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Phencyclidine-like *in vivo* effects of methoxetamine in mice and rats

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Abstract

Methoxetamine (MXE) is a novel drug of abuse that is structurally similar to phencyclidine (PCP). In the present study, rats were trained to discriminate PCP from saline and substitution tests were performed with arylcyclohexylamines PCP, eticyclidine (PCE), tenocyclidine (TCP), and MXE. PCP and PCE engendered PCP-lever selection in all subjects, whereas MXE and TCP produced PCP-lever selection in animals that did not display behavioral disruption. Last, the substituted tryptamine dipropyltryptamine (DPT) produced moderate PCP-lever selection and elicited behavioral disruption in all subjects at the highest dose tested. Immediately following the final substitution test in the drug discrimination experiment, the same rats and a separate group of experimentally-naïve rats were implanted with osmotic mini-pumps delivering continuous PCP infusions for 11 days. Consistent with PCP withdrawal, disruption of food-maintained operant responding was observed when the pumps were removed, but cumulative MXE administration dose-dependently reversed this effect. A third group of rats self-administered several unit doses of PCP and MXE. Results of the self-administration tests revealed that MXE was a less effective reinforcer than PCP. Lastly, mice were implanted with radiotelemetry probes to simultaneously monitor thermoregulatory and locomotor responses following injections of PCP, PCE, or MXE. All three arylcyclohexylamines elicited dose-dependent hypothermic effects, but only PCP produced increases in locomotor activity. Together, these findings indicate that MXE elicits PCP-like interoceptive effects, but reduced reinforcing and locomotor stimulant effects *in vivo*.

Keywords

methoxetamine; MXE; phencyclidine; PCP; eticyclidine; PCE; tenocyclidine; TCP

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1. Introduction

Methoxetamine (MXE; 2-(3-methoxyphenyl)-2-(ethylamino)-cyclohexanone) is a novel psychoactive substance first identified in the United Kingdom in September 2010 (EMCDDA, 2013), and its development as a designer drug of abuse is well-documented (Morris, 2011; Morris & Wallach, 2014). Its structure places it in the arylcyclohexylamine chemical class, alongside related compounds PCP and ketamine. Similar to the recent emergence of other designer drugs of abuse, such as the synthetic cathinones, opioids, and cannabinoids, MXE and related arylcyclohexylamines are widely available through internet distributors and local “head shops” (Wood *et al.*, 2012; Corazza *et al.* 2012; De Paoli *et al.* 2013). Common motivations for using MXE include ease of access and affordability, a belief that it possesses relatively low hepatotoxicity and urotoxicity, and its ketamine- and PCP-like psychotomimetic and anesthetic effects (Hofer *et al.* 2011; Kjellgren & Jonsson, 2013; Winstock *et al.*, 2016). For review of the history and recreational use of novel arylcyclohexylamines, see Morris and Wallach (2014).

MXE and related compounds eticyclidine (PCE; *N*-ethyl-1-phenylcyclohexylamine) and tenocyclidine (TCP; 1-(1-(2-thienyl)cyclohexyl)piperidine) possess an arylcyclohexylamine backbone similar to PCP (Figure 1). In addition to structural similarities between MXE and PCP, MXE is also a high affinity ligand for the PCP-site on the glutamate *N*-methyl-D-aspartate (NMDA) receptor with an affinity value ($pK_i = 6.59$ nM) comparable to that of PCP itself ($pK_i = 7.23$ nM (Roth *et al.*, 2013). Moreover, MXE is a high affinity reuptake inhibitor at monoamine transporters (especially the serotonin transporter [SERT]) as measured *in vitro* (Hondebrink *et al.*, 2017), stimulates the firing rate of dopamine neurons in the ventral tegmental area and increases extracellular dopamine levels in the nucleus accumbens of awake, freely-moving Sprague-Dawley rats (Mutti *et al.*, 2016), and increases extracellular dopamine and serotonin concentrations in the medial prefrontal cortex of awake, freely-moving ddY mice (Fuchigami *et al.*, 2015). Similar to MXE, PCP increases extracellular concentrations of dopamine, serotonin, and norepinephrine in the medial prefrontal cortex of Sprague-Dawley and Wistar rats (Quarta & Large, 2011; Etou *et al.*, 1998)

In addition to recent characterizations of MXE’s neuropharmacological effects, previous studies have reported that MXE produces abuse-related behavioral effects that are similar to those of ketamine. For example, rats initially trained to self-administer ketamine also self-administered MXE during substitution trials, although the reinforcing effects of MXE were less robust than those of ketamine in both studies (Botanas *et al.*, 2015; Mutti *et al.*, 2016). However, MXE produced a conditioned place preference comparable to that observed with ketamine (Botanas *et al.*, 2015), and substituted for 7.5 mg/kg ketamine in a drug discrimination procedure (Chiamulera *et al.*, 2016). Nevertheless, there is currently only a single study that has directly compared the *in vivo* effects of MXE to PCP (Halberstadt *et al.*, 2016). Thus, several rodent procedures were used in the present study in an effort to further characterize the behavioral and thermoregulatory effects of MXE and related arylcyclohexylamines. A drug discrimination procedure was used to characterize the interoceptive effects of MXE, PCE, and TCP in rats trained to discriminate PCP from saline. A rodent model of drug withdrawal was used to evaluate whether MXE would attenuate

PCP-withdrawal-related reductions in an operant responding. The reinforcing effects of MXE were also investigated in rats using intravenous self-administration procedures. Finally, the locomotor and thermoregulatory effects of MXE, PCE, and PCP were compared in mice. Together, these experiments indicate that MXE has some PCP-like *in vivo* effects, which may support its abuse liability observed in human users.

2. Materials and Methods

2.1 Drugs

All drugs were dissolved in 0.9% physiological saline. For mice, injections were administered intraperitoneally (IP) in a volume of 0.01 ml/g. For rats in drug discrimination and PCP withdrawal studies, injections were administered IP in a volume of 1.0 ml/kg. For rats in intravenous self-administration experiments, drug solutions were delivered intravenously (IV) by a single-speed infusion pump at a flow rate of 0.055 ml/s for a duration of 2 seconds through 3 French (0.036 inch) heparin-coated polyurethane round tip catheters. PCP, TCP, and PCE were obtained through the NIDA Drug Supply Program as hydrochloride salts. Because only limited quantities of TCP and PCE were available, the *in vivo* effects of these compounds were tested in mice only. Purified MXE HCl (molecular weight = 283.79) was authenticated via single proton nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy and gas chromatography–mass spectrometry, tested to be a $99.26 \pm 0.20\%$ pure via quantitative NMR, and provided as a generous gift by the Drug Enforcement Administration Special Testing & Research Laboratory Reference Materials Program (Dulles, VA). All other laboratory reagents and supplies were obtained from standard commercial sources.

2.2 Animals

Fourteen male Sprague-Dawley rats (Harlan Sprague-Dawley) weighing 220–240g on delivery were housed two animals per cage ($23.75 \times 45.40 \times 17.78 \text{ cm}^3$), and fourteen male NIH Swiss mice (Harlan Sprague-Dawley) weighing 20–25g on delivery were housed three animals per cage ($15.24 \times 25.40 \times 12.70 \text{ cm}$). All animals were housed in a temperature- and humidity-controlled vivarium in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility. Room conditions were maintained at $22 \pm 2^\circ\text{C}$ and 45–50% humidity, with lights set to a 12 h/12 h light/dark cycle. During experimental testing, rats were maintained at 340–360g by food presentations earned during the experiments (45mg chocolate flavor Dustless Precision Pellets no. F0299, Bio-Serv, Frenchtown, NJ, USA) and supplemental feeding following experimental sessions (Lab Diet rodent chow Laboratory Rodent Diet no. 5001, PMI Feeds, St Louis, MO, USA). Mice were free-fed throughout testing (Lab Diet rodent chow Laboratory Rodent Diet no. 5001, PMI Feeds, St Louis, MO, USA). For all animal subjects, water was available *ad libitum* in the home cages throughout testing, and all subjects were drug naïve (with the exception of surgical anesthetics, where applicable) before testing. Aside from the 24 hour radiotelemetry and locomotor testing sessions (see below), all experiments were conducted during the animals' light cycle. All studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals (2013). All experimental protocols were approved by the

Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

2.3 Drug discrimination

Rats ($n = 5$) were trained in two-lever operant chambers (Model ENV-008-VP; Med Associates) that were enclosed in light- and sound-attenuating cabinets (Model G7211, Gerbrands) equipped with an exhaust fan for air circulation and masking of ambient laboratory noise. The operant chambers were equipped with stimulus lights over the retractable levers and a house light located near the chamber ceiling. A pellet dispenser delivered 45mg chocolate flavor pellets (Dustless Precision Pellets no. F0299, Bio-Serv, Frenchtown, NJ) into a food magazine centered between the levers. All programming and recording were controlled through a MED Associates interface with MED-PC software (V4, Fairfax VT, USA) by a computer located in an adjacent room.

Lever press training—Responding on extended levers was initially reinforced under a fixed ratio 1 (FR1) schedule in daily sessions that lasted for 60 minutes or until 60 reinforcers were earned, whichever occurred first. During lever press training sessions, the house light and stimulus lights were illuminated to signal reinforcer availability. Every 20th reinforcer earned incremented the response requirement by 2, and the final FR value carried across sessions until the rats displayed stable responding under a terminal FR20 schedule. To attenuate lever bias, both levers would extend into the chamber at the start of each session, but completion of the response requirement on one lever would deliver a reinforcer and retract that lever until the animal was reinforced for completing the next response requirement on the remaining lever. After a brief timeout (10 sec), both levers would extend into the chamber. In this manner, each subject entered the discrimination training phase with a history of receiving equivalent reinforcement on each of the two response levers.

Discrimination training—Once shaped to the terminal FR20 schedule, rats were trained to discriminate 3.0 mg/kg PCP from saline. The 3.0 mg/kg PCP training dose was selected given that this dose is within the range commonly used in rat drug discrimination experiments (*e.g.*, Wiley, 1995; West *et al.*, 2000; Winter *et al.*, 2004;) Prior to the start of each discrimination training session, rats received an intraperitoneal (IP) injection of either saline or the training dose of PCP. Daily sessions began with a 10 min timeout period, with lights off and levers retracted, followed by a session lasting up to 60 min, with a maximum of 60 food pellets available under an FR20 schedule. During discrimination training sessions, both levers extended and their associated stimulus lights were illuminated. When saline was administered, completion of the response requirement on one lever (saline-appropriate lever) resulted in food delivery. When the training dose of PCP was administered, completion of the response requirement on the other lever (PCP-appropriate lever) resulted in food delivery. The positions of the saline- and PCP-appropriate levers were counterbalanced across rats. Responding on the injection-inappropriate lever reset the FR on the injection-appropriate lever, and had no other programmed consequences. Discrimination training sessions were conducted 5–7 days per week and continued until the following three criteria were met for three consecutive sessions: 1) percentage of injection appropriate responding before delivery of the first reinforcer was >85%; 2) percentage of injection-

appropriate responding for the entire session was >90%; and 3) all available food pellets were earned during saline training sessions. If a rat did not pass all three of the previously stated criteria, then the same training injection (saline or 3.0 mg/kg PCP) was repeated for the next training session, with the constraint that rats did not receive saline or training drug injections for more than three days in a row. Saline or training drug sessions were randomly ordered (for example, where S = saline injection and D = training drug, five consecutive training sessions could be SSSDS, SDDDS, DDDSD, or DSDSD).

Substitution testing—After five consecutive training days wherein discriminative performance met the training criteria, rats were tested in extinction, and then returned to the regular training schedule on the following day. Approximately half of the test sessions were conducted the day after saline training sessions with the remainder following drug training sessions. Test sessions were identical to training sessions except that doses of test compounds were administered using a multiple-component cumulative-dosing procedure and no responses were reinforced. Each component lasted up to 10 min and 20 consecutive responses on either lever ended the session (if 10 min had not elapsed.) Rats were removed from the operant chamber, injected with the next cumulative dose, and returned to the chamber. Levers extended into the chamber 10 min later. The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted on both levers by the elapsed time prior to 20 total responses on either lever. Table 1 presents the injected and cumulative doses of the test compounds used for substitution testing.

2.4 Continuous PCP administration and spontaneous withdrawal

The PCP-induced withdrawal model used in the present study is similar in approach to that used by Wessinger and Owens (1991). After completing the drug discrimination experiments described above, these same rats ($n = 5$) and a separate group of experimentally-naïve rats ($n = 5$) responded under an FR20 schedule of food reinforcement. Following an approximately 3-week “wash out” period wherein animals received saline injections, but no drug was administered, rats were anesthetized with isoflurane anesthesia and subcutaneously implanted with osmotic mini-pumps (Alzet Osmotic Pumps model 2ML2, 5.1 cm length, 1.4 cm diameter, reservoir volume of 2 ml; Durect Corporation, Cupertino, CA.) Pumps delivered 3.0 mg/kg PCP per day for 11 consecutive days. During this 11-day period of continuous PCP infusion, rats responded in daily sessions under the FR20 schedule of food reinforcement. Changes in response rates (as compared to the 10 days prior to PCP infusions) were measured to determine PCP-elicited disruption of ongoing operant behavior, and return to baseline rates was interpreted as tolerance to PCP-induced rate suppression. Immediately after the 11th session, rats were again anesthetized with inhaled isoflurane and pumps were removed. On the following day, a final operant session was conducted wherein rats received multiple injections (IP) of saline or cumulative doses of 0.03, 0.30, and 3.0 mg/kg MXE (corresponding to injected doses of 0.03, 0.27, 2.7 mg/kg MXE) 10 min prior to four discrete 10 min sessions under the FR20 schedule. Reductions in response rate following saline injections (as compared to the last 3 days of PCP infusion) were interpreted

as PCP withdrawal and the capacity of MXE to normalize response rates was interpreted as alleviation of PCP withdrawal.

2.5 Intravenous self-administration

Rats ($n = 4$) were trained to lever press for 5s access to a palatable liquid reinforcer (evaporated milk diluted 1:1 with water) during daily 1 hour sessions. All training and testing sessions were conducted in custom-built, operant conditioning chambers (base chamber models are Med Associates, Inc., Fairfax, VT) equipped with response levers, auditory and visual feedback stimuli, a food pellet dispenser, and an infusion pump. Sessions began at approximately 0700 hours. At the start of each session, the house light was illuminated, a retractable lever extended into the chamber, and lever pressing behavior was reinforced on an FR1 schedule of milk reinforcement. Every 20th reinforcer earned increased the ratio requirement by two responses until rats reached a terminal FR10 schedule of reinforcement. All sessions terminated automatically once 60 reinforcers were delivered or 1 hour had elapsed, whichever occurred first. After 5 consecutive sessions in which all 60 milk reinforcers were earned, rats were outfitted with a silastic harness and allowed to habituate 5–7 sessions prior to self-administration training. Heparin coated polyurethane round tip catheters were then implanted in external jugular veins under inhaled isoflurane anesthesia as previously described (Norwood *et al.*, 2014). Briefly, the scruff of the neck and mid-scapular region of each rat was shaved and sanitized. A small incision was made along the lateral aspect of the neck, and a blunt dissection with sterile forceps was performed to expose the external jugular vein. After isolating the vessel, the distal end of the vein was tied off with silk suture, and a small cut opened the vein. A pre-measured, heparin coated polyurethane round tip catheter was then introduced into the vessel and passed to the approximate level of the atrium. The catheter was secured to the vein with silk suture, and then passed subcutaneously using a sterile trocar. The catheter exited in the midscapular region, and terminated at an injection port attached to the rat with a silastic harness. Following surgery, all catheterized rats were individually housed in Plexiglas cages for the duration of these experiments. Surgeries were performed 7 days before initiation of experiments, allowing time for incisions to heal and for rats to recover normal body weights.

Immediately following post-surgical recovery, rats resumed responding for milk reinforcers until stable responding occurred (*i.e.*, 3 consecutive days of 55 milk reinforcers earned during a training session). All rats met this criterion within 4 training sessions. Following these training sessions, rats self-administered various unit doses of PCP (0.03–0.3 mg/kg/inf), MXE (0.01–0.1 mg/kg/inf), or saline under an FR 1 schedule of reinforcement in 60 min sessions. Self-administration of these unit doses was determined in triplicate in random order across test drugs. Sessions maintained by milk reinforcement or saline occurred intermittently. Substitution trials occurred daily and a single substitution dose was tested in each trial, in three separate sessions per dose. Following self-administration sessions, catheters were infused with 0.1 ml of a sterile heparin sodium (2 USP units/ml) and gentamicin (10 mg/ml). If pressure was noted, catheter patency was further tested with 0.5–0.1 ml mixture of ketamine-midazolam (15 + 0.75 mg/ml), although this did not occur during the studies described herein.

2.6 Radiotelemetry of thermoregulation and locomotor activity

Three groups of mice ($n = 4\text{--}5$ per group) were used in these studies, and glass-encapsulated radiotelemetry probes were implanted IP under inhaled isoflurane anesthesia as previously described (Fantegrossi *et al.*, 2013). Briefly, the abdominal area of each mouse was shaved and sanitized with iodine swabs. A rostral-caudal cut approximately 1.5 cm in length was made with sterile skin scissors, providing access to the intraperitoneal cavity. A cylindrical glass-encapsulated radiotelemetry probe (model ER-4000 E-Mitter; Mini Mitter, Bend, OR) was then inserted, and the incision was closed using absorbable 5–0 chromic gut suture material. Surgeries were carried out at least 7 days before initiation of experimental conditions, allowing time for incisions to heal and for mice to recover normal body weights. Following surgery, all implanted mice were individually housed in $15.24 \times 25.40 \times 12.70$ cm³ cages for the duration of all telemetry experiments.

Implanted transmitters produced activity- and temperature-modulated signals that were sent to a receiver (model ER-4000 Receiver; Mini Mitter) underneath each cage. These receivers were situated inside standard light- and sound-attenuating chambers (Model ENV-022M; Med Associates) to minimize environmental variability during tests. Every 5 min, the computer collected two data updates from the probes – core temperature (in °C) on one channel and locomotor counts on the other. Each chamber was equipped with a house light (to establish a photoperiod) and an exhaust fan. After at least 60 min of baseline data collection, mice were removed from the chambers, injected IP with saline, returned to the home cage, and then returned to the chambers for 24 hours of data collection. Only data collected 8 hours post-injection were included for statistical analysis because that marked the end of the animals' light cycle in the testing chambers. In the same manner, mice were assigned to treatment groups in which they received injections of PCP (10–100 mg/kg), PCE (3–30 mg/kg), or MXE (10–56 mg/kg), in an ascending dose order across testing days, and each group of mice was injected once per week.

2.7 Statistical analysis

Drug discrimination—Results of stimulus substitution tests were expressed as the mean ($\pm SE$) percent PCP-lever selection. Estimates of median effective dose (ED₅₀) values were determined for test compounds that produced >50% PCP-lever selection in individual rats that completed the FR requirement. To estimate ED₅₀ values for each rat, a non-linear regression analysis was performed that included no more than a single dose producing <50% PCP-lever selection and no more than a single dose producing >50% PCP-lever selection (Hill Slope constrained to 1.0 and normalized range set a 0–100%). Response rate during stimulus substitution tests are plotted as the mean ($\pm SE$) number of responses per second. Dunnett's multiple comparisons tests were performed to compare the lowest dose of a test drug to the successively higher doses tested within the multi-component cumulative-dosing test session.

Self-administration—Results of stimulus substitution tests are expressed as the mean ($\pm SE$) number of infusions per session. Doses of test compounds that produced the maximal number of injections per session were analyzed using a one-way repeated-measures analysis

of variance (RMANOVA) (sphericity assumed) followed by Holm-Sidak multiple comparisons tests to compare test drugs and saline.

PCP withdrawal procedure—Response rate data following repeated saline injections or PCP infusions are plotted for visual analysis as the mean ($\pm SE$) responses per second recorded during a testing session. Response rate data following injections of saline or cumulative doses of MXE are plotted for visual analysis as mean ($\pm SE$) responses per second percent of control during a testing session. Percent of control was computed as the average of the response rate data observed during the final three sessions of PCP infusion. Dose-response curves (or injection number-response curves for saline-treated rats) comprising the responses per second as percent of control measure observed during the cumulative dosing phase were analyzed using a simple linear regression procedure. The estimated slope values determined for each regression line were compared using a comparison of fits (extra sum-of-squares) *F*-test.

Thermoregulation—Thermoregulatory effects of MXE, PCP, and PCE were plotted as means ($\pm SE$) over the 8 hour testing session (data collected beyond 8 hours post-injection were not plotted). The maximal hypothermic effects produced by doses of each drug were plotted as dose-response curves using the means ($\pm SE$) and the doses of each drug that produced the greatest hypothermic effect were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests.

Locomotor activity—Total horizontal counts of MXE, PCP, and PCE were plotted as means ($\pm SE$) over the 8 hour testing session. Dose-response curves were truncated when lethal effects were observed. One-way RMANOVAs were performed to compare doses within a treatment group followed by Dunnett's multiple comparisons tests to compare drug doses to saline control.

Statistical significance was declared at $p < .05_{2\text{-tail}}$ for all statistical analyses. All statistical analyses and creation of figures were performed using GraphPad Prism software (V7.03, La Jolla, CA, USA).

3. Results

3.1 MXE, TCP and PCE produce PCP-like interoceptive effects in subsets of sample

Figure 2 displays the results of drug substitution tests with PCP, MXE, TCP, PCE, and DPT. The percentage of PCP-lever responses for test compounds is displayed in the upper graphs and response rate is displayed in the lower graphs. Rats learned to reliably discriminate the 3.0 mg/kg PCP training dose from saline (Fig. 2, left black circle and white square, respectively). All substitution test compounds engendered $>50\%$ PCP-lever selection (see Table 2 for average peak effects of the test compounds and each compound's ED_{50} value only in the few subjects that completed the FR requirement during testing sessions.) Based only on the performances of rats that did not display complete behavioral disruption, the relative potency for the three arylcyclohexylamines to engender PCP-lever selection was $PCE > MXE > TCP$. DPT produced moderate levels of PCP-lever selection up to a dose that suppressed response rates.

3.2 Discontinuation of chronic PCP infusion elicits withdrawal-like reductions in operant responding that are dose-dependently reversed by cumulative MXE administration

Figure 3A displays the results of continuous PCP administration followed by spontaneous withdrawal in rats trained to press a lever for food reinforcement. Compared to the final three sessions of saline, the first session following implantation of PCP pumps was characterized by a reduction in response rates, and rates steadily recovered to pre-PCP levels by the 3rd session following pump implantation. Similarly, the first session following removal of the osmotic pumps (Fig. 3B) was characterized by a reduction in response rates, as compared to the final three sessions of PCP infusion. Cumulative administration of MXE reversed this withdrawal-related reduction in response rate as evidenced by a statistically significant difference in slope (MXE estimated slope value = 9.17; saline estimated slope = -0.10) (*i.e.*, the increase in responses per second percent of control associated with a one unit increase in dose of drug or injection number) between MXE- and saline-treated rats [$F(2, 5) = 20.92, p = .004$].

3.3 MXE functions as reinforcer in rats trained to self-administer PCP

Figure 4 displays the results of drug substitution tests with PCP, MXE, and saline. Substitution tests with saline engendered relatively few infusions per session, whereas 0.1 mg/kg/inj PCP and 0.03 mg/kg/inj MXE produced an eightfold and fourfold increase in the number of infusions per session, respectively. A RMANOVA revealed a statistically significant difference in peak effects between substitution compounds [$F(2, 6) = 16.97, p = .003$], with PCP maintaining more self-administration behavior than MXE. Results of Holm-Sidak tests are displayed in Figure 4. Although the maximal reinforcing effect of PCP was greater than that of MXE, the maximally effective unit dose of MXE was lower than the maximally effective unit dose of PCP.

3.4 PCP, MXE, and PCE elicit hypothermic effects in mice

Figure 5A–C displays the thermoregulatory effects of PCP (Fig. 5A), MXE (Fig. 5B), and PCE (Fig. 5C), measured over 8 hr. PCP produced a biphasic thermoregulatory effect evidenced by transient hyperthermia following 30 mg/kg PCP injection, but sustained hypothermia at 100 mg/kg PCP. In addition, 100 mg/kg PCP produced lethality in 2 of 4 subjects. In contrast, MXE and PCE produced only hypothermic effects, and 56 mg/kg MXE produced lethality in 1 of 5 subjects and 30 mg/kg PCE in 2 of 5 subjects. An ANOVA revealed a statistically significant difference in maximal hypothermic effects between PCP, MXE, and PCE [$F(2, 7) = 5.47, p = .037$]. Results of Tukey's multiple comparisons tests are presented in Figure 5D.

3.5 PCP, but not MXE or PCE, elicits locomotor stimulant effects in mice

Figure 6 displays the total horizontal counts of non-lethal doses of PCP, MXE, and PCE measured over the 8 hour testing session. A statistically significant effect of dose was only observed in mice injected with PCP [$F(4, 16) = 12.59, p < .0001$]. There were no differences in onset of action between drugs and no noteworthy differences in locomotor activity following non-lethal doses of drugs as a function of session time (data not shown).

4. Discussion

This is the first study to demonstrate that MXE produces interoceptive, hypothermic, and reinforcing effects that are similar to those of PCP, and that MXE can alleviate withdrawal-related reductions in operant responding induced by PCP. Although MXE tended to produce qualitatively similar effects to those of PCP, quantitative differences were observed with PCP typically eliciting more robust effects than MXE. Chiamulera *et al.* (2016) observed that 1 mg/kg MXE fully substituted (*i.e.*, >80% generalization) for 7.5 mg/kg ketamine in rats. In the present study, 3 mg/kg MXE substituted for 3 mg/kg PCP in only 2 of 5 rats and also produced a reduction in response rate, indicating that the present results should be interpreted with caution. Perhaps interestingly, when 1 mg/kg MXE substituted for 7.5 mg/kg ketamine, it did not reduce response rate (Chiamulera *et al.*, 2016). In that study, reinforced single-dose test sessions were used as opposed to cumulative-dosing test sessions under extinction, as used in the present study. In cumulative-dosing testing sessions wherein subjects respond under extinction, rate-decreasing effects of drugs may be obscured by reductions in response rate that result from multiple-component test sessions, as evidenced by vehicle injections across successive components (*e.g.*, Fantegrossi *et al.*, 2013). As such, several rats in the present study may have displayed rate suppression as a result of a complex interaction of pharmacological and procedural variables. Nevertheless, the few subjects that completed the FR requirement following ascending doses of MXE displayed progressive increases in percent PCP-lever selection, indicating that the interoceptive effects of MXE may share similarities with those of PCP. Future drug discrimination experiments are necessary to support this possibility. The partial substitution of DPT for PCP may be related to the common mechanism of increased synaptic serotonin previously noted for these compounds (Quarta & Large, 2011; Etou *et al.*, 1998; Nagai *et al.*, 2007).

In rats receiving chronic PCP via osmotic minipumps, abrupt discontinuation of PCP has been shown to lead to prolonged suppression of response rates in food-controlled responding before returning to higher steady rates (Wessinger, 1987; Beardsley & Balster, 1987; Wessinger & Owens, 1991). In those studies, response rates recovered over several consecutive days making it unlikely that the presently-observed withdrawal-induced suppression of response rates was ameliorated by the passage of time (approximately 1 hour) as opposed to cumulative MXE administration. Moreover, cumulative saline injections did not alleviate PCP-withdrawal-induced suppression of response rates in the present study. These findings indicate that abrupt cessation of exposure to an arylcyclohexylamine may produce symptoms of withdrawal that can be alleviated following administration of other pharmacologically-related compounds. This may have important implications for continued drug-seeking behavior among individuals who frequently use arylcyclohexylamines.

It is well-established that PCP is readily self-administered by various species under a variety of testing conditions (*e.g.*, Balster & Woolverton, 1980; Marquis *et al.*, 1989; Carlezon & Wise, 1996). In the present study, rats self-administered unit doses of PCP and MXE. Consistent with findings reported in previous studies in which subjects were trained to self-administer ketamine (Bontanas *et al.*, 2015; Mutti *et al.*, 2016), MXE maintained operant responding above the levels observed when contingent saline was available. Although MXE more potently produced its own maximal number of infusions than PCP, PCP was a more

effective reinforcer overall. This finding is also consistent with previous studies (Bontanas *et al.*, 2015; Mutti *et al.*, 2016) which reported that ketamine produced greater maximal infusion levels compared to MXE.

In the present study, PCP produced a dose-dependent biphasic effect on core body temperature. This is partially consistent with a previous study by Hiramatsu *et al.* (1986) who reported that 5 and 10 mg/kg PCP produced an increase in rectal temperature in mice, but 40 mg/kg produced a sustained hypothermic effect. In that study, male ddY mice were used and rectal temperatures were recorded, while the present studies used male NIH Swiss-Webster mice and recorded core body temperature via telemetry probes. Nevertheless, this is the first report to demonstrate that, similar to PCP and the related arylcyclohexylamine PCE, MXE dose-dependently produces sustained decreases in core body temperature that descend far below the normal physiological range. In the same mice exhibiting these hypothermic effects, MXE and PCE failed to elicit increases in unconditioned motor activity. These results are surprising given that MXE produces increases in dopamine neuron firing and extracellular dopamine concentrations *in vivo* (Mutti *et al.*, 2016), and given previous reports that doses of 5–10 mg/kg MXE increase locomotor activity in Sprague-Dawley rats (Halberstadt *et al.*, 2016; Horsley *et al.*, 2016.) However, the present findings are perhaps consistent with an earlier study in which doses of 1.25, 2.5, and 5 mg/kg MXE failed to increase locomotor activity in rats undergoing place preference conditioning (Bontanas *et al.*, 2015). Nevertheless, in the present study, MXE doses up to those inducing some lethality were assessed and failed to produce significant locomotor activity in mice, perhaps indicating a species difference in this regard.

5. Conclusions

The studies presented herein demonstrate that the novel arylcyclohexylamine MXE produces behavioral and physiological effects that are similar to those of the prototypical dissociative drug of abuse PCP. MXE may produce PCP-like discriminative stimulus effects, functions as a positive reinforcer, and substitutes for PCP in an assay of withdrawal alleviation. Differences in locomotor and thermoregulatory effects between MXE and PCP may be due to a species difference, or, given the lethality observed in the present study, may simply be masked by general MXE-induced toxicity. As such, MXE may possess relatively high abuse potential among individuals who use arylcyclohexylamines for non-medical purposes, and is likely to remain a drug of concern until its pharmacology is more fully understood.

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Abbreviations

ANOVA	analysis of variance
DPT	dipropyltryptamine

MXE	methoxetamine; 2-(3-methoxyphenyl)-2-(ethylamino)-cyclohexanone
NMDA	<i>N</i> -methyl-D-aspartate
PCE	eticyclidine; <i>N</i> -ethyl-1-phenylcyclohexylamine)
PCP	phencyclidine
RMANOVA	repeated-measures analysis of variance
TCP	tenocyclidine; 1-(1-(2-thienyl)cyclohexyl)piperidine)

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Highlights

- The arylcyclohexylamines MXE, PCE, and TCP produce interoceptive effects that are similar to PCP.
- MXE substitutes for and supports self-administration in rats trained to self-administer PCP.
- MXE reverses spontaneous withdrawal following discontinuation of PCP administration.
- MXE, PCE, and PCP produce hypothermic effects in mice.
- PCP, but not MXE and PCE, elicits locomotor stimulant effects in mice.

Phencyclidine (PCP)

Tenocyclidine (TCP)

Eticyclidine (PCE)

Methoxetamine (MXE)

Figure 1.
Chemical structures of the drugs used in these studies: PCP, TCP, PCE, and MXE.

Figure 2.

Results of stimulus substitution tests with doses of PCP (●), MXE (△), PCE (▲), TCE (▽), and DPT (◆) in rats ($n = 5$) trained to discriminate 3 mg/kg PCP from saline. S = saline control and TD = training dose refer to the percent PCP-lever selection that was observed during training conditions. Mean ($\pm SE$) percent PCP-lever selection (upper graphs) and mean ($\pm SE$) responses per second (lower graphs). Rats that did not complete FR during a test session were not included in the percent PCP-lever selection data (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 5). * $p < .05$ compared to lowest dose within test drug.

Figure 3.

Effects of chronic PCP infusions on response rate in rats who would subsequently tested with repeated saline injections (○; $n = 5$) or cumulative MXE injections (●; $n = 5$) are displayed in panel A. The mean responses per second ($\pm SE$) is presented along the ordinate and consecutive sessions along the abscissa. Horizontal lines above the final three sessions of saline or PCP exposure depicts stable response rate among rats, and the final three points in the PCP data set were used as percent control for the cumulative MXE administration session. Saline or MXE after a hyphen indicates the treatment those rats received during the withdrawal/PCP pump removal phase. Panel B displays the results of cumulative MXE administration (▲) or saline (△) on responses per second in rats that received abrupt removal of PCP; S = saline injection; S1-S4 indicates the injection number of saline within a cumulative dosing series.

Figure 4.

Results of drug substitution tests with PCP (●), MXE (△), and saline (□) in rats ($n = 4$ per group). The mean number of infusions per session (determined in triplicate) ($\pm SE$) is presented along the ordinate and dose of drug (mg/kg/inf) is presented along the abscissa; S = saline infusion. * $p < .05$ compared to saline, # $p < .05$ compared to 0.03 mg/kg MXE.

Figure 5.

Results of thermoregulation experiments in which groups of mice received ascending doses of PCP (panel A), MXE (panel B), or PCE (panel C). Doses for each drug are indicated by symbols above each panel. Core temperature is depicted as means ($\pm$ SE) in degrees Celsius along the ordinate and post-injection time in minutes is depicted along the abscissa. Panel D presents the dose-response curves depicting the maximal hypothermic effect (in degrees Celsius) observed in mice treated with PCP (●; $n = 4$), MXE (△; $n = 5$), or PCE (▲; $n = 4$); S = saline. The number of mice is indicated in parenthesis, otherwise the number of mice was equal to 5). * $p < .05$ compared to 100 mg/kg PCP.

Figure 6.

Results of locomotor activity measures observed in mice injected with PCP (●; $n = 4$), MXE (△; $n = 5$), or PCE (▲; $n = 4$); S = saline control. Total horizontal counts observed over the 8 hour testing session are expressed as means ($\pm SE$) along the ordinate. Doses of test drugs are expressed along the abscissa. Only presented are doses of test compounds that did not produce lethality within a treatment group. * $p < .05$ compared to horizontal counts following a saline injection.

Table 1

Injected and cumulative doses of test compounds used in drug discrimination experiment

Test Compound	Injected (Bolus) Doses (mg/kg)	Cumulative Doses (mg/kg)
PCP	0.1, 0.2, 0.7, 2	0.1, 0.3, 1, 3
MXE	0.03, 0.07, 0.2, 0.7, 2	0.03, 0.1, 0.3, 1, 3
PCE	0.03, 0.07, 0.2, 0.7, 2	0.03, 0.1, 0.3, 1, 3
TCP	0.1, 0.2, 0.7, 2	0.1, 0.3, 1, 3
DPT	0.3, 0.7, 2, 7	0.3, 1, 3, 10

Table 2

Results of stimulus substitution tests in rats trained to discriminate 3.0 mg/kg PCP

Test Compound	Mean maximal effect ($\pm SE$) <i>n</i>	ED ₅₀ (mg/kg) ($\pm SE$)
PCP	74.5 (10.71) 5	1.25 (0.90)
MXE	100 (0) 2	0.25 (0.10)
PCE	80 (20) 4	0.17 (0.06)
TCP	73.92 (23.84) 3	0.63 (0.38)
DPT	59.34 (18.18) 4	0.71 (0.17)