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Sex Differences in Abuse-Related Neurochemical and Behavioral Effects of 3,4-methylenedioxymethamphetamine (MDMA) in Rats

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Abstract

3,4-methylenedioxymethamphetamine (MDMA) is a substrate for the dopamine (DA), norepinephrine and serotonin (5HT) transporters that produces greater pharmacological effects on certain endpoints in females than males in both clinical and rodent preclinical studies. To evaluate potential for sex differences in abuse-related MDMA effects, the present study compared MDMA effects on intracranial self-stimulation (ICSS) and on *in vivo* microdialysis measurements of DA or 5HT in the nucleus accumbens (NAc) in female and male Sprague-Dawley rats. For ICSS studies, electrodes were implanted in the medial forebrain bundle and rats trained to press for electrical stimulation over a range of frequencies (56–158 Hz, 0.05 log increments) under a fixed-ratio 1 schedule, and the potency (0.32–3.2 mg/kg, 10 min pretreatment) and time course (3.2 mg/kg, 10–180 min pretreatment) of MDMA effects were determined. For *in vivo* microdialysis, rats were implanted with bilateral guide cannulae targeting the NAc, and the time course of MDMA effects (1.0–3.2 mg/kg, 0–180 min) on DA and 5HT was determined. MDMA produced qualitatively similar effects in both sexes on ICSS (both increases in low ICSS rates maintained by low brain-stimulation frequencies and decreases in high ICSS rates maintained by high brain-stimulation frequencies) and microdialysis (increases in both DA and 5HT). The duration and peak levels of both abuse-related ICSS facilitation and increases in NAc DA were longer in females. MDMA was also more potent to increase 5HT in females. These results provide evidence for heightened sensitivity of females to abuse-related behavioral and neurochemical effects of MDMA in rats.

Keywords

Sex difference; MDMA; ICSS; Microdialysis

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1. INTRODUCTION

3,4-methylenedioxymethamphetamine (MDMA) is an abused drug that functions as a substrate that passes through dopamine (DA), norepinephrine (NE) and serotonin (5HT) transporters (DAT, NET and SERT, respectively) and ultimately promotes monoamine release. Clinical evidence suggests potential sex differences in MDMA abuse patterns. For example, a recent 10-year (1999–2008) epidemiological study reported MDMA abuse in the United States was consistently higher in adolescent (12–17 year old) females than males (Wu et al., 2010). Furthermore, the tendency for greater MDMA use in females differed from the trend for males to show greater use of other abused drugs such as marijuana. Moreover, human laboratory studies in adults have also identified sex differences in subjective and physiological effects of MDMA (Liechti et al., 2001, McCann et al., 1994, Pardo-Lozano et al., 2012, Verheyden et al., 2002). For example, when administered at equivalent doses, MDMA was more effective in women than men to produce perceptual changes (e.g. increased scores on an Altered States of Consciousness rating scale), motor effects (e.g. jaw clenching) and subjective effects including anxiety, sedation and dizziness (Liechti et al., 2001; Pardo-Lozano et al., 2012).

Preclinical models have also been useful for interrogating sex differences in effects of abused drugs (Becker and Koob, 2016) and in particular, sex differences in MDMA effects appear dependent upon the experimental endpoint. For example, female rats were more sensitive than males to MDMA-induced locomotor activity (Palenicek et al., 2005, Walker et al., 2007) and memory impairment (Soleimani Asl et al., 2015). However, in other studies, female rodents were less sensitive than males to MDMA-induced hyperthermia (Wallinga et al., 2011, Wyeth et al., 2009), and neurotoxicity (Fonsart et al., 2008; Miller and O'Callaghan, 1995). Pharmacokinetic studies have indicated that MDMA may have a smaller volume of distribution and is less extensively metabolized to 3,4-methylenedioxyamphetamine (MDA) in females than in males, suggesting a potential pharmacokinetic mechanism of sex differences in MDMA effects (Fonsart et al., 2009, Hirt et al., 2010). However, the endpoint-specific nature of these sex differences suggests that these differences cannot be solely attributed to pharmacokinetics. Although the preclinical literature cited above suggests the existence of sex differences in MDMA effects on a range of endpoints, there is a paucity of preclinical research determining the role of sex in MDMA's abuse-related behavioral or neurochemical effects.

Accordingly, the present study goal was to systematically compare MDMA effects in female and male rats using (1) intracranial self-stimulation (ICSS) and (2) in vivo microdialysis procedures. ICSS is a behavioral procedure in which operant responding is maintained by electrical brain stimulation, and many abused drugs, including MDMA, increase (or "facilitate") ICSS responding (Bauer et al., 2013, Carlezon and Chartoff, 2007, Hubner et al., 1988, Negus and Miller, 2014). Microdialysis is an in vivo neurochemical procedure used to assess drug-induced changes in brain neurochemistry (Ungerstedt, 1991), and many abused drugs increase extracellular nucleus accumbens (NAc) DA levels (Cadoni et al., 2005, Di Chiara and Imperato, 1988, Nash et al., 1990). Some monoamine releasers, including MDMA, release 5HT as well as DA, and neurochemical selectivity of monoamine releasers to promote DA vs. 5HT release correlates with in vivo selectivity to facilitate ICSS

and produce other abuse-related behavioral effects (Bauer et al., 2013, Baumann et al., 2011, Bonano et al., 2015, Suyama et al., 2016, Wee et al., 2005). Based on the clinical evidence for sex differences in abuse-related effects of MDMA, we hypothesized that MDMA would facilitate ICSS to a greater extent in female vs. male rats, and these behavioral results would also be reflected in MDMA sex differences in NAc DA and 5HT level changes.

2. MATERIALS AND METHODS

2.1. Drugs

(±) 3,4-methylenedioxymethamphetamine HCl (National Institute on Drug Abuse Supply Program; Bethesda, MD) was dissolved in sterile saline and administered at a volume of 1 ml/kg intraperitoneal (IP).

2.2. Subjects and Surgeries

Studies were conducted in a total of 35 adult Sprague-Dawley rats (Harlan, Frederick, MD, USA). Six female and six male rats were used in ICSS studies, and 11 female and 12 male rats were used in microdialysis studies. All rats had ad libitum food and water access in their home cages and were housed individually on a 12 hour light-dark cycle (lights on from 6 a.m. – 6 p.m.) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Male rats weighed between 300 and 350 g at the time of surgery, whereas females weighed between 250 and 300 g. Estrous cycle was not monitored in females because repeated lavage to assess vaginal cytology and identify cycle phase is known to alter DA transporter-mediated drug effects (Walker et al., 2002). In addition, recent guidelines have suggested that estrous cycle monitoring is not essential for initial sex difference studies, because variability in many dependent variables across the estrous cycle in females is not significantly greater than variability in males (Beery and Zucker, 2011, Meziane et al., 2007, Mogil and Chanda, 2005, Prendergast et al., 2014), and sex differences when they exist are often sufficiently robust to be detectable without reference to estrous cycle phase (Becker and Koob, 2016). Animal maintenance accorded with The National Institutes of Health guidelines on care and use of research animals, and experimental protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Rats were anesthetized with 2.5–3.0% isoflurane in oxygen until unresponsive to toe pinch and secured in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). For ICSS studies, the cathode of a stainless steel electrode (0.25 mm diameter and insulated except at the flattened tip; MS303/1-AIU/SPC, Plastics One, Roanoke, VA, USA) was stereotaxically implanted into the left medial forebrain bundle at the level of the lateral hypothalamus using previously published coordinates for females and males (Lazenka et al., 2016, Paxinos and Watson, 1998, Stratmann and Craft, 1997). Specifically, female coordinates were 3.8 mm posterior to bregma, 1.6 mm lateral to the midsagittal suture, and 8.7 mm ventral to the skull, and male coordinates were 2.8 mm posterior to bregma, 1.7 mm lateral to the midsagittal suture, and 8.8 mm ventral to the skull. For microdialysis studies, guide cannulae (0.5 mm outer diameter; CXG-8, Eicom, San Diego, CA, USA) were implanted bilaterally and terminated 1 mm above the nucleus accumbens (NAc; coordinates for both sexes were:

1.5 mm anterior to bregma, 1.8 mm lateral to midsagittal suture, 6.0 mm ventral to dura). A dummy cannula (CXD-8, Eicom) was inserted into each guide cannula to maintain cannula patency. Electrodes/guide cannulae were secured to the skull using screws (Plastics One, Inc., Roanoke, VA, USA) and orthodontic resin (Butler Schein, Dublin, OH, USA), and for ICSS studies, the anode of the electrode (0.125 mm diameter, un-insulated) was wrapped around one of the screws to act as a ground. Animals were allowed at least seven recovery days prior to initiation of ICSS training or microdialysis testing.

2.3. Intracranial Self-Stimulation

2.3.1. Apparatus—Operant chambers consisted of sound-attenuating boxes containing modular acrylic and metal test chambers (29.2×30.5×24.1 cm). Each chamber had a response lever (4.5 cm wide, 2.0 cm deep, 3.0 cm off the floor), three stimulus lights (red, yellow, and green) centered 7.6 cm above the response lever, a 2 W house light, and an ICSS stimulator (Med Associates, St. Albans, VT, USA). Bipolar cables routed through a swivel-commutator connected the stimulator to the electrode (Model SL_{2C}, Plastics One, Roanoke, VA, USA). Med-PC IV computer software controlled all programming parameters and data collection (Med Associates, St. Albans, VT, USA). Throughout training and testing, daily ICSS sessions in female and male rats were conducted concurrently in one room equipped with a total of 12 chambers, and each rat was assigned to one chamber for the entire study.

2.3.2. Training—The behavioral procedure was similar to that described previously (Bauer et al., 2013, Negus and Miller, 2014). The house light was illuminated during behavioral sessions, and lever pressing under a fixed-ratio 1 (FR 1) schedule of reinforcement produced delivery of a 0.5 sec train of square-wave cathodal pulses (0.1 msec/pulse). During brain stimulation, stimulus lights over the lever were illuminated and responding had no scheduled consequences. Stimulation intensity was then individually manipulated for each rat to identify an intensity that maintained a rate of reinforcement greater than 30 stimulations/min. Once an appropriate intensity was identified, frequency manipulations were introduced during sessions consisting of three consecutive 10 min components, and each component consisted of ten 60 sec trials. The stimulation frequency was 2.2 Log Hz for the first trial of each component, and frequency decreased in 0.05 log unit steps during the subsequent nine trials to a final frequency of 1.75 Log Hz. Each trial began with a 10 sec time-out period, during which responding had no scheduled consequences, and five non-contingent stimulations at the designated frequency were delivered at 1 sec intervals during the last 5 sec of the time out. During the remaining 50 sec of each trial, responding was reinforced under an FR 1 schedule and produced both electrical brain stimulation and stimulus light illumination. Training continued until frequency-rate curves were not statistically different over three consecutive training days as indicated by lack of a significant effect of “day” in a two-way ANOVA with frequency and day as the two variables (see data analysis). Training was completed within five weeks of surgery.

2.3.3. Testing—Dose-effect studies were conducted in five female and six male rats, and test sessions consisted of three consecutive “baseline” components followed by a 10 min timeout period and then by three consecutive “test” components. Saline or a single MDMA dose (0.32, 1.0 or 3.2 mg/kg) was administered IP at the beginning of the time-out period,

and the dose order was counterbalanced across subjects within each sex using a pseudo Latin-square design. After completion of dose-effect studies, the time courses of effects produced by saline and 3.2 mg/kg MDMA were determined in test sessions that consisted of three consecutive baseline components followed first by IP injection of either saline or 3.2 mg/kg MDMA and then by pairs of test components that began 10, 30, 100 or 180 min after injection. Time-course studies were conducted in the same rats used in dose-effect studies with one exception. One female lost its electrode and was replaced with a drug-naïve female. Saline and MDMA were tested in a counterbalanced order across rats. Test sessions were conducted on Tuesdays and Fridays, and three-component baseline training sessions were conducted on all other weekdays.

2.3.4. Data Analysis—The primary dependent measure was the reinforcement rate in stimulations/trial. Raw reinforcement rates were normalized to the maximum control rate (MCR) for each subject on each day, where MCR was defined as the mean of the maximal rates observed during the second and third “baseline” components for that day. Therefore, %MCR was equal to [(response rate during a frequency trial) / (maximum control rate)] x 100. Data for each frequency were averaged across baseline and test components for each rat and then across rats to yield group mean baseline and test “frequency-rate” curves for each experimental manipulation. Separate three-way analyses of variance (ANOVAs) were performed using SPSS statistics version 22 (IBM, Armonk, NY) for dose-effect and time-course studies, with sex as a between-subjects factor, and either frequency and dose or frequency and time as within-subjects factors. Following a significant interaction, planned pairwise comparisons were conducted using the Holm-Sidak post hoc test (GraphPad Prism 6, GraphPad Software, Inc., La Jolla, CA). In many cases, Mauchly’s Test of Sphericity indicated a significant violation of sphericity for the repeated measures ANOVAs; therefore, degrees of freedom were corrected with Greenhouse-Geisser sphericity estimates. Student’s t-tests were used to compare (a) the amplitudes of brain stimulation required to maintain ICSS, and (b) the maximum control rates of ICSS, in female and male rats. The criterion for statistical significance was set at the 95% confidence level ($p < 0.05$).

2.4. Microdialysis

2.4.1. Procedure—Microdialysis procedures were identical to those described previously (Suyama et al., 2016). On test days, rats were briefly anesthetized with 3.0% isoflurane in oxygen, one of the dummy cannulae was removed, and a microdialysis probe (10 mm long, CX-I-8-2, Eicom) with a 2 mm artificial cellulose “cuprophane” membrane (50kDa molecular weight cut-off) at its tip was inserted into an 8 mm guide cannula such that it extended 2 mm beyond the end of the guide cannula and into the NAc. The probe was connected to a two-channel liquid swivel (TCS2–23, Eicom), and the rat was placed into an acrylic experimental cage (30 cm³). Microdialysis probes were perfused with artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂) at a rate of 1 µL/min. Mobile phase consisted of 2% methanol (EMD, Gibbstown, NJ, USA), 100mM phosphate buffer (Sigma Chemicals, St. Louis, MO, USA), 500 mg/L 1-decane sodium sulfonate (TCI America, Montgomeryville, PA, USA), and 50 mg/L EDTA-2Na⁺ (Dojindo Laboratories, Kumamoto, Japan).

Dialysate samples were collected into a 50 μ L injector loop at 10 min intervals using an online auto-injector (EAS-20s, Eicom) and immediately analyzed for DA and 5HT concentrations by high-pressure liquid chromatography (HPLC) coupled to electrochemical detection (HTEC-500, Eicom). DA and 5HT were separated using a C18-reverse phase column (PP-ODS II, Eicom) and detected using a graphite working electrode and an Ag vs. AgCl reference electrode with an applied potential of +450 mV. Preliminary experiments conducted by probe immersion into a known standard concentration of DA indicated a lag time of ~20 min for dialysate to traverse the tubing from the probe to the electrochemical detector at the 1 μ L/min flow rate. DA and 5HT were identified according to characteristic standard solution retention times, and concentrations were quantified by comparison with peak heights of the standard concentration curve (0.01–100 pg per 10 μ L) generated prior to drug administration in each microdialysis experiment. The lower neurotransmitter detection limit was 0.1 pg. DA and 5HT levels were determined to be stable after six consecutive baseline samples were obtained with <25% variability around the running mean of both neurotransmitters. Once DA and 5HT levels stabilized, rats were treated with saline or MDMA (1.0 or 3.2 mg/kg, IP), and dialysate samples were collected for 180 min after drug administration. Rats were tested no more than three times (no more than twice per cannula; at least one week between re-accessing a given site).

Although MDMA also acts at NET, NE was not assessed in microdialysis studies because (1) procedures described above for simultaneous detection of DA and 5HT cannot be used to also detect NE, and (2) prevailing evidence suggests only a minor role for NE relative to DA and 5HT for abuse-related effects of monoamine releasers (Banks et al., 2014; Rosenberg et al., 2013).

2.4.2. Data Analysis—The primary dependent variables were extracellular DA and 5HT concentrations in each dialysate fraction expressed as a percent of the average of the six mean baseline concentrations before MDMA or saline administration for each experiment. These individual normalized DA and 5HT concentrations were then averaged across rats to yield group mean results for graphical presentation. Separate three-way ANOVAs were performed for MDMA effects on DA and 5HT, with sex and dose as between-subjects factors and time as a within-subjects factor in both analyses. Other details of three-way ANOVA methods were identical to those described above. In addition, peak DA and 5HT increases (defined as maximum effect at any time point) produced by vehicle and each MDMA dose were compared in females and males using a two-way ANOVA with dose and sex as the two factors. A significant ANOVA was followed by the Holm-Sidak post hoc test. Baseline DA and 5HT levels in females and males were compared by Student's t-test. The criterion for statistical significance was set at the 95% confidence level ($p < 0.05$).

2.5. Verification of Cannula and Electrode Placements

ICSS electrode and microdialysis probe placements were verified by gross dissection as described previously (Bert et al., 2004, Lazenka et al., 2016, Suyama et al., 2016). Following experiment termination, rats were sacrificed by decapitation, and brains were removed and suspended in 10% (v/v) formalin (Fisher Scientific; Waltham, MA) for 48 h at 4°C. After this fixation period, brains were sliced using a carbon steel razor blade (Ted Pella, Redding,

CA) at the level of electrode or cannula entry, and the most ventral aspect of the ICSS electrode or microdialysis probe track was recorded by viewing the tissue with a Fisher Scientific™ Stereomaster™ dissecting microscope with 10X eyepiece and 4X objective. Locations were verified by comparison to figures from Paxinos and Watson (2007).

3. RESULTS

3.1. Intracranial Self-Stimulation

Mean $\pm$ SEM brain stimulation intensities required to maintain ICSS were $125.8 \pm 9.8 \mu\text{A}$ and $166.2 \pm 26.8 \mu\text{A}$ in females and males, respectively, and these intensities were not significantly different. Mean $\pm$ SEM maximum control reinforcement rates (MCRs) were 64.0 ± 1.2 and 59.9 ± 4.5 stimulations per trial in females and males, and again, these results were not significantly different. Figure 1 compares the effects of each MDMA dose with effects of saline on ICSS frequency-rate curves in female and male rats. As described in Methods, all data were analyzed in a single three-way ANOVA, but results are segregated by sex for clarity of display. MDMA produced dose-dependent changes in ICSS in both sexes as indicated by a significant Dose $\times$ Frequency interaction ($F_{5,26, 47.32}=12.99$, $p<0.0001$), and there was no difference in MDMA effects between sexes ($p>0.05$ for main effect of sex and all interactions between sex and other variables). Post-hoc analysis comparisons within sex indicated that lower doses of 0.32 and 1.0 mg/kg MDMA produced significant increases in ICSS at one or two isolated frequencies in both sexes, but effects were small. The highest dose of 3.2 mg/kg MDMA produced biphasic effects in both sexes expressed as an increase in low ICSS rates maintained by low brain-stimulation frequencies and a more prominent decrease in high ICSS rates maintained by high brain-stimulation frequencies.

In time-course studies, saline had no effect on ICSS frequency-rate curves at any time in either sex (data not shown). Three-way ANOVA indicated a significant main effect of frequency ($F_{1,86, 24.28}=104.13$, $p<0.0001$) but no other significant main effects or interactions. Figure 2 shows the time course of effects produced by 3.2 mg/kg MDMA. As described in Methods, all data were analyzed in a single three-way ANOVA that revealed a significant Sex $\times$ Time $\times$ Frequency interaction ($F_{5,84, 52.59}=2.34$, $p<0.05$), but results are segregated by time point for clarity of display. In general, MDMA produced biphasic effects in both sexes where rate-increasing effects had a longer duration than rate-decreasing effects in both sexes and rate-increasing effects lasted longer in females than males. Specifically, 3.2 mg/kg MDMA produced biphasic ICSS rate-increasing and rate-decreasing effects in both sexes after 10 min, but at later time points, rate-decreasing effects at high brain-stimulation frequencies dissipated more quickly than rate-increasing effects at low brain-stimulation frequencies in both sexes. As a result, the profile of MDMA effects featured more prominent abuse-related ICSS facilitation after 100 min than after 10 min. With regard to sex differences in MDMA effects over time, females and males responded similarly to MDMA after 10 min, but at later time points, females showed significantly greater facilitation than males at lower frequencies of ICSS. Specifically, the facilitation of low ICSS rates maintained by low brain-stimulation frequencies was greater in females than in males after 30 min (63 Hz), 100 min (63–79 Hz) and 180 min (79–89 Hz).

3.2. Microdialysis

Prior to saline or MDMA injections, baseline DA levels were 0.48 ± 0.12 (mean $\pm$ SEM) and 0.85 ± 0.09 nM, and baseline 5HT levels were 0.35 ± 0.08 and 0.49 ± 0.12 nM, in female and male rats, respectively. Basal DA, but not 5HT, levels were significantly lower in female rats compared to male rats ($t_{28}=2.47$, $p=0.02$). Figure 3 shows the time course of effects produced by saline or MDMA expressed as a percent of baseline levels of DA (top panels) or 5HT (middle panels). As described in Methods, the data for DA in the top two panels were analyzed in one three-way ANOVA, and data for 5HT in the middle two panels were analyzed in a separate three-way ANOVA, but results are segregated by sex for clarity of display. In general, MDMA produced a dose- and time-dependent increase in both DA and 5HT levels, and DA increases were more sustained in females. For MDMA effects on DA levels, the Sex $\times$ Dose $\times$ Time interaction was not significant ($F_{540, 64.75}=1.99$, $p=0.08$); however, there was a significant Dose $\times$ Time interaction ($F_{540, 64.75}=7.92$, $p<0.0001$) and a significant Sex $\times$ Time interaction ($F_{270, 64.75}=8.33$, $p<0.0001$). More specifically, significant increases in NAc DA levels were not observed until 170 min after 1.0 mg/kg MDMA in females. Conversely, in males, 1.0 mg/kg MDMA produced an early and transient increase in DA from 90–100 min after its administration. The higher dose of 3.2 mg/kg MDMA increased DA levels with similar onsets in both females (80 min) and males (90 min), but DA levels continued to rise for the remainder of the sampling period in females. At this dose in males, DA levels peaked 110 min after MDMA administration, but did not continue to increase.

There was also not a significant Sex $\times$ Dose $\times$ Time interaction for MDMA effects on 5HT levels ($F_{4.61, 55.28}=0.81$, $p=0.54$); however, as with DA, there was a significant Dose $\times$ Time interaction ($F_{4.61, 55.28}=13.68$, $p<0.0001$). Both 1.0 and 3.2 mg/kg MDMA significantly increased NAc 5HT levels in females following post-hoc analysis, whereas only 3.2 mg/kg MDMA significantly increased 5HT levels in males. In contrast to results with DA, there was not a significant Sex $\times$ Time interaction ($F_{230, 55.28}=1.60$, $p=0.21$).

As an additional measure of MDMA neurochemical effects, the peak effect (defined as the maximum effect at any time point) of each MDMA dose on DA and 5HT levels was determined, and these results are also shown in Figure 3 (bottom panels). Again, MDMA dose-dependently increased peak extracellular DA levels (main effect of Dose [$F_{2,24}=12.5$, $p<0.001$]) and females exhibited significantly greater peak DA levels that were especially evident at the highest MDMA dose (main effect of sex [$F_{1,24}=6.7$, $p=0.016$]); however, the Sex $\times$ Dose interaction did not achieve the criterion for statistical significance ($F_{2,24}=3.282$, $p=0.055$). MDMA also produced a dose-dependent increase in peak 5HT levels (main effect of Dose [$F_{2,24}=27.08$, $p<0.001$]). However, there was not a main effect of sex ($F_{1,24}=0.25$, $p=0.62$) or a significant Sex $\times$ Dose interaction ($F_{2,24}=2.33$, $p=0.12$). In particular, 1.0 mg/kg MDMA produced larger increases in mean 5HT levels in females than males, but this effect did not approach significance ($p=0.15$).

3.3. Cannula and electrode placements

Figure 4 shows probe placements from microdialysis studies and electrode placements for ICSS studies. All microdialysis probes were located in the NAc. All electrode tips were placed in the medial forebrain bundle.

4. DISCUSSION

This study compared MDMA abuse-related behavioral and neurochemical effects in female and male rats. There were two main findings. First, MDMA produced qualitatively similar profiles of behavioral and neurochemical effects in both females and males. In ICSS studies, MDMA administration to both sexes produced a mixed profile of dose- and time-dependent effects that included both (a) abuse-related facilitation of low ICSS rates maintained by low brain-stimulation frequencies, and (b) abuse-limiting decreases in high ICSS rates maintained by high brain-stimulation frequencies. Similarly, in microdialysis studies, MDMA produced dose- and time-dependent increases in NAc DA and 5HT levels in both sexes. These findings are consistent with other evidence to suggest that ICSS rate-increasing effects are associated with DA release, ICSS rate-decreasing effects are associated with 5HT release, and drugs that promote release of both DA and 5HT can concurrently produce both ICSS rate-increasing and rate-decreasing effects (Bauer et al., 2013; Bonano et al., 2015; Suyama et al., 2016). Second, subtle but significant sex differences were apparent in both MDMA abuse-related behavioral and neurochemical effects. Of particular importance, a dose of 3.2 mg/kg MDMA produced more sustained ICSS facilitation and a more sustained period of increasing DA release in females than males. These results add to a growing body of evidence for sex differences in MDMA effects and suggest that sustained abuse-related behavioral and neurochemical effects in females may contribute to higher abuse liability of MDMA in females.

4.1. Intracranial Self-Stimulation

The present study did not identify sex differences in amplitudes of brain stimulation required to maintain ICSS or in expression of ICSS in female and male rats. This agrees with a lack of significant sex differences in ICSS reported in previous studies (Russell et al., 2014, Stratmann and Craft, 1997), although as in the present study, these previous studies also observed trends for females to be slightly more sensitive than males to electrical brain stimulation. Estrous cycle was not monitored here, but previous studies have reported either no effect (Russell et al., 2014, Stratmann and Craft, 1997) or significant but small effects (Bless et al., 1997) of estrous cycle phase on ICSS in rats. Overall, these results suggest little or no effect of sex or estrous phase on baseline ICSS.

Effects of MDMA on ICSS in male rats in the present study are consistent with effects reported previously in male rats (Bauer et al., 2013, Hubner et al., 1988). For example, Bauer et al. (2013) found that the racemate and isomers of MDMA produced dose- and time-dependent changes in ICSS that included both facilitation of low ICSS rates maintained by low brain-stimulation frequencies and depression of high ICSS rates maintained by high brain-stimulation frequencies. The present study extended this biphasic profile of MDMA effects to females while also revealing subtle sex differences in MDMA effects that were

most evident during time-course studies. In particular, 3.2 mg/kg MDMA produced a more sustained facilitation of ICSS in females than males. To our knowledge, this is the first study to examine sex differences for MDMA effects on ICSS in rats. However, in general agreement with the present results, MDMA was also found to be more potent and/or effective to stimulate locomotor activity (Palenicek et al., 2005; Walker et al., 2007) and to maintain acquisition of drug self-administration (Vandewater et al., 2015) in female than male rats. Taken together, these results converge in suggesting that female rats are more sensitive than males to MDMA effects thought to result from MDMA-induced increases in DA release, and this issue will be discussed further below. Moreover, these results are also consistent with other evidence to suggest that female rats are more sensitive than males to abuse-related effects of other drugs (e.g. cocaine and methamphetamine) that act at DA transporters and increase extracellular DA levels (Lynch and Carroll, 1999, Reichel et al., 2012, Roth and Carroll, 2004a, b); however, for drugs like MDMA, these differences are not large (Vandewater et al., 2015) and can depend on conditions of the procedure (Caine et al., 2004). Lastly, these results are consistent with human laboratory studies to show that women are more sensitive than men to certain MDMA effects (Liechti et al., 2001, McCann et al., 1994, Pardo-Lozano et al., 2012, Verheyden et al., 2002). For example, Liechti et al. (2001) examined subjective and physiological effects of MDMA in women and men with little or no prior MDMA experience. The dose of MDMA tested (~1.7 mg/kg) produced stronger subjective effects in women, and sex differences were identified for both “positive” effects that might contribute to abuse and be related to ICSS facilitation reported here (e.g. increases in “Positive Basic Mood”) as well as other “negative” effects that may not contribute to MDMA use (e.g. “Fear of Loss of Body Control”). Results of that study were interpreted as evidence for increased susceptibility of women to the 5HT-releasing effects of MDMA (Liechti et al., 2001), but in view of the present study and other preclinical results reported above, females may also be more sensitive to the DA releasing effects of MDMA that might contribute to positive subjective effects.

4.2. Microdialysis

In the present study, baseline NAc DA levels were significantly lower in female compared to male rats, whereas there was no sex difference in baseline 5HT levels. The present DA results are generally consistent with previous studies demonstrating a trend toward higher NAc DA levels in both intact male vs. female rats (Holly et al., 2012) and in male vs. ovariectomized females administered estradiol (Cummings et al., 2014). Differences in basal striatal DA levels as a function of estrous cycle in rats (Xiao and Becker, 1994) and menstrual cycle in nonhuman primates (Czoty et al., 2009) have also been previously reported. In particular, basal DA levels in the striatum were significantly lower in female rats during diestrus than in males (Xiao and Becker, 1994). In contrast to this literature on determinants of DA levels, there is less evidence available with regards to sex differences in the 5HT system. Striatal 5HT levels were higher in the metestrus/diestrus phase of female mice compared to males (Yang et al., 2015), and ventromedial prefrontal cortex 5HT levels were higher in female rats compared to males (Staiti et al., 2011). However, to the best of our knowledge, no studies have examined sex differences in NAc 5HT. Repeated lavage to assess estrous cycle phase was not conducted in this study because it is known to alter effects of monoaminergic transporter drug effects (e.g. cocaine) (Walker et al., 2002).

However, the relatively low baseline DA levels observed here suggest oversampling of the diestrus phase in the current study due to either the longer diestrus duration relative to other estrous cycle phases or because of estrous cycle absence.

MDMA significantly increased both extracellular NAc DA and 5HT levels in both female and male rats in the present study in a dose- and time-dependent function. The present MDMA neurochemical results in male rats are consistent with previous *in vivo* microdialysis studies in both male rats (Baumann et al., 2005, Baumann et al., 2008, Kankaanpää et al., 1998) and male monkeys (Murnane et al., 2010). Moreover, the slower and more sustained effects of MDMA on NAc DA vs. 5HT in the present study are consistent with other non-selective monoamine releaser effects on NAc DA vs. 5HT levels in male rats (Suyama et al., 2016). As with ICSS behavioral studies, the present results extended this profile to females and revealed evidence of subtle sex differences in MDMA effects.

The most robust sex difference in MDMA effects detected in the present neurochemical studies was that MDMA produced more sustained increases in DA culminating in greater peak DA increases in females compared to males. This greater effect of 3.2 mg/kg MDMA on NAc DA levels in females may have contributed to the more sustained ICSS facilitation by 3.2 mg/kg MDMA in females, and this conclusion is consistent with other evidence to suggest that facilitation of ICSS by monoamine releasers correlates with increases in NAc DA release (Suyama et al., 2016). This sex difference in MDMA effects on NAc DA levels is also consistent with previous reports that both amphetamine (Virdee et al., 2014) and cocaine (Holly et al., 2012) also produce greater increases in NAc DA levels in female compared to male rats. However, amphetamine-induced DA release does not differ significantly between females and males in dorsal striatum (Becker, 1999, Becker and Cha, 1989, Castner et al., 1993).

In addition to the sex difference in MDMA effects on NAc DA levels, a dose of 1.0 mg/kg MDMA significantly increased 5HT levels in females but not males, although this effect did not achieve criteria for significance in the comparison of peak 5HT levels after MDMA administration. Monoamine releaser-induced increases in NAc 5HT levels generally correlate with depression of ICSS; however, ICSS depression does not emerge until NAc 5HT reaches relatively high levels (500% of baseline; Suyama et al., 2016). With MDMA, a dose of 3.2 mg/kg MDMA was required to increase NAc 5HT levels above 500% of baseline and to depress high rates of ICSS in both females and males. Although sex differences in MDMA potency to increase 5HT levels was not associated with sex differences in behavior in the present study, it may be related to the higher sensitivity of women to subjective effects of MDMA thought to be mediated by 5HT (Liechti et al., 2001).

4.3. Experimental Design Issues with Female Subjects

For the purposes of this study, estrous cycle in females was not monitored or controlled to avoid interactions between drug effects and repeated vaginal lavage (Walker et al., 2002) and to facilitate preliminary assessment of sex differences in MDMA effects. One issue with sex difference research and a lack of estrous cycle monitoring is whether variance among the female population is greater due to estrous cycle influence (Becker and Koob, 2016,

Prendergast et al., 2014). An increase in variance might preclude findings of a significant difference when one actually exists (Type II error) due to either violation of the homogeneity of variance or because larger variances are likely to reduce the effect size and therefore power (Sullivan, 2012). In this set of studies, the difference in either facilitation of ICSS or increase in extracellular DA and 5HT levels following MDMA administration was robust enough such that variance was not a major factor, and differences were detected using a standard criterion for significance of $p < 0.05$. Nonetheless, the lack of estrous cycle monitoring or control is a limitation of the present study, and it remains possible that hormonal fluctuations associated with the estrous cycle could further influence MDMA effects in females relative to males. For example, basal and drug-altered extracellular 5HT levels in rats were influenced by sex and estrous cycle phase in rats (Manswood et al., 1999), and effects on pre-pulse inhibition in rats also varied across the estrous cycle (Bubenikova et al., 2005). Future studies would be required to address interactions between MDMA effects and ovarian hormones, but the present study was conducted in the spirit of recommendations to encourage studies in females without the additional requirement of controlling for estrous cycle phase (Becker and Koob, 2016).

One other consideration in these studies in female and male rats is that placement of ICSS electrodes and microdialysis probes differed slightly across sexes. Specifically, Figure 4 shows overlapping but generally more caudal placement of ICSS electrodes in females and of microdialysis probes in males. These differences likely resulted from the smaller body size of females and the use of more caudal coordinates for ICSS electrodes in females as described in Methods. It is possible that these differences in rostrocaudal distribution contributed to differences in MDMA effects between males and females. However, three factors suggest that this effect is likely to be minimal. First, rostrocaudal distribution of both electrode and probe placements overlapped in females and males. Second, this overlap was less prominent for ICSS electrodes, but the medial forebrain bundle has a long rostro-caudal axis (from -0.92 mm to -4.8 mm relative to Bregma, Paxinos and Watson, 1998), and prevailing evidence suggests that stimulation anywhere along this rostro-caudal axis activates the same neural substrate (Negus and Miller, 2014). Third, there was more extensive overlap of microdialysis probes, all of which resided in the anatomically defined nucleus accumbens within a 0.9 mm rostro-caudal range from $+1.6$ mm to $+2.5$ mm relative to bregma. Moreover, we are not aware of any studies to show differences in effects of systemic drug administration on dopamine or serotonin release along the rostro-caudal extent of nucleus accumbens. Reverse perfusion of drugs has been reported to produce effects that vary along this dimension (Bowers et al., 2000; Heidbreder and Feldon, 1998), but regions considered too “posterior” in these analyses are more caudal than the most caudal placements observed in this study.

5. CONCLUSION

In conclusion, MDMA produced qualitatively similar behavioral and neurochemical effects in female and male rats; however, a dose of 3.2 mg/kg MDMA produced more sustained increases in both ICSS and in NAc DA in females. These results provide additional support for a relationship between increases in NAc DA and expression of ICSS facilitation in rats.

Moreover, these results provide evidence for sex differences in MDMA effects that might contribute to sex differences in patterns of MDMA abuse.

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Abbreviations

aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
DA	dopamine
FR	fixed ratio
HPLC	high-pressure liquid chromatography
ICSS	intracranial self-stimulation
IP	intraperitoneal
MCR	maximum control rate
MDMA	3,4-methylenedioxymethamphetamine
NAc	nucleus accumbens
NE	norepinephrine
SEM	standard error of the mean
5HT	serotonin

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HIGHLIGHTS

- Time course of 3.2 mg/kg MDMA rate-increasing effects in ICSS greater in females
- MDMA more potent to increase serotonin in nucleus accumbens of females
- 3.2 mg/kg MDMA produces greater DA increases in nucleus accumbens of females

Figure 1. Effects of MDMA on ICSS in female and male rats

Abscissae: Brain stimulation frequency in Hz (log scale). Ordinates: ICSS rate expressed as Percent Maximum Control Reinforcement Rate (%MCR). Left panel shows mean ICSS results for females and right panel shows mean ICSS results for males. The gray line represents ICSS after saline administration, and symbols represent ICSS after the specified test dose of MDMA (0.32 mg/kg: triangles, inverted triangles: 1.0 mg/kg, squares: 3.2 mg/kg). Filled points indicate a significant difference from saline within a sex ($p < 0.05$). All points represent mean $\pm$ SEM in five female rats and six male rats.

Figure 2. MDMA (3.2 mg/kg) time-course effects on ICSS in female and male rats

Abscissae: Brain stimulation frequency in Hz (log scale). Ordinates: ICSS rate expressed as Percent Maximum Control Reinforcement Rate (%MCR). Baseline data (top left panel) were collected and effects of 3.2 mg/kg MDMA administration beginning 10, 30, 100 and 180 minutes in females (triangles) and males (squares) are presented in the bottom four panels. Filled points represent significant differences from baseline responding in either females or males and asterisks represent significant differences between females and males.

All points represent mean $\pm$ SEM in five female and six male rats with significance set at $p < 0.05$.

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Figure 3. Time course of saline and MDMA effects on NAc DA and 5HT levels

Top and middle panels: Abscissae: Time in minutes relative to injection of saline or MDMA.

Ordinates: Percent baseline DA (top panels) or 5HT levels (middle panels) in female rats (left panels) or male rats (right panels). Note that the first two post-injection data points (10 and 20 min) show DA or 5HT levels that were collected before injection, but were not analyzed until after injection due to the time required for these samples to traverse dead space in tubing connecting the microdialysis probe to the electrochemical detector. Filled points indicate a significant difference from saline within a sex ($p < 0.05$). Bottom panels:

Abscissae: dose of MDMA in mg/kg. Ordinates: Peak change in % Baseline DA (left panel) or 5HT (right panel) observed at any time after each injection in females (open bars) and males (filled bars). **Indicates a significant ($p < 0.05$) sex difference. All points represent mean $\pm$ SEM in five female and five male rats.

Figure 4. Electrode and cannula placements

Left panel shows ICSS electrode placements in females (triangles) and males (circles). Right panel shows microdialysis probe placements in females (dashed lines) and males (solid lines). Figures were produced based on comparisons to Paxinos and Watson, 2007.