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Behavioral sensitization following concurrent exposure to 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxymethamphetamine (MDMA) in male Sprague-Dawley rats

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Behavioral sensitization following concurrent exposure to 4-methylmethcathinone (4-MMC) and 3, 4-methylenedioxymethamphetamine (MDMA) in male Sprague-Dawley rats

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Abstract: Recreational use of a new class of stimulant drugs known as synthetic cathinones is a recent public health concern. Although the Drug Enforcement Administration placed several of the most common of these substances permanently on schedule 1, their use is still prevalent as they remain low cost, accessible, and potent. Concomitant use of cathinone derivatives with other psychostimulant drugs is commonly reported by recreational users. Despite the prevalence of synthetic cathinone abuse, there is currently a paucity of scientific research regarding the behavioral and neurochemical effects of these drugs in mixtures with other drugs of abuse. The behavioral sensitization paradigm is a preclinical tool that can be used to assess the influence of prior drug exposure on sensitivity to the behavioral effects of another drug. Utilizing this paradigm, the present study assessed the combined locomotor stimulant effects of 3,4-methylenedioxymethamphetamine (MDMA) and a common bath salt constituent, 4-methylmethcathinone (4-MMC). Male Sprague-Dawley rats (N=120) were randomly assigned to one of 15 treatment groups (N=8) and administered intraperitoneal injections of one of the following treatments once per day for seven consecutive days: saline, 3 mg/kg MDMA, 4-MMC (1 or 5 mg/kg), 3 mg/kg MDMA+4-MMC (1.0 or 5.0 mg/kg). A 10 day drug-free incubation period followed, after which rats were challenged with a single I.P. injection of either saline, 4-MMC (1.0 or 5.0 mg/kg), or 3 mg/kg MDMA. On treatment days 1 and 7, and during the post-incubation challenge test, locomotor activity was monitored for one hour immediately before and one hour immediately after injections. Results indicate 4-MMC alone failed to induce behavioral sensitization, but when combined with MDMA, the induction of sensitization was greater than that produced by MDMA alone. These findings suggest the possibility of increased abuse liability of 4-MMC when used concurrently with MDMA or following prior MDMA use.

Key Words: Behavioral sensitization, synthetic cathinones, bath salts, 3, 4-methylenedioxymethamphetamine, 4-methylmethcathinone,

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For centuries, native populations in Eastern Africa and Arabia have chewed the leaves and shoots of the *Catha edulis* (khat) plant to experience its intoxicating effects (Patel, 2000). The active compound of this plant, cathinone, is similar in structure and pharmacology to the central nervous system (CNS) stimulant, amphetamine (Patel, 2000).

The recent emergence of a new class of illicit substances known as synthetic cathinones has received considerable attention in the media and scientific literature (The DAWN Report, 2013; European Monitoring Centre for Drugs and Drug Addiction, 2012; Torrance & Cooper, 2010; Wood et al., 2010). For some time, these substances were widely available through internet sources, in convenience stores and gas stations, and in head shops among other places. They were labeled “bath salts” or “not for human consumption” to avoid detection by drug enforcement agencies and became popular for their status as a “legal high” (Corazza, Demetrovics, Brink, & Schifano, 2013).

Psychoactive “bath salts” are derived from cathinone and are reported to produce a variety of subjective effects in humans similar to the effects of other psychostimulants (Zawilska & Wojcieszak, 2013). These effects include but are not limited to euphoria, increased alertness, sociability, intensified sensory experiences and sexual arousal. Synthetic cathinones also exhibit a similar neurochemical profile to other psychostimulants. Baumann et al. (2013) describe the cocaine-like effects of a principal bath salt constituent, 3, 4-methylenedioxypyrovalerone (MDPV). MDPV selectively blocks the reuptake of catecholamine neurotransmitters in the nucleus accumbens with preferential effects on the dopamine transporter (Baumann et al., 2013). 4-Methylmethcathinone, also known as mephedrone (4-MMC, MEPH) is another major

constituent of illicit bath salts, and is reported to increase extracellular levels of dopamine in the nucleus accumbens, though as a dopamine releaser rather than a reuptake blocker (Cameron, Kolanos, Verkariya, De Felice, & Glennon, 2013; Baumann et al., 2012; Cameron, Kolanos, Solis, Glennon, & De Felice, 2012).

Scientific research has responded promptly to the widespread popularity of psychoactive "bath salts". Many studies have been conducted to establish the pharmacological activities, neurochemical profile, behavioral effects, and abuse liability of these drugs. Despite much scientific research on these substances, most of the currently published preclinical studies fail to model the patterns of use in humans.

Concurrent use of more than one drug is reportedly common among recreational "bath salts" users. For example, the Drug Abuse Warning Network (DAWN) Report (2013) indicates that in over 52% of the emergency department visits related to synthetic cathinones, they were being used in combination with at least one other drug of abuse. Yet, the majority of published preclinical research has only examined individual substances. In an effort to understand the behavioral effects of synthetic cathinones, it is essential that preclinical scientific research model the typical patterns of human drug use, including mixtures of these substances with other drugs of abuse.

The behavioral sensitization paradigm is a common preclinical drug screening tool that can be easily modified to assess the effects of drug mixtures. This experimental procedure involves repeated or intermittent treatment with a drug followed by a brief period of abstinence and then reintroduction of the original compound or another drug. Specific research methods vary with respect to the number of drug treatments and the period of abstinence. Assessments of behavior are typically conducted before and following repeated treatments and again after the

period of abstinence. Increased responsiveness to a drug following repeated drug exposure is indicative of behavioral sensitization (Pierce & Kalivas, 1997). Robinson & Berridge (2008) suggest that repeated exposure to potentially addictive substances persistently modifies neurons and neural circuitry that would normally classify stimuli as null or salient, changing them into addictive stimuli.

It is well established that prototypical psychomotor stimulants, such as amphetamine and cocaine readily establish behavioral sensitization to the drugs' locomotor effects (de Jong, Inge, Steenbergen, Peter, & de Kloet, 2009; Magdenzo & Bustos, 2003). Given their similarities to psychomotor stimulants, it is not surprising that synthetic cathinones also establish behavioral sensitization. At the present time, only a few published studies have explored the effects of 4-MMC in this experimental paradigm. For example, Gregg, Tallarida, Reitz, McCurdy, & Rawls (2013b) reported that high doses of 4-MMC (15-30 mg/kg) produce sensitization of repetitive behaviors, though they did not find sensitization to ambulatory activity. They suggested that sensitization to repetitive behavior may be related to the actions of 4-MMC at 5-HT transporters. Lisek et al. (2012) reported that repeated exposure to a low dose (0.5 mg/kg) of 4-MMC produced sensitization to the drug's motor stimulant effects following a drug washout period. The authors suggested a role of dopamine D_1 and D_2 receptors in in the expression of sensitization to 4-MMC. In another study, Gregg, Tallarida, Reitz, & Rawls (2013a) reported that prior exposure to a higher dose of 4-MMC (15 mg/kg) produced cross-senstization to the motor stimulant effects of cocaine following a drug washout period. Gregg et al. (2013a) suggest that this may occur due to separate, but additive effects of the drugs on neuronal extracellular dopamine.

MDMA has also been shown to produce motor stimulant sensitization at multiple doses after repeated exposure (Kalivas, Duffy, & White, 1998; Modi, Yang, Swann, & Dafny, 2006). Kalivas et al. (1998) further observed multiple doses of MDMA to increase extracellular dopamine in the nucleus accumbens and reported cross sensitization between MDMA and cocaine.

While behavioral sensitization has been observed following repeated exposure to mephedrone and MDMA, to date, no published studies have evaluated these drugs in combination. Therefore, the aim of the current study was to determine if low doses of 4-MMC and MDMA produce behavioral sensitization when administered concurrently. A secondary aim was to assess if cross-sensitization occurred between these substances. MDMA was selected due to its pharmacological similarity to 4-MMC (Cameron et al., 2013a; Cameron et al., 2013b; Baumann et al., 2012) and because these drugs are frequently co-abused by humans.

Methods

Subjects

One hundred twenty experimentally naïve male Sprague-Dawley rats (Charles River Laboratories Inc., Wilmington, MA) were pair housed in polycarbonate cages in a temperature- and humidity-controlled vivarium maintained on a 12:12-h light-dark cycle with lights on at 05:00. Animals were approximately 60 days old at the onset of the study and weighed between 240 and 360 grams during the study. Each animal had access to food and water *ad libitum* in their home cages. Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Western Michigan University (WMU).

Apparatus

Eight custom-designed acrylic test chambers (40.5 × 40.5 × 40.5 cm) were housed within an Accuscan activity monitoring system (Accuscan Instruments Inc., Columbus, Ohio, USA) for the assessment of each animal's movements during behavioral assessments. Each test chamber was equipped with 16 infrared beams in the X and Y planes and 16 infrared beams in the Z plane. The chambers were connected to a computer running Versamax® software to analyze infrared beam breaks on horizontal and vertical axes.

Procedures

Behavioral assessments occurred between the hours of 08:00 and 16:00. Rats were randomly assigned to one of the following treatment groups for the seven day repeated exposure period: 1.0 mg/kg 4-MMC (n=16), 5.0 mg/kg 4-MMC (n=16), 3.0 mg/kg MDMA (n=24), 1.0 mg/kg 4-MMC+3.0 mg/kg MDMA (n=16), 5.0 mg/kg 4-MMC+3.0 mg/kg MDMA (n=16), or saline (n=32). Animals were administered one of these treatments daily for seven consecutive days and locomotor activity was monitored on days 1 and 7. On test days, rats were placed individually in the test chambers for a 60 min habituation period, after which they were injected and immediately placed back into the test chamber for an additional 60 min. On treatment days 2 through 6, animals were injected and immediately returned to their home cages.

Following day 7, rats experienced a 10-day drug washout period. On day 17, rats were returned to the activity monitoring chambers for a 60 min habituation period and then injected with 4-MMC (1 or 5 mg/kg), MDMA (3.0 mg/kg), or saline and immediately placed into test chambers for an additional 60 min. Treatment groups are displayed in Table1.

Data Analysis

Locomotor activity data were processed by the Versamax® software as the number of horizontal beam breaks. Horizontal activity counts were plotted as treatment group means (± *SEM*) for each five minute time interval over each 120 minute assessment period. Using SPSS Data Analytics Software, a two-way split-plot ANOVA with a Huynh-Feldt correction was completed separately for each treatment group with time interval and test day as within-subjects factors. If an ANOVA revealed a significant interaction, simple main effects tests were completed using a Sidak adjustment. Cumulative measures of horizontal activity following injections were also calculated for each treatment group on day 1 and day 7. These post-injection activity totals were analyzed with a two-way split-plot ANOVA with Huynh-Feldt correction, with test day as a within subjects factor and treatment as a between subjects factor. If statistical tests of post-injection totals revealed a significant interaction between day and treatment, simple main effects tests were run with a Sidak adjustment.

Cumulative measures of activity during the post-washout test session were analyzed using separate one-way ANOVA tests for each of the three challenge day treatments, followed by multiple comparisons tests with a Sidak adjustment. The comparisons made in these three tests were established *a priori*.

Results

Sensitization Induction

The time course of horizontal activity prior to and immediately after injections on day 1 and day 7 are displayed in figure 1 for each treatment group. Each data point depicts the mean (± *SEM*) number of horizontal beam breaks per five minute interval. In all except the 4-MMC 1 treatment group, statistical analyses indicate higher levels of activity on day 1 compared to day 7

at selected time intervals before injections. Additionally, in all treatment groups except the saline control group and the 4-MMC 1 group, activity levels were higher on day 7 compared to day 1 at selected time intervals after injections.

As shown in Figure 1, 4-MMC produced a rapid, dose-dependent increase in locomotor activity that peaked at approximately 10 min post-injection and returned to levels comparable to that of the saline-treated animals within 30 min. A similar rapid onset of drug-induced activity was observed with 4-MMC + MDMA mixtures, although increased activity was sustained by 60 min post-injection, particularly with the 5 mg/kg 4-MMC + MDMA mixture.

Table 2 depicts results of the two-way repeated measures ANOVA on horizontal activity counts, conducted separately for each treatment group. In saline-treated rats, there was a significant main effect of time interval, test day, and a significant time interval by test day interaction. As seen in Figure 1, saline-treated rats were slightly more active on day 1 compared to day 7 for the first 25 min of habituation, but these rats were more active on day 7 compared to day 1 only during one post-injection time interval, at approximately 50 minutes.

Similar repeated measures two-way ANOVA tests on the activity of either 4-MMC 1 or 4-MMC 5-treated rats showed a significant main effect of time interval, no significant difference between test days, and a significant time interval by test day interaction. As shown in Figure 1, 4-MMC 1-treated rats did not display any significant differences in activity between day 1 and day 7 at any time intervals. In contrast, the 4-MMC 5-treated rats displayed greater activity on day 7 compared to day 1 at approximately 10 to 15 minutes post-injection.

A two way-repeated measures ANOVA on the horizontal activity of MDMA-treated rats also indicated a significant main effect of time, but not test day, though there was a significant test day by time interval interaction. Sidak post-hoc tests indicated that horizontal activity in

MDMA-treated rats was significantly greater on day 1 compared to day 7 for the first 25 minutes of the habituation phase, and activity was greater on day 7 compared to day 1 at all time intervals between 10 and 55 minutes post-injection.

In the treatment groups given either 4-MMC + MDMA mixture, each repeated measures two-way ANOVA indicated significant main effects of time interval, test day, as well as significant time interval by test day interactions. Post hoc analyses indicated that, in animals treated with 4-MMC 1 + MDMA 3, activity was elevated on day 7 compared to day 1 between 5 and 45 minutes post-injection. Post-tests also indicated that animals treated with 4-MMC 5 + MDMA 3 displayed greater horizontal activity on day 7 compared to day 1 between 10 and 60 minutes post-injection.

A mixed model two-way ANOVA was also conducted on cumulative horizontal activity following injections with treatment group as a between subjects variable and test day as a within subjects variable. This analysis indicated a significant treatment effect [F (5, 114) =32.38, $p<.05$], a significant test day effect [F (1, 114) =70.13, $p<.05$], and a significant treatment by test day interaction [F (5, 114) =11.16, .05]. These results are displayed in Figure 2. As noted by the symbols, Sidak post-hoc tests indicated a statistically significant difference in cumulative measures of drug-induced activity between day 1 and day 7 only in the animals treated with MDMA, 4-MMC 1 + MDMA, and 4-MMC 5 + MDMA.

Expression of Sensitization to MDMA or 4-MMC

On day 17, rats were challenged with saline, 3.0 mg/kg MDMA, 1.0 mg/kg 4-MMC, or 5.0 mg/kg 4-MMC to test for expression of sensitization and cross-sensitization between MDMA and 4-MMC. These data are displayed in Figure 3 as post-injection sums (± SEM) of horizontal beam breaks by treatment group and in Figure 4 the number of horizontal beam breaks (± SEM)

at each 5 min interval during the two hour assessment period. Three separate analyses were conducted on cumulative measures of horizontal activity on day 17. For each of these analyses, a one-way ANOVA was conducted to compare the activity of rats that received the same drug treatment during the post-washout challenge test. The saline-saline treated group was also included in each of these three analyses.

A one way ANOVA of the activity produced by MDMA indicated that rats pretreated with MDMA or an MDMA+4-MMC mixture displayed significantly higher levels of activity than did rats challenged with saline [F (6, 49) =6.71, $p<.05$]. However, pretreatment with MDMA or a 4-MMC+MDMA mixture did not produce increased activity upon subsequent challenge with MDMA compared to animals pretreated with saline and challenged with MDMA.

When challenged with 1.0 mg/kg 4-MMC, none of the treatment groups displayed significantly greater activity compared to the saline-saline treatment group. When challenged with 5.0 mg/kg 4-MMC all treatment groups had significantly greater cumulative measures of activity than saline-saline animals [F (4, 35) =6.92, $p<0.05$]. However, none of the treatment groups that received drug during the induction phase (days 1 through 7) displayed higher activity when challenged with 4-MMC 5 compared to those rats that received saline during the induction phase.

Discussion

The goal of this study was to assess the induction of sensitization in Sprague-Dawley rats when they were repeatedly dosed with MDMA, 4-MMC, or mixtures of these two substances. The possibility was also assessed that prior exposure to MDMA, 4-MMC, or mixtures of these substances would influence the expression of sensitization to either MDMA or 4-MMC when these substances were delivered individually after a delay.

The results of this study indicate the induction of sensitization to MDMA and to MDMA+4-MMC mixtures, although sensitization was not induced in animals that received only 4-MMC. The current findings regarding sensitization to MDMA are in agreement with previous research completed by Kalivas et al. (1998) and Modi et al. (2006). Failure of 4-MMC to induce sensitization to ambulatory behavior is also in agreement with results reported by Gregg et al. (2013b), though several methodological differences between these studies are worth noting. Also important to note is that Gregg et al. (2013b) did report evidence of sensitization to repetitive behaviors, but similar measures of activity were not included in the present study.

Both Kalivas et al. (1998) and Modi et al. (2006) offer several explanations for sensitization to MDMA's motor stimulant effects following repeated exposure. Much of what they suggest is related to the mesocorticolimbic structures, especially the nucleus accumbens, neural pathways heavily implicated to play a role in behavioral sensitization (Pierce & Kalivas, 1997). For example, Kalivas et al. (1998) analyzed neurochemical data from the nucleus accumbens and found that repeated administration of MDMA increased extracellular dopamine in a manner similar to what has been observed with other drugs known to produce locomotor sensitization. An increase in extracellular dopamine in this brain region is thought not only to play a role in the locomotor effects of stimulant drugs, but also to contribute to the addictive properties of stimulants (Pierce & Kalivas, 1997).

Gregg et al. (2013b) proposed several reasons that repeated administration of 4-MMC did not produce sensitization to the ambulatory effects of this drug. They proposed that the high doses (15-30mg/kg) and dosing pattern used in their study preferentially sensitized repetitive behavior (Gregg et al., 2013b). When those variables were controlled for in the present research using a constant dosing pattern and a lower dose of 4-MMC, sensitization of ambulatory activity

was still not observed, although a there was a trend toward ambulatory sensitization seen in rats repeatedly dosed with 5 mg/kg. Considering the pharmacological similarities of 4-MMC and MDMA (Baumann et al., 2012), 4-MMC may produce ambulatory sensitization at a dose that has not yet been tested, however this seems unlikely as the dose-response functions for MDMA and 4-MMC at NET, DAT, and SERT are nearly identical (Baumann et al., 2012). Alternatively, Ball, Budreau, & Rebec (2006) observed that there may be a context-dependent component that influences sensitization. Therefore, the context in which 4-MMC is delivered may have a role in the induction of ambulatory sensitization to this substance.

Finally, there may be an important methodological difference between the report of ambulatory activity in Gregg et al. (2013b) and that of the present research. Whereas Gregg et al. (2013b) report ambulatory behavior as the total number of consecutive beam breaks in the horizontal plane, the present study did not discriminate between consecutive and repeated beam breaks in the horizontal plane in reporting ambulatory activity. Future research should define ambulatory activity along one of these parameters and retest 4-MMC for ambulatory sensitization.

Induction of sensitization was observed in both 4-MMC+MDMA treatment groups. As this is the first study to examine a mixture of MDMA and 4-MMC, it is difficult to say why concurrent treatment with these substances induced sensitization to horizontal activity, although the pharmacological properties of each substance alone are well established, which allows for some explanations to be offered.

Considering the time course of horizontal activity for the 4-MMC 1+ MDMA treatment group, it appears these two drugs may simply have additive effects, as the increase in horizontal activity looks like the combination of the increased horizontal activity observed in the 4-MMC 1

and MDMA 3 treatment groups. However, analyzing the mean horizontal activity of the 4-MMC 5 + MDMA treatment group suggests the effects of this mixture is not simply an addition of the effects of the two drugs. Not only did the 4-MMC 5+ MDMA drug mixture produce greater sensitization, but the increase in activity was also sustained at the same magnitude through the duration of the post-injection monitoring period, an observation not observed when either 4-MMC 5 or MDMA were given individually. Further research on the interaction of these drugs is required to elucidate the results obtained from the current study.

Although sensitization was induced in rats that were repeatedly exposed to MDMA or 4-MMC+MDMA mixtures, the results of the present study indicate that only those rats pretreated with MDMA or 4-MMC 5+MDMA expressed sensitization to MDMA. Additionally, animals pretreated with 4-MMC alone did not exhibit expression of sensitization to 4-MMC or cross-sensitization to MDMA. This observation differs from observations made by Lisek et al. (2012), who observed expression of sensitization in a low dose (0.5 mg/kg) of 4-MMC, and Gregg et al. (2013a), who observed that prior exposure to 4-MMC enhanced subsequent response on a cocaine challenge.

Several factors may have contributed to these discrepant results. In regards to Lisek et al. (2012), it is possible that drug delivery context may have contributed to their observation of ambulatory sensitization (Ball et al., 2006), although their methods do not make it clear whether drug was administered in a context-dependent or context-independent manner. Kalivas & Duffy (1993) show that length of the forced abstinence period may also influence the level to which sensitization is expressed; however, both the present study and Lisek et al. (2012) utilized the same period of 10 days of forced abstinence. Further research should aim to clarify whether expression of sensitization is observed in animals with prior exposure to low doses of 4-MMC.

Gregg et al. (2013a) observed that prior exposure to 4-MMC enhanced subsequent response to cocaine; however the present study did not replicate those findings when rats were challenged with MDMA. This discrepancy could be due to the pharmacological differences between MDMA and cocaine (Baumann et al., 2013; Cameron et al., 2013), although there are other explanations to consider, as the mechanisms underlying 4-MMC cross-sensitization remain poorly understood (Gregg et al., 2013a). Prior exposure to MDMA also did not influence cross-sensitization to 4-MMC challenge. Given the pharmacological similarities between these drugs (Baumann et al., 2013; Cameron et al., 2013), it seems likely that the mechanism by which they produce cross-sensitization would be similar. Further research should elucidate this mechanism.

In conclusion, the major results of this experiment are that repeated exposure to mixtures of 4-MMC and MDMA induce sensitization to the locomotor stimulant effects of the drug mixture. This is suggestive of increased abuse liability of mixtures of MDMA and 4-MMC, although further research should utilize additional preclinical trials, such as conditioned place preference and self-administration, to further evaluate this possibility. These conclusions are limited because the neuropharmacological interactions of 4-MMC and MDMA, especially after repeated administration, are unknown. Future research should also focus on clarifying how the neurochemical actions of these drugs interact.

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Treatment Groups		
Induction Phase Treatment (Days 1-7)	Post Washout Challenge Treatment (Day 17)	Number of Animals
Saline	Saline	8
Saline	MDMA 3	8
Saline	4-MMC 1	8
Saline	4-MMC 5	8
MDMA 3	MDMA 3	8
MDMA 3	4-MMC 1	8
MDMA 3	4-MMC 5	8
4-MMC 1	MDMA 3	8
4-MMC 1	4-MMC 1	8
MDMA 3+4-MMC 1	MDMA 3	8
MDMA 3+4-MMC 1	4-MMC 1	8
4-MMC 5	MDMA 3	8
4-MMC 5	4-MMC 5	8
MDMA 3+4-MMC 5	MDMA 3	8
MDMA 3+4-MMC 5	4-MMC 5	8

Table 1: Treatment Groups. During the induction phase, six treatments were used: SAL, MDMA 3, 4-MMC 1, 4-MMC 5, MDMA 3+4-MMC 1, or MDMA 3+4-MMC 5 (*n*=32, 24, or 16). During the post washout challenge, each pretreatment group was split into subgroups such that a set of eight animals from each of the pretreatment groups would receive one of the three drug treatments tested post washout.

Note: Numbers after drug names in the above table refer to drug dose in mg/kg

Two-way Repeated Measures ANOVA Results			
Treatment Group	Time	Day	Time*Day Interaction
Saline (n=32)	[$F(23, 713)=98.81, p<.05$]	[$F(1, 31)=10.64, p<.05$]	[$F(23, 713)=6.01, p<.05$]
MDMA 3 (n=24)	[$F(23, 529)=44.03, p<.05$]	[$F(1, 23)=.02, p>.05$]	[$F(23, 529)=14.17, p<.05$]
4-MMC 1 (n=16)	[$F(23, 345)=52.35, p<.05$]	[$F(1, 15)=.06, p>.05$]	[$F(23, 345)=1.65, p<.05$]
4-MMC 5 (n=16)	[$F(23, 345)=45.43, p<.05$	[$F(1, 15)=.03, p>.05$]	[$F(23, 345)=6.80, p<.05$
4-MMC 1+MDMA 3 (n=16)	[$F(23, 345)=33.48, p<.05$]	[$F(1, 15)=4.85, p<.05$]	[$F(23, 345)=9.87, p<.05$
4-MMC 5+MDMA 3 (n=16)	[$F(23, 345)=37.92, p<.05$]	[$F(1, 15)=12.48, p<.05$]	[$F(23, 345)=17.16, p<.05$]

Table 2: In the above table are the statistical values obtained from each two-way repeated measures ANOVA comparing the mean number of horizontal beam breaks per treatment group on day 1 and day 7.

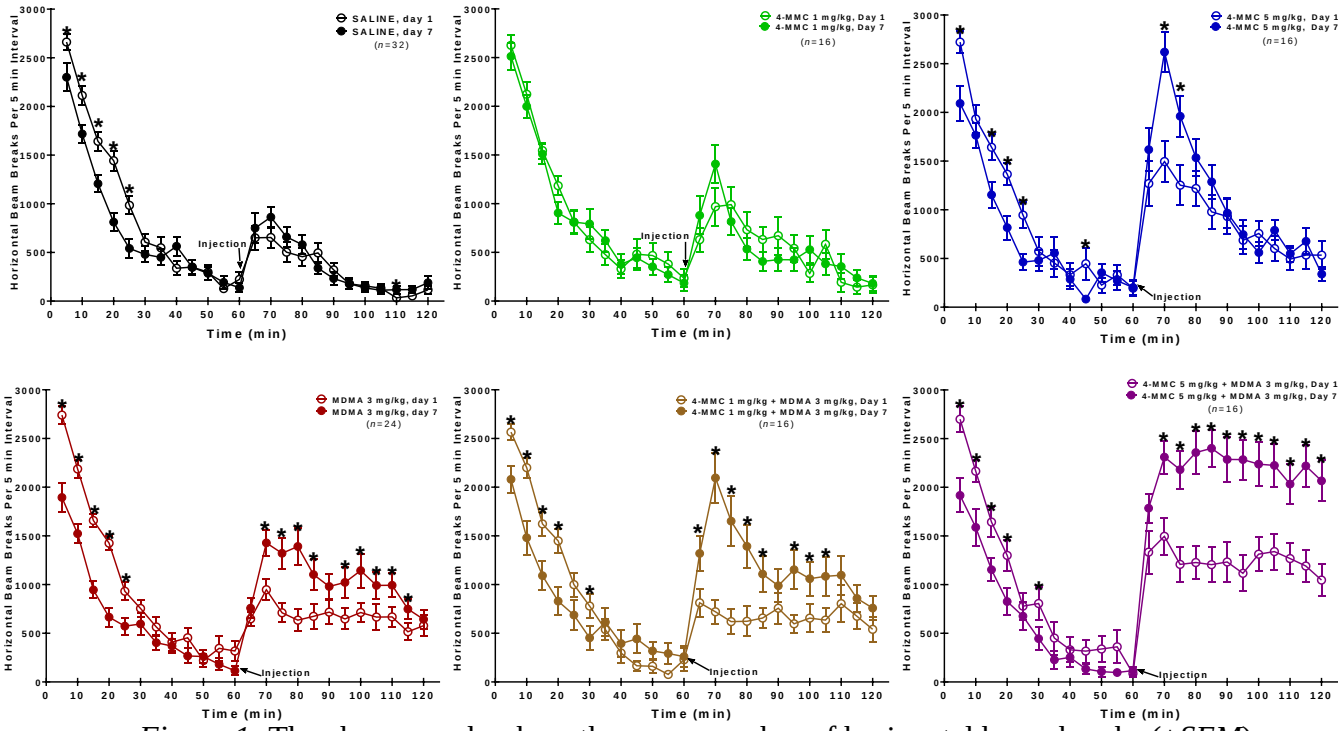

Figure 1: The above graphs show the mean number of horizontal beam breaks (±*SEM*) per treatment group on day1 and day 7. Each line indicates 60 minutes of habituation and 60 minutes post-injection. Each data point shows the mean number of horizontal beam breaks for the treatment group ± *SEM*. Asterisks above individual data points signify that average treatment group activity was statistically different between day 1 and day 7.

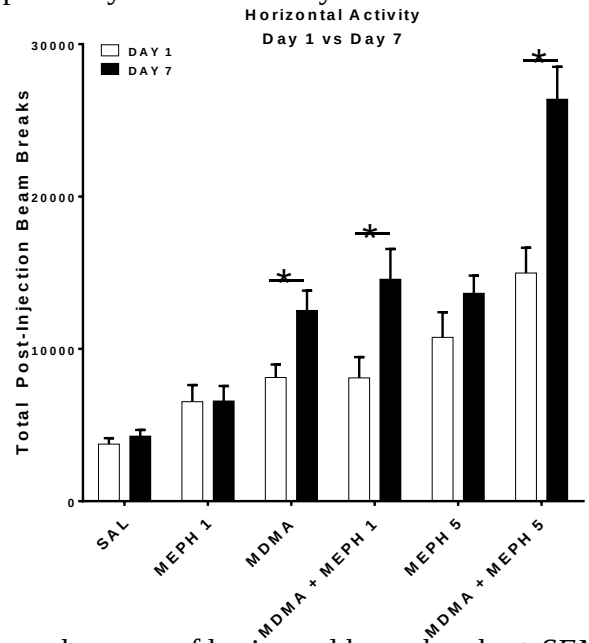

Figure 2: The above graph shows the sums of horizontal beam breaks ± *SEM* 60 minutes post injection on day 1 and day 7. Asterisks indicate statistically greater activity day 7 compared to day 1.

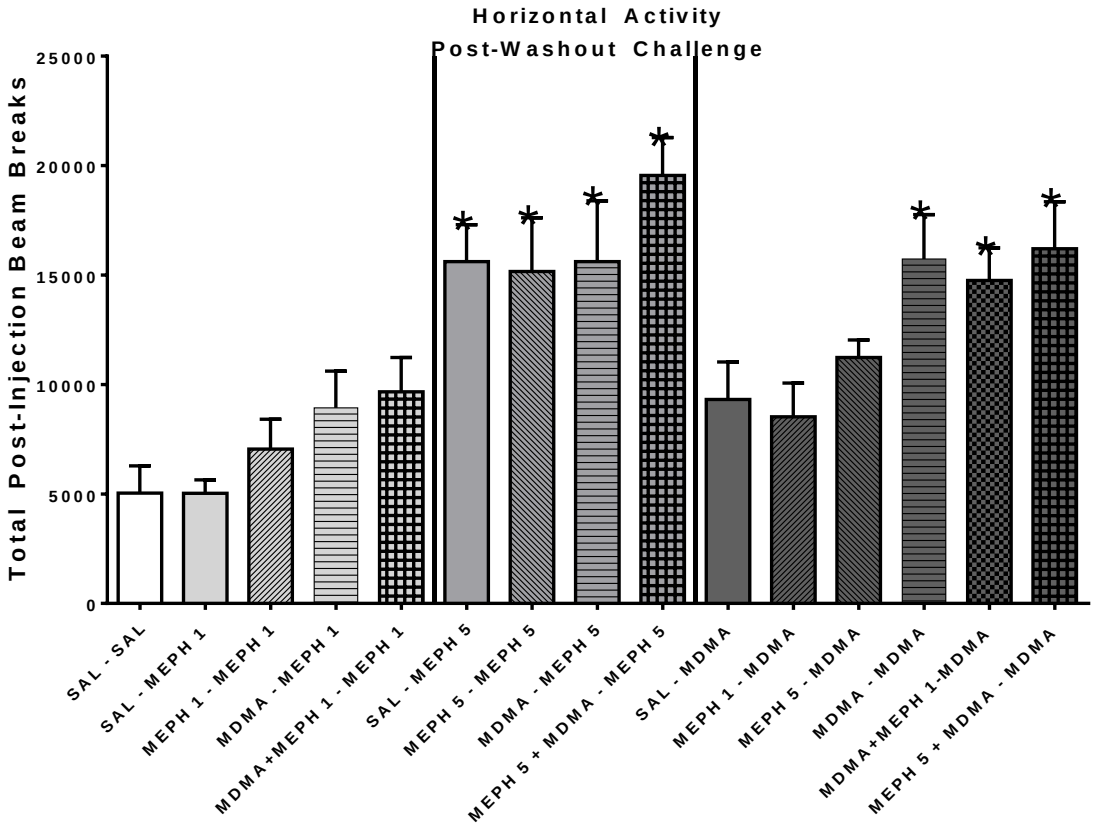

Figure 3: The above graph shows the average total horizontal beam breaks ± *SEM* 60 minutes post-injection on the day 17 challenge. Asterisks designate treatment groups significantly different from the saline-saline treatment group. No significant differences were observed between groups that received drug in both pretreatment and challenge and those animals that received saline pretreatment and drug challenge.

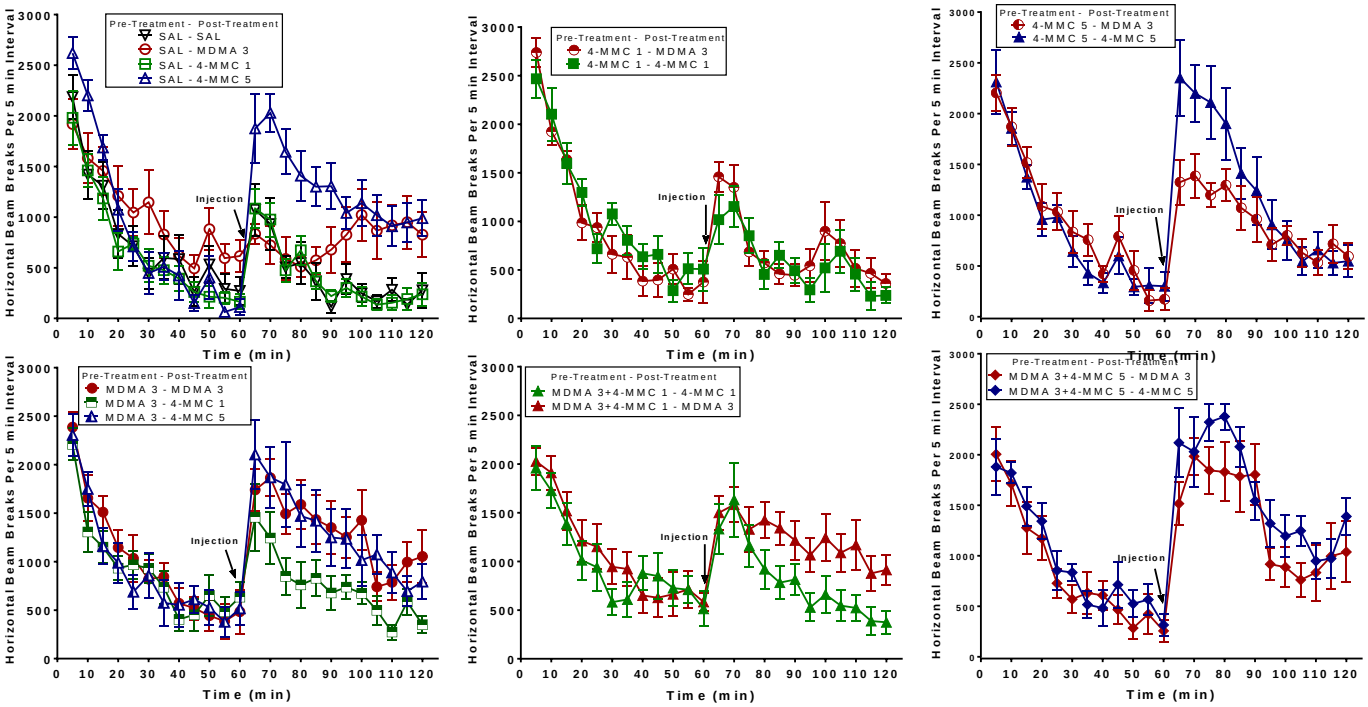

Figure 4: The above graphs show the time course of data of post washout treatment groups. Each data point represents the average number of horizontal beam breaks per treatment group ± *SEM*

Note: Statistical analyses were not performed on these data.