexynyl, and the like.

[0081] The terms "aryl" or "ar" as used herein alone or as part of another group denote optionally substituted homocyclic aromatic groups, preferably monocyclic or bicyclic groups containing from 6 to 12 carbons in the ring portion, such as phenyl, biphenyl, naphthyl, substituted phenyl, substituted biphenyl or substituted naphthyl. Phenyl and substituted phenyl are the more preferred aryl.

[0082] The phrase "decreasing the concentration of (+)methamphetamine, (+)amphetamine, or (+)MDMA in the brain" may refer to either decreasing the amount of (+)methamphetamine, (+)amphetamine, or (+)MDMA in the brain, or changing the rate of entry of (+)methamphetamine, (+)amphetamine, or (+)MDMA into the brain.

[0083] The term "hapten" refers to a partial or incomplete antigen. Haptens are protein-free substances that generally are not capable of stimulating antibody formation, but may react with antibodies. Amphetamine, methamphetamine, and their derivatives are haptens.

[0084] The terms "heterocyclo" or "heterocyclic" as used herein alone or as part of another group denote optionally substituted, fully saturated or unsaturated, monocyclic or bicyclic, aromatic or nonaromatic groups having at least one heteroatom in at least one ring, and preferably 5 or 6 atoms in each ring.

[0085] The heterocyclo group preferably has 1 or 2 oxygen atoms, 1 or 2 sulfur atoms, and/or 1 to 4 nitrogen atoms in the ring, and may be bonded to the remainder of the molecule through a carbon or heteroatom. Exemplary heterocyclo groups include heteroaromatics such as furyl, thienyl, pyridyl, oxazolyl, pyrrolyl, indolyl, quinolinyl, or isoquinolinyl and the like. Exemplary substituents include one or more of the following groups: hydrocarbyl, substituted hydrocarbyl, keto, hydroxy, protected

hydroxy, acyl, acyloxy, alkoxy, alkenoxy, alkynoxy, aryloxy, halogen, amido, amino, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[0086] The term "heteroaromatic" as used herein alone or as part of another group denotes optionally substituted aromatic groups having at least one heteroatom in at least one ring, and preferably 5 or 6 atoms in each ring. The heteroaromatic group preferably has 1 or 2 oxygen atoms, 1 or 2 sulfur atoms, and/or 1 to 4 nitrogen atoms in the ring, and may be bonded to the remainder of the molecule through a carbon or heteroatom. Exemplary heteroaromatics include furyl, thienyl, pyridyl, oxazolyl, pyrrolyl, indolyl, quinoliny, or isoquinoliny and the like. Exemplary substituents include one or more of the following groups: hydrocarbyl, substituted hydrocarbyl, keto, hydroxy, protected hydroxy, acyl, acyloxy, alkoxy, alkenoxy, alkynoxy, aryloxy, halogen, amido, amino, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[0087] The terms "hydrocarbon" and "hydrocarbyl" as used herein describe organic compounds or radicals consisting exclusively of the elements carbon and hydrogen. These moieties include alkyl, alkenyl, alkynyl, and aryl moieties. These moieties also include alkyl, alkenyl, alkynyl, and aryl moieties substituted with other aliphatic or cyclic hydrocarbon groups, such as alkaryl, alkenaryl and alkynaryl.

[0088] The term "subject" as used herein refers to a mammal. Suitable mammals may include mice, rats, dogs, non-human primates, and humans.

[0089] The "substituted hydrocarbyl" moieties described herein are hydrocarbyl moieties which are substituted with at least one atom other than carbon, including moieties in which a carbon chain atom is substituted with a hetero atom such as nitrogen, oxygen, silicon, phosphorous, boron, sulfur, or a halogen atom. These substituents may include one or more of the following groups: halogen, carbocycle, carboxy, aryl, heterocyclo, alkoxy, alkenoxy, alkynoxy, aryloxy, hydroxy, protected hydroxy, keto, acyl, acyloxy, nitro, amino, amido, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[0090] As various changes could be made in the above compounds, products and methods without departing from the scope of the invention, it is intended

that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

EXAMPLES

[0091] The following examples illustrate various iterations of the invention.

Example 1. Hapten Design and Antibody Selection.

[0092] When generating monoclonal antibodies (mAb, plural and singular) against small molecules, the chemical composition and molecular orientation of the drug-like hapten on the antigen is a crucial determinant. This is especially important when attempting to discover therapeutic mAb against the drugs of abuse (+)methamphetamine ((+)METH), (+)amphetamine ((+)AMP) and the related compound (+)3,4-methylenedioxymethamphetamine ((+)MDMA, the plus isomer in the racemic mixture known as MDMA or ecstasy). The goal of these studies was to design and synthesize (+)METH-like haptens with structural attributes that would make them effective for generating monoclonal antibodies for treating medical problems associated with these stimulant drugs of abuse.

[0093] For these studies, hapten spacers between (+)METH and the carrier protein were progressively lengthened from 4 to 10 atoms to increase the potential for greater interaction of the hapten with the antibody binding site and/or to increase flexibility of the spacer between the (+)METH backbone structure and the carrier. It was hypothesized that a progressive lengthening and flexibility of the spacer arm would lead to increased affinity and specificity due to increased access to the entire (+)METH-like structure. As a secondary strategy, the location of the linker attachment to the (+)METH structure (e.g., para and meta attachments) was varied in an attempt to elicit antibodies with different conformational selectivity for (+)METH-like compounds.

[0094] Chemicals and Drugs. All chemicals and protein antigens were purchased from Sigma (St. Louis, MO), unless otherwise noted. Enzymes and *E. coli* strains were purchased from Invitrogen (Carlsbad, CA). (+)-[2',6'-³H(n)]-methamphetamine ([³H]-(+METH; 23.5 Ci/mmol) and (±)-[2,6-³H(n)]-amphetamine ([³H]- (±)AMP; 45 Ci/mmol) were obtained from the National Institute on Drug Abuse

(Bethesda, MD) after synthesis at the Research Triangle Institute (Research Triangle Park, NC). Other METH-like drugs used in this study were also obtained from the National Institute on Drug Abuse.

[0095] $[^3\text{H}]$ -(+)-METH was used as sent, but the $[^3\text{H}]$ -(\pm)-AMP was chromatographically separated to obtain $[^3\text{H}]$ -(+)-AMP for use in our studies of (+)-AMP specificity. The separation was performed on a 150 x 4 mm (i.d.) 5 μm CrownPak CR(+) column (Chiral Technologies Inc., Exton, PA). The mobile phase consisted of 0.1 M perchloric acid (Fisher Scientific) containing 10% (v/v) methanol. The column temperature was maintained at 15°C. The flow rate was 1.0 ml/min and the injection volume was 50 μL . Chromatographic peaks were detected using ultraviolet absorption detection at a wavelength of 210 nm. The retention times for $[^3\text{H}]$ -(+)-AMP and $[^3\text{H}]$ -(-)-AMP were 20.1 min and 24.4 min, respectively.

[0096] Haptens and hapten-protein conjugation. Five different stereospecific (+)-isomer (+)-METH-like haptens were synthesized. All haptens were synthesized as HCl salts to aid in solubility, and stored as solids or powders until used. The chemical structures are shown in Table 1. The complete synthesis of one of the haptens ((+)-METH P6) was previously reported (Byrnes-Blake *et al.*, 2001, Int Immunopharmacol 1:329-338). The chemical names and abbreviations of the five haptens are:

- (S)-(+)-4-(3-carboxypropyl)methamphetamine, (+)-METH P4
- (S)-(+)-4-(5-carboxypentyl)methamphetamine, (+)-METH P6
- (S)-(+)-4-(5-carboxypentyloxy)methamphetamine, (+)-METH P06
- (S)-(+)-3-(5-carboxypentyloxy)methamphetamine, (+)-METH M06
- (S)-(+)-3-(9-carboxynonyloxy)methamphetamine, (+)-METH M010

[0097] Each hapten was initially covalently bound to at least 2-3 different protein antigens and used for immunization of mice to test for anti-METH IgG response. The individual mouse and hapten-protein antigen combination that yielded the highest anti-(+)-METH IgG titers was chosen for production of monoclonal antibodies (see details below). The following is a list of the hapten-protein conjugates that produced the mAb listed in Table 1: (+)-METH P4 and (+)-METH P6 conjugated to bovine serum

albumin; (+)METH PO6 and (+)METH MO6 conjugated to Inject Supercarrier Immune Modulator (cationized BSA (cBSA), Pierce Biotech, Rockford, IL); (+)METH MO10 conjugated to ovalbumin (OVA).

[0098] All chemical reactions for covalent binding of the haptens to protein antigens followed the same general procedure. The haptens were first solubilized in either 0.1 M 2-[*N*-morpholino]ethanesulfonic acid buffer (pH 4.5) or dimethylformamide and then adjusted to pH 4.5 with HCl. All haptens were coupled to their respective protein antigens by a carbodiimide reaction using the cross-linker 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (Pierce Biotech). This chemical synthesis forms a peptide bond between the carboxyl group of the hapten linker arm and free amino groups of lysine side chains in the respective proteins. The reactions were conducted with continuous stirring under dark conditions at room temperature for 18 hrs. At the end of the reaction, all antigens were purified as described by Byrnes-Blake *et al.* (2003, Eur J Pharmacol 461:119-128). This purification involved dialysis against distilled water, phosphate-buffered saline (pH 7.4), and a final purification of the soluble fraction on a gel filtration column in phosphate-buffered saline (pH 7.4). Purified antigens were stored at -20°C until needed.

[0099] Immunization, screening, and hybridoma generation. Female BALB/c mice (Charles River Laboratories, Wilmington, MA) were used for all immunizations. For production of the (+)METH P6 mAb, mice were immunized subcutaneously in the hindquarters with 100 µg of the (+)METH P6 antigen emulsified 1:1 (v/v) in TiterMax adjuvant (CytRx Corporation, Norcross, GA) and boosted monthly with 50 µg of the antigen until a favorable titer was reached. For all other antigen immunizations, the mice were initially immunized in the hindquarters subcutaneously with 20-100 µg of antigen emulsified in Freund's complete adjuvant. The initial immunization was followed by a boost with 20-50 µg of antigen emulsified in Freund's incomplete adjuvant three weeks later followed by three boosts at six week intervals, until a favorable titer level was reached. Serum samples were taken via tail bleed periodically to measure anti-(+)METH IgG. Titers were measured by ELISA (enzyme-linked immunosorbant assay) using 96-well microtiter plates coated with the original

hapten conjugated to a different protein. For example, if the original antigen was (+)METH-MO6-cBSA, (+)METH-MO6 conjugated to thyroglobulin was used to avoid selecting carrier protein-reactive antibodies. The screening for anti-(+)METH IgG response was conducted by ELISA or a [³H]-(+METH radioimmunoassay (RIA), using (+)METH and (+)AMP as the inhibitors. After sufficient anti-(+)METH IgG titers were achieved, conventional hybridoma technology was utilized as described previously (Valentine *et al.*, 1994, J Pharmacol Exp Ther 269:1079-1085). The hybridoma fusion partner for mouse B cells was cell line P3X63Ag8.653 (American Type Culture Collection, Manassas, VA). IgG isotype and light chain identity was determined with a mouse antibody isotyping kit (Boehringer Mannheim, Indianapolis, IN).

[0100] Production and purification. Monoclonal antibodies were produced in either a Cell-*Pharm* System 2500 hollow fiber bioreactor (Valentine *et al.*, 1996, J Pharmacol Exp Ther 278:709-716; Unisyn Technologies, Inc., Hopkinton, MA) or in a Biostat B 10 liter bioreactor (Sartorius Corp, Edgewood, NY). All antibodies were harvested and stored at -80°C until purification. mAb were purified either by affinity chromatography using Protein-G Sepharose (Amersham Biosciences, Piscataway, NJ), or ion exchange chromatography using SP Sepharose (Amersham Biosciences, Piscataway, NJ) as described in Hardin *et al.* (1998, J Pharmacol Exp Ther 285:1113-1122), or a combination of the two methods. Following purification, all antibodies were concentrated and buffer exchanged into 15 mM sodium phosphate containing 150 mM sodium chloride (pH 6.5-7.5) as described in McMillan *et al.* (2002, Behav Pharmacol 13:465-473).

[0101] Determination of immunochemical specificity. The cross-reactivity profiles of each mAb for methamphetamine, and structurally related and unrelated compounds, were determined by RIA in a manner similar to that described by Owens *et al.* (1988, J Pharmacol Exp Ther 246:472-478). An IC₅₀ value for inhibition of [³H]-(+METH binding (and [³H]-(+AMP for the mAb generated against the (+)METH MO10 hapten) was determined for each ligand after fitting a sigmoidal curve to the data points. K_D values for mAb were determined by the method of Akera and Cheng (1977, Biochim Biophys Acta 470:412-423).

[0102] Results. For these studies, the hapten linkers were progressively lengthened from 4-10 atoms to increase the potential for greater interaction of the METH-like structures with the antibody binding site and to increase the flexibility of the spacer. It was hypothesized that a progressive lengthening of the spacer arm would lead to increases in affinity due to improved access to the entire METH-like structure; and the different immobilized conformations would elicit antibodies having different conformational selectivity for (+)METH-like compounds.

[0103] The haptens were conjugated to the terminal amino groups of lysines on all carrier proteins by carbodiimide chemistry, which forms a peptide bond with the available carboxylic acid on the hapten. There were 59 lysines in bovine serum albumin, 20 in each of the four subunits of ovalbumin and even more conjugation sites were available on cationized bovine serum albumin (i.e., Imject Supercarrier Immune Modulator). However not all of the lysines or conjugation sites were available at the surface of the protein for coupling to the haptens. Preliminary optimization experiments showed that a ratio of hapten to protein of 30:1 to 90:1 yielded the best incorporation rates for the syntheses. While the hapten incorporation rate for the antigens could not be precisely determined, initial mass spectrophotometry studies indicated that an average of 4 haptens were conjugated to each molecule of protein (data not shown).

[0104] Because the primary goal was to select for high-affinity mAb, the antigen dose was kept relatively low (e.g., 10-20 µg). While immunization with higher hapten-antigen doses (e.g., 50-100 µg) sometimes led to higher titers, the affinity for (+)METH was often too low. Thus a minimum dose of antigen was typically used. This strategy routinely led to immunological response in only 40–70% of animals. In more recent studies, it was discovered that a primary reason for <100% immunological response was the low incorporation rate of the hapten on protein antigens, which in part was overcome by judicious use of Freund's complete and incomplete adjuvants to boost and sustain immunological response.

[0105] Each mouse serum from each group of immunizations was routinely screened (typically 6-10 mice) after each boost to determine the maturity of the immune response and the relative immunochemical characteristics of the polyclonal

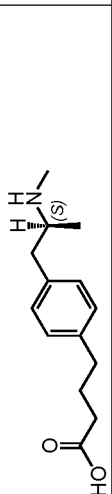
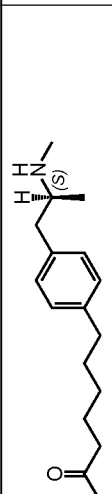
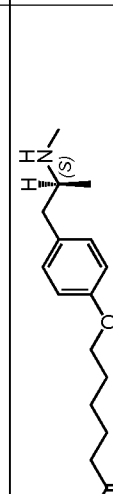
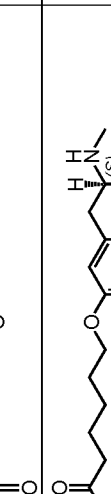
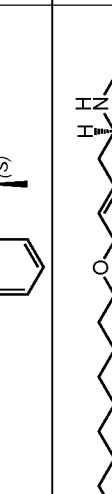
serum (titer, affinity and specificity). For this, a [³H]-(+)-METH RIA was used. The screening assay always involved inhibitions of [³H](+)-METH binding with increasing doses of (+)-METH and (+)-AMP to determine the relative affinities for each ligand. The final choice of a specific mouse for use in generating hybridomas was based primarily on the animal with the highest titer and affinity for (+)-METH. From this process of screening immune serum, 3-10 unique monoclonal antibodies were generally found from each fusion. Most importantly, a polyclonal antiserum that was positive for (+)-AMP was not discovered until the MO10 hapten was used.

[0106] For producing the hybridomas, mice were chosen that had been immunized with Freund's complete adjuvant and boosted with Freund's incomplete adjuvant. The one exception was the immunizations with (+)-METH P6, which used Titermax as the adjuvant. In preliminary optimization experiments, immunizations with alum precipitated antigens, Titermax adjuvant and Ribi's adjuvant were tried on several occasions. While these adjuvants generally produced high titers, it was found that the highest affinity antibodies were generated with Freund's adjuvants.

Example 2. MAb Cross Reactivity Studies.

[0107] After screening over 25,000 potential hybridoma cell lines for mAb production, five mAb with the most favorable immunochemical characteristics were extensively studied for molecular properties and preclinical efficacy (see Table 1). The rest of the hybridoma cell lines were stored frozen in case of future need. The selection of a mAb for more extensive *in vitro* and *in vivo* testing was based on the desire to have a range of affinities, a range of drug specificities, and a high level of mAb production from the parent hybridoma cell line. This final criterion was needed to increase the feasibility of large scale mAb production for *in vivo* testing. In most cases there was one or more similar affinity or specificity mAb that were produced from the same fusion. For instance, the separate fusions that produced mAb6H4 and mAb4G9 (see Table 1) also produced mAb with virtually the same affinity and specificity, but slightly different amino acid sequences. These two particular antibodies were chosen because the parent hybridoma cell line produced significantly more mAb.

Table 1. Chemical Structure of Haptens, the Resulting mAb, and K_D Values for Key Drugs

Hapten Structure	Hapten Name	mAb Name (isotype and light chain)	Key Psychostimulants		
			(+)METH K _D (nM)	(+)AMP K _D (nM)	(+)MDMA K _D (nM)
	(+)METH P4	mAb6H8 (IgG ₁ κ)	250	41,000	106
	(+)METH P6	mAb6H4 (IgG ₁ κ)	11	4000	4
	(+)METH PO6	mAb6H7 (IgG _{2b} κ)	95	47,000	87
	(+)METH MO6	mAb9B11 (IgG ₁ λ)	41	5000	123
	(+)METH MO10	mAb4G9 (IgG _{2b} κ)	34	120 (51 nM with [³ H]-(+)/AMP)	140

Results

[0108] RIA was used to determine the relative affinity and cross-reactivity profile of each mAb (Tables 1 and 2). Only one of five haptens generated mAb with the desired therapeutic potential. Immunization with the MO10 hapten resulted in production of mAb (mAb4G9) with high-affinity binding to (+)METH, (+)AMP, and (+)MDMA; little or no cross-reactivity with (-)METH-like isomers; and no significant cross-reactivity with endogenous compounds or structurally similar common medications (Tables 1 and 2).

Table 2. Characterization of the binding specificities of three important prototype anti-METH/MDMA or anti-METH/MDMA/AMP mAb.

Drug	Antibody Specificity (IC ₅₀ ratio as percent) ^a			
	mAb6H4 (11 nM) ^b	mAb6H8 (250 nM) ^b	mAb4G9 (34 nM) ^b	mAb2F11
(+)METH	100%	100%	100%	100%
(+)AMP	0.1%	2.3%	34%	33%
(+)MDMA	125%	340%	29%	41%
(-)METH	3%	1.1%	10.2%	2.2%
(-)AMP	<0.1%	0.3%	6.3%	5.3%
(-)MDMA	0.7%	1.8%	1.1%	0.6%
(+)MDA	0.1%	2.4%	9.0%	nyd
(-)MDA	<0.1%	<0.1%	0.2%	nyd
4-OH-METH	58.8%	29.4%	10.6%	nyd
(+)pseudoephedrine	<0.1%	1.8%	0.4%	nyd
(+)norpseudoephedrine	<0.1%	<0.1%	<0.1%	nyd
<i>l</i> -phenylphrine	0.1%	<0.1%	<0.1%	nyd
(+)ephedrine	<0.1%	<0.1%	<0.1%	nyd
(+)phenylpropanolamine	<0.1%	<0.1%	<0.1%	nyd
β-phenylethylamine	<0.1%	<0.1%	0.1%	nyd
tyramine	<0.1%	<0.1%	<0.1%	nyd
dopamine	<0.1%	<0.1%	<0.1%	nyd
norepinephrine	<0.1%	<0.1%	<0.1%	nyd
serotonin	<0.1%	<0.1%	<0.1%	nyd
epinephrine	<0.1%	<0.1%	<0.1%	nyd

^a IC₅₀ ratio = (RIA IC₅₀ value for METH/RIA IC₅₀ value for test ligand).
See Table 1 for the structures of the haptens used to generate these antibodies.
^b IC₅₀ value for METH binding from Table 1.
nyd: value not yet determined

[0109] Since mAb4G9 was the only of the chosen five mAb to significantly cross-react with (+)AMP (Tables 1 and 2), its affinity for (+)AMP was examined in more detail. For this, a RIA analysis was conducted using [³H]-(+AMP) (in addition to a RIA with [³H]-(+METH) and AMP as the inhibitor. These data showed the actual affinity for AMP was 51 nM (Table 1), demonstrating that this mAb has virtually the same K_D value for AMP and METH. [³H]-(+MDMA) was not available for determining a more accurate K_D value for (+)MDMA binding, but it seems likely that the true K_D value would be significantly lower than the value indicated by MDMA inhibition of [³H]-(+METH) binding in the RIA.

[0110] Attaching the linker of the hapten distal to the chiral center of the molecule yielded a refined specificity for (+)-isomers (Table 2). The relatively short length of spacer arms of haptens (+)METH P4 and (+)METH P6 (4- and 6-carbon linkers, respectively), coupled with attachments at the para-carbon of the (+)METH phenyl ring (Table 1), hindered the flexibility of haptens. This likely forced the immune system to recognize the presence of the methyl group on the nitrogen molecule of (+)METH and (+)MDMA and its absence in (+)AMP. Thus, mAb affinity was high for (+)METH and (+)MDMA, but low for (+)AMP. The hapten (+)METH PO6, like (+)METH P6, was designed with a linker attached to the para-carbon of the phenyl ring, but an oxygen was included to influence localized charge and solubility and mimic the presence of one of two oxygen atoms at the para and meta positions of the methylenedioxy group of (+)MDMA (Table 1). An oxygen attached to the phenyl ring structure was included in two other haptens, (+)METH MO6 and (+)METH MO10, but linkers were attached to the meta-carbon of the phenyl ring of (+)METH. This strategy was designed to present the oxygen of the (+)MDMA-like structure along the same spatial plane as the (+)METH molecule's chiral center. The longer (+)METH MO10 spacer was used to allow more flexibility in the hapten on the protein in hopes of discovering mAb with broader recognition of (+)METH-like structures. These combined strategies resulted in the best balance of affinity and specificity.

[0111] From these studies, it was learned that 1) linkers located distal to the chiral center of this very small molecule favor generation of stereospecific

antibodies, 2) a longer flexible linker arm like (+)METH MO10 favors generation of antibodies with broader selectivity for (+)METH-like compounds, and 3) spacers ≥ 6 atoms produce higher affinity mAb. Importantly, discovery of mAb4G9 was not an isolated event, as other MO10-derived mAb with similar specificities for (+)METH and (+)AMP have since been discovered.

Example 3. Antibody Sequence Analysis.

[0112] To gain a better molecular understanding of how the primary amino acid sequence affected mAb affinity for (+)METH, related and unrelated sequence features in each mAb variable region was analyzed. Three of the mAb were IgG1 subclass and two were IgG2 (Table 1). Except for anti-METH/MDMA mAb9B11 (λ light chain), all of the mAb possessed a κ light chain.

[0113] cDNA cloning and sequencing of mAb. For these studies, five prototype anti-METH mAb ranging in METH affinities from 11 to 250 nM were analyzed (Table 1). A single prototype mAb resulting from each of the haptens was chosen for detailed studies. The light chain (LC, singular and plural) cDNA of the mAb were cloned by RT-PCR using Superscript II reverse transcriptase (Invitrogen) with an exact reverse primer matching the C-terminus of the LC named MLEND1. Not (5'-GGG GCG GCC GCG CGT CTC AGG ACC TTT GTC TCT AAC-3') (SEQ ID NO:1). The LCs of mAb6H4, mAb6H8, and mAb6H7 were amplified in the forward direction with the degenerate primer ML2, and the LC of mAb4G9 was amplified in the 5' direction with the degenerate primer ML4 (Coloma *et al.*, 1992). The LC of mAb9B11 was amplified in the forward direction with the primer sequence 5'-ATGGCCTGGA(T/C)TTCACCTTATACTCTCTCTCCTGGCTCTC-3' (SEQ ID NO:2). The resulting cDNA was blunt-ligated into the *Sma* I site of the cloning vector pGEM-3Z.

[0114] The heavy chain cDNA of all IgG1 (from (+)METH P4, (+)METH P6 and (+)METH MO6) mAb were amplified using RT-PCR as described above with an exact reverse primer to the C-terminus of the heavy chain, named MHEND. NotI 5' GGG GCG GCC GCA GGG CTC CAA GGA CAC TGG GAT CAT TT 3' (SEQ ID NO:3), and a mixture of three degenerate primers based on the MHALT primers from Coloma *et al.*

(1992, J Immunol Methods 152:89-104). The primers were modified from the originally published sequence only by the substitution of a Nhe I restriction site for the original restriction site. The IgG2 mAb (from (+)METH PO6 and (+)METH MO10) were amplified with the reverse primer 5'-CTCCCGGTCTCCGGGTAAATGA-3' (SEQ ID NO:4).

[0115] The forward sequence of the heavy chain of mAb6H8 was amplified with primer MHALT1 (Coloma *et al.*, 1992, J Immunol Methods 152:89-104). The forward primers for mAb6H4, mAb6H7, mAb9B11, and mAb4G9 were designed from the results of N-terminal sequencing of the mature proteins (see Fig. 1A and B for protein sequences). The primer sequences used were: 5'-GAGTGCAGCTTCAGGAGTCAGGACCTAGC-3' (SEQ ID NO:5) for mAb6H4, 5'-GATGTAAACTTCAGGAGTCAGGACCTGGCCTCGTGAAACCTTCTCAGTC-3' (SEQ ID NO:6) for mAb6H7, 5'-GAGGTGCAGCTTCCGGAGTCAGGACCTAGC-3' (SEQ ID NO:7) for mAb9B11, and 5'-GAGTACCAGCTCCAGCAGTCTGGGAC-3' (SEQ ID NO:8) for mAb4G9. The cDNA was then blunt-ligated into the *Sma* I site of cloning vector pGEM-3Z. The resulting plasmids of all mAb cloning were transformed into *E. coli* strain DH5 α and sequenced at University of Arkansas for Medical Sciences DNA Core Sequencing Facility.

[0116] All sequences were submitted to the GenBank database. The GenBank-assigned accession numbers for the following antibodies are listed here in parentheses (light chain, heavy chain): mAb6H8 (DQ381551, DQ381550), mAb6H4 (DQ381543, DQ381542); mAb6H7 (EF392838, EF392837), mAb9B11 (EF392840, EF392839) and mAb4G9 (EF392842, EF392841). The germ-line usage of the different mAb was determined by comparing the DNA sequences to those in the IMGT database using the web-based program V-QUEST tools (Internet address: <http://imgt.cines.fr>) and by visual examination of the sequences (Giudicelli *et al.*, 2004, Nuc Acid Res 32:W435-440).

[0117] Results. Alignments of the amino acid sequences of the variable region of the mAb are presented in Fig. 2. An analysis of complementarity determining regions revealed a high degree of diversity in both composition and length. The first light chain CDRs (L1) varied in length from 10-14 residues, and with the exception of

mAb4G9, possessed a large number of serine residues (Fig. 2B). The only conserved residue in L1 CDR, or any of the light chain CDRs, was the serine at position L26. The L2 CDRs were 7 residues in length except for mAb6H7, which possessed only 5 amino acids. The L3 CDRs were all 9 residues in length except mAb4G9 which had 10 residues. The CDRs of the heavy chains (Fig. 2A) exhibited similar lengths in the H1 and H2 CDRs, but little homology. The H1 CDR had a conserved threonine at position H30 and either a tryptophan or tyrosine at position H33. The H3 CDRs differed in length from 8-16 residues. Although not immediately apparent from the alignment, all H3 regions possessed two tyrosine residues spaced five residues apart, with the second tyrosine before the tryptophan at H103.

[0118] While comparisons of CDR sequences are important, differences in CDRs can be attributed to differences in germ-line sequences of particular V-region genes, and to somatic mutation within the CDRs of these V-region genes. To better understand the relative importance of the germ-line and somatic mutations, the sequenced genes were analyzed using the IMGT database (Giudicelli *et al.*, 2004, Nuc Acid Res 32:W435-440). The analysis showed that each antibody was unique and not clonal. That is, rather than coming from one germ-line gene arrangement early in B cell development, they resulted from unique V(D)J recombination events. These unique germ-line gene rearrangements then underwent somatic DNA mutations, that were often silent, but some resulted in amino acid changes that differed from the original germ-line gene. Thus, no clear pattern of response was found.

[0119] This sequence analysis elucidated unique sequence differences in the antibody CDRs. A common feature was a conserved proline at position 95 or 95a of all L3 CDR regions, except for mAb9B11 (Fig. 2B), which had a serine residue. Because of their ability to form "hinges," proline residues often lend flexibility in main chain protein sequences. This proline/serine was immediately followed by either a hydrophobic amino acid (*i.e.* leucine or valine as in mAb6H4 and mAb9B11, respectively) or an aromatic residue. It is possible that these residues could be important for interaction with the phenyl ring of (+)METH-like compounds via

hydrophobic or pi-pi interactions, and the preceding proline could lend flexibility to adapt to different conformations.

Example 4. Molecular Modeling and Docking.

[0120] Based on the results of the primary sequence alignment, three mAb (mAb6H4, mAb6H8 and mAb4G9) were chosen for structural modeling. Each CDR was assigned and given a canonical classification (Al-Lazikani *et al.*, 1997, J Mol Biol 273:927-948), except for the H3 CDRs, which do not possess canonical classes.

[0121] IgG variable region structural modeling and analysis. Molecular modeling of the three dimensional structure of the variable regions of three of the mAb was performed using the WAM antibody modeling algorithm (Whitelegg and Rees, 2000, Protein Eng 13:819-824). mAb6H4, mAb6H8, and mAb4G9 were chosen for more detailed analysis because they exhibited the full range of affinities for (+)METH and a broad range of ligand specificities for other important METH-like drugs. The primary amino acid sequences of the variable regions of the HC (heavy chain) and LC were first submitted to the WAM antibody modeling site for alignment. The program aligned the sequences against known sequences in the database and searched for canonical classes of complementarity determining regions (CDR). Based on these classifications, the program assigned a 3-dimensional structure to the framework regions and CDRs by fitting the main chain to that of the closest known structures.

[0122] Ligand docking. For docking simulation, the FlexX (Tripos) program was used. First, a deep pocket was identified at the interface of the CDRs from surface modeling and electrostatic calculations in Pymol (Delano Scientific, San Carlos, CA) and Sybyl (Tripos). To define this region as a putative active site, residues within an area 6 Å around F L94 (for mAb6H4) or Y L94 (for mAb4G9) were selected. The METH ligand was assigned formal charges by Sybyl and the molecule was allowed partial flexibility. The program was set to find the 30 best docking conformations and return these in a consensus scoring table.

[0123] Results. The three-dimensional models exhibited classical antibody β -sheet fold conformation (Fig. 3). In general, all models showed conformity with

geometrical constraints throughout the structures. The analysis indicated that less than 2% of residues had main chain Ψ and Φ angles in outlier regions. All three models appeared to conform reasonably well to known protein structural features and constraints, and they presented an appropriate foundation to conduct base docking analysis.

[0124] All CDRs fell within canonical classes except L3 of mAb4G9 and the H3 CDRs, which do not have canonical classes. The H3 CDRs of all three antibodies were predicted to form a kinked or "hairpin," rather than extended conformation. Comparison of the models revealed conserved structural elements and some potentially important differences in the root mean square deviations (RMSD) of the CDR loop configurations (Fig. 3). The loop structure of mAb6H4 was arbitrarily chosen as a reference point to compare the differences from the other two antibodies, because it had the highest affinity for (+)METH. The L2 CDRs of all three antibodies occupied nearly the same spatial positions. The L3 regions of mAb6H4 and mAb6H8 were very similar, even though they differed in affinity for (+)METH by about 25-fold.

[0125] Based on the modeling results, docking simulation was performed with mAb6H4 and mAb4G9. According to the models (Fig. 4), a deep pocket was formed by the interaction of the H1, H2, L1 and L3 CDR loops for both antibodies, with a wider pocket formed in the binding region of mAb4G9 due to a shorter H3 CDR. A theoretical docking of (+)METH was created into these mAb pockets and identified residues within 8 angstroms of the ligand as possible sites for ligand-mAb interaction (Fig. 4). The results of this FlexX-based docking indicated that the METH molecule was generally oriented with the hydrophobic phenyl group toward the interior of the pocket. In mAb6H4 and mAb4G9, the charged nitrogen of METH was in close proximity to a histidine at position L32 and H35 respectively.

[0126] Based on the molecular modeling analyses, the interface between (+)METH and the mAb was relatively small, (the surface area of (+)METH is 174 \AA^2) with small shifts in protein conformation producing large changes in binding. As can be seen in Fig. 3, the most striking deviations appeared in the H3 CDR region, with over 6 Å and 7 Å RMSD in mAb6H8 and mAb4G9, respectively. The diversity in the positions

of the CDR suggests that each of these antibodies exhibited a binding paradigm to (+)METH-like drugs that was somewhat independent of loop configuration. The surface rendering of the models exposed a deep pocket at the CDR interface of mAb6H4. This pocket appeared to be approximately the size of (+)METH and would likely accommodate docking of the ligand. By contrast, the potential binding pocket of mAb4G9 was wider and shallower. It is hypothesized that the longer linker arm of (+)METH MO10 combined with the changed dihedral angle of an oxygen at the meta position of the phenyl ring contributed to the formation of a larger pocket. Analysis indicated that only five of the six CDR loops, might be directly involved in binding of (+)METH-like drugs, with L2 showing little contact. The (+)METH docking simulation with FlexX indicated that the potential binding pockets were dominated by aromatic residues with some capable of making hydrogen bonds (i.e., histidine and tyrosine).

Example 5. Anti-(+)METH mAb Alter (+)METH Pharmacokinetics in Rats.

[0127] The ability of anti-(+)METH mAb6H4 (generated against (+)METH P6 hapten, see Table 1) to alter (+)METH brain concentrations was examined in two different models of (+)METH abuse (Byrnes-Blake *et al.*, 2005, *Eur J Pharmacol* 521:86-94).

[0128] The overdose model was designed to mimic a drug abuser taking a high *iv* (+)METH dose and treated with (+)METH mAb in the emergency room. In this model, rats received 1 mg/kg (+)METH (*iv*) followed 30 min later by an anti-(+)METH mAb dose. The mAb pretreatment model was designed to mimic an abuser in drug treatment administered an anti-(+)METH mAb medication at the start of behavioral modification therapy who relapses to (+)METH use. In this model, rats were pretreated with anti-(+)METH mAb6H4 and received a 1 mg/kg *iv* (+)METH dose the following day. This dose (without mAb) produced about 2.5 hrs of locomotor effects. Rats (3/time point) were sacrificed at varied times after (+)METH administration to determine (+)METH brain concentrations. mAb6H4 decreased (+)METH brain concentrations in both models. Indeed, (+)METH brain concentrations in both models were virtually superimposable at comparable times after 30 min—the time of mAb administration in

the overdose model. Both studies clearly show that antibodies against (+)METH can significantly reduce (+)METH brain concentrations over time.

[0129] Next, the “functional” half-life of each of the afore-mentioned anti-(+)METH mAb (see Table 1) was determined. This “functional” assay compared (+)METH concentrations in the absence and presence of mAb. By this measure, the best antibodies are those with the highest and longest increases in serum (+)METH and (+)AMP concentrations. First, it was determined that the pharmacokinetic properties of the mAb were not different (results not shown). For instance, they all had a serum half-life of about 7–8 days, which ruled out the possibility that one or more of them were quickly cleared and thus inactivated through elimination. It also showed the potential to produce a long-acting anti-(+)METH therapy by passive or active immunization.

[0130] To conduct the “functional” studies, male rats (n=4/group) were given 14-day continuous (+)METH infusions at 5.6 mg/kg/day by sc osmotic minipumps. After achieving steady state (+)METH concentrations (at 24 hrs), each rat was treated with a dose of mAb that was equimolar in binding sites to the steady-state body burden of (+)METH. Only a single dose of mAb was administered at this time point, but (+)METH was continuously infused at a rate of 50% of the body burden per hour to maintain a (+)METH steady state. Serum samples were collected pre-mAb and at time points after mAb administration. All anti-(+)METH mAb caused significant acute increases in serum (+)METH concentrations compared with pre-mAb controls. However, there were substantial differences in serum (+)METH concentration vs. time curves for the five mAb (Fig. 5, open symbols). Most anti-(+)METH mAb appeared to be partially inactivated to differing degrees over time, as judged by their inability to maintain high concentrations of (+)METH in serum over time. This inactivation was particularly striking for the highest affinity mAb6H4 ($K_D = 11$ nM). However, mAb4G9 ((+)METH and (+)AMP, $K_D = 34$ and 51 nM, respectively) was still very effective after about 2 wks. It was also the only mAb that maintained significantly increased concentrations of (+)AMP (closed circles) and (+)METH (open circles) over time compared to pre-mAb concentrations (square symbols with “A” and “M” inside).

[0131] It was originally hypothesized that mAb affinity was the primary driving force for therapeutic efficacy. However, these studies revealed that the duration of action and function of the anti-(+)METH mAb *in vivo* was decreased, while the mAb protein pharmacokinetics were not different (similar $t_{1/2}$, clearance, and volume of distribution values). This was unanticipated. The first generation of haptens (*e.g.*, (+)METH P6 and (+)METH P4) were purposely designed to produce mAb specific for (+)METH, with virtually no cross reactivity with (+)AMP. When the second generation of haptens (*e.g.*, (+)METH MO10) were produced, it was discovered that the resulting mAb, with specificity for (+)METH and (+)AMP, had the clinically important advantages of increased duration of action and efficacy.

[0132] For instance (see Figure 7 and 8) mAb4G9 substantially alters the disposition of AMP, unlike mAb6H4 (generated by the P6 hapten). As shown in Fig. 8, mAb4G9 reduces both METH and AMP concentrations in the brain, which is medically important. It also repartitions AMP into the serum from other compartments (Figure 7). mAb6H4 has minimal or no effect on AMP in either brain or serum because it is not broadly specific *in vivo*.

[0133] Furthermore, mAb4G9 has a longer functional half-life than mAb generated by previous haptens. Figure 7 shows mAb6H4 has significantly less *in vivo* functionality (increased serum levels of METH/AMP is an indication of activity) by the 24 h timepoint. In contrast, mAb4G9 maintains significantly elevated serum METH and AMP concentrations 24 h after administration, indicating extended *in vivo* functionality.

Example 6. Behavioral Assays

[0134] The *in vivo* effects of mAb4G9 were examined using a drug discrimination model. mAb4G9 appeared to be an effective and long-lasting antagonist of METH effects in this model (Figure 6A). The mAb4G9 (600 mg/kg) shifted the dose response curves for at least 7 days. On the first and second day after mAb treatment, the dose response curve was shifted about 4- to 6-fold. When tested again at 7 days after mAb4G9 treatment, the dose response curve was still shifted about 2.5 fold.

[0135] We also tested the ability of mAb4G9 to block (+)AMP self-administration in a model in which rats responded under a fixed interval (60 sec) paradigm for a dose of 0.03 mg/kg (+)AMP per infusion. As shown in Figure 6B, the number of (+)AMP injections per session was significantly reduced after mAb4G9 administration (100 mg/kg). Thus, mAb4G9 dramatically reduced responding for (+)AMP. These studies suggest that a single mAb could help prevent relapse to (+)METH and/or (+)AMP use.

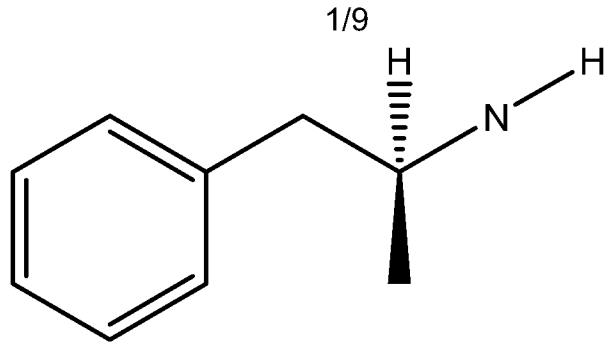
CLAIMS

What is claimed is:

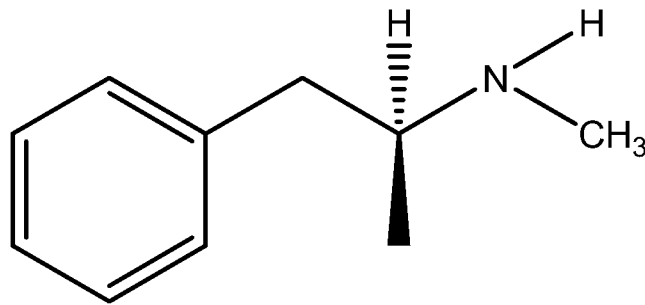
1. A monoclonal antibody that recognizes each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine.
2. The monoclonal antibody of claim 1, wherein the antibody does not substantially recognize each compound of the group consisting of (-)amphetamine, (-)methamphetamine, and (-)3,4-methylenedioxymethamphetamine.
3. The monoclonal antibody of claim 1, wherein the antibody has an IC₅₀ ratio of greater than about 20% for each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine.
4. The monoclonal antibody of claim 3, wherein the antibody has an IC₅₀ ratio of less than about 12% for each compound of the group consisting of (-)amphetamine, (-)methamphetamine, and (-)3,4-methylenedioxymethamphetamine.
5. The monoclonal antibody of claim 1, wherein the antibody has an IC₅₀ ratio of greater than about 25% for each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine.
6. The monoclonal antibody of claim 5, wherein the antibody has an IC₅₀ ratio of less than about 12% for each compound of the group consisting of (-)amphetamine, (-)methamphetamine, and (-)3,4-methylenedioxymethamphetamine.

7. The monoclonal antibody of claim 1, wherein the heavy chain of the antibody comprises the amino acid sequence of SEQ ID NO:25, and the light chain of the antibody comprises the amino acid sequence of SEQ ID NO:26.
8. The monoclonal antibody of claim 1, wherein the heavy chain of the antibody comprises the amino acid sequence of SEQ ID NO:19, and the light chain of the antibody comprises the amino acid sequence of SEQ ID NO: 21.
9. The monoclonal antibody of claim 1, wherein the antibody is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a chimeric antibody, a recombinant antibody, and an antibody fragment.
10. A method of treating drug use in a subject, the method comprising administering a monoclonal antibody of claim 1 to the subject, wherein the monoclonal antibody decreases the concentration of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine in the brain of the subject.
11. The method of claim 10, wherein the subject is selected from the group comprising a rodent, a non-human primate, and a human.
12. An assay for detecting the presence of at least one compound in a sample selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine, the assay comprising contacting the sample with a monoclonal antibody of claim 1 and detecting the association of the monoclonal antibody in the sample with at least one compound selected from the group consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine.
13. The assay of claim 12, wherein the sample is a biological sample or a chemical sample.

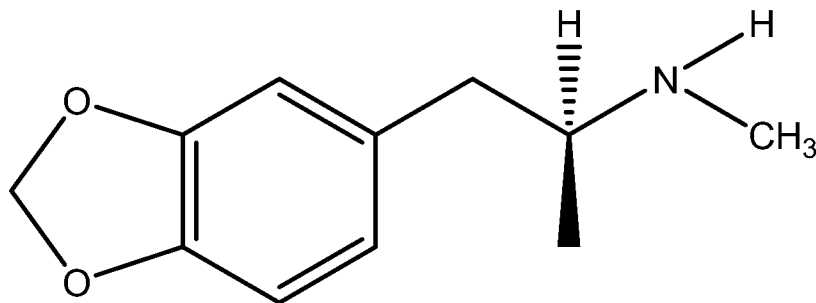
14. The assay of claim 13, wherein the biological sample is a biological fluid.
15. The assay of claim 12, wherein the association is detected by a means selected from the group consisting of ELISA, immunochromatographic assay, and immunoprecipitation assay.
16. The assay of claim 12, wherein the assay further comprises quantifying the amount of a compound selected from the group of compounds consisting of (+)methamphetamine, (+)amphetamine, and (+)3,4-methylenedioxymethamphetamine in the sample.
17. A kit, wherein the kit comprises a monoclonal antibody that recognizes each compound of the group consisting of (+)amphetamine, (+)methamphetamine, and (+)3,4-methylenedioxymethamphetamine.



(+) Amphetamine



(+)Methamphetamine

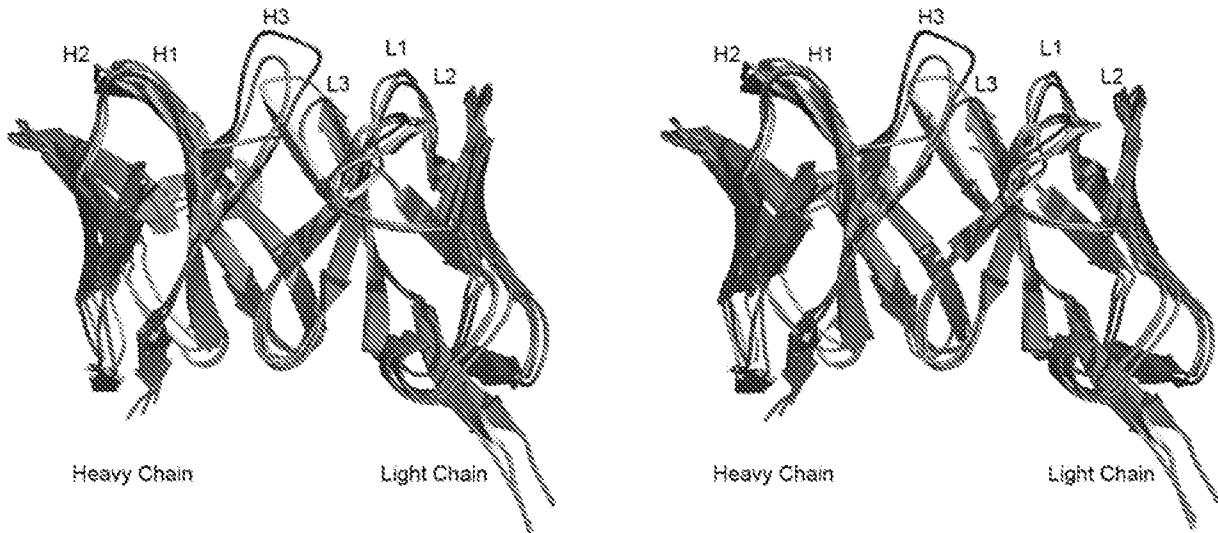


(+)-3,4-Methylenedioxyamphetamine

FIG. 1

mAb	KD	(nM)	FR1	CDR H1	FR2
6H8	250		QVQLQQPGAE L V K P G A S M K L S C K A S G Y T F T S F W M H - W V K Q R P G Q G L E W I G E		
6H4	10		E V Q L Q E S G P S L V K P S Q T L S L T C S V T G D S V T S G Y W S - W I R Q F P G N K L D Y M G Y		
6H7	41		D V K L Q E S G P G L V K P S Q S L S L T C S V T G S S I T S A Y Y W N W N R Q F P G N K L E W M G Y		
9B11	41		E V Q L P E S G P G L V A P S Q S L S I T C T V S G F S L T D G Y G V N W V R Q P P G K G L E W L G M		
4G9	40		E Y Q L Q Q S G T V L A R P G A S V K M S C K A S G Y T F T S Y W M H - W V K Q R P G Q G L E W I G G		
6H8	250		INPSNGRNK Y N E K F K N K A T L T V D K S S S T A Y M Q L S S L T S E D S A V Y Y C T R D S F		
6H4	10		I S - Y R G S T Y N P S L K S R I S I T R D T S K N Q V Y L Q L K S V S S E D T A T Y Y C S Y F D S		
6H7	41		I R Y D G Y N N - Y N P S L K N R I S I T R D T S K N Q F F L K L N S V T T E D T A T Y Y C A R D D Y		
9B11	41		I W - D D G D T D Y S S V L K S R L S I T K D N S K N Q V F L K M N R L Q T D D T A R Y F C A R D T L		
4G9	40		I Y P G N S D T T Y N Q K F K G K A K L T A V T S T S T A Y M E L S S L T N E D S A V Y Y C L Y G N Y		
6H8	250		GGSYDGFYSMDYWGQGTSVTVS		
6H4	10		DD-YAMEY- - -WGQGTSTVTVS		
6H7	41		DEAY- - - -WGQGTLLVTVS		
9B11	41		YTSYAMDY- - -WGQGTSTVTVS		
4G9	40		DFDY- - - -WGQGTLLTVS		

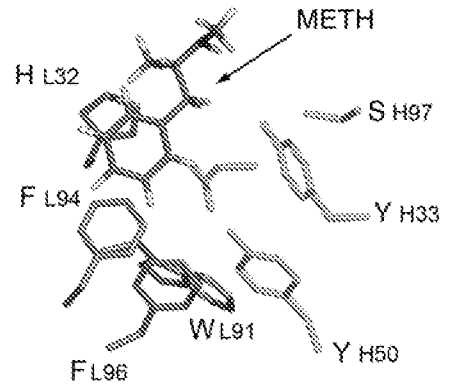
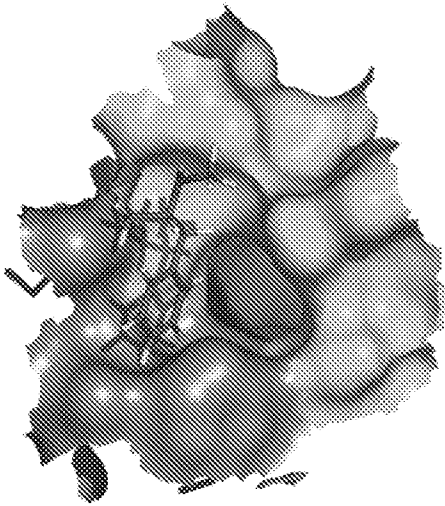
FIG. 2A



mAb Name	CDR Regions and RMSD (Å) from mAb6H4					
	L1	L2	L3	H1	H2	H3
mAb6H8	2.27	0.84	0.67	1.85	3.36	6.67
mAb4G9	2.44	0.88	3.72	1.39	3.59	7.22

FIG. 3

mAb6H4



mAb4G9

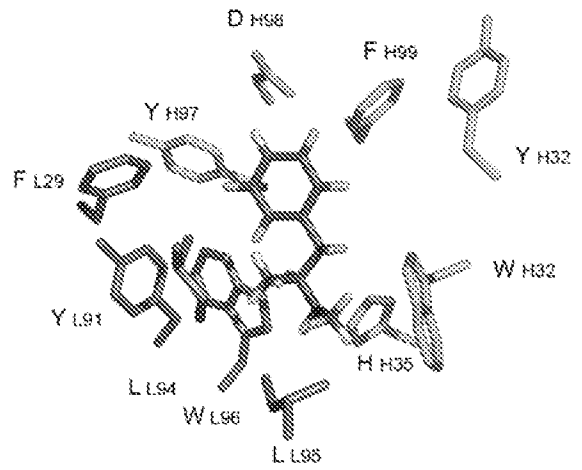
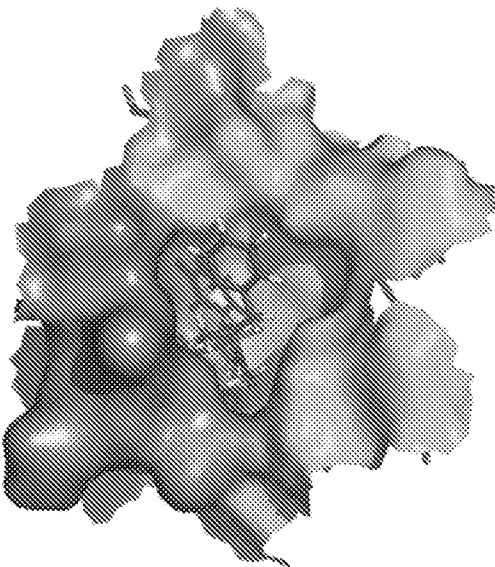


FIG. 4

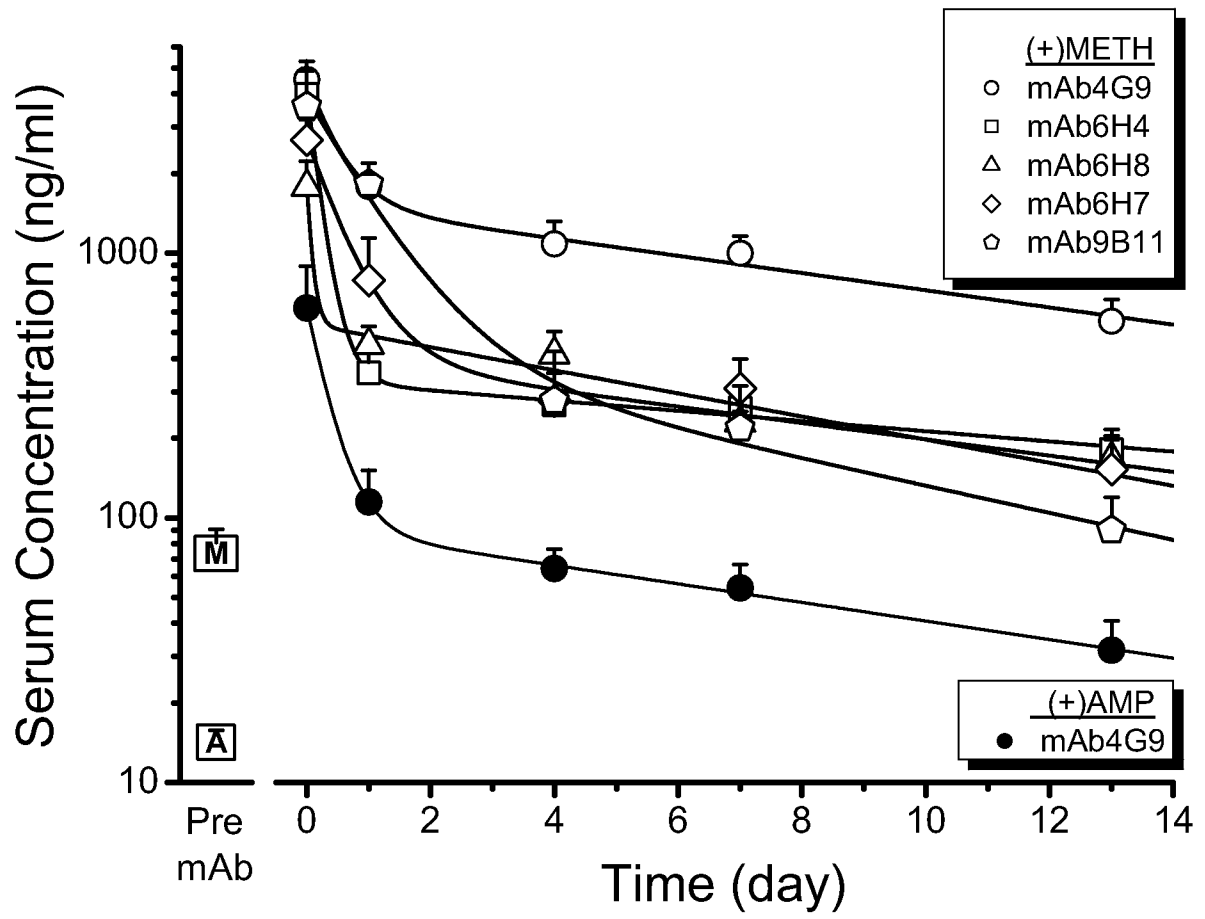


FIG. 5

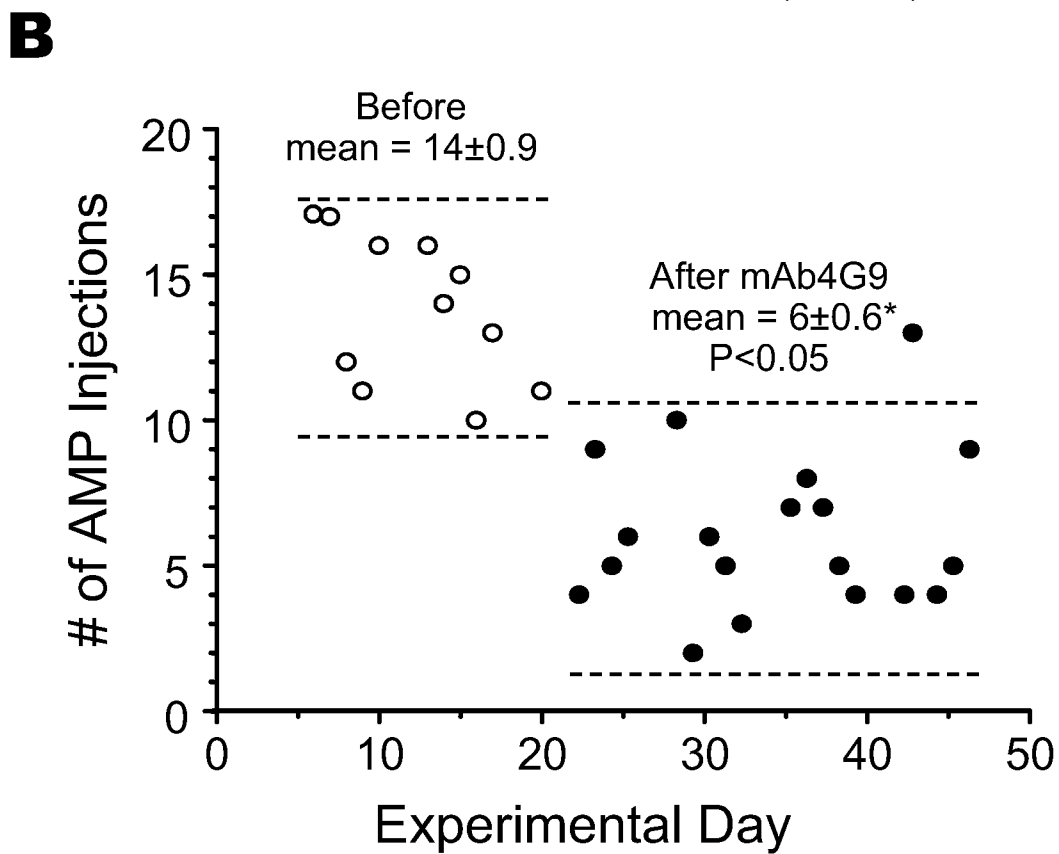
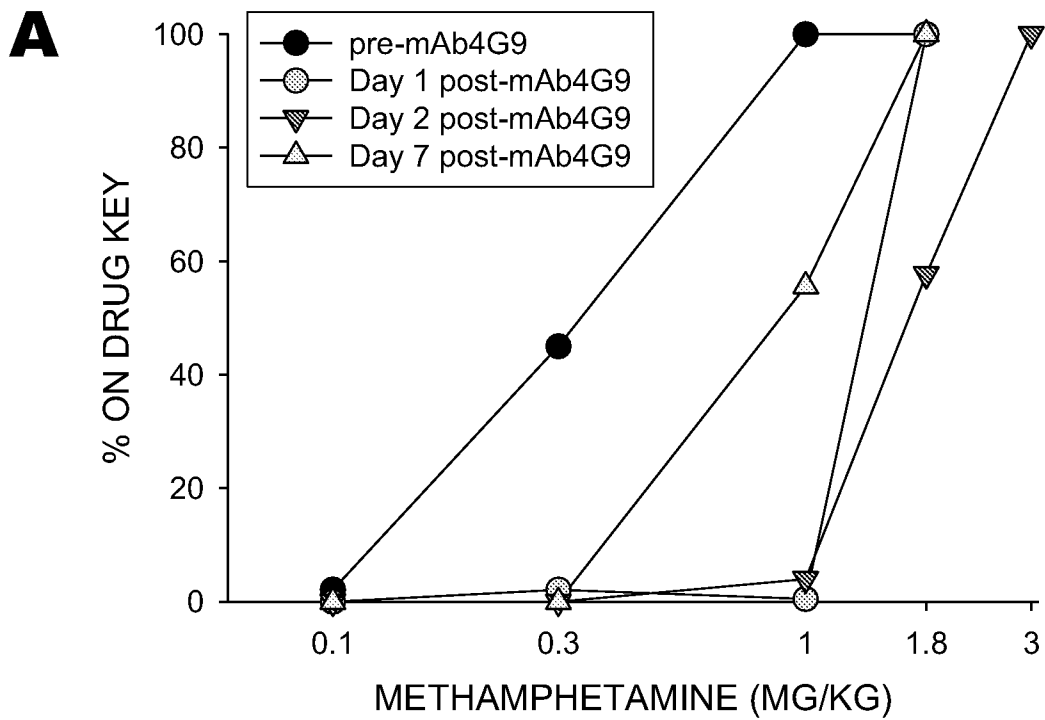
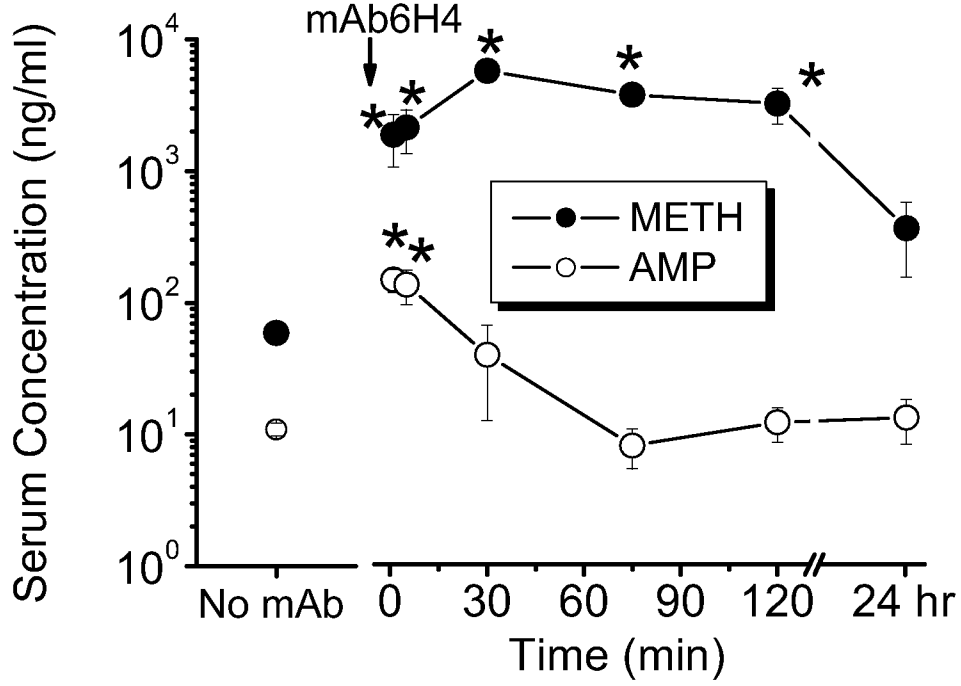


FIG. 6

A



B

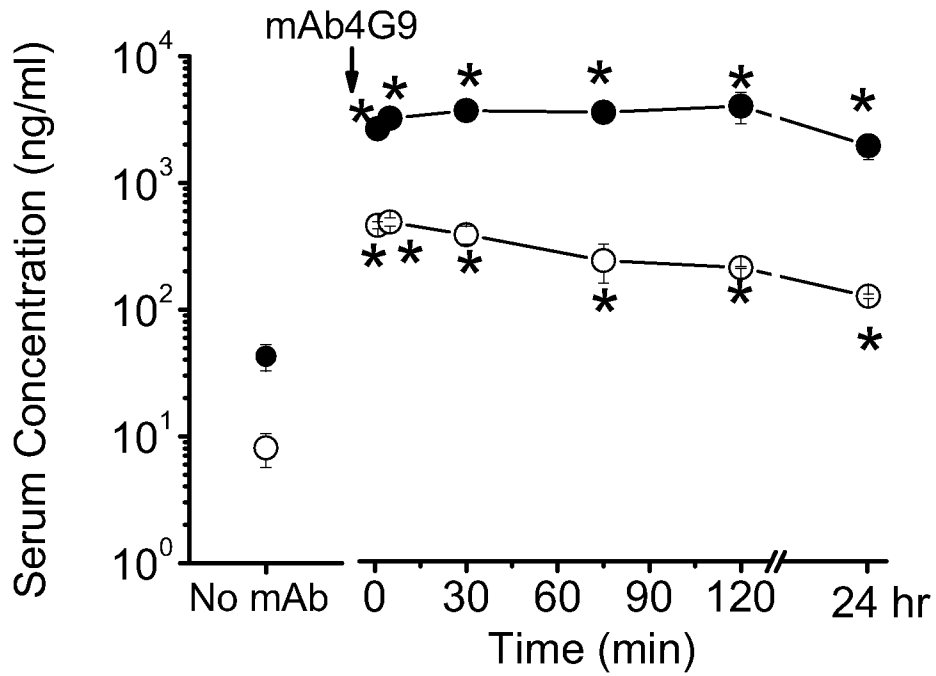
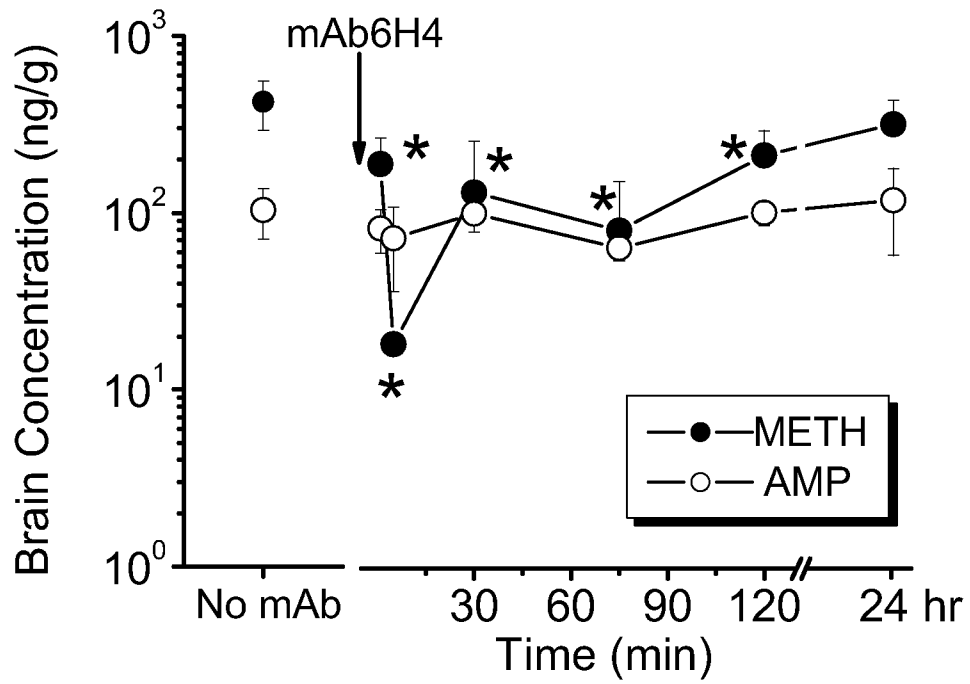


FIG. 7

A



B

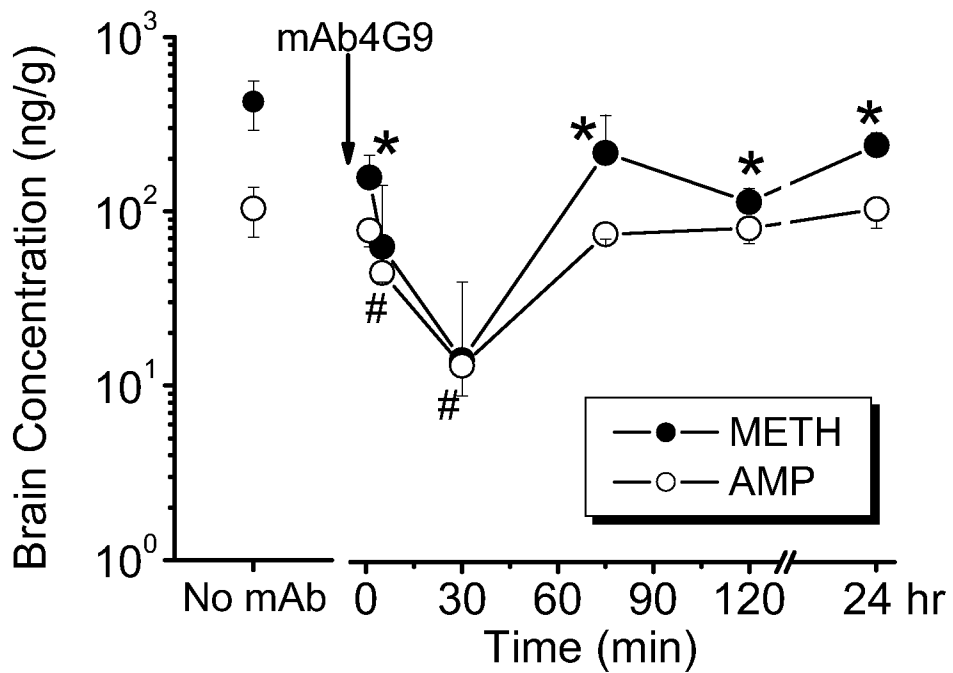


FIG. 8