1521-0103/390/1/14–28$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

dx.doi.org/10.1124/jpet.123.001942
J Pharmacol Exp Ther 390:14–28, July 2024

Special Section on Nanotherapeutics in Cancer Research

Phencyclidine-Like Abuse Liability and Psychosis-Like Neurocognitive Effects of Novel Arylcyclohexylamine Drugs of Abuse in Rodents

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Received September 28, 2023; accepted January 2, 2024

ABSTRACT

Abuse of novel arylcyclohexylamines (ACX) poses risks for toxicities, including adverse neurocognitive effects. In vivo effects of ring-substituted analogs of phencyclidine (PCP), eticyclidine (PCE), and ketamine are understudied. Adult male National Institutes of Health Swiss mice were used to assess locomotor effects of PCP and its 3-OH, 3-MeO, 3-Cl, and 4-MeO analogs, PCE and its 3-OH and 3-MeO analogs, and ketamine and its deschloro and 2F-deschloro analogs, in comparison with those of methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), and two benzofuran analogs of MDMA. PCP-like interoceptive effects for all of these ACXs were determined using a food-reinforced drug discrimination procedure in adult male Sprague Dawley rats. A novel operant assay of rule-governed behavior incorporating aspects of attentional set-shifting was used to profile psychosis-like neurocognitive effects of PCP and 3-Cl-PCP in rats, in comparison with cocaine and morphine. PCP-like ACXs were more effective locomotor stimulants than the amphetamines, PCE-like ACXs were as effective as the amphetamines, and ketamine-like ACXs were less effective than the amphetamines. Addition of -Cl, -OH, or -OMe at the 3-position on the aromatic ring did not impact locomotor effectiveness, but addition of -OMe at the 4-position reduced locomotor effectiveness. Lethal effects were induced by drugs with -OH at the 3-position or -OMe at the 3- or 4-position. All novel ACXs substituted at least partially for PCP, and PCP and 3-Cl-PCP elicited dose-dependent psychosis-like neurocognitive deficits in the rule-governed behavior task not observed with cocaine or morphine. Novel ACXs exhibit substantial abuse liability and toxicities not necessarily observed with their parent drugs.

SIGNIFICANCE STATEMENT

Novel arylcyclohexylamine analogs of PCP, PCE, and ketamine are appearing on the illicit market, and abuse of these drugs poses risks for toxicities, including adverse neurocognitive effects. These studies demonstrate that the novel ACXs exhibit PCP-like abuse liability in the drug discrimination assay, elicit varied locomotor stimulant and lethal effects in mice, and induce psychosis-like neurocognitive effects in rats.

Introduction

Arylcyclohexylamine (ACX) drugs were developed as anesthetics in the 1950s (Collins et al., 1960) and became widespread drugs of abuse by the mid-1960s (Balster, 1989). As the recreational popularity of the prototypical ACX phencyclidine (PCP) waned, its structural analog ketamine became associated with 1990s dance culture (Smith et al., 2002), and remains a common "club drug" to this day (Palamar et al., 2023). Recently, novel ACXs have appeared on the illicit market, driven by their relative ease of synthesis and by the

This work was supported, in part, by a contract with the US Department of Justice, Drug Enforcement Administration [15DDHQ20P00000859] (to W.E.F.) and by the University of Arkansas for Medical Sciences Division for Diversity, Equity and Inclusion (to D.R.P.). None of the funding sources participated in study design, in the collection, analysis, or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

No author has an actual or perceived conflict of interest with the contents of this article.

dx.doi.org/10.1124/jpet.123.001942.

ABBREVIATIONS: 5-EAPB, 1-(benzofuran-5-yl)-N-ethylpropan-2-amine; 6-EAPB, 1-(benzofuran-6-yl)-N-ethylpropan-2-amine; ACX, arylcyclohexylamine; CL, confidence limit; E_{max}, maximal substitution; FR, fixed ratio; MDMA, 3, 4-methylenedioxymethamphetamine; METH, methamphetamine; NIH, National Institutes of Health; PCE, eticyclidine; PCP, phencyclidine; RGB, rule-governed behavior.

scaffold's versatility, allowing clandestine chemists to produce new analogs with tailored pharmacological profiles. For example, PCP and ketamine are primarily non-competitive NMDA receptor antagonists (Roth et al., 2013), while the more elaborated analog bromadol is a potent μ-opioid receptor agonist (Itzhak and Simon, 1984). Structural modifications to the base ACX structure thus yield radically distinct pharmacological actions, and mechanisms cannot necessarily be assumed based solely on chemical structure.

The introduction of functional group substitutions on aromatic rings is a common structural modification among synthetic psychoactive drugs of abuse. Amphetamines were first modified by lengthening the alkyl chain (amphetamine to methamphetamine to ethyl-amphetamine), then functional groups were added to various positions on the phenyl ring (Fuller, 1978). One resulting compound, para-chloroamphetamine, elicited unexpected toxicity and has since become known as the prototypical serotonin neurotoxin (Sanders-Bush and Sulser, 1970). Opioids have been similarly modified, with various ring-substituted fentanyl analogs now proliferating on the illicit market (Liu et al., 2018; Duffy et al., 2019; Liu et al., 2022). Abused synthetic cannabinoid receptor agonists are also extensively substituted (Ti et al., 2021; Watanabe et al., 2023; Xiang et al., 2023), and "bath salts" cathinone analogs have followed the template established for the amphetamines, with various constituents added to the phenyl ring (Mochizuki et al., 2021; Wojcieszak et al., 2020). Most recently, simple ring-substituted PCP-like and ketamine-like ACXs have emerged as drugs of abuse (Davidsen et al., 2020; Quinn et al., 2020; Frison et al., 2021; Castro et al., 2022), and have been involved in overdose fatalities (Ameline et al., 2019; Arbouche et al., 2021; Copeland et al., 2022; Chaves et al., 2023; Lo Faro et al., 2023). These compounds consist of analogs with variations in either the amine moiety (cyclic versus non-cyclic) or substitutions on the aromatic ring, or a combination of both. Common substitutions on the aromatic ring include hydroxy (-OH) and methoxy (-OMe) groups at the 3 and 4 positions, and halogenated derivatives at the 2, 3, and 4 positions (-Cl and -F).

To date, there have been surprisingly few assessments of abuse liability of novel ACXs. We (Berquist et al., 2018) and others (Botanas et al., 2015; Chiamulera et al., 2016; Mutti et al., 2016) have profiled the PCP-like effects of the novel ACX methoxetamine using locomotor activity, drug discrimination, and intravenous self-administration in rodents, prior to its placement into Schedule I under the Controlled Substances Act in June of 2022. Aside from those studies and the intravenous self-administration testing of a limited dose range of 3-MeO-PCP and 4-MeO-PCP (Abiero et al., 2020), there are no in vivo studies pertaining to abuse liability of novel PCP-like substituted ACXs. In addition to its abuse potential, chronic use of PCP induces persistent psychosis-like neuropsychological deficits, which can last from weeks to months (Rainey and Crowder, 1975; Allen and Young, 1978; Cosgrove and Newell, 1991). Worryingly, clinical reports of psychosis elicited by other abused ACXs, including methoxetamine (de Jong et al., 2014; Moccia et al., 2019; Caloro et al., 2018) and dextromethorphan (Martinak et al., 2017; Journey et al., 2022), have also been accumulating in the literature, suggesting that novel ring-substituted ACXs may also induce lasting schizophrenia-like symptoms in users.

Here we assessed locomotor effects of PCP and its 3-OH, 3-MeO, 3-Cl, and 4-MeO analogs; eticyclidine (PCE) and its 3-OH and 3-MeO analogs; and ketamine and its deschloro and 2F-deschloro analogs in adult male NIH Swiss mice. Because locomotor effects of the ACXs are less well-characterized than those of dopaminergic stimulants, we compared their effects to those of methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), and the novel MDMA analogs 1-(benzofuran-5-yl)-N-ethylpropan-2-amine (5-EAPB) and 1-(benzofuran-6-yl)-N-ethylpropan-2-amine (6-EAPB). In adult male Sprague Dawley rats, we determined PCP-like interoceptive effects for 3-OH-PCP, 3-MeO-PCP, 3-Cl-PCP, 4-MeO-PCP, PCE, 3-OH-PCE, 3-MeO-PCE, ketamine, deschloroketamine, and 2F-deschloroketamine using a food-reinforced drug discrimination procedure. Finally, we developed a novel operant assay of rule-governed behavior (RGB) which incorporates aspects of attentional set-shifting (Robbins, 1998) and used it to profile psychosis-like neurocognitive effects of PCP and 3-Cl-PCP in adult male Sprague Dawley rats.

Materials and Methods

Drugs

Phencyclidine HCl, (*S*)-methamphetamine HCl, and 3,4-methylenedioxymethamphetamine HCl (racemic) were provided by the NIDA Drug Supply Program (Rockville, MD, USA). 3-OH-phencyclidine HCl, 3-MeO-phencyclidine HCl, 3-Cl-phencyclidine HCl, 4-MeO-phencyclidine HCl, 3-OH-eticyclidine HCl and 3-MeO-eticyclidine HCl, deschloroketamine (racemic), 2F-deschloroketamine (racemic), 1-(benzofuran-5-yl)-N-ethylpropan-2-amine HCl (5-EAPB, racemic), and 1-(benzofuran-6-yl)-N-ethylpropan-2-amine (6-EAPB, racemic) were received from the United States Drug Enforcement Administration (DEA) Special Testing and Research Laboratory (Sterling, VA, USA). Eticyclidine HCl was provided by RTI International (Research Triangle Park, NC, USA). Ketaset (racemic ketamine HCl injection, USP) was purchased from the University of Arkansas for Medical Sciences Hospital Pharmacy (Little Rock, AR, USA). All drugs except for ketamine were weighed as HCl salts, dissolved in 0.9% physiologic saline, and stored in glass vials at 3–5°C until the time of use. Ketamine solutions were diluted in 0.9% physiologic saline from the 10 mg/mL Ketaset stock and were stored as described above until use. Mice received intraperitoneal drug injections in a volume of 0.1 ml/10 g, and rats received intraperitoneal injections in a volume of 0.1 ml/100 g.

Animals

Adult male NIH Swiss mice (Charles River Laboratory, Wilmington, MA) weighing approximately 30 g upon delivery and adult male Sprague Dawley rats (Harlan Laboratories, Inc., Indianapolis, IN) weighing approximately 350 g upon delivery were used as subjects. Mice were housed in groups of three and rats were pair-housed in species-appropriate polycarbonate cages within separate temperature-controlled rooms in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited vivarium. The vivarium was maintained at 22°C ± 2°C and 45–50% humidity, with lights set to a 12-hour light/dark cycle (lights on at 6:00 AM, CST). Mice were given ad libitum access to standard rodent feed (Laboratory Rodent Diet no. 5001; PMI Feeds, St. Louis, MO) and water except during testing. After one week of acclimation to the vivarium, including ad libitum access to food and water, rats were food restricted for the duration of these studies to increase the likelihood of higher response rates. Rat body weights were maintained at ~85% of their previously established free-feeding weight with appropriate corrections based on the Sprague-Dawley growth curve as rats aged, and with supplemental feedings after the completion of daily behavioral sessions. All

subjects were drug-naive before the initiation of testing, and any observable signs following treatments with the vehicle, test substance, or the reference drug were recorded. Behavioral procedures were carried out in accordance with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the NIH. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

Equipment

Locomotor Activity in Mice. Locomotor studies occurred in clear acrylic chambers (43.2 × 43.2 × 29.8 cm) equipped with arrays of infrared light emitters and detectors spaced 2.5 cm apart along two perpendicular walls (Med Associates, Inc., St. Albans, VT, USA). One activity count was registered each time a subject interrupted a light beam. Exhaust fans mounted within the enclosing chambers masked extraneous sounds, and ambient illumination was provided by a 4W bulb mounted above each arena within each enclosed chamber. Following each session, testing chamber surfaces were rinsed with tap water, sprayed and wiped with Coverage Plus NPD (a one-step cleaner, disinfectant, and deodorizer concentrate) (Steris Healthcare, Mentor, OH, USA), and dried with paper towels.

Groups of adult male NIH Swiss mice (n = 8 per group) were randomly assigned to receive a single test drug. Animals were transported from the vivarium to the laboratory and allowed to habituate for at least 30 minutes before testing. After this post-transport habituation period, mice were removed from their home cage and placed into the center of the arena and enclosed within the lighted, noise masking cabinet. After 60 minutes exploration and habituation to the testing arenas (only the last 30 minutes of which was analyzed), animals were removed from the locomotor boxes, weighed, and administered an intraperitoneal injection of saline or a dose of the assigned test compound. Immediately after injection, mice were returned to the center of the testing arena and activity was recorded for 6 hours post-injection as infrared beam breaks and converted to distance traveled using Activity Monitor 7 Software (Med Associates, Inc., St. Albans, VT, USA). Each group of mice was tested once per week, and doses were increased in half-log increments if motor activity remained at saline-like levels, or quarter-log increments if increased motor activity was observed. Subjects were thus injected until dose-effect curves became biphasic or until any adverse or lethal effects were observed.

Drug Discrimination in Rats. All drug discrimination training and testing sessions were conducted in custom-built operant conditioning chambers equipped with two response levers, auditory and visual feedback stimuli, and a food pellet dispenser. Chambers were sited on enclosed shelves in two rows of four. Each row was shielded from laboratory lights with an opaque shade, and equipped with a speaker playing white noise to mask ambient laboratory sounds. Each row of chambers was also furnished with a fan to ensure airflow. On the front wall of each chamber, there were two response levers, 5.0 cm from the midline and 4.0 cm above the floor bars. A downward displacement of a lever with a force approximately 0.20 N through approximately 1.0 cm defined a response. Two incandescent lights were located in a row above each lever. An aperture for the delivery of food pellet (45 mg grain-based food pellets; Bio-Serv, Frenchtown, NJ, USA) was mounted outside the chamber behind a 5.0 x 5.0 cm opening in the front wall midline between the two levers and 2.0 cm above the floor. All data collection and programming of behavioral contingencies were accomplished with software from Med Associates, Inc. (Med-PC version 5).

Groups of adult male Sprague Dawley rats (n = 8 per group) were first trained to depress response levers for food presentation under continuous reinforcement (fixed-ratio 1, FR1) conditions for three sessions, each lasting 60 minutes or until 60 pellets were earned. Each lever press and pellet delivery produced an audible click. Beginning on the fourth session, the FR value increased by two every 20th reinforcer earned. In this manner, rats were incremented from FR1 to the terminal FR10 schedule across successive sessions. Following acquisition of the terminal FR10 schedule, rats began training sessions to establish stimulus control on each lever. At the start of each training session, rats were injected intraperitoneally with 0.9% physiologic saline or the training drug of 3.0 mg/kg PCP, then placed into the experimental chamber. Fifteen minutes after placement into the chamber, house lights at the back of the experimental space and stimulus lights mounted above the levers were illuminated, signaling the start of the session. Training sessions lasted for 60 minutes or until 60 reinforcers were earned. Only responses on the lever paired with the injection for that session (i.e., saline versus PCP) resulted in the delivery of a food pellet, and a resetting schedule in which the response requirement reset to 0 if a rat switched to the other lever before completing the FR10 was employed. Rats were considered ready for substitution testing once they achieved five consecutive discrimination training sessions wherein the percentage of responses emitted during the first FR (i.e., prior to delivery of the first reinforcer) and the percentage of responses for the entire session were ≥80% on the injection-appropriate lever. Stimulus substitution test sessions were similar to discrimination training sessions except no food pellets were delivered upon completion of the FR10 and the sessions ended upon completion of the first FR10 or after 5 minutes had elapsed, whichever occurred first. Failure to complete the response requirement prior to the end of the 5-minute substitution session was recorded as a complete suppression of responding. Rats were required to complete at least one saline and one PCP discrimination session wherein they met the training criteria described above in between substitution test sessions. Test substances were first administered at 3.0 mg/kg, assuming equal potency to PCP. Dose-effect curves were completed using smaller or larger doses in half-log or quarter-log increments, to generate a curve with the smallest dose eliciting saline-like responding, and the largest dose eliciting 80% or more responding the PCP lever, or a complete suppression of responding. Half-log dose increments were used if the previously tested dose elicited <20% PCP-appropriate responding or >80% PCP-like responding; otherwise, quarter-log dose increments were used.

RGB in rats. All training and testing sessions were conducted in Med Associates modular operant conditioning chambers (model ENV-008, Med Associates Inc., St. Albans, VT, USA) equipped with two lighted nose-poke response keys (model ENV-123A-O lighted pigeon keys, Med Associates, Inc., St. Albans, VT, USA) mounted at the front of the chamber, a single response lever mounted at the back of the chamber, auditory and visual feedback stimuli, and a food pellet dispenser. Chambers were enclosed within light- and sound-attenuating cubicles (each furnished with a fan to ensure airflow) in three rows of two, sited on a dedicated rack. On the front wall of each chamber, there were two lighted nose-poke keys, 5.0 cm from the midline and 4.0 cm above the floor. An inward displacement of a plastic disc with a force approximately 0.20 N through approximately 0.5 cm defined a response. Blue LED lights (EverBrightt 24V, 6W, YM E-Bright, amazon.com) were located above each nose-poke key, and a red LED light (EverBrightt 24V, 6W, YM E-Bright, amazon.com) was mounted above the rear lever. An aperture for the delivery of food pellets (45 mg grain-based food pellets; Bio-Serv, Frenchtown, NJ, USA) was mounted outside the chamber behind a 5.0 x 5.0 cm opening in the front wall midline between the two nose-poke keys and 2.0 cm above the floor. All data collection and programming of behavioral contingencies were accomplished with software from Med Associates, Inc. (Med-PC version 5).

Adult male Sprague Dawley rats (n = 6) were first trained to respond for food and discriminate PCP from saline as described above. Upon completion of all discrimination experiments, rats were retrained to respond for food (FR1) on the left and right nose-poke keys, whichever was lit. An orienting response requirement (FR1) was then imposed on the lit back lever. Completion of this response would produce a series of three audible clicks and light one of the two nose poke keys (randomly selected by the controlling computer with the constraint that the same lever could not be selected more than three times in a row.) A single response on the lit key was reinforced. Once rats performed this task with >80% accuracy for five consecutive

sessions, a new condition was imposed such that the orienting response to initiate each trial could produce either the series of clicks (which signaled reinforcement on the lit nose-poke key) or a new series of three beeps, which signaled reinforcement on the unlit nose-poke key. After five correct trials, the controlling computer randomly selected a new rule set (clicks versus beeps; left versus right lever lit). Thus, during these behavioral sessions, rats attended to the auditory stimulus then responded appropriately to the rule. As the rule set continually changed throughout the session, rats performed both intradimensional and extradimensional shifts in their performance (left versus right lever, lit versus unlit lever). The primary endpoint of interest in this procedure was "trials to shift," which is the mean number of trials performed before a new rule set is selected. A rat exhibiting perfect performance (zero incorrect trials across the entire session) would score a 5 in this measure since five correct trials were required to prompt a new rule selection. Additional measures for this procedure included latency to respond (i.e., time elapsed between trial initiation and selection of a lever at the front of the test chamber) and new trial latency (i.e., time elapsed between one completed trial and initiation of the next trial by making a single response on the lit nose-poke key at the rear of the chamber). The effects of PCP, 3-Cl-PCP, morphine, and cocaine were determined in this procedure, and dose-effect curves were generated by advancing in quarter-log increments, as dictated by test results. Testing continued until a curve was generated with the smallest dose eliciting saline-like responding and the largest dose eliciting response suppression. At least five "recovery" sessions with no injection were interposed between individual compounds, and three sessions with saline injection were performed prior to initiation of drug testing to provide a baseline against which to test the effects of the individual drugs.

Data Analysis

For locomotor dose-effect functions, activity (ambulatory distance) was summed across the 6-hour session for each animal, then presented as group mean (±S.E.M.) for each dose. For locomotor time-activity curves, activity is presented in 30-minute bins (mean ± S.E.M.) across the 6-hour session. Statistical analyses were performed on dose-effect data by one-way repeated measures ANOVA on group means or on ranked data with Dunnett or Bonferroni post-hoc tests used for pairwise comparisons to saline, as appropriate for data normality and equality of variance across groups. In all cases, $P < 0.05$ was considered statistically significant. Time-activity data were not statistically analyzed but are presented so that qualitative trends in activity can be visually assessed. ED_{50} potency values were estimated from the ascending limbs of locomotor dose-effect functions using linear regression, but because lethal effects were obtained for many drugs, these values should be interpreted with some caution. Apparent maximal substitution (E_{max}) values and fold-changes relative to saline and (*S*)-methamphetamine are presented in Table 1.

The percentage of drug-appropriate responding was calculated by dividing the total number of responses on the drug-appropriate lever by the total number of responses emitted. Rate of responding was calculated by dividing the total number of responses by the session time (excluding the post-response TO periods). These data are shown as mean values (±S.E.M.) for groups of subjects at each drug dose. If subjects were tested repeatedly under a single condition, the data were pooled for an individual subject and then averaged into a group mean. Full substitution is operationally defined as: 1) 80% or more of the group responses on the drug-appropriate lever and 2) the group mean significantly different from that of vehicle. For all analyses, $P < 0.05$ was considered statistically significant, and was assessed by one-way repeated measures ANOVA on group means or by Friedman's repeated measures ANOVA on ranked data, as appropriate for data normality and equality of variance across groups. In both cases, post-hoc pairwise comparisons were made using Tukey's HSD test. Dose–effect curves for percent drug-appropriate responding were analyzed using standard linear regression techniques, from which ED_{50} values (50% effective dose for the substitution) with 95% confidence limit (95%CL) values were calculated. Only points on the linear part of the ascending portions of the curves were used. For test substances that did not fully substitute for the training drug, standard ANOVA was conducted to determine whether drug substitution differed from vehicle controls. The maximal substitution was also compared, and relative efficacy of test compounds to PCP was calculated as (E_{max} test compound/E_{max} PCP) * 100. Response rate data from drug substitution sessions were calculated as responses per second and were statistically compared with saline substitution rates using the Holm-Sidak method following a one-way repeated-measures ANOVA.

Data from the rule-governed behavior task were shown as mean values (±S.E.M.) for subjects at each dose of each drug. For all analyses, $P < 0.05$ was considered statistically significant, and was assessed by one-way repeated-measures ANOVA and Tukey's HSD test versus saline control trials.

Results

Locomotor Activity in Mice. PCP and its analogs elicited dose-dependent (Fig. 1A) and time-dependent (Fig. 2) locomotor stimulant effects in mice. Administration of PCP resulted in significant locomotor stimulant effects in mice ($F = 56.675$,

TABLE 1

ED_{50} (in mg/kg) and E_{max} values for locomotor effects of all drugs (in meters/6 hours), with 95% confidence intervals. Relative effectiveness as compared with METH and saline are also presented as fold changes. ED_{50} values for 4-MeO-PCP, ketamine, and 2F-deschloroketamine were not determined because the half-maximal locomotor effects for these compounds were not different from those observed following saline injection (*t* test).

Drug	ED_{50} values (mg/kg)	95%CL (mg/kg)	E_{max} values (m/6 hrs)	fold change METH	fold change SAL
PCP	11.63	9.75–13.51	872.27	1.21	10.49
3-Cl-PCP	28.05	17.35–38.12	1063.94	1.48	11.59
3-OH-PCP	8.10	3.90–12.30	626.78	0.87	7.88
3-MeO-PCP	9.44	5.17–13.71	554.36	0.77	5.78
4-MeO-PCP	ND	ND	281.31	0.39	4.02
PCE	6.13	4.38–7.88	588.99	0.82	9.23
3-OH-PCE	2.69	1.54–3.84	618.89	0.86	6.74
3-MeO-PCE	6.89	3.58–10.20	689.29	0.96	7.39
Ketamine	ND	ND	198.46	0.28	2.15
Deschloroketamine	8.58	3.50–13.65	409.92	0.57	3.94
2F-Deschloroketamine	ND	ND	174.29	0.24	1.86
S-METH	1.36	1.26–1.45	719.28	1.00	8.92
MDMA	18.18	8.94–27.42	608.93	0.85	7.65
5-EAPB	19.97	8.45–31.50	791.49	1.10	8.49
6-EAPB	2.66	1.22–4.10	704.97	0.98	7.86

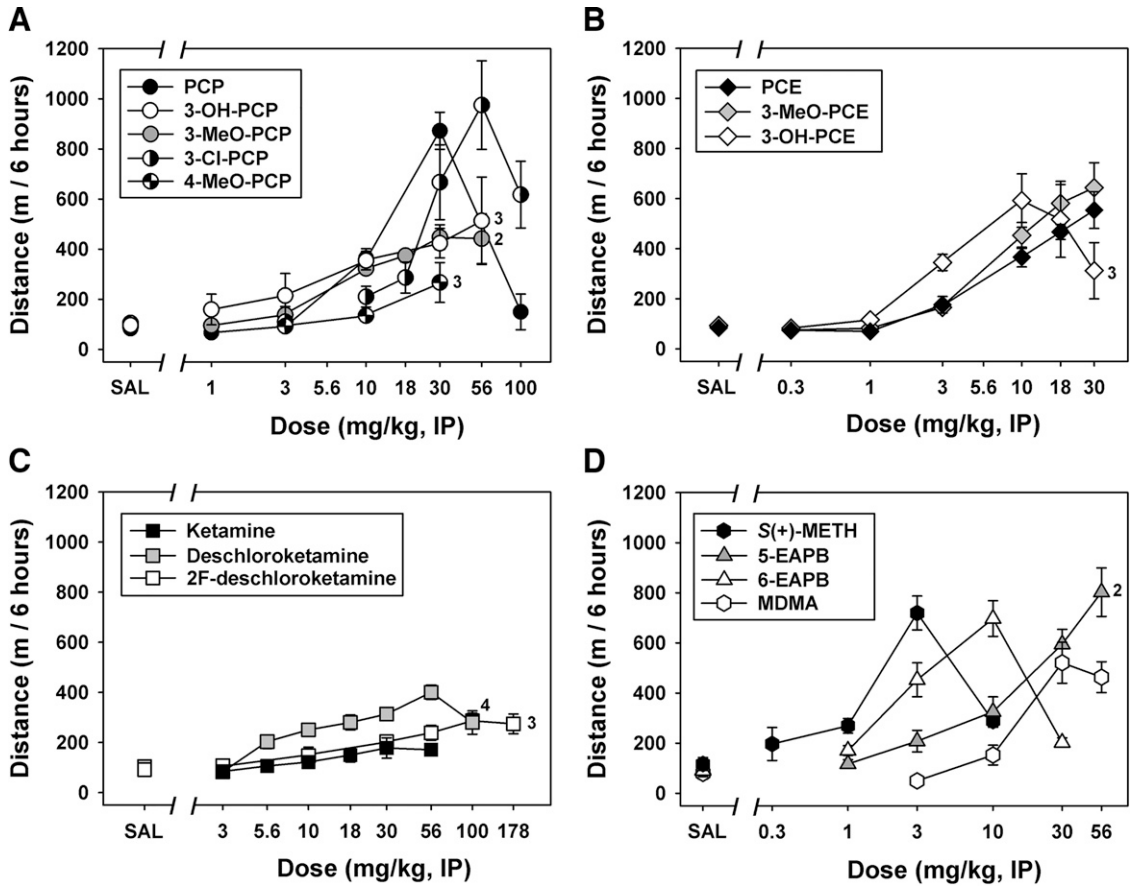

Fig. 1. Effects of PCP and its analogs 3-OH-PCP, 3-MeO-PCP, 3-Cl-PCP, and 4-MeO-PCP (panel A), PCE and its analogs 3-OH-PCE and 3-MeO-PCE (panel B), ketamine and its analogs deschloroketamine and 2F-deschloroketamine (panel C), and *S*-methamphetamine, MDMA, and its analogs 5-EAPB and 6-EAPB (panel D) on locomotor activity in adult male NIH Swiss mice. Numerals adjacent to points indicate the number of animals that died during the session. Data from animals that did not survive are not included in the group mean for that point. *Abscissae:* dose of drug, expressed in mg/kg on a log scale and administered intraperitoneally immediately before placement into the locomotor boxes. 'SAL' represents injection of saline. *Ordinates:* Total distance traveled, in meters, over the duration of the 6-hour session. Time-activity curves for each dose of each drug are presented in Figs. 2–5. ED_{50} potency and E_{max} effectiveness values and their associated 95% confidence intervals for each drug are presented in Table 1.

df = 5, $P < 0.05$), with a maximal effect that was 1.21-fold greater than that of METH (see Table 1). The dose-effect function for PCP was biphasic, with doses of 10 mg/kg (q = 4.758, $P < 0.05$) and 30 mg/kg (q = 13.360, $P < 0.05$) inducing significantly more motor activity than saline. Similarly, 3-Cl-PCP (F = 13.103, df = 6, $P < 0.05$), 3-OH-PCP (F = 3.088, df = 5, $P < 0.05$), and 3-MeO-PCP ($\chi^2 = 27.571$, df = 6, $P < 0.05$) also produced significant increases in motor activity. For 3-Cl-PCP, a biphasic dose-effect function was also generated, with doses of 30 mg/kg (q = 4.394, $P < 0.05$), 56 mg/kg (q = 6.746, $P < 0.05$), and 100 mg/kg (q = 4.015, $P < 0.05$) eliciting significantly more activity than saline. The maximal effect for 3-Cl-PCP was 1.48-fold greater than that of METH, while 3-OH-PCP and 3-MeO-PCP elicited less motor activity than METH (see Table 1). For both 3-OH-PCP and for 3-MeO-PCP, lethal effects were observed at 56 mg/kg, which precluded testing of larger doses. For mice that survived administration of 56 mg/kg 3-OH-PCP (t = 3.043, $P < 0.05$) or 56 mg/kg 3-MeO-PCP (q = 2.806, $P < 0.05$), significant locomotor stimulant effects were observed. In addition, doses of 18 mg/kg (q = 2.673, $P < 0.05$) and 30 mg/kg (q = 3.207, $P < 0.05$) 3-MeO-PCP also elicited significantly more motor activity than saline. In contrast to PCP and all other PCP analogs, 4-MeO-PCP did not induce significant locomotor stimulant effects in mice (F = 3.127, $P = 0.057$), producing a maximal effect that was 0.39-fold that of METH (see Table 1).

PCE and its analogs elicited dose-dependent (Fig. 1B) and time-dependent (Fig. 3) locomotor stimulant effects in mice. Administration of PCE resulted in significant locomotor stimulant effects in mice ($\chi^2 = 32.643$, df = 6, $P < 0.05$) with a maximal effect that was 0.82-fold that of METH (see Table 1). The dose-effect function for PCE was biphasic, with doses of 18 mg/kg (q = 3.207, $P < 0.05$) and 30 mg/kg (q = 3.875, $P < 0.05$) inducing significantly more motor activity than saline. Similarly, 3-OH-PCE (F = 14.143, df = 6, $P < 0.05$) and 3-MeO-PCE ($\chi^2 = 32.000$, df = 6, $P < 0.05$) also produced significant increases in motor activity. For 3-OH-PCE, significant locomotor stimulant effects were observed at doses of 3 mg/kg (t = 3.310, $P < 0.05$), 10 mg/kg (t = 6.550, $P < 0.05$), and 18 mg/kg (t = 5.233, $P < 0.05$), and the maximal effect was 0.86-fold that of METH (see Table 1). Lethal effects were observed at 30 mg/kg, which precluded testing of larger doses of 3-OH-PCE. For mice that received 3-MeO-PCE, doses of 18 mg/kg (q = 2.940, $P < 0.05$) and 30 mg/kg (q = 3.074, $P < 0.05$) elicited significant locomotor stimulant effects. The maximal effect of 3-MeO-PCE on motor activity was equivalent to

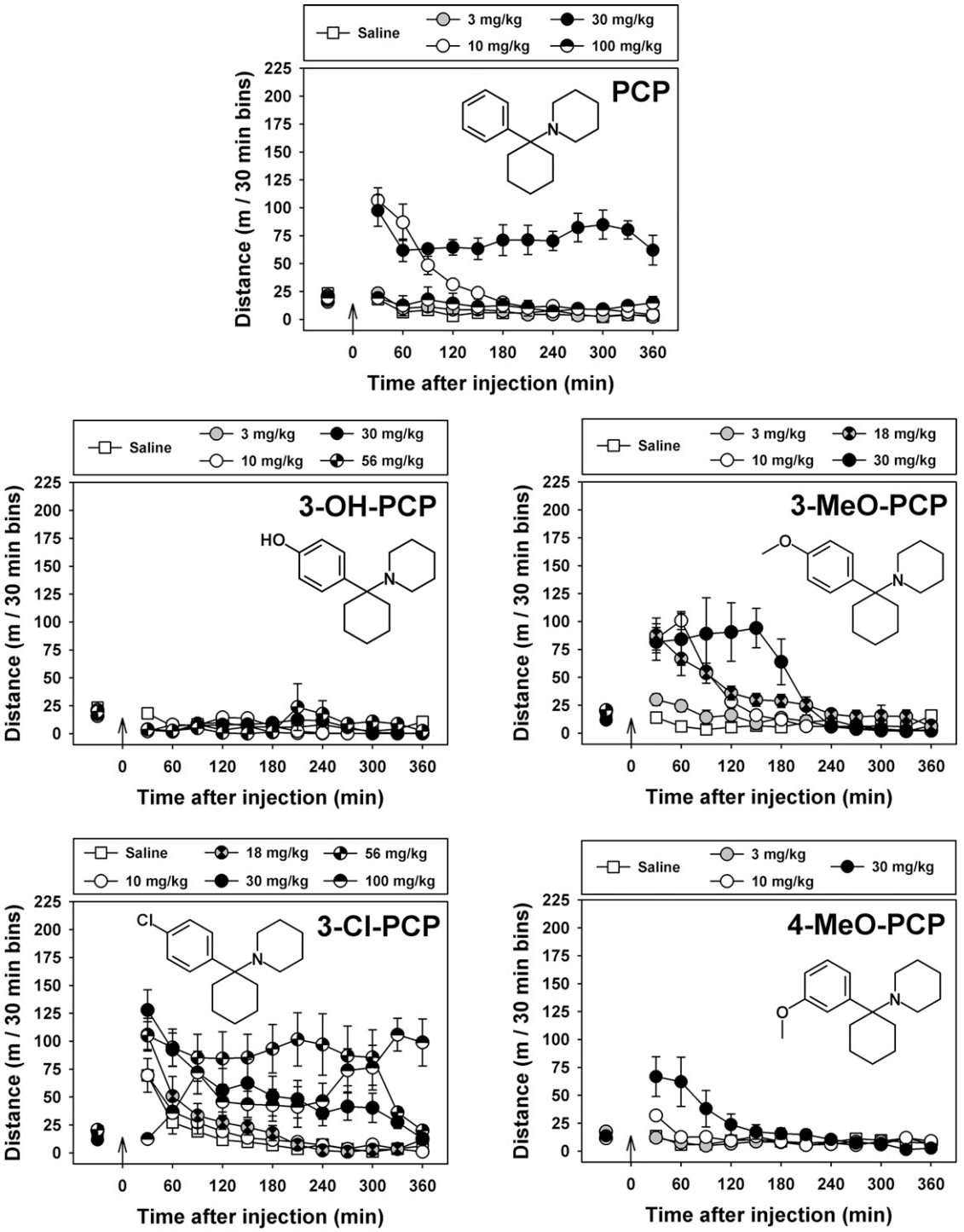

Fig. 2. Effects of individual doses of PCP and its analogs 3-OH-PCP, 3-MeO-PCP, 3-Cl-PCP, and 4-MeO-PCP on locomotor activity in adult male NIH Swiss mice, and chemical structures for each drug. *Abscissae:* time after injection, in minutes. Points to the left of 0 indicate activity observed during the last 30 minutes of the 60-minute habituation period prior to injection. *Ordinates:* Distance traveled, in meters, during each 30-minute bin of the 6-hour session. Dose-effect curves for each drug are presented in Fig. 1A. ED_{50} potency and E_{max} effectiveness values and their associated 95% confidence intervals for each drug are presented in Table 1.

that of METH (see Table 1). Lethality rapidly occurred in the first three mice administered 56 mg/kg 3-MeO-PCE, so no further animals were injected, and no locomotor data were obtained with this toxic dose.

Ketamine itself did not elicit significantly more motor activity than saline ($F = 0.572$, $df = 6$, $P = 0.686$), but reductions in ambulatory speed were quantified by the activity software (data not shown) and motor incoordination was noted by technicians during visual inspections. Indeed, the maximal effect of ketamine on motor activity was only 0.28-fold that of METH (see Table 1). Both ketamine analogs also elicited comparatively weak locomotor stimulant effects, which were low in magnitude (Fig. 1C) and short in duration (Fig. 4). Deschloroketamine elicited dose-dependent increases in activity

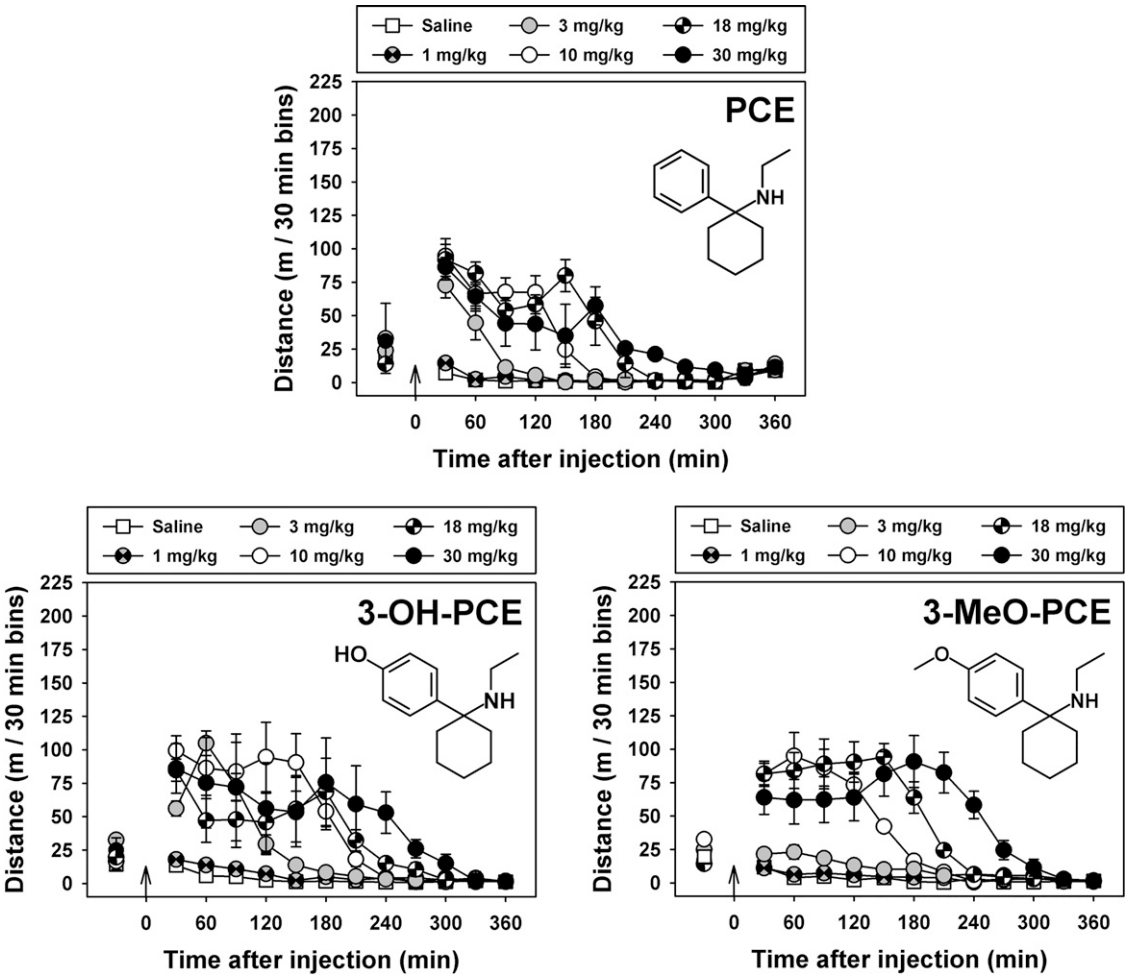

Fig. 3. Effects of individual doses of PCE and its analogs 3-OH-PCE and 3-MeO-PCE on locomotor activity in adult male NIH Swiss mice, and chemical structures for each drug. *Abscissae:* time after injection, in minutes. Points to the left of 0 indicate activity observed during the last 30 minutes of the 60-minute habituation period prior to injection. *Ordinates:* Distance traveled, in meters, during each 30-minute bin of the 6-hour session. Dose-effect curves for each drug are presented in Fig. 1B. ED_{50} potency and E_{max} effectiveness values and their associated 95% confidence intervals for each drug are presented in Table 1.

(F = 6.899, df = 6, $P < 0.05$) with doses of 18 mg/kg (q = 2.825, $P < 0.05$), 30 mg/kg (q = 4.223, $P < 0.05$), and 56 mg/kg (q = 3.881, $P < 0.05$), all producing significantly more activity than saline. The maximal effect of deschloroketamine on motor activity was about half that of METH (see Table 1). Lethal effects were observed at a dose of 100 mg/kg deschloroketamine, precluding the testing of larger doses. For 2F-deschloroketamine, significant locomotor stimulant effects (F = 3.433, df = 7, $P < 0.05$) were observed only at the dose of 100 mg/kg (t = 4.189, $P < 0.05$), and the maximal effect was only 0.24-fold that of METH (see Table 1). Lethal effects were observed soon after administration of 178 mg/kg 2F-deschloroketamine, so no further doses were tested.

(*S*)-METH, MDMA, and its analogs elicited dose-dependent (Fig. 1D) and time-dependent (Fig. 5) locomotor stimulant effects in mice. Administration of (*S*)-METH resulted in significant locomotor stimulant effects in mice (F = 25.768, df = 4, $P < 0.05$). The dose-effect function for (*S*)-METH was biphasic, with doses of 3 mg/kg (q = 9.232, $P < 0.05$) and 10 mg/kg (q = 2.625, $P < 0.05$) inducing significantly more motor activity than saline. Similarly, MDMA (χ^2 = 24.700, df = 4, $P < 0.05$), 5-EAPB (χ^2 = 32.357, df = 5, $P < 0.05$), and 6-EAPB (χ^2 = 27.100, df = 5, $P < 0.05$) also produced significant increases in motor activity. For MDMA, a biphasic dose-effect curve was obtained, with doses of 30 mg/kg (q = 3.637, $P < 0.05$) and 56 mg/kg (q = 3.162, $P < 0.05$) eliciting significantly more locomotor activity than saline. The maximal effect of MDMA on motor activity was 0.85-fold that of METH (see Table 1). For 5-EAPB, doses of 10 mg/kg (q = 2.806, $P < 0.05$), 30 mg/kg (q = 4.143, $P < 0.05$), and 56 mg/kg (q = 4.543, $P < 0.05$) elicited significantly greater locomotor activity than saline. The maximal effect of 5-EAPB on motor activity was equivalent to that of METH (see Table 1). Lethal effects were obtained in two mice following administration of 56 mg/kg 5-EAPB, so no further doses were tested. Finally, the effects of 6-EAPB on ambulatory activity were also biphasic, and animals administered doses of 3 mg/kg (q = 3.795, $P < 0.05$) and 10 mg/kg (q = 4.585, $P < 0.05$) exhibited significantly more activity than mice injected with saline. The maximal effect of 6-EAPB on motor activity was equivalent to that of METH (see Table 1).

Drug Discrimination in Rats. PCP dose-dependently and fully substituted for its training dose, producing a maximum of >80% PCP-appropriate responding 15 minutes after injection of 3.0 mg/kg during test sessions (see Fig. 6A). PCP

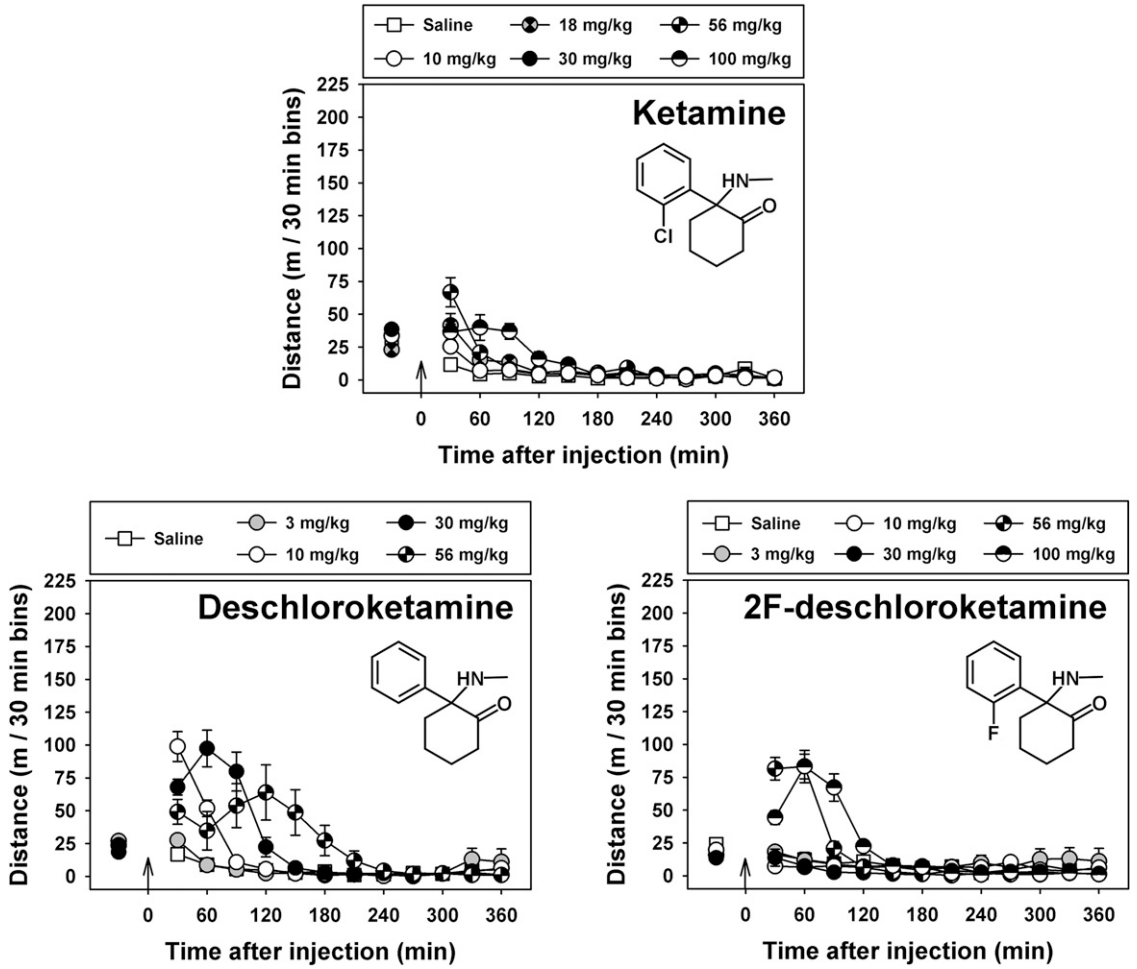

Fig. 4. Effects of individual doses of ketamine and its analogs deschloroketamine and 2F-deschloroketamine on locomotor activity in adult male NIH Swiss mice, and chemical structures for each drug. *Abscissae:* time after injection, in minutes. Points to the left of 0 indicate activity observed during the last 30 minutes of the 60-minute habituation period prior to injection. *Ordinates:* Distance traveled, in meters, during each 30-minute bin of the 6-hour session. Dose-effect curves for each drug are presented in Fig. 1C. ED_{50} potency and E_{max} effectiveness values and their associated 95% confidence intervals for each drug are presented in Table 1.

elicited significant PCP-like interoceptive effects ($\chi^2 = 29.260$, df = 4, $P < 0.05$), and doses of 1.8 mg/kg (q = 7.196, $P < 0.05$) and 3.0 mg/kg (q = 14.149, $P < 0.05$) were different from saline. There were no systematic effects of PCP on response rates during substitution test sessions, but the largest tested dose (3.0 mg/kg) significantly decreased rates as compared with saline substitution sessions (t = 3.368, $P < 0.05$) (see Table 3). The prototypical psychostimulant cocaine was also substituted to determine the pharmacological selectivity of the discrimination and never elicited discriminative performance different from saline (F = 1.227, df = 4, $P = 0.325$) up to a dose that elicited complete response suppression in half the subjects (see Fig. 6A). In contrast, 3-OH-PCP (F = 34.049, df = 4, $P < 0.05$), 3-Cl-PCP (F = 5.533, df = 4, $P < 0.05$), 3-MeO-PCP (F = 58.293, df = 3, $P < 0.05$), and 4-MeO-PCP (F = 9.420, df = 3, $P < 0.05$) all produced significant PCP-like discriminative effects. 3-OH-PCP dose-dependently and fully substituted for PCP, producing a maximum of >80% PCP-appropriate responding 15 minutes following 3.0 mg/kg (see Fig. 6A). The relative efficacy of 3-OH-PCP to PCP was 98.37%, such that 3-OH-PCP was equally efficacious to PCP (see Table 2, below). There were no significant effects of 3-OH-PCP on response rates during substitution test sessions (see Table 3), and no significant observations were noted during substitution tests with 3-OH-PCP, at any dose. 3-Cl-PCP dose-dependently and fully substituted for PCP, producing a maximum of >80% PCP-appropriate responding 15 minutes following 3.0 mg/kg (see Fig. 6A). The relative efficacy of 3-Cl-PCP to PCP was 87.01%, such that 3-Cl-PCP was equally efficacious to PCP (see Table 2, below). Injection of 3-Cl-PCP dose-dependently reduced response rates during substitution test sessions (see Table 3) as compared with saline control, with doses of 1.0 mg/kg (t = 3.522, $P < 0.05$), 1.8 mg/kg (t = 3.493, $P < 0.05$), and 3.0 mg/kg (t = 4.057, $P < 0.05$) eliciting response rates significantly different from those observed after saline injection. No significant observations were noted during substitution tests with 3-Cl-PCP, at any dose. 3-MeO-PCP dose-dependently and fully substituted for PCP, producing a maximum of >80% PCP-appropriate responding 15 minutes following 10.0 mg/kg (see Fig. 6A). The relative efficacy of 3-MeO-PCP to PCP was 98.87%, such that 3-MeO-PCP was equally efficacious to PCP (see Table 2, below). Response rate was decreased to less than 25% of vehicle control following 10.0 mg/kg 3-MeO-PCP, and this effect was statistically

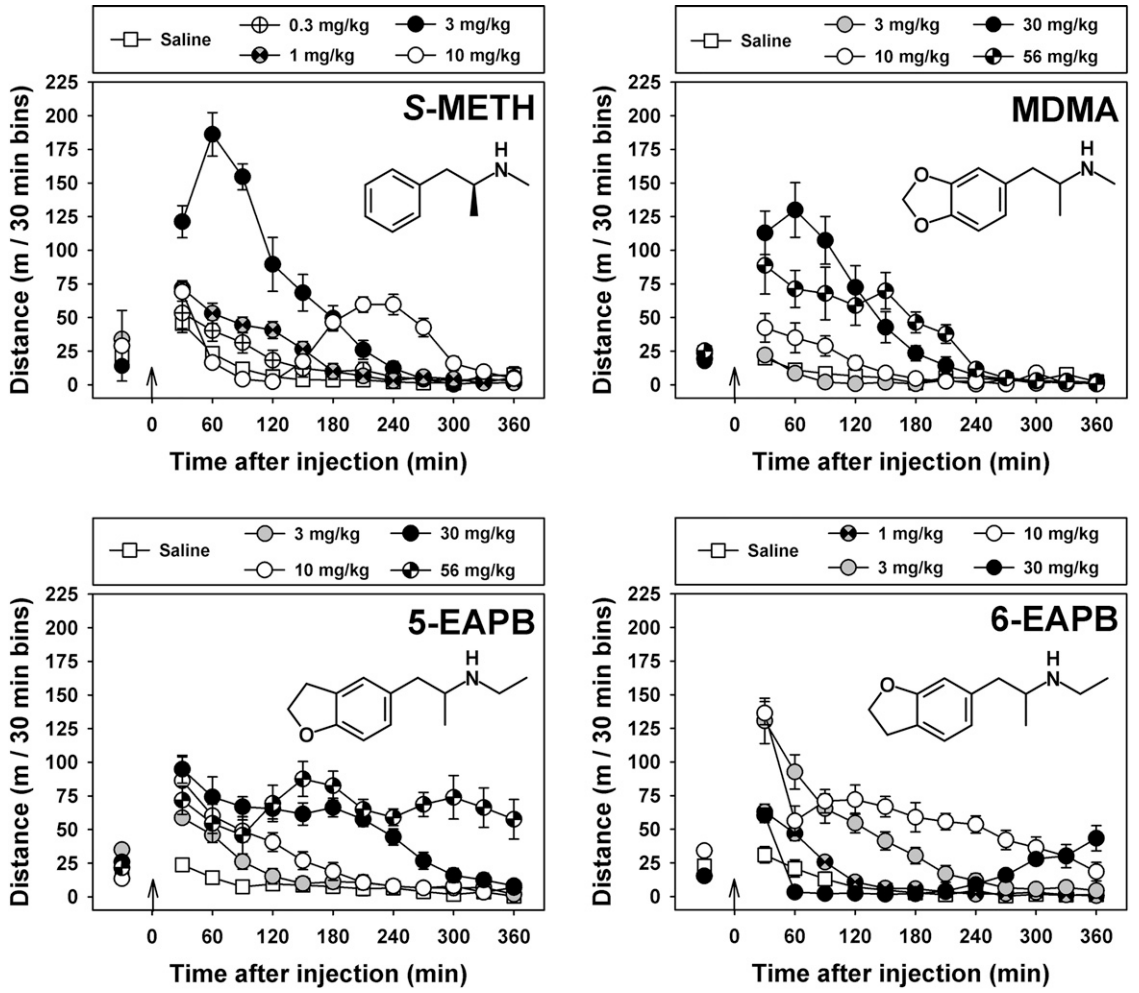

Fig. 5. Effects of individual doses of *S*-methamphetamine, MDMA, and its analogs 5-EAPB and 6-EAPB on locomotor activity in adult male NIH Swiss mice, and chemical structures for each drug. *Abscissae:* time after injection, in minutes. Points to the left of 0 indicate activity observed during the last 30 minutes of the 60-minute habituation period prior to injection. *Ordinates:* Distance traveled, in meters, during each 30-minute bin of the 6-hour session. Dose-effect curves for each drug are presented in Fig. 1D. ED_{50} potency and E_{max} effectiveness values and their associated 95% confidence intervals for each drug are presented in Table 1.

significant ($t = 5.401$, $P < 0.05$) (see Table 3). One of eight animals was behaviorally suppressed at the highest tested dose of 3-MeO-PCP (10.0 mg/kg), failing to emit a single response during testing. The largest dose at which this animal responded (5.6 mg/kg) engendered less than 50% PCP-like responding, so this animal was therefore not included in the ED_{50} calculations for this compound. 4-MeO-PCP dose-dependently and partially substituted for PCP, producing a maximum of <80% PCP-appropriate responding 15 minutes following 18.0 mg/kg (see Fig. 6A). The relative efficacy of 3-MeO-PCP to PCP was 61.15%, such that 4-MeO-PCP was less efficacious than PCP (see Table 2, below). Response rate was significantly decreased to less than 35% of vehicle control following 18.0 mg/kg ($t = 4.381$, $P < 0.05$) and was completely suppressed following administration of 30.0 mg/kg, which precluded testing of larger doses (see Table 3). Two rats failed to respond during testing of 18.0 mg/kg 4-MeO-PCP, and all eight animals were behaviorally suppressed at 30.0 mg/kg 4-MeO-PCP. For 3 of these subjects, the largest dose at which these animals responded engendered less than 75% PCP-like responding. Therefore, these animals were not included in the ED_{50} calculations for this compound.

PCE dose-dependently and fully substituted for PCP, producing significant PCP-like interoceptive effects ($F = 48.507$, $df = 4$, $P < 0.05$) with a maximum of >80% PCP-appropriate responding 15 minutes following 1.0 mg/kg (see Fig. 6B). The relative efficacy of PCE to PCP was 102.12%, such that PCE was equally efficacious to PCP (see Table 2, below). Administration of PCE did not significantly alter response rates as compared with saline control at any dose (see Table 3), and no significant observations were noted during substitution tests with PCE, at any dose. 3-OH-PCE dose-dependently and fully substituted for PCP, producing significant PCP-like interoceptive effects ($F = 81.527$, $df = 4$, $P < 0.05$), with a maximum of >80% PCP-appropriate responding 15 minutes following 1.8 mg/kg (see Fig. 6B). The relative efficacy of 3-OH-PCE to PCP was 99.09%, such that 3-OH-PCE was equally efficacious to PCP (see Table 1, below). During test sessions, 3-OH-PCE elicited dose-dependent reductions in response rates (see Table 3), with 1.0 mg/kg ($t = 3.545$, $P < 0.05$), 1.8 mg/kg ($t = 3.366$, $P < 0.05$), and 3.0 mg/kg ($t = 4.122$, $P < 0.05$) engendering rates significantly lower than those observed during saline test sessions. No significant observations were noted during

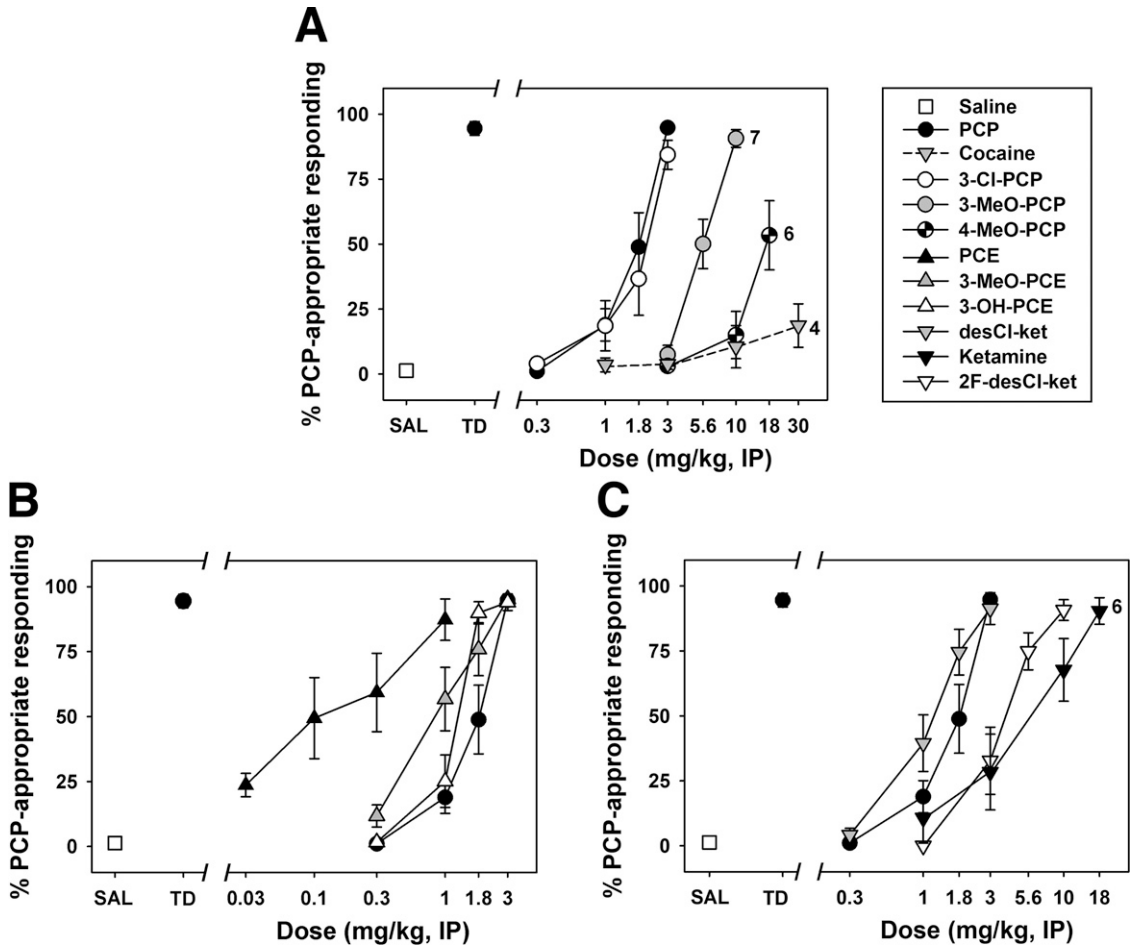

Fig. 6. Discriminative stimulus effects of PCP, 3-OH-PCP, 3-Cl-PCP, 3-MeO-PCP, 4-MeO-PCP, and cocaine (panel A), PCE, 3-OH-PCE, and 3-MeO-PCE (panel B), and ketamine, deschloroketamine, and 2F-deschloroketamine (panel C) in adult male Sprague Dawley rats trained to discriminate 3.0 mg/kg PCP from saline. Points represent group means (n = 8 rats) and error bars represent standard error of the mean. Points without error bars indicate that the variability is contained within the point. Numerals adjacent to points indicate the number of rats completing the test session, if less than 8. *Abscissae:* Dose of drug substituted during test sessions, expressed in mg/kg on a log scale. 'SAL' represents data from saline substitution while 'TD' represents data from test sessions with the training dose of PCP (3.0 mg/kg). *Ordinates:* Percent of total responses emitted on the PCP-appropriate lever. ED_{50} potency and E_{max} effectiveness values and their associated 95% confidence intervals for each drug are presented in Table 2, while corresponding response rate data are presented in Table 3.

substitution tests with 3-OH-PCE, at any dose. 3-MeO-PCE dose-dependently and fully substituted for PCP, producing significant PCP-like interoceptive effects ($F = 21.111$, $df = 4$, $P < 0.05$) with a maximum of >80% PCP-appropriate responding 15 minutes following 3.0 mg/kg (see Fig. 6B, below). The relative efficacy of 3-MeO-PCE to PCP was 98.30%, such that 3-MeO-PCE was equally efficacious to PCP (see Table 2, below). There were no systematic effects of 3-MeO-PCE on response rates during substitution test sessions (see Table 3), but 1.0 mg/kg 3-MeO-PCE elicited significantly

TABLE 2

ED_{50} (in mg/kg) and E_{max} values for interoceptive effects of all drugs (as % PCP-appropriate responding), with 95% confidence intervals. Some rats were excluded from determination of ED_{50} values if the largest dose at which these animals responded engendered less than 75% PCP-like responding (see text of Results).

Drug	ED_{50} values (mg/kg)	95%CL (mg/kg)	E_{max} values (%PCP lever)	95%CL (%PCP lever)
PCP-1	1.15	0.870–1.422	97.92	94.63–100.0
PCP-2	1.62	1.21–2.03	91.68	82.57–100.0
3-OH-PCP	1.34	0.992–1.686	96.32	91.40–100.0
3-Cl-PCP	1.83	1.455–2.209	85.20	71.77–98.63
3-MeO-PCP	5.76	4.50–7.02	90.64	81.72–99.56
4-MeO-PCP	12.66	8.38–16.94	56.06	26.90–85.22
Ketamine	6.42	4.53–8.31	90.27	82.13–98.41
Deschloro-ketamine	1.19	0.86–1.51	91.31	77.83–100.0
2F-deschloro-ketamine	4.17	2.94–5.41	91.68	82.57–100.0
PCE	0.26	0.12–0.40	93.62	86.85–100.0
3-OH-PCE	1.22	1.00–1.43	97.03	93.29–100.0
3-MeO-PCE	1.03	0.73–1.34	96.25	93.35–99.16

TABLE 3
Mean response rates (in responses/second) ± SEM during discrimination substitution tests. Asterisks indicate significant differences from rates observed during saline substitution, dashes indicate doses that were not tested, and "supp" indicates complete behavioral suppression.

Drug	0.03	0.1	0.3	1.0	1.8	3.0	5.6	10.0	18.0	30.0
Saline	1.144 ± 0.134									
PCP	—	—	1.095 ± 0.203	1.353 ± 0.148	1.158 ± 0.284	0.486 ± 0.056*	—	—	—	—
3-OH-PCP	—	—	0.855 ± 0.115	0.895 ± 0.086	0.848 ± 0.274	0.918 ± 0.207	—	—	—	—
3-Cl-PCP	—	—	1.116 ± 0.102	0.668 ± 0.081*	0.673 ± 0.131*	0.579 ± 0.073*	supp	—	—	—
3-MeO-PCP	—	—	—	—	—	1.031 ± 0.141	0.709 ± 0.124	0.311 ± 0.064*	—	—
4-MeO-PCP	—	—	—	—	—	1.139 ± 0.109	—	1.000 ± 0.130	0.484 ± 0.052*	supp
PCE	1.056 ± 0.055	0.815 ± 0.184	0.808 ± 0.166	0.749 ± 0.205	—	—	—	—	—	—
3-OH-PCE	—	—	0.751 ± 0.128	0.688 ± 0.103	0.687 ± 0.080*	0.600 ± 0.104*	—	—	—	—
3-MeO-PCE	—	—	0.818 ± 0.089	0.683 ± 0.138*	0.843 ± 0.185	0.782 ± 0.068	—	—	—	—
S-Ketamine	—	—	—	1.031 ± 0.182	—	0.984 ± 0.176	—	0.568 ± 0.163*	0.318 ± 0.081*	0.185 ± 0.111*
S-deschloro-ketamine	—	—	0.826 ± 0.139	0.880 ± 0.185	0.905 ± 0.105	0.861 ± 0.239	—	—	—	—
S-2F-deschloro-ketamine	—	—	—	1.110 ± 0.162	—	0.743 ± 0.125	0.721 ± 0.048	0.701 ± 0.140	—	—

different rates from those obtained following saline administration (t = 3.811, $P < 0.05$). No significant observations were noted during substitution tests with 3-MeO-PCE, at any dose.

Ketamine dose-dependently and fully substituted for PCP, producing significant PCP-like interoceptive effects (χ^2 = 20.871, df = 4, $P < 0.05$) with a maximum of >80% PCP-appropriate responding 15 minutes following 18.0 mg/kg (see Fig. 6C). The relative efficacy of ketamine to PCP was 92.30%, such that ketamine was equally efficacious to PCP (see Table 2, below). Response rates were significantly different from those obtained following saline administration following treatment with 10.0 mg/kg (t = 3.886, $P < 0.05$), 18.0 mg/kg (t = 4.700, $P < 0.05$), and 30.0 mg/kg (t = 5.442, $P < 0.05$) ketamine (see Table 3). Persistent motor incoordination was noted when removing rats from operant chambers after treatment with 18.0 and 30.0 mg/kg ketamine, precluding the testing of larger doses. DesCl-Ket dose-dependently and fully substituted for PCP, producing significant PCP-like interoceptive effects (F 3 5.830, df = 4, $P < 0.05$) with a maximum of >80% PCP-appropriate responding 15 minute following 3.0 mg/kg (see Fig. 6C). The relative efficacy of desCl-Ket to PCP was 93.25%, such that desCl-Ket was equally efficacious to PCP (see Table 2, below). There were no significant effects of desCl-Ket on response rates during substitution test sessions (see Table 3), and no significant observations were noted during substitution tests with desCl-Ket, at any dose. 2F-desket dose-dependently and fully substituted for PCP, producing significant PCP-like interoceptive effects (χ^2 = 29.280, df = 4, $P < 0.05$) with a maximum of >80% PCP-appropriate responding 15 minutes following 10.0 mg/kg (see Fig. 6C). The relative efficacy of 2F-desket to PCP was 100%, such that 2F-desket was equally efficacious to PCP (see Table 2, below). There were no significant effects of 2F-desket on response rates during substitution test sessions (see Table 3), and no significant observations were noted during substitution tests with 2F-desket, at any dose.

RGB in Rats. Administration of PCP (F = 39.786, df = 4, $P < 0.05$) and 3-Cl-PCP (F = 30.929, df = 4, $P < 0.05$) significantly altered the number of trials required to shift the rule set, but injections of cocaine (F = 1.001, df = 3, $P = 0.426$) and morphine (F = 3.250, df = 2, $P = 0.093$) had no significant effects on this measure (see Fig. 7A). Doses of 3.0 mg/kg (q = 3.660, $P < 0.05$) and 5.6 mg/kg (q = 10.797, $P < 0.05$) PCP disrupted rule-governed behavior in rats such that significantly more trials were required to prompt a new rule. These same doses also elicited significant effects on latencies to respond (F = 22.733, df = 4, $P < 0.05$) (Fig. 7B), with 3.0 mg/kg (q = 3.745, $P < 0.05$) and 5.6 mg/kg (q = 7.508, $P < 0.05$) increasing latency as compared with saline trials. Injection of PCP also significantly increased the latency to initiate a new trial (χ^2 = 11.918, df = 4, $P < 0.05$) (Fig. 7C), with 5.6 mg/kg eliciting a longer latency as compared with saline trials (q = 2.800, $P < 0.05$). Similarly, treatment with 5.6 mg/kg (q = 4.815, $P < 0.05$) and 10.0 mg/kg (q = 8.900, $P < 0.05$) 3-Cl-PCP elicited significant increases in the number of trials required to shift the rule set (Fig. 7A). However, distinct from PCP, there was no significant effect of 3-Cl-PCP on latency to respond (χ^2 = 4.522, df = 4, $P = 0.340$) (Fig. 7B), but there was a significant effect (F = 9.232, df = 4, $P < 0.05$) on latency

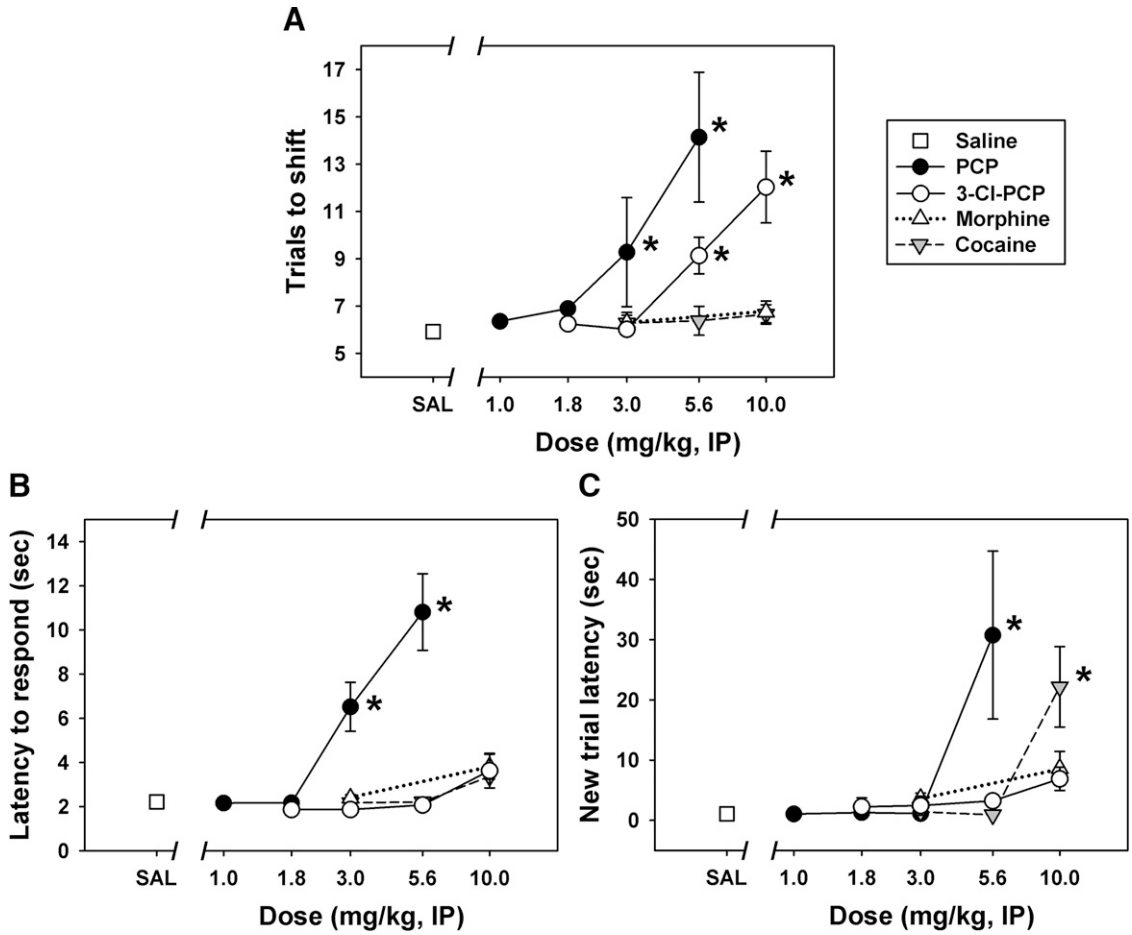

Fig. 7. Effects of saline, PCP, 3-Cl-PCP, morphine, and cocaine on RGB in adult male Sprague Dawley rats trained to perform a food-reinforced operant task. Data are presented for trials to shift the rule set (panel A), latency to respond (panel B) and latency to initiate a new trial (panel C). Asterisks indicate significant differences from saline control. *Abscissae:* Dose of drug administered 15 minutes prior to test sessions, expressed in mg/kg on a log scale. 'SAL' represents data from saline control sessions. *Ordinates:* (A) Number of trials required to shift to a new rule set; (B) latency to respond correctly or incorrectly after auditory cues were presented, in seconds; (C) latency to initiate a new trial after the visual cue was presented, in seconds.

to initiate a new trial (Fig. 7C) at only the largest tested dose of 10.0 mg/kg ($q = 5.662$, $P < 0.05$). Cocaine administration also produced a significant effect on latency to initiate a new trial ($F = 12.000$, $df = 3$, $P < 0.05$) at 10.0 mg/kg ($q=4.928$, $P < 0.05$) (Fig. 7C), but did not elicit any significant effects on latency to respond (Fig. 7B) at any tested dose ($F = 1.393$, $df = 3$, $P = 0.292$). Injection of morphine did not significantly affect latency to respond ($F = 2.516$, $df = 2$, $P = 0.142$) (Fig. 7B) or latency to initiate a new trial ($F = 4.191$, $df = 2$, $P = 0.057$) (Fig. 7C).

Discussion

The tricyclic structure of the PCP molecule allows for numerous chemical modifications which yield new biologically active analogs. Some substitutions on the aromatic ring have previously been shown to alter both effectiveness and relative potency (Kalir et al., 1984; Cone et al., 1984), while alkyl substitutions on the nitrogen atom impact only drug potency (Jasinski et al., 1981; Shannon, 1981; Shannon and DeGregorio, 1981; Risner, 1982). Altering of the size of the cyclohexyl ring (McQuinn et al., 1981; Shannon et al., 1983) or substitutions with –OH (Carroll et al., 1981) or alkyl groups (Vincent et al., 1979) impacts both the efficacy and relative potency of the resulting compounds. The present studies focused on several novel psychoactive substances currently emerging as drugs of abuse, all of which contain substitutions to the aromatic ring of the prototypical ACXs PCP, PCE, or ketamine. The major finding of these studies is that these ACXs generally elicited PCP-like interoceptive and neurocognitive effects in rats, but varied substantially in their locomotor stimulant effects in mice.

Because locomotor effects of PCP-like compounds are less well-characterized than those of traditional psychostimulants in mice, we determined effects of methamphetamine and some related compounds on motor activity and compared these effects to the ACXs. Among the amphetamine analogs, all drugs were generally similar in terms of their locomotor effects, but MDMA was the least effective compound studied. The finding that MDMA was a less effective locomotor stimulant in mice than (*S*)-METH is consistent with previous studies (Gatch et al., 2019; Gannon et al., 2023). While 5-EAPB has previously been shown to elicit locomotor sensitization in mice across a limited dose range (Sayson et al., 2020), we are not aware of any reports of locomotor stimulant effects of its

positional isomer 6-EAPB, and our finding that 6-EAPB is more potent than 5-EAPB appears to be novel.

Perhaps surprisingly, PCP was a more effective locomotor stimulant than the amphetamines. Careful examination of time-activity curves (Figs. 2 and 5) suggests that differences in induction of motor stereotypy may at least partially explain this apparent effectiveness difference. The pattern of motor activity indicative of stereotypy is an initial increase in distance traveled observed immediately after injection (as the drug is absorbed), rapidly followed by a persistent decline in activity counts as the animals enter focused stereotypy, then later in the session, as the drug is cleared, metabolized, and eliminated, a "late phase" of increased activity is observed. This pattern is readily apparent for the largest doses of the amphetamines in Fig. 5, but is much less pronounced for the PCP-like compounds in Fig. 2, suggesting that these drugs induced briefer and weaker stereotypies than the amphetamines. Interestingly, addition of -Cl, -OH, or -MeO at the 3-position of the aromatic ring on the PCP scaffold did not impact locomotor effectiveness, but addition of -MeO at the 4-position reduced locomotor effectiveness. Moreover, addition of -OH at the 3-position or -MeO at either the 3- or 4-position of the aromatic ring of the PCP scaffold introduced lethal effects not observed with either the parent drug or with the 3-Cl analog. PCE and its analogs were generally less effective than the PCP-like drugs in the locomotor activity assay but were still approximately as effective as (*S*)-METH. Fig. 3 again shows much less of a stereotypy signal for the PCE-like drugs than observed with the amphetamines. Addition of -OH at the 3-position of the aromatic ring on the PCE scaffold increased potency but also elicited lethal effects not observed with the parent drug. As expected, ketamine and its analogs were not particularly effective locomotor stimulants, eliciting marginal and short-lived stimulant effects. At large doses, all of these agents reduced ambulatory velocity and produced motor incoordination. Importantly, both analogs elicited lethal effects not observed with the parent drug.

In rats trained to discriminate PCP from saline, all of the novel ACXs substituted – at least partially – for PCP. It is widely recognized that there is a strong correlation between discriminative stimulus effects of drugs in nonverbal species and subjective effects reported by humans (Schuster and Johanson, 1988; Brauer et al., 1997); thus, all of the novel ACXs tested here would likely elicit PCP-like effects in humans. For the PCP-like drugs, addition of -MeO at the 4-position on the aromatic ring reduced both potency and effectiveness as compared with PCP, while the 3-MeO compound exhibited reduced potency, but still fully substituted for PCP. This is a similar effect (e.g., reducing potency, not effectiveness) previously identified following methylation of the 3- or 4-position of the piperidine ring (Cone et al., 1984). Interestingly, the 3-chloro substitution on PCP did not impact potency or effectiveness. This contrasts with a previous study in rats trained to discriminate 3.0 mg/kg PCP from saline, which found that the similarly halogenated 3-fluoro-phencyclidine (3-F-PCP) exhibited significantly reduced potency as compared with PCP (Cone et al., 1984). More recently, 4-fluoro-phencyclidine (4-F-PCP) was shown to elicit abuse-relevant effects of conditioned place preference and locomotor sensitization in mice (Ortiz et al., 2021). The effects of halogenation at various ring positions of the PCP structure should be investigated further.

For the PCE-like drugs, PCE itself was about 4-fold more potent than PCP in the discrimination task. Addition of either -OH or -MeO at the 3-position on the aromatic ring of PCE reduced potency but did not change effectiveness. Despite this reduction in potency as compared with PCE, 3-OH-PCE and 3-MeO-PCE were still as potent as PCP. In squirrel monkeys trained to discriminate PCP from saline, PCE and PCP substituted with equal potency (Brady and Balster, 1981), but previous studies in rats have demonstrated that PCE is more potent than PCP in assays of food-maintained responding under multiple schedule control (McMillan et al., 1992) and in PCP discrimination (Berquist et al., 2018).

Ketamine was the least potent drug that fully substituted for PCP, and both of its analogs were more potent than their parent. Previous studies in squirrel monkeys (Brady and Balster, 1981), rats (Brady and Balster, 1982; Koek et al., 1990), and mice (Middaugh et al., 1988) all confirm that ketamine is less potent than PCP in drug discrimination experiments, but the present results suggest that removal of the 2-Cl group present on the ketamine molecule substantially improves discriminative potency, as the ED_{50} values for deschloroketamine and PCP obtained in these experiments were essentially equivalent. Interestingly, replacement of the –Cl with a –F at the 2-position also improved potency to substitute for PCP, although a recent paper reported drug discrimination results indicating that 2-F-deschloroketamine substitutes for ketamine in rats with a potency equivalent to that of ketamine itself (Li et al., 2022).

Schizophrenia is characterized by a variety of diagnostic symptoms, including hallucinations and delusions, disordered thoughts, deficits in social interaction, emotional expression and motivation, and cognitive dysfunction in the realms of impaired attention and working memory (Pearlson, 2000). The glutamatergic neuronal dysfunction hypothesis of schizophrenia (Carlsson et al., 1997) originated from the observation that acute PCP administration elicits transient psychosis in normal volunteers (Luby et al., 1959) and exacerbates symptoms in schizophrenic patients (Javitt and Zukin 1991). Animal models of psychosis-like neurocognitive impairment thus include pre-pulse inhibition of the startle response (Swerdlow et al., 2008; Swerdlow et al., 2016), which measures deficits in sensorimotor gating, and attentional set-shifting procedures which model decrements in rule-governed behavior (Bubeníková-Valesová et al., 2008). In a typical rodent test of intradimensional/extradimensional shift, animals are trained to dig for food in vessels distinguished by their odor and texture. Animals then perform a series of discrimination tests, including intradimensional shift (within odor or texture) and extradimensional shift (from odor to texture, or vice versa). These procedures are slow to train, have low throughput, and require a significant investment in hands-on technician time. Our development of a novel operant assay of rule-governed behavior, which incorporates aspects of attentional set-shifting, allows reasonably rapid training of animals, is relatively high throughput, is largely "hands off" for the technician, and, as the present results demonstrate, is selectively sensitive to PCP-like drugs. We found that PCP and 3-Cl-PCP elicited dose-dependent psychosis-like neurocognitive deficits in the RGB task, which were not observed with any doses of the psychostimulant cocaine or the μ-opioid morphine. PCP disrupted rule-governed behavior at doses that also increased latency to respond and latency to initiate the next trial, but 3-Cl-PCP impaired RGB at doses

which did not impact either of these endpoints. Importantly, PCP elicited significant disruption of RGB performance at the same dose used to establish discriminative control, but 3-Cl-PCP did not significantly impact RGB performance at any doses that substituted for PCP. These results may suggest that 3-Cl-PCP is less likely to induce psychosis-like neurocognitive impairments at the doses in which it is likely to be abused than is PCP, although further study of novel ACXs using this procedure is necessary to make definitive statements in this regard.

In summary, the continuing emergence of new psychoactive substances as drugs of abuse challenges scientists and policymakers alike. The multitude of sites amenable to chemical modification on the PCP structure suggests that novel ACXs will remain public health concerns in the years to come. Based on the present findings, many ring-substituted analogs may be expected to exhibit greater toxicity and lethality than their parent drugs, and may also elicit persistent psychosis-like neurocognitive impairments in users. A greater understanding of this relatively understudied pharmacological class is needed.

Acknowledgments

The authors thank the University of Arkansas for Medical Sciences Department of Laboratory Animal Medicine for expert husbandry and veterinary services.

Data Availability

The authors declare that all the data supporting the findings of this study are contained within the paper.

Authorship Contributions

Participated in research design: Gannon, Fitzgerald, Johnson, Fantegrossi.

Conducted experiments: Shaw, Patel, Gannon, Fitzgerald, Fantegrossi.

Contributed new reagents or analytic tools: Carbonaro, Johnson.

Performed data analysis: Gannon, Fantegrossi.

Wrote or contributed to the writing of the manuscript: Shaw, Gannon, Carbonaro, Johnson, Fantegrossi.

References

Abiero A, Botanas CJ, Custodio RJ, Sayson LV, Kim M, Lee HJ, Kim HJ, Lee KW, Jeong Y, Seo JW et al. (2020) 4-MeO-PCP and 3-MeO-PCMo, new dissociative drugs, produce rewarding and reinforcing effects through activation of mesolimbic dopamine pathway and alteration of accumbal CREB, deltaFosB, and BDNF levels. *Psychopharmacology (Berl)* **237**:757–772.

Allen RM and Young SJ (1978) Phencyclidine-induced psychosis. *Am J Psychiatry* **135**:1081–1084.

Ameline A, Greney H, Monassier L, Raul JS, and Kintz P (2019) Metabolites to parent 3-MeO-PCP ratio in human urine collected in two fatal cases. *J Anal Toxicol* **43**:321–324.

Arbouche N, Kintz P, Zagdoun C, Gheddar L, Raul JS, and Ameline A (2021) Determination of 3-MeO-PCP in human blood and urine in a fatal intoxication case, with a specific focus on metabolites identification. *Forensic Sci Res* **6**:208–214.

Balster RL (1989) Behavioral pharmacology of PCP, NMDA and sigma receptors. *NIDA Res Monogr* **95**:270–274.

Berquist MD, Hyatt WS, Bauer-Erickson J, Gannon BM, Norwood AP, and Fantegrossi WE (2018) Phencyclidine-like in vivo effects of methoxetamine in mice and rats. *Neuropharmacology* **134**(Pt A):158–166.

Botanas CJ, de la Peña JB, Dela Peña IJ, Tampus R, Yoon R, Kim HJ, Lee YS, Jang CG, and Cheong JH (2015) Methoxetamine, a ketamine derivative, produced conditioned place preference and was self-administered by rats: Evidence of its abuse potential. *Pharmacol Biochem Behav* **133**:31–36.

Brady KT and Balster RL (1981) Discriminative stimulus properties of phencyclidine and five analogues in the squirrel monkey. *Pharmacol Biochem Behav* **14**:213–218.

Brady KT and Balster RL (1982) Discriminative stimulus properties of ketamine stereoisomers in phencyclidine-trained rats. *Pharmacol Biochem Behav* **17**:291–295.

Brauer LH, Goudie AJ, and de Wit H (1997) Dopamine ligands and the stimulus effects of amphetamine: animal models versus human laboratory data. *Psychopharmacology (Berl)* **130**:2–13.

Bubeníková-Valesová V, Horácek J, Vrajová M, and Höschl C (2008) Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev* **32**:1014–1023.

Caloro M, Calabrò G, de Pisa E, Rosini E, Kotzalidis GD, Lonati D, Locatelli CA, Papa P, Schifano F, and Girardi P (2018) Combined NMDA Inhibitor Use in a Patient With Multisubstance-induced Psychotic Disorder. *J Addict Med* **12**:247–251.

Carlsson A, Hansson LO, Waters N, and Carlsson ML (1997) Neurotransmitter aberrations in schizophrenia: new perspectives and therapeutic implications. *Life Sci* **61**:75–94.

Carroll FI, Brine GA, Boldt KG, Cone EJ, Yousefnejad D, Vaupel DB, and Buchwald WF (1981) Phencyclidine metabolism: resolution, structure, and biological activity of the isomers of the hydroxy metabolite, 4-phenyl-4-(1-piperidinyl)cyclohexanol. *J Med Chem* **24**:1047–1051.

Castro AL, da Costa MJCSP, Tarelho S, Sousa L, Russo F, and Franco JM (2022) Intoxication by 3-MeO-PCP and O-desmethyltramadol: an unusual NPS mix. *Int J Legal Med* **136**:1297–1301.

Chaves TV, Wilffert B, and Sanchez ZM (2023) Overdoses and deaths related to the use of ketamine and its analogues: a systematic review. *Am J Drug Alcohol Abuse* **49**:141–150.

Chiamulera C, Armani F, Mutti A, and Fattore L (2016) The ketamine analogue methoxetamine generalizes to ketamine discriminative stimulus in rats. *Behav Pharmacol* **27**(2–3 Spec Issue):204–210.

Cone EJ, McQuinn RL, and Shannon HE (1984) Structure-activity relationship studies of phencyclidine derivatives in rats. *J Pharmacol Exp Ther* **228**:147–153.

Copeland CS, Hudson S, Treble R, and Hamnett HJ (2022) The First Fatal Intoxication with 3-MeO-PCP in the UK and a Review of the Literature. *J Anal Toxicol* **46**:461–470.

Collins VJ, Gorospe CA, and Rovenstine EA (1960) Intravenous nonbarbiturate, nonnarcotic analgesics: preliminary studies. 1. Cyclohexylamines. *Anesth Analg* **39**:302–306.

Cosgrove J and Newell TG (1991) Recovery of neuropsychological functions during reduction in use of phencyclidine. *J Clin Psychol* **47**:159–169.

Davidsen AB, Mardal M, Johansen SS, Dalsgaard PW, and Linnet K (2020) In vitro and in vivo metabolism and detection of 3-HO-PCP, a synthetic phencyclidine, in human samples and pooled human hepatocytes using high resolution mass spectrometry. *Drug Test Anal* **12**:987–993.

de Jong B, van Vuren AJ, Niesink RJ, and Brunt TM (2014) [A patient in a methoxetamine-induced dissociative psychosis]. *Ned Tijdschr Geneeskd* **158**:A7358.

Duffy J, Urbas A, Niemitz M, Lippa K, and Marginean I (2019) Differentiation of fentanyl analogues by low-field NMR spectroscopy. *Anal Chim Acta* **1049**:161–169.

Frison G, Zancanaro F, Frasson S, Quadretti L, Agnati M, Vlassich F, Gagliardi G, Salerno TMG, Donato P, and Mondello L (2021) Analytical Characterization of 3-MeO-PCP and 3-MMC in Seized Products and Biosamples: The Role of LC-HRAM-Orbitrap-MS and Solid Deposition GC-FTIR. *Front Chem* **8**:618339.

Fuller RW (1978) Structure-activity relationships among the halogenated amphetamines. *Ann N Y Acad Sci* **305**:147–159.

Gannon BM, Fitzgerald LR, Godwin CO, Hughes-Meredith HD, Rice KC, and Fantegrossi WE (2023) Effects of ambient temperature on locomotor activity and place conditioning elicited by abused psychostimulants in mice: Role of 3,4-methylenedioxy moiety. *Drug Alcohol Depend* **250**:110917.

Gatch MB, Dolan SB, and Forster MJ (2019) Locomotor activity and discriminative stimulus effects of five novel synthetic cathinone analogs in mice and rats. *Drug Alcohol Depend* **199**:50–58.

Itzhak Y and Simon EJ (1984) A novel phencyclidine analog interacts selectively with mu opioid receptors. *J Pharmacol Exp Ther* **230**:383–386.

Jasinski DR, Shannon HE, Cone EJ, Vaupel DB, Risner ME, McQuinn RL, Su T-P, and Pickworth WB (1981) Interdisciplinary studies on phencyclidine, in *PCP (Phencycidine): Historical and Current Perspectives* (Domino EF ed) pp 331–400, NPP Books, Ann Arbor, Michigan.

Javitt DC and Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* **148**:1301–1308.

Journey JD, Agrawal S, and Stern E (2022) Dextromethorphan Toxicity, in *StatPearls*. StatPearls Publishing, Treasure Island, Florida.

Kalir A, Teomy S, Amir A, Fuchs P, Lee SA, Holsztynska EJ, Rocki W, and Domino EF (1984) N-allyl analogues of phencyclidine: chemical synthesis and pharmacological properties. *J Med Chem* **27**:1267–1271.

Koek W, Woods JH, and Colpaert FC (1990) N-methyl-D-aspartate antagonism and phencyclidine-like activity: a drug discrimination analysis. *J Pharmacol Exp Ther* **253**:1017–1025.

Li F, Du H, Wu B, Wei J, Qiao Y, Lai M, Zhou W, Shen H, Wang Y, Xu P et al. (2022) 2-Fluorodeschloroketamine has similar abuse potential as ketamine. *Addict Biol* **27**:e13171.

Liu C, Li T, Han Y, Hua Z, Jia W, and Qian Z (2018) The identification and analytical characterization of 2,2′-difluorofentanyl. *Drug Test Anal* **10**:774–780.

Liu CM, Song CH, Jia W, Hua ZD, and Liao Q (2022) The application of ^{19}F NMR spectroscopy for the analysis of fluorinated new psychoactive substances (NPS). *Forensic Sci Int* **340**:111450.

Lo Faro AF, Berardinelli D, Cassano T, Dendramis G, Montanari E, Montana A, Berretta P, Zaami S, Busardò FP, and Huestis MA (2023) New Psychoactive Substances Intoxications and Fatalities during the COVID-19 Epidemic. *Biology (Basel)* **12**:273.

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, and Kelley R (1959) Study of a new schizophrenomimetic drug; sernyl. *AMA Arch Neurol Psychiatry* **81**:363–369.

Martinak B, Bolis RA, Black JR, Fargason RE, and Birur B (2017) Dextromethorphan in Cough Syrup: The Poor Man's Psychosis. *Psychopharmacol Bull* **47**:59–63.

McMillan DE, Wright DW, and Wenger GR (1992) Effects of phencyclidine-like drugs on responding under multiple fixed ratio, fixed interval schedules. *Behav Pharmacol* **3**:143–147.

McQuinn RL, Cone EJ, Shannon HE, and Su TP (1981) Structure-activity relationships of the cycloalkyl ring of phencyclidine. *J Med Chem* **24**:1429–1432.

Middaugh LD, Favara JP, Boggan WO, and Stringer AJ (1988) Discriminative properties of phencyclidine in mice: generalization to ketamine and monohydroxy metabolites. *Psychopharmacology (Berl)* **96**:381–384.
Moccia L, Tofani A, Mazza M, Covino M, Martinotti G, Schifano F, Janiri L, and Di Nicola M (2019) Dorsolateral Prefrontal Cortex Impairment in Methoxetamine-Induced Psychosis: An ^{18}F-FDG PET/CT Case Study. *J Psychoactive Drugs* **51**:254–259.
Mochizuki A, Adachi N, and Shojo H (2021) Detection of 4-FMC, 4-MeO-α-PVP, 4-F-α-PVP, and PV8 in blood in a forensic case using liquid chromatography-electrospray ionization linear ion trap mass spectrometry. *Forensic Sci Int* **325**:110888.
Mutti A, Aroni S, Fadda P, Padovani L, Mancini L, Collu R, Muntoni AL, Fattore L, and Chiamulera C (2016) The ketamine-like compound methoxetamine substitutes for ketamine in the self-administration paradigm and enhances mesolimbic dopaminergic transmission. *Psychopharmacology (Berl)* **233**:2241–2251.
Ortiz DM, Custodio RJP, Abiero A, Botanas CJ, Sayson LV, Kim M, Lee HJ, Kim HJ, Jeong Y, Yoon S et al. (2021) The dopaminergic alterations induced by 4-F-PCP and 4-Keto-PCP may enhance their drug-induced rewarding and reinforcing effects: Implications for abuse. *Addict Biol* **26**:e12981.
Palamar JJ, Wilkinson ST, Carr TH, Rutherford C, and Cottler LB (2023) Trends in Illicit Ketamine Seizures in the US From 2017 to 2022. *JAMA Psychiatry* **80**:750–751.
Pearlson GD (2000) Neurobiology of schizophrenia. *Ann Neurol* **48**:556–566.
Quinn M, Brettell T, Joshi M, Bonetti J, and Quarino L (2020) Identifying PCP and four PCP analogs using the gold chloride microcrystalline test followed by raman microspectroscopy and chemometrics. *Forensic Sci Int* **307**:110135.
Rainey Jr JM and Crowder MK (1975) Prolonged psychosis attributed to phencyclidine: report of three cases. *Am J Psychiatry* **132**:1076–1078.
Risner ME (1982) Intravenous self-administration of phencyclidine and related compounds in the dog. *J Pharmacol Exp Ther* **221**:637–644.
Robbins TW (1998) Homology in behavioural pharmacology: an approach to animal models of human cognition. *Behav Pharmacol* **9**:509–519.
Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, and Iversen L (2013) The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. *PLoS One* **8**:e59334.
Sanders-Bush E and Sulser F (1970) P-chloroamphetamine: in vivo investigations on the mechanism of action of the selective depletion of cerebral serotonin. *J Pharmacol Exp Ther* **175**:419–426.
Sayson LV, Custodio RJP, Ortiz DM, Lee HJ, Kim M, Jeong Y, Lee YS, Kim HJ, and Cheong JH (2020) The potential rewarding and reinforcing effects of the substituted benzofurans 2-EAPB and 5-EAPB in rodents. *Eur J Pharmacol* **885**:173527.
Schuster C and Johanson C (1988) Relationship between the discriminative stimulus properties and subjective effects of drugs, in *Transduction mechanisms of drug stimuli* (Colpaert F and Balster R, eds) pp 161–175, Springer, Berlin.
Shannon HE (1981) Evaluation of Phencyclidine Analogs on the Basis of Their Discriminative Stimulus Properties in the Rat. *J Pharmacol Exp Ther* **216**:543–551.
Shannon HE and DeGregorio CM (1981) Effects of N-substituted analogs and metabolites of phencyclidine on avoidance behavior in the rat. *J Pharmacol Exp Ther* **218**:55–62.
Shannon HE, McQuinn RL, Vaupel DB, and Cone EJ (1983) Effects of cycloalkyl ring analogs of phencyclidine on behavior in rodents. *J Pharmacol Exp Ther* **224**:327–333.
Smith KM, Larive LL, and Romanelli F (2002) Club drugs: methylenedioxymethamphetamine, flunitrazepam, ketamine hydrochloride, and gamma-hydroxybutyrate. *Am J Health Syst Pharm* **59**:1067–1076.
Swerdlow NR, Weber M, Qu Y, Light GA, and Braff DL (2008) Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology (Berl)* **199**:331–388.
Swerdlow NR, Braff DL, and Geyer MA (2016) Sensorimotor gating of the startle reflex: what we said 25 years ago, what has happened since then, and what comes next. *J Psychopharmacol* **30**:1072–1081.
Ti L, Tobias S, Maghsoudi N, Milloy MJ, McDonald K, Shapiro A, Beriault D, Stefan C, Lysyshyn M, and Werb D (2021) Detection of synthetic cannabinoid adulteration in the unregulated drug supply in three Canadian settings. *Drug Alcohol Rev* **40**:580–585.
Vincent JP, Kartalovski B, Geneste P, Kamenka JM, and Lazdunski M (1979) Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes. *Proc Nat Acad Sci* **76**:4678–4682.
Watanabe S, Baginski S, Iwai T, Matsushita R, Takatsu M, Nakanishi T, Lindbom K, Mckenzie C, Vikingsson S, Kronstrand R et al. (2023) Systematic In Vitro Metabolic Profiling of the OXIZID Synthetic Cannabinoids BZO-4en-POXIZID, BZO-POXIZID, 5F-BZO-POXIZID, BZO-HEXOXIZID and BZO-CHMOXIZID. *J Anal Toxicol* **47**:455–463.
Wojcieszak J, Kuczyńska K, and Zawilska JB (2020) Four Synthetic Cathinones: 3-Chloromethcathinone, 4-Chloromethcathinone, 4-Fluoro-α-Pyrrolidinopentiophenone, and 4-Methoxy-α-Pyrrolidinopentiophenone Produce Changes in the Spontaneous Locomotor Activity and Motor Performance in Mice with Varied Profiles. *Neurotox Res* **38**:536–551.
Xiang J, Wen D, Zhao J, Xiang P, Shi Y, and Ma C (2023) Study of the Metabolic Profiles of "Indazole-3-Carboxamide" and "Isatin Acyl Hydrazone" (OXIZID) Synthetic Cannabinoids in a Human Liver Microsome Model Using UHPLC-QE Orbitrap MS. *Metabolites* **13**:576.

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