Published in final edited form as:

Behav Pharmacol. 2017 August ; 28(5): 394–400. doi:10.1097/FBP.0000000000000310.

Characterization of the discriminative stimulus effects of 3,4-methylenedioxypyrovalerone (MDPV) in male Sprague-Dawley rats

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Abstract

Recreational use of 3,4-methylenedioxypyrovalerone (MDPV) in the early 2000s prompted numerous scientific investigations of its behavioral and neurochemical effects. The purpose of this study was to further characterize the interoceptive stimulus effects of MDPV using a validated *in vivo* drug-detection assay. Male Sprague-Dawley rats were trained to discriminate 0.3 mg/kg MDPV from saline under a fixed ratio 20 (FR 20) schedule of food reinforcement. After stimulus control was established with MDPV (approximately 35 training sessions), substitution tests commenced with drugs from several chemical classes, including drugs with predominantly dopaminergic actions (MDPV, *d*-amphetamine, (+)-methamphetamine, (–)-cocaine), drugs with predominantly serotonergic actions ((+)-LSD, (+)-fenfluramine), and drugs with both serotonergic and dopaminergic actions (MDMA, 4-MMC). Full substitution for the 0.3 mg/kg MDPV cue was observed with *d*-amphetamine, (+)-methamphetamine, and (–)-cocaine. Surprisingly, the 5-HT releaser (+)-fenfluramine fully substituted in half the subjects, but completely suppressed responding in the remaining subjects. MDMA, 4-MMC, and (+)-LSD failed to fully substitute for MDPV. These results indicate that the MDPV cue is similar to cues produced by drugs with predominantly dopamine-increasing effects, and perhaps serotonin-releasing effects among individual subjects. Given these findings, further research is warranted to directly assess the contributions of dopamine and serotonin receptor isoforms to the discriminative stimulus functions of MDPV.

Keywords

methylenedioxypyrovalerone; MDPV; methylmethcathinone; 4-MMC; mephedrone; drug discrimination; discriminative stimulus; rat

Introduction

Recreational use of the synthetic cathinone, 3,4-methylenedioxypyrovalerone (MDPV), has prompted numerous experimental investigations into its neurochemical and behavioral

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Conflicts of Interest

The authors declare no conflict of interest.

effects. Recent studies using *in vitro* receptor binding techniques revealed that MDPV potently blocks reuptake of dopamine and norepinephrine at the dopamine and norepinephrine transporters, respectively, but produces relatively weaker effects on serotonin uptake at the serotonin transporter (Baumann et al. 2013; Simmler et al. 2013; Eshleman et al. 2013). In addition, MDPV produces dose-dependent and statistically significant increases in extracellular dopamine in the nucleus accumbens of conscious male Sprague-Dawley rats (Schindler et al. 2016; Baumann et al., 2013). Moreover, these effects are 10-fold more potent than the dopamine-increasing effects of cocaine (Baumann et al., 2013). Further, the results of the aforementioned studies are consistent with electrophysiological studies demonstrating that MDPV produces outward hyperpolarizing currents in hDAT-expressing *Xenopus laevis* oocytes (Cameron et al. 2013a;b), similar to a non-substrate protein receptor blocker. Considered together, these findings indicate MDPV produces effects similar to cocaine at monoamine transporters, but with comparatively weaker effects at SERT.

In addition to the foregoing neurochemical and electrophysiological evidence, preclinical behavioral pharmacology studies indicate MDPV may possess a high abuse potential. Several recent studies have investigated the reinforcing and rewarding effects produced by MDPV in nonhuman models of abuse liability. For example, MDPV maintains self-administration (e.g., Schindler et al. 2016; Aarde et al. 2013, 2015; Watterson et al. 2012), lowers intracranial self-stimulation thresholds (Kolanos et al. 2015; Bonano et al. 2014a;b; Watterson et al. 2012), and produces conditioned place preference in rats (King et al. 2015a; b). Based on these preclinical findings, it is clear that MDPV can serve as a positive reinforcer or a rewarding stimulus that may lead to excess use and behavioral dependencies in users.

Relatively fewer studies have characterized MDPV as an antecedent source of control over responding using drug discrimination methods. Presently, only one published study trained animals to discriminate MDPV (Fantegrossi et al. 2013). In that study, male NIH Swiss mice were trained to discriminate 0.3 mg/kg MDPV from saline under an FR 10-10-s time-out schedule of evaporated milk reinforcement. Using a cumulative dosing test procedure, MDPV, MDMA, and methamphetamine fully substituted for the 0.3 mg/kg MDPV cue. Contrariwise, morphine and a cannabinoid CB₁ and CB₂ receptor agonist, JWH-018, produced minimal partial substitution.

A few studies have assessed MDPV for stimulus substitution in rats trained to discriminate other drugs. For example, Gatch et al. (2013) reported that MDPV dose-dependently produced full substitution in Long-Evans rats trained to discriminate 10 mg/kg cocaine or 1 mg/kg methamphetamine from saline. Harvey and Baker (2016) observed full substitution with MDPV in Sprague-Dawley rats trained to discriminate a drug mixture consisting of 0.5 mg/kg *d*-amphetamine and 1.5 mg/kg MDMA, but MDPV produced no more than partial substitution in rats trained to discriminate 1.5 mg/kg MDMA from saline. Finally, Gannon et al. (2016) demonstrated that the (*S*)(+)-MDPV isomer fully substituted for 10 mg/kg cocaine more potently than the (*R*)(-)-MDPV isomer, but not compared to the racemic mixture or cocaine. Together, these findings suggest that MDPV produces interoceptive cues similar to prototypical psychostimulant drugs that increase extracellular dopamine levels.

In light of the foregoing drug discrimination studies, additional investigations are warranted to further evaluate the interoceptive stimulus effects of MDPV in animals trained to discriminate this substance. Although MDPV shares structural similarities to substituted phenethylamines, such as MDMA, amphetamine, and methamphetamine, and comparable pharmacological mechanisms of action to cocaine (Baumann et al., 2013), knowledge of a drug's chemical structure and mechanisms of action determined *in vitro* cannot fully predict its interoceptive effects. However, this information is useful for selecting drugs for stimulus substitution for imperfectly understood training drugs (e.g., emerging drugs of abuse). As such, *d*-amphetamine, (+)-methamphetamine, MDMA, and (-)-cocaine were selected to assess substitution to MDPV in the present study. Moreover, the synthetic cathinone, 4-methylmethcathinone (4-MMC), was also included as a test compound, given its similar pharmacological effects to MDMA at monoamine transporters measured *in vitro* and *in vivo* (e.g., Baumann et al., 2012). The 5-HT releaser, (+)-fenfluramine, and the ergoline derivative, (+)-LSD, were also included in the substitution test series and were predicted to serve as negative controls.

Methods

Subjects

Eight male Sprague-Dawley rats weighing 350–400g (Charles River Laboratories, Wilmington, MA, USA) were individually housed in polycarbonate cages with corncob bedding in a temperature- and humidity-controlled colony maintained on a 12/12h light/dark schedule (lights on at 0800h). Rats received daily food rations (LabDiet®, PMI® Nutrition International, LLC, Brentwood, MO, USA) and were maintained at 85–90% free-feeding weights. Access to water was unrestricted in home cages. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (2013) and were approved by the Institutional Animal Care and Use Committee of Western Michigan University.

Apparatus

Eight computer-operated rat operant conditioning chambers (ENV-100; MED Associates, St. Albans, VT, USA), contained in sound-attenuating cabinets equipped with fans, were used for all training and testing sessions. All chambers were equipped with three retractable levers, a food magazine, and a 10 V house light. Two levers were equidistant from the center lever that was located directly beneath the food magazine along the front wall. Food reinforcers consisted of 45 mg powderless food pellets (Bioserv, Frenchtown, NJ, USA). Experimental events were recorded using Med-PC software Version IV (MED Associates, St. Albans, VT, USA).

Training Procedures

On the first day of training, all subjects were placed in the operant conditioning chambers for a single 60-min session, with food delivery occurring under a VT 60-s schedule of reinforcement. All subjects consumed all pellets delivered to the food magazine and proceeded to lever press training.

During lever-press training, each rat was placed in an operant chamber with only the center lever extended. Food deliveries were programmed under an FR schedule of reinforcement. All subjects were required to earn five pellets under a given FR schedule before the FR requirement increased by one. Lever press training sessions comprised the following five phases in which the FR schedule was gradually incremented: 1) FR 1 to FR 5; 2) FR 5 to FR 10; 3) FR 10 to FR 15; 4) FR 15 to FR 20; and 5) FR 20. These sessions were programmed to engender reliable lever pressing under the terminal FR 20 schedule. If a subject did not display reliable responding under some FR schedule, the response requirement was reduced for remedial training. Each session lasted 30-min or after the delivery of 60 food pellets, whichever occurred first. All subjects proceeded to errorless training after 7 to 10 lever press training sessions.

During errorless training sessions, rats were injected (I.P.) with either 0.3 mg/kg MDPV (drug, D) or 0.9% bacteriostatic saline (vehicle, V) and placed into the chamber with only the condition-appropriate lever present (left or right). Four subjects were assigned the left lever for D injections and the right lever for V injections. Conditions were reversed for the remaining four subjects. Lever press responses were reinforced under an FR 20 schedule of reinforcement and all rats were exposed to six 20-min errorless training sessions in the following order with one session per day: V, V, D, D, V, D. All rats proceeded to discrimination training after the sixth errorless training session.

During discrimination training sessions, rats were injected with either D or V and placed in the chamber with both levers extended. These sessions lasted 20 min and were conducted once per day, five to six days per week. D and V injections were implemented in a pseudorandom order, under the restriction that no subject received more than two consecutive D or V training sessions throughout the study. Consistent with errorless training, the D and V lever assignments remained constant for each subject and counterbalanced across subjects. The criteria for discrimination training required each subject to emit 80% condition-appropriate responding prior to the delivery of the first reinforcer and for the remainder of the training session for at least 8 of 10 consecutive sessions.

Once a subject met the discrimination training criteria, stimulus substitution tests commenced. A test session occurred after a subject completed at least one D training session and one V training session in which it met the aforementioned training criteria. If a subject failed to meet the training criteria, training sessions continued until it met the criteria for two consecutive sessions. Doses of substitution test compounds were counterbalanced across subjects in each training group. Substitution tests occurred no more than two times per week. During a stimulus substitution test, subjects were injected with a dose of one of the test compounds, returned to their home cages, and subsequently placed into the experimental chamber after the 15-min injection interval had elapsed. Test sessions were conducted under extinction and ended when a subject completed an FR 20 on either lever or after 20 min elapsed, whichever occurred first. In addition, vehicle (V) substitution tests were included in the MDPV, 4-MMC, *d*-amphetamine, (+)-methamphetamine, (–)-cocaine, and MDMA substitution test series. Doses for all substitution test compounds were selected based on previous drug discrimination studies completed in-house that included testing in the same species via the same (i.p.) route of drug administration.

Drugs

($\pm$)3,4-methylenedioxypyrovalerone hydrochloride (HCl) (MDPV), ($\pm$) mephedrone HCl, (+)-methamphetamine HCl, (–)-cocaine HCl, (+)-lysergic acid diethylamide tartrate, and (+)-fenfluramine HCl were provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD, USA). *d*-Amphetamine hemisulfate was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Each drug solution was prepared by dissolving salt in 0.9% (wt/vol) bacteriostatic saline. All doses are expressed as weight of salt. Drug injections were performed via i.p. injections with a 15 min pre-session injection interval in a 1 ml/kg injection volume.

Data Analysis

Acquisition of drug stimulus control was determined by the number of discrimination training sessions required to reach the training criteria. A learning curve was created to display averaged percent MDPV-lever selection values during the first FR as a function of training sessions. Percent MDPV-lever selection was determined by dividing the number of responses on the D-lever by the total number of responses emitted up to completion of the first FR 20 on either lever. Response rate was recorded as the number of responses per second. For drug substitution tests, the percent MDPV-lever selection and responses per second were reported as group means ($\pm$ *SE*) in dose-response curves. Full substitution was considered to occur at 80% MDPV-lever selection, partial substitution was considered to occur at > 20% but < 80% MDPV-lever selection, and 20% MDPV-lever selection was considered no substitution. To compare potencies of drugs that produced full substitution, median effective dose values (ED_{50} ; with a range set from 0–100%) were calculated by fitting linear regression functions to the dose-response curves using a least squares regression analysis. Linear portions of dose-response curves, including not more than a single dose producing < 20% MDPV-lever selection and not more than a single dose producing > 80% MDPV-lever selection, were used to compute regression equations. Estimated ED_{50} values were then computed for each test compound using the calculated regression equations and 95% confidence intervals were attached to each estimated value. Further, ED_{50} values were only computed if, in addition to a test compound producing full-substitution, at least four of the eight subjects completed the FR 20 on either lever during a test sessions. For all dose-response curves, the percent substitution value for the V test was determined by calculating the average of all five V substitution tests (see above).

Response rates are presented as the number of responses emitted per second divided by the total test session time. Response rate data associated with each substitution test drug were analyzed by one-way repeated-measures analysis of variance (RMANOVA) followed by a Dunnett's multiple comparisons test that compared each dose of a test drug to the response rate observed under V. Similar to the percent substitution value for V, the response rate value for V was determined by calculating the average of all response rates obtained during V substitution tests. Statistical significance was identified at $p < .05$. All graphic displays and statistical analyses were conducted using PRISM GraphPad Version 7 software (La Jolla, CA, USA).

Results

A major goal of this study was to establish MDPV stimulus control over lever-press responding in rats. Acquisition of drug stimulus control was acquired in an average of 35.38 sessions $\pm$ 4.77 (*SE*). Figure 1 displays the learning curve of rats trained to discriminate 0.3 mg/kg MDPV from saline. Each data point represents the mean ($\pm$ *SE*) for each percent MDPV-lever selection prior to the delivery of the first reinforcer under the FR 20 schedule of reinforcement. Note that both D and V sessions are plotted in Figure 1 as percent MDPV-lever selection. As evident in Figure 1, as training sessions progressed, the separation between D-injection values occurring above the 80% dashed line and V-injection values occurring below the 20% dashed line become more evident, reflecting the development of stimulus control by MDPV.

To further characterize the interoceptive stimulus effects of MDPV, stimulus substitution was assessed with a broad range of pharmacological agents. For all substitution tests, individual dose-response curves of drugs with similar pharmacological effects (e.g., dopamine releaser) are grouped into single figures. The percent MDPV-lever selection values and response rates obtained with (A) MDPV (0.01 – 0.3 mg/kg), (B) (-)-cocaine (0.1 mg/kg – 10 mg/kg), (C) (+)-methamphetamine (0.03 – 1.0 mg/kg), and (D) *d*-amphetamine (0.03 – 1.0 mg/kg) are shown in Figure 2. MDPV dose-dependently increased MDPV-lever selection up the 0.3 mg/kg MDPV training dose, which was the only dose of MDPV to produce full-substitution ($100\% \pm 0$ *SE*) ($ED_{50} = 0.06$ mg/kg; 95% confidence interval [CI] = 0.03 – 0.11 mg/kg). There was a statistically significant effect of MDPV on responses per second [$F(4, 28) = 3.06, p < .05$] but Dunnett's multiple comparisons test failed to detect where the statistical differences occurred among the response rate values.

Dose-dependent MDPV-lever selection was evident following (-)-cocaine administration and full substitution was produced by the 10 mg/kg dose ($97.92\% \pm 2.08$) ($ED_{50} = 0.73$ mg/kg; 95% CI = 0.37 – 1.47 mg/kg). Cocaine did not significantly decrease response rate [$F(5, 35) = 1.13, NS$]. (+)-Methamphetamine produced full-substitution at both the 0.3 ($86.87\% \pm 11.22$) and 1.0 ($97.86\% \pm 2.14$) mg/kg doses ($ED_{50} = 0.12$ mg/kg; 95% CI = 0.07 – 0.22 mg/kg). Methamphetamine did not significantly decrease response rate [$F(4, 28) = 2.59, p = .058$]. Finally, *d*-amphetamine produced full-substitution at both the 0.3 ($80.46\% \pm 12.84$) and 1.0 (100 ± 0) mg/kg doses ($ED_{50} = 0.14$ mg/kg; 95% CI = 0.08 – 0.24 mg/kg). *D*-amphetamine did not significantly decrease response rate [$F(4, 28) = 2.34, p = .08$].

The percent MDPV-lever selection values and response rates under (A) 4-MMC (0.03 – 10.0 mg/kg), (B) MDMA (0.1 – 3.0 mg/kg), (C) (+)-LSD (0.01 – 0.1 mg/kg), and (D) (+)-fenfluramine (0.03 – 3.0 mg/kg) are presented in Figure 3. 4-MMC dose-dependently produced increases in percent MDPV-lever selection, and approached full-substitution in six of eight subjects ($77.26\% \pm 12.46$). There was a significant main effect of 4-MMC on response rate [$F(6, 42) = 15.22, p < .001, \eta^2 = 0.49$]: the 3 and 10 mg/kg doses of 4-MMC decreased response rate compared to V. MDMA produced low partial substitution up to the 3 mg/kg ($n = 7$) dose ($26.62\% \pm 14.52$). There was a significant effect of MDMA on response rate [$F(4, 28) = 5.57, p < .002, \eta^2 = 0.25$]: the 3 mg/kg dose of MDMA decreased response rate compared to V. (+)-LSD produced low partial substitution ($n = 7$) at the 0.1 mg/kg dose

(28.80% $\pm$ 15.01), with no significant effect on response rate [$F(3, 21) = 2.40, p = .10$]. Finally, (+)-fenfluramine produced a low percentage of responses on the MDPV-lever at the three lowest doses tested. However, 3 mg/kg (+)-fenfluramine produced full-substitution in four of the eight subjects (89.88% $\pm$ 10.12) ($ED_{50} = 1.39$ mg/kg; 95% CI = 0.9697 – 2.385). Responding in the remaining four subjects was completely suppressed by this dose. There was a statistically significant effect of fenfluramine on response rate [$F(5, 35) = 6.28, p < .001, \eta^2 = 0.37$]: the 0.03 and 3 mg/kg doses of fenfluramine each decreased response rates compared to V.

Discussion

Discriminative stimulus control by 0.3 mg/kg MDPV was established within an average of approximately 35 discrimination training sessions in the present study. These results demonstrate that 0.3 mg/kg MDPV can serve as an antecedent source of control over lever-press responding in rats. Only one previous study has demonstrated discriminative stimulus control by MDPV, using male NIH Swiss mice responding under an FR 10 –10-s time-out schedule of evaporated milk reinforcement (Fantegrossi et al., 2013). The number of training sessions required to reach discrimination acquisition criteria was not reported in that study, precluding comparisons between these two studies regarding the time required to establish stimulus control with MDPV. Notably, the number of training sessions required to establish stimulus control with MDPV in the present study was comparable to that observed in previous drug discrimination studies conducted in the same laboratory with structurally similar training drugs. For example, stimulus control was established with 1.5 mg/kg MDMA in an average of 29 training sessions (Harvey and Baker, 2016), with 10 mg/kg cocaine in an average of 40 sessions (Kueh and Baker, 2007), and in an average of 16 or 30 sessions with 1 or 0.3 mg/kg *d*-amphetamine, respectively (Quisenberry et al., 2013). Thus, it appears that 0.3 mg/kg MDPV produces stimulus control over responding relatively quickly under standard, two-lever discrimination procedures and this occurs at a rate that is comparable to structurally- or pharmacologically-related substances.

The substituted phenethylamines, methamphetamine and amphetamine, fully substituted for 0.3 mg/kg MDPV. However, the structurally similar entactogen, MDMA, did not. These data are only partially consistent with the findings reported by Fantegrossi et al. (2013), who reported both MDMA and methamphetamine produced full substitution in mice trained to discriminate 0.3 mg/kg MDPV from saline. Species differences may account for the variable effects of MDMA on MDPV-percent lever selection between the present study and that of Fantegrossi et al. (2013). However, additional drug discrimination studies directly comparing the species under identical testing procedures would be necessary to evaluate this speculation. Moreover, previous research has demonstrated comparatively greater neurotoxic effects of MDMA on dopaminergic functioning in mice relative to rats, and greater neurotoxic effects of MDMA on serotonin functioning in rats relative to mice (for review, Easton and Marsden, 2006). Thus, it is possible that the relative contribution of dopaminergic versus serotonergic actions of MDMA may differentially influence substitution for MDPV in mice and rats.

As noted above, MDMA and 4-MMC failed to fully substitute in 0.3 mg/kg MDPV trained rats in the present study; however, 4-MMC approached full substitution at the highest dose tested. Although only a single drug discrimination study has been published using MDPV as the training drug (Fantegrossi et al., 2013), other studies have tested 4-MMC for substitution in rats trained to discriminate a DA releaser, methamphetamine, or a monoamine transporter blocker, cocaine, from saline. For example, Gatch et al., (2013) trained groups of rats to discriminate 1 mg/kg methamphetamine or 10 mg/kg cocaine from saline and tested 4-MMC for substitution. In that study, 4 MMC equipotently produced full substitution in both training groups. It is noteworthy that Gatch et al. (2013) used an approximately 33 fold higher dose of cocaine (10 mg/kg) for training than the dose used for MDPV (0.3 mg/kg) in the present study. Previous research has demonstrated that MDPV is ~10 times more potent than cocaine at producing locomotor activation (e.g., Baumann et al. 2013). A comparison of the ED₅₀ values of MDPV (ED₅₀ = 0.06 mg/kg) and (-)-cocaine (ED₅₀ = 0.73 mg/kg) obtained in the present study reveals that MDPV is approximately 12 times more potent than (-)-cocaine at producing the 0.3 mg/kg MDPV cue, a relative potency difference that is somewhat consistent with that reported by Baumann et al. (2013). In any event, future studies including a higher training dose of MDPV may be necessary for substrates at monoamine transporters, such as MDMA and 4-MMC, to fully substitute in male rats.

The 5-HT releaser (+)-fenfluramine and the ergoline derivative, (+)-LSD, were initially included in the present study as negative controls given the aforementioned research indicating that MDPV produces relatively weak effects at SERT (Baumann et al., 2013). Surprisingly, 3 mg/kg fenfluramine produced full substitution in four of the subjects, while responding was completely suppressed in the other four subjects. It is presently unknown if the discriminative effects of MDPV (including downstream effects) are mediated by stimulation of serotonergic mechanisms, although a recent report revealed that the rewarding and locomotor-stimulant effects of cocaine (a drug with pharmacological actions similar to MDPV) are attenuated by 5-HT_{2C} receptor stimulation (Craig and Unterwald, 2013). Moreover, alterations in serotonergic neurotransmission can modulate the discriminative stimulus effects of cocaine (for review, Walsh and Cunningham, 1997). Thus, it is possible that the interoceptive effects of MDPV may involve serotonergic mechanisms. Further drug discrimination research including drugs with selective actions on serotonergic targets is warranted to evaluate this possibility.

Conclusion

MDPV produced discriminative stimulus effects similar to those of some structurally- and pharmacologically-related compounds, but not others. The current findings provide evidence that drugs that primarily produce increases in extracellular dopamine may also produce similar discriminative cues to those of MDPV. Although, it is worth noting that MDPV also increases extracellular norepinephrine levels, which may contribute to its discriminative stimulus effects. Based on these findings, further drug discrimination studies that include NET inhibitors may identify if norepinephrine contributes to the MDPV interoceptive cue.

Acknowledgments

We acknowledge Rachel L. Burroughs, Eric L. Harvey, and Nathyn A. Thompson for their assistance with conducting some of the behavioral testing for this study. The National Institute on Drug Abuse drug control supply program generously provided most of the drugs used in the study. This research was supported by a grant from the National Institutes of Health (R15DA038295).

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Figure 1.

Learning curve of rats trained to discriminate 0.3 mg/kg MDPV from saline. Filled circles (●) represent the effect of 0.3 mg/kg MDPV and open circles (○) represent response to vehicle (means $\pm$ SE). After session 20, the sample size is less than 8 (indicated by number in parenthesis) on some training sessions, owing to some subjects having met the discrimination criteria and undergoing substitution tests during those sessions.

Figure 2. Results of substitution tests with (A) MDPV, (B) cocaine, (C) (+)-methamphetamine, and (D) (d)-amphetamine, in rats trained to discriminate 0.3 mg/kg MDPV from saline. Mean ($\pm$ SE) percent MDPV-lever selection (left vertical axis) and mean ($\pm$ SE) responses per second (right vertical axis).

Figure 3.

Results of substitution tests with doses of (A) 4-MMC, (B) MDMA, (C) (+)-LSD, and (D) (+)-fenfluramine in rats trained to discriminate 0.3 mg/kg MDPV from saline. Mean ($\pm$ SE) percent MDPV-lever selection (left vertical axis) and mean ($\pm$ SE) responses per second (right vertical axis). Rats that did not complete the FR during a test session were not included in the mean percent drug-lever selection (the number of rats is indicated in parenthesis; otherwise the number of rats was equal to 8). For response rate, statistical differences from V are indicated by asterisks (** $p < .01$ **** $p < .0001$).