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ORIGINAL INVESTIGATION

An in vivo microdialysis assessment of concurrent MDMA and cocaine administration in Sprague–Dawley rats

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Abstract
Background and rationale Despite the popularity of polysubstance abuse among recreational methylendioxymethamphetamine (MDMA) users, relatively few controlled experimental studies have documented the neurobehavioral effects of MDMA in combination with other abused substances.
Objective In this study, the combined acute effects of MDMA and cocaine were examined by conducting in vivo microdialysis in the rat nucleus accumbens while simultaneously monitoring locomotor activity.
Methods Male Sprague–Dawley rats were administered cocaine (10 or 20 mg/kg, i.p.), MDMA (1.5 or 3.0 mg/kg, i.p.), or one of four combinations of cocaine and MDMA during microdialysis experiments. Locomotor activity was monitored, and dialysis samples were collected every 30 min for 3 h prior to injections, for one 30-min period following saline injections, and for an additional 3-h period following drug injections. Samples were analyzed for dopamine content by high-performance liquid chromatography with electrochemical detection.
Results Significant differences in locomotor activity and dopamine efflux were found among treatment groups, with some MDMA/cocaine combinations producing significantly greater increases compared to single doses of cocaine or MDMA within the first 30 min after injection.
Conclusion Considering the popularity of polysubstance use among recreational MDMA users, the clinical implications of the current findings warrant further investigation.

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Despite widespread media coverage of the health risks associated with recreational methylendioxymethamphetamine (MDMA or "Ecstasy") use, abuse of this controlled substance continues to be a significant health concern. Of particular concern is the relatively high incidence of polysubstance use (the use of two or more substances in combination) among recreational users of MDMA (Barret et al. 2006; Gouzoulis-Mayfrank and Daumann 2006; Montgomery et al. 2005; Wish et al. 2006) and the potential negative health consequences of chronic excessive use, such as anxiety, obsessive-compulsive behavior, memory deficits, and impaired decision making (Montgomery et al. 2005; Parrott et al. 2001; Reay et al. 2006). Survey studies indicate that polysubstance use among MDMA users is fairly common, especially MDMA in combination with psychostimulant drugs, such as amphetamine, methamphetamine, or cocaine (Khorana et al. 2004; Riley et al. 2001; Williams et al. 1998; Winstock et al. 2001). Lua et al. (2003) analyzed urine samples from police detainees in Taiwan and found evidence for a high rate of MDMA use in combination with other illicit drugs. Scholey et al. (2004) reported significantly greater psychoactive drug use among experienced MDMA users compared to nonusers based on an internet survey. These data revealed that cocaine use was greatest among heavy Ecstasy users (81%) compared to novice (44%) and moderate (61%) MDMA users. Wish et al. (2006) conducted a self-report-based study with a population of college students. Their results indicated that MDMA users are more likely to have used cocaine, heroin, lysergic acid diethylamide, other hallucinogens, and inhalants. In relation to psychostimulant use, MDMA preceded cocaine use in 46% of the individuals surveyed.

Although polysubstance abuse among MDMA users is well documented, a review of the experimental literature revealed a significant paucity of research involving animal models of polysubstance use. Indeed, a number of researchers investigating the psychopathology of drug abuse have called for further investigations of polysubstance use to include both human (Barret et al. 2006; Gouzoulis-Mayfrank and Daumann 2006; Wish et al. 2006) and animal studies (Gouzoulis-Mayfrank and Daumann 2006). A few preclinical studies have examined the neurochemical and behavioral effects of MDMA pretreatment regimens prior to cocaine administration (Aberg et al. 2007; Cole et al. 2003; Horan et al. 2000; Morgan et al. 1997a; Kalivas et al. 1998). MDMA pretreatment regimens in these studies consisted of those known to produce serotonergic neurotoxicity (e.g., 10–20 mg/kg twice a day for 4 days) or regimens considered subneurotoxic (repeated administration of doses less than 10 mg/kg). A number of these studies have investigated the effects of MDMA pretreatment on cocaine-induced conditioned place preference (CPP) in rodents (Aberg et al. 2007; Cole et al. 2003; Horan et al. 2000). In addition, in vivo microdialysis studies have demonstrated that MDMA pretreatment enhances dopamine (DA) efflux in the nucleus accumbens (Morgan et al. 1997a). Results of these studies suggest that prior MDMA exposure modulates the neurobehavioral effects of psychostimulant drugs, which may influence vulnerability to the abuse liability of these drugs.

Although several studies have examined the effects of repeated MDMA exposure on subsequent effects of cocaine, only a few studies have examined the behavioral effects of concurrent administration of these two drugs in rats (Diller et al. 2007) or adolescent mice (Daza-Losada et al. 2009). The latter study also examined postmortem monoamine levels in the striatum, cortex, and hippocampus, in mice euthanized 25 min following acute injections. There are currently no published reports on the acute in vivo neurochemical effects of low dose MDMA and cocaine administered concurrently. The present study examined the effects of concurrent MDMA and cocaine administration on locomotor activity and nucleus accumbens DA levels using in vivo microdialysis sampling techniques in rats.

Method

Animals

Subjects consisted of 84 drug naïve male Sprague–Dawley rats (Charles River Laboratories, Portage, MI, USA) aged 5–7 months and weighing approximately 500 g. Animals were individually housed in polycarbonate cages with corn cob bedding and free access to water and standard rodent diet. Prior to surgery and during recovery from surgery, animals were housed in an animal colony that was maintained under constant temperature (20±2°C) and humidity (50±5%) conditions and on a 12-h/12-h light/dark cycle (lights on at 0700 hours and off at 1900 hours). All procedures were approved by Western Michigan University's Institutional Animal Care and Use Committee. In accordance with animal welfare policy, all efforts were made to minimize pain and distress and the number of animals used.

Drugs

Cocaine-hydrochloride and 3,4-methylenedioxymethamphetamine (MDMA) were obtained from the National Institute on Drug Abuse (Rockville, MD, USA). Sodium pentobarbital was purchased from Sigma-Aldrich (St. Louis, MO, USA), and atropine sulfate was purchased from Calbiochem (San Diego, CA, USA). All drugs were dissolved in 0.9% sterile saline and administered by intraperitoneal injection. Drug doses were calculated based on the weights of the salts.

Surgical procedures

Rats were injected with 1.0 mg/kg atropine sulfate (Calbiochem, San Diego, CA, USA) 15 min prior to being anesthetized with 51.0 mg/kg sodium pentobarbital (Sigma-Aldrich, St. Louis, MO, USA). Animals were then placed in a Kopf small animal stereotaxic device (David Kopf Instruments, Tujunga, CA, USA) and maintained at 37.5°C using a Gaymar T/PUMP heat therapy pump (Gaymar Industries Inc, Orchard Park, NY, USA). Removable guide cannulae (Bioanalytical Systems Inc, West Lafayette, IN, USA) were stereotaxically implanted in the nucleus accumbens (AP +1.70, ML −1.50, and DV −6.20) with the incisor bar adjusted to achieve the flat skull position (Paxinos and Watson 1998). Guide cannulae were secured in place with three small jewelers' screws (Bioanalytical Systems Inc, West Lafayette, IN, USA) and dental cement (PERM, Hygenic Corp, Akron, OH, USA). Animals were allowed to recover from surgery for at least 4 days prior to experimental testing.

Microdialysis and locomotor activity sampling procedures

Locomotor activity assessment and in vivo microdialysis procedures were conducted simultaneously in six identical custom designed Plexiglas chambers (40.5 cm L×40.5 cm W×40.5 cm H) equipped with a Versamax® Activity Monitoring System (Accuscan Instruments Inc., Columbus, OH, USA). All locomotor data were collected in 30-min intervals that coincided with microdialysis sample collection times (see below). The primary locomotor activity measure included in the data analysis was the total dis-

tance traveled (centimeters) during each 30-min sampling period.

BAS BR-2 microdialysis probes with a 2-mm membrane (Bioanalytical Systems Inc, West Lafayette, IN, USA) were flushed with artificial cerebral spinal fluid (147.2 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, and 1.0 mM $MgCl_2$) at a flow rate of 0.5 µl/min 12 h prior to calibration using a BAS Bee Hive syringe pump controller (MD-1020), BAS Baby Bee syringe pumps (MD-1001), and 1.0 ml BAS Bee Stinger gas tight syringes (MDN-0100). At 0600 hours, probes were placed in a calibration solution containing 15 nM DA, and a 30-min calibration sample was collected at a flow rate of 1.5 µl/min. Between 0700 and 0800 hours, microdialysis probes were inserted into guide cannulae, and rats were tethered to Instech fluid swivels (Plymouth Meeting, PA, USA) attached to counter-balanced arms and placed in the activity monitoring chambers. Microdialysis flow rates were maintained at 1.5 µl/min for the duration of each experiment. Following insertion, the probes were allowed to equilibrate for 3 h prior to testing. At approximately 1100 hours, microdialysis sample collection began. Samples were collected at 30-min intervals at a constant flow rate of 1.5 µl/min to produce a 45-µl sample volume. The microdialysis sampling regimen consisted of six 30-min baseline samples, one 30-min sample following saline injections, and six additional 30-min samples following drug injections, for a total of 13 samples per animal. Immediately following collection, samples were flash frozen and stored in a −80°C freezer until high-performance liquid chromatography (HPLC)-electrochemical detection analysis.

Drug treatments consisted of cocaine (10 or 20 mg/kg), MDMA (1.5 or 3.0 mg/kg), or one of the following drug combinations: cocaine 10 mg/kg+MDMA 1.5 mg/kg (C10/M1.5), cocaine 10 mg/kg+MDMA 3.0 mg/kg (C10/M3.0), cocaine 20 mg/kg+MDMA 1.5 mg/kg (C20/M1.5), or cocaine 20 mg/kg+MDMA 3.0 mg/kg (C20/M3.0). All animals received two saline injections immediately before the saline sampling period and two injections immediately before the drug sampling period. For those animals administered only one drug, the second injection prior to the drug sampling period consisted of saline. Experiments were conducted simultaneously in six rats per day, with random assignment of animals to the treatment groups. Initial experiments were conducted with MDMA (1.5 or 3.0 mg/kg) in combination with 10 mg/kg cocaine prior to investigating these MDMA doses in combination with 20 mg/kg cocaine. Eight rats were excluded from the study for various reasons, including surgical complications, improper probe placement, or signs of infection upon histological examination. Data from an additional six animals were excluded from the statistical analyses due to insufficient sample volumes in one or more sampling periods.

High-performance liquid chromatography-electrochemical detection

Within 4 days after initial freezing, each batch of 26–52 samples was removed from the −80°C freezer, placed in a −4°C refrigerated autosampler, and analyzed over a 6.5–13 h period. DA was detected by reverse phase HPLC coupled to electrochemical detection using an ESA Coulochem II Model 5200 detector, an ESA 582 solvent delivery module, an MD-150/RP-C18 column (particle size 3 µm, 3.0×150 mm i.d.), a model 5014 analytical cell, and a PC running ESA 501 chromatography software (ESA, Chelmsford, MA, USA). The detector settings were as follows: guard cell, 350 mv; CH1, −175 mv; and CH2, 250 mv; all filter settings were set to 5 s. A commercially prepared mobile phase, MD-TM (ESA, Chelmsford, MA, USA) was used consisting of 75 mM sodium dihydrogen phosphate monohydrate, 1.7 nm 1-octanesulfonic acid, 100 µl/L triethylamine, 25 µm EDTA, 10% acetonitrile, and adjusted to a pH=3.0 with phosphoric acid. Probe recovery data were calculated by HPLC analysis of the 30-min calibration samples collected prior to probe insertion. Probe recoveries averaged 22.23% (±0.63).

Histology

Immediately following the completion of microdialysis procedures, rats were euthanized by injection of a solution containing sodium pentobarbital (50 mg/kg) dissolved in a 60% ethanol and then perfused at a constant pressure of 300 mm/hg with a 10% sucrose solution followed by a 10% formalin solution using a Perfusion One pressurized perfusion system (myNeuroLab, St Louis, MO, USA). The microdialysis guides were removed after the perfusion, and the brains were removed from the skulls and stored in 10% formalin. A Vibratome 1500 sectioning system (Ted Pella Inc, Redding, CA, USA) was used to slice coronal sections at a thickness of 70 µm. Coronal sections were mounted on microscope slides, and probe placements were confirmed. Probe placements were within the nucleus accumbens target for all 70 animals included in the statistical analyses (see Fig. 1).

Data analysis

The primary dependent measures analyzed were total distance traveled (centimeters) and DA concentrations in dialysate during each 30-min sampling period. For graphic and statistical analyses, DA concentrations were expressed as a percentage of average baseline levels. Averages were calculated from the six baseline microdialysis samples, and data points were graphed as a percentage of this average. The data for both dependent measures were graphed as follows: group means (±SEM) for the average of six baseline sampling periods (AVBL), group means for the

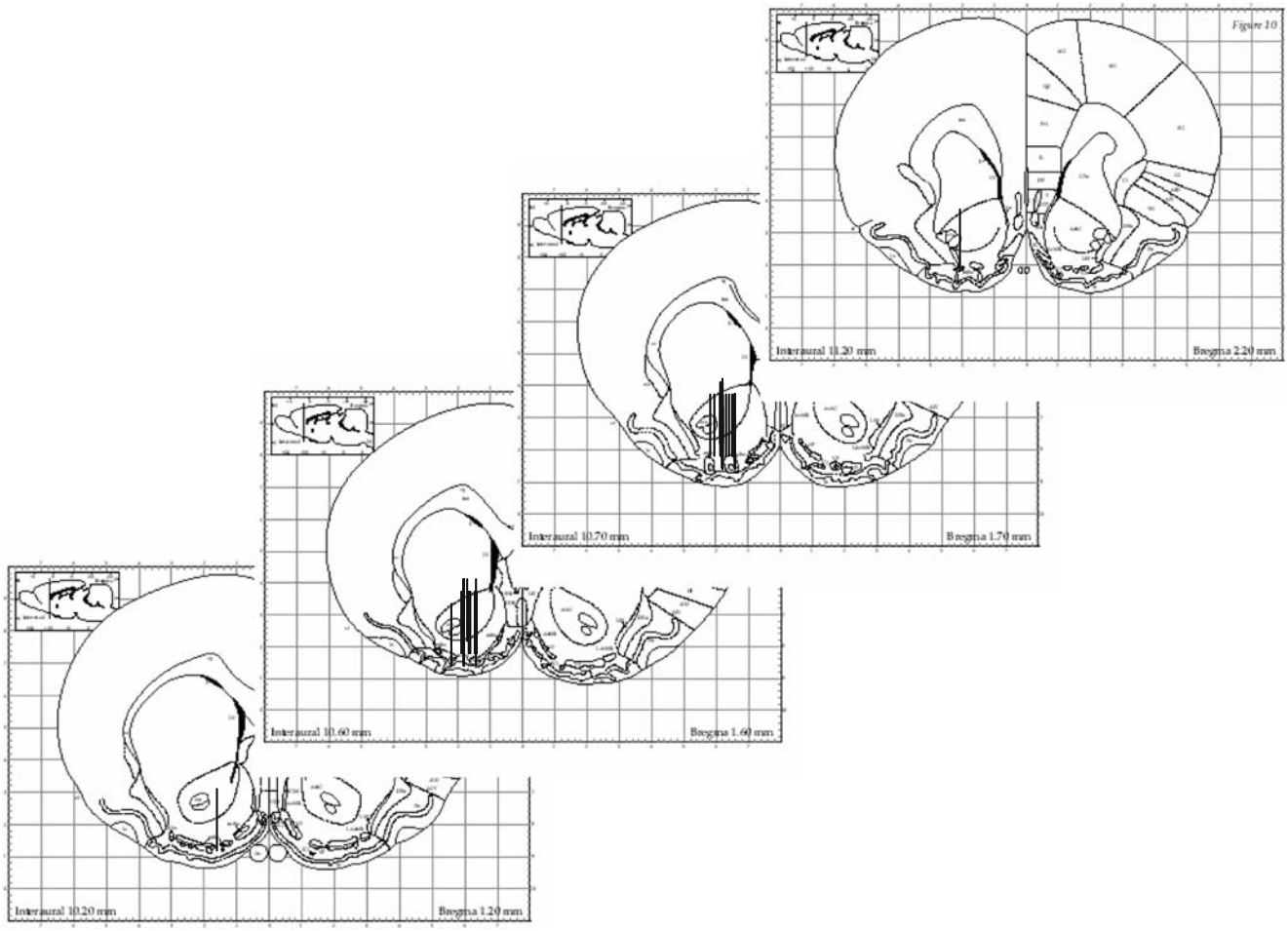

Fig. 1 Probe placements for all animals included in the data analyses were within the shell or lateral shell of the nucleus accumbens

30-min sampling period following the saline injection, and group means for each of the six sampling periods following drug injections (PI 30–180 min). These data were statistically analyzed using a two-factor repeated measures analysis of variance (ANOVA) with treatment group as a between-subjects factor and sampling period as a within-subjects factor. In addition, Bonferroni post-test comparisons were conducted among all treatment groups. A Pearson *r* correlational analysis was also conducted to assess the relationship between DA and distance traveled during the first post-injection sampling period.

Results

This study represents the first in vivo microdialysis investigation of concurrent acute administration with MDMA and cocaine in which locomotor activity and extracellular DA levels in the nucleus accumbens were sampled simultaneously in rats. Drug induced increases in locomotor activity, and extracellular DA levels during the first post-injection period appeared to be positively correlated. A Pearson correlation test indicated a linear relationship between DA and distance traveled during the first post-injection sample ($R=0.446$, $p<0.05$).

The effects of each drug alone and each MDMA/cocaine dose combination on locomotor activity are displayed in Fig. 2, which depicts the total distance traveled during each 30-min sampling period over a period of 3 h after the administration of cocaine (10 or 20 mg/kg), MDMA (1.5, 3.0 mg/kg), or each of the four dose combinations (C10/M1.5, C10/M3.0, C20/M1.5, or C20/M3.0). For each treatment group, the average distance traveled during pre-drug baseline sessions (AVBL) and the distance traveled following saline injections administered 30 min before the drug injections are also displayed in these graphs for comparison. For illustration purposes, data for the cocaine-only treatment groups are plotted in both the left graph and right graph in Fig. 1. As expected, both cocaine doses produced significant increases in locomotor activity well above baseline levels, with greater increases produced by 20 mg/kg cocaine. In contrast, both MDMA doses

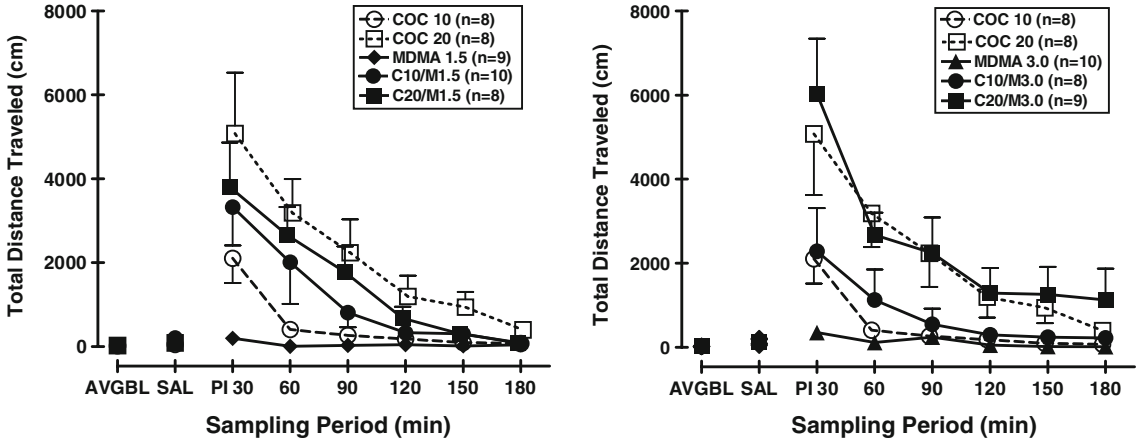

Fig. 2 Effects of methylendioxymethamphetamine (MDMA)/cocaine combinations on locomotor activity. Group means (±SEM) are plotted for the average distance traveled over six baseline sampling periods (AVBL), the distance traveled during a single 30-min sampling period following saline injections (SAL), and during six consecutive 30-min sampling periods following drug injections (PI 30–180 min). The *left graph* depicts treatment groups administered MDMA (1.5 mg/kg) and cocaine (10, 20 mg/kg) alone or in combination. The *right graph* depicts treatment groups administered MDMA (3.0 mg/kg) and cocaine (10 or 20 mg/kg) alone or in combination. The following post hoc comparisons were statistically significant at the PI 30-min sampling period only: C10 vs. C20/M3.0*, M1.5 vs. C20**, M1.5 vs. C10/M1.5*, M1.5 vs. C20/M1.5*, M1.5 vs. C20/M3.0**, M3.0 vs. C20**, M3.0 vs. C20/M1.5*, M3.0 vs. C20/M3.0**, and C10/M3.0 vs. C20/M3.0* (*$p<0.05$, **$p<0.01$)

produced only modest increases in activity compared to baseline levels. Visual inspection of the graphed data suggests that 1.5 mg/kg MDMA enhanced the locomotor effects of 10 mg/kg cocaine, particularly at earlier time intervals (PI 30–PI 90), but suppressed the effects of 20 mg/kg cocaine at all post-injection intervals (see Fig. 2, left). In contrast, 3.0 mg/kg MDMA appears to have had minimal effects when combined with either dose of cocaine (see Fig. 2, right). A two-way repeated measures ANOVA on these data showed statistically significant effects of treatment group [$F(7,434)=5.24$, $p<0.001$], sampling period [$F(7,434=51.67$, $p<0.001$], and the interaction [$F(49,434)=3.85$, $p<0.001$] between treatment group and sampling period. Bonferroni post-tests revealed significant differences in locomotor activity only during the first 30 min post-drug injection sampling period for the following group comparisons: C10 vs. C20/M3.0 ($p<0.05$), M1.5 vs. C20 ($p<0.01$), M1.5 vs. C10/M1.5 ($p<0.05$), M1.5 vs. C20/M1.5 ($p<0.05$), M1.5 vs. C20/M3.0 ($p<0.001$), M3.0 vs. C20 ($p<0.01$), M3.0 vs. C20/M1.5 ($p<0.05$), M3.0 vs. C20/M3.0 ($p<0.001$), and C10/M3.0 vs. C20/M3.0 ($p<0.05$).

Figure 2 depicts extracellular DA levels in the nucleus accumbens represented as a percentage of average baseline levels. Group means are plotted for the average baseline (AVBL), the 30-min post-saline injection sampling period, and for six subsequent 30-min post-drug injection sampling periods (PI 30–180 min). When administered alone, 10 mg/kg cocaine increased nucleus accumbens DA levels to 254% of baseline, and 20 mg/kg cocaine increased DA levels to 431% of baseline during the first 30-min sampling period. When administered alone, MDMA produced modest increases in nucleus accumbens DA levels, with the greatest increase observed during the second post-injection sampling period (60 min). MDMA 1.5 mg/kg increased DA levels to 128% and 151% of baseline in the 30- and 60-min post-injection sampling periods, respectively. MDMA 3.0 mg/kg increased DA levels to 163% and 194% of baseline in the 30- and 60-min post-injection sampling periods, respectively. The combined administration of 10 mg/kg cocaine and 1.5 mg/kg MDMA (C10/M1.5) increased nucleus accumbens DA levels to 370% of baseline. The combination of 20 mg/kg cocaine and 1.5 mg/kg MDMA (C20/M1.5) increased nucleus accumbens DA levels to 417% of baseline. The 10-mg/kg cocaine+3.0 mg/kg MDMA combination (C10/M3.0) increased nucleus accumbens DA levels by 283%, and the 20-mg/kg cocaine+3.0-mg/kg MDMA combination (C20/M3.0) increased these levels by 707%. Statistical analyses showed significant effects of treatment group [$F(7,434=5.40$, $p<0.001$], sampling period [$F(7,434)=61.78$, $p<0.001$], and a significant interaction [$F(49,434)=4.63$, $p<0.001$] between treatment group and sampling period. Bonferroni post-tests showed significant differences for the following group comparisons: C10 vs. C20/M3.0 for both the 30-min ($p<0.01$) and 60-min post-injection samples ($p<0.05$), M1.5 vs. C20/M1.5 for the 60-min post-injection sample ($p<0.05$), M1.5 vs. C20/M3.0 for both the 30-min ($p<0.001$) and 60-min post-drug injection samples ($p<0.01$), M3.0 vs. C20/M3.0 for both the 30-min ($p<0.001$) and 60-min post-drug injection samples ($p<0.05$), and C10/M3.0 vs. C20/M3.0 for the 30-min post-injection sample ($p<0.05$). There were no significant differences among treatment groups at later post-injection times.

Discussion

The current findings are in accordance with the well-established actions of psychomotor stimulants on the mesocorticolimbic DA system (Bozarth 1986; Bozarth and Wise 1986). Also consistent with previous findings, cocaine produced locomotor activation and increased DA efflux to a greater extent than MDMA (Koch and Galloway 1997). Moreover, the present results support recent reports that the combined acute administration of MDMA and cocaine may produce greater locomotor activation and enhance dopaminergic responses to a greater extent than either drug administered separately, but the combined effects of these drugs depend on the particular dose combination. A review of the published literature revealed only two previous studies that have explored the combined acute effects of MDMA and cocaine (Diller et al. 2007; Daza-Losada et al. 2009). Diller et al. (2007) assessed the concurrent administration of cocaine and MDMA on CPP in adult male Sprague–Dawley rats. In that study, MDMA (0, 5.0, or 10 mg/kg) was administered 25 min prior to cocaine (0, 2.5, or 5.0 mg/kg) injections, in order to allow for the peak actions of cocaine and MDMA to occur simultaneously. Results indicated that 5.0 mg/kg MDMA enhanced the effects of 2.5 mg/kg cocaine, but actually diminished the effects of 5.0 mg/kg cocaine. Furthermore, the addition of either 2.5 or 5.0 mg/kg cocaine systematically increased CPP induced by 10 mg/kg MDMA, indicating that decreased reward properties of high levels of MDMA are reversible by cocaine administration. Although the present study examined lower MDMA doses (1.5 or 3.0 mg/kg) and higher cocaine doses (10 or 20 mg/kg) and assessed acute locomotor activation and dopaminergic responses of these drug combinations rather than drug-induced place preference, the current findings are generally congruent with those of Diller et al. (2007) in that some, but not all, MDMA/cocaine dose combinations produced greater behavioral activation and greater increases in DA efflux than either drug administered alone.

Using several behavioral screening assays of anxiolytic activity (elevated plus maze, locomotor activity, social interaction test, and a passive avoidance test), Daza-Losada et al. (2009) investigated the effects of concurrent MDMA (5, 10, or 20 mg/kg) and cocaine (25 mg/kg) administration in adolescent mice. Cocaine, MDMA, and cocaine/MDMA combinations increased motor activity and reduced social contacts. In the elevated plus maze, cocaine/MDMA combinations significantly increased time spent in the open arms compared to either drug alone. In separate experiments, these investigators also analyzed postmortem brain tissue content of biogenic amines 25 min after treatment with these drug combinations. They reported an increase in striatal DA turnover as indicated by increased ratios of DOPAC/DA and HVA/DA in animals that were administered MDMA/cocaine combinations, indicative of increased DA availability at the synapse. These findings suggest concurrent administration of MDMA, and cocaine may increase DA efflux to a greater extent than either drug alone. The current results are generally consistent with those of Daza-Losada et al. (2009) and extend these findings to the in vivo neurochemical effects of MDMA/cocaine combinations.

The individual effects of cocaine and MDMA on extracellular DA efflux are well documented. Cocaine increases DA efflux in the nucleus accumbens shell (Morgan et al. 1997a, b;

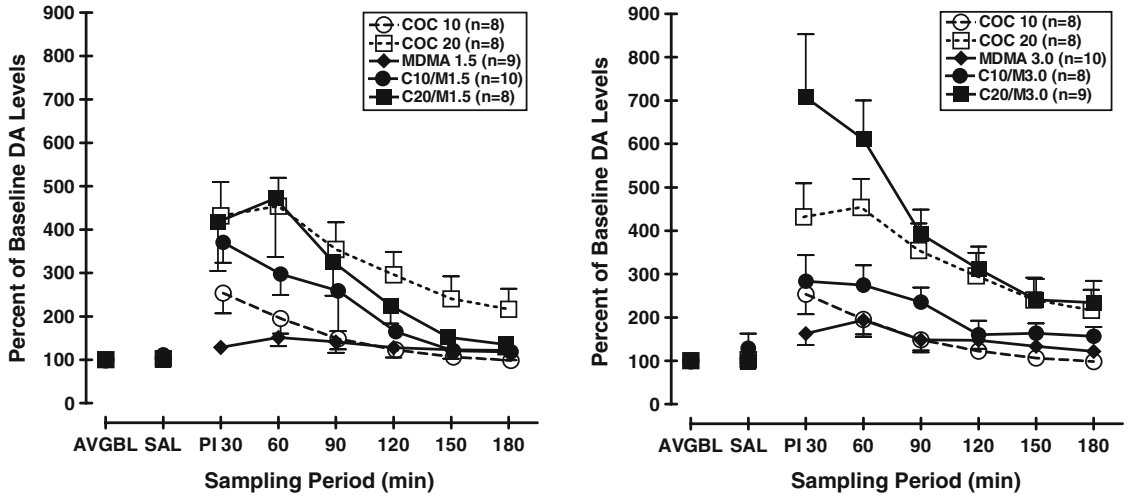

Fig. 3 Percentage increase in extracellular dopamine levels in the nucleus accumbens. DA levels are expressed as a percentage of average baseline levels. Data points represent group means (±SEM). See Fig. 2 for additional details. The following post hoc comparisons were statistically significant: C10 vs. C20/M3.0 at PI 30 min** and 60 min*, M1.5 vs. C20/M1.5 at PI 60 min*, M1.5 vs. C20/M3.0 at PI 30 min** and PI 60 min**, M3.0 vs. C20/M3.0 at PI 30 min** and PI 60 min*, and C10/M3.0 vs. C20/M3.0 at PI 30 min* (*$p<0.05$, **$p<0.01$)

Shimada et al. 1996; Bradberry 1994, 2002). MDMA has also been demonstrated to enhance DA transmission (Cadoni et al. 2005; Green et al. 2003; Koch and Galloway 1997), although the mechanisms related to the efflux of extracellular DA levels differ. Cocaine's blockade of DA transporters generates an increase in extracellular levels of DA, whereas amphetamine analogs, including MDMA, have been shown to reverse DA transporter activity and in turn increase DA efflux (Metzger et al. 1998). MDMA appears to modulate DA release by more than one mechanism (1) by producing actions analogous to amphetamine on DA terminals (Cadoni et al. 2005) and (2) by releasing serotonin by acting on 5-HT_2 receptors (Bradberry 1994; Cadoni et al. 2005; Koch and Galloway 1997). Furthermore, it has been hypothesized that enhanced 5-HT release may act via 5-HT_{2c} receptors to suppress the psychomotor effects of cocaine (Burmeister et al. 2004), although this suppression of the psychomotor effects may be negated after DA efflux reaches a critical point (Fletcher et al. 2006). In consideration of this hypothesis, the relatively low doses of MDMA examined in the current study may have been insufficient to suppress the behavioral activation induced by cocaine because the DA efflux induced by 10 and 20 mg/kg cocaine was already substantially high such that MDMA's suppressive effects were negligible. The present data indicate that MDMA (1.5 or 3.0 mg/kg) did not suppress the psychomotor stimulant effects of cocaine (10 or 20 mg/kg), although the 1.5-mg/kg MDMA dose did produce a modest suppression of the behavioral activation induced by 20 mg/kg cocaine. However, this effect was not statistically significant. In contrast, the 3.0-mg/kg MDMA dose appeared to slightly enhance the locomotor stimulant effects of 20 mg/kg cocaine. Similarly, 1.5 mg/kg MDMA slightly enhanced the locomotor effects of 10 mg/kg cocaine, but 3.0 mg/kg MDMA failed to do so. It is conceivable that higher MDMA doses are required to modulate the psychomotor stimulant effects of cocaine. As noted above, Diller et al. (2007) reported that 5.0 mg/kg MDMA diminished the effects of 5.0 mg/kg cocaine in a CPP assay.

Regarding the combined effects of MDMA and cocaine on DA efflux in the current study, the greatest increase was observed following the C20/M3.0 combination. Although there was a trend toward enhanced DA efflux by MDMA/cocaine combinations compared to the effects of cocaine (10 or 20 mg/kg) alone and the ANOVA comparing all groups indicated a statistically significant treatment effect, Bonferroni post-test comparisons indicated that only some group comparisons were statistically significant. The post-test comparisons of interest (C10 vs. C10/M1.5, C10 vs. C10/M3.0, C20 vs. C20/M1.5, and C20 vs. C20/M3.0) were not among those that were statistically significant. See Fig. 3 legend for the post-test comparisons that were statistically significant. The MDMA doses examined in the current study were selected because they more closely approximate the doses typically used by humans. Further investigations of the psychomotor stimulant and neurochemical effects of a wider range of MDMA/cocaine dose combinations are needed to supplement the current findings and those of Diller et al. (2007) and Daza-Losada et al. (2009).

Recent investigations regarding the prevalence of polysubstance abuse have demonstrated the popularity of MDMA use in conjunction with cocaine (Chinet et al. 2007; Marsden et al. 2006). Both cocaine and MDMA produce forward locomotion and elevations in extracellular DA in experimental animal models. The results of the present study showed that in combination, these drugs produce a significant increase in extracellular DA levels and moderate increases in locomotor activity, and some, but not all, MDMA/cocaine dose combinations were greater than the effects of either drug administered separately. These preclinical data may be relevant to understanding the heightened abuse liability associated with polysubstance use. Considering the dearth of preclinical investigations of MDMA/cocaine combinations, extensive studies of the neurochemical, neurobehavioral, and neurotoxic actions of this drug combination are warranted. The next phase of preclinical investigations of concurrent MDMA/cocaine administration ought to involve drug self-administration procedures to determine the relative reinforcing efficacy of this drug combination in comparison to that of each drug alone. The clinical implications of the current findings also warrant further investigation.

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