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Title Page

4-Methylmethcathinone (Mephedrone): Neuropharmacological Effects of a Designer Stimulant of Abuse

Gregory C. Hadlock, Katy M. Webb, Lisa M. McFadden, Pei Wen Chu,

Jonathan D. Ellis, Scott C. Allen, David M. Andrenyak, Paula L. Vieira-Brock, Christopher L. German,

Kevin M. Conrad, Amanda J. Hoonakker, James W. Gibb, Diana G. Wilkins,

Glen R. Hanson, and Annette E. Fleckenstein

Department of Pharmacology & Toxicology, University of Utah, 30 S 2000 E, Rm. 201, Salt Lake City, UT 84112

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Running Title Page

Running Title: Mephedrone and Monoaminergic Neuronal Function

Corresponding Author:

Annette E. Fleckenstein, Ph.D.

Professor

University of Utah

Department of Pharmacology & Toxicology

30 South 2000 East, Room 201

Salt Lake City, UT 84112

e-mail: fleckenstein@hsc.utah.edu

telephone: 801-585-7474

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Abbreviations: 1-(4-Methylphenyl)-2-methylaminopropane-1-one 4-methylmethcathinone, mephedrone; 1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methylpropan-2-amine, methylenedioxymethamphetamine, MDMA; 2-(methylamino)-1-phenyl-propan-1-one, methcathinone, MCAT; 5-hydroxytryptamine, serotonin, 5HT; dopamine, DA; N-methyl-1-phenylpropan-2-amine, methamphetamine, METH; rotating disc electrode, RDE.

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Abstract

The designer stimulant, 4-methylmethcathinone (mephedrone), is among the most popular of the derivatives of the naturally occurring psychostimulant, cathinone. Mephedrone has been readily available for legal purchase both online and in some stores, and has been promoted by aggressive web-based marketing. Its abuse in many countries, including the United States, is a serious public health concern. Owing largely to its recent emergence, there are no formal pharmacodynamic or pharmacokinetic studies of mephedrone. Accordingly, the purpose of this study was to evaluate effects of this agent in a rat model. Results revealed that, similar to methylenedioxymethamphetamine, methamphetamine and methcathinone, repeated mephedrone injections (4 x 10 - 25 mg/kg/injection, s.c., 2-h intervals, administered in a pattern used frequently to mimic psychostimulant “binge” treatment) cause a rapid decrease in striatal dopamine (DA) and hippocampal serotonin (5-hydroxytryptamine; 5HT) transporter function. Mephedrone also inhibited both synaptosomal DA and 5HT uptake. Like methylenedioxymethamphetamine, but unlike methamphetamine or methcathinone, repeated mephedrone administrations also caused persistent serotonergic, but not dopaminergic, deficits. However, mephedrone caused DA release from a striatal suspension approaching that of methamphetamine, and was self-administered by rodents. A method was developed to assess mephedrone concentrations in rat brain and plasma, and mephedrone levels were determined 1 h after a “binge” treatment. These data demonstrate that mephedrone has a unique pharmacological profile with both abuse liability and neurotoxic potential.

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Introduction

The designer drug, 4-methylmethcathinone (mephedrone), is among the most popular of the derivatives of the naturally occurring psychostimulant, cathinone (Cressey, 2010; ACMD, 2010; Morris, 2010). Its structure is closely related to the phenylethylamine-family of illicit agents including methamphetamine (METH) and methylenedioxymethamphetamine (MDMA), differing by a keto group at the beta carbon. Mephedrone has been readily available for purchase both on- and off-line, and its circulation has been promoted by web-based marketing. Its rise in popularity in the United Kingdom received international attention, and led to its ban in 2010. Also in 2010, there were increasing reports of the abuse and seizure liability of mephedrone in regions other than Europe, including South-East Asia, Australia, and North America. Its abuse in the United States, particularly in the form of "Ivory Wave" (e.g., its combination with the stimulant, 3,4-methylenedioxypyrovalerone) has become a health concern.

Owing largely to its recent emergence, there are very few formal pharmacodynamic or pharmacokinetic studies of mephedrone. A single report by Kehr et al. (in press) indicates that mephedrone causes DA release in the nucleus accumbens. Beyond this, clinical and anecdotal reports are the primary source of information concerning mephedrone. This lack of information is problematic for public health policy makers and law enforcement organizations as they attempt to develop and implement appropriate strategies for dealing with the recreational use and abuse of mephedrone and related drugs. Of further concern, it has been suggested that mephedrone may resemble dangerous drugs such as methcathinone (MCAT), MDMA, or METH. This is problematic, as it is well established that high-dose administration of these stimulants can cause long-lasting monoaminergic deficits in humans (McCann et al., 1998; Sekine et al., 2001; Reneman et al., 2001) and non-human models (Ricaurte et al., 1980; Wagner et al., 1980; Guilarte et al., 2003; Hotchkiss et al., 1979; Sparago et al., 1996; Nash and Yamamoto, 1992; Gygi et al., 1997; Mereu et al., 1983). The potential clinical relevance of understanding these changes is underscored by findings that stimulant (e.g., METH) abusers often display general persistent impairment across several

neurocognitive domains, including deficits in executive function and memory (Volkow et al., 2001; Scott et al., 2007).

As noted, there are currently very few published studies describing the pharmacological or toxicological impact of mephedrone. Thus, the present study addresses this issue. Results revealed that, similar to MDMA, METH and MCAT (Fleckenstein et al., 1999; Metzger et al., 2000; Haughey et al., 2000; Hansen et al., 2002), repeated mephedrone injections rapidly decrease dopamine (DA) and serotonin (5-hydroxytryptamine; 5HT) transporter function. Like MDMA, but unlike METH (Stone et al., 1986; Schmidt and Kehne, 1990; McCann et al., 1994; Reneman et al., 2001; Krasnova and Cadet, 2009) repeated mephedrone administrations cause persistent serotonergic, but not dopaminergic, deficits. Of note, mephedrone causes DA release from a striatal suspension approaching that of METH, and is self-administered by rodents. These data demonstrate important similarities and differences among mephedrone and other related stimulants of abuse. Moreover, these data demonstrate that mephedrone has a unique pharmacological profile with both abuse liability and neurotoxic potential.

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Methods

Animals. Male Sprague-Dawley rats (290-400 g; Charles River Laboratories, Raleigh, NC) were maintained under controlled lighting and temperature conditions with constant access to food and water. With the exception of rats in the self-administration experiments, which were singly housed, rats were housed 3-4 animals per cage during treatment. Rats were maintained in a warmer ambient environment during treatment (e.g., ≥ 27˚C) to ensure that the mephedrone-treated rats attained hyperthermia. Temperatures were assessed at 1-h intervals beginning 30 min before the first saline or mephedrone injections. Rats were sacrificed by decapitation. All procedures were conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Utah Institutional Animal Care and Use Committee.

Drugs and Chemicals. Mephedrone hydrochloride, METH hydrochloride, cocaine hydrochloride, and MDMA hydrochloride were supplied by the Research Triangle Institute (Research Triangle Park, NC). Fluoxetine hydrochloride, cefazolin, and heparinized saline were purchased from Sigma Aldrich (St. Louis, MO). Mephedrone-d_3 was purchased from Cerilliant (Round Rock, TX). Mephedrone, METH, cocaine, and MDMA were dissolved in 0.9% saline vehicle prior to administration. Drug doses were calculated as the free base.

Synaptosomal [^{3}H]DA and [^{3}H]5HT uptake. [^{3}H]DA and [^{3}H]5HT uptake were determined using a rat striatal (DA) or hippocampal (5HT) synaptosomal preparation as previously described (Kokoshka et al., 1998a). In brief, synaptosomes were prepared by homogenizing freshly dissected striatal or hippocampal tissue in ice-cold 0.32 M sucrose pH 7.4, and centrifuged (800 x g, 12 min; 4°C). In some experiments, small sections of the left anterior stiatum and left anterior hippocampus were quickly frozen on dry ice and retained for determining DA and 5HT content. The supernatants were centrifuged (22,000 x g, 15 min; 4°C) and the resulting pellets were resuspended in ice-cold assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 CaCl2, 16 sodium phosphate, 1.4 MgSO4, 11 glucose and 1 ascobic acid; pH 7.4) and 1 μM pargyline. For the IC_{50} experiments, cocaine, MDMA, or mephedrone

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(1 nM - 5 μM) was present in the assay tubes. Samples were incubated for 10 min at 37 °C and the assays initiated by the addition of [^{3}H]DA or [^{3}H]5HT (0.5 nM or 5 nM final concentration, respectively). Following incubation for 3 min, samples were placed on ice to stop the reaction. Samples were then filtered through GF/B filters (Whatman, Florham Park, NJ) soaked previously in 0.05% polyethylenimine. Filters were rapidly washed three times with 3 ml of ice-cold 0.32M sucrose buffer using a filtering manifold (Brandel, Gaithersburg, MD). For [^{3}H]DA uptake, nonspecific values were determined in the presence of 50 μM cocaine. For [^{3}H]5HT uptake, nonspecific values were determined in the presence of 10 μM fluoxetine. Radioactivity trapped in filters was counted using a liquid scintillation counter. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories Inc., Hercules, CA).

DA and 5HT Concentrations. Striatal and hippocampal tissues were quickly frozen on dry ice and stored at -80°C until sonication (Branson Sonifier 250; Branson Ultrasonics Corporation, Danbury, CT) in 1 ml of tissue buffer (50 mM sodium phosphate dibasic, 30 mM citric acid with 10% (v/v) methanol, pH 2.5), then centrifuged at 18,800 x g for 15 min at 4 °C to separate the supernatant from the protein. The supernatant was centrifuged at 18,800 x g for 10 min at 4°C, and 25 μl was injected onto a high-performance liquid chromatography system (Dynamax AI-200 Autosampler and SD-200 pump; Varian, Walnut, CA) coupled to an electrochemical detector (Eox = +0.70 V; Varian Star 9080) to quantitate the concentrations of DA and 5HT. Monoamines were separated on a Whatman PartiSphere C-18 column (250 × 4.6 mm, 5 μm; Whatman, Clifton, NJ) in mobile phase consisting of 25% (v/v) MeOH, 0.04% (w/v) sodium octyl sulfate, 0.1 mM EDTA, 50 mM sodium phosphate dibasic, and 30 mM citric acid, at a pH of 2.65 and a flow rate of 0.75 ml/min. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories Inc., Hercules, CA).

Mephedrone Concentrations. Rat brains were homogenized separately by weighing each brain sample and homogenizing with 10 ml of MilliQ water. For the extraction, 0.5 ml of each plasma and brain homogenate was transferred to separate glass tubes. Mephedrone-d_3 (30 mg) was added as

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the internal standard. A 0.1 ml volume of ammonium hydroxide and 4 ml of 1-chlorobutane: acetonitrile (4:1, v:v) was added to each tube. After mixing and centrifugation, the upper organic layers were transferred to separate culture tubes and evaporated to dryness at 40 °C under air. A 0.1 ml volume of 0.2 % formic acid: methanol (75:25, v:v) was added to each extract. The reconstituted extracts were transferred to separate polypropylene autosampler vials. The extracts were analyzed on a Waters Acuity liquid chromatograph interfaced with a Waters Premier XE Quattro tandem mass spectrometer (Waters, Waltham , MA). Chromatographic conditions used a Synergi MAX RP 150 x 2 mm column (Phenomenex, Torrance, CA). The mobile phase consisted of 0.2 % formic acid: methanol (75:25, v:v) at a 0.2 ml/minute flow rate. Positive ion electrospray was used for the ionization. Selection reaction monitoring was used to monitor the peak areas for mephedrone (*m/z* 178→160) and mephedrone-d_3 (*m/z* 181→163). For both compounds, a cone voltage of 25 V and a collision energy of 15 V were utilized. Calibration standards (1 to 500 ng/ml) and quality control samples (8, 80, and 240 ng/ml) were prepared by fortification of a known concentration of drug to analyte-free matrix and were concurrently analyzed with the study samples. The study samples were diluted appropriately so that measured mephedrone concentrations were within the range of the calibration curve.

Rotating Disk Electrode (RDE) Voltammetry Analysis. RDE voltammetry was used to measure drug-stimulated DA release using a modification of previously published procedures used to measure potassium-stimulated DA release from rat striatal suspensions (Volz et al., 2007) and METH-induced vesicular DA efflux (Volz et al., 2006). Striatal suspensions were placed in the glass chamber at 37°C and a detection current baseline was established as described previously (Volz et al., 2007). The striatal suspensions were then preloaded with 10.2 µl of 20 µM DA solution (resulting in a 400 nM [DA] inside the chamber) and, within 3 minutes, the detection current returned to the original baseline. After the DA was preloaded onto the striatal suspensions, a small quantity of assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 CaCl2, 16 sodium phosphate, 1.4 MgSO4, 11 glucose, pH 7.4) containing 0.25 mM mephedrone, MDMA or METH (resulting in 5 µM concentrations inside the glass chamber) was added

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to the glass chamber to stimulate DA release. The resulting current outputs caused by DA release were recorded and converted to extravesicular [DA] versus time profiles by calibrating with known [DA] described previously (Volz et al., 2006). The initial velocities of mephedrone-stimulated DA release were calculated from the first 3 s of release and normalized to striatal wet weight as described previously (McElvain and Schenk, 1992; Volz et al., 2007).

Food Training. Rats were restricted to approximately 90% of their free-feeding food quantity and then placed in operant chambers connected to a PC computer running Graphic State software (Coulbourn Instruments, Whitehall, PA). Each chamber was equipped with two retractable levers, one of which was the “active” lever resulting in the delivery of a food pellet, whereas the other lever had no programmed consequences. Training consisted of an overnight 14 h schedule of food reinforcement (45 mg of Rodent Grain food pellets; Bio-Serv Delivering Solutions, Frenchtown, NJ) at FR 1, with only the stimulus-appropriate (drug) lever eliciting the reward

Catheter Implantation. After food training, rats were anesthetized with 90 mg/kg ketamine and 7 mg/kg xylazine (i.p.) and indwelling catheters consisting of a threaded connecter, Silastic tubing (10 cm, o.d. 0.51 mm), ProLite polypropylene surgical mesh and dental cement were implanted subcutaneously proximal to the scapula. The distal end of the catheter tubing was inserted into the right jugular vein and was secured to the surrounding tissue with sutures. To maintain catheter patency animals were infused daily with 0.1 ml antibiotic solution containing cefazolin (10.0 mg/ml) dissolved in heparinized saline (70 U/ml; Sigma-Aldrich, St Louis, MO), followed by 0.05 ml infusion of heparin and 0.05 ml of heparinzed glycol to lock the catheter.

Self-Administration. After 3 d of recovery, animals were randomly assigned to self-administer either mephedrone (0.24 mg/ 10 µl infusion), METH (0.24 mg/ 10 µl infusion) or saline (10 µl infusion) for 7 or 8 (4 h/d; room temperature 29°C). For each active lever press, an infusion pump (Coulbourn Instruments), connected to a liquid swivel (Coulbourn Instruments) suspended outside the operant

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chamber delivered 10 ul infusion over a 5-s duration through a polyethylene tubing located within a spring leash (Coulbourn Instruments) tethered to the rat. During this period, both levers were retracted. Following the infusion, the levers remained retracted for an additional 20 s. The active lever was counterbalanced within each group. Pressing the inactive lever resulted in no programmed consequences, although it was recorded. Rectal temperatures were measured using a digital thermometer (Physiotemp Instruments, Clifton, NJ) approximately 30 min after the end of each session.

Data Analysis. Statistical analyses between two groups were performed using a two-tailed Student's t-test. Statistical analyses among multi-group data were conducted using one-way ANOVA, followed by a Newman-Keul's post hoc test. Differences among groups were considered significant if the probability of error was less than or equal to 5%. IC_{50} values were determined using a least squares, non-linear regression fit with a minimum of eight data points (determined in triplicate) per curve, and competing drug concentrations ranging from 1 nM to 5 μM. Lever pressing and mephedrone intake during self-administration was analyzed using a two-way repeated-measures ANOVA with Bonferroni multiple comparisons posthoc analysis. RDE DA release velocities during the first 3 sec were calculated using linear regression. IC_{50} values were determined and all statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

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Results

Results presented in Fig. 1 reveal that repeated mephedrone injections (4 x 10 or 25 mg/kg/injection, s.c., 2-h intervals, administered in a pattern used frequently in rodent models to mimic psychostimulant "binge" treatment) cause a rapid (within 1 h) decrease in DA and 5HT transporter function, as assessed in striatal and hippocampal synaptosomes, respectively. This decrease was not likely due to residual drug introduced by the original s.c. injections, as other studies have demonstrated that the preparation of synaptosomes "washes" drug from the preparation (Fleckenstein et al., 1997; Kokoshka et al., 1998b). Of note, the IC_{50} value of mephedrone for inhibiting DA uptake was similar to that of METH, while the IC_{50} value for 5HT uptake was similar to that of MDMA (Table 1). Further, mephedrone increased core body temperatures throughout the course of treatment from an average of 37.8 ± 0.1* ˚C for saline-treated rats to an average of 39.5 ± 0.1* and 40.0 ± 0.1* ˚C for the rats treated with 10 and 25 mg/kg mephedrone, respectively (*, indicates significant difference from all other groups ($p < 0.05$)). Administration of 4 x 1 mg/kg/injection, or 4 x 3 mg/kg/injection, s.c., 2-h internals, was without effect on DAT or 5HT transporter function, as assessed 1 h after treatment. Specifically, striatal [^{3}H]DA uptake levels were 1.7 ± 0.2, 1.8 ± 0.1 and 1.4 ± 0.1 fmol/µg protein for saline-, 4 x 1 mg/kg mephedrone-, and 4 x 3 mg/kg mephedrone-treated rats, respectively (n=7-8; $p \geq 0.05$). Hippocampal [^{3}H]5HT uptake levels were 0.8 ± 0.1, 0.7 ± 0.1 and 0.9 ± 0.1 fmol/µg protein for saline-, 4 x 1 mg/kg mephedrone-, and 4 x 3 mg/kg mephedrone-treated rats, respectively (n=7-8; $p \geq 0.05$). Both doses of mephedrone increased core body temperatures throughout the course of treatment from an average of 37.4 ± 0.1* ˚C for saline-treated rats to an average of 38.3 ± 0.1* and 39.0 ± 0.1* ˚C for the rats treated with 4 x 1 and 4 x 3 mg/kg mephedrone, respectively (*, indicates significant difference from all other groups ($p < 0.05$)).

Data presented in Fig. 2 demonstrate that repeated mephedrone administrations (4 x 10 or 25 mg/kg/injection, s.c., 2-h intervals) also caused persistent decreases in hippocampal 5HT transporter function (Fig. 2A) and 5HT (Fig. 2B) levels, as assessed 7 d after treatment. These mephedrone treatments decreased striatal 5HT levels as well (4.0 ± 0.6, 3.5 ± 0.5 and 2.1 ± 0.2* pg/µg protein for

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saline, 4 x 10 and 4 x 25 mg/kg, respectively (n = 6 – 10); *, indicates significant difference from saline-controls, $p < 0.05$). In contrast, mephedrone treatment was without effect on striatal DA transporter function (Fig. 2C), DAT immunoreactivity (data not shown), or DA (Fig. 2D) concentrations as assessed 7 d after treatment. Mephedrone increased core body temperatures throughout the course of treatment from an average of 37.7 ± 0.1* ˚C for saline-treated rats to an average of 39.2 ± 0.2* and 39.7 ± 0.1* ˚C for the rats treated with 10 and 25 mg/kg mephedrone, respectively (*, indicates significant difference from all other groups ($p < 0.05$)). Administration of 4 x 1 mg/kg/injection or 4 x 3 mg/kg/injection of mephedrone, s.c., 2-h internals, did not decrease DA or 5HT levels, as assessed 7 d after treatment. Specifically, striatal DA content was 110.9 ± 6.2, 119.8 ± 4.5 and 131.3 ± 7.3 pg/µg protein, for the saline-, 4 x 1 mg/kg/injection mephedrone-, and 4 x 3 mg/kg/injection mephedrone-treated rats, respectively (n=8; $p = 0.08$, with DA content being slightly increased after 4 x 3 mg mephedrone/kg/injection). Hippocampal 5HT content was 5.7 ± 0.6, 6.7 ± 1.5 and 6.6 ± 0.8 pg/µg protein, in the saline-, 4 x 1 mg/kg/injection mephedrone-, and 4 x 3 mg/kg/injection mephedrone-treated rats, respectively (n=7-8; $p \geq 0.05$). Both doses of mephedrone significantly increased core body temperatures throughout the course of treatment from an average of 37.4 ± 0.1* ˚C for saline-treated rats to an average of 38.8 ± 0.2* and 39.0 ± 0.1* ˚C for the rats treated with 1 and 3 mg/kg mephedrone, respectively (*, indicates significant difference from the saline-treated rats ($p \leq 0.05$)).

In order to determine brain and plasma mephedrone levels, a method was developed using liquid chromatography-mass spectrometry. Using this assay, results revealed plasma levels of 384.2 ± 62.2, and 1294.3 ± 145.5 ng mephedrone/ml plasma as assessed 1 h after 4 x 10 or 25 mg/kg/injection (s.c., 2-h intervals), respectively. Whole brain levels of 2.1 ± 0.2 and 7.8 ± 0.9 ng mephedrone/mg tissue were found 1 h after 4 x 10, 25 mg/kg/injection (s.c., 2-h intervals), respectively.

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DA release was assessed after application of 5.0 μM of METH, mephedrone, or MDMA onto striatal suspensions that were preloaded with DA. Results revealed that the initial velocities (determined over the first 3 s) were 0.29* ± 0.01, 0.25 ± 0.01* and 0.16 ± 0.01* nmol DA/(s*g wet weight tissue) for METH, mephedrone and MDMA, respectively ($F(2, 4071) = 85.2509$, *, indicates significant difference from all other groups, $p \leq 0.05$). The maximal values for DA release for METH, mephedrone and MDMA were 3.8 ± 0.6*, 2.7 ± 0.2* and 1.7 ± 0.2* nmol DA/g weight tissue, respectively (*, indicates significant difference from all other groups ($p \leq 0.05$)).

Fig. 4A demonstrates that rodents self-administer mephedrone. Whereas saline self-administering animals (n=10) decreased pressing from day 1 of self-administration to day 8, mephedrone self-administering rats (n=13) increased pressing (drug x day interaction: $F(7, 147)=24.88$, $p<0.05$; Figure 4A). Mephedrone self-administering animals increased daily drug intake from 1.77 ± 0.15 mg on day 1 to 6.78 ± 1.00 mg on day 8 ($F(7, 84)=17.59$, $p<0.05$). Discrimination of the reinforced lever from the inactive lever increased from a ratio of 2.65: 1 reinforced presses per inactive press on day 1 to 10.71: 1 reinforced presses per inactive press on day 8 in mephedrone self-administering rats. Approximately 85% of mephedrone self-administering rats increased drug intake on 3 or more consecutive days. Mephedrone self-administration also increased core body temperature (assessed 30 min after the end of each daily session) from an average of 37.3 ± 0.1 °C for saline-controls to an average of 38.0 ± 0.1* °C for mephedrone self-administering rats (*, indicates significant difference from saline-controls ($p < 0.05$)).

For comparison with data presented in Fig. 4A, animals were allowed to self-administer METH under identical conditions (e.g., same dosing, duration of sessions, etc.) as were utilized to study mephedrone self-administration (Fig. 4B). Again saline self-administering animals (n=8) decreased pressing from day 1 of self-administration to day 7 while METH self-administering rats (n=8) rapidly acquired stable lever-pressing behavior ($F(6,84)=23.63$, $p<0.05$). Daily drug intake averaged 2.55 ± 0.06 mg METH/session across the 7 d of treatment). Discrimination of the reinforced lever from the

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inactive lever averaged a ratio of 10.1:1 reinforced presses per inactive press in the METH self-administering animals. METH self-administration also increased core body temperature (assessed 30 min after the end of each daily session) from an average of 37.6 ± 0.1 °C for saline-controls to an average of 38.2 ± 0.2* °C for METH self-administering rats (*, indicates significant difference from saline-controls ($p < 0.05$)).

Discussion

The stimulant/hallucinogen, mephedrone, has received recent international attention. Most abusers report that in terms of its subjective effects, the agent most resembles MDMA (Carhart-Harris et el., 2011). However, some abusers also liken its subjective effects to those of cocaine (Carhart-Harris et al., 2011). Of further interest are reports that, unlike MDMA (First and Tasman, 2010), some mephedrone abusers tend to binge on mephedrone (Schifano et al., 2010; but see also, Carhart-Harris et al., 2011).

The present study demonstrates that mephedrone has several pharmacological characteristics in common with other well-characterized psychostimulants such as MDMA and METH. First, the IC_{50} value for inhibition of striatal synaptosomal DA uptake resembles that of METH, while the IC_{50} values for inhibition of hippocampal synaptosomal 5HT uptake resembles that of MDMA. Second, and like METH and MDMA (Fleckenstein et al., 1999; Metzger et al., 2000; Haughey et al., 2000; Hansen et al., 2002), repeated high-dose injections of mephedrone, administered in a regimen designed to mimic “binge” use in humans, causes rapid decreases in DA and 5HT transporter function. Third, each of these agents promotes stimulant-induced hyperthermia.

Although METH and MDMA share many characteristics, one important factor that distinguishes METH and MDMA is that the latter causes persistent serotonergic deficits in rat and human models, while largely sparing dopaminergic neurons (Stone et al., 1986; Schmidt and Kehne, 1990; McCann et al., 1994; Reneman et al., 2001). In this respect, mephedrone more closely resembles MDMA. This is of interest, as most individuals who abuse mephedrone report subjective effects reminiscent of MDMA (Schifano et al., 2010; Carhart-Harris et al., 2011), suggesting similarities in the underlying mechanisms of action of these agents.

Despite the similarities noted above with the effects of MDMA, mephedrone causes greater DA release as assessed in a striatal suspension preloaded with equimolar concentrations of DA. In fact,

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in response to application of 5 μM drug (a concentration selected based, in part, upon studies by Clausing et al. (1995) wherein extracellular brain levels in the μM range were demonstrated after amphetamine administration) the *in vitro* DA-release capacity of mephedrone approaches that of METH. Although this study is limited in that only a single drug concentration was employed, its results are consistent with recent microdialysis findings by Kehr et al. (in press) that mephedrone caused DA release (albeit the present study examined DA release from a striatal suspension) and mephedrone caused greater DA release than MDMA. These data are also consistent with reports by some users that the subjective effects of mephedrone resemble METH, or like a combination of MDMA and cocaine (Carhart-Harris et al., 2011). Finally, these data are consistent with our finding that mephedrone is readily self-administered by rats (Fig. 4). Of note, METH is a potent DA-releasing agent (Bowyer et al., 1993; Kuczenski et al., 1995; Tata and Yamamoto, 2007) and its high-dose administration causes persistent dopaminergic deficits (for review, see Hanson et al., 2004; Yamamoto and Bankston, 2005; Tata and Yamamoto; 2007, and references therein). Since mephedrone has DA-releasing capability resembling METH and yet does not cause dopaminergic deficits, it is of significant interest in terms of studying the differential mechanisms understanding the long-term damage caused by these stimulants.

Of note, mephedrone concentrations were evaluated and detected in both rat plasma and brain samples following the controlled administration of mephedrone. Mean whole brain levels of 2.1 ± 0.2 ng mephedrone/mg tissue were found 1 h after 4 x 10 mg/kg/injection (s.c., 2-h intervals). This value compares with mean brain levels of 4.3 ± 0.5 ng/mg tissue as reported 1 h after 4 x 5 mg/kg/injection of METH (s.c., 2-h intervals; Truong et al., 2005). However, any comparison between these METH and mephedrone data must be made very cautiously as studies designed specifically to compare pharmacokinetics are necessary to address differences and similarities between the drugs.

Given the DA-releasing capacity of mephedrone, the finding that mephedrone readily penetrates the blood-brain barrier, mephedrone is readily self-administered by rats, and that the reinforcing effects of

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psychostimulants are associated with increases in brain DA levels (Volkow et al., 1999), it is reasonable to speculate that mephedrone may have significant abuse liability. Indeed, results presented in Fig. 4A demonstrate that mephedrone is readily self-administered, as assessed over 8 d of exposure to 4-h sessions (0.24 mg/kg/infusion). For comparison, the ability of METH to elicit self-administration under identical experimental conditions was assessed. Results confirmed numerous reports that, like mephedrone, METH is readily self-administered. However, and in contrast to effects of mephedrone, lever-pressing behavior did not increase over the 8-d duration of the experiment, possibly due to the increased stereotypy associated with this high infusion dose. Several factors likely account for this differential response including differences in pharmacokinetics, DA-releasing capabilities, and potential long-term consequence of repeated exposures (e.g., repeated high-dose METH administrations cause persistent dopaminergic damage, whereas data presented in Figs. 2C and 2D reveal that repeated high-dose mephedrone administrations do not cause such deficits).

In summary, mephedrone is a unique psychostimulant of abuse that shares pharmacological properties similar to, and yet distinct from, both METH and MDMA. Its ability to cause subjective effects resembling MDMA reportedly likely contributes to its abuse. However, its ability to cause DA release greater than MDMA may be particularly problematic in that, in comparison to MDMA, this drug may have enhanced abuse liability more resembling that of DA-releasing agents such as METH. Prior to this report, clinical and anecdotal reports have been the primary source of information concerning the stimulant. As noted above, this lack of reliable information is particularly problematic for public health policy makers and law enforcement organizations as they attempt to develop and implement appropriate strategies for dealing with the escalating recreational use of this substance and products that contain mephedrone and related drugs. In fact, the US Drug Enforcement Administration recently appealed for information concerning mephedrone and its analogs. Thus, additional studies are needed both to further investigate the impact of mephedrone, but also the various synthetic analogs that are an important public health concern.

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Authorship Contributions

Participated in research design: Hadlock, Webb, Chu, McFadden, Gibb, Hanson, and Fleckenstein.

Conducted experiments: Hadlock, Webb, Chu, McFadden, Ellis, Allen, Andrenyak, Veira-Brock, German, Conrad, and Hoonakker.

Contributed new reagents or analytical tools: Andrenyak and Wilkins.

Performed data analysis: Hadlock, Webb, Chu, McFadden, Andrenyak, Veira-Brock, German, Wilkins, and Fleckenstein.

Wrote or contributed to the writing of the manuscript: Hadlock, Webb, Gibb, Hanson, and Fleckenstein.

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FOOTNOTES

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Figure Legends

Fig. 1. Repeated mephedrone injections rapidly (within 1 h) decrease hippocampal synaptosomal (A) 5HT uptake and striatal synaptosomal (B) DA uptake. Rats received mephedrone (4 x 10 or 25 mg/kg/injection; s.c.; 2-h intervals) or saline (1 ml/kg/injection; s.c.: 2-h intervals) and were sacrificed 1 h after the final injection. Columns represent means and vertical lines 1 SEM determinations in 6-10 rats. *, indicates significant difference from saline controls ($p < 0.05$).

Fig. 2 Repeated mephedrone injections cause persistent decreases in (A) hippocampal synaptosomal 5HT uptake and (B) hippocampal 5HT content, but not in (C) striatal synaptosomal DA uptake or (D) striatal DA content as assessed 7 d after treatment. Rats received mephedrone (4 x 10 or 25 mg/kg/injection; s.c.; 2-h intervals) or saline vehicle (1 ml/kg/injection; s.c.: 2-h intervals) and were sacrificed 7 d later. Tissues were assayed as described in "DA and 5HT concentrations," and "Synaptosomal [^{3}H]DA and [^{3}H]5HT uptake" (see **Methods** above). Columns represent the means and vertical lines 1 SEM determinations in 6-10 rats. *, indicates significant difference from all other groups ($p < 0.05$).

Fig. 3. Mephedrone causes DA release from a striatal suspension. 5.0 µM of METH, mephedrone, or MDMA were applied to a striatal suspension that was preloaded with DA (see Methods, n = 7–11). The initial velocity of DA release (determined over the first 3 s) for METH, mephedrone and MDMA were 0.29 ± 0.01*, 0.25 ± 0.01* and 0.16 ± 0.01* nmol/(s*g wet weight tissue), respectively (*, indicates significant difference from all other groups, $p < 0.05$, $f(2, 4071)=85.2509$). The maximal DA release for METH, mephedrone and MDMA were 3.8 ± 0.6*, 2.7 ± 0.2* and 1.7 ± 0.2* nmol/g weight weight tissue, respectively. *, indicates significant difference from all other groups ($p < 0.05$).

Fig. 4. (A) Mephedrone and (B) METH are self-administered by rats. Rats were food-trained as described in Methods. After catheter implantation, rats were given access (4-h sessions; 7-8 days) to saline (10 µl/infusion), mephedrone (0.24 mg/ 10 µl infusion), or METH (0.24 mg/ 10 µl infusion)

according to an FR1 schedule of reinforcement as descried in Methods. * indicates significant difference from saline controls ($p < 0.05$).

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TABLE LEGEND

Table 1. IC_{50} values for striatal DA uptake and hippocampal 5HT uptake in synaptomes. IC_{50} values represent the means of at least three independent experiments, and were obtained as described in Methods. N/A – not assessed. [a], values reported previously (Fleckenstein et al., 1997).

Drug	DA uptake IC50 (nM)	5HT uptake IC50 (nM)
Mephedrone	467 ± 17	558 ± 48
MDMA	1216 ± 263	291 ± 36
Cocaine	1032 ± 96	1036 ± 137
METH	291 ± 4[a]	N/A

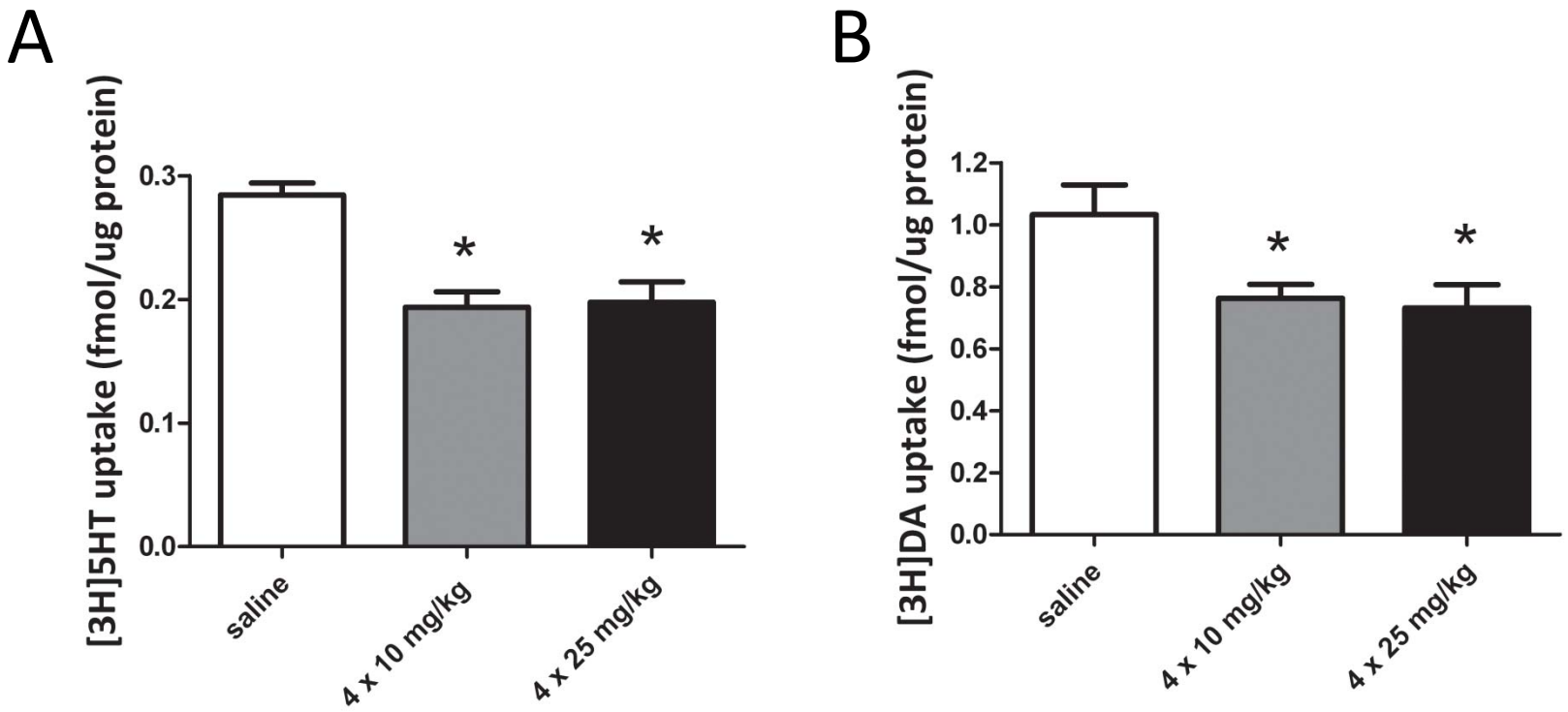

Figure 1

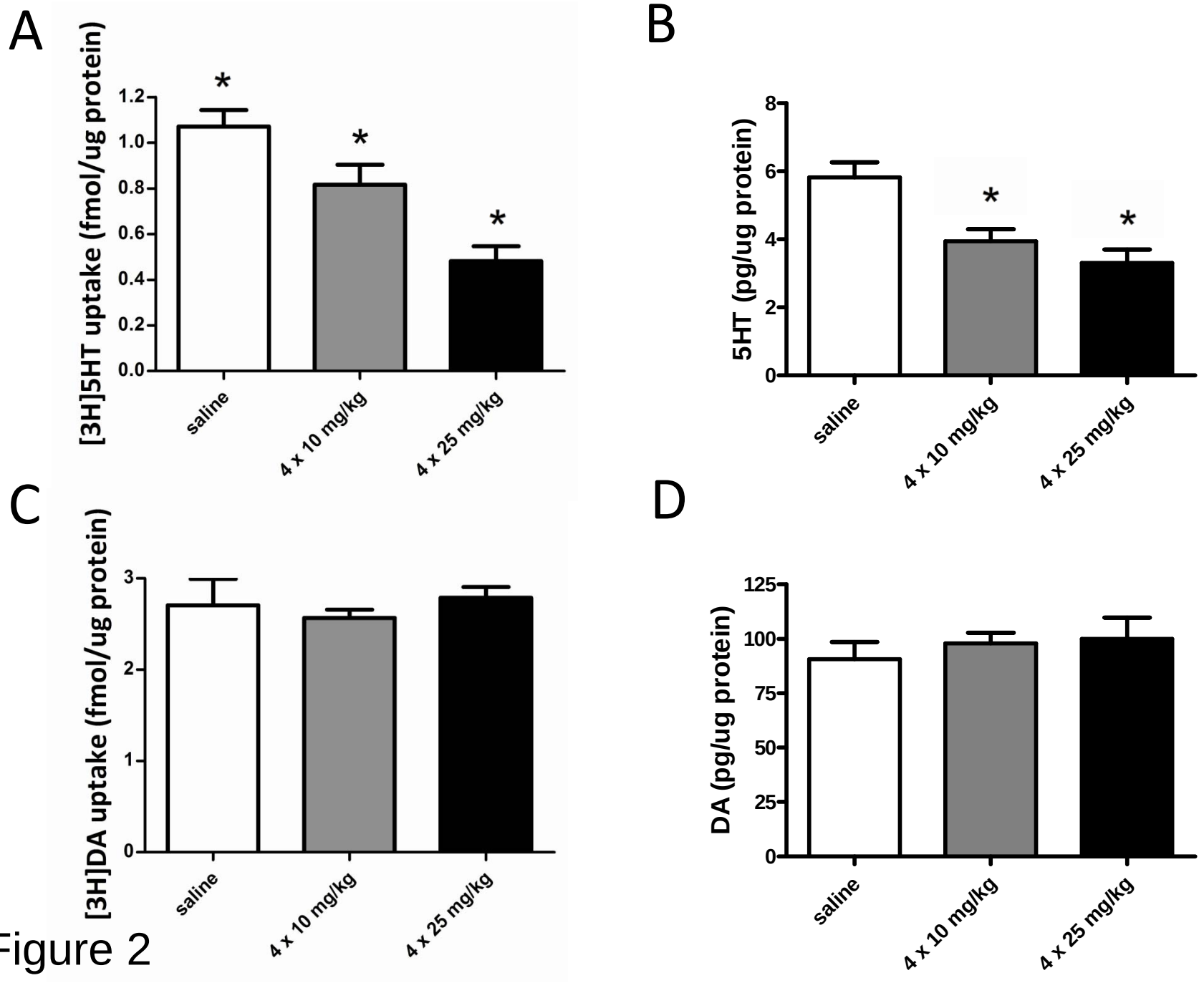

Figure 2

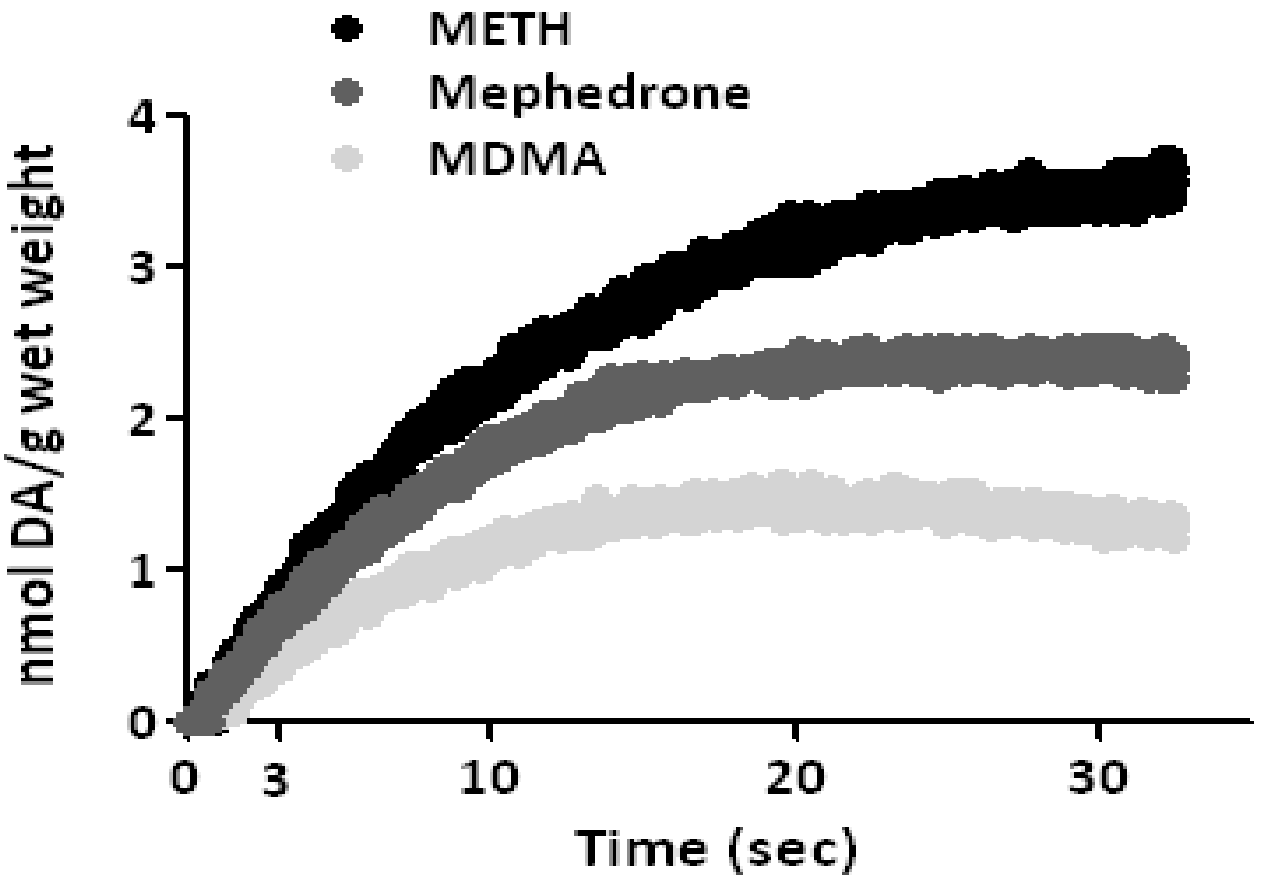

Figure 3

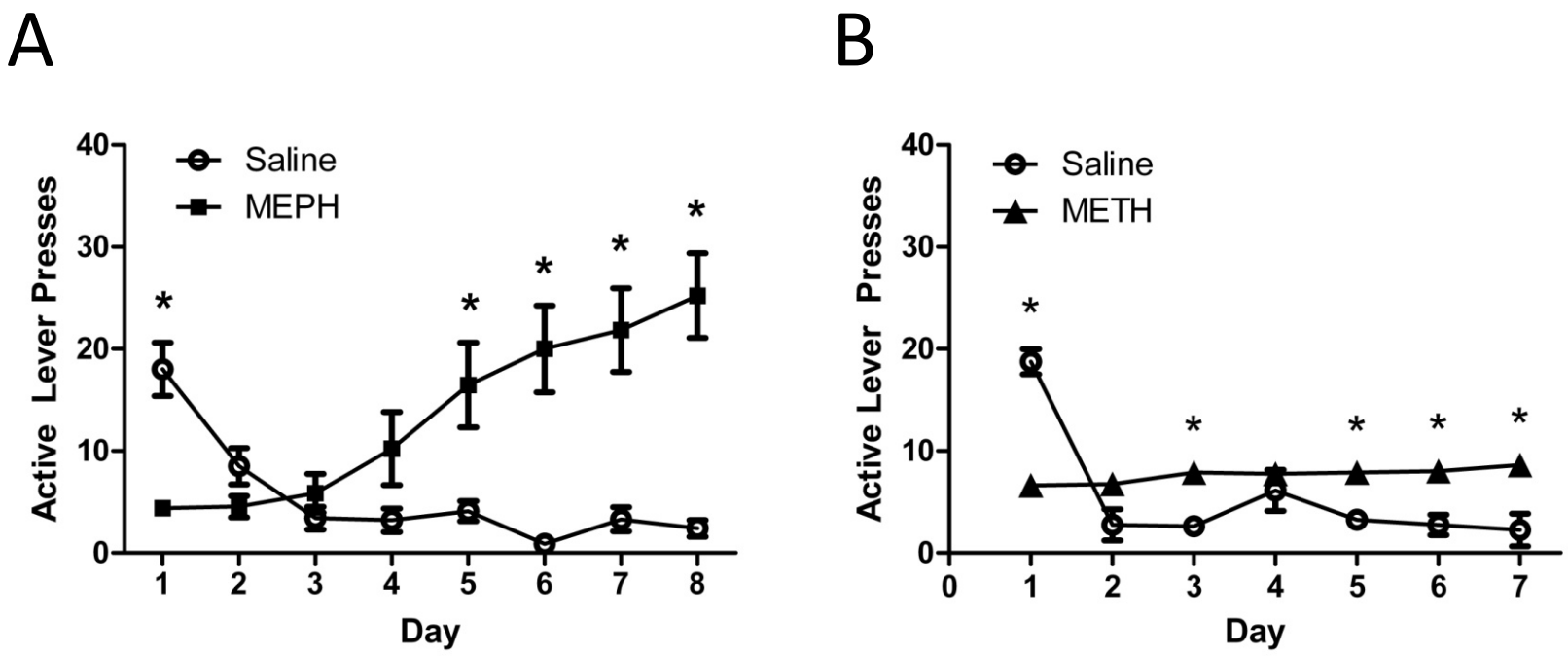

Figure 4