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Stereoselective differences between the reinforcing and motivational effects of cathinone-derived 4-methylmethcathinone (mephedrone) in self-administering rats

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Abstract

Mephedrone (4-methylmethcathinone (4-MMC)) (MEPH) is a new psychoactive substance (NPS) of the synthetic cathinone class. MEPH has a chiral center and exists as two enantiomers (*R*-, *S*-MEPH), yet stereospecific effects of MEPH have not been extensively investigated in preclinical assays. Because significant behavioral and neurochemical differences can exist between enantiomers, probing effects of stereochemistry on biological activity enables separation of adverse and therapeutic effects. Our prior work showed that *R*-MEPH, relative to *S*-MEPH, produced greater locomotor activation, place preference and facilitation of brain reward thresholds in rodents. The present study sought to determine if MEPH enantiomers display stereospecific reward and reinforcement in rat self-administration assays. In Experiment 1, rats were trained to self-administer racemic MEPH (0.50 mg/kg/inf), and dose substitution effects of *R*-MEPH (0.50 mg/kg/inf) and *S*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) were examined. In Experiment 2, separate rats were trained to self-administer *R*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) or *S*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) and were thereafter evaluated under progressive-ratio access conditions. Within this cohort, 50-kHz ultrasonic vocalizations (USVs) were recorded to measure potential differences in subjective positive affect associated with MEPH enantiomer self-administration. We identified enantiomer- and dose-dependent effects on infusions earned during self-administration following acquisition of racemic MEPH, with greatest infusions under low-effort, fixed-ratio 1 access conditions from low-dose *S*-MEPH self-administration. When taxed with progressive-ratio access conditions, rats trained to self-administer *R*-MEPH showed higher breakpoints than those

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of rats trained to self-administer *S*-MEPH. Additionally, *R*-MEPH elicited greatest rates of 50-kHz USVs compared to *S*-MEPH. Taken together, these data suggest that the *R*-enantiomer of MEPH is primarily responsible for the rewarding, reinforcing, and motivational properties of racemic MEPH, which increases our understanding of stereospecific preferences pertaining to MEPH abuse.

Keywords

addiction; stereoselective; mephedrone; self-administration; synthetic cathinone; ultrasonic vocalizations

Introduction

Mephedrone (4-methylmethcathinone, MEPH) is categorized as a new psychoactive substance (NPS) and is specifically among the class of synthetic cathinone (i.e. β -keto amphetamine) drugs [1-2]. Although MEPH is now illegal in the United States (Schedule I) and Europe, these compounds initially entered the drug market and were sold via the internet labeled as ‘not for human consumption’—labels included terms such as “plant food,” “bath salts,” and “research chemicals” with the ultimate intent of bypassing illegalization by the Drug Enforcement Agency [3-4]. These new “legal” high alternative drugs were quickly found to present a public health concern as they provided a relatively inexpensive and easy-to-manufacture drug-of-abuse option for many clandestine laboratories nationwide. While MEPH is currently scheduled as an illicit substance, it is still involved in 50% of all emergency hospital presentations related to NPS misuse in Europe, particularly in England where the number of individuals requiring treatment for MEPH more than doubled from 953 in 2010–2011 to 2,024 in 2014–2015 [5-6]. In humans, MEPH produces empathogenic effects, analogous to those reported in 3,4-methylenedioxymethamphetamine (MDMA, ‘Ecstasy’) users. This similarity of MEPH with MDMA may be due to enhanced levels of serotonin (5-HT) in the nucleus accumbens more so than its effect on elevating extrasynaptic dopamine (DA) [7-8]. Preclinical studies using place conditioning and self-administration show that MEPH produces rewarding and reinforcing effects in part by enhancing monoamine transmission [9-10]. Pharmacologically, MEPH functions as a non-selective monoamine transporter substrate to release DA, 5-HT and norepinephrine (NE) [2, 7, 11].

MEPH, similar to other controlled substances such as methamphetamine (METH), cocaine and MDMA, has a chiral center and stereochemical composition that includes *R* and *S* enantiomers (Figure 1). This stereochemical signature enables separation of addictive and therapeutic effects as enantiomers of established psychostimulants can produce vastly different pharmacological effects. In one study examining stereoselective effects of METH, the *S*-enantiomer demonstrated 25-fold greater potency than *R*-enantiomer in rats trained in a drug discrimination task [12]. Similarly, *S*-methcathinone has a 3-fold greater ability to substitute for a discriminative stimulus paired with racemic cocaine relative to *R*-methcathinone [13]. Using self-administration in non-human primates, Wang and colleagues (2007) [14] demonstrated more consistent reinforcement from *S*-MDMA and racemic MDMA relative to *R*-MDMA. For MDPV, crystal structures of enantiomers have been

described in prior work, and stereoselective effects include potent DAT uptake inhibition and facilitation of brain reward thresholds following systemic *S*-MDPV relative to racemate *R*-MDPV or mixture [15-16].

For MEPH, Gregg et al. (2015) [17] showed that both enantiomers have similar potency as substrates at DAT, but *S*-MEPH is about 50-fold more potent than *R*-MEPH in promoting 5-HT release by its substrate action at SERT. Dose-response experiments using intracranial self-stimulation (ICSS) to compare rewarding effects in rats demonstrated that *R*-MEPH produces greater maximal facilitation of ICSS than equivalent doses of *S*-MEPH [17]. In rat CPP studies, *R*-MEPH produces place preference whereas equivalent doses of *S*-MEPH do not. More recent work indicates that *S*-MEPH displays anxiolytic and antidepressant effects in cocaine-withdrawn rats, suggesting therapeutic potential for the *S* enantiomer [18].

An initial understanding of stereospecific abuse liability of novel psychoactive compounds such as MEPH can plausibly unlock an enantiomer with behavioral effects that give support for subsequent testing for clinical utility. For example, compounds used to treat depression and anxiety, including bupropion and venlafaxine, have chiral centers and exert stereospecific effects at monoamine transporters [for review, see 19]. While racemic MEPH maintains self-administration behavior under fixed-ratio (FR) and progressive-ratio (PR) schedules of reinforcement, it is unknown if the *R*- and *S*-enantiomers contribute equally or differently to facilitate reinforcement [10, 20]. Here, we examined stereospecific reinforcing effects of MEPH in rats during self-administration (fixed-ratio and progressive-ratio schedules of reinforcement). Additionally, 50-kHz ultrasonic vocalizations (USVs) were recorded as an analog measure of subjective positive affect—several groups find that psychostimulant self-administration elicits robust quantities of USVs that relate in part to their reinforcing properties [21-23; for review, see 24]. Mesolimbic DA transmission supports psychostimulant-evoked 50-kHz USVs [25-26], although readers should note that exposure to novel environments and stimuli can also elicit 50-kHz USVs from recorded subjects [e.g., 27]. Findings from the current study suggest that that *R*-enantiomer is primarily responsible for the rewarding, reinforcing, and motivational effects of racemic MEPH.

Results and discussion

Racemic MEPH is readily self-administered by rodents yet its enantiomers exert varying degrees of reward and reinforcement. Rats from Experiment 1 were trained to self-administer racemic MEPH after which stereoselective effects were examined—low- and moderate-dose *S*-MEPH was taken most readily whereas high-dose *S*-MEPH and *R*-MEPH were taken at comparable levels as racemic MEPH. Rats acquired responding for racemic MEPH over the course of training [$F(13, 420) = 16.58, p < 0.001$] (Figure 2A). Following acquisition, dose-substitution studies were conducted using a within-subjects design to assess reinforcing efficacies of *R*-MEPH (0.50 mg/kg/inf) and *S*-MEPH (0.25, 0.50, 2.00 mg/kg/inf). A one-day buffer wherein rats received racemic MEPH (0.50 mg/kg/inf) was included prior to each 5-day test session set at which point stereospecific effects were determined—responding remained stable on buffer days across test session sets (mean $\pm$ *S.E.M.* ranging from 32.8 $\pm$ 3.3 to 35.4 $\pm$ 2.6 infusions). Results indicate no significant

interaction between Drug Group across Session and no significant main effect of Session. However, a significant effect of Drug Group [$F(5, 450) = 68.83, p < 0.001$] was detected. Post-hoc comparisons further showed that rats self-administered more *S*-MEPH (0.25 and 0.50 mg/kg/inf) relative to racemic MEPH (p 's < 0.001) (Figure 2B). These findings suggest that rats with previous exposure to racemic MEPH may increase drug intake to a greater magnitude when self-administering a low-reward reinforcer (e.g., low- and moderate-dose *S*-MEPH)—a pattern of responding that could be an effort to compensate for a relative decrease in reinforcement when self-administering the *S*-enantiomer. An alternative explanation for enhanced intake of *S*-MEPH following racemic MEPH acquisition centers on principles of sensitization from prior exposure to either the same or different drug of abuse as is taken in later sessions [e.g., 28]. Rats that have been previously sensitized to cocaine, for example, acquire cocaine self-administration faster and inject greater quantities compared to saline-pretreated control rats [29]. Additionally, pretreatment with either cocaine or amphetamine produces sensitization and accelerated escalation of cocaine during self-administration [30-31].

When examining infusions earned from *S*-MEPH self-administering rats in Experiment 2, two-way ANOVAs revealed a significant interaction between Drug Group and Session [$F(6.34, 66.55) = 2.77, p < 0.05$] with main effects of each variable [Drug Group: $F(2, 21) = 4.84, p < 0.05$; Session: $F(3.17, 66.55) = 19.08, p < 0.001$]. Post-hoc tests revealed that moderate-dose *S*-MEPH was infused significantly more than high-dose [$p < 0.05$] and that, across drug groups, more infusions were earned from sessions 5 through 10 relative to session 1 [all p 's < 0.05]. Similar effects were observed when examining active lever presses during *S*-MEPH self-administration [Interaction: $F(7.78, 81.71) = 2.06, p = 0.052$; Drug Group: $F(2, 21) = 4.48, p < 0.05$; Session: $F(3.89, 81.71) = 12.93, p < 0.001$ —rats pressed significantly more for moderate-dose *S*-MEPH compared to high-dose [$p < 0.05$], and, across drug groups, more active lever presses were performed from sessions 6 through 10 relative to session 1 [all p 's < 0.05].

When examining infusions earned for *R*-MEPH self-administering rats, a significant interaction [$F(7.98, 83.74) = 4.24, p < 0.001$] as well as main effects of Drug Type [$F(2, 21) = 39.94, p < 0.001$] and Session [$F(3.99, 83.74) = 29.49, p < 0.001$] were found. Post-hoc tests showed that both low- and moderate-dose *R*-MEPH were infused at greater quantity compared to high-dose [all p 's < 0.01]. Collapsing drug groups across sessions, rats infused more *R*-MEPH from sessions 3 to 10 relative to session 1 [all p 's < 0.001]. Finally, when examining active lever presses for *R*-MEPH drug groups, a significant interaction was found [$F(6.03, 63.28) = 3.29, p < 0.01$] as well as main effects for Drug Group [$F(2, 21) = 40.61, p < 0.001$] and Session [$F(3.01, 63.28) = 16.07, p < 0.001$]. Post-hoc tests showed that low-dose *R*-MEPH was pressed for at greater rate compared to high-dose *R*-MEPH [$p < 0.001$]. Upon collapsing across drug groups, more active lever presses were found from sessions 3 through 10 compared to session 1 [all p 's < 0.001] (Figure 3).

Following acquisition under FR access conditions, all rats were shifted to a PR schedule of reinforcement to assess drug-seeking reinforcement and motivation. When examining active lever presses, a marginally significant main effect of Drug Group within *R*-MEPH self-administering rats was found [$F(2, 20) = 3.44, p = 0.052$], and a significant effect was found

within the *S*-MEPH self-administering rats [$F(2, 21) = 4.47, p < 0.05$]. Post-hoc comparisons revealed that rats pressed more for moderate-dose *R*-MEPH when compared to high-dose *R*-MEPH [$p = 0.054$], and that high-dose *S*-MEPH self-administering rats pressed significantly more than low-dose *S*-MEPH self-administering rats [$p < 0.05$]. Analysis of breakpoint revealed a significant effect of Drug Group [*S*-MEPH: $F(2, 21) = 5.14, p < 0.05$; *R*-MEPH: $F(2, 20) = 3.67, p < 0.05$], and post-hoc tests additionally verified significantly higher breakpoints during self-administration of moderate-dose *R*-MEPH compared to high-dose *R*-MEPH [$p < 0.05$] as well as higher breakpoints from high-dose *S*-MEPH self-administering rats relative to low-dose [$p < 0.05$] (Figure 4). Moderate-dose *R*-MEPH led to enhanced responding on the inactive lever during the first day of PR self-administration but not thereafter which we interpret as an artifact of shifting schedules of reinforcement and action-outcome response contingencies. Given that *R*-MEPH, but not *S*-MEPH, induces place preference in rats [17], our hypothesis held that higher breakpoints would be observed from *R*-MEPH self-administering rats—an effect attested to by the present data. Interestingly, our finding parallels published enantiomeric effects observed with MDMA. For example, in a study examining MDMA and its enantiomers under a PR schedule of reinforcement in rhesus monkeys, the reinforcing efficacies of racemic MDMA and *S*-MDMA were substantially higher than *R*-MDMA [14].

S-MEPH displayed a dose-dependent increase in motivation to work for infusions during PR testing but not greater motivation than *R*-MEPH which itself showed an inverted-U profile and exerted greatest reinforcing effect at moderate-dose (0.50 mg/kg/inf). It should be noted that comparable rates of effort were exerted for high-dose *S*-MEPH and low-dose *R*-MEPH—an effect that suggests significantly greater potency of *R*-MEPH in the context of drug-seeking motivation. Moderate-dose *R*-MEPH (0.50 mg/kg/inf) self-administering rats tended to respond with high variability when taxed under progressive ratio access conditions which may be an effect of individual differences in bodyweight or ability to metabolize MEPH. Further studies better interrogating mechanisms and endophenotypes underlying individual differences in motivation to self-administer MEPH are needed. Studies suggest a negative relationship between serotonergic activity and the reinforcing efficacies of psychostimulants [32–33]. Wee (2005) [32] examined if 5-HT activity affects the reinforcing efficacy of amphetamine analogs that had similar potencies *in vitro* as DA releasers but varied in 5-HT releasing potency. In that study, PAL 313, PAL 314, PAL 303, PAL 353, and *d*-amphetamine were all positive reinforcers when assessed under PR self-administration. PAL 313, the most potent 5-HT releaser, was still less potent than *d*-amphetamine, the least potent 5-HT releaser. Taken together, there is a correlation between DA/5-HT releasing potency and reinforcing potency, suggesting that the dual DA/5-HT actions of a compound influences its potency as a reinforcer. In the context of our findings, the relatively lower responding observed with the *S*-enantiomer may be due to a greater 5-HT releasing potency compared to DA. Interestingly, both enantiomers of MEPH display similar potencies as substrates at DAT, but *S*-MEPH is 50-fold more potent than *R*-MEPH in producing 5-HT release through its substrate action at SERT [17]. Based on these findings, differences in the DA/5-HT releasing potencies of the MEPH enantiomers are likely responsible for the greater reinforcing efficacy of *R*-MEPH versus *S*-MEPH.

USV recordings were taken following self-administration of MEPH enantiomers to disentangle subjective rewarding effects from reinforcing efficacies and demonstrate for the first time that positive affect follows MEPH self-administration. A one-way ANOVA examining 50-kHz USVs by Drug Group during early acquisition of self-administration did not reach statistical significance [$F(3, 13) = 2.83, p = 0.08$]. During late acquisition, however, a significant main effect of Drug Group was observed [$F(3, 13) = 4.54, p < 0.05$], and contrasts revealed that moderate-dose *R*-MEPH self-administration yielded significantly greater 50-kHz USVs compared to equivalent dose of self-administered *S*-MEPH [0.50 mg/kg/inf; $p < 0.05$] as well as high-dose *S*-MEPH [2.00 mg/kg/inf; $p < 0.05$] (Figure 5). Our findings are consistent with other groups that have shown elicitation of 50-kHz USVs following systemically and self-administered cocaine or amphetamine [34-37]. Moreover, 50-kHz USVs are supported in part by mesolimbic DA transmission as optical stimulation of DA terminals within NAcc from VTA projection neurons transiently evokes 50-kHz USVs [26]. Recently, we demonstrated that the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV), which blocks DA and NE uptake with approximately 10-fold greater potency compared to cocaine yet has negligible affinity at SERT, elicits a strong bout of 50-kHz USVs following self-administration [38]. Additionally, Simmons and colleagues reported that MDPV-elicited 50-kHz USVs were more persistent relative to cocaine-elicited USVs, but that USVs decreased to near-zero levels following load-up (*i.e.*, initial infusions in beginning of session) of either compound [23, 38].

Results from the present study suggest positive affect is experienced following initial infusions of MEPH but, similar to other psychostimulants, is relatively sparse after rats titrate to high drug levels. Our results additionally support the idea that augmented DA transmission from self-administered *R*-MEPH likely underlies the positive subjective response experienced during load-up. Self-administration of *S*-MEPH, which more strongly blocks 5-HT reuptake, elicited fewer 50-kHz USVs most notably at higher doses. Interestingly, *S*-MDPV was shown to elicit more potent locomotor activating and cocaine discriminating effects in mice relative to *R*-MDPV and the racemic mixture suggesting diverging cathinone-specific stereospecific effects [39]—an effect additionally observed with enantiomers of MDMA in rhesus monkeys [40]. Further studies utilizing pharmacological agents targeting 5HT receptor subtypes and/or transporters would enhance our understanding as to whether relatively low reinforcing effects of *S*-MEPH can be augmented by mitigating 5HT influence. Collectively, these findings suggest that the 5-HT system may have minor influence on drug taking behavior, and that DA transmission underlies in larger part drug-seeking behavior and the experience of positive affect following reward receipt. Future studies should address whether augmented 5-HT transmission, as is observed most closely with *S*-MEPH self-administration, may dampen the efficacy of DAT blockade and in turn diminish positive affect and attenuate drug-seeking behavior.

In summary, our results show that rats pre-exposed to racemic MEPH have enhanced *S*-MEPH consumption whereas a typical inverted U-shaped curve was observed when drug-naïve rats began *S*-MEPH self-administration. This study indicates the importance of environmental factors and provides evidence supporting the notion that behavioral sensitization is involved during the early stages of drug addiction and provide insight into the rapid progression of addiction. Furthermore, progressive-ratio experiments revealed that

the motivation to work for reinforcer was significantly greater in *R*-MEPH-trained rats than *S*-MEPH-trained rats at any dose of *S*-MEPH tested. Our data suggest *R*-MEPH is primarily responsible for the rewarding and reinforcing effects observed in racemic MEPH. This phenomenon of stereospecific reinforcing properties provides greater insight into the addictive properties of MEPH.

Methods

Animals

Male Sprague-Dawley rats (initial weight 275-300 g, N=64) from Harlan Laboratories (Indianapolis, IN) were housed two per cage and maintained in a controlled environment on a reverse 12-h light/dark cycle (lights off at 9:00AM). Rats were individually housed after catheter implantation. Food and water were provided *ad libitum* except during experimental procedures (drug self-administration). All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and Temple University's Institutional Animal Care and Use Committee.

Drugs

Racemic MEPH and (*R,S*)-MEPH were synthesized by Fox Chase Chemical Diversity Center, Doylestown, PA. Racemic MEPH and (*R,S*)-MEPH were dissolved in physiological saline (0.9%) and were filtered via 0.45 μ m cellulose acetate membrane (Corning). Drugs were delivered by intravenous injection at a volume of 50 μ L/inf. Body weights were averaged across rats to calculate required concentration for drug solution approximately once per week. The chiral center of MEPH is subject to base-promoted racemization. However, we have found that only ~5% racemization occurs over a period of 90 min in rat plasma at 37°C [17].

Surgery

Rats were anesthetized with isoflurane/oxygen vapor mixture (isoflurane 5 percent induction, 2–3 percent maintenance) and prepared for surgery with indwelling jugular vein catheter (PlasticsOne; SAI Infusion Technologies). Prior to surgery, rats were injected with an analgesic (meloxicam, 1 mg/kg, i.p. dissolved in saline). The catheter tubing was subcutaneously passed from the back to the right shoulder of each rat and was then inserted into the right jugular vein. The tubing was secured using suture thread 9-mm surgical staples were used to close incision sites. Following surgery, rats were placed into individual cages to recover and transferred to a clean home cage where they remained individually-housed. Rats had ~7 days of recovery before self-administration sessions began. Catheters were flushed with 0.3 ml of heparinized saline with enrofloxacin daily to maintain catheter patency and reduce infection risk.

MEPH self-administration

Acquisition—Sound-attenuating rat operant chambers (Med-Associates) were used to conduct all drug self-administration experiments. The rat was placed in the chamber, and the catheter located on the rat's back was connected to the tubing enclosed in a protective spring leash attached to the operant chamber. To begin each session, house lights were turned on

and two retractable levers extended into the chamber. Rats were trained on a fixed ratio-1 (FR-1) schedule to lever press for drug infusions during daily 2-hour sessions. During the sessions, a response on the right lever (active lever) resulted in a 3-s drug infusion and simultaneous light cue+tone stimulus. Responses on the left lever (inactive lever) had no consequences but were recorded to monitor for non-specific motor behavior. A 20-s time out period followed each infusion to limit possibility of overdose. Data collection and operant response contingencies (i.e. drug delivery, light cue+tone) were controlled from MED-PC IV software (Med Associates).

Rats in Experiment 1 (N=16) were trained to self-administer an established dose of racemic MEPH (0.50 mg/kg/inf) for 14 days [Hadlock, 2011]. Upon completion of acquisition, MEPH-trained rats were given opportunity to self-administer *R*-MEPH (0.50 mg/kg/inf) or *S*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) using a within-subjects design with drug assignment balanced according to Latin square. Doses were given in blocks of 6 consecutive sessions.

For Experiment 2, rats (N=48) were trained to self-administer *S*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) or *R*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) during daily 2-h sessions for 10 days to establish enantiomeric MEPH self-administration. Following acquisition of *R*- and *S*-MEPH self-administration, the paradigm for drug infusion changed to a progressive-ratio (PR) schedule of reinforcement for each enantiomer. Rats in all groups remained on the same dose per acquisition under FR-1 access conditions. PR sessions proceeded for 7 days and lasted for at least 2 hours or following 30 min of inactivity (i.e. no lever presses). Following the first drug infusion, all subsequent infusions were contingent upon progressively-increasing response requirement; the sequence of increasing response ratio is described by Richardson and Roberts [Richardson, 1996]. Breakpoint was defined as the final ratio completed to obtain a reinforcement before the session ended.

Ultrasonic vocalization (USV) recording and analysis

Ultrasonic emissions were detected via UltraMic200K aluminum condenser microphones (Dodotronic; Italy) with integrated digital-to-analog conversion which suspended above Plexiglas self-administration chambers as described in prior reports [23, 38]. Audio was sampled at 192-kHz and was recorded in “.wav” file format using RavenPro software (Cornell Lab of Ornithology, Bioacoustics Research Program; Ithaca, NY, USA) on a nearby personal computer. Recording sessions were initiated on single days during early (days 3-5) and late (days 8-10) acquisition of fixed-ratio self-administration. Analysis was conducted offline by trained experimenters, and putative USVs were collected after verification based on spectrographic appearance and audio playback. Only USVs with mean frequency between 38- and 80-kHz and with duration > 15 ms were included in analysis.

Data analysis

All statistical analyses were conducted using either GraphPad Prism5 or SPSS (IBM). In Experiment 1, behavioral data were analyzed using a one-way ANOVA with Session as within-subjects factor. For dose-substitution, a mixed ANOVA was used to examine behavioral responses across Session and between Drug Group (Racemic MEPH [days 10-14 of acquisition], *R*-MEPH 0.50, *S*-MEPH 0.25, *S*-MEPH 0.50, *S*-MEPH 2.00) during days

2-6 of 6-d blocks permitting day 1 as buffer. For Experiment 2, active lever press and breakpoint data were analyzed using two-way mixed ANOVAs with Session as within-subjects and Drug Group as between-subjects factors (0.25, 0.50, 2.00) for each enantiomer family of comparisons (*R*-MEPH, *S*-MEPH). When violations in sphericity were detected for repeated-measures factor, a Greenhouse-Geisser correction was applied to normalize respective dependent measure data. Additionally, total 50-kHz USVs were analyzed using one-way ANOVAs with Drug Group as between-subjects factor during early and late acquisition phases. Post-hoc analyses proceeded using Bonferroni corrections, and familywise α was established at 0.05 for each analysis.

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Abbreviations

MDMA	3,4-methylenedioxymethamphetamine
MDPV	3,4-methylenedioxypyrovalerone
5HT	5-hydroxytryptamine (serotonin)
ANOVA	Analysis of variance
DA	Dopamine
DAT	Dopamine transporter
FR	Fixed-ratio
MEPH	Mepehdrone

NE	Norepinephrine
NAcc	Nucleus accumbens
PR	Progressive-ratio
USV	Ultrasonic vocalization
VTa	Ventral tegmental area

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S-Mephedrone R-Mephedrone

Figure 1.
Chemical structures mephedrone enantiomers.

Figure 2.

Acquisition of racemic MEPH. Rats were trained to self-administer racemic MEPH (0.50 mg/kg/inf) during daily 2-h sessions for 14 days (A) followed by blocks of *R*- or *S*-MEPH self-administration (B). Data points show mean + *S.E.M.*. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to racemic MEPH (days 10-14 of acquisition). $N=16$.

Figure 3.

Acquisition of *R*- and *S*-MEPH self-administration. Rats were trained to self-administer either (A) *S*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) or (B) *R*-MEPH (0.25, 0.50, 2.00 mg/kg/inf) during 2-h daily sessions for 10 days. The number of infusions, active lever presses, and inactive lever presses are shown. Data points show mean + *S.E.M.* † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ comparing moderate to high doses. ## $p < 0.01$ comparing low to high doses. * $p < 0.05$, ** $p < 0.01$ compared to Session 1. N's = 8/group.

Figure 4. (A) S- and (B) R-MEPH self-administration under progressive ratio (PR) access conditions. Data shows the number of active lever presses, breakpoint, and inactive lever presses. Data points show mean + $S.E.M.$ † $p < 0.05$ comparing moderate to high doses. # $p < 0.05$ comparing low to high doses. N's = 7-8/group.

Figure 5.

Characterization of 50-kHz USVs during self-administration of S- and R-MEPH. Total 50-kHz USVs are shown in (B) as mean + *S.E.M.* during early and late acquisition. USVs across late acquisition 2-h sessions of dose-equivalent MEPH enantiomer self-administration sessions are depicted in (C). * $p < 0.05$ compared to R-MEPH (0.50 mg/kg/inf) group. Scale bar in right panel of (A) is 100 ms. N's = 6-8/group.