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Self-administration of the synthetic cathinone MDPV enhances reward function via a nicotinic receptor dependent mechanism

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Abstract

Methylenedioxypyrovalerone (MDPV) is an addictive synthetic drug with severe side effects. Previous studies have shown that MDPV has positive reinforcing properties. However, little is known about the effect of MDPV self-administration on the state of the brain reward system and the neuronal mechanisms by which MDPV mediates its effects. The goal of the present studies was to determine the effect of MDPV self-administration on reward function and the role of cholinergic neurotransmission in the reinforcing effects of MDPV. To study the effect of MDPV self-administration on the brain reward system, rats were prepared with intravenous catheters and intracranial self-stimulation electrodes (ICSS). For 10 days, the reward thresholds were assessed immediately before (23 h post prior session) and after 1 h of MDPV self-administration. The reward thresholds were decreased immediately after MDPV self-administration, which is indicative of a potentiation of brain reward function. The reward thresholds 23 h after MDPV intake gradually increased over time, which is indicative of anhedonia. Pretreatment with the nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine decreased the self-administration of MDPV and completely prevented the decrease in reward thresholds. A control study with palatable chocolate pellets showed that responding for a natural reinforcer does not affect the state of the brain reward system. Furthermore, mecamylamine did not affect responding for food pellets. In conclusion, the self-administration of MDPV potentiates reward function and nAChR blockade prevents the reward enhancing effects of MDPV self-administration. Preventing the MDPV-induced increase in cholinergic neurotransmission might be a safe approach to diminish MDPV abuse.

Keywords

MDPV; Self-administration; ICSS; Reward; Anhedonia; Nicotinic receptors; Rats

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1. Introduction

During the last decade there has been a strong increase in the abuse of synthetic cathinones (German et al., 2014). The alkaloid cathinone is the main psychoactive compound of the khat plant (*Catha edulis*), which has been used as a stimulant in parts of Africa and the Arabian peninsula for hundreds of years. Methylenedioxypyrovalerone (MDPV) was first synthesized in 1929 and is structurally closely related to cathinone (German et al., 2014; Koppe et al., 1969). MDPV was initially considered as a treatment for chronic fatigue but its high risk for abuse and dependence halted its clinical usage. In the late 2000s, MDPV was rediscovered by illicit drug users and since then there has been a rapid and worldwide increase in MDPV abuse (Fuwa et al., 2007). MDPV is often the main ingredients of bath salts, which are a mixture of synthetic cathinones and other drugs of abuse. Bath salts are typically snorted but are also taken orally, smoked, or injected (Zawilska and Wojcieszak, 2017). MDPV induces strong feeling of euphoria and chronic MDPV use leads to dependence, paranoia, hallucinations, agitation, and weight loss (Karila et al., 2018). Some of the adverse psychiatric side effects of MDPV might be due to the fact that it disrupts the communication between brain sites (Colon-Perez et al., 2016).

MDPV blocks the uptake of dopamine and norepinephrine and to a lesser degree serotonin (Baumann et al., 2013). Animal studies indicate that MDPV is 10 times more potent than cocaine in producing locomotor and cardiovascular effects (Baumann et al., 2013). MDPV is readily self-administered by rats under fixed and progressive ratio schedules (Aarde et al., 2013; Watterson et al., 2014). The noncontingent administration of MDPV lowers brain reward thresholds in the intracranial self-stimulation (ICSS) procedure (i.e., enhanced reward function) and induces conditioned place preference (CPP) (Bonano et al., 2014; King et al., 2015; Watterson et al., 2014). At this point, little is known about the neuronal mechanisms that mediate the stimulant and positive reinforcing properties of MDPV. The neuropeptide orexin plays a critical role in motivated behavior and facilitates central dopamine release in response to drugs of abuse (España, 2012; Sakurai, 2014). Therefore, Simmons et al. investigated the effects of orexin receptor blockade on the self-administration of MDPV. Blockade of orexin (OX) receptors with the nonselective OX1/OX2 receptor antagonist suvorexant did not affect the self-administration of MDPV in rats (Simmons et al., 2017). In contrast, blockade of OX receptors diminishes the self-administration of nicotine and cocaine (Gentile et al., 2017; Hollander et al., 2008). Furthermore, a recent study showed that pre-treatment with monoamine reuptake inhibitors (fluoxetine, desipramine, bupropion) or monoamine synthesis inhibitors (*para*-chlorophenylalanine, p-CPA; α -methyl-*para*-tyrosine (α -MPT) does not affect the increase in locomotor activity after MDPV administration in mice (Gannon et al., 2018).

Previous studies reported that rats self-administer MDPV and that noncontingent MDPV administration potentiates reward function. However, little is known about the effects of chronic MDPV self-administration on the state of the brain reward systems. Therefore, the first goal of the present study was to evaluate the effect of MDPV self-administration on the state of the brain reward system. Rats were prepared with ICSS electrodes in the medial forebrain bundle and intravenous catheters. The ICSS method provides an objective measure of the state of the reward system (Der-Avakian and Markou, 2012). Acute noncontingent

drug administration increases the sensitivity to rewarding electrical stimuli and lowers the brain reward threshold (Igari et al., 2013). In contrast, high and aversive doses of drugs of abuse or cessation of chronic drug administration decreases the sensitivity to rewarding electrical stimuli and elevates brain reward thresholds (Bruijnzeel et al., 2006). We measured the reward thresholds immediately after the MDPV self-administration session and 23 h after MDPV self-administration. Therefore, we were able to determine the acute rewarding effects of MDPV self-administration and whether repeated MDPV self-administration leads to elevations in reward thresholds after a brief period of abstinence (23 h). Drug withdrawal leads to elevations in reward thresholds and therefore an increase in the reward thresholds would be indicative of the development of dependence (Ahmed et al., 2002). At this point, there is no treatment for MDPV abuse and there have not been any drugs identified that diminish the acute reinforcing and/or rewarding effects of MDPV. Central cholinergic systems play a critical role in mediating the rewarding effects of drugs of abuse (Mark et al., 2011). Many studies have shown that nicotinic acetylcholine receptors (nAChRs) play a role in the reinforcing effects of nicotine (Harrison et al., 2002; Picciotto and Kenny, 2013; Watkins et al., 1999). However, more recent studies have reported that nAChR activation also plays a role in the reinforcing effects of powerful stimulants. Blockade of nAChRs decreases the self-administration of cocaine and inhibition of cholinergic interneurons in the nucleus accumbens prevents cocaine-induced CPP (Levin et al., 2000; Witten et al., 2010). It is not known if cholinergic transmission plays a role in MDPV abuse. Therefore, the second goal of the present study was to explore the role of the cholinergic neurotransmission in the positive reinforcing effects of MDPV. This was done by investigating the effects of the nonselective nAChR antagonist mecamylamine on the self-administration of MDPV and changes in reward thresholds induced by MDPV self-administration. After completing the MDPV experiment, a control study was conducted to determine whether responding for palatable chocolate pellets and nAChR blockade in nondependent animals affects the state of the brain reward system.

2. Materials and methods

2.1. Animals

Male Long-Evans rats (200–225 g, Charles River, Raleigh, NC) were housed in a climate-controlled vivarium on a reversed 12 h light-dark cycle. The animals were housed individually to protect the catheters. Food and water were available ad libitum. The experimental protocols were approved by the University of Florida (UF) Institutional Animal Care and Use Committee.

2.2. Drugs

MDPV hydrochloride and mecamylamine hydrochloride were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). MDPV and mecamylamine were dissolved in sterile saline (0.9% sodium chloride). Mecamylamine was administered subcutaneously (sc) in a volume of 1 ml/kg body weight. Drug doses are expressed as salt.

2.3. Experimental design

2.3.1. Experiment 1: MDPV self-administration and brain reward function—

First, we investigated the effect of MDPV self-administration on the state of the brain reward system. Second, it was investigated if nAChR blockade affects MDPV self-administration and changes in reward function immediately afterwards. Adult rats ($n = 11$) were prepared with ICSS electrodes in the medial forebrain bundle and trained on the ICSS procedure (Marcinkiewicz et al., 2009). After completing ICSS training, the rats were prepared with intravenous catheters and trained to respond for food pellets (45 mg, nutritionally complete purified, F0021, Bio-Serv, Frenchtown, NJ) under a FR5-T020s schedule (Yamada and Bruijnzeel, 2011). Eleven rats were prepared with both an electrode and intravenous catheter, but 2 electrodes were damaged during the experiment. Therefore, 11 rats self-administered MDPV and the reward thresholds and response latencies in 9 of these rats were determined. After the ICSS and food training sessions had been completed, the rats were allowed to self-administer MDPV for 10 days (0.03 mg/kg/inf, 1 h per session), 5 days per week. This dose was based on a previous study that investigated the acquisition and extinction of MDPV self-administration in rats (Schindler et al., 2016). Prior to the onset of MDPV self-administration, we obtained a 5-day baseline for the reward thresholds and response latencies. After the onset of MDPV self-administration, reward thresholds and response latencies were assessed immediately before and after ten daily MDPV self-administration sessions. The ICSS test session that was conducted immediately prior to MDPV self-administration was 23 h after the end of the last MDPV self-administration session. The reward thresholds were determined at these two time points in order to be able to determine the acute rewarding effects of MDPV self-administration (immediately after MDPV self-administration) and to determine if the self-administration of MDPV would lead to early signs of dependence as reflected by elevated reward thresholds after a brief period of abstinence (23 h post MDPV self-administration). On day 6, the ICSS session immediately before MDPV self-administration was conducted after the rats had had 2-days off. Therefore, this ICSS session was conducted 71 h after the last MDPV self-administration session. After 10 days of MDPV self-administration, the role of nAChRs in the reward enhancing effects of MDPV self-administration was investigated. The nAChR antagonist mecamylamine (0, 1, 3 mg/kg) was administered according to a Latin-square design and therefore all rats received all 3 doses. Mecamylamine was administered 10 min before the onset of the MDPV self-administration session. The ICSS parameters were assessed before the mecamylamine injection and after the MDPV self-administration session. In our previous studies, we reported that these doses of mecamylamine do not elevate the reward thresholds of drug naive control rats (Qi et al., 2014, 2016). The animals self-administered MDPV 5 days per week and were treated with mecamylamine before every other session. Therefore, the self-administration conditions on the pre-test day were the same for each dose. The half-life of mecamylamine is 1.2 h and therefore all mecamylamine would have been metabolized at the onset of the next test session (Debruyne et al., 2003).

2.3.2. Experiment 2: operant responding for food pellets and brain reward function—

This experiment was conducted to determine if operant responding for palatable chocolate pellets (45 mg, F0299, Bio-Serv, Frenchtown, NJ) affects the state of the brain reward system. It was also determined whether nAChR blockade affects operant responding

for this natural reinforcer. With a few exceptions, the design of this study was the same as the design of the MDPV self-administration study. The rats ($n = 8$) were prepared with ICSS electrodes and trained on the ICSS procedure. After completing the ICSS training, the rats were trained to respond for the chocolate pellets under the FR5-TO20s schedule. During the food training the rats received 17e20 g per day and during the remainder of the experiment 20 g per day. The animals received the food immediately after the operant sessions were completed. Prior to the sessions that combined ICSS and operant responding for food pellets, baseline (5-day) reward thresholds and response latencies were determined. The rats were then allowed to respond for chocolate pellets 1 h per day and the reward thresholds and response latencies were assessed immediately before and after the ICSS test sessions. The combined food responding/ICSS sessions were conducted 5 days per week. It was then investigated if mecamylamine (0, 1, 3 mg/kg, sc) affects responding for food pellets and reward thresholds. Mecamylamine was administered according to a Latin-square design 10 min before operant responding for food pellets. The rats were tested in the ICSS setup immediately after operant responding for food pellets. In the MDPV experiment, mecamylamine was administered before the 1 h self-administration session and ICSS testing was conducted immediately after this. Therefore, the interval between mecamylamine administration and ICSS testing was the same in the MDPV and food experiment.

2.4. Surgical procedures

2.4.1. Intravenous catheter implantation—Rats were anesthetized with an isoflurane-oxygen vapor mixture (1e3% isoflurane) and prepared with a catheter in the right jugular vein (Bruijnzeel et al., 2009; Yamada and Bruijnzeel, 2011; Zislis et al., 2007). Catheters consisted of silastic tubing (length 13.5 cm, 0.51 mm inside diameter, 0.94 mm outside diameter, Dow Corning, Midland, MI) that was connected to a 22 gauge stainless steel guide cannula, which was molded onto a durable polyester fiber mesh (Plastics One, Roanoke, VA). The tubing was passed subcutaneously from the mid scapular region to the ventral thorax, inserted into the jugular vein (4.0 cm), and secured with silk suture thread. After the implantation of the catheters, the animals were allowed to recover for 1 week. During the recovery period, the rats received daily intravenous infusions with the antibiotic Gentamycin (2 mg/kg, 0.2 ml, Sigma-Aldrich, St. Louis, MO). To ensure long-term patency of the catheters, 0.04 ml glycerol (Amresco Inc., Solon, Ohio) with 100 units of heparin per ml [Sagent Pharmaceuticals, Schaumburg, IL]) was infused into the catheter after the administration of the antibiotic. When the catheters were not being used, they were capped with a piece of Tygon tubing (Saint-Gobain Performance Plastics, Valley Forge, PA) that was plugged with monofilament.

2.4.2. Electrode implantations and ICSS procedure—Rats were anesthetized with an isoflurane and oxygen vapor mixture (2% isoflurane) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 3.3 mm below the interaural line (flat skull). The coordinates for the electrodes were based on previous studies (Bruijnzeel and Markou, 2004; Marcinkiewicz et al., 2009). The electrodes (11 mm in length, Plastics One, Roanoke, VA, USA) were implanted in the medial forebrain bundle with the incisor bar set 5 mm above the interaural line (AP -0.5 mm, ML ± 1.7 mm, DV -8.3 mm from dura). After the intracranial surgeries, the rats were allowed to recover for at least one

week. Rats were then trained on a modified discrete-trial ICSS procedure (Kornetsky and Esposito, 1979), as described previously (Bruijnzeel et al., 2009; Marcinkiewicz et al., 2009; Qi et al., 2015). The rats were tested in twelve operant conditioning chambers that are placed in sound-attenuating chambers (Med Associates, Georgia, VT, USA). The rats were trained to turn a response wheel on a fixed-ratio (FR) 1 schedule of reinforcement. Each quarter turn of the wheel resulted in the delivery of a 0.5 s train of 0.1 ms cathodal square-wave pulses at a frequency of 100 Hz. After the acquisition of responding (100 reinforcements within 10 min), each trial began with the delivery of a non-contingent electrical stimulus, followed by a 7.5 s response window during which the animal could respond to receive a second contingent stimulus that was identical to the initial stimulus. A response during this 7.5 s window was labeled a positive response and the lack of a response was labeled a negative response. During the 2 s period immediately after a positive response, additional responses had no consequences. The inter-trial interval (ITI) that followed a positive response or the end of the response window (in the case of a negative response) had an average duration of 10 s. Responses that occurred during the ITI resulted in a further 12.5 s delay of the onset of the next trial. During the training period, the duration of the ITI and delay periods induced by time-out responses were gradually increased until animals performed consistently. Then brain reward thresholds were assessed by using a modification of the psychophysical method of limits. Test sessions consisted of four alternating series of descending and ascending current intensities starting with a descending series. Blocks of three trials were presented to the rats at a given stimulation intensity, and the intensity was altered systematically between blocks of trials by 5 μ A steps. The initial stimulus intensity was set 40 μ A above the baseline current-threshold for each animal. Each test session typically lasted 30 min and provided two variables: brain reward thresholds and response latencies. The brain reward threshold was defined as the midpoint between stimulation intensities that supported responding (i.e., positive responses on at least two of the three trials) and stimulation intensities that failed to support responding. Four threshold estimates were recorded and the mean of these values was taken as the final threshold. The time interval between the beginning of the non-contingent stimulus and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency on all trials during which a positive response occurred. Elevations in brain reward thresholds reflect anhedonia (Barr et al., 2002). Drugs that have sedative effects or induce motor impairments increase the response latency and stimulants decrease the response latency (Igari et al., 2013; Liebman, 1985).

2.5. Operant responding for food and MDPV self-administration

The drug self-administration sessions in the first experiment were conducted as described before (Zislin et al., 2007; Bruijnzeel et al., 2009; Yamada and Bruijnzeel, 2011). First the rats were trained to respond for food pellets (45 mg, nutritionally complete purified, F0021, Bio-Serv, Frenchtown, NJ). Food training sessions were conducted because it facilitates the acquisition of nicotine self-administration. For the duration of the food training the rats were fed 17–20 g (75–85% of baseline ad libitum calories) of lab chow per day (Fernandez-Galaz et al., 2001). The rats were trained to respond on the active lever to receive food pellets. Responding on the inactive lever did not have any scheduled consequences. Instrumental training started under a fixed-ratio 1, time-out 1 s (FR1 TO1-s) reinforcement schedule and

the training schedule was progressively increased until the rats responded under a FR5 TO20-s reinforcement schedule. After completion of the food training sessions, the rats were fed 20 g of lab chow per day. The rats were then allowed to self-administer MDPV at the 0.03 mg/kg/inf dose for 1 h per day for 10 days. Responding on the active lever resulted in the delivery of a MDPV infusion (0.1 ml infused over a 5.6-s time-period). The initiation of the delivery of an infusion was paired with a cue light, which remained illuminated throughout the 20-s timeout period. Responding on the inactive was recorded but did not have any scheduled consequences. Food training for the second experiment was conducted the same way with the only exception that these rats received chocolate-flavored sucrose pellets (45 mg, F0299, Bio-Serv, Frenchtown, NJ). Both the food and MDPV self-administration sessions were 1 h.

2.6. Statistics

The effect of time (days 1–10) on operant responding for MDPV or food was analyzed with one-way, repeated-measures analyses of variance (ANOVA). The ICSS parameters were expressed as a percentage of the 5-day baselines. The ICSS parameters were then analyzed by two-way ANOVAs with time (days 1e10) and treatment (before and after MDPV or food) as within subjects factors. The effect of mecamlamine on operant responding for MDPV or food was analyzed with two-way ANOVAs with mecamlamine dose and treatment (before and after MDPV or food) as within subjects factors. Statistically significant results in the ANOVA's were followed by the Bonferroni post-hoc test. The data were analyzed with GraphPad Prism 7 software for Windows.

3. Results

3.1. Experiment 1: MDPV self-administration and brain reward function

The rats self-administered MDPV (1 h) for 10 days and the brain reward thresholds were assessed before and after the self-administration of MDPV. The ICSS test session that were conducted immediately before MDPV self-administration were 23 h after the last MDPV self-administration session. There was an effect of time on responding on the active lever ($F_{9, 90} = 8.188$, $P < 0.0001$, Table 1), inactive lever ($F_{9, 90} = 2.222$, $p < 0.05$, Table 1), and the number of MDPV infusions received (Infusions: $F_{9, 90} = 9.105$, $p < 0.0001$, Fig. 1A). During the first session, the maximum number of infusions was capped at 24 and all the rats reached the maximum number of infusions. During the second session, the maximum number of infusions was 100 and only 1 rat reached this high level of intake. After the second session, there was a decline in MDPV intake and MDPV intake stabilized at about 20 infusions per session. The self-administration of MDPV decreased the brain reward thresholds (Treatment: $F_{1,8} = 223$, $p < 0.0001$, Fig. 1B) and the response latencies (Treatment: $F_{1,8} = 30.46$, $p < 0.001$, Fig. 1C) of the rats. This indicates that the self-administration of MDPV potentiates brain reward function and shortens the response time. In a separate ANOVA analysis, it was investigated if the reward thresholds that were measured 23 h after MDPV self-administration changed over time. The ANOVA analysis indicated that there was an effect of time on the reward thresholds ($F_{9, 72} = 2.94$, $p < 0.01$, Fig. 2A). The posthoc analysis indicated that at day 10 the reward thresholds were higher than at day 1. This indicates that the reward thresholds between self-administration sessions

gradually increased over time. The response latencies did not change over time (Fig. 2B). We then investigated the effects of mecamlamine on the self-administration of MDPV and changes in reward thresholds induced by MDPV self-administration. Mecamlamine decreased responding on the active lever ($F_{2,20} = 4.63$, $p < 0.05$, Table 2) and the number of MDPV infusions received ($F_{2,20} = 5.18$, $p < 0.05$, Fig. 3A), but did not affect responding on the inactive lever (Table 2). The self-administration of MDPV decreased the brain reward thresholds ($F_{1,8} = 9.97$, $p < 0.05$, Fig. 3B) and the response latencies ($F_{1,8} = 8.14$, $p < 0.05$, Fig. 3C). The MDPV self-administration induced decrease in the reward thresholds and response latencies was prevented by pretreatment with mecamlamine as indicated by significant MDPV $\times$ mecamlamine interactions (Thresholds $F_{2,16} = 3.76$, $p < 0.05$; Latencies $F_{2,16} = 6.89$, $p < 0.01$). The posthoc analyses indicated that the self-administration of MDPV decreased the brain reward thresholds and this was prevented by pretreatment with 1 and 3 mg/kg of mecamlamine. The reward thresholds after MDPV self-administration were significantly higher in the animals that received 3 mg/kg of mecamlamine than in the animals that received vehicle. Posthoc analysis also indicated that 3 mg/kg of mecamlamine prevented the MDPV self-administration induced decrease in the response latencies.

3.2. Experiment 2: operant responding for food pellets and brain reward function

In this experiment, the rats responded for food pellets for 10 days and the reward thresholds were assessed immediately before and after responding for food pellets. The ICSS sessions immediately before operant responding for food were 23 h after the previous food sessions. Responding on the active lever (Table 1), inactive lever (Table 1), and the number of food pellets received (Fig. 4A) did not change over time. There was no effect of operant responding for food pellets on the reward thresholds (Fig. 4B) or the response latencies (Fig. 4C). Furthermore, the reward thresholds did not change during the 10-day period that the rats responded for food pellets. There was an effect of time on the response latencies ($F_{9,63} = 2.071$, $p < 0.05$). The posthoc test did not reveal significant differences between the response latencies on specific test days. However, a close look at the data in Fig. 4C indicates that the response latencies were slightly lower at the beginning and at the end of the 10-day period. This effect was, however, very small and most likely is due to normal day to day variability in the response latencies. It was then investigated if the nAChR antagonist mecamlamine affects responding for food pellets and the brain reward thresholds. Mecamlamine was administered immediately before operant responding for food and reward thresholds were assessed immediately after operant responding for food. Pretreatment with mecamlamine did not affect responding on the active lever, inactive lever, or the number of food pellets received (Table 2, Fig. 5A). Furthermore, the administration of mecamlamine before operant responding for food did not affect the reward thresholds and response latencies immediately after operant responding for food (Fig. 5B and C).

4. Discussion

The first goal of the present studies was to investigate the effect of MDPV self-administration on the state of the brain reward system. The self-administration of MDPV had a dramatic effect on the brain reward system. During the 10-day self-administration

period, MDPV lowered the brain reward thresholds by 20–30%. A separate analysis on the brain reward thresholds before each MDPV self-administration session showed that the reward thresholds increased over time. This suggests that the rats experienced affective withdrawal signs between the self-administration sessions and thus that the rats were starting to display early signs of the development of dependence. We then investigated the role of nAChRs in MDPV self-administration and MDPV-induced changes in the state of the brain reward system. Pretreatment with the highest dose of mecamylamine decreased the self-administration of MDPV and prevented the effect of MDPV self-administration on the state of the brain reward system. In the second experiment, we investigated the effect of operant responding for food pellets on the state of the brain reward system. Operant responding for food pellets did not affect the brain reward thresholds of the rats. Furthermore, blockade of nAChRs before the food operant sessions did not affect the brain reward thresholds of the rats. These findings indicate that the self-administration of MDPV potentiates brain reward function via a nAChR dependent mechanism.

The present study provides new insight into the effect of the self-administration of a powerful psychostimulant on brain reward function. The self-administration of MDPV led to a large decrease in brain reward thresholds and this effect was relatively stable over time. Previous studies have shown that noncontingent MDPV administration lowers brain reward thresholds (Bonano et al., 2014; Watterson et al., 2014), but to our knowledge this is the first observation of a decrease in reward thresholds after MDPV self-administration. Several studies have investigated the relationship between drug self-administration and brain reward thresholds. Ahmed and colleagues investigated the effects of short (1 h/day) and long (6 h/day) access cocaine self-administration on reward thresholds (Ahmed et al., 2002). In both groups of rats, the reward thresholds were assessed 3 h or 17–22 h after each self-administration session. Interestingly, in the short access group the self-administration of cocaine did not affect the reward thresholds. The half-life of cocaine in rodents is only about 20 min and therefore 3 h after the self-administration session almost all cocaine would have been metabolized (Benuck et al., 1987; Nayak et al., 1976). Therefore, 3 h after the self-administration session would have been too late to detect a reward enhancing effect of cocaine self-administration. In the present MDPV study, we measured the brain reward thresholds 23 h after the self-administration session. The reward thresholds 23 h after MDPV self-administration gradually increased over time. Chronic drug administration leads to the development of dependence and cessation of drug administration in dependent animals leads to elevations in brain reward thresholds (Bruijnzeel et al., 2006; Epping-Jordan et al., 1998). Drug withdrawal leads to elevations in reward thresholds because the animals become less sensitive to the rewarding electrical stimuli (i.e., anhedonia) (Der-Avakian and Markou, 2012). Therefore, our observation that the reward thresholds increased over time suggests that the rats started to develop dependence and displayed signs of withdrawal between the self-administration sessions. At one test day (Day 6), the reward thresholds were assessed 71 h after the last MDPV self-administration session (Fig. 2A). It is interesting to note that at this time point the reward thresholds were not elevated above baseline levels. The reward thresholds might have been lower, although not significantly, at Day 6 than at the other days because spontaneous withdrawal signs have dissipated at this time point. Previous work has shown that the elevations in reward thresholds peak 24 h after

the cessation of drug administration and the thresholds are often back to baseline at the 71 h time point (Igari et al., 2013; Schulteis et al., 1995). Affective withdrawal symptoms play a critical role in the continuation of drug taking in humans. We found that the reward thresholds 23 h after the self-administration sessions gradually increased over time but that the reward thresholds immediately after MDPV self-administration remained stable (percent decrease relative to 5-day baseline). This indicates that at the onset of MDPV self-administration, MDPV intake is driven by positive reinforcement processes but at the end of the 10-day self-administration period it is driven by positive and negative reinforcement processes. The effect of short access to MDPV in the present study is somewhat similar as long access to cocaine. Ahmed and colleagues showed that long access to cocaine leads to a gradual increase in reward thresholds and the thresholds were elevated 3 h and 17–22 h after cocaine self-administration. Noncontingent administration of cocaine to the long access rats decreased the reward thresholds by about 15 percent, but they remained more than 10 percent above baseline levels. Therefore, the work by Ahmed and colleagues indicates that long access to cocaine leads to the development of dependence and negative mood states and even after exposure to cocaine the long access animals remain in a negative mood state. This pattern of results suggests that drug intake in dependent subjects is driven by negative reinforcement processes. Drug intake diminishes the negative mood state associated with withdrawal but does not lead to euphoria. In contrast, in the present study we showed that MDPV self-administration in dependent animals prevents the anhedonia associated with withdrawal but also induces euphoria. This suggests that in dependent subjects, the positive reinforcing properties of drugs continue to play a role in drug taking. This is in line with clinical studies showing that pharmacological treatments that decrease the positive reinforcing properties of drugs of abuse, decrease drug intake in dependent subjects (Davidson et al., 1999). A prime example of this is the use of naltrexone as a treatment for alcohol addiction. Naltrexone decreases the euphoria induced by alcohol and decreases the desire to drink in people who are addicted to alcohol (Littleton and Ziegler, 2003).

In the present MDPV study, we also started exploring the neurobiological mechanisms that mediate the positive reinforcing properties of MDPV. The cholinergic system has been shown to play a role in the positive reinforcing properties of drugs (Levin et al., 2000; Spealman and Goldberg, 1982). Therefore we investigated the effects of nAChR blockade on the self-administration of MDPV and the effects of MDPV self-administration on the state of the brain reward system. The rats received the nonselective, noncompetitive nAChR antagonist mecamylamine before the self-administration of MDPV. Mecamylamine decreased the self-administration of MDPV and completely blocked the decrease in reward thresholds induced by the self-administration of MDPV. This indicates that mecamylamine completely blocks the reward enhancing effects of MDPV self-administration. MDPV is a stimulant and in general stimulants decrease the response latencies in the ICSS procedure (Igari et al., 2013; Kenny et al., 2005). In the present study, MDPV decreased the response latencies by about 10 percent and this was blocked by pretreatment with mecamylamine. We also performed studies to rule out the possibility that mecamylamine decreases the intake of natural reinforcers or induces a negative mood state. Our study showed that mecamylamine did not decrease operant responding for the chocolate flavored pellets. A close look at Fig. 5A, shows that mecamylamine slightly decreased operant responding for food pellets but

this did not reach statistical significance. Mecamylamine did not affect the reward thresholds of the rats. This control study indicates that mecamylamine does not affect responding for a natural reinforcer and does not increase the reward thresholds in drug-naïve control rats. The present finding that mecamylamine decreases operant responding for MDPV is in line with previous studies that investigated the role of nAChRs in drug intake. Numerous studies have reported that nonselective nAChR antagonists decrease the self-administration of nicotine and they decrease the reward thresholds after the noncontingent administration of nicotine (Igari et al., 2013; Watkins et al., 1999). However, it has also been shown that nAChR blockade affects the self-administration of other drugs of abuse. Levin and colleagues showed that low doses of mecamylamine decreased operant responding for cocaine and did not affect operant responding for pellets (Levin et al., 2000). A high dose of mecamylamine was not more effective in decreasing operant responding for cocaine than lower doses but induced a decrease in operant responding for food. This confirms that relatively low doses of mecamylamine that do not decrease responding for natural reinforcers decrease operant responding for powerful psychostimulants. Mecamylamine has also been shown to decrease alcohol self-administration in rats. Interestingly, a clinical study reported that mecamylamine attenuated euphoria induced by alcohol and the desire to consume alcohol (Chi and Wit, 2003).

Mecamylamine blocks central nAChRs but it has also been reported that mecamylamine blocks peripheral ganglionic receptors and NMDA receptors (O'Dell and Christensen, 1988; Shytle et al., 2002). However, it is unlikely that blockade of the ganglionic receptors or NMDA receptors contributed to the effects of mecamylamine on the self-administration of MDPV and the reward enhancing effects of MDPV self-administration. Mecamylamine readily crosses the blood brain barrier after systemic administration and mediates its central effects at doses that are 3–10 times lower than at which it mediates peripheral effects (Nickell et al., 2013; Shytle et al., 2002). Papke and colleagues compared the efficacy and potency of mecamylamine as inhibitors of central nAChRs and NDMA receptors (Papke et al., 2001). A very low dose of mecamylamine induced a prolonged inhibition of nAChRs. A 10 times higher dose of mecamylamine was needed to inhibit NMDA receptors, and in contrast to the nAChRs, the NMDA receptors were only briefly inhibited. In the present studies, we used relatively low doses of mecamylamine and higher doses would have been needed to observe effects of ganglionic or NMDA receptor blockade. Therefore, it is highly unlikely that blockade of ganglionic or NMDA receptors contributed to the effects of mecamylamine on MDPV self-administration.

The present study shows that cholinergic neurotransmission plays a role in MDPV self-administration in rats. In this study we did not explore the specific cholinergic pathways that might play a role in MDPV self-administration. However, based on studies with other stimulants it is speculated that cholinergic input into the ventral tegmental (VTA) is important for MDPV self-administration. The VTA is innervated by cholinergic neurons that originate in the laterodorsal tegmental (LTDg) (Picciotto et al., 2012). Cocaine and methamphetamine induce a prolonged increase in acetylcholine levels in the VTA (Bechtholt and Mark, 2002; Dobbs and Mark, 2008). Furthermore, inhibiting the cholinergic projection from the LTDg to the VTA decreases cocaine self-administration and cocaine-induced CPP (Shabani et al., 2010; Shinohara et al., 2014). There is evidence that acetylcholine release in

the VTA increases glutamatergic transmission in this brain site and increases the likelihood of burst firing of dopamine projection neurons (Picciotto et al., 2012). Therefore, it is speculated that MDPV increases cholinergic input from the LDTg to the VTA and thereby enhances dopamine release in the nucleus accumbens and the positive reinforcing properties of MDPV. Although in our studies we used a nonselective nAChR antagonist, we suggest that some of the effects of mecamylamine on MDPV self-administration might have been mediated by blockade of $\alpha 6\beta 2^*$ nAChRs (*denotes assembly with other subunits) in the nucleus accumbens. In the nucleus accumbens, the $\alpha 6\beta 2^*$ nAChRs are located on dopamine terminals and blockade of these receptors inhibits dopamine release (Champtiaux et al., 2003; Exley et al., 2008). Interestingly, cocaine-induced CPP is diminished in $\alpha 6$ nAChR knockout mice and in wild-type mice after pretreatment with the selective $\alpha 6\beta 2^*$ nAChR antagonist α -Conotoxin MII (Sanjakdar et al., 2015). The expression of $\alpha 6\beta 2^*$ nAChRs in the brain is very restricted and therefore $\alpha 6\beta 2^*$ nAChR antagonists might have fewer side effects than nonselective nAChR antagonists. Future studies are needed to determine the role of $\alpha 6\beta 2^*$ nAChRs in the abuse of synthetic cathinones. Cholinergic systems play a critical role in reward signaling and habit formation (Amaya and Smith, 2018; Witten et al., 2010). Therefore, treatments that diminish the increase in cholinergic transmission in the reward system may decrease the rewarding effects of drugs of abuse and thereby decrease the likelihood of developing an addiction.

In conclusion, the present study shows that the self-administration of MDPV is highly rewarding. A relatively brief period of MDPV self-administration also led to the development of dependence as indicated by elevated reward thresholds between self-administration sessions. After the first few days of MDPV self-administration, MDPV intake is driven by positive (euphoria) and negative (diminished anhedonia) reinforcement processes. The present study also showed that nAChR blockade decreased the self-administration of MDPV and prevented the increase in brain reward function. Nicotinic receptor blockade did not affect operant respond in a control study with chocolate flavored food pellets. There are currently no treatments for MDPV addiction. Nicotinic receptor blockade might be a safe and effective option to decrease MDPV intake in people with an MDPV addiction.

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Fig. 1.

MDPV self-administration potentiates brain reward function. MDPV (0.03 mg/kg/inf) was self-administered 1 h per day for 10 days (A). During the first and second day the maximum number of infusions were capped at 24 and 100. After day 2, the rats had unlimited access to MDPV. Reward thresholds (B) and response latencies (C) were assessed immediately before and after the self-administration of MDPV and expressed as a percentage of the 5-day pre-MDPV baseline. The pre-MDPV baseline brain reward threshold was $112.0 \pm 9.6 \mu\text{A}$ and the baseline response latency was $3.0 \pm 0.1 \text{ s}$. Asterisks (**, $p < 0.01$; *, $p < 0.05$) indicate lower reward thresholds or shorter latencies after the self-administration session. Data in B and C are expressed as a percentage of the 5-day baseline. $N = 11$ (A), $N = 9$ (B, C), data are expressed as means $\pm$ SEM.

Fig. 2.

Development of anhedonia during abstinence from MDPV. Reward thresholds (A) 23 h after the self-administration of MDPV gradually increased over time. Elevations in reward thresholds reflect anhedonia and this suggests that the animals develop dependence. The response latencies (B) did not change over time. Asterisk (*, $p < 0.05$) indicates elevated reward thresholds compared to Day 1. Data in A and B are expressed as a percentage of the 5-day baseline. $N = 9$, data are expressed as means $\pm$ SEM.

Fig. 3.

Blockade of nicotinic receptors diminishes the rewarding effects of MDPV self-administration. The nAChR antagonist mecamylamine was administered 10 min before the self-administration of MDPV (0.03 mg/kg/inf, 1 h session, A). Reward thresholds (B) and response latencies (C) were assessed before and after the MDPV self-administration session and expressed as a percentage of the 5-day pre-MDPV baseline. The pre-MDPV baseline brain reward threshold was $112.0 \pm 9.6 \mu\text{A}$ and the baseline response latency was $3.0 \pm 0.1 \text{ s}$. Pre-treatment with mecamylamine decreased the self-administration of MDPV, the reward enhancing effects of the self-administration session, and increased the response latencies. Asterisks (**, $p < 0.01$) indicate a decrease in MDPV infusions and lower reward thresholds or shorter latencies after the self-administration session. Plus signs (+ $p < 0.05$; ++, $p < 0.01$) indicate higher reward thresholds or longer latencies compared to thresholds or latencies immediately after MDPV self-administration and pre-treatment with vehicle. Data in B and C are expressed as a percentage of the 5-day baseline. $N = 11$ (A), $N = 9$ (B, C), data are expressed as means $\pm$ SEM.

Fig. 4.

Operant responding for food pellets does not affect the state of the brain reward system. The rats were allowed to respond for food pellets for 10 days (A). Reward thresholds (B) and response latencies (C) were assessed immediately before and after operant responding for food pellets (1 h session). Data in B and C are expressed as a percentage of the 5-day baseline. $N = 8$, data are expressed as means $\pm$ SEM.

Fig. 5.

Blockade of nicotinic receptors does not affect operant responding for food pellets or the state of the brain reward system. Rats responded for food pellets for 1 h (A) and reward thresholds (B) and response latencies (C) were assessed before and after responding for food pellets. ICSS parameters were assessed immediately before and after food responding. Mecamylamine was administered after the first ICSS session and 10 min before operant responding for food. Thresholds and latencies are expressed as a percentage of the 5-day baseline. $N = 8$, data are expressed as means $\pm$ SEM.

Table 1

Operant responding for MDPV and food pellets during a 10-day period.

Day	MDPV		Food	
	Active lever	Inactive lever	Active lever	Inactive lever
1	123 ± 1	1.5 ± 0.7	498 ± 46	34 ± 0.9
2	272 ± 47	9.9 ± 7.5	501 ± 46	46 ± 2.1
3	230 ± 46	1.2 ± 0.7	519 ± 40	4.3 ± 2.5
4	160 ± 31	0.0 ± 0.0	477 ± 46	3.8 ± 1.4
5	132 ± 18	0.5 ± 0.5	454 ± 54	8.1 ± 2.0
6	100 ± 15	0.6 ± 0.3	429 ± 51	5.9 ± 3.8
7	105 ± 12	0.1 ± 0.1	494 ± 44	8.8 ± 4.2
8	116 ± 19	0.4 ± 0.2	506 ± 46	2.1 ± 1.0
9	109 ± 16	0.2 ± 0.1	473 ± 43	2.1 ± 1.1
10	107 ± 18	0.5 ± 0.3	522 ± 43	5.3 ± 2.5

Table 2

Operant responding for MDPV and food after mecamylamine administration.

Mecamylamine (mg/kg, sc)	MDPV		Food	
	Active lever	Inactive lever	Active lever	Inactive lever
0	126 ± 38	0.6 ± 0.5	428 ± 70	2.4 ± 1.3
1	75 ± 18	2.2 ± 1.1	420 ± 45	3.0 ± 1.3
3	48 ± 16*	3.2 ± 2.0	324 ± 41	5.4 ± 3.7

Asterisk indicates decrease in operant responding compared to vehicle group (*p < 0.05).