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Effects of intrastriatal dopamine D1 or D2 antagonists on methamphetamine-induced egocentric and allocentric learning and memory deficits in Sprague-Dawley rats

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Abstract

Rationale: Methamphetamine (MA) is an abused psychostimulant that causes cognitive deficits after chronic use. Neostriatal dopamine receptors play a role in MA monoamine neurotoxicity. Blocking dopamine receptors prior to MA exposure in adult rats attenuates monoamine reductions and reactive gliosis.

Objectives: We tested whether blocking dopamine receptors protects against cognitive deficits.

Methods: First, we determined the effects of MA alone versus MA in combination with the dopamine receptor D1 antagonist SCH-23390 or the dopamine receptor D2 antagonist sulpiride on cFos expression and monoamines at the age when rats in the cognitive experiment were to begin testing and monoamines in rats after cognitive testing.

Results: SCH-23390 infused into the neostriatum prior to systemic administration of MA attenuated MA-induced cFos activation while sulpiride induced cFos activation. Two weeks after MA, rats had dopamine and serotonin reductions that were attenuated by each antagonist. Other rats treated the same way, were tested for egocentric learning and memory in the Cincinnati water maze, for navigational strategy in a star water maze, and spatial learning and memory in a Morris water maze. Pretreatment with SCH-23390 or sulpiride attenuated the effects of MA on egocentric and spatial learning and memory. MA-treated rats showed a shift from an egocentric to a disorganized strategy in the Star maze that was less disorganized in groups receiving MA and an antagonist. Post-behavior monoamine reductions remained but were attenuated by the antagonists but not identically to what was seen in rats not behaviorally tested.

Conclusions: The results show for the first time that dopamine receptors are mediators of MA-induced cognitive deficits.

Keywords

methamphetamine; dopamine D1 antagonist; dopamine D2 antagonist; SCH-23390; sulpiride; egocentric learning; spatial learning; monoamines; rat

INTRODUCTION

Methamphetamine (MA) is an addictive psychostimulant that when abused can result in impairments in attention, learning, memory, and executive function (Hoffman et al. 2006; Moon et al. 2007; Simon et al. 2010; Simon et al. 2000; Thompson et al. 2004; Zhong et al. 2016). MA-induced cognitive impairments are accompanied by changes in dopaminergic and serotonergic biomarkers (Chang et al. 2007; Thompson et al. 2004; Volkow et al. 2001c). Despite characterization of the cognitive effects of MA, the role of striatal dopamine (DA) receptors in MA-induced cognitive deficits is unknown.

Reductions in DA and serotonin (5-HT) transporters (DAT and SERT, respectively) (Kish et al. 2009; Volkow et al. 2001b) as well as changes in levels of DA receptors D1 (DRD1) and D2 (DRD2) are seen in chronic MA abusers (Volkow et al. 2001a; Worsley et al. 2000). Similar to human findings, after neurotoxic regimens of MA, rodents exhibit reductions in striatal DA and 5-HT and hippocampal 5-HT (Broening et al. 2005; Cappon et al. 2000; Friedman et al. 1998; Fukumura et al. 1998; Gutierrez et al. 2017; Herring et al. 2010; Herring et al. 2008a; Herring et al. 2008b; Wallace et al. 2001), in DAT in the neostriatum and SERT in the hippocampus (Ares-Santos et al. 2012; Friend and Keefe 2013; Gross et al. 2011), and in expression of striatal DRD1 and DRD2 (McCabe et al. 1987). In rodents, pharmacologically inhibiting DRD1 or DRD2 prior to MA attenuates reductions in striatal DA, 5-HT, and of DAT and SERT binding (Broening et al. 2005; Gross et al. 2011; Sonsalla et al. 1986).

Egocentric navigation, assessed in the Cincinnati water maze (CWM), is impaired after MA (Herring et al. 2010; Herring et al. 2008a; Vorhees et al. 2011) as is allocentric navigation in the Morris water maze (MWM) after 4×10 mg/kg MA given 2 h apart (Gutierrez et al. 2017) or after a single 40 mg/kg dose of MA (Gutierrez et al. 2018). It is known that CWM egocentric learning is dependent on striatal DA (Braun et al. 2015; Braun et al. 2016; Braun et al. 2012) and MWM learning is dependent on hippocampus (Morris et al. 1982; Morris et al. 1986a; O'Keefe and Nadel 1978).

We established that a single dose of MA at 40 mg/kg induces neurotoxicity and impairs learning and memory comparable to that induced by 4×10 mg/kg given at 2 h intervals. In order to test the effects of intrastriatal DRD1 or DRD2 antagonists at interfering with MA-induced cognitive deficits, we used the single dose model to prevent complications from having to handle and anesthetize rats four separate times prior to four subcutaneous injections of MA at 2 h intervals. Repeated infusion every 2 h adds stress to the rats and might interfere with the effects of the antagonists. Since we demonstrated that the single dose model induced the same neurotoxic and cognitive deficits (Gutierrez et al. 2018) and also avoids the potential problems of repeated intrastriatal infusions, the single dose model was used here.

We pretreated male rats with SCH-23390, a DRD1 antagonist, or sulpiride, a DRD2 antagonist, 15 min prior to MA. The antagonists were infused directly into the neostriatum prior to systemic injection of 40 mg/kg MA. To determine the effectiveness of the antagonists we used cFos expression as a marker for MA-induced activation. We then

measured how the antagonists modified cFos expression from MA exposure. Next, we determined the effects of MA $\pm$ antagonists on monoamines two weeks after treatment at the time when cognitive testing was to be started in the main experiments. Cognitive tests included the CWM, the MWM, and the star water maze. After behavioral testing, we determined monoamine levels in the rats that were cognitively tested to see if levels were different compared with rats examined 2 weeks after treatment.

METHODS

Subjects.

Adult male Sprague-Dawley CD IGS rats (251–275 g; 60–70 days old) were purchased from Charles River (strain 001, Charles River, Raleigh, NC). Rats were housed 2/cage with HEPA filtered air (30 air changes/h [Alternative Design, Siloam Spring, AR]) in polysulfone cages with woodchip bedding for 1–2 weeks prior to an experiment. The protocol was approved by the Institutional Animal Care and Use Committee of Cincinnati Children's Research Foundation and adhered to guidelines on animal use from the National Institutes of Health. Rats were maintained on NIH-07 diet (LabDiet Inc., Richmond, IN) with reverse osmosis filtered/UV sterilized water (SE Lab Group, Napa, CA) in an AAALAC International accredited vivarium. The vivarium was maintained on a 14–10 h light-dark cycle (lights on at 600 h; at $19 \pm 1^\circ\text{C}$ and humidity $50 \pm 10\%$) in cages with a dome-shaped enclosure as enrichment (Vorhees et al. 2008).

Surgery.

Rats were anesthetized with 5% isoflurane and 95% oxygen, placed in a Kopf stereotaxic, and implanted stereotaxically with a cannula on each side. Each cannula was positioned to be centered in each caudate (Fig. 1S, right) using coordinates of: AP, +0.26 mm relative to Bregma; ML, ± 3.0 mm; DV, -5.2 mm (Paxinos and Watson 1998). A guide cannula and obturator was used and was 22 gauge and 6.5 mm in length (World Precision Instruments, Sarasota, FL). Each cannula was secured using dental cement and the incision sutured. Obturators were inserted and left in place until treatment. Rats were given 9–10 days of recovery before treatment. On the day of treatment, a 22 gauge injector cannula 7.0 mm in length (World Precision Instruments, Sarasota, FL) was attached to infusion tubing and inserted into the cannula on each side.

Drug treatment.

Temperature transponders (IPTT-300; BMDS, Seaford, DE) were implanted under light isoflurane anesthesia 3 days prior to MA treatment. Baseline body temperatures were taken prior to infusion of SCH-23390 (Sigma; 2 μg in 1 μL saline; (Gross and Marshall 2009; Steiner and Kitai 2000) or sulpiride (Sigma; 200 ng in 1 μL saline; (Gross et al. 2011; Gross and Marshall 2009; Marshall et al. 2001). Drugs were infused at a rate of 0.1 $\mu\text{L}/\text{min}$ for 10 min, and the injector was left in place for 5 min. Rats were moved to a holding cage and received a subcutaneous injection of saline (0.9% NaCl) or 40 mg/kg (+)-MA HCl (>98% pure and calculated as the freebase; Sigma) in saline in a volume of 3 mL/kg. Each cage was placed in an infant incubator maintained at 24°C . Core body temperatures were recorded at 30 min intervals for 8 h. If core body temperature rose to 41.0°C , the rat was placed in a

cage with shallow water until its temperature went below 40.5 °C. Treatment groups were saline-saline (S/S), saline-MA (S/MA), SCH-23390-saline (SCH/S), and SCH-23390-MA (SCH/MA) or sulpiride-saline (SUL/S), sulpiride-MA (SUL/MA).

Experimental Design:

There were six experiments, three for the DRD1 antagonist SCH-23390 and three for the DRD2 antagonist sulpiride. Each experiment had four groups: S/S, antagonist/S, S/MA, and antagonist/MA. For SCH-23390: **Exp-1**: Striatal cFos expression; **Exp-2**: monoamines in rats not behaviorally tested; **Exp-3**: cognitive testing and monoamines after behavior. Experiments for sulpiride followed the same pattern: **Exp-4**: striatal cFos expression; **Exp-5**: monoamines in rats not behaviorally tested; **Exp-6**: cognitive testing and monoamines after behavior. Group sizes are shown in figure captions.

Drug Doses:

Antagonist doses were based on previous studies demonstrating that MA-induced cFos upregulation is blocked by SCH-23390 infused prior to 4 mg/kg MA, whereas cFos expression is enhanced after sulpiride prior to a single dose of 4 mg/kg MA (Gross and Marshall 2009). These doses attenuate MA reductions of DAT and SERT binding in striatum (Gross et al. 2011).

cFos Immunohistochemistry:

Rats were handled 3 times per day for 15 min for 7 days before cFos assessment; antagonist was given 15 min prior to S or MA. Ninety minutes following MA, rats were given an overdose of sodium pentobarbital (60 mg/kg) and transcardially perfused with cold phosphate buffer saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. Brains were collected and stored in PFA overnight at 4 °C and transferred to a 30% sucrose solution for 48 h before coronal slices were taken every 50 µm and stained for cFos. The primary antibody was rabbit polyclonal cFos (sc-52; Santa Cruz Biotechnology) diluted 1:250. The secondary antibody was goat anti-rabbit IgG-FITC (sc-2012; Santa Cruz Biotechnology) diluted 1:300. Free-floating sections were washed three times in PBS for 15 min/wash and then incubated for 1 h in a blocking solution made of 5% goat serum and 0.3% Triton X-100 in PBS. After another wash, slices were placed in wells with the primary antibody consisting of the antibody in blocking buffer and left in wells on a rocker overnight at 4 °C. Slices were washed three times for 15 min/wash in PBS and incubated in secondary antibody in blocking buffer for 30 min. Sections were re-washed and mounted on slides. Slides were dried for 1 h and cover-slipped using ProLong Gold Antifade Mountant (ThermoFischer, P36931). Image acquisition was performed with a Nikon AI Inverted microscope at 20X magnification. Image analysis was performed using NIS-Elements software (Nikon Instruments) by an experimenter blinded to treatment conditions. One section per subject was used for analysis, and six subjects were used for each treatment condition (six sections per condition). A template outlining regions of interest (ROI) in the dorsolateral (610 × 610 µm²) and dorsomedial (638 × 638 µm²) striatum was created. For each section, the template was unilaterally applied to a hemisphere selected at random for analysis. Binary objects were determined by automatically setting intensity thresholds that were then adjusted for linearity.

Total binary objects within the ROI were quantified automatically by the software. Counts were averaged across rats and expressed as a percentage of counts found in control (S/S) rats.

Monoamines:

Monoamines were analyzed at two ages in four groups (two for each antagonist). For each antagonist, one cohort was assayed two weeks after treatment at the age when rats in subsequent experiments would start behavioral testing. Monoamines were also determined in rats after behavioral testing. Rats were decapitated, brains removed and dissected using a brain block into dorsal striatum, nucleus accumbens, and hippocampus (Braun et al. 2016; Williams et al. 2007) and rapidly frozen on dry ice, placed in conical tubes, and stored at -80°C . Tissues were weighed, sonicated in ice-cold 0.1 N perchloric acid, and then centrifuged at 20,800 RCF for 13 min at 4.0°C . Supernatant was collected, loaded onto the autosampler (20 μL injection volume), and run at a rate of 0.5 mL/min as described (Gutierrez et al. 2018). The HPLC-ECD system consisted of a Dionex UltiMate® 3000 Analytical Autosampler (Thermo Fisher Scientific) with an ESA 5840 pump and a Coulochem III electrochemical detector (Thermo Fisher Scientific) set to -150 mV for E1 and $+250\text{ mV}$ for E2. A guard cell set to $+350\text{ mV}$ was used. The column was an LC-18 Supelco Supelcosil™ column (15 cm x 4.6 mm, 3 μm ; Sigma-Aldrich Co.). MD-TM Mobile Phase (Thermo Fisher Scientific) consisting of 89% water, 10% acetonitrile, and 1% sodium phosphate monobasic (monohydrate) was used. Monoamine standards for norepinephrine (NE), DA, and 5-HT were pre-calculated, prepared, and serially diluted to create standard curves.

Behavior

Straight channel—Rats were adapted to swimming in a straight water channel ($244 \times 15 \times 50\text{ cm}$) with a platform at one end for four trials. These trials acclimate rats to swimming, assess swim speed, and provide experience with the escape platform. Latency to reach the platform was recorded.

Cincinnati water maze

The CWM is a multiple-T labyrinthine maze used to test egocentric learning and memory (Vorhees and Williams 2014a; b; Vorhees and Williams 2016). The maze is made of gray PVC and has 10 T-shaped cul-de-sacs that branch from a central corridor. It is 50 cm deep and filled with water to a depth of 25 cm. A schematic of the maze is shown in the upper panel of Fig. 2. The escape platform is submerged 1.5 cm below the water surface. Rats were tested for 18 days, 2 trials/day under infrared lighting and allowed a maximum of 5 min per trial. If the rat found the platform within 5 min, it was given trial 2 within 30 s. If the rat did not find the platform within 5 min, it was placed in a holding cage for at least 5 min before trial 2. After trial 2, rats were placed in cages with thick absorbent material to wick away water to dry and before being returned to the vivarium. Latency to escape and errors were recorded (defined as head and front leg entry into the stem of a T or into an arm of a cul-de-sac; reentries to the start arm were also counted as errors).

Star water maze

The maze was adapted from the Starmaze (Rondi-Reig et al. 2006). The maze is 210 cm in diameter, 51 cm deep, filled with water to a depth of 20 cm with an octagonal hub in the center. Testing was done over 20 days and consisted of 4 blocks of 4 training days with a probe trial on the 5th day. A schematic of the maze is shown in the lower left panel of Fig. S2. During training, two proximal cues were placed on the center hub facing the start arm (arm-0), one was white on the right side of the facing wall, and the other was checkered and on the left side of the facing wall. On probe trials, these cues were placed facing arm-5 where rats were started.

Training.

Rats were given 2 trials per day for 4 days with a time limit of 90 s per trial. Each trial began with the rat placed in arm-0 with the escape platform in arm-3. Once the platform was found, the rat was allowed to remain for 10–15 s before trial-2. If a rat failed to find the platform within 90 s, it was placed there for 10–15 s.

Probe.

On probe trial days, one training trial was given before the probe trial. Rats were started in arm-5 on probe trials. If a rat used a guidance strategy (G) it would follow the wall signs and make a right turn from arm-5, skip the next arm and enter arm-2. If the rat used an allocentric strategy (A) by relying on distal cues, it would swim directly to arm-3. If a rat used an egocentric strategy (E) by relying on internal cues, it would turn left from arm-5, make a right, followed by another right, and then a left into arm-0. This sequence would be identical to the sequence of turns used during training. Any solution other than these could not be classified and was designated undetermined (U). Following a probe trial, the rat was given one additional training trial. Probe data were used if the rat met a criterion of 4 correct out of 8 trials in block-1 or 5 out of 8 in blocks 2–4. A trial was scored correct if the rat made not more than one error to reach the goal.

Morris water maze

The MWM is a hippocampal and entorhinal cortex-dependent test of spatial learning and memory (Hafting et al. 2005; McNamara and Skelton 1993; Morris et al. 1982; Morris et al. 1986b). The test was conducted in phases: acquisition, reversal, shift, and cued-random (Vorhees and Williams 2006). Each spatial phase consisted of 6 days, 4 trials/day, to find the submerged platform followed by 1 probe trial on the seventh day with the platform removed. The time limit was 2 min on learning trials and 45 s on probe trials. The pool was 244 cm in diameter, 51 cm deep, made of black polypropylene, and filled halfway with water. A schematic of the maze is shown in the upper panel of Fig. 3. The diameter of the platform during acquisition was 10 cm, 7 cm during reversal, and 5 cm during shift. For acquisition the platform was in the SW quadrant; for reversal in the NE quadrant, and for shift in the NW quadrant. During cued-random testing, black curtains were closed around the maze to block distal cues and rats were given 4 trials/day for 2 days with the platform and start moved on every trial. To make the platform visible, a small yellow ball mounted on a steel

rod was mounted 10 cm above the platform. Performance was tracked using ANY-maze software (Stoelting, Wood Dale, IL).

Statistical Procedures

Analyses were conducted using mixed linear ANOVA models (SAS Proc Mixed, SAS Institute, Cary, NC, v9.3 TS Level 1M2) with an autoregressive (ar1) covariance structure. Degrees of freedom were estimated with the Kenward-Rogers method. Data were analyzed with treatment group as the independent variable. Slice-effect ANOVAs were conducted where significant interactions were found at each level of the repeated measure factor. Dunnett's tests were used where significant F-ratios were obtained to compare experimental groups with S/S controls. Significance was set at $p = 0.05$ (2-tailed where no *a priori* prediction was made, 1-tailed where we had previous findings). Star maze probe trial data were frequencies and were analyzed using Fisher's exact test. Behavioral data are presented as least square (LS) mean $\pm$ SEM. cFos data are expressed as percentage of controls and analyzed by one-way ANOVA.

RESULTS

Experiments with DRD1 antagonist SCH-23390

Exp-1: cFos—For SCH-23390 there was a significant main effect for cFos ($F(3,20)=143.4$, $p<0.0001$). *Post hoc* analysis revealed that the S/MA group had significantly increased cFos compared with the S/S group (Fig. 1A and Fig. 1S, left). MA increased the number of cFos-positive cells while no increases were seen in the SCH/MA group supporting the idea that SCH-23390 interferes with MA-induced cFos upregulation.

Exp-2: Monoamines in rats not behaviorally tested—In neostriatum for the SCH-23390 experiment, there were no effects on NE (Table 1). There were, however, reductions in neostriatal DA ($F(3,22)=7.1$, $p<0.01$) and 5-HT ($F(3,22)=4.4$, $p=0.01$; Table 1). *A posteriori* group comparisons revealed significant decreases in DA in the S/MA group compared with S/S controls and a trend in the SCH/MA group. For 5-HT, there was a significant decrease in the S/MA and SCH/MA groups compared with S/S controls. In nucleus accumbens, there were no significant effects on NE (Table 1). However, a significant main effect was found for DA ($F(3,22)=4.1$, $p<0.05$) and 5-HT ($F(3,22)=3.2$, $p<0.05$; Table 1). MA significantly reduced DA and 5-HT in the S/MA and SCH/MA groups compared with S/S controls. In hippocampus, there was no significant effect on NE (Table 1). However, there was a significant effect on 5-HT ($F(3,22)=16.5$, $p<0.0001$; Table 1). 5-HT was reduced in both the S/MA and SCH/MA groups.

Exp-3: Post-Behavior Monoamines—In neostriatum there were effects on NE ($F(3,49)=4.1$, $p<0.01$), DA ($F(3,48)=20.2$, $p<0.0001$), and 5-HT ($F(3,50)=2.4$, $p<0.05$; Table 1). NE levels were elevated in S/MA rats compared with S/S controls. For DA and 5-HT, the S/MA group had significantly lower neurotransmitter levels compared with S/S controls. Nucleus accumbens had main effects for NE ($F(3,47)=2.9$, $p<0.05$), DA ($F(3,49)=3.3$, $p<0.05$), and 5-HT ($F(3,51)=3.1$, $p<0.05$; Table 1). NE was elevated and DA decreased in the S/MA group compared with S/S controls. While there was an effect on 5-HT, the differences

were trends and not significant. Analysis of hippocampal NE showed no differences. For hippocampal 5-HT, there was a main effect ($F(3,50)=15.6$, $p<0.0001$; Table 1). Both the S/MA and SCH/MA groups showed reduced 5-HT compared with S/S controls.

Behavior

Temperature.—One of the hallmarks of MA-induced neurotoxicity in adult rats is hyperthermia (Cappon et al. 1997). Therefore, core body temperature was monitored in rats scheduled for behavior as a proxy for neurotoxicity. Analysis of temperature readings from the implanted transponders revealed a group main effect ($F(3,106)=229.1$, $p<0.0001$) and a group $\times$ time interaction ($F(48,736)=4.0$, $p<0.0001$; Figure 1C). The S/MA and SCH/MA groups both differed from S/S controls at all intervals except the pre-drug interval at time-0. As can be seen, SCH-23390 did not significantly alter MA-induced hyperthermia.

Straight Channel—No significant differences were found from MA exposure with or without exposure to SCH-23390 on straight channel swim latencies (not shown).

Cincinnati water maze—The CWM test was analyzed in blocks because the data in block-1 were censored from truncation of the range caused by the 5 min time limit and most rats reached the time limit on early days of the test. Block-1 was days 1–9 and Block-2 was days 10–18.

There were no significant effects on Block-1 errors. There was a significant group main effect on block-2 errors ($F(3,70)=4.4$, $p<0.01$; Figure 2A, B). Post hoc comparisons showed that the S/MA and SCH/MA groups performed worse than S/S controls. A similar main effect was found for latency ($F(3,65.1)=4.0$, $p<0.05$; Figure 2C,D). However, on latency only the S/MA group had longer latencies than S/S controls.

Star water maze—Successful trials were compared for each block of trials. There was a main effect only on block-3 ($F(3,52)=2.7$, $p=0.05$; Figure S2A). The S/MA group had fewer successful trials compared with S/S controls. Analysis of latency and errors showed no significant effects (Figure S2B,C). For probe trials, only strategies used within each group were analyzed, but not between groups. On day-5 probe trial, significant effects for strategy were found for the S/S, SCH/S, and S/MA groups but not for the SCH/MA group (Figure S2D). On the day-10 probe trial, differences were found for all groups (Figure S2E). On day-15 probe trial, significant effects were found for SCH/S and S/MA groups (Figure S2F). On day-20 probe, no differences were found (Figure S2G).

Morris water maze—A group main effect was found on acquisition latency ($F(3,74.7)=3.2$, $p<0.05$; Figure 3A,B). *Post hoc* analysis revealed longer latencies in the S/MA group compared with S/S controls, whereas the SCH/MA group showed protection. There was a main effect for path efficiency ($F(3,83.5)=2.8$, $p<0.05$; Figure 3C), but no group differed from S/S. For reversal latency there were no differences (Figure 3D,E). Reversal path efficiency revealed a main effect of group ($F(3,72.6)=2.2$, $p<0.05$; Figure 3F). The S/MA group was significantly less efficient compared with S/S controls whereas the SCH/MA showed a protective effect. No significant differences were found in the shift phase (Figure 3G,H,I). There were no effects on probe trials for any phase (not shown).

Experiments with DRD2 antagonist Sulpiride

Exp-4: cFos

For the DRD2 antagonist sulpiride experiment there was a significant main effect for cFos ($F(3,19)=13.9$, $p<0.0001$). In this case, each treated group differed from S/S (Figure 1B) since sulpiride induces cFos similarly to MA.

Exp-5: Monoamines in rats not behaviorally tested

Analysis of the neostriatum for sulpiride showed no effects on NE. There were, however, main effects on DA ($F(3,23)=4.2$, $p<0.05$) and 5-HT ($F(3,22)=4.4$, $p<0.01$; Table 2). *Post hoc* analysis showed significant reductions of DA in the S/MA group whereas the SUL/MA group showed partial protection. 5-HT was decreased in the S/MA and SUL/MA groups compared with S/S controls. In the nucleus accumbens there were no effects on NE even though the SUL/MA mean was higher in this group, but the standard errors were also higher. Effects were found on DA ($F(3,23)=9.7$, $p<0.001$) and 5-HT ($F(3,23)=6.8$, $p<0.01$; Table 2) in the nucleus accumbens. Further analysis showed significant reductions in DA and 5-HT in the S/MA and SUL/MA groups compared with S/S controls. In the hippocampus there were no effects on NE, but there was a main effect on 5-HT ($F(3,23)=22.22$, $p<0.0001$; Table 2). 5-HT was lower in the S/MA and SUL/MA groups compared with the S/S group.

Exp-6: Post-behavior monoamines

Analysis of post-behavior neostriatum showed significant main effects on NE ($F(3,53)=2.5$, $p<0.05$), DA ($F(3,52)=23.9$, $p<0.0001$), and 5-HT ($F(3,54)=2.6$, $p<0.05$; Table 2). Both DA and 5-HT were reduced in the S/MA group, whereas the SUL/MA groups showed protective effects. NE was increased in the S/MA group compared with S/S controls. In the nucleus accumbens, there was no effect on NE. There were main effects for DA ($F(3,53)=2.6$, $p<0.05$) and 5-HT ($F(3,52)=4.1$, $p<0.05$; Table 2), however, none of the groups differed from S/S controls. In the hippocampus, there was no effect on NE but there was on 5-HT ($F(3,55)=9.9$, $p<0.0001$; Table 2). *A posteriori* comparisons revealed that 5-HT was reduced in the S/MA and SUL/MA groups compared with S/S controls.

Behavior

Temperature.—As before, core body temperatures were recorded during treatment as a marker of MA-induced neurotoxicity. Analysis of core body temperature for the DRD2 experiment showed a main effect of group ($F(3,112)=218.0$, $p<0.0001$) and a group x time interaction ($F(48,824)=4.8$, $p<0.0001$; Figure 1D). Both the S/MA and the SUL/MA group were significantly different from the S/S controls at all time points except before the first dose (time-0).

Straight Channel

No significant differences were found on straight channel swim latencies (not shown).

Cincinnati water maze

There were no effects on Block-1 errors or latency. There was a main effect of group for block-2 errors ($F(3,74.5)=6.88$, $p<0.001$; Figure 4A,B) and latency ($F(3,71)=7.33$, $p<0.001$;

Figure 4C,D). On both measures, the S/MA group performed worse than S/S controls whereas the SUL/MA groups showed partial protection and did not differ from S/S controls. There were no interactions.

Star water maze

Comparison of successful trials for sulpiride showed no group differences (Figure S3A). No effects on latency or errors were found (Figure S3B,C). For day-5 probe trial, analyses of strategy showed effects in the S/S, SUL/S, and S/MA groups (Figure S3D). For the day-10 probe trial, differences were found for S/S, S/MA, and SUL/MA groups (Figure S3E). Day-15 analysis showed differences in the S/MA and SUL/MA groups (Figure S3F). For the day-20 probe, a trend was observed for the S/MA group ($p < 0.06$) (Figure S3G).

Morris water maze

A significant main effect was found on acquisition latency ($F(3,87)=4.0$, $p < 0.01$; Figure 5A,B) and path efficiency ($F(3,92.3)=3.4$, $p < 0.05$; Figure 5C). The S/MA group differed from S/S controls for latency whereas the SUL/MA group showed a protective effect. Although there was a main effect on path efficiency, none of the comparisons with S/S controls were significant. For reversal, there were no effects on latency (Figure 5D,E). There was a main effect of group on path efficiency ($F(3,76.6)=2.4$, $p < 0.04$; Figure 5F) as well as a group $\times$ day interaction ($F(15,278)=1.7$, $p < 0.05$). The S/MA group had significantly lower path efficiency compared with S/S controls; while the SUL/MA group showed a small protective effect. Analysis of the interaction revealed that the S/MA and SUL/MA groups differed from S/S controls on days 3 and 4 (not shown). For shift, the main effect of group for latency was not significant but there was a group $\times$ day interaction ($F(15,270)=1.8$, $p < 0.05$; Figure 5G,H). The S/MA group had longer latencies compared with the S/S group on days 1 and 6 (Figure 5G) whereas the SUL/MA groups did not differ from S/S controls. There was a main effect of group on path efficiency ($F(3,70.9)=2.8$, $p < 0.05$; Figure 5I). The S/MA group found the goal less efficiently compared with S/S controls, whereas the SUL/MA groups showed a partial protective effect. There were no effects on probe trials for any phase (not shown).

DISCUSSION

In order to determine if blocking DA receptors prior to MA protected against cognitive deficits while preventing the temperature regulation effects of peripheral antagonist administration, we infused a DRD1 or DRD2 antagonist into the neostriatum of adult male rats prior to MA and tested for neurotoxicity and learning and memory. Prior studies showed that MA-induced monoamine neurotoxicity is attenuated by prior exposure to DA antagonists; for example, one experiment injected SCH-23390 i.p. prior to MA and saw attenuated reductions in DA and 5-HT, but they did not control for the temperature modifying effects of SCH-23390 or sulpiride on MA-induced hyperthermia (Sonsalla et al. 1986). In a different experiment, to circumvent this confound, SCH-23390 or eticlopride were given subcutaneously 15 min before each of 4 doses of MA in a room maintained at 24 or 33 °C. At 24 °C, both antagonists lowered body temperature, but at 33 °C, no effects of the antagonists were seen on MA-induced hyperthermia but the antagonists attenuated the

effects of MA on striatal DA reductions, with eticlopride being more effective than SCH-23390 (Broening et al. 2005). Subsequently, the efficacy of SCH-23390 was assessed by infusion into the striatum. In this experiment, MA-induced neurotoxicity on DAT was blocked by SCH-23390 infusion while holding the body temperature of the rats at 40 °C, the body temperature often found when using a neurotoxic dosing regimen of MA (Friend and Keefe 2013). While these data support that DRD1 and DRD2 are involved in MA-induced neurotoxicity, none of these studies determined whether the neuroprotective effects of DA receptor antagonists provided protection against the cognitive deficits induced by MA. Here we tested whether DA receptor antagonists infused into the neostriatum at doses that protect against MA-induced neurotoxicity protect three types of learning and memory.

As expected, we found that antagonizing DRD1 or DRD2 receptors prior to MA attenuated MA-induced monoamine reductions and relevant to our hypothesis, the antagonists partially protected against egocentric learning and memory deficits in the CWM and spatial learning and memory deficits in the MWM. An unexpected finding was that after behavioral testing, MA-treated rats without antagonists had increased levels of neostriatal NE compared with controls. Neurochemically, the DA and 5-HT reductions seen prior to behavioral testing were more severe in some brain regions than at the later assessment done after behavioral testing. However, the design of the study does not permit a determination of what factors contributed to the early vs. late differences. The partial recoveries could be the result of time since treatment since the early assessment was done two weeks post-treatment and the late assessment was done 11 weeks post-treatment. Since the late assessment was done after behavioral testing, it could also be that the partial recovery was caused by behavioral testing itself, or it could be the product of both time and behavioral testing. The only way to fully resolve this would have been to have a third group treated and brain neurotransmitters assessed 11 weeks post-treatment in rats not behaviorally tested, but this was not part of the hypothesis being tested. Moreover, while several partial recoveries occurred by 11 weeks post-treatment, the more striking aspect of the late assessment was how persistent the monoamine reductions were.

Overall, the data show that MA-induced monoamine neurotoxicity is long lasting but can be attenuated by intra-striatal exposure to DRD1 or DRD2 antagonists. Further, the data suggest that the protective effects of the antagonists were not uniform, but rather the DRD1 antagonist was more effective than the DRD2 antagonist. However, because of differences in antagonist doses, differences in each drug's receptor affinity, clearance, spread, receptor density (e.g., DRD1 being postsynaptic and DRD2 being both pre-and postsynaptic), exact comparisons of efficacy are tentative. The larger point is that the data support a role of DRD1 and DRD2 in mediating MA-induced cognitive deficits even though the protective effects were incomplete. Nevertheless, these are the first data to show that DA receptors are involved in MA-induced egocentric and allocentric learning and memory impairments.

Egocentric learning and memory is known to be impaired by 4×10 mg/kg MA (Herring et al. 2010; Herring et al. 2008a; Herring et al. 2008b; Vorhees et al. 2011), and we recently showed that this is also the case after a single 40 mg/kg dose (Gutierrez et al. 2018). Using the latter model, we replicated our previous findings on egocentric navigation but with a few differences. In the previous single-dose study, rats learned the CWM faster than in the

current study and made fewer errors. The reason for this may be because rats in the present experiment underwent exposure to isoflurane during surgery and implantation of a cannula bilaterally in the center of the caudate that caused a track of damage around each cannula, unlike in the study establishing the effects of the single 40 mg/kg MA dose on learning and memory where no cannula were used. Exposure to isoflurane can produce cognitive changes on its own under some conditions (Callaway et al. 2012; Culley et al. 2004; Lin and Zuo 2011; Zhang et al. 2017). Here, rats were anesthetized for approximately 45 min; whether this is long enough to impair egocentric learning is not known. Secondly, cannula placement damaged tissue as it penetrated the neostriatum. Since egocentric performance depends on striatal function (Braun et al. 2015; Braun et al. 2016; Braun et al. 2012; Fouquet et al. 2013; Kesner et al. 1989; Packard and Teather 1997), especially dorsal striatum, damage to this area likely affected egocentric navigation. Irrespective of absolute differences in performance between the present and previous experiments using the 40 mg/kg MA dose, the data show a clear link between DRD1 and DRD2 and MA-induced egocentric navigation deficits.

We sought to determine whether MA alters search strategy in a task intended to test multiple strategies simultaneously. For this we used a SWM (Fouquet et al. 2013; Rondi-Reig et al. 2006). This task, modified for rats, showed previously that a single-dose of MA (40 mg/kg) resulted in MA rats using an egocentric strategy more often than other strategies (Gutierrez et al. 2018). However, in alignment with the slower rate of learning in the present experiment in the CWM after cannula implantation, we found few differences in strategy between groups, and those differences that did occur did not differentiate groups. Across all groups, rats showed little strategic consistency. Differences in the number of successful trials on the third phase were lower than in our previous study. Among rats that did show a strategy preference, slightly more allocentric navigation was seen compared with what we saw in our previous 40 mg/kg experiment.

The results from the MWM demonstrated that impaired MA-induced allocentric navigation deficits were also attenuated by DRD1 or DRD2 antagonists. Both antagonists protected against MA-induced latency deficits on acquisition and path efficiency during reversal trials. The DRD2 antagonist provided protection in the shift phase for path efficiency, suggesting a stronger effect from DRD2 antagonism than for the DRD1 antagonist. Antagonist treatment did not protect against hippocampal 5-HT reductions, as expected.

The monoamine data suggest some recovery between 2 weeks after treatment and the end of behavioral testing 11 weeks later, e.g., hippocampal 5-HT in the SCH/MA group. This may explain some of the protection seen in the MWM, although this was a small effect and direct comparisons between the groups could be the result of time since treatment, the effect of behavioral testing or a combination of both. While allocentric learning and memory is critically dependent on the hippocampus, the striatum plays some role (D'Hooze and De Deyn 2001; Whishaw et al. 1987) and we know that large reductions in striatal DA also produce MWM deficits (Braun et al., 2012). These effects are presumably the result of interconnections between striatum and hippocampus.

The protective effects of DA receptor antagonists were significant but incomplete. This may be because (1) the antagonists did not spread sufficiently to protect all MA-affected neurons, (2) the dose of the antagonists was not optimal, (3) MA neurotoxicity affects other brain regions the antagonist did not reach but are important for learning and memory, or (4) MA neurotoxicity involves neurotransmitters and receptors other than those of DA (e.g., 5-HT, glutamate, GABA), thereby limiting the range of protection possible from DA antagonists alone. Previous studies testing the effects of DA antagonists used smaller doses of MA divided over several hours (Friend and Keefe 2013; Gross et al. 2011; Gross and Marshall 2009), whereas we used a single dose. How other divided antagonist dosing regimens might affect learning and memory is not known. We also gave the antagonists 15 min before MA; whether this is the optimal pretreatment interval is unclear but was based on published data on MA neurotoxicity using these same antagonists. Future experiments should try higher doses of the antagonists or continuous infusion using osmotic mini-pumps, or combining the antagonists to see if a combination further enhances neurocognitive protection. Another explanation for incomplete protection stems from how MA interacts with DA receptors. For instance, blocking DRD1 prior to MA treatment attenuates the loss of monoaminergic activity by inhibiting excitotoxicity from over-stimulation of recurrent cortical-striatal loops (Gross and Marshall 2009; Mark et al. 2004). Blocking DRD2, on the other hand, protects by preventing changes that result from decreased DAT activity through prevention of oxidative stress and/or changes to protein-protein interactions (Baucum et al. 2004; Dickinson et al. 1999; Fleckenstein et al. 1997a; Fleckenstein et al. 1997b; LaVoie and Hastings 1999; Lee et al. 2007; Metzger et al. 2000). In both cases the protection occurs at presynaptic dopaminergic terminals that may underlie the similarities in the effects of the two antagonists on monoamines and behavior. The effect of having a local target, as in the case of the DRD2 antagonist, rather than involvement of multiple intermediate regions and signaling systems, as with the DRD1 antagonist, may explain the protective effects that sulpiride had on striatal based performance in the CWM. This was also seen in the MWM during the shift phase, where sulpiride had protective effects on path efficiency. Interestingly, neither antagonist prevented 5-HT depletions in the hippocampus suggesting that the source of protection was striatally mediated. Whether a different range of doses would have produced greater protection was not tested here. It is likely, however, that the behavioral and neurochemical protections were largely attributable to preservation of the striatal monoaminergic system.

It should be noted that the effects of the antagonists in combination were not tested here. The possibility exists that more complete protection might be achieved by administering both antagonists together. The rationale for a combination of antagonists would require evidence that each antagonist individually provided partial protection, which is what was found but we did not know this *a priori*. Had protection been obtained from one antagonist but not the other, for example, then giving a combination would be unproductive. Similarly, a combination would make little sense if both antagonists failed to provide even partial protection. As it turned out each antagonist produced partial effects making a combination logical but only in hindsight. The results indicate that the next step would be either to test higher doses of each antagonist, and/or to give both simultaneously. An additional consideration is that the behavioral tests were not counterbalanced: all rats received the tests

in the same order. Therefore, tests further removed in time from treatment may have been less affected. Had, for example, the MWM been given earlier perhaps the effect would have been somewhat different. This too warrants further investigation.

In summary, DRD1 and DRD2 antagonists partially protect against MA-induced cognitive deficits and striatal monoamine reductions. The CWM data reveal that MA-induced egocentric learning and memory deficits are attenuated by DRD1 and DRD2 antagonists. The MWM data reveal that MA-induced allocentric learning and memory deficits are also attenuated by DRD1 and DRD2 antagonists presumably by striatal, hippocampal connections since we only infused the antagonists into the dorsal striatum. The data represent a step forward in deciphering how MA-induced cognitive deficits are mediated. A fuller understanding of these mechanisms may provide targets for future interventions to alleviate some of the long-term adverse effects from chronic MA abuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Striatal c-Fos expression.

A, Quantification of cFos for the DRD1 experiment. S/MA treatment greatly increased cFos expression in the neostriatum compared with S/S controls, whereas this effect was blocked in the SCH/MA group. Group sizes: S/S=6, S/MA=6, SCH/S=6, SCH/MA=6. **B**, Quantification of cFos for the DRD2 experiment. As before, S/MA greatly increased neostriatal cFos expression, as did the DRD2 antagonist, sulpiride in the groups that received it: SUL/S and SUL/MA compared with the S/S group; this is a known effect of sulpiride. Group sizes: S/S=6, S/MA=6, SUL/S=6, SUL/MA=6. **C, DRD1 core body temperature.** Temperature recordings from implanted transponders before, during and 8.5 h after MA treatment. Both the S/MA and SCH/MA groups exhibited significant MA-induced hyperthermia. Group sizes: S/S=12, SCH/S=15, SCH/MA=13, S/MA=14. *** $P < 0.001$ vs. S/S. **D, DRD2 Core body temperature.** Temperature recordings from implanted transponders for the DRD2 experiment. The two groups receiving MA (S/MA and SUL/MA) both exhibited significant hyperthermia. Data are LS Mean $\pm$ SEM. Group sizes: S/S=12, SUL/S=15, SUL/MA=16, S/MA=14. ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ vs. S/S.

Cincinnati water maze: DRD1

Figure 2. DRD1 Experiment: Cincinnati water maze (maze schematic shown in upper panel). **A:** Errors shown by day **B:** Errors averaged across days. **C:** CWM latency shown by day. **D:** Latency averaged across days. The S/MA group made the most errors and had the longest latencies compared with the S/S group. The SCH/MA group was similar to the S/MA group for errors, but did not differ significantly from the S/S group for latency. Data expressed as LS Mean $\pm$ SEM. Group sizes: S/S=14, SCH/S=14, SCH/MA=12, S/MA=15. * $P < 0.05$; ** $P < 0.01$ vs. S/S.

Morris water maze: DRD1

Figure 3. DRD1 Experiment: Morris water maze (maze schematic shown in upper panel): **A:** Acquisition latency by day. **B:** Acquisition latency averaged across days. **C:** Path efficiency averaged across days. **D:** Reversal latency by day. **E:** Reversal latency averaged across days. **F:** Path efficiency averaged across days; **G:** Shift latency by day. **H:** Shift latency averaged across days. **I:** Path efficiency averaged across days. The S/MA group showed significantly longer latencies during acquisition compared with the S/S group, but this was not seen in the SCH/MA group. The S/MA group had significantly reduced path efficiency on reversal compared with the S/S group that was not seen in the SCH/MA group. There were no significant group differences on shift trials. Data represented as LS Mean $\pm$ SEM. Group sizes: S/S=14, SCH/S=14, SCH/MA=12, S/MA=15. * $P < 0.05$ vs. S/S.

Cincinnati water maze: DRD2

Figure 4. DRD2 Experiment: Cincinnati water maze.

A: Errors shown by day. **B:** Errors averaged across days. **C:** Latency shown by day. **D:** Latency averaged across days. For both errors and latency, the S/MA group performed significantly worse than the S/S group. The SUL/MA group did not differ from the S/S group. Data expressed as LS Mean $\pm$ SEM. Group sizes: S/S=14, SUL/S=15, SUL/MA=16, S/MA=15. ** $P < 0.01$ vs. S/S.

Morris water maze: DRD2

Figure 5. DRD2 Experiment: Morris water maze

A: Acquisition latency by day. **B:** Acquisition latency averaged across days. **C:** path efficiency averaged across days. **D:** Reversal latency by day. **E:** Latency averaged across days. **F:** Reversal path efficiency averaged across days. **G:** Shift latency by day. **H:** Shift latency averaged across days. **I:** Shift path efficiency averaged across days. The S/MA group had longer latencies on acquisition compared with the S/S controls, whereas the SUL/MA group did not differ from the S/S group. The S/MA group had reduced path efficiency on reversal and shift compared with the S/S group, whereas the SUL/MA group did not differ from the S/S group. Data are LS Mean $\pm$ SEM. Group sizes: S/S=14, SUL/S=15, SUL/MA=16, S/MA=15. * $P < 0.05$ vs. S/S.

TABLE 1

DRD1 (SCH-23390)

REGION	Monoamine	Group	Pre-behavior	Post-behavior
Neostriatum	NE	S/S	238.0 ± 69.5 (7)	150.2 ± 32.7 (7)
		SCH/S	218.4 ± 69.5 (7)	161.3 ± 30.3 (7)
		S/MA	185.2 ± 75.1 (6)	284.8 ± 30.3 (6) *
		SCH/MA	299.1 ± 75.1 (6)	174.0 ± 31.4 (6)
	DA	S/S	9535.2 ± 1214.7 (7)	11387.0 ± 776.5 (7)
		SCH/S	12027.0 ± 1214.7 (7)	12318.0 ± 748.2 (7)
		S/MA	4769.5 ± 1312.1 (6) *	4417.0 ± 808.2 (6) *
		SCH/MA	5841.0 ± 1312.1 (6) †	8693.3 ± 776.5 (6)
	5-HT	S/S	3011.6 ± 530.7 (7)	2004.9 ± 306.1 (7)
		SCH/S	3040.0 ± 530.7 (7)	1909.9 ± 295.0 (7)
		S/MA	921.3 ± 573.2 (6) *	1048.8 ± 295.0 (6) *
		SCH/MA	1118.6 ± 573.2 (6) *	1969.4 ± 306.1 (6)
N. Accumbens	NE	S/S	671.4 ± 219.1 (6)	505.7 ± 240.0 (12)
		SCH/S	714.2 ± 202.9 (7)	1033.0 ± 230.6 (13)
		S/MA	660.7 ± 219.1 (6)	1405.9 ± 230.6 (14) *
		SCH/MA	605.6 ± 219.1 (6)	1285.4 ± 230.6 (13)
	DA	S/S	9574.3 ± 1246.4 (7)	9239.4 ± 1057.8 (13)
		SCH/S	8786.5 ± 1246.4 (7)	7973.8 ± 1057.8 (13)
		S/MA	4631.7 ± 1346.2 (6) *	5549.6 ± 1057.8 (14) *
		SCH/MA	4654.2 ± 1346.2 (6) *	9087.6 ± 1057.8 (13)
	5-HT	S/S	10307.0 ± 1467.3 (7)	11765.0 ± 2093.8 (13)
		SCH/S	8745.9 ± 1467.3 (7)	13956.0 ± 1949.2 (15)
		S/MA	3263.4 ± 1584.9 (6) **	7773.2 ± 2093.8 (14)
		SCH/MA	3325.7 ± 1584.9 (6) *	15339.0 ± 2093.8 (13)
Hippocampus	NE	S/S	617.0 ± 55.3 (7)	602.5 ± 42.0 (14)
		SCH/S	679.3 ± 55.3 (7)	596.9 ± 40.6 (15)
		S/MA	515.9 ± 59.7 (6)	552.9 ± 42.0 (14)
		SCH/MA	640.3 ± 59.7 (6)	633.0 ± 45.4 (12)
	5-HT	S/S	3806.8 ± 439.6 (7)	3789.5 ± 373.3 (14)
		SCH/S	3530.2 ± 439.6 (7)	3982.6 ± 373.3 (14)
		S/MA	470.9 ± 474.8 (6) ****	877.4 ± (373.3 (14) ****
		SCH/MA	444.2 ± 474.8 (6) ****	2064.8 ± 403.2 (12) **

Values are Mean ± SEM expressed as ng/mg tissue.

S = saline, SCH = SCH-23390; MA = methamphetamine; SUL = sulpiride

* P < 0.05

**
P < 0.01

P < 0.0001

[†]
P < 0.10 (trend) vs. S/S group.

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TABLE 2

DRD2 (Sulpiride)

REGION	Monoamine	Group	Pre-behavior	Post-behavior
Neostriatum	NE	S/S	238.0 ± 33.3 (7)	150.2 ± 37.8 (7)
		SUL/S	170.7 ± 33.3 (7)	234.6 ± 33.8 (7)
		S/MA	185.2 ± 36.0 (6)	284.8 ± 35.0 (6) *
		SUL/MA	147.8 ± 33.3 (6)	254.8 ± 32.7 (7)
	DA	S/S	9535.2 ± 1196.5 (7)	11387.0 ± 734.2 (7)
		SUL/S	10378.0 ± 1196.5 (7)	12603.0 ± 683.5 (7)
		S/MA	4769.5 ± 1292.37 (6) *	4417.0 ± 764.2 (6) *
		SUL/MA	7054.18 ± 1196.5 (7)	10288.0 ± 661.8 (7)
	5-HT	S/S	3011.6 ± 471.7 (7)	2004.9 ± 323.4 (7)
		SUL/S	1639.1 ± 471.8 (7)	2076.0 ± 301.1 (7)
		S/MA	921.3 ± 509.5 (6) *	1048.8 ± 311.6 (6) *
		SUL/MA	794.7 ± 509.5 (7) *	1368.0 ± 291.5 (7)
N. Accumbens	NE	S/S	671.4 ± 267.9 (6)	505.7 ± 364.6 (12)
		SUL/S	405.0 ± 248.1 (7)	1554.7 ± 326.2 (15)
		S/MA	660.7 ± 267.9 (6)	1405.9 ± 350.3 (13)
		SUL/MA	1296.2 ± 248.1 (7)	1175.8 ± 315.8 (16)
	DA	S/S	9574.3 ± 996.3 (7)	9239.4 ± 1422.8 (13)
		SUL/S	10279 ± 996.3 (7)	10479.0 ± 1324.5 (15)
		S/MA	4631.7 ± 1076.1 (6) **	5549.6 ± 1422.8 (13)
		SUL/MA	4339.1 ± 996.3 (7) **	10125.0 ± 1282.5 (16)
	5-HT	S/S	10307.0 ± 1343.8 (7)	11765.0 ± 1374.8 (13)
		SUL/S	5740.66 ± 1343.8 (7)	13706.0 ± 1324.7 (14)
		S/MA	3263.44 ± 1451.4 (6) **	7773.2 ± 1374.8 (13)
		SUL/MA	2481.79 ± 1343.8 (7) **	13397.0 ± 1239.2 (16)
Hippocampus	NE	S/S	617.0 ± 60.4 (7)	602.5 ± 37.9 (7)
		SUL/S	540.7 ± 60.42 (7)	588.1 ± 36.6 (7)
		S/MA	515.9 ± 65.3 (6)	552.9 ± 37.9 (6)
		SUL/MA	536.6 ± 60.4 (7)	618.5 ± 35.4 (7)
	5-HT	S/S	3806.8 ± 428.4 (7)	3789.5 ± 442.9 (7)
		SUL/S	5010.6 ± 428.4 (7)	3454.9 ± 427.8 (7)
		S/MA	470.9 ± 462.8 (6) ****	877.4 ± 442.9 (6) ****
		SUL/MA	1502.2 ± 428.4 (7) **	1794.4 ± 414.3 (7) **

Values are Mean ± SEM expressed as ng/mg tissue.

S = saline, SCH = SCH-23390; MA = methamphetamine; SUL = sulpiride

*
P < 0.05

**
P < 0.01

P < 0.0001 vs. S/S group.

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