Detrimental effects of the 'bath salt' Methylenedioxypyrovalerone on social play behavior in male rats

Running title: MDPV and social play behavior

Sara Schiavi[1], Francesca Melancia[1], Emilia Carbone[1], Valeria Buzzelli[1], Antonia Manduca[1], Patricia Jiménez Peinado[1], Clemens Zwergel[2], Antonello Mai[2,3], Patrizia Campolongo[4,5], Louk J.M.J. Vanderschuren[6], Viviana Trezza[1]

[1]Dept. of Science, University "Roma Tre", Rome, Italy;
[2]Dept. of Drug Chemistry & Technologies, Sapienza University of Rome, Rome, Italy;
[3]Istituto Pasteur- Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome, Italy;
[4]Dept. of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy;
[5]Neurobiology of Behavior Laboratory, Santa Lucia Foundation, 00143 Rome Italy;
[6]Dept. of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands;

Corresponding author:
Viviana Trezza, PhD
Department of Science, Section of Biomedical Sciences and Technologies
University "Roma Tre"
Viale G. Marconi 446
00146 Rome, Italy
tel: +39-0657336343
fax: +39-0657336365
email: viviana.trezza@uniroma3.it

Abstract

Methylenedioxypyrovalerone (MDPV) is the most popular synthetic cathinone found in products marketed as 'bath salts', widely abused among teenagers and young adults. Synthetic cathinones have pharmacological effects resembling those of psychostimulants, which are known to disrupt a variety of social behaviors. However, despite the popular use of MDPV by young people in social contexts, information about its effects on social behavior is scarce. To investigate the impact of MDPV on social behavior at young age, and the underlying neurobehavioral mechanisms, we focused on social play behavior. Social play behavior is the most characteristic social behavior displayed by young mammals and it is crucial for neurobehavioral development. Treatment with MDPV reduced social play behavior in both juvenile and young adult male rats, and its play-suppressant effect was subject to tolerance but not sensitization. As the behavioral effects of MDPV have been ascribed to dopaminergic and noradrenergic neurotransmission, and given the role of these neurotransmitters in social play, we investigated the involvement of dopamine and noradrenaline in the play-suppressant effects of MDPV. The effects of MDPV on social play were blocked by either the α2 adrenoceptor antagonist RX821002 or the dopamine receptor antagonist flupenthixol, given alone or together at sub-effective doses. In sum, MDPV selectively suppresses the most vigorous social behavior of developing rats through both noradrenergic and dopaminergic mechanisms. This study provides important preclinical evidence of the deleterious effects of MDPV on social behavior, and as such increases our understanding of the neurobehavioral effects of this popular cathinone.

Introduction

Methylenedioxypyrovalerone (MDPV) is a designer drug belonging to the class of synthetic cathinones, a group of psychoactive substances that emerged in the mid-2000s as alternatives to illicit psychostimulants such as amphetamine, MDMA or cocaine (1, 2). Currently, MDPV is a popular cathinone found in products marketed as 'bath salts', which are easily available online. Its use is predominant among youth populations particularly in social contexts, such as festivals and rave parties (3-5). Due to their high abuse potential, the constantly growing market and the lack of any registered medicinal use, most of the common cathinones, including MDPV, have been classified as Schedule I controlled substances in the United States (Drug Enforcement Administration, 2011) and they are monitored by the European Monitoring Center for Drugs and Drugs Addiction (EMCDDA) (6). Still, MDPV continues to be available and its use by young people is a matter of concern (6).

It has been recently reported that synthetic cathinones are β-keto analogs of amphetamine with pharmacological effects resembling those of cocaine and amphetamines (7). In particular, the behavioral effects of MDPV have been mainly ascribed to dopaminergic and noradrenergic neurotransmission, as it acts in the brain as a potent uptake inhibitor at plasma membrane transporters for dopamine (DAT) and noradrenaline (NET) (8). However, despite the fact that the use of synthetic cathinones among young people is a growing public health concern, their impact on brain and behavior is still a matter of debate. Users report MDPV-related positive psychoactive effects resembling those of cocaine, such as euphoria, talkativeness, sexual arousal, alertness, motor excitation, and increased concentration, sociability, productivity, motivation and libido (1, 6). However, several adverse effects have also been reported following acute intoxication, such as hallucinations, psychosis, delirium, depressed mood, anxiety, agitation, disorganized thoughts and cognitive alteration, that also occur in users of amphetamine and cocaine (1, 9, 10).

Preclinical findings in rodents have shown that MDPV induces dose-dependent stereotyped behaviors, alterations in thermoregulation and that it has abuse potential (8, 11-13).

Psychostimulants are known to disrupt a variety of social behaviors (14-18) and it has recently been shown that MDPV enhances aggressive behavior in adult mice with greater potency than cocaine (19). Surprisingly, however, despite the popular use of synthetic cathinones by young people in social contexts, it is not known whether MDPV produces psychostimulant-like disruptions of social behavior at young age.

One characteristic social behavior displayed by the young of most mammalian species, including rats and humans, is social play behavior. This form of social behavior is thought to be of great importance for the development of cognitive and social competence (20-24). Abnormalities in social play are also observed in childhood psychiatric disorders such as autism, early-onset schizophrenia and attention deficit/hyperactivity disorder (25-29). Therefore, because of the scarce information about the effects of MDPV on social behavior and the importance of social play for behavioral development, we here investigated the effects of MDPV on social play in male rats, and the underlying neurobehavioral mechanisms.

Materials and Methods

Animals

Male Wistar rats (Charles River Laboratories, Italy) arrived in our animal facility at 21 days of age and were housed in groups of five in Macrolon cages (43 × 26 × 20 cm) under controlled conditions (temperature 21±1 °C, 60±10% relative humidity and 12/12 h light cycle with lights on at 07:00 a.m). Food and water were available *ad libitum*. After one week of acclimation, animals were tested at post-natal days (PND) 28-30. One experiment (*experiment 2*) was performed in male Wistar rats at PND 80 to evaluate whether the effects of MDPV could be also extended to young adult animals. Overall, a total number of 392 juvenile rats (weighting around 70-100g) and a total number of 69 young adult rats (weighting around 300-350g) were used. Animals were experimentally naive and were used only once. Sample size (n) for each experiment is indicated in the figure legends. The experiments were approved by the Italian Ministry of Health (Rome, Italy) and performed in agreement with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) (30) guidelines, and the guidelines of the Italian Ministry of Health (D.L. 26/14) and the European Community Directive 2010/63/EU.

Drugs

3,4-Methylenedioxypyrovalerone (MDPV) (0.025, 0.05, 0.1, 0.25 and 0.5 mg/kg), flupenthixol-dihydrochloride (0.125-0.06 mg/kg) and RX821002-hydrochloride (0.2-0.1 mg/kg), were dissolved in saline and given intraperitoneally (i.p.). Solutions were freshly prepared on the day of the experiment and were administered in a volume of 2 ml/kg in juvenile rats and 1 ml/kg in young adult rats. MDPV was administered 30 min before testing while both RX821002 and flupenthixol were administered 15 min prior to MDPV administration. Saline was used as control treatment for all the drugs used.

Experimental design

Eight experiments were performed. To assess the effects of MDPV on social play behavior, in *experiment 1* juvenile rats (PND 28-30) were treated with a broad range of doses of MDPV (0.025, 0.05, 0.1, 0.25, or 0.5 mg/kg) or saline (control group). As MDPV is widely used by young adults, in *experiment 2* we tested the effects of MDPV (0.025, 0.05, 0.1, 0.25, or 0.5 mg/kg) on social behavior in young adult rats (PND 80).

Social play behavior is influenced by the level of social activity of the partner (32-35). Therefore, in *experiment 3* we investigated whether the effects of MDPV on social play in juvenile rats depended on the behavior of the test partner and/or if the social repertoire of saline-treated rats was influenced by MDPV-treated partners. To this aim, we treated none, one, or both members of a test pair with MDPV (0.5 mg/kg), and behavior of both test partners was scored separately. Next, we investigated whether tolerance (*experiment 4*) or sensitization (*experiment 5*) would occur to the effect of MDPV on social play behavior after repeated treatment. To this aim, animals were pretreated with either MDPV (0.5 mg/kg) or saline for 5 consecutive days (postnatal days 25-29). On day 30 (i.e. one day after the last pretreatment injection), animals were isolated for 3.5 h. Next, half of both pretreatment groups (MDPV or saline) was treated 30 min before testing with either saline or MDPV, given at the effective dose of 0.5 mg/kg to assess the occurrence of tolerance (*experiment 4*), or at the sub-effective dose of 0.1 mg/kg to assess the occurrence of sensitization (*experiment 5*).

MDPV acts as a potent uptake inhibitor for dopamine and noradrenaline, but not serotonin (8). Both dopaminergic and noradrenergic neurotransmission have been implicated in the modulation of social play (36, 37). Furthermore, we have previously shown that the play-suppressant effects of methylphenidate and amphetamine are mediated through alpha-2 noradrenergic but not dopaminergic receptors (14, 38-40). Therefore, to clarify whether MDPV exerts its effects on social play through dopaminergic (*experiment 6*) or noradrenergic (*experiment 7*) neurotransmission, animals were treated with either the dopamine receptor antagonist flupenthixol (0.125 mg/kg) or the α-2 adrenoceptor antagonist RX821002 (0.2 mg/kg) before MDPV (0.5 mg/kg). Last, in *experiment*

8 we investigated whether dopaminergic and noradrenergic neurotransmission are simultaneously involved in the effect of MDPV on social play, by treating the animals with either sub-effective doses of both RX821002 (0.1 mg/kg) and flupenthixol (0.06 mg/kg), or with saline (control group) before treatment with MDPV (0.5 mg/kg).

Social play behavior in juvenile rats

All the experiments were performed in a sound-attenuated chamber under dim light conditions. The testing arena consisted of a Plexiglas cage (40×40×60 cm) with approximately 2 cm of wood shavings covering the floor. Rats were tested starting at PND 28-30 and social play behavior was assessed as previously described (41). Rats were individually habituated to the test cage for 10 min on 2 days prior to testing. On the test day, rats were socially isolated for 3.5 h before testing. This isolation period has been shown to induce a half-maximal increase in the amount of social play behavior (42). At the appropriate time before testing, pairs of animals were treated with drugs or vehicle. In all experiments except for *experiment 3*, both animals of a pair received the same drug treatment. The test consisted of placing two animals into the test cage for 15 min. The animals in a test pair did not differ more than 10 g in body weight and had no previous common social experience (i.e., they were not cagemates). A pair of rats was considered as one experimental unit and the behavioral parameters were therefore scored per pair of animals, except for *experiment 3,* in which the behavioral parameters were scored per each individual animal of a test pair. The Observer 3.0 software (Noldus Information Technology BV, Wageningen, The Netherlands) was used to score behaviors related to play. In rats, a bout of social play behavior starts with one rat soliciting ('pouncing') another animal, by attempting to nose or rub the nape of its neck. The animal that is pounced upon can respond in different ways. If the animal that is pounced upon fully rotates to its dorsal surface, 'pinning' is the result, i.e., one animal lying with its dorsal surface on the floor with the other animal standing over it. From this position, the supine animal can initiate another play bout, by trying to gain access to the other animal's neck. Thus, during social play, pouncing is

considered an index of play solicitation, while pinning can be regarded as the terminal component of a single play bout as well as a releaser of a prolonged play bout (43). Pinning and pouncing frequencies can be easily quantified and they are considered to be the most characteristic parameters of social play behavior in rats (36). During the social encounter, animals may also display social behaviors not directly associated with play, such as sniffing or grooming the partner's body.

The following parameters were scored:

Social behaviors directly related to play:

- Frequency of pinning.
- Frequency of pouncing.

Social behaviors unrelated to play:

- Time spent in social exploration: the total amount of time (s) spent in non-playful forms of social interaction (i.e., one animal sniffing or grooming any part of the partner′s body).

Locomotor activity during the social encounter:

- Crossing: a grid, dividing the arena into equally sized squares (6×6 cm), was projected over the recordings, and the number of line crossings made by the animal was recorded.

Social interaction in young adult rats

The test was performed as previously described (44). Eighty-day-old rats were individually habituated to the test cage for 5 min on each of the two days prior to testing. Before testing, animals were socially isolated for 24 hours to enhance their social motivation and thus facilitate the expression of social behaviors during testing. The animals of each pair were similarly treated, did not differ more than 10 g in body weight and were not cage mates. The test was performed between 9 a.m. and 2 p.m. under low light conditions. Behavior was assessed per pair of animals and analyzed by a trained observer who was unaware of treatment condition using the The Observer 3.0 software (Noldus Information Technology BV, Wageningen, The Netherlands).

The total number of play-related behaviors (pouncing, pinning and boxing) and the total time spent in social exploration (sniffing any part of the body of the test partner, social grooming, following/chasing, crawling under/over and kicking) were scored for 10 min (45). Moreover, locomotor activity during the social encounter was assessed by a grid dividing the arena into equally sized squares (6×6 cm) projected over the recordings and the number of line crossings made by the animal was counted.

Statistical analysis

Data are expressed as mean ± SEM and statistical significance was set at $p < 0.05$. To assess the effects of single or combined treatments on social play behavior, data were analyzed using one-way or two-way analysis of variance, respectively, followed by Student-Newman-Keuls *post hoc* tests where appropriate.

Results

MDPV suppresses social play behavior.

The results of *experiment 1* show that systemic administration of MDPV inhibited social play behavior in juvenile rats (pinning: $F_{5,47}$ =5.70, p<0.001; pouncing: $F_{5,47}$ =3.18, p=0.015). MDPV, administered at the highest tested dose (0.5 mg/kg), reduced pinning (p<0.01, Figure 1a) and pouncing (p<0.05, Figure 1b), whereas lower doses (0.025-0.05-0.1-0.25 mg/kg) had no effect on play behavior. General social exploration (i.e., sniffing and grooming any part of the body of the test partner, including the anogenital area) was not affected by MDPV treatment ($F_{5,47}$ =1.72, n.s., Figure 1c). The effects of MDPV on social play were not secondary to changes in locomotor activity, since at the dose that decreased social play behavior (0.5 mg/kg), MDPV did not affect the number of crossings ($F_{5,47}$ =1.57, n.s., Figure 1d). Thus, MPDV selectively suppresses social play behavior in juvenile rats. To support the idea that MDPV induces selective effects on social play behavior, the results of *experiment 2* show that MDPV, administered at the dose of 0.5 mg/kg, reduced the total frequency of play-related behaviors ($F_{5,27}$=2.95, p=0.03, Figure 1e) in young adult rats (PND 80) without affecting general social exploration ($F_{5,27}$=1.62, n.s., Figure 1f) and locomotor activity ($F_{5,27}$=0.13, n.s., data not shown), confirming that MDPV selectively suppresses play-related behaviors even in young adult rats.

An additional experiment (*experiment 3*) was performed in to investigate whether the effects of MDPV on social play behavior in juvenile (e.g., PND 28-30) rats depended on the behavior exhibited by the test partner and/or if the social repertoire of saline-treated rats was influenced by MDPV-treated animals. None, one, or both members of a test pair were treated with MDPV (0.5 mg/kg) and behavior of both test partners was scored separately. The two-way ANOVA analysis gave the following results: pinning $F_{(subject)1,54}$ =9.06, p=0.004; $F_{(partner)1,54}$ =4.14, p=0.05; $F_{(subject\ x\ partner)1,54}$ =5.19, p=0.03; pouncing $F_{(subject)1,54}$ =2.28, p=0.03; $F_{(partner)1,54}$ =4.02, p=0.05; $F_{(subject\ x\ partner)1,54}$ =1.72, n.s.; social exploration $F_{(subject)1,54}$ =0.00, p=n.s.; $F_{(partner)1,54}$=0.83, n.s.; $F_{(subject\ x\ partner)1,54}$ =0.16, n.s. Post-hoc analyses revealed that pinning (p<0.001, Figure 2a) and pouncing

($p<0.05$, Figure 2b) were reduced when both members of a test pair were treated with MDPV. When only the scored animal of a test pair was treated with MDPV, there was also a decrease in pinning ($p<0.001$) and pouncing ($p<0.05$) events, indicating that MDPV-treated rats pin and pounce their partner less, regardless of whether the partner is treated with saline or MDPV. Interestingly, saline-treated rats interacting with MDPV-treated partners also showed reduced pinning ($p<0.01$) and pouncing ($p<0.05$) compared to pairs in which both animals were treated with saline. These results suggest that MDPV-treated rats displayed both reduced initiation to play and responsiveness to play solicitation, indirectly decreasing playfulness in their saline-treated partners as well.

The effects of MDPV on social play are subject to tolerance but not sensitization.

Experiment 4 evaluated whether tolerance occurs to the effects of MDPV on social play behavior after repeated treatment. To this aim, juvenile rats were treated with the dose of MDPV that reduced social play in the previous experiments (0.5 mg/kg, i.p.) or saline (control group) once daily for 5 consecutive days. On the sixth day, half of both pretreatment groups were tested after acute treatment with MDPV (0.5 mg/kg, i.p.), or saline (control group). A two-way ANOVA analysis gave the following results: pinning: $F_{(repeated)1,26}$ =0.45, n.s; $F_{(acute)1,26}$ =9.20, p=0.005; $F_{(repeated\ x\ acute)1,26}$ =1.32, n.s; pouncing: $F_{(repeated)1,26}$ =0.01, n.s; $F_{(acute)1,26}$ =9.90, p=0.004; $F_{(repeated\ x\ acute)1,26}$ =2.17, n.s). Post-hoc analyses revealed that acute administration of MDPV (0.5 mg/kg, i.p.) decreased pinning ($p<0.01$, Figure 3a) and pouncing ($p<0.01$, Figure 3b) in animals repeatedly treated with saline but not in animals repeatedly pretreated with MDPV (0.5 mg/kg, i.p.), indicating that tolerance to the effect of MDPV on social play occurs. Social exploration was not affected by either the repeated or acute treatment ($F_{(repeated)1,26}$ =0.80, n.s.; $F_{(acute)1,26}$ =0.91, n.s.; $F_{(repeated\ x\ acute)1,26}$ =0.072, n.s., Figure 3c).

Next, to evaluate whether sensitization occurs to the effects of MDPV on social play behavior after repeated treatment, animals were treated with MDPV (0.5 mg/kg, i.p.) or saline once daily for 5 consecutive days. On the sixth day, half of both pretreatment groups were treated with a dose of

MDPV that does not affect social play by itself (0.1 mg/kg, i.p.), or saline (*experiment 5*). Acute administration of a low dose of MDPV (0.1 mg/kg, i.p.) did not affect social play in rats repeatedly treated with either saline or MDPV (0.5 mg/kg, i.p), indicating that sensitization to the effect of MDPV on social play behavior had not occurred after repeated treatment (pinning: $F_{(repeated)1,26}$ =0.14, n.s.; $F_{(acute)1,26}$ =1.51, n.s.; $F_{(repeated\ x\ acute)1,26}$ =0.68, n.s., Figure 4a; pouncing: $F_{(repeated)1,26}$ =0.01, n.s.; $F_{(acute)1,26}$ =1.16, n.s.; $F_{(repeated\ x\ acute)1,26}$ =1.47, n.s., Figure 4b). Social exploration was not affected by either the repeated or acute treatment ($F_{(repeated)1,26}$ =0.77, n.s.; $F_{(acute)1,26}$ =1.55, n.s.; $F_{(repeated\ x\ acute)1,26}$ =1.05, n.s., Figure 4c).

Pharmacological mechanisms underlying the effects of MDPV on social play behavior.

It has recently been shown that certain behavioral effects of MDPV are mediated by activation of dopaminergic receptors, as a result of DAT blockade by MDPV (12, 46, 47). To investigate whether the effects of MDPV on social play in juvenile rats also depend on dopaminergic neurotransmission (*esperiment 6*), we administered the dopamine receptor antagonist cis-(Z)-flupenthixol, at a dose (0.125 mg/kg i.p.) that does not affect social play by itself (40), before administration of MDPV (0.5 mg/kg i.p.). Systemic administration of flupenthixol blocked the effects of MDPV on social play (pinning: $F_{(pretreatment)1,26}$ =0.025, n.s.; $F_{(treatment)1,26}$ =4.57, p=0.042; $F_{(pretreatment\ x\ treatment)1,26}$ =0.95, p=n.s., Figure 5a; pouncing: $F_{(pretreatment)1,26}$ =0.33, n.s.; $F_{(treatment)1,26}$ =5.11, p=0.032; $F_{(pretreatment\ x\ treatment)1,26}$ =0.62, n.s., Figure 5b). Indeed, post-hoc analysis revealed that MDPV reduced both pinning ($p<0.05$) and pouncing ($p<0.05$) in animals pretreated with saline but not in animals pretreated with flupenthixol (Figure 6a, b). No differences were found in the time spent in social exploration ($F_{(pretreatment)1,26}$ =1.03, n.s.; $F_{(treatment)1,26}$ =1.01, n.s.; $F_{(pretreatment\ x\ treatment)1,26}$ =1.15, n.s., Figure 5c).

We have previously shown that psychostimulants like amphetamine and methylphenidate exert their play-suppressant effects through stimulation of alpha-2 noradrenergic receptors (14, 38, 40), and MDPV has been shown to block the NET (48). Therefore, to investigate whether alpha-2

adrenoceptors are also involved in the reduction of social play induced by MDPV, juvenile rats were treated with the α-2 adrenoceptor antagonist RX821002 (0.2 mg/kg, i.p.) before administration of MDPV (0.5 mg/kg, i.p.) (*experiment 7*). Systemic administration of the α-2 adrenoceptor antagonist RX821002 (0.2 mg/kg, i.p.) 15 min before MDPV administration counteracted the effects of MDPV on social play behavior (pinning: $F_{(pretreatment)1,20}$ =7.35, p=0.013; $F_{(treatment)1,20}$ =4.25, p=n.s; $F_{(pretreatment\ x\ treatment)1,20}$ =4.98, p=0.04; pouncing: $F_{(pretreatment)1,20}$ =13.2, p=0.002; $F_{(treatment)1,20}$ =6.71, p=0.02; $F_{(pretreatment\ x\ treatment)1,20}$ =4.68, p=0.04). Post-hoc analysis revealed that MDPV reduced both pinning ($p<0.01$) and pouncing ($p<0.01$) in animals pretreated with saline but not in animals pretreated with RX821002 (Figure 5d, e). No differences were found in social exploration ($F_{(pretreatment)1,20}$ =0.21, n.s.; $F_{(treatment)1,20}$ =0.56, n.s.; $F_{(pretreatment\ x\ treatment)1,20}$ =0.16, n.s., Figure 5f).

To test whether dopaminergic and noradrenergic neurotransmission are simultaneously involved in the play-suppressant effects of MDPV (*experiment 8*), we treated juvenile rats with either a combination of sub-effective doses of both the alpha-2 noradrenergic receptor antagonist RX821002 (0.1 mg/kg i.p.) and the dopamine receptor antagonist flupenthixol (0.06 mg/kg i.p.), or with saline (control group) 15 min before treatment with MDPV. The combined administration of sub-effective doses of RX821002 and flupenthixol antagonized the play-suppressant effects of MDPV (pinning: $F_{(pretreatment)1,26}$ =1.22, n.s.; $F_{(treatment)1,26}$ =0.93, n.s.; $F_{(pretreatment\ x\ treatment)1,26}$ =4.61, p=0.04, Figure 5g; pouncing: $F_{(pretreatment)1,26}$ =1.27, n.s.; $F_{(treatment)1,26}$ =0.64, n.s.; $F_{(pretreatment\ x\ treatment)1,26}$ =14.2, $p<0.001$, Figure 5 h). Post-hoc analyses revealed that MDPV reduced both pinning ($p<0.05$) and pouncing ($p<0.01$) in animals pretreated with saline but not in animals pretreated with sub-effective doses of both RX821002 and flupenthixol (Figure 5 g, h). No differences were found in social exploration ($F_{(pretreatment)1,26}$ =0.63, n.s.; $F_{(treatment)1,26}$ =0.95, n.s.; $F_{(pretreatment\ x\ treatment)1,26}$ =0.016, n.s., Figure 5 i).

Discussion

Synthetic cathinones are widely abused by young people, often in a social setting (3-5). However, scarce information is available about their direct effect on social behavior. Here, we provide evidence that the widely abused cathinone, MDPV, suppresses social play behavior, a characteristic and rewarding form of social interaction displayed by young mammals (22, 36, 49). The play-suppressant effect of MDPV in juvenile rats was behaviorally specific: it was the result of reduced play initiation and diminished response to play solicitation, but it was not associated with changes in social exploratory behavior or locomotor activity during social interaction. Interestingly, MDPV reduced play-related behaviors without affecting social exploration and locomotor activity also in young adult rats, suggesting that the detrimental effects of MDPV on social play are not exclusive to juvenile animals, i.e., are not age-dependent. The play-suppressant effects of MDPV were subject to tolerance but not sensitization, and were mediated by dopaminergic and noradrenergic neurotransmission. Together, these data demonstrate that MDPV has selective detrimental effects on social play behavior and shed light on the pharmacological mechanisms involved.

To dissect the effects of MDPV on either play initiation or the responsiveness to play solicitation, we performed an experiment in which MDPV was injected to none, one, or both partners of the test dyad. Pouncing, which is an index of play solicitation, and pinning, which is the most characteristic response to play initiation, were significantly reduced in all MDPV-treated rats, irrespective of the treatment of their test partners. Interestingly, the altered behavior of MDPV-treated rats also affected the behavior of saline-treated partners. Indeed, saline-treated rats interacting with MDPV-treated partners also showed reduced pinning and pouncing frequencies compared to test pairs in which both animals were treated with saline. These results support the notion that reciprocity is necessary to maintain appropriate levels of playfulness and they are consistent with findings showing that social play behavior is influenced by the level of social activity of the partner (32-35, 40).

In humans, the development of tolerance, dependence and withdrawal has been reported after the frequent use of high doses of MDPV (50). A recent study showed that the aggressive responses induced by MDPV in adult mice are subject to sensitization, being enhanced after repeated administration (19). Therefore, we tested whether tolerance or sensitization, or both, would occur to the effect of MDPV on social play behavior after repeated treatment. We found that the reduction in social play induced by systemic administration of MDPV was suppressed following repeated treatment, indicating that tolerance to the effects of MDPV in social play had occurred. Conversely, social play was unaffected when animals were repeatedly pretreated with MDPV and acutely treated with a sub-effective dose of MDPV on the test day, showing that sensitization to the effects of MDPV on social play behavior had not occurred. Therefore, the play-suppressant effects induced by MDPV are different by those induced by methylphenidate, effects of which on social play are not subject to tolerance or sensitization (40).

In the brain, MDPV acts as a potent uptake inhibitor at plasma membrane transporters for both dopamine (DAT) and noradrenaline (NET), while it lacks significant activity at the serotonin transporter (SERT) and it is not considered a substrate releaser (18, 48). In particular, in vitro studies reported that MDPV is 50-fold more potent at DAT, 10-fold more potent at NET and 10-fold less potent at SERT than cocaine (1, 8). On this basis, we hypothesized that changes in dopaminergic and/or noradrenergic neurotransmission underlie the play-suppressant effects of MDPV. To test this possibility, we treated rats with the α-2 adrenoceptor antagonist RX821002 or the dopamine receptor antagonist flupenthixol prior to MDPV. We found that both the α-2 adrenoceptor antagonists RX821002 and the dopamine receptor antagonist flupenthixol, given alone, counteracted the effects of MDPV on social play. Furthermore, combined administration of sub-effective doses of both RX821002 and flupenthixol also antagonized the play-suppressant effects of MDPV, indicating that the effects of MDPV on social play are mediated by activation of both α-2 adrenoceptors and dopamine receptors. Together, these data show that MDPV and other psychostimulants suppress social play through distinct, but overlapping neurochemical mechanisms

(14, 40). That is, both amphetamine and methylphenidate exert their play-suppressant effects through stimulation of alpha-2 noradrenergic receptors but not dopamine receptors (14, 38-40). On the other hand, cocaine reduces social play by simultaneous increases in dopamine, noradrenaline, and serotonin neurotransmission (14).

Dopaminergic and noradrenergic neurotransmission have dissociable roles in social play behavior (37). Systemic administration of drugs that either stimulate or reduce dopaminergic neurotransmission can disrupt social play, likely by altering dopaminergic neurotransmission in a variety of brain regions, leading to a dysbalance in dopamine signalling between different circuits (37). On the other hand, it has been shown that dopamine stimulates the motivation for social play (39), acting in the nucleus accumbens (NAc) (41). Therefore, the dopaminergic mechanisms that underlie the reduction in social play induced by MDPV may either occur through the integration with other brain structures and neurotransmitters not primarily involved in the motivational aspect of social play, or may be related to dopaminergic control of more cognitive components of social behavior, such as adaptive behavior and decision making (51, 52). In support of this possibility, it has recently been shown that the effects of MDPV on memory consolidation and associative memory are also related to activation of dopamine receptors (12). Alongside dopamine, noradrenergic neurotransmission is also involved in the play-suppressant effects of MDPV. Increases in noradrenergic neurotransmission have been found to negatively modulate the motivation for social play behavior and its expression (39). Furthermore, noradrenaline is involved in cognitive processes, such as learning, attention, impulse control, decision making and behavioral flexibility (53), that all need to be optimally aligned for the proper performance of social play (37). Thus, although the experimental setup used in the present study does not allow to separate the incentive-motivational and pleasurable properties of social play, the fact that the play-suppressant effects of MDPV are mediated through both dopaminergic and noradrenergic neurotransmission leads to hypothesize that MDPV may reduce social play by affecting cognitive processes that are necessary for the proper execution of social play. For instance, MDPV may alter the ability of the

animals to interpret and adequately reciprocate social stimuli, or to adjust their social behavior to the changing circumstances in the social and physical environment.

Notably, MDPV (and bath salts in general) is often used in social contexts to facilitate social interactions/approaches (1). However, we here found that MDPV reduced play-related behaviors in rats without altering general social exploration. These results suggest that the acute effects of MDPV on social play behavior are comparable to the effects of other psychostimulants which are also abused in social settings, such as amphetamine, methylphenidate or cocaine, that also exert play-suppressant effects in male rats (14, 24, 40). Importantly, sex-dependent differences in the effects of MDPV (54-56) and in the structure and intensity of social play behavior (23) have been documented. Therefore, investigating the effects of MDPV on social play behavior in female rats, and the neural mechanisms involved, is an intriguing issue which deserves further investigation.

Collectively, our data provide evidence of the deleterious effects of MDPV on social play behavior in both juvenile and young adult male rats and they also indicate potential neural mechanisms for the social dysfunctions induced by this popular cathinone. Social play behavior is critical for proper neurobehavioral development, and altered or reduced patterns of social play may lead to enduring behavioral deficits (37). Since MDPV can induce a wide range of behavioral alterations in humans (1, 6), more research into the persistent behavioral effects following both acute and chronic exposure to this synthetic cathinone at early ages is warranted.

Founding sources

This work was supported by Marie Curie Career Reintegration Grant PCIG09-GA-2011-293589 (VT) and by Excellence Departments, MIUR-Italy, Grant/Award Numbers: ARTICOLO 1, COMMI 314- 337 LEGGE 232/2016, ARTICOLO 1.

Disclosure/Conflicts of interest

The authors declare that, except for income received from their primary employers, no financial support or compensation has been received from any individual or corporate entity over the past five years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Author contribution

S.S., F.M., E.C and P.J.P. performed the experiments. S.S., A.Man. and E.C. analysed and contributed to the design of the behavioral experiments. E.C. V.B. and A.Man. contributed to data analysis. C.Z., A.Mai, P.C. and L.J.M.J.V. contributed to the design of the experiments and edited the manuscript. S.S. wrote the manuscript. V.T. supervised the project, designed the experiments and wrote the manuscript.

References

1. Karila L, Lafaye G, Scocard A, Cottencin O, Benyamina A. MDPV and alpha-PVP use in humans: The twisted sisters. Neuropharmacology. 2018;134(Pt A):65-72. doi:10.1016/j.neuropharm.2017.10.007
2. Valente MJ, Guedes de Pinho P, de Lourdes Bastos M, Carvalho F, Carvalho M. Khat and synthetic cathinones: a review. Archives of toxicology. 2014;88(1):15-45. doi:10.1007/s00204-013-1163-9
3. Ashrafioun L, Bonadio FA, Baik KD, Bradbury SL, Carhart VL, Cross NA, et al. Patterns of Use, Acute Subjective Experiences, and Motivations for Using Synthetic Cathinones ("Bath Salts") in Recreational Users. Journal of psychoactive drugs. 2016;48(5):336-43. doi:10.1080/02791072.2016.1229875
4. German CL, Fleckenstein AE, Hanson GR. Bath salts and synthetic cathinones: an emerging designer drug phenomenon. Life sciences. 2014;97(1):2-8. doi:10.1016/j.lfs.2013.07.023
5. Zawilska JB, Wojcieszak J. Designer cathinones--an emerging class of novel recreational drugs. Forensic science international. 2013;231(1-3):42-53. doi:10.1016/j.forsciint.2013.04.015
6. European Monitoring Centre for Drugs and Drug Addiction (2014), EMCDDA–Europol Joint Report on a new psychoactive substance: MDPV (3,4-methylenedioxypyrovalerone), Joint Reports, Publications Office of the European Union, Luxembourg.
7. Angoa-Perez M, Anneken JH, Kuhn DM. Neurotoxicology of Synthetic Cathinone Analogs. Current topics in behavioral neurosciences. 2017;32:209-30. doi:10.1007/7854_2016_21
8. Baumann MH, Partilla JS, Lehner KR. Psychoactive "bath salts": not so soothing. European journal of pharmacology. 2013;698(1-3):1-5. doi:10.1016/j.ejphar.2012.11.020
9. Froberg BA, Levine M, Beuhler MC, Judge BS, Moore PW, Engebretsen KM, et al. Acute Methylenedioxypyrovalerone Toxicity. Journal of medical toxicology : official journal of

the American College of Medical Toxicology. 2015;11(2):185-94. doi:10.1007/s13181-014-0446-8

10. Weaver MF, Hopper JA, Gunderson EW. Designer drugs 2015: assessment and management. Addiction science & clinical practice. 2015;10:8. doi:10.1186/s13722-015-0024-7

11. Aarde SM, Huang PK, Creehan KM, Dickerson TJ, Taffe MA. The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: self-administration and locomotor activity in rats. Neuropharmacology. 2013;71:130-40. doi:10.1016/j.neuropharm.2013.04.003

12. Atehortua-Martinez LA, Masniere C, Campolongo P, Chasseigneaux S, Callebert J, Zwergel C, et al. Acute and chronic neurobehavioral effects of the designer drug and bath salt constituent 3,4-methylenedioxypyrovalerone in the rat. J Psychopharmacol. 2019;33(3):392-405. doi:10.1177/0269881118822151

13. Glennon RA, Young R. Neurobiology of 3,4-methylenedioxypyrovalerone (MDPV) and alpha-pyrrolidinovalerophenone (alpha-PVP). Brain research bulletin. 2016;126(Pt 1):111-26. doi:10.1016/j.brainresbull.2016.04.011

14. Achterberg EJ, Trezza V, Siviy SM, Schrama L, Schoffelmeer AN, Vanderschuren LJ. Amphetamine and cocaine suppress social play behavior in rats through distinct mechanisms. Psychopharmacology. 2014;231(8):1503-15. doi:10.1007/s00213-013-3272-9

15. Miczek KA, Haney M, Tidey J, Vatne T, Weerts E, DeBold JF. Temporal and sequential patterns of agonistic behavior: effects of alcohol, anxiolytics and psychomotor stimulants. Psychopharmacology. 1989;97(2):149-51. doi:10.1007/bf00442237

16. Moro M, Salvador A, Simòn VM. Changes in the Structure of the Agonistic Behavior of Mice Produced by d-Amphetamine. Pharmacology Biochemistry and Behavior. 1997;56:47-54.

17. Schiorring E. Social isolation and other behavioral changes in groups of adult vervet monkeys (Cercopithecus aethiops) produced by low, nonchronic doses of d-amphetamine. Psychopharmacology. 1979;64(3):297-302. doi:10.1007/bf00427513

18. Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, et al. Pharmacological characterization of designer cathinones in vitro. British journal of pharmacology. 2013;168(2):458-70. doi:10.1111/j.1476-5381.2012.02145.x

19. De-Giorgio F, Bilel S, Ossato A, Tirri M, Arfe R, Foti F, et al. Acute and repeated administration of MDPV increases aggressive behavior in mice: forensic implications. International journal of legal medicine. 2019;133(6):1797-808. doi:10.1007/s00414-019-02092-3

20. Bateson P. Playfulness and creativity. Current biology : CB. 2015;25(1):R12-6. doi:10.1016/j.cub.2014.09.009

21. Nijhof SL, Vinkers CH, van Geelen SM, Duijff SN, Achterberg EJM, van der Net J, et al. Healthy play, better coping: The importance of play for the development of children in health and disease. Neuroscience and biobehavioral reviews. 2018;95:421-9. doi:10.1016/j.neubiorev.2018.09.024

22. Pellis SM, Pellis VC. The Playful Brain. Oxford OX2 England: Oneworld Publications 2009.

23. Spinka M, Newberry RC, Bekoff M. Mammalian play: training for the unexpected. The Quarterly review of biology. 2001;76(2):141-68. doi:10.1086/393866

24. Manduca A, Campolongo P, Palmery M, Vanderschuren LJ, Cuomo V, Trezza V. Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats. Psychopharmacology. 2014;231(8):1661-73. doi:10.1007/s00213-013-3337-9

25. Helgeland MI, Torgersen S. Stability and prediction of schizophrenia from adolescence to adulthood. European child & adolescent psychiatry. 2005;14(2):83-94. doi:10.1007/s00787-005-0436-0

26. Jones P, Rodgers B, Murray R, Marmot M. Child development risk factors for adult schizophrenia in the British 1946 birth cohort. Lancet. 1994;344(8934):1398-402. doi:10.1016/s0140-6736(94)90569-x

27. Jordan R. Social play and autistic spectrum disorders: a perspective on theory, implications and educational approaches. Autism : the international journal of research and practice. 2003;7(4):347-60. doi:10.1177/1362361303007004002

28. Moller P, Husby R. The initial prodrome in schizophrenia: searching for naturalistic core dimensions of experience and behavior. Schizophrenia bulletin. 2000;26(1):217-32. doi:10.1093/oxfordjournals.schbul.a033442

29. Alessandri SM. Attention, play, and social behavior in ADHD preschoolers. Journal of abnormal child psychology. 1992;20(3):289-302. doi:10.1007/bf00916693

30. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. British journal of pharmacology. 2010;160(7):1577-9. doi:10.1111/j.1476-5381.2010.00872.x

31. Lopez-Arnau R, Lujan MA, Duart-Castells L, Pubill D, Camarasa J, Valverde O, et al. Exposure of adolescent mice to 3,4-methylenedioxypyrovalerone increases the psychostimulant, rewarding and reinforcing effects of cocaine in adulthood. British journal of pharmacology. 2017;174(10):1161-73. doi:10.1111/bph.13771

32. Pellis SM, McKenna M. What do rats find rewarding in play fighting? An analysis using drug-induced non-playful partners. Behavioural brain research. 1995; 68:65-73.

33. Pellis SM, McKenna MM. Intrinsic and extrinsic influences on play fighting in rats: effects of dominance, partner's playfulness, temperament and neonatal exposure to testosterone

propionate. Behavioural brain research. 1992;50(1-2):135-45. doi:10.1016/s0166-4328(05)80295-5

34. Trezza V, Vanderschuren LJ. Cannabinoid and opioid modulation of social play behavior in adolescent rats: differential behavioral mechanisms. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2008;18(7):519-30. doi:10.1016/j.euroneuro.2008.03.001

35. Varlinskaya EI, Spear LP, Spear NE. Social Behavior and Social Motivation in Adolescent Rats: Role of Housing Conditions and Partner's Activity Physiology & behavior. 1999;67:475–82.

36. Trezza V, Baarendse PJ, Vanderschuren LJ. The pleasures of play: pharmacological insights into social reward mechanisms. Trends in pharmacological sciences. 2010;31(10):463-9. doi:10.1016/j.tips.2010.06.008

37. Vanderschuren LJ, Achterberg EJ, Trezza V. The neurobiology of social play and its rewarding value in rats. Neuroscience and biobehavioral reviews. 2016;70:86-105. doi:10.1016/j.neubiorev.2016.07.025

38. Achterberg EJ, van Kerkhof LW, Damsteegt R, Trezza V, Vanderschuren LJ. Methylphenidate and atomoxetine inhibit social play behavior through prefrontal and subcortical limbic mechanisms in rats. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2015;35(1):161-9. doi:10.1523/JNEUROSCI.2945-14.2015

39. Achterberg EJ, van Kerkhof LW, Servadio M, van Swieten MM, Houwing DJ, Aalderink M, et al. Contrasting Roles of Dopamine and Noradrenaline in the Motivational Properties of Social Play Behavior in Rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2016;41(3):858-68. doi:10.1038/npp.2015.212

40. Vanderschuren LJ, Trezza V, Griffioen-Roose S, Schiepers OJ, Van Leeuwen N, De Vries TJ, et al. Methylphenidate disrupts social play behavior in adolescent rats.

Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2008;33(12):2946-56. doi:10.1038/npp.2008.10

41. Manduca A, Lassalle O, Sepers M, Campolongo P, Cuomo V, Marsicano G, et al. Interacting Cannabinoid and Opioid Receptors in the Nucleus Accumbens Core Control Adolescent Social Play. Frontiers in behavioral neuroscience. 2016;10:211. doi:10.3389/fnbeh.2016.00211

42. Niesink RJ, Van Ree JM. Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. Neuropharmacology. 1989;28(4):411-8. doi:10.1016/0028-3908(89)90038-5

43. Pellis SM, Pellis VC. Play-Fighting Differs From Serious Fighting in Both Target of Attack and Tactics of Fighting in the Laboratory Rat Rattus norvegicus. Aggressive Behavior. 1987;13:227-42.

44. Manduca A, Morena M, Campolongo P, Servadio M, Palmery M, Trabace L, et al. Distinct roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in social behavior and emotionality at different developmental ages in rats. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2015;25(8):1362-74. doi:10.1016/j.euroneuro.2015.04.005

45. Bara A, Manduca A, Bernabeu A, Borsoi M, Serviado M, Lassalle O, et al. Sex-dependent effects of in utero cannabinoid exposure on cortical function. Elife. 2018;7. doi:10.7554/eLife.36234

46. Cameron KN, Kolanos R, Solis E, Jr., Glennon RA, De Felice LJ. Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. British journal of pharmacology. 2013;168(7):1750-7. doi:10.1111/bph.12061

47. Bernstein DL, Nayak SU, Oliver CF, Rawls SM, Rom S. Methylenedioxypyrovalerone (MDPV) impairs working memory and alters patterns of dopamine signaling in

mesocorticolimbic substrates. Neuroscience research. 2019. doi:10.1016/j.neures.2019.07.003

48. Baumann MH, Bukhari MO, Lehner KR, Anizan S, Rice KC, Concheiro M, et al. Neuropharmacology of 3,4-Methylenedioxypyrovalerone (MDPV), Its Metabolites, and Related Analogs. Current topics in behavioral neurosciences. 2017;32:93-117. doi:10.1007/7854_2016_53

49. Panksepp J, Siviy S, Normansell L. The psychobiology of play: theoretical and methodological perspectives. Neuroscience and biobehavioral reviews. 1984;8(4):465-92.

50. Andrabi S, Greene S, Moukaddam N, Li B. New Drugs of Abuse and Withdrawal Syndromes. Emergency medicine clinics of North America. 2015;33(4):779-95. doi:10.1016/j.emc.2015.07.006

51. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. Trends in neurosciences. 2007;30(5):194-202. doi:10.1016/j.tins.2007.03.006

52. Fernandez M, Mollinedo-Gajate I, Penagarikano O. Neural Circuits for Social Cognition: Implications for Autism. Neuroscience. 2018;370:148-62. doi:10.1016/j.neuroscience.2017.07.013

53. Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology. 2007;191(3):391-431. doi:10.1007/s00213-006-0578-x

54. Hambuchen MD, Hendrickson HP, Gunnell MG, McClenahan SJ, Ewing LE, Gibson DM, et al. The pharmacokinetics of racemic MDPV and its (R) and (S) enantiomers in female and male rats. Drug Alcohol Depend. 2017;179:347-54. doi:10.1016/j.drugalcdep.2017.07.011

55. McClenahan SJ, Hambuchen MD, Simecka CM, Gunnell MG, Berquist MD, Owens SM. Cardiovascular effects of 3,4-methylenedioxypyrovalerone (MDPV) in male and female Sprague-Dawley rats. Drug Alcohol Depend. 2019;195:140-7. doi:10.1016/j.drugalcdep.2018.12.006

56. King HE, Wakeford A, Taylor W, Wetzell B, Rice KC, Riley AL. Sex differences in 3,4-methylenedioxypyrovalerone (MDPV)-induced taste avoidance and place preferences. Pharmacology, biochemistry, and behavior. 2015;137:16-22. doi:10.1016/j.pbb.2015.07.013

Figure legends

Figure 1. Acute administration of MDPV (0.5 mg/kg) suppresses social play behavior without affecting general social exploration and locomotor activity in both juvenile and young adult rats. MDPV (0.5 mg/kg) suppressed pinning (a) and pouncing (b) behaviors in juvenile (i.e., 28-30-day-old) rats. Social exploration (c) and locomotor activity (i.e., the number of crossings (d)) were not affected by MDPV. Similarly, MDPV reduced play-related behaviors (e) without affecting social exploration (f) in young adult (i.e., 80-day-old) rats (juveniles: n SAL= 10, n MD0.025= 7, n MD0.05= 7, n MD0.1= 11, n MD0.25= 8, n MD0.5= 10; young adults: n SAL= 6, n MD0.025= 6, n MD0.05= 5, n MD0.1= 5, n MD0.25= 6, n MD0.5= 5). Data represent mean values ± SEM; *p<0.05, **p<0.01 vs SAL group (Student–Newman–Keuls post hoc test).

Figure 2. MDPV reduces social play behavior regardless of the treatment received by the test partner, affecting social play in vehicle-treated animals as well. When behavior of the members of a pair was scored separately, there was a reduction in pinning (a) and pouncing (b) either when one or both rats in a pair were treated with MDPV. In addition, MDPV-treated rats affected behavior of their saline-treated partners: saline-treated rats interacting with MDPV-treated partners showed indeed reduced pinning (a) and pouncing (b) frequency. These results suggest that MDPV-treated rats displayed both reduced initiation to play and responsiveness to play solicitation, indirectly decreasing playfulness in their saline-treated partners as well. Social exploration was unaffected (c). n SAL-SAL= 16 , n SAL-MD= 12, n MD-SAL= 12, n MD-MD= 18). Data represent mean values ± SEM; *p<0.05, **p<0.01, *** p<0.001 vs SAL-SAL group (Student–Newman–Keuls post hoc test). ‘Subject’ represents the treatment of the animal whose behavior was scored; ‘Partner’ represents the treatment of its test partner.

Figure 3. Following repeated treatment, tolerance occurs to the play-suppressant effects of MDPV. Acute administration of MDPV (0.5 mg/kg, i.p.) decreased the amount of pinning (a) and

pouncing (b) in animals pretreated with saline (SAL-MD0.5 group) but not in animals pretreated with MDPV (MD0.5-MD0.5 group), indicating that tolerance to the effect of MDPV had occurred. Social exploration was not affected by either the repeated and acute treatment (c) (n SAL-SAL= 7, n SAL-MD0.5= 8, n MD0.5-SAL= 7, n MD0.5-MD0.5= 8). Data represent mean values ± SEM; **p<0.01, vs SAL-SAL group (Student–Newman–Keuls post hoc test).

Figure 4. Following repeated treatment, sensitization to the play-suppressant effects of MDPV does not occur. Acute administration of a low doses of MDPV (0.1 mg/kg, i.p.) did not affect pinning (a) and pouncing (b) in rats repeatedly pretreated with an effective dose of MDPV (0.5 mg/kg, i.p.), revealing that sensitization to the effects of MDPV on social play behavior had not occurred. Social exploration was not affected by either the repeated and acute treatment (c) (n SAL-SAL= 7, n SAL-MD0.1= 8, n MD0.5-SAL= 7, n MD0.5-MD0.1= 8). Data represent mean values ± SEM.

Figure 5. The effects of MDPV on social play depend on both dopaminergic and noradrenergic neurotransmission. The effects of MDPV on on pinning (a, d) and pouncing (b, e) were blocked by either the dopamine receptor antagonist flupenthixol (0.125 mg/kg i.p.; n SAL-SAL= 9, n SAL-MD= 8, n FLU-SAL= 7, n FLU-MD= 6) or the α-2 adrenoceptor antagonist RX821002 (0.2 mg/kg i.p.; n SAL-SAL= 6, n SAL-MD= 7, n RX-SAL= 5, n RX-MD= 6). Moreover, combined administration of sub-effective doses of the alpha-2 noradrenergic receptor antagonist RX821002 (0.1 mg/kg i.p.) and the dopamine receptor antagonist flupenthixol (0.06 mg/kg i.p.) antagonized the effects of MDPV on pinning (g) and pouncing (h) (n SAL-SAL= 6, n SAL-MD= 8, n FLU/RX-SAL= 8, n FLU/RX-MD= 8). Social exploration was not affected by treatments (c, f, i). Data represent mean values ± SEM; *p<0.05, **p<0.01, vs SAL-SAL group (Student–Newman–Keuls post hoc test).

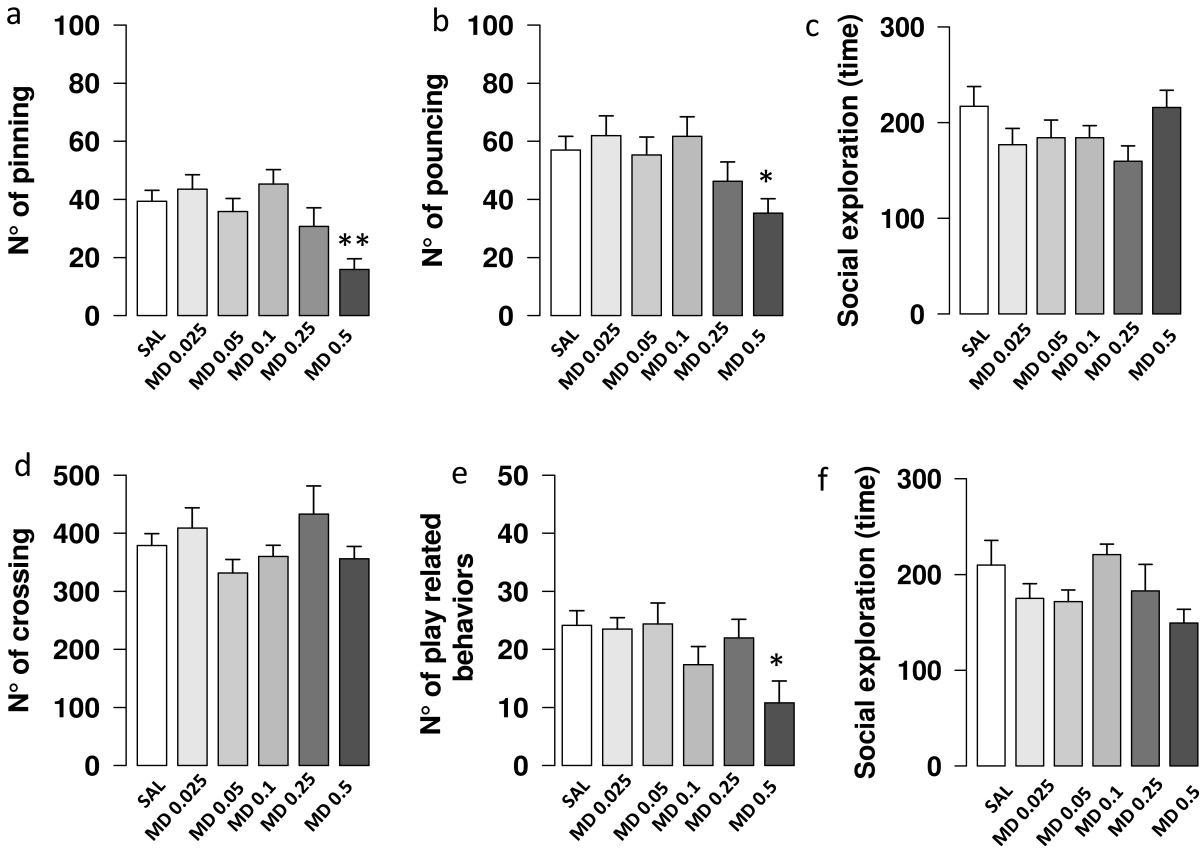
a
N° of pinning
100
80
60
40
20
0
**
b
N° of pouncing
100
80
60
40
20
0
*
c
Social exploration (time)
300
200
100
0
d
N° of crossing
500
400
300
200
100
0
e
N° of play related behaviors
50
40
30
20
10
0
*
f
Social exploration (time)
300
200
100
0
SAL
MD 0.025
MD 0.05
MD 0.1
MD 0.25
MD 0.5

Figure 1

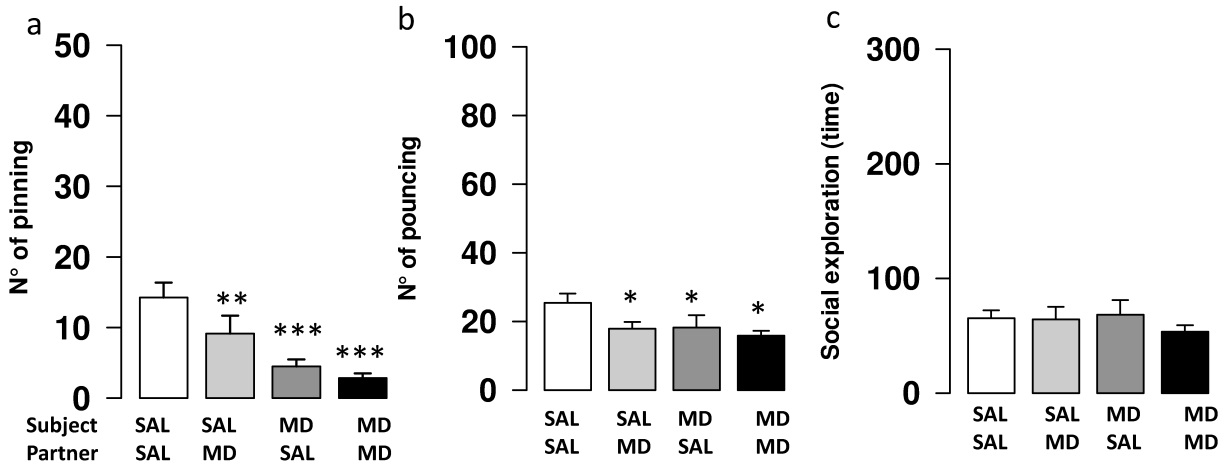
a
N° of pinning
50
40
30
20
10
0
**
***
***
Subject
Partner
SAL
SAL
SAL
MD
MD
SAL
MD
MD
b
N° of pouncing
100
80
60
40
20
0
*
*
*
SAL
SAL
SAL
MD
MD
SAL
MD
MD
c
Social exploration (time)
300
200
100
0
SAL
SAL
SAL
MD
MD
SAL
MD
MD

Figure 2

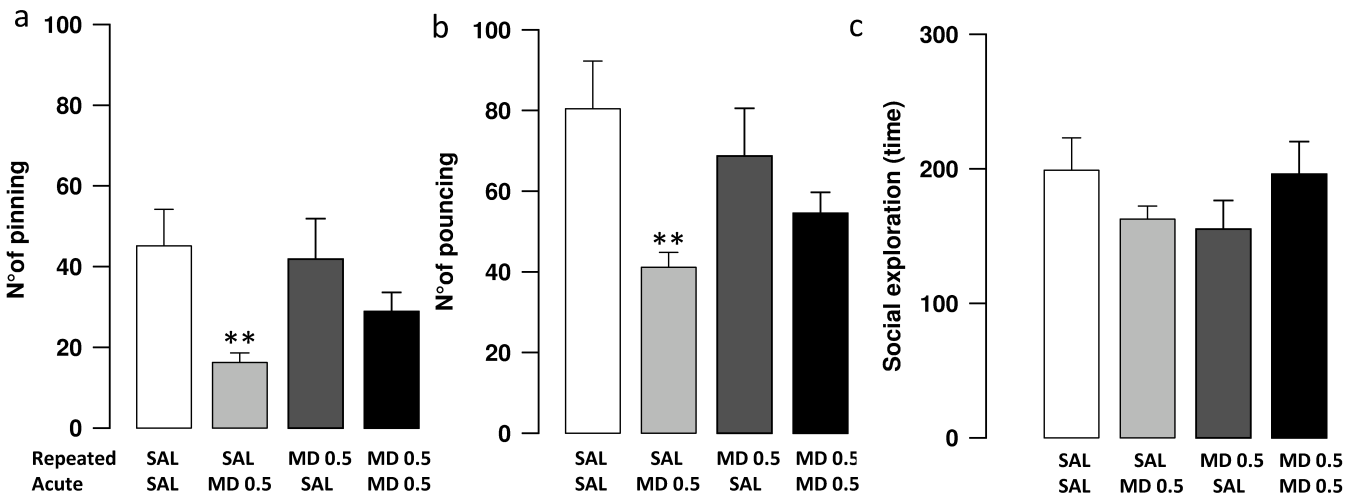
a
N°of pinning
100
80
60
40
20
0
**
Repeated
Acute
SAL
SAL
SAL
MD 0.5
MD 0.5
SAL
MD 0.5
MD 0.5
b
N°of pouncing
100
80
60
40
20
0
**
SAL
SAL
SAL
MD 0.5
MD 0.5
SAL
MD 0.5
MD 0.5
c
Social exploration (time)
300
200
100
0
SAL
SAL
SAL
MD 0.5
MD 0.5
SAL
MD 0.5
MD 0.5

Figure 3

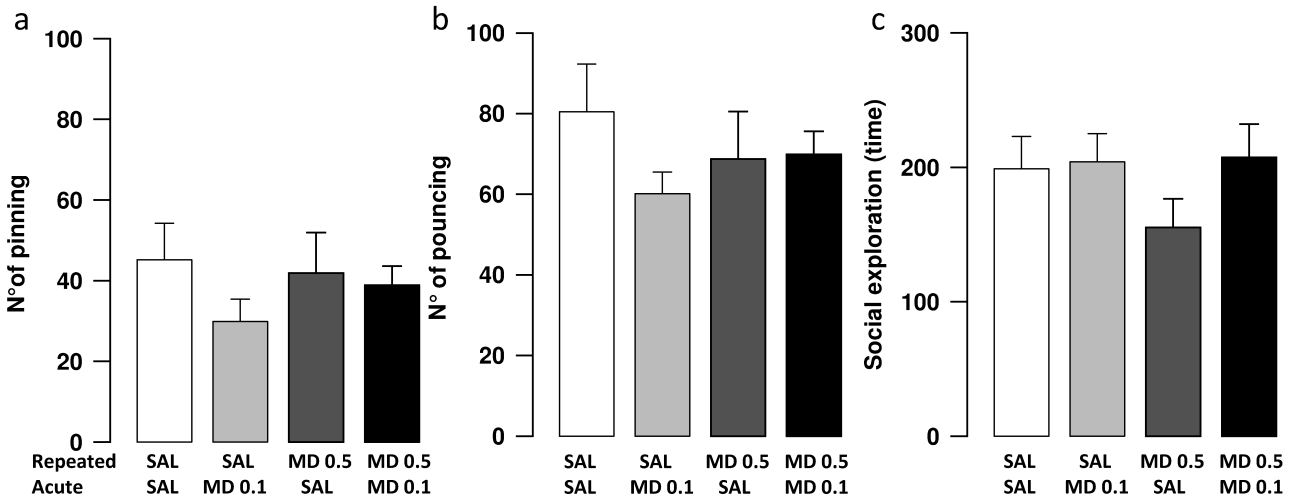
a
N°of pinning
100
80
60
40
20
0
Repeated
Acute
SAL
SAL
SAL
MD 0.1
MD 0.5
SAL
MD 0.5
MD 0.1
b
N° of pouncing
100
80
60
40
20
0
SAL
SAL
SAL
MD 0.1
MD 0.5
SAL
MD 0.5
MD 0.1
c
Social exploration (time)
300
200
100
0
SAL
SAL
SAL
MD 0.1
MD 0.5
SAL
MD 0.5
MD 0.1

Figure 4

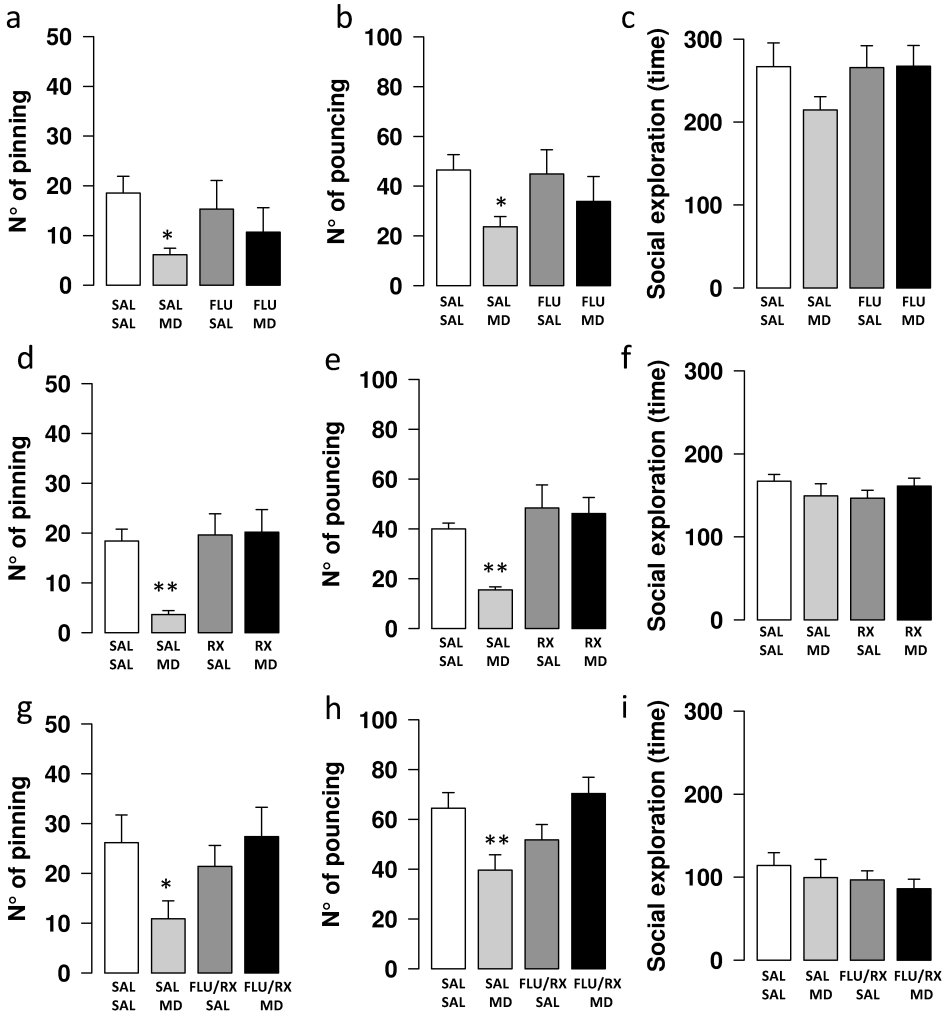
a
N° of pinning
50
40
30
20
10
0
*
SAL SAL
SAL MD
FLU SAL
FLU MD
b
N° of pouncing
100
80
60
40
20
0
*
SAL SAL
SAL MD
FLU SAL
FLU MD
c
Social exploration (time)
300
200
100
0
SAL SAL
SAL MD
FLU SAL
FLU MD
d
N° of pinning
50
40
30
20
10
0
**
SAL SAL
SAL MD
RX SAL
RX MD
e
N° of pouncing
100
80
60
40
20
0
**
SAL SAL
SAL MD
RX SAL
RX MD
f
Social exploration (time)
300
200
100
0
SAL SAL
SAL MD
RX SAL
RX MD
g
N° of pinning
50
40
30
20
10
0
*
SAL SAL
SAL MD
FLU/RX SAL
FLU/RX MD
h
N° of pouncing
100
80
60
40
20
0
**
SAL SAL
SAL MD
FLU/RX SAL
FLU/RX MD
i
Social exploration (time)
300
200
100
0
SAL SAL
SAL MD
FLU/RX SAL
FLU/RX MD

Figure 5