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Research Article

Sensitization to the Motor Stimulant Effects of 3,4-Methylenedioxypyrovalerone (MDPV) and Cross-Sensitization to Methamphetamine in Rats

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Abstract *Background.* In recent years, there has been a dramatic increase in abuse of the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV), often in combination with other illicit stimulants. *Purpose.* We sought to determine if repeated exposure to MDPV would produce sensitization to the motor stimulant effects of the drug, and whether cross-sensitization would develop with the stimulant effects of methamphetamine (METH). *Study design.* Male Sprague-Dawley rats were administered MDPV (1 mg/kg or 5 mg/kg) or saline once daily for five days at 24 h intervals, or were administered MDPV (1 mg/kg) or saline once daily for five days at 48 h intervals. For cross-sensitization experiments, rats were administered METH (1 mg/kg) or MDPV (1 mg/kg or 5 mg/kg) once daily for five days at 48 h intervals and, following a five-day incubation period, were given an acute challenge injection of either MDPV (0.5 mg/kg) or METH (0.5 mg/kg), respectively. *Results.* Rats repeatedly administered MDPV (1 mg/kg) every 48 h, but not every 24 h, demonstrated increased motor activity when given either a subsequent challenge of MDPV (0.5 mg/kg IP) or METH (0.5 mg/kg), indicating the development of behavioral sensitization and cross-sensitization, respectively. Moreover, rats repeatedly administered METH (1 mg/kg) every 48 h did not exhibit cross-sensitization to the motor stimulating effects of a subsequent challenge with MDPV (0.5 mg/kg). *Conclusion.* These results suggest that specific patterns of MDPV administration may lead to lasting changes in behavioral responses to subsequent METH exposure.

Keywords psychostimulant; synthetic cathinone; methamphetamine; sensitization; cross-sensitization; MDPV

1. Introduction

Synthetic cathinones are designer psychostimulants that are often falsely marketed as "bath salts" or "legal high" alternatives to traditional illicit psychostimulants such as methamphetamine (METH), cocaine, or 3,4-methylenedioxymethamphetamine (MDMA). Synthetic cathinones have become increasingly popular drugs of abuse in recent years. In the United States, mephedrone, methylone, and 3,4-methylenedioxypyrovalerone (MDPV) initially emerged as the most prominent "bath salts" constituents, comprising the vast majority of all synthetic cathinones obtained in drug seizures prior to their permanent classification as Schedule I substances in 2012 [1]. Of these three, MDPV was the most commonly abused in the USA [2] and identified in numerous case reports of synthetic cathinone-related toxicity, bizarre behaviors, and death [3,4,5,6,7,8].

Despite its placement into Schedule I status in the USA in 2012, MDPV continues to appear in drug markets, and recent reports of MDPV addiction have emerged [9]. While MDPV-related toxicity is now well established, the scientific assessment of abuse liability is still in its infancy [10,11]. Preclinical animal studies have revealed that MDPV has potent reinforcing [12,13,14] and rewarding effects [14,15,16], fully substitutes for cocaine and methamphetamine in drug discrimination tests [17,18], and elevates locomotor activity in a manner indicative of psychostimulants [12,17,19,20]. In humans, concurrent use of MDPV and other illicit stimulants is prevalent and evidence suggests that prior stimulant use enhances severity of adverse sympathomimetic effects during acute MDPV use [7]. Users of synthetic cathinones tend to be young individuals that may subsequently engage in intake of other illicit stimulants such as cocaine or METH, and conversely, users of traditional psychostimulants often transition to concurrent use of synthetic cathinones due to their increased availability and/or reduced cost [21,22,23,24]. However, despite mounting preclinical literature suggesting an abuse potential of MDPV, and human literature suggesting enhanced vulnerability to MDPV toxicity with prior amphetamine use, there are currently no published reports detailing whether MDPV exposure alters behavioral sensitivity and responsiveness, and thus potentially abuse vulnerability to traditional illicit

psychostimulants (e.g., METH). Conversely, it has not yet been determined if use of traditional psychostimulants such as METH enhances behavioral sensitivity to subsequent MDPV exposure. One method for assessing lasting changes in behavioral sensitivity and responsiveness is via motor sensitization, in which repeated exposure to a drug leads to a progressive and enduring enhancement of motor behavior (e.g., locomotion, stereotypy, etc.) elicited by a subsequent drug challenge [25].

With regards to synthetic cathinones, it has been previously demonstrated that prior exposure to mephedrone produces locomotor sensitization in rats when given a subsequent mephedrone or cocaine challenge [26,27, 28,29]. Furthermore, repeated oral administration of cathinone, the parent compound of synthetic cathinones and the primary psychoactive alkaloid found in *Catha edulis*, also leads to locomotor sensitization in rats [30, 31]. However, to our knowledge, the phenomenon of behavioral sensitization has not yet been established for MDPV. Therefore, the purpose of the present study was to assess the ability of repeated MDPV administration to produce motor sensitization. We also sought to determine if cross-sensitization between MDPV and METH could be observed.

2. Methods

2.1. Subjects

Male Sprague-Dawley rats (Harlan Laboratories, Livermore, CA, USA), weighing approximately 250–275 g upon arrival, were housed in a humidity- and temperature-controlled colony, maintained on a 12:12 reversed light/dark cycle, and were provided ad libitum access to food and water except during locomotor testing procedures. All experimentation was conducted during the dark phase (7:00 AM–7:00 PM), which corresponds to the active phase in humans. All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University and in accordance with the principles of the National Research Council's *Guide for the Care and Use of Laboratory Animals* (2011).

2.2. Drugs

MDPV was purchased from Laboratory Supply USA (San Diego, CA, USA). A 10 mg sample of MDPV was analyzed by liquid chromatography-mass spectrometry for purity at the Research Triangle Institute (Durham, NC, USA) and determined to have an apparent purity of $> 95\%$, as previously reported [14]. Methamphetamine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO, USA). All drugs were dissolved in sterile saline and administered via the intraperitoneal (IP) route in a volume of 1 mL/kg.

2.3. Motor testing procedures

Motor activity was assessed using a Rotorat system apparatus (Med Associates, St Albans, VT, USA). This apparatus measures rotational ambulation and locomotor behavior quantified by quarter turns (90 ° rotations) in a bowl-shaped arena as we have previously described [32,33], and offers the advantage of measuring bodily rotations as an index of psychostimulant effects that can go undetected when animals are tested in an open field environment, which primarily measures forward horizontal motor activity [34, 35,36]. In addition, rotation can be measured in rotational increments as small as 2 °, which allows for customization of the threshold for assessing motor activity. For all experiments, drug or saline injections were administered immediately prior to being placed into the arena for 90 min. Prior to commencement of drug administration procedures, all rats underwent two days of acclimation to the testing apparatus. On the first acclimation day, rats were placed into and allowed to freely explore the arena for 90 min during which no locomotor activity was recorded. On the second day, rats were first fitted with a plastic neck collar, followed by administration of saline immediately prior to placement into the arena for 90 min. Rotational activity was recorded by a rotating actuator mounted at the top of the arena that was connected to the rat via a stainless steel spring tether and a metal clamp affixed to the plastic collar. For all subsequent sessions, activity was assessed in identical 90 min sessions, as prior studies have revealed peak effects of MDPV on locomotor activity during this time period [12, 17,20,37].

2.4. Experiments

2.4.1. Experiments 1a and 1b—MDPV-induced motor sensitization with 24 h intertreatment intervals

Initial doses of MDPV of 1 mg/kg and 5 mg/kg were chosen based upon previously published findings by our laboratory and others of lowered thresholds for intracranial self-stimulation at similar doses [14,15,16] as well as acute locomotor stimulant properties of MDPV [12,17,18,20]. In Experiment 1a, rats were injected with either MDPV 1 mg/kg ($n = 8$) or vehicle ($n = 8$) and placed into the testing apparatus for 90 min sessions once daily for five consecutive days, 24 h apart (thus sessions 1–5 = days 1–5). Experiment 1b followed the same timeline, but rats were injected with either MDPV 5 mg/kg ($n = 8$) or vehicle ($n = 8$) once daily for five consecutive days, 24 h apart prior to placement into the test apparatus for 90 min. Following a five-day incubation period where rats remained in the home cage, rats that previously received the 1 mg/kg dose of MDPV or vehicle received a 0.5 mg/kg challenge dose prior to the final activity test session. For rats receiving the 5 mg/kg dose of MDPV, a challenge dose of 1 mg/kg was administered prior to the final activity test session.

2.4.2. Experiment 2—MDPV-induced motor sensitization with 48 h intertreatment intervals

For Experiment 2, rats received either MDPV 1 mg/kg ($n = 10$) or saline ($n = 8$) immediately prior to 90 min sessions once daily for five days, 48 h apart (thus sessions 1–5 = days 1, 3, 5, 7, and 9). Following a five-day incubation period where rats remained in the home cage, all rats regardless of drug history received a saline injection immediately prior to a 90 min test session to assess for any residual nonspecific activity (e.g., context-related locomotion). On the next day, all rats received a 0.5 mg/kg challenge dose of MDPV prior to the final activity test session.

2.4.3. Experiment 3—cross-sensitization of METH-induced motor sensitization to MDPV

For Experiment 3, rats were treated with either 1 mg/kg METH ($n = 16$) or saline ($n = 16$) immediately prior to daily 90 min sessions for five days, 48 h apart (thus sessions 1–5 = days 1–9). The 48 h intertest interval was chosen to be consistent with Experiment 2. Following a five-day incubation period where rats remained in the home cage, all rats regardless of drug history received a saline injection immediately prior to a test session to assess for any residual nonspecific activity. On the next day, all rats received a challenge injection of 0.5 mg/kg MDPV dose prior to the final activity test session.

2.4.4. Experiment 4—cross-sensitization of MDPV-induced motor sensitization to METH

For Experiment 4, rats were administered either 1 mg/kg MDPV ($n = 10$), 5 mg/kg MDPV ($n = 8$), or saline ($n = 8$) immediately prior to daily 90 min sessions for five days, 48 h apart (thus sessions 1–5 = days 1, 3, 5, 7, and 9). The 48 h intertest interval was chosen to be consistent with Experiments 2 and 3 in which sensitization to the motor stimulant effects of MDPV were observed (see Section 3). Following a five-day incubation period, all rats regardless of drug history received a saline injection immediately prior to a test session to assess for any residual nonspecific activity effects. On the next day, all rats received a 0.5 mg/kg challenge of METH dose prior to the final activity test session.

2.5. Data analysis

Data analyses were conducted using Prism 5 software (GraphPad, La Jolla, CA, USA). For all experiments, the dependent measure was total number of quarter (90 °) turns defined as the sum of both clockwise and counterclockwise rotations that were recorded during each of the five 90 min sessions, as well as during saline and challenge test sessions. For Experiments 1–3, activity across the five repeated treatment sessions were analyzed with 2×5 mixed-model ANOVAs, with dose (saline or drug) as the between-subjects factor and session as the within-subjects factor. One-way repeated measures ANOVAs were always conducted for saline and drug groups individually with post hoc pairwise comparisons to compare session effects. For Experiment 4, activity across the five repeated treatment sessions was analyzed with a 3×5 mixed-model ANOVA, with dose (saline, MDPV 1 mg/kg, MDPV 5 mg/kg) as the between-subjects factor and session as the within-subjects factor. For both saline and drug challenge tests, activity measures were analyzed with independent samples t-tests (Experiments 1–3) and Dunnett's test (Experiment 4). Numerical results are displayed as mean $\pm$SEM, where appropriate. P-values $< .05$ were considered significant for all tests.

3. Results

3.1. Baseline and drug-stimulated rotational activity

3.1.1. Experiment 1

In Experiment 1a, statistical analyses revealed a significant difference in baseline activity, such that rats subsequently assigned to the saline treatment group displayed more quarter turns (874.88 ± 148.33) than rats subsequently assigned to the 1 mg/kg MDPV group (468.76 ± 91.56), $t(7) = 2.60$, $P < .05$. There was a significant main effect of session ($F(4,56) = 4.30$, $P < .05$), dose ($F(1,14) = 88.05$, $P < .001$), and a dose $\times$ session interaction ($F(4,56) = 7.49$, $P < .05$). Post hoc analyses revealed that number of total quarter turns were significantly greater during sessions 1 and 2 than session 5 ($P < .05$; see Figure 1(a)). No other session differences were observed. No significant differences were found across sessions in rats receiving saline. Post hoc tests also revealed significantly increased activity in rats receiving 1 mg/kg MDPV as compared to rats receiving saline across all treatment sessions ($P < .05$; Figure 1(a)).

In Experiment 1b, statistical analyses did not reveal a significant difference in number of turns for the initial acclimation session, such that rats subsequently assigned to the saline treatment group displayed similar numbers of quarter turns (763.89 ± 169.21) as compared to rats subsequently assigned to the 5 mg/kg MDPV group (640.63 ± 85.13). There was neither a significant main effect of session nor a significant dose $\times$ session interaction. However, there was a significant main effect of dose ($F(1,15) = 21.12$, $P < .001$), with rats receiving 5 mg/kg MDPV displaying more quarter turns as compared to saline treated animals (Figure 1(b)). For the results of sensitization tests, no significant differences in motor activity following the MDPV 0.5 mg/kg challenge dose were observed between rats with a history of 1 mg/kg MDPV or saline (see Figure 1(a)). In Experiment 1b, rats with a history of saline treatment showed increased motor activity following administration of 1 mg/kg MDPV as compared to rats with a history of 5 mg/kg MDPV treatment ($t(7) = 2.63$, $P < .05$, see Figure 1(b)).

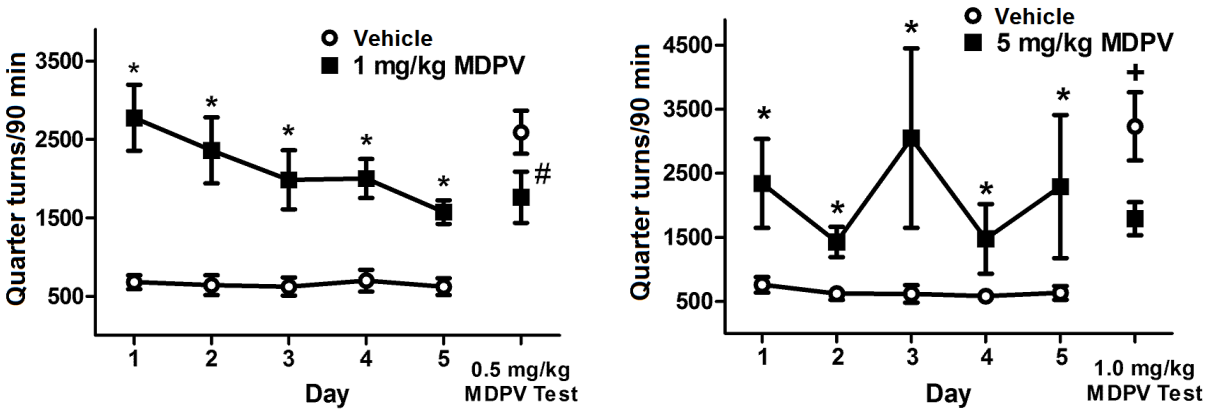


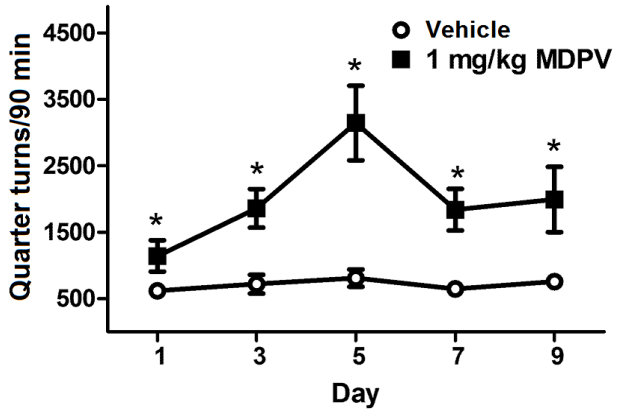
Figure 1: Effects of five repeated MDPV administrations separated by 24 h on rotational activity and behavioral sensitization. (a) For Experiment 1a, 1 mg/kg MDPV (filled squares, $n = 8$) or saline vehicle (open circles, $n = 8$) were administered across five treatment sessions separated by 24 h intervals. (b) For Experiment 1b, 5 mg/kg MDPV (filled squares, $n = 8$) or saline vehicle (open circles, $n = 8$) were administered across five treatment sessions separated by 24 h intervals. Across the five treatment sessions, animals receiving 1 mg/kg MDPV (Experiment 1a) or 5 mg/kg MDPV (Experiment 1b) displayed more quarter turns compared to rats receiving saline ($^{*}P < .05$ vs. saline treated animals on the corresponding day). For Experiment 1a sensitization tests, the number of quarter turns in rats with a history of saline exposure was significantly increased following administration of 0.5 mg/kg MDPV ($^{\#}P < .05$ vs. day 5), yet such increases were not observed in rats with a history of 1 mg/kg MDPV exposure. Similarly, for Experiment 1b sensitization tests, the number of quarter turns in rats with a history of saline exposure was significantly increased following administration of 1 mg/kg MDPV ($^{+}P < .05$ vs. day 5), yet such increases were not observed in rats with a history of 5 mg/kg MDPV exposure.

3.1.2. Experiment 2

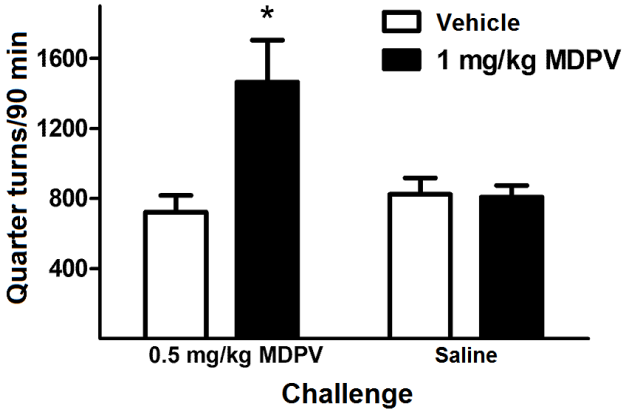
We did not observe a significant difference in activity during the initial acclimation session, such that rats subsequently assigned to the saline treatment group showed a similar number of quarter turns (524.00 ± 86.75) as compared to animals subsequently assigned to the 1 mg/kg MDPV group (510.50 ± 75.24). A two-way mixed ANOVA revealed a significant main effect of session ($F(4,64) = 4.12$, $P < .01$), dose ($F(1,16) = 87.825$, $P < .001$), and a significant dose $\times$ session interaction ($F(4,64) = 2.75$, $P < .05$). Post hoc analyses revealed a significant difference in activity across treatment sessions in the 1 mg/kg MDPV group ($F(4,36) = 4.35$, $P < .01$), with activity levels in session 3 significantly greater than all other sessions (P-values $< .05$; see Figure 2(a)). No significant differences in activity levels were found across sessions in rats receiving saline. Furthermore, the number of quarter turns in rats receiving 1 mg/kg MDPV was significantly higher than rats receiving saline across all treatment sessions ($P < .05$, Figure 2(b)). Rats with a history of repeated treatment with 1 mg/kg MDPV demonstrated an elevated motor response to the 0.5 mg/kg challenge of MDPV as compared to rats with a history of saline treatment ($t(7) = 3.04$, $P < .05$). In contrast, no significant differences between treatment groups were observed in the motor response to a saline challenge ($P > .05$; see Figure 2(b)).

3.1.3. Experiment 3

There were no significant differences in the number of quarter turns between rats subsequently assigned to the saline treatment group (463.00 ± 72.30) as compared to those subsequently assigned to receive 1 mg/kg METH (442.75 ± 49.70). A two-way mixed ANOVA revealed a significant main effect of session ($F(4,120) = 3.42$, $P < .05$), dose ($F(1,30) = 28.246$, $P < .001$), and a dose $\times$ session interaction ($F(4,120) = 3.31$, $P < .05$). Post hoc analyses revealed a significant difference in activity levels across treatment sessions in the 1 mg/kg METH group ($F(4,60) = 4.14$, $P < .01$), with activity in sessions 1, 2, and 3 being significantly lower than session 5, and session 2 being significantly lower than session 4 (Figure 3(a)). No significant differences in activity were found across sessions in rats receiving saline. Rats treated with 1 mg/kg METH showed significantly greater activity than rats receiving saline across all treatment conditions ($P < .05$; Figure 3(b)). There were no significant differences in the motor response to the 0.5 mg/kg MDPV challenge between rats with a history of repeated METH versus saline treatment ($P > .05$). Furthermore, there were no significant differences in motor response to the saline challenge between rats with a history of METH versus saline treatment ($P > .05$; see Figure 3(b)).

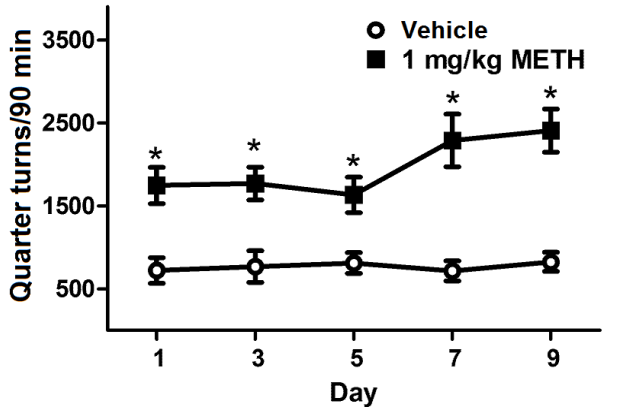


(a)

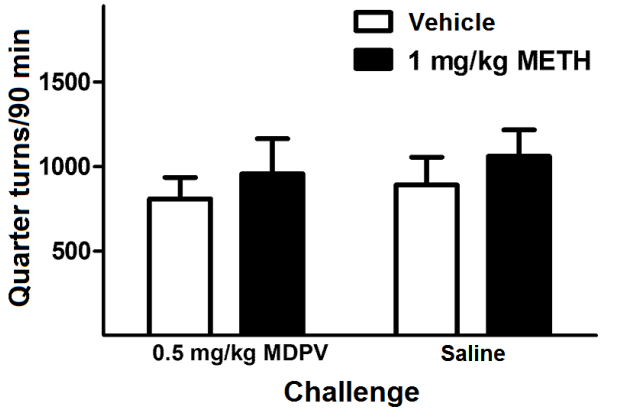


(b)

Figure 2: Effects of five repeated MDPV administrations separated by 48 h on rotational activity and behavioral sensitization. For Experiment 2, 1 mg/kg MDPV (filled squares, $n = 10$) or saline (open circles, $n = 8$) was administered across five treatment sessions separated by 48 h intervals. (a) Across the five treatment sessions, animals receiving 1 mg/kg MDPV displayed more quarter turns compared to rats receiving saline ($^*P < .05$ vs. saline on corresponding day). (b) For sensitization tests, there were no differences in the number of quarter turns following saline administration in rats with a history of 1 mg/kg MDPV or saline exposure. However, administration of 0.5 mg/kg MDPV induced a significantly greater number of quarter turns in rats with a history of 1 mg/kg MDPV versus animals receiving saline ($^*P < .05$).



(a)



(b)

Figure 3: Effects of repeated METH administration separated by 48 h on rotational activity and cross-sensitization with MDPV. For Experiment 3, 1 mg/kg METH (filled squares, $n = 16$) or saline (open circles, $n = 16$) was administered across five treatment sessions separated by 48 h intervals. (a) Across the five treatment sessions, animals receiving 1 mg/kg METH displayed more quarter turns compared to rats receiving saline ($^*P < .05$ vs. saline on corresponding day). (b) For sensitization tests, there were no differences observed in the number of quarter turns following administration of saline or 0.5 mg/kg MDPV between rats with a history of 1 mg/kg METH or saline exposure.

3.1.4. Experiment 4

There were no significant differences in baseline number of quarter turns between rats subsequently assigned to the saline treatment group (497.88 ± 96.47), 1 mg/kg MDPV group (513.90 ± 91.62) or 5 mg/kg MDPV group (529.30 ± 90.46). A two-way mixed ANOVA did not reveal a significant main effect of *session* but did reveal a significant main effect of dose ($F(1,21) = 5.07$, $P < .05$) and a significant dose $\times$ session interaction ($F(8,84) = 2.614$, $P < .05$). Pairwise comparison revealed significant differences between the saline and MDPV 1 mg/kg groups as well as between the saline and MDPV 5 mg/kg groups ($P < .05$; Figure 4(a)). One-way ANOVA revealed significant differences between sessions 1 and 3 ($P < .05$). For sessions 1 and 3, activity in saline treated group was significantly lower than that in the 5 mg/kg

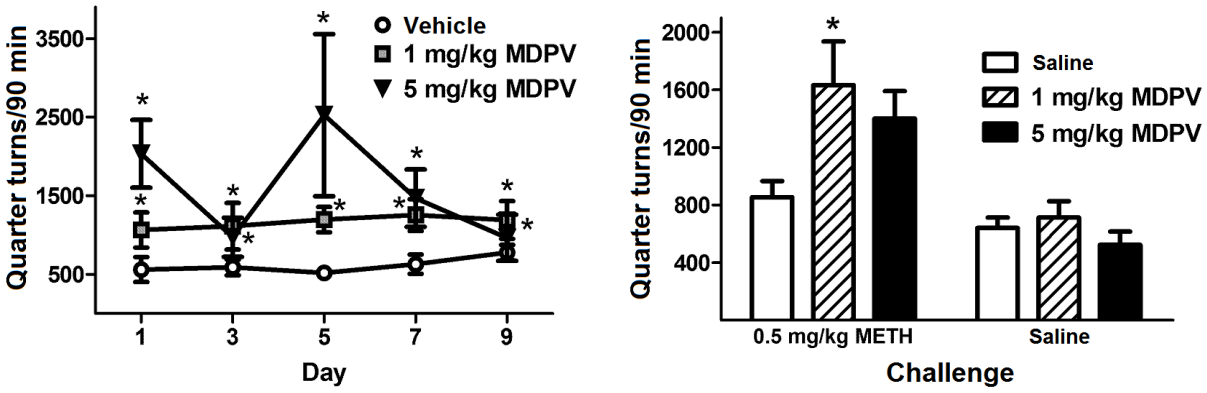


Figure 4: Effects of repeated MDPV administration separated by 48 h on rotational activity and cross-sensitization with METH. For Experiment 4, 1 mg/kg MDPV (filled squares, $n = 10$), 5 mg/kg MDPV (filled triangles, $n = 6$), or saline (open circles, $n = 8$) were administered across five treatment sessions separated by 48 h intervals. (a) Across the five treatment sessions, animals receiving 1 mg/kg MDPV and 5 mg/kg MDPV displayed more quarter turns compared to rats receiving saline ($^*P < .05$ vs. saline on the corresponding day). (b) For sensitization tests, there were no differences in the number of quarter turns following saline administration in rats with a history of 1 mg/kg MDPV, 5 mg/kg MDPV or saline exposure. However, a significantly greater number of quarter turns was observed following challenge with 0.5 mg/kg METH in rats with a history of 1 mg/kg MDPV versus animals with a history of saline ($^*P < .05$).

MDPV group ($P < .05$; Figure 4(b)). Rats with a history of repeated treatment of 1 mg/kg MDPV exhibited increased motor activity in response to a 0.5 mg/kg METH challenge as compared to saline treated animals ($P < .05$). However, rats with a history of treatment with 5 mg/kg MDPV did not exhibit increased motor activity in response to the 0.5 mg/kg MDPV challenge ($P = .13$). There were also no significant differences in locomotor responses following saline challenge between rats with a history of METH 1 mg/kg versus saline treatment or MDPV 5 mg/kg versus saline (see Figure 4(b)). It should be noted that $n = 2$ animals receiving the 5 mg/kg dose of MDPV displayed signs of behavioral toxicity (lethargy, altered posture, piloerection), and data from these animals were excluded from analysis.

4. Discussion

The present study revealed that acute systemic administration of MDPV leads to increased motor activity, and that repeated administration at intermittent (48 h) intervals leads to an enhancement of motor activity when subjects were subsequently tested with a challenge dose of either MDPV or METH. These findings indicate the development of motor sensitization to MDPV and cross-sensitization to METH, respectively. However, when MDPV treatments were separated by 24 h, sensitization to the motor stimulant effects of an MDPV challenge was not observed. Furthermore, rats receiving intermittent administration of METH did not display cross-sensitization to a subsequent MDPV challenge. To our knowledge, this is the first report of motor sensitization to MDPV, as well as cross-sensitization to METH, following repeated MDPV administration. In all of these experiments, enhanced motor activity was not observed following a subsequent saline challenge, suggesting that drug-induced hyperactivity was drug-specific and not driven by contextual conditioning factors such as re-exposure to the testing environment [38, 39].

The augmented motor response seen following repeated exposure to psychostimulants is a robust and common phenomenon observed in laboratory animals [40, 41, 42]. The expression of behavioral sensitization is thought to reflect lasting neural adaptations that develop with repeated drug exposure [25]. Furthermore, evidence suggests that these neuroadaptations may, at least in part, contribute to the transition to compulsive drug use [40, 43], as repeated drug exposure also potentiates the development of drug self-administration and reward [44, 45, 46]. In this regard, our findings suggest that prior MDPV exposure may facilitate the rewarding or reinforcing effects of illicit psychostimulants such as METH.

The primary mechanism of action of MDPV is similar to cocaine in its ability to inhibit presynaptic plasma membrane dopamine and norepinephrine transporters (DAT and NET, resp.), with little effects on presynaptic serotonin transporters (SERT) [19, 47, 48]. Compared to cocaine, however, the potency of MDPV at inhibiting DAT and NET are 50 and 10 times greater, respectively [19]. Furthermore, like cocaine, MDPV induces outward (hyperpolarizing) electrical currents in human DAT-expressing cells, whereas inward DAT currents are produced by METH and other synthetic cathinones such as mephedrone and

methylone [49,50]. Systemic administration of MDPV elevates extracellular dopamine levels in the nucleus accumbens with at least 10 times greater potency than cocaine and with much longer lasting effects [19,20]. In addition, MDPV-induced hyperactivity is correlated with levels of the drug in the striatum [51]. Thus, consistent with previous studies on cocaine and amphetamines [44,45, 52], the ability of MDPV to induce locomotor sensitization is likely mediated by its ability to augment extracellular dopamine levels in limbic and motor regions via potent DAT inhibition.

In the present study, doses of MDPV of 1 mg/kg and 5 mg/kg were chosen based upon previous findings by our laboratory and others of lowered thresholds for intracranial self-stimulation at similar doses [14,15,16] as well as acute locomotor stimulant properties [12,17,18,20]. However, it is unclear how these doses translate to equivalent doses abused by humans, especially given the complexity and difficulty of interspecies dose extrapolation [53]. Using a body surface area as a conversion factor [54], it could be estimated that a 5 mg/kg dose of MDPV would be approximately equivalent to 50 mg in humans, which is larger than doses of $\sim$ 10 mg frequently reported by human users [21,55,56]. However, it should be noted that synthetic cathinone products often contain impurities, adulterants, and even other psychoactive substances, including cocaine and other amphetamine-type stimulants [57,58,59], and it is therefore extremely difficult to precisely estimate dose-response relationships in humans. Additional studies are needed to ascertain behavioral sensitization across a wider range of doses of MDPV and/or METH.

Cross-sensitization has been shown to occur with both cocaine and amphetamines as well as other drug classes [52, 60,61], and is thought to occur when two drugs share overlapping mechanisms of action, albeit often differing from their primary mechanism of action [42]. Thus, while MDPV increases extracellular dopamine through DAT inhibition, and METH increases extracellular dopamine through monoamine substrate releasing effects, the net common effect of increased dopamine transmission on postsynaptic dopamine receptors is a likely possibility [52]. Furthermore, repeated exposure to both cocaine and amphetamines also enhance glutamate signaling in corticolimbic circuits [42, 62], which may also play a role in the locomotor sensitizing effects of MDPV and its cross-sensitization to METH.

However, we noted that animals with a history of treatment of 5 mg/kg MDPV did not show evidence of cross-sensitization to METH. We hypothesize that these observations may be a result of increasing levels of stereotypic behavior at higher doses, which has been reported by others [12,17,37]. In addition, this lack of cross-sensitization with high doses of MDPV may also be a result of increased formation of metabolites 3,4-dihydroxypyrovalerone and 4-hydroxy-3-methoxypyrovalerone, which have recently been shown to be negatively correlated with locomotor activity [37], although it has not yet been determined if these metabolites are active or brain penetrant.

The lack of cross-sensitization to MDPV in animals with a history of METH exposure was also puzzling, given that across-sensitizing effect of METH was observed in rats with a history of MDPV exposure. Many experimental variables may have contributed to these negative observations, including drug dose, number of exposures, dosing schedule, route of administration, and species/strain effects [36]. Thus, the possibility remains that bidirectional cross-sensitization between MDPV and METH may occur under certain experimental conditions. Given the mechanistic similarity between cocaine and MDPV, we predicted full cross-sensitization for both drugs. It is possible, however, that the lack of bidirectional cross-sensitization may be related to known effects of METH on serotonergic transmission, which is known to modulate rewarding and reinforcing effects of various psychostimulants [63]. Clearly, further studies would be needed to dissect the precise monoaminergic mechanisms underlying cross-sensitization, or lack thereof, between MDPV and METH. In addition, it should be noted that our assessment of motor activation was conducted for 90 min following injection, and it is possible that some residual aspects of MPDV- or METH-induced increases in motor activity may not have been detected in this time frame. Thus the possibility still exists for latent cross-sensitizing effects of MDPV in animals with a history of prior METH exposure. Finally, given the similar neurochemical mechanisms of action of MDPV and cocaine, it is important for future studies to determine the degree of cross-sensitization between these two substances.

The lack of locomotor sensitization to MDPV observed in Experiments 1a and 1b, where the intertreatment interval was 24 h, is not without precedent. In this particular experiment only, we noted higher baseline levels of activity in animals subsequently assigned to receive saline. The reasons for these observations are unknown, but we do not believe this influenced the results, as previous work has revealed that intermittent drug administration with longer interdose intervals (e.g., 48 h or longer) produces greater locomotor sensitization for methamphetamine, cocaine, and morphine [64,65,66]. As mentioned above, it has recently been demonstrated that MDPV-induced hyperactivity is positively correlated with plasma MDPV levels, but inversely related to plasma levels of the MDPV metabolites 3,4-dihydroxypyrovalerone and 4-hydroxy-3-methoxypyrovalerone [37]. Thus, the possibility exists that excess accumulation of potentially inhibitory MDPV metabolites by shorter interdose intervals may actually limit drug-induced hyperactivity, and this is an important area of future study.

5. Conclusions

The findings presented here suggest that repeated use of MDPV can increase the sensitivity and behavioral responsivity to the drug, which may underlie the increased propensity to develop psychostimulant-induced psychosis or toxicity with subsequent use [7,56]. While the present study only evaluated cross-sensitization of the locomotor stimulant effects of MDPV to that of METH, evaluation of MPDV cross-sensitization to other psychostimulants (e.g., MDMA or cocaine) or drug classes are needed to determine the enduring changes in behavioral responsivity induced by this novel psychoactive substance.

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Authors' contributions Lucas R. Watterson and Peter R. Kufahl contributed equally to this work.

Conflict of interest The authors declare that they have no conflict of interest.

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