GENDER-SPECIFIC MODULATION OF

COCAINE-INDUCED PLACE PREFERENCE

BY 3,4-METHYLENEDIOXYMETHAMPHETAMINE

A Senior Honors Thesis

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ABSTRACT

Gender-Specific Modulation of Cocaine-Induced Place

Preference by 3,4-Methylenedioxymethamphetamine. (April 2004)

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In contemporary North-American culture, two frequently combined drugs possessing abuse liability are cocaine and "ecstasy" (+/- 3,4-methylenedioxy-methamphetamine [MDMA]). Each drug is a stimulant acting on brain dopamine sites, i.e., cocaine acts to block reuptake of dopamine while ecstasy increases voltage-gated release of the neurotransmitter. With recurrent use, disturbances in dopamine regulation may alter the reward properties of these drugs when presented singularly or concurrently. Because an increasing number of drug abusers are female (currently, females comprise nearly one-third of the drug-abusing population), possible differential drug sensitivity in females has emerged as an issue and could affect treatment programs. This is the first study to test for behavioral alterations across gender with concurrent administration of these frequently abused drugs. It was hypothesized that elevated levels of extracellular dopamine associated with a combined exposure regimen would increase CPP relative to the case where each drug was administered alone, and it was predicted this effect would be amplified in females relative to males. Adult male and female rats received one of three doses of cocaine (0 mg/kg, 2.5 mg/kg, 5 mg/kg) followed by MDMA (0 mg/kg, 5 mg/kg, 10 mg/kg) and were then tested in a CPP paradigm. Specifically, conditioned place preference (CPP), a commonly used model for studying

the role of contextual cues in drug reward and drug-seeking, was employed in this investigation that sought to explore possible synergism between cocaine and MDMA (ecstasy) in male and female rats. These results, however, suggest a biphasic effect of MDMA reward and a linear correlation between cocaine dose and reward properties. Concurrent administration displays antagonism of each drug. Females displayed less overall sensitivity than males except at the highest level of MDMA. This suggests that chronic drug abuse potential may vary according to both drug dose and gender.

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INTRODUCTION

Poly-drug abuse and "being female" correlate with negative psychological experiences among MDMA users (Topp et al., 1999). Though 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") is an illegal substance, it is gaining global popularity as a recreational drug, especially in youth culture and at all-night dance parties called "raves." The drug's increasing appeal may be attributable to positive subjective effects such as euphoria, mental stimulation, emotional tenderness, and decreased anxiety (Schenk et al., 2003; Zhou et al., 2003), which in humans are experienced concomitantly with MDMA use or within the first 24-hour period following drug exposure. Adverse psychological effects of MDMA such as severe depression, loss of appetite and cognitive impairment, which are attenuated by polydrug abuse and gender, were reported in humans more than 24-hours after taking the drug (Verheyden et al., 2003). Positive effects and residual adverse effects of MDMA may reflect the initial rush of 5-HT (5-hydroxytryptamine, [serotonin]) followed by attenuation of 5-HT levels (Verheyden et al., 2003).

Drug effects neurochemically and behaviorally have been attributed primarily to the mesolimbic dopamine system, which runs from the ventral tegmental area to the nucleus accumbens (Higgins and Fletcher, 2003). Dopamine has been implicated as mediating the stimulatory effects of cocaine, which acts as a dopamine transport inhibitor (blocking reuptake and increasing dopamine availability at D1 and D2

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postsynaptic receptors) (Riley, 1995). Cocaine and MDMA vary in the time onset of their observed effects. Cocaine simultaneously increases extracellular concentrations of serotonin and dopamine in contrast to MDMA, which causes an immediate increase of serotonin following injection and delayed extracellular increase of dopamine (White et al., 1994). Rodent studies suggest that cocaine may enhance neurotoxic potential of MDMA by increasing dopamine release (Horan et al., 2002; Fletcher et al., 2001).

Several studies additionally link drug potentiation to other neurotransmitter systems, which modify dopamine function or act independently of the dopaminergic system (Higgins and Fletcher, 2003; Bardo, 1998). Dopamine outside of the nucleus accumbens can also affect drug reward mechanisms; for instance, increases in dopamine activity in the medial prefrontal cortex (mPFC) have been linked with the reinforcing effects of cocaine in the conditioned place preference paradigm (Zavala et al., 2003).

Cocaine and MDMA both affect serotonin levels at the synapse. Though long associated with food reward, central serotonin centers similarly influence other reward-associated behaviors, especially those of drugs of abuse (Higgins and Fletcher, 2003). Serotonin has a role in motor control, pain perception, and sympathetic nervous system outflow. At the human level, excessive levels of serotonin can cause a multitude of symptoms commonly known as "Serotonin Syndrome" manifested as mental alterations such as confusion or agitation, autonomic changes such as hyperthermia and hypertension, and neuromuscular variations observable through muscle jerks and hyperactivity. Serotonin neurons innervate the dopaminergic system, which is associated with the rewarding and potentially addictive properties of drug use. Mounting evidence indicates that activating some serotonin receptor subtypes facilitates dopamine effects (Slikker et al., 1989).

MDMA and cocaine both block reuptake transporters for serotonin, dopamine, and norepinephrine, acting, however, through different mechanisms (Slikker et al., 1989). Cocaine selectively inhibits reuptake transporters and increases serotonin release whereas MDMA inhibits monoamine oxidase (Leonardi & Azmitia, 1994), which increases synaptic levels of monoamines (serotonin and norepinephrine). MDMA acts

primarily by stimulating the release of and successively blocking the reuptake of serotonin, thereby considerably amplifying the availability of the neurotransmitter in the synapse (Schenk et al., 2003; White et al., 1994).

Increased levels of serotonin neurotransmitter coincides with a reduction in self-administration of cocaine and suggests a decrease in the drug's rewarding properties. In other instances, lowered serotonin neurotransmitter availability enhances drug-reward behavior. However, serotonin studies have not consistently defined the relationship between serotonin levels and drug-reward behavior (Higgins and Fletcher, 2003).

In animals, at low doses (1-5 mg/kg), MDMA has limited neurotoxic potential and acts primarily as an indirect serotonin agonist (Zhou et al., 2003). Additionally, MDMA administration also acts to increase synaptic levels of dopamine by directly inhibiting the dopamine transporter, which can act in addition to serotonin release (Schenk et al. 2003). Repeated or high dose (>20 mg/kg) administrations of MDMA to rats are neurotoxic to serotonin-containing axons (Green et al., 1995).

Serotonergic mechanisms have been suggested in behavioral effects of MDMA such as hyperactivity, as stimulus properties of MDMA generalized to serotonin agonists and some effects of MDMA were attenuated by prior administration of serotonin uptake inhibitors or pharmacological manipulations of serotonergic systems (Bankston and Cunningham, 2002; Callaway et al., 1990; Liechti and Vollenweider; 2000Schenk et al., 2003). Furthermore, recent studies with Rhesus monkeys implicate serotonergic mechanisms for MDMA's reward properties as pretreatment with the 5-HT_2 antagonist, ketanserin, decreased MDMA self-administration (Fantegrossi et al. 2002). Though MDMA has been implicated as a monoamine oxidase inhibitor responsible for decreasing serotonin metabolism, it also has been found to increase serotonin release.

The lethality of MDMA is attributed to its hyperthermic effect, an increase in core body temperature, that shortly follows hyperactivity. Unlike normal exercise-induced hyperthermia marked by increased cutaneous blood flow, MDMA contributes to hyperthermia by reducing cutaneous blood flow through vasoconstriction, diminishing normal heat transfer from the body to the environment. Further evidence of MDMA's

ability to impair heat dissipation is seen in studies that report a failure of tail skin temperatures to increase. This hyperthermic effect also exacerbates dopamine neurotoxicity. Relatively small, physiologically relevant changes in temperature significantly lower dopamine transporter function. The hyperthermic effects of MDMA are dose-dependent, and cocaine also has the propensity to cause hyperthermia. Cocaine activates neuromuscular junctions to speed the body's metabolism, which increases heat production. This hyperthermic effect is seen most often in hot conditions, and may impair thermoregulatory adjustments that mediate heat dissipation; even small doses in humans have been shown to impair sweating and dilation of cutaneous vessels as well as disrupting heat perception. This can be seen at levels as low as 2 mg/kg in rats.

Both cocaine and MDMA have been demonstrated separately to possess abuse liability (Schenk et al., 2003). Previous studies pre-exposed animals to MDMA at a single dose and later tested them with CPP or self-administration. These studies exhibit lasting neurological alterations produced by repeated MDMA exposure as no MDMA was in the animals at the time of testing with cocaine (delay between MDMA exposure and CPP or self-administration study was longer than the half-life of MDMA). Consistent with concurrent polydrug abuse trends among rave attendees, this is the first study of its nature to test the behavioral effects of near-simultaneous exposure to both MDMA and cocaine.

The present research is also novel in its appraisal of the behavioral effects of cocaine and ecstasy between gender. Male rat studies have traditionally dominated areas of neuropscyhopharmacology research. However, women are comprising an increasingly larger percentage of the drug abusing population and rave participants. Reports indicated that of all reproductive-age women, approximately 10% engage in regular use of illegal drugs, 10% of which abuse cocaine (King et al., 1990). A new push has been made to understand the perplexing differences seen in drug abuse patterns as well as differential responses to drug therapy between men and women. For instance, the patterns of cocaine abuse between men and women differ, wherein women experience an enhanced response and longer duration "high." In rats, females exhibit

increased hyperactivity after cocaine exposure and develop self-administration behavior more quickly than do males (Zhou et al., 2003).

Bowman and Kuhn report that gender differences in cocaine responding emerge only after puberty, suggesting that ovarian hormones may influence the observed gender differences (1996). Additional studies show that hyperactivity following cocaine exposure is greater during proestrus and estrus (when estrogen and other ovarian hormones are highest) than during diestrus (Sell et al., 2000), and when estrogen is administered to gonadectomized female mice (Chen et al., 2003). Female rats implanted with 17β-estradiol exhibited greater locomotor hyperactivity following injections of both cocaine and (+)-MDMA than did ovariectomized females. The enhanced response to cocaine appeared within 5 minutes following drug injection whereas the enhanced response to (+)-MDMA was delayed for approximately 30 minutes (Zhou et al., 2003). These studies indicate that estrogen is a major contributor to gender differences in the behavioral response to cocaine and MDMA. Changes in estrogen hormone level cause variation in hyperactivity and dopamine release (Becker & Cha, 1989).

Long-term cocaine abuse in humans can cause sexual dysfunction (Cocores et al., 1986) in addition to causing irregularities in the menstrual cycle by altering hypothalamic amine release (King et al., 1990). Though female rats have regular, 4-day estrous cycles, within 7 days of cocaine exposure, female rats have shown repetitive days of estrous along with an absence of proestrus and enhanced periods of diestrus (King et al., 1990).

Previous animal models of drug potentiation have had profound implications with respect to defining drug abuse potential. Considerable evidence exists to demonstrate the importance of drug-related environmental stimuli in the reinforcing properties of drugs (Cervo et al., 2002; Hoffman et al., 1989; Knapp et al., 2002). An appropriate paradigm to study the influence of context on drug use is conditioned place preference (CPP). In this model, a drug is administered to the animal immediately before placement in an environment with unique contextual stimuli (olfactory, visual, tactile). Following alternating daily pairings of the drug with the unique context, and a

distinctly different context with no drug (vehicle), the animal is tested for preference by being allowed free-choice access to the drug-paired or the vehicle-paired context. Place preference is then defined by some measure of preference for one environment over another. Numerous drugs of abuse have been shown to produce a place preference, including cocaine and MDMA (Bardo et al., 1995; Hoffman et al., 1989).

While CPP is considered a valid model of drug reward and drug-seeking, it is further believed that augmentation in the development or expression of a CPP is a reflection of the enhancement of the neural mechanisms of drug reward. Conditioned place preference is commonly studied to ascertain a relative degree of reward and incentive motivation elicited by drugs and drug-paired stimuli (Zavala et al., 2003). The CPP paradigm is based on classical (Pavlovian) conditioning principles, as contextual cues acquire secondary reinforcing properties through temporal pairing with the drug which functions as the unconditioned stimulus [US] (Calcagnetti et al., 1993). Polydrug abuse (e.g., combined cocaine and MDMA) has been demonstrated to disrupt the acquisition of learned tasks (Block et al., 2002), perhaps through modulation of neural loci associated with learning and potential cognitive impairments (Cory-Slechta, 1995; Lasley et al., 1996). Thus, it is reasonable to suspect that any effects of xenobiotic substances on drug CPP may be due to alterations in learning and conditioning mechanisms. Moreover, mounting evidence indicates there may be pronounced gender differences in CPP to various drugs of abuse.

The purpose of this research project was to characterize the dose-effect pattern produced by cocaine or MDMA in a CPP preparation, and more importantly to determine if synergism occurs when the two drugs are presented concurrently, as clinical consumption patterns verify (Block et al., 2002; Gross et al., 2002; Parry et al., 2002; Smit et al., 2002). In addition, gender (male, female) differences in drug responsiveness were assessed.

MATERIALS AND METHODS

Apparatus

Place conditioning and testing were conducted in seven 20×60×20 cm wooden shuttle boxes with wooden tilt floors. At each end of each box was a microswitch interfaced to an IBM compatible computer. A BASIC computer program was written to continuously record the number of times and duration the switch was activated via a tilt of the floor.

One compartment of the apparatus had smooth white walls and floor, and the other compartment had black walls with a black sandpaper floor. For conditioning sessions, the boxes were divided into two equal-sized compartments by removable partitions. On test sessions (pretest and posttest), the partitions were removed and a 20×10×5 cm wooden platform was installed 2 cm above the floor to divide the two compartments but allow free access to either compartment by rats. In earlier investigations, subjects showed a strong preference for the black compartment (Miller et al., 1999; Miller et al., 2000; Miller and Nation, 1997; Smith and Nation, 2003). To counteract this preference, a 40 W light was positioned 50 cm above the black compartment of each apparatus. These seven lamps provided the only illumination in the testing room. Following each conditioning and test session the apparatus was cleaned with a mild soap solution. The apparatus was located in a sound-resistant room with a 40 dB white noise generator operating continuously. All conditioning and testing sessions were conducted during the light phase of the cycle.

Drugs

The Research Technology Branch of the National Institutes on Drug Abuse (NIDA) provided cocaine HCl and 3,4-MDMA gratis. Cocaine HCl and MDMA HCl were dissolved in a 0.9% w/v saline vehicle, and the doses were expressed as the salt. The (+)- isomer of MDMA was selected for this study because of its enhanced ability to

produce hyperactivity relative to the (-)- isomer, and because it has been used extensively in similar studies. All cocaine was administered intraperitoneally (i.p.) and all MDMA was administered subcutaneously (s.c.) such that 1 mL/kg delivered the desired dose.

Experiment 1: Male CPP

Animals

The animal housing and testing facility was approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International), and all animal maintenance and research design and administration was conducted in accordance with guidelines provided by the University Laboratory Animal Care Committee (ULACC). All aspects of the research followed the guidelines outlined in Principles of Laboratory Animal Care (NIH Publication No. 85–23). The health of the animals was monitored by the campus veterinarian throughout the duration of the project.

Adult male Sprague-Dawley rats (n = 63), weighing approximately 250 - 300g at the start of the experiment, were obtained from a commercial source (Harlan, Houston, TX). All animals were provided rat chow and tap water *ad libitum* throughout the course of the experiment, and were housed individually in plastic cages throughout the study. A 12h/12h light-dark cycle was used throughout the study. Beginning one week prior to the start of the experiment, animals were handled daily to limit any confounding variables caused by handling stress during the conditioning or experimental procedures.

Procedures

The present study involved a biased conditioning procedure. Body weights were recorded daily throughout the experiment. CPP experiment was started following one

week of acclimation to the laboratory environment. On day 1 of the CPP experiment, animals were transferred from the colony room to the testing chambers for 20 minutes in an effort to habituate the animals to transportation and the sound and illumination of the room. Animals were placed in the apparatus an allowed free access between compartments during the 20 minute period to overcome freezing effects from exposure to a novel environment. Data recorded from the first day was not used in calculating preferred side. On day 2, animals were placed in the CPP apparatus and allowed free access to either compartment for 15 minutes. Pretest data were measured by the amount of time animals spent in each compartment, and these data were used to determine animals' pretest preference for the white or the black compartment.

On four alternate days (days 3, 5, 7, and 9), each animal received a daily subcutaneous injection of MDMA (0, 5, or 10 mg/kg body weight) 25 minutes before an intraperitoneal cocaine injection (0, 2.5, or 5 mg/kg body weight). The time delay between MDMA and cocaine injections was based on the slow onset of the physiological effects of ecstasy (see above). The resulting interaction of 3 doses of MDMA x 3 doses of cocaine created 9 groups for each gender experiment; 0C (0 mg/kg cocaine) – 0M (0 mg/kg MDMA) (n = 7), 0 C - 5M (n = 7), 0C –10M (n = 7), 2.5C – 0M (n = 7), 2.5C – 5M (n = 7), 2.5C – 10M (n = 7), 5C – 0M (n = 7), 5C –5M (n = 7), and 5C – 10M (n = 7). See Table 1 below.

Table 1: Drug Conditions. For both males and females, three doses of cocaine were interacted with 3 doses of MDMA creating 9 conditions of drug exposure, each containing 7 rats, n=63 per gender experiment.

		MDMA		
Cocaine	0 COC - 0 MDMA	0 COC - 5 MDMA	0 COC - 10 MDMA
	2.5 COC - 0 MDMA	2.5 COC - 5 MDMA	2.5 COC - 10 MDMA
	5 COC - 0 MDMA	5 COC - 5 MDMA	5 COC - 10 MDMA

Animals were confined to the least-preferred compartment (defined as the compartment in which the animal spent less amount of time on the day 2 pretest) for 20 minutes immediately after injection of cocaine and/or MDMA. On the other four alternate days (days 4, 6, 8, and 10), all animals received a daily i.p. vehicle (saline) injection and were confined to the most-preferred compartment (defined as the compartment in which the animal spent more amount of time on the day 2 pretest) for 20 minutes immediately after injection. All injections were given at a volume of 1 ml/kg. Animals were run in squads of seven, counterbalanced by group assignments. On day 11, posttest data were obtained using the same procedure as the day 2 pretest; in the absence of an injection, subjects were permitted free access between the cambers. Weight-sensitive flooring in the place preference apparatus recorded the amount of time spent in each chamber in addition to the frequency of threshold crossings.

Experiment 2: Female CPP

Animals

Adult female Sprague-Dawley rats (n = 63), weighing approximately 250-300g at the start of the experiment, were obtained from a commercial source (Harlan, Houston, TX). All animals were provided rat chow and tap water *ad libitum* throughout the course of the experiment, and were housed individually in plastic cages throughout the study. A 12h/12h light-dark cycle was used throughout the study. Beginning one week prior to the start of the experiment, animals were handled daily to limit any confounding variables caused by handling stress during the conditioning or experimental procedures.

Procedures

The procedure for Experiment 2 was precisely as described for Experiment 1, with the following exceptions. To lessen the likelihood that all female rats (upon

matching their estrus cycles before or during the course of the experiment) would experience confounds with increased estrogen during drug exposure or testing days female animals were allowed 10 days of acclimation to the laboratory environment before starting in the CPP environment. The reproductive cycles of female humans and animals living in close contact frequently align so their estrus cycles are occurring simultaneously. Because we had no way to determine which stage of the estrous cycle female rats were on without introducing unwanted stress, we had to counterbalance our testing days with the possibility that all or some of the female rats would be cycling together. To accomplish this, we staggered CPP start days so that 1/3 of the animals (3 squads of 7 animals each, total n=21) began testing on each one of three consecutive days. Animals in each squad (n=7) were randomly chosen from each drug exposure condition (see Table 1 for drug conditions) so that in the event of estrous cycle synchronization, rats in the same condition would still be receiving drug or vehicle injections on alternate days of the estrous cycle. This randomized drug exposure schedule decreased the likelihood that sensitivity to CPP or to cocaine and/or MDMA from estrous would cause alterations in data trends. Thus, randomizing conditions to have slightly altered experiment schedules eliminated estrous as a possible confounding variable to CPP. Varying start days permitted the combination of all data and the observation of gender-based (not estrogen-based) trends by limiting possible confounding variables attributed the synchronization of the ovulatory cycle with presentation of cocaine and/or ecstasy.

Females were vaginally lavaged immediately following the final test run and on the following three days, for a total of 4 consecutive samples of vaginal cells (the estrous cycle in rats is only 4 days). This was necessary to ensure that drug exposure and preference testing in each of the 9 drug conditions was indeed counterbalanced. Microscope slides were stained and scored for estrous.

Although drugs of abuse, e.g. cocaine, have been shown to disrupt estrous cycles in females (King, et.al. 1990), the vaginal lavage procedure itself has been shown to create a CPP (Walker et al., 2002). The additional stress caused by vaginal lavage

could not have been equilibrated across gender, and would thus have presented a potential confound for comparisons between males and females if performed prior to or during the course of the experiment and data collection. All other aspects of the study proceded as described on Experiment 1.

Statistical Analysis

The pretest data from day 2 and the posttest data from day 11 were examined. The conditioning scores in both experiments were defined by the number of minutes spent on the drug-conditioned compartment on posttest trial minus the number of minutes spent on the same compartment in the pretest trial. For each gender subset, subject weight was analyzed using a repeated measures ANOVA test with weekly body weight averages serving as the within factor. The behavioral data were analyzed by a 3 doses of cocaine (0 mg/kg, 2.5 mg/kg, 5 mg/kg) X 3 doses of MDMA (0 mg/kg, 5 mg/kg, 10 mg/kg) X 2 gender (female, male) completely factorial ANOVA. T-tests between individual groups were used for post hoc analysis. Thus a total of 126 animals (N=18 groups, N=7/group) were required for this investigation.

RESULTS

Results from the three-way ANOVA indicate a significant interaction at the overall level, i.e. gender x MDMA x cocaine ($F(4,108)=3.39$, $p<0.05$). A further interaction effect was revealed between MDMA and cocaine ($F(4,108)=6.99$, $p<0.001$). The gender by MDMA interaction approached significance ($F(2, 108)=2.80$, $p=0.065$) [refer to figures 1 and 2].

Experiment 1: Male CPP

In males, cocaine administered alone produced a clear dose-effect curve; significant differences from saline were evident at both 2.5 mg/kg and 5 mg/kg; $p<0.01$. A biphasic effect was seen across the three MDMA doses in males; $p<0.01$ when they were administered singularly, wherein reward properties increased at the medium dose (5 mg/kg MDMA) and diminished at the highest dose (10 mg/kg). The rewarding properties of 5 mg/kg MDMA presented alone were diminished by the addition of cocaine. Similarly, there was a reversal of the aversive properties of 10 mg/kg MDMA with the addition of cocaine.

Male rats receiving 10 mg/kg MDMA injections showed increased excitation and ejaculation at the time of cocaine i.p. administration and placement within their non-preferred chamber for conditioning. These excitatory signs were not observed at the 5 mg/kg or 0 mg/kg level of MDMA.

Two male rats died on day 5 of the research project due to hyperthermia following conditioning runs with 5C – 10M and 2.5C – 10M. A third male rat in the 0C – 10M condition was detected showing symptoms of the early stages of hyperthermia (decreased muscle tone, non-responsiveness, rapid heart rate, shallow breathing, frothing at the mouth, and temporary paralysis). To quickly lower core body temperature, he was placed in a walk-in freezer at 45°C for approximately 20 minutes. Within 10 minutes following cooling, the rat was responsive to nerve tests and breathing was more normal.

Within 30 minutes after having been placed in the freezer, the rat was pulling himself around his home cage although his extremities remained unusable for several hours.

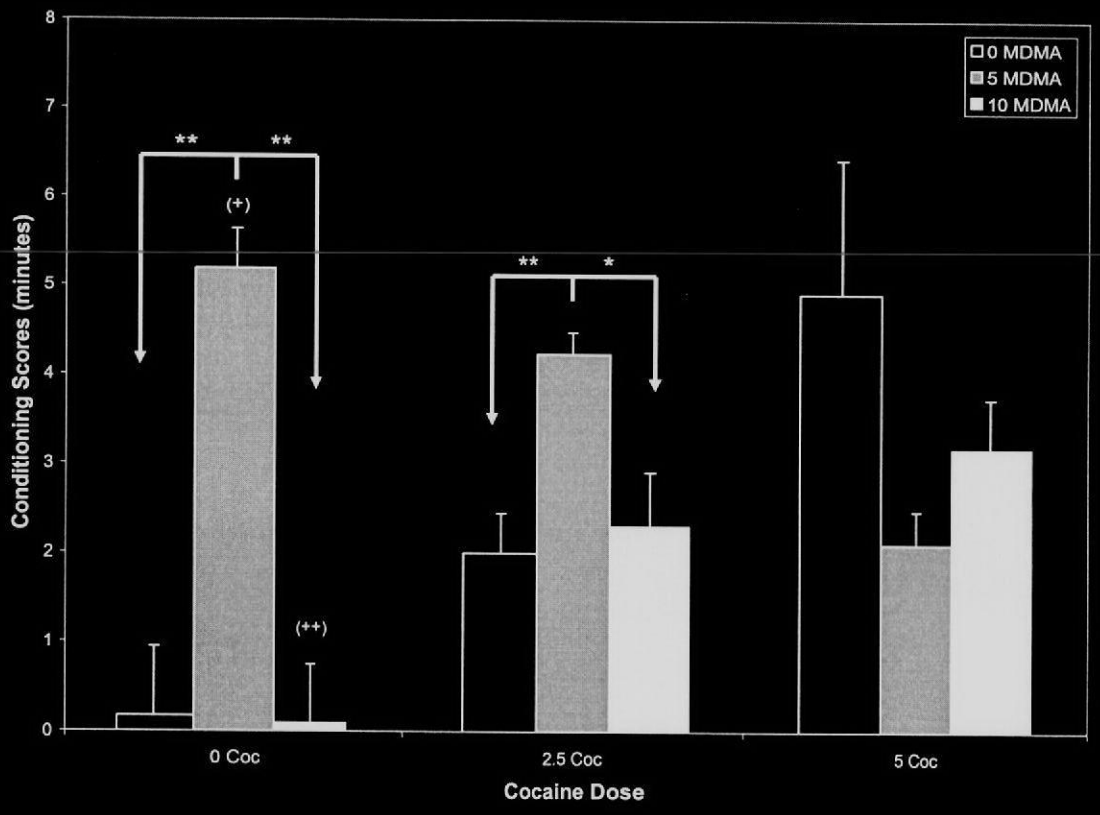


Figure 1: Effect by Cocaine in Males. Mean (±SEM) conditioning scores (measured in minutes) of male animals for 0 mg/kg cocaine-injected animals, 2.5 mg/kg cocaine-injected animals, and/or 5 mg/kg cocaine-injected animals CPP produced by 0 mg/kg, 5 mg/kg, and/or 10 mg/kg MDMA. Presence of an asterisk (*) indicates that the CPP conditioning scores produced by 5 mg/kg and/or 10 mg/kg MDMA were significantly different from CPP conditioning scores produced by 0 mg/kg MDMA (vehicle) within their respective cocaine dose ($p < 0.05$). Asterisks (**) indicate that the CPP conditioning scores produced by 5 mg/kg MDMA and/or 10 mg/kg MDMA were significantly different from CPP conditioning scores produced by 0 mg/kg MDMA (vehicle) within their respective cocaine dose ($p<0.01$). Presence of a plus sign (+) indicates that the CPP conditioning scores at 0 mg/kg cocaine and/or 5 mg/kg MDMA were significantly different between gender ($p<0.05$). The plus signs (++) indicate that the CPP conditioning scores at 0 mg/kg cocaine and 10 mg/kg MDMA were also significantly different between gender ($p<0.01$).

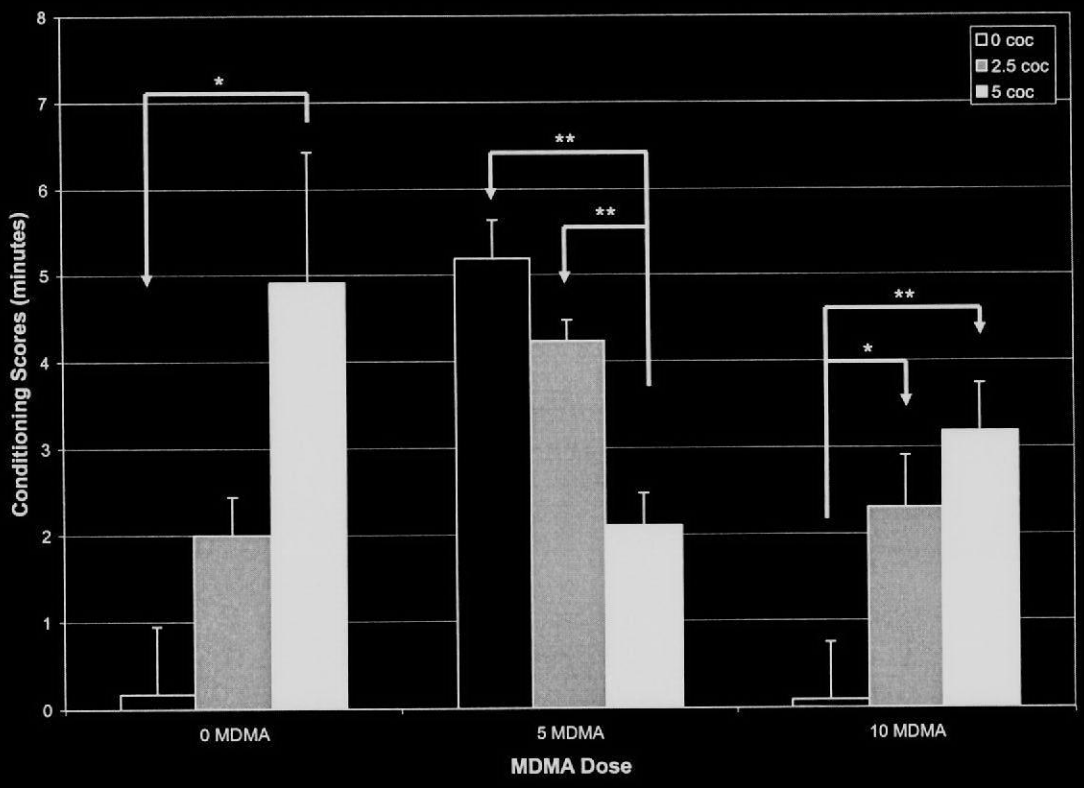


Figure 2: Effect by MDMA in Males. Mean (±SEM) conditioning scores (measured in minutes) of male animals for 0 mg/kg MDMA-injected animals, 5 mg/kg MDMA-injected animals, and/or 10 mg/kg MDMA-injected animals CPP produced by 0 mg/kg, 2.5 mg/kg, and/or 5.0 mg/kg cocaine. Presence of an asterisk (*) indicates that the CPP conditioning scores produced by 2.5 mg/kg and/or 5 mg/kg cocaine were significantly different from CPP conditioning scores produced by 0 mg/kg cocaine (vehicle) within their respective MDMA dose ($p < 0.05$). Asterisks (**) indicate that the CPP conditioning scores produced by 2.5 mg/kg cocaine and/or 5 mg/kg cocaine were significantly different from CPP conditioning scores produced by 0 mg/kg cocaine (vehicle) within their respective MDMA dose ($p<0.01$).

Results from post hoc t-tests between individual groups of male rats are summarized in Tables 2 & 3.

Table 2: Cocaine Dose in Males. Independent sample t-test on mean (±SEM) conditioning scores (measured in minutes) between 0 mg/kg, 5 mg/kg, and 10 mg/kg MDMA-exposed animals within their respective cocaine dose. Asterisks (*) indicate that the CPP conditioning scores between the two groups were significantly different ($p < 0.05$). Double asterisks (**) indicate that differences in mean conditioning scores between two groups were significant ($p<0.01$).

Cocaine Dose	Compared MDMA Doses (and mean CPP scores ± SEM, minutes)	t Value	Probability
0 mg/kg	0 coc-0 mg/kg MDMA (0.17 ± 0.77) < 0 coc-5 mg/kg MDMA (5.19± 0.45)	t(12) = 5.59	p < 0.01**
	0 coc-10 mg/kg MDMA (0.09 ± 0 65) < 0 coc-5 mg/kg MDMA (5.19 ± 0 45)	t(12) = 6.41	p < 0 01**
2 5 mg/kg	2.5 coc-0 mg/kg MDMA (2 00 ± 0 45) < 2.5 coc-5 mg/kg MDMA (4.23 ± 0 24)	t(12) = 4 39	p < 0.01**
	2.5 coc-10 mg/kg MDMA (2 30 ± 0.61) < 2.5 coc-5 mg/kg MDMA (4 23 ± 0 24)	t(12) = 2 96	p < 0.05*

Table 3: MDMA Dose in Males. Independent sample t-test on mean (±SEM) conditioning scores (measured in minutes) produced by 0 mg/kg, 2.5 mg/kg, and 5 mg/kg cocaine within their respective MDMA dose. Presence of an asterisk (*) indicates that mean conditioning scores between two groups were significantly different ($p < 0.05$), and asterisks (**) indicate that differences in mean conditioning scores between two groups were significant ($p<0.01$)

MDMA Dose	Compared Cocaine Doses (and mean CPP scores ± SEM, minutes)	t Value	Probability
0 mg/kg	0 coc -0 mg/kg MDMA (0.17 ± 0 77) < 2 5 coc -0 mg/kg MDMA (2 00 ± 0.45)	t(12) = 2.05	p = 0.06
	0 coc -0 mg/kg MDMA (0 17 ± 0.77) < 5 coc -0 mg/kg MDMA (4 91 ± 1.72)	t(12) = 2 51	p < 0.05*
5 mg/kg	5 coc -5 mg/kg MDMA (2.10 ± 0 37) < 0 coc -5 mg/kg MDMA (5.19 ± 0 45)	t(12) = 5 27	p < 0 01**
	5 coc -5 mg/kg MDMA (2 10 ± 0.37) < 2.5 coc -5 mg/kg MDMA (4.23 ± 0 24)	t(12) = 4 78	p < 0 01**
10 mg/kg	0 coc -10 mg/kg MDMA (0 09 ± 0.65) < 2.5 coc -10 mg/kg MDMA (2 30 ± 0.60)	t(12) = 2.48	p < 0 05*
	0 coc -10 mg/kg MDMA (0 09 ± 0 65) < 5 coc -10 mg/kg MDMA (3 18 ± 0 56)	t(12) = 3.59	p < 0 01**

Thus, for males, individual comparisons indicated that at the 0 mg/kg cocaine dose, group differences existed between 0 coc – 0 MDMA and 0 coc – 5 MDMA

($p<0.01$) as well as between 0 coc – 10 MDMA and 0 coc – 5 MDMA ($p<0.01$). At the 2.5 mg/kg cocaine dose, group differences existed between 2.5 coc – 0 MDMA and 2.5 coc – 5 MDMA ($p<0.01$) as well as between 2.5 coc – 10 MDMA and 2.5 coc – 5 MDMA ($p<0.05$). Individual comparisons indicated that at the 0 mg/kg MDMA dose, group differences existed between 0 coc – 0 MDMA and 2.5 coc – 0 MDMA ($p<0.05$). At the 5 mg/kg MDMA dose, group differences existed between 0 coc – 0 MDMA and 5 coc – 0 MDMA ($p<0.01$) as well as between 5 coc – 5 MDMA and 2.5 coc – 5 MDMA ($p<0.01$). At the 10 mg/kg MDMA dose, group differences existed between 0 coc – 10 MDMA and 2.5 coc – 10 MDMA ($p<0.05$) as well as between 0 coc – 10 MDMA and 5 coc – 10 MDMA ($p<0.01$).

Experiment 2: Female CPP

Females, like males, showed a dose-effect curve with cocaine exposure wherein the highest dose of cocaine produced more rewarding effects and heightened CPP (see figures 3 and 4). Although not rewarding alone, 5 mg/kg MDMA did produce an enhanced place preference in combination with a low level of cocaine ($p<0.05$), which was reversed in combination with a high level of cocaine. Although the 10 mg/kg MDMA presentation was highly rewarding to females, when in combination with 5 mg/kg cocaine (which was also rewarding when presented alone), this level of MDMA becomes aversive.

Reported trends for female subjects are collapsed across estrus cycle to depict overall female performance. Estrus, however, is suspected to be responsible, at least in part, for the high variance in conditioning. Upon completion of testing, 19 female rats (approximately 1/3) were determined to be in estrous, as determined with vaginal lavage. The other 44 female rats were either in proestrus, diestrus, or meta-estrus on their final testing day.

During experimentation with females, no animals died from complications with hyperthermia and only one rat developed hyperthermic symptoms (see above), including

eye and nose bleeds. One rat was discovered with hyperthermia on days 7 and 9 (both drug conditioning days). On day 7, she was limp and nonresponsive when taken out of the CPP chamber. She was immediately placed in a 45°C walk-in type freezer and remained there approximately 40 minutes. Approximately 1 hour after cooling core body temperature, the rat was able to stand independently, was more responsive, and had regained slight motility. On day 9 of the experiment (final day of drug conditioning), the same animal displayed extreme hyperactivity upon completion of the 20-minute conditioning session. Within 5 to 10 minutes of returning to its homecage, the animal once again had developed symptoms of hyperthermia and was cooled for 30 minutes.

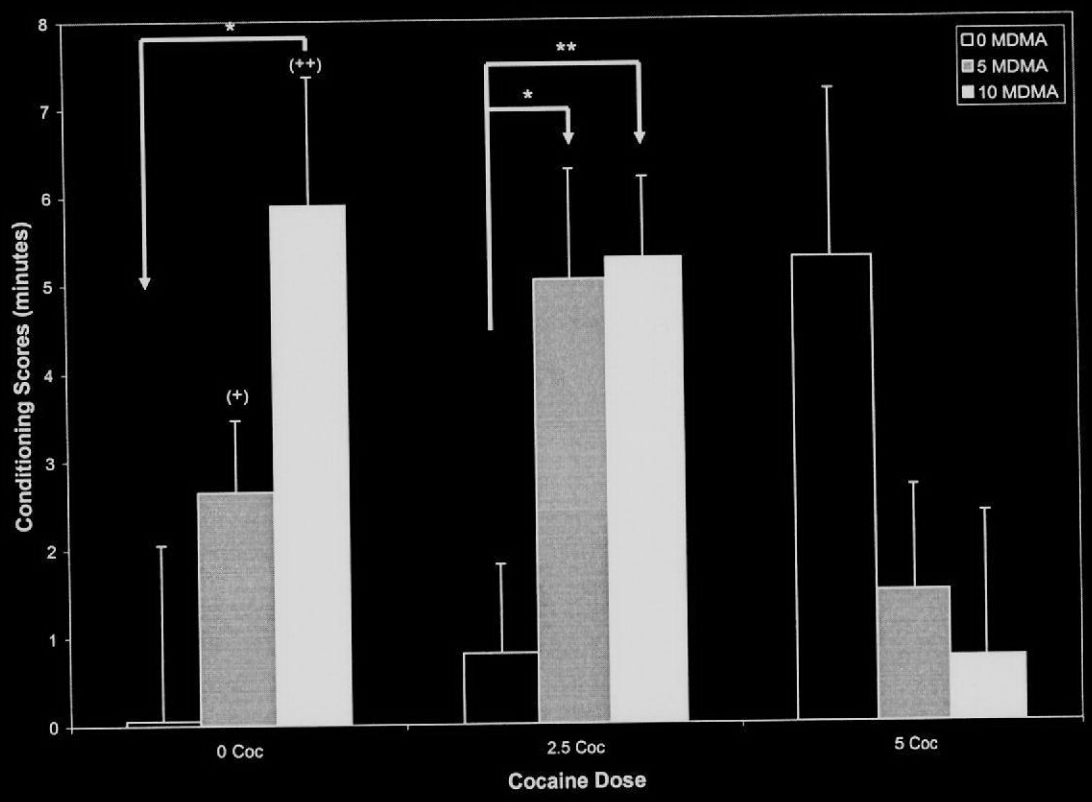


Figure 3: Effect by Cocaine in Females. Mean (±SEM) conditioning scores (measured in minutes) of female animals for 0 mg/kg cocaine-injected animals, 2.5 mg/kg cocaine-injected animals, and/or 5 mg/kg cocaine-injected animals CPP produced by 0 mg/kg, 5 mg/kg, and/or 10 mg/kg MDMA. Presence of an asterisk (*) indicates that the CPP conditioning scores produced by 5 mg/kg and/or 10 mg/kg MDMA were significantly different from CPP conditioning scores produced by 0 mg/kg MDMA (vehicle) within their respective cocaine dose ($p < 0.05$). Asterisks (**) indicate that the CPP conditioning scores produced by 5 mg/kg MDMA and/or 10 mg/kg MDMA were significantly different from CPP conditioning scores produced by 0 mg/kg MDMA (vehicle) within their respective cocaine dose ($p<0.01$). Presence of a plus sign (+) indicates that the CPP conditioning scores at 0 mg/kg cocaine and/or 5 mg/kg MDMA were significantly different between gender ($p<0.05$). The plus signs (++) indicate that the CPP conditioning scores at 0 mg/kg cocaine and/or 10 mg/kg MDMA were also significantly different between gender ($p<0.01$).

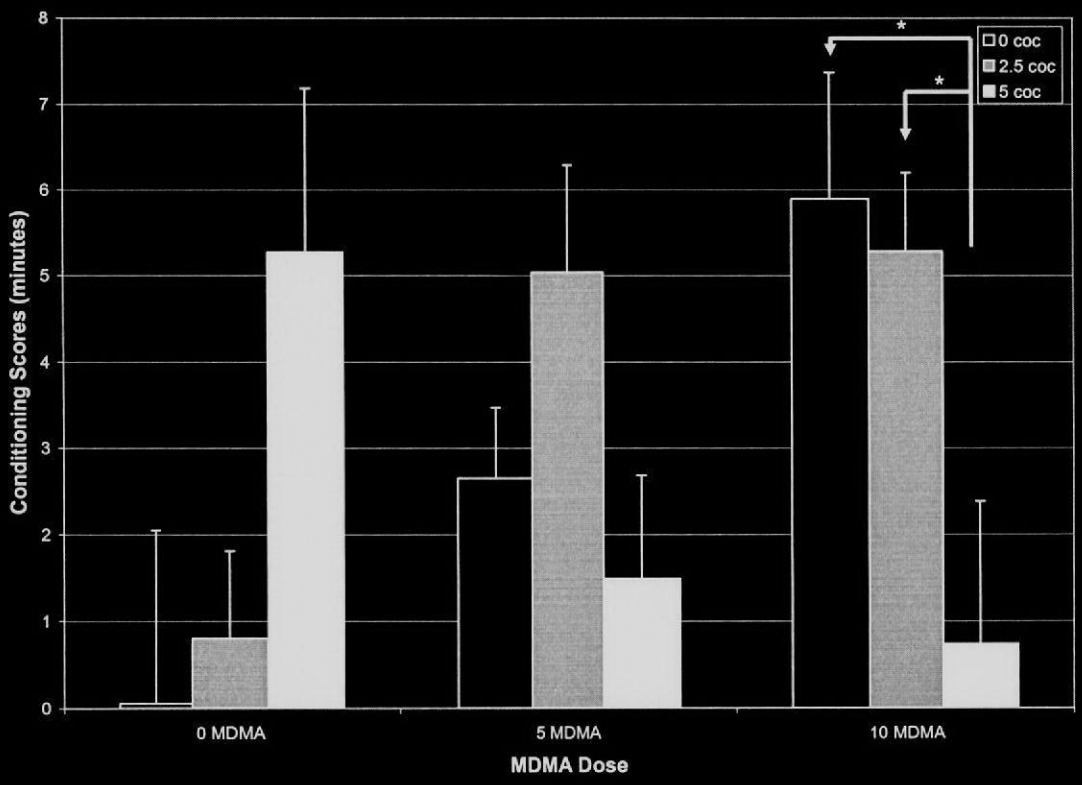


Figure 4: Effect by MDMA in Females. Mean (±SEM) conditioning scores (measured in minutes) of female animals for 0 mg/kg MDMA-injected animals, 5 mg/kg MDMA-injected animals, and/or 10 mg/kg MDMA-injected animals CPP produced by 0 mg/kg, 2.5 mg/kg, and/or 5.0 mg/kg cocaine. Presence of an asterisk (*) indicates that the CPP conditioning scores produced by 2.5 mg/kg and/or 5 mg/kg cocaine were significantly different from CPP conditioning scores produced by 0 mg/kg cocaine (vehicle) within their respective MDMA dose ($p < 0.05$).

Results from post hoc t-tests between individual groups of female rats are summarized in Tables 4 & 5.

Table 4: Cocaine Dose in Females. Independent sample t-test on mean (±SEM) conditioning scores (measured in minutes) between 0 mg/kg, 5 mg/kg, and 10 mg/kg MDMA-exposed animals within their respective cocaine dose. Asterisks (*) indicate that the CPP conditioning scores between the two groups were significantly different ($p < 0.05$). Double asterisks (**) indicate that differences in mean conditioning scores between two groups were significant ($p<0.01$).

Cocaine Dose	Compared MDMA Doses (and mean CPP scores ± SEM, minutes)	t Value	Probability
0 mg/kg	0 coc-0 mg/kg MDMA (0 059 ± 1 99) < 0 coc-10 mg/kg MDMA (5.90± 1.99)	t(12) = 2.36	p < 0.05*
2.5 mg/kg	2 5 coc-0 mg/kg MDMA (0.80 ± 1.01) < 2 5 coc-5 mg/kg MDMA (5.04 ± 1 25)	t(12) = 2 64	p < 0.05*
	2.5 coc-0 mg/kg MDMA 0.80 ± 1 01) < 2.5 coc-10 mg/kg MDMA (5.28 ± 0.92)	t(12) = 3 29	p < 0.01**

Table 5: MDMA Dose in Females. Independent sample t-test on mean (±SEM) conditioning scores (measured in minutes) produced by 0 mg/kg, 2.5 mg/kg, and 5 mg/kg cocaine within their respective MDMA dose. Presence of an asterisk (*) indicates that mean conditioning scores between two groups were significantly different ($p < 0.05$).

MDMA Dose	Compared Cocaine Doses (and mean CPP scores ± SEM, minutes)	t Value	Probability
10 mg/kg	5 coc-10 mg/kg MDMA (0.74 ± 1 64) < 0 coc-10 mg/kg MDMA (5 90 ± 1 47)	t(12) = 2 34	p < 0 05*
	5 coc-10 mg/kg MDMA (0.74 ± 1.64) < 2.5 coc-10 mg/kg MDMA (5 28 ± 0.92)	t(12) = 2 42	p < 0.05*

Thus, for females, individual comparisons indicated that at the 0 mg/kg cocaine dose, group differences existed between 0 coc – 0 MDMA and 0 coc – 10 MDMA ($p<0.05$). At the 2.5 mg/kg cocaine dose, group differences existed between 2.5 coc – 0 MDMA and 2.5 coc – 5 MDMA ($p<0.05$) as well as between 2.5 coc – 0 MDMA and 2.5 coc – 10 MDMA ($p<0.01$). Individual comparisons indicated that at the 10 mg/kg

MDMA dose, group differences existed between 5 coc – 10 MDMA and 0 coc – 10 MDMA ($p<0.05$) as well as between 5 coc – 10 MDMA and 2.5 coc – 10 MDMA ($p<0.05$).

Gender Differences

Also notable is the difference between males and females at the 10 mg/kg dose of MDMA (see Table 6). Whereas males showed aversion to single administrations of the drug and increased preference when paired with cocaine, females showed a preference of single administration of 10 mg/kg MDMA and decreased preference when paired with cocaine. Females seemed to express a shift right in the dose-effect curve (decreased senstitivity) for MDMA (e.g., while 10 mg/kg MDMA alone is aversive to males, it is not aversive to females until combined with 5 mg/kg cocaine).

Table 6: Gender Differences. Independent sample t-test on mean (±SEM) conditioning scores (measured in minutes) between gender within their respective drug dose. A plus sign (+) indicates that the CPP conditioning scores between the two groups were significantly different ($p < 0.05$). Double plus signs (++) indicate that differences in mean conditioning scores between two groups were significant ($p<0.01$).

Drug Dose	Compared Gender (and mean CPP scores ± SEM, minutes)	t Value	Probability
0 coc-5 mg/kg MDMA	Female (2.65 ± 0 82) < Male (5 19 ± 0 45)	t(12) = 2 71	p < 0 05+
0 coc-10 mg/kg MDMA	Male (0.09 ± 0.65) < Female (5 90 ± 1.47)	t(12) = 3.60	p < 0 01++

Thus, individual comparisons indicated that at the 0 coc – 5 MDMA dose, group differences existed between males and females ($p<0.05$). At the 0 coc – 10 MDMA dose, group differences existed between males and females ($p<0.01$).

DISCUSSION

Experiment 1: Male CPP

In males, a clear and significant place preference for cocaine was demonstrated between 0 mg/kg, 2.5 mg/kg, and 5 mg/kg cocaine combined with 0 mg/kg MDMA. The rewarding properties of cocaine at these doses has been well demonstrated and is consistent with our findings (Busse et al., 2003). Increases in cocaine dose modulate neural reward mechanisms through the dopamine system. The reward value of 5 mg/kg MDMA presented singularly produced a significant CPP similar to what was produced by 5mg/kg cocaine alone. The combination of a highly rewarding dose of MDMA (5 mg/kg) with a moderately rewarding dose of cocaine (2.5 mg/kg) actually starts to become less rewarding. The most rewarding doses of MDMA (5 mg/kg) and cocaine (5 mg/kg) produce the highest levels of CPP when presented separately, yet when combined actually become significantly more aversive. This is in opposition to the rationale that formed the basis for our research, i.e. that the combination of the rewarding drugs would actually produce an even greater reward neurochemically and a more pronounced place preference. Ostensibly, in combination these highly rewarding drugs appear to actually become aversive. The aversive properties of MDMA may be in effect more prevalently at higher doses. Male rats showed no CPP for 10 mg/kg MDMA. Experimentation with MDMA is frequently done at the 10 mg/kg dose and has shown to induce CPP similar to saline even in adolescent subjects (Achat-Mendes, C. 2003), and MDMA at 20 mg/kg is considered neurotoxic. 10 mg/kg MDMA is not neurotoxic enough entirely deplete drug-reward systems, which allows a resurgence of reward-conditioning when this dose is paired with rewarding doses of cocaine.

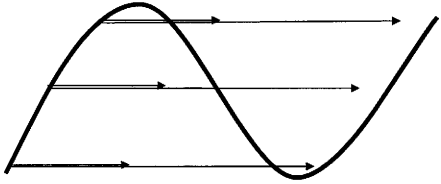
While cocaine at these levels show a linear relationship between dose and reward, MDMA at these levels depicts a biphasic effect wherein low doses are highly rewarding and higher doses are more aversive. When these drugs are combined, the properties of one drug antagonize the rewarding or aversive properties of the other. A

possible explanation for this phenomenon may rest with a cubic function which may characterize the interactive effects of the two commonly abused drugs. That is, relatively small drug doses possess rewarding characteristics. As the potency of the drug increases or larger doses are administered the aversive properties of the drug cause a downward shift in CPP, as rewarding effects are lost or eclipsed by more aversive drug effects. The cubic trend proposes that pushing past the aversiveness of high drug doses with exceptionally potent doses may once again stimulate neural reward mechanisms (perhaps by potentiating change in secondary or alternate reward systems). Exceptionally high doses of a single drug or synergistic effects in poly-drug conditions may show reward agonism, however, such strong drug dosages or multiple drug combinations are typically associated with high rates of lethality and thus are contraindicated.

Consistent with the theory of a cubic trend of poly-drug effect elicited by concurrent combinations of cocaine and MDMA, what would initially be on the ascending limb of a cubic-dose-effect curve would be altered unidirectionally by increases in drug dose (see figure 5).

The addition of cocaine antagonizes (offsets) the biphasic effect produced by MDMA. Although MDMA at 5 mg/kg is even more rewarding than the highest dose of cocaine (10 mg/kg), the highly rewarding effects are progressively decreased as the amount of added cocaine is increased. Inversely, although MDMA at 10 mg/kg is aversive, it becomes increasingly more rewarding in combination with high doses of cocaine. Whereas cocaine is highly rewarding at the 5 mg/kg level, subsequent pairings with higher levels of MDMA result in significantly decreased conditioned place preference.

Figure 5: Cubic trend for males. Short arrows denote change in CPP with increase from 0 MDMA to 5 MDMA. Long arrows denote change in CPP with increase from 0 MDMA to 10 MDMA.



The reversal by high doses of cocaine of the aversive properties of 10mg/kg MDMA is explained by a cubic trend with respect to defining the character of cocaine/MDMA interactions (refer to figure 5). The interactive effects of the drug hint at enhanced reward properties with increasing drug dose above the aversive level.

Experiment 2: Female CPP

Reported trends for female subjects are collapsed across estrus cycle to depict overall female performance. Estrus, however, is suspected to be responsible, at least in part, for the high variance in conditioning. Estrus has traditionally been noted to cause alterations in drug sensitivity at both the animal and human level (Sell et al., 2000). Previous studies have shown that estrous enhances cocaine sensitivity and creates a shift in the dose-response curve for cocaine (Bowman & Kuhn, 1996; Chen et al., 2003; Zhou et al., 2003). Females show a decreased sensitivity to MDMA and cocaine relative to males and show large variance due to estrus cycles. Estrus has traditionally been noted to cause alterations in drug sensitivity at both the animal and human level. Previous studies have shown that estrous enhances cocaine sensitivity and creates a shift in the

dose-response curve for cocaine (Bowman & Kuhn, 1996; Chen et al., 2003; Zhou et al., 2003).

Females exhibit a dose-dependent cocaine reward effect. MDMA's ability to reverse those effects are delayed (i.e., a higher MDMA dose is required to offset the reward properties associated with cocaine use). MDMA at the 10 mg/kg level is still highly rewarding (whereas MDMA at the 5 mg/kg level was most rewarding in males). Decreased sensitivity in females would thus require higher drug levels to achieve the same behavioral effects, whether rewarding or aversive. Similarly, while 10 mg/kg MDMA alone is aversive in males, this high dose of MDMA must be combined with 5 mg/kg coc before this aversiveness is seen in females.

Aversiveness from each drug's hyperthermic effect may also be one of the underlying reasons for the reduction in preference at 5 mg/kg MDMA with subsequent pairings of cocaine.

Conclusions

Our results show that cocaine and MDMA do interact, but in an antagonistic fashion, rather than a synergistic one. These findings could provide insight to deeper understanding of the interactive effects of combining drugs of abuse as well as provide new insight and potential therapeutic strategies for drug dependence in men and women.

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- Content Mastery; Help One Student To Succeed (HOSTS) reading enrichment

Aggie Scholars Promote Incentive, Resources & Excellence (ASPIRE) *Aug. 2000 – May 2002*
- Executive Committee, Communications and Technology Coordinator, Mentor, Scholar Liaison

DISTINGUISHED AWARDS AND ACADEMIC RECOGNITIONS:

Texas A&M University Scholar
Dean's List for Academic Excellence
Terry Foundation Scholar
Senator Robert C. Byrd Scholar
Supplemental Instructor of the Year Award
Tau Kappa Outstanding Service Award

HONOUR SOCIETIES:

Golden Key International Honour Society *Nov. 2002 – present*
- President, Vice-President, Regional and International Conference Representative

Texas Iota Chapter - Alpha Epsilon Delta Pre-Medical Honor Society *Sept 2002 – present*
Tau Kappa Junior Honor Society *Aug. 2002 – May 2003*
Lambda Sigma *Aug. 2001 – May 2002*
- Service Chair