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The novel ketamine analog methoxetamine produces dissociative-like behavioral effects in rodents

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Abstract

Methoxetamine (MXE) is a ketamine analogue sold online that has been subject to widespread abuse for its dissociative and hallucinogenic effects. Previous studies have shown that MXE has high affinity for the phencyclidine (PCP) binding site located within the channel pore of the NMDA receptor (NMDAR), but little is known about its behavioral effects. Dissociative anesthetics such as ketamine and PCP produce a characteristic behavioral profile in rats that includes locomotor hyperactivity and disruption of prepulse inhibition of acoustic startle (PPI). The goal of the present investigation was to determine whether MXE produces PCP-like effects in Sprague-Dawley rats using the PPI paradigm and the Behavioral Pattern Monitor (BPM), which enables analyses of patterns of locomotor activity and investigatory behavior. PPI studies were conducted with several other uncompetitive NMDAR antagonists that produce dissociative effects in humans, including PCP, the *S*-(+)- and *R*-(-)- isomers of ketamine, and *N*-allylnormetazocine (NANM; SKF-10,047). MXE disrupted PPI when administered at 3 and 10 mg/kg SC. The rank order of potency of MXE and the other test compounds in the PPI paradigm (PCP > MXE > *S*-(+)-ketamine > NANM > *R*-(-)-ketamine) parallels their affinities for the PCP binding site reported in the literature. When tested in the BPM, 10 mg/kg MXE induced locomotor hyperactivity, reduced the number of rearings, increased the roughness of locomotor paths, and produced perseverative patterns of locomotion. Administration of PCP (2.25 and 6.75 mg/kg, SC) produced a similar profile of effects in the BPM. These results indicate that MXE produces a behavioral profile similar to that of other psychotomimetic uncompetitive NMDAR antagonists. Our findings support the classification of MXE as a dissociative drug and suggest that it likely has effects and abuse potential similar to that of PCP and ketamine.

The arylcyclohexylamines phencyclidine (PCP) and ketamine belong to a commonly abused class of substances that act by blocking the NMDA receptor (NMDAR). These

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arylcylohexylamines interact with a specific PCP binding site located within the channel pore of the NMDAR and act as uncompetitive antagonists. PCP and ketamine produce dissociation and hallucinations at subanesthetic doses (Luby et al 1959; Meyer et al 1959; Vollenweider et al., 1997), effects thought to mimic acute schizophrenic symptomatology (Javitt and Zukin, 1991; Halberstadt, 1995; Javitt et al., 2012). Use of ketamine has increased significantly internationally in recent years (Li et al., 2011). Ketamine acts as a rapidly acting antidepressant agent (Coyle and Laws, 2015), and some individuals may be using ketamine to relieve the symptoms of depression.

Adding to the complexity of this existing problem is the availability of several new “research chemicals” or “legal highs” that are designed to mimic the dissociative effects of PCP and ketamine. One example is methoxetamine (2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone), also known as MXE, an analog of ketamine that has been subject to widespread abuse in North America and Europe since its emergence in 2010 (Corazza et al., 2012; Morris and Wallach, 2014). Like ketamine, MXE binds to the PCP site in the NMDAR (Roth et al., 2013). MXE displaces [³H]MK-801 from the PCP binding site with higher affinity ($K_i = 259$ nM) than ketamine ($K_i = 659$ nM), but lower affinity than PCP ($K_i = 59$ nM). According to Botanás and colleagues, MXE is self-administered by rats and induces a conditioned place preference, suggesting it has reinforcing effects (Botanás et al., 2015). Interestingly, those investigators also reported that MXE does not alter locomotor activity when administered to Sprague-Dawley rats at doses up to 5 mg/kg. The lack of an effect of MXE is surprising because locomotor hyperactivity is a prominent component of the behavioral syndrome induced by NMDAR antagonists in rodents (Sturgeon et al., 1979; Mattia et al., 1986; Lehmann-Masten and Geyer, 1991; Danysz et al., 1994; Imre et al., 2006; Adams et al., 2013). The locomotor stimulation produced by NMDAR antagonists is often used as a preclinical measure of their psychotomimetic effects. Furthermore, the ability of a drug to produce psychomotor stimulation is often viewed as a predictor of positive reinforcing effects (Wise and Bozarth, 1987; Wise, 1988). Therefore, the failure of MXE to increase locomotor activity suggests it may lack the characteristic effects and abuse potential of PCP and other uncompetitive NMDAR antagonists, but additional confirmation is needed.

Prepulse inhibition (PPI), another behavioral paradigm used to test NMDAR antagonists in rodents, has particular relevance to the sensory disturbances induced by dissociative drugs. PPI refers to the phenomenon where the startle response is attenuated if the startle-inducing stimulus is preceded by a weak non-startling prepulse, and serves as an operational measure of sensorimotor gating or filtering (Braff et al., 2001; Powell et al., 2012). Psychotomimetic uncompetitive NMDAR antagonists disrupt PPI in rats (reviewed: Geyer et al., 2001). The hallucinogenic effects of PCP are believed to result, at least in part, from loss of subcortical filtering mechanisms, resulting in cortical sensory overload (Vollenweider and Geyer, 2001). Therefore, the PPI disruption produced by PCP-like drugs may reflect the same information processing deficits that contribute to their hallucinogenic effects.

The present studies were conducted to determine whether MXE produces behavioral effects in rats that are similar to those of other NMDAR antagonists. Because PPI is a cross-species measure with a strong conceptual link to human phenomenology, we compared the effects of MXE and several other uncompetitive NMDAR antagonists on PPI. In addition to testing

PCP and the *R*(-)- and *S*(+)-isomers of ketamine, we also assessed whether PPI is disrupted by *N*-allylnormetazocine (NANM, SKF-10,047). NANM, an NMDAR antagonist from a discrete structural class (benzomorphan derivatives), has been shown in clinical trials to produce dissociative effects in humans (Keats and Telford, 1964). Finally, we tested whether MXE produces PCP-like effects in the Behavioral Pattern Monitor (BPM), which assesses both the amount and patterns of locomotor activity and investigatory behavior in rats (Geyer et al., 1986). PCP and other psychotomimetic NMDAR antagonists produce a unique profile of effects in the BPM (Lehmann-Masten and Geyer, 1991; Krebs-Thomson et al., 1998). These studies confirmed that MXE produces PCP-like behavioral effects, supporting its classification as a dissociative drug.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats from Harlan Industries (Indianapolis, IN, USA; $n=211$; initial weight 250–275 g) were housed in pairs under a 12-h reverse light/dark cycle (lights off at 0700 hours). The use of a reversed light/dark cycle enabled behavioral testing during the animals' awake phase. Food and water were available *ad libitum*. Animals were acclimatized for approximately 1 week after arrival prior to behavioral testing and maintained in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facilities that meet all federal and state guidelines. The age of the animals at the time of behavioral testing ranged from 2–5 months. Animals were sometimes used for multiple experiments but were only tested 1–2 times with NMDAR antagonists and testing was separated by at least two weeks to avoid carryover effects. Procedures were approved by the University of California San Diego institutional animal care and use committee. Principles of laboratory animal care were followed as well as specific laws of the USA.

Drugs

The following drugs were used: phencyclidine hydrochloride (PCP), ($\pm$)-*N*-allylnormetazocine hydrochloride (NANM), *S*(+)-ketamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA); *R*(-)-ketamine hydrochloride (Cayman Chemical, Ann Arbor, MI, USA); and methoxetamine hydrochloride (MXE) (Lipomed AG, Cambridge, MA, USA). Drug doses are expressed as the salt form. All drugs were dissolved in saline and administered subcutaneously. The injection volume was 1 ml/kg.

Apparatus

Startle—Eight startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA, USA) were used to measure startle reactivity in rats (Mansbach et al., 1989, 1991). The startle test chambers are sound-attenuated, lighted, and ventilated enclosures containing a clear nonrestrictive cylindrical Plexiglas stabilimeter, 8.2 cm in diameter. A high-frequency loudspeaker mounted 24 cm above the Plexiglas cylinder produces all acoustic stimuli. The peak and average amplitudes of the startle response are detected by a piezoelectric accelerometer. At the onset of the startling stimulus, 100 1-ms readings are recorded, and the average amplitude is used to determine the magnitude of the startle response (measured in

arbitrary units). A dynamic calibration system is used to ensure comparable stabilimeter sensitivity across test chambers, and sound levels are measured using the dB(A) scale.

Behavioral Pattern Monitor (BPM)—Locomotor activity and investigatory behavior was measured in the BPM, a 30.5 × 61.0 × 28.0 cm black Plexiglas chamber equipped with seven wall holes (three per side wall, one on the back wall) and three floor holes, each 2.5 cm in diameter (see: Geyer et al., 1986). Photocells in each hole detect investigatory nosepokes (holepokes). Rearings are detected when subjects make contact with a metal touchplate located 15.2 cm above the floor. A 4 × 8 grid of infrared photobeams is used to detect the animal's position in X–Y coordinates. Every 55 ms, a microprocessor system recorded the status of all beams and stored this information for subsequent off-line analysis.

Acoustic startle test sessions

Acoustic startle test sessions consisted of startle trials (pulse-alone) and prepulse trials (prepulse + pulse). The pulse-alone trial consisted of a 40-ms 120-dB pulse of broadband white noise. Prepulse + pulse trials consisted of a 20-ms acoustic prepulse, an 80-ms delay, and then a 40-ms 120-dB startle pulse (100 ms onset–onset). There was an average of 15 s (range = 6–22 s) between trials. During each inter-trial interval, the movements of the animals were recorded once to measure responding when no stimulus was present (data not shown). Each startle session began with a 5-min acclimation period to a 65-dB broadband noise that was present continuously throughout the session. One week after arrival, animals were tested in a brief baseline startle/PPI session to create treatment groups matched for levels of startle and PPI. The startle test session contained 12 pulse-alone trials and 36 prepulse + pulse trials (12 prepulses each of 68, 71, and 77 dB [equivalent to 3, 6, and 12 dB above background]) presented in a pseudo-randomized order. Six pulse-alone trials were presented at the beginning and the end of the test session but were not used in the calculation of PPI values.

Experimental Design

On the testing day, rats were brought to the testing room and allowed to sit for 60 min before receiving injections. Test injections were administered under red lights in the testing room. Animals were tested during the dark phase in darkness. Animals were placed in the startle chambers 15 min after treatment with NANM, 5 min after treatment with MXE or PCP, and immediately after treatment with ketamine. Animals were placed in the BPM 10 min after treatment with PCP, and 5 min after treatment with MXE. The BPM test sessions lasted for 60-min (for MXE) or 120-min (for PCP). The experimental parameters used for the studies with PCP and ketamine (doses, preinjection time, number of subjects per group) were chosen based on the results of our previous dose-response studies (Mansbach and Geyer, 1989, 1991; Lehmann-Masten and Geyer, 1991; Krebs-Thomson et al., 1998).

Analysis

PPI—The amount of PPI was calculated as a percentage score for each prepulse + pulse trial type: $\%PPI = 100 - \{[(\text{startle response for prepulse} + \text{pulse trial}) / (\text{startle response for pulse-alone trial})] \times 100\}$. Startle magnitude was calculated as the average response to all of the pulse-alone trials. PPI data were analyzed with two-factor analysis of variance (ANOVA)

with treatment as the between-subjects factor and trial type (prepulse intensity) as a repeated measure. For experiments in which there was no significant interaction between drug and prepulse intensity, PPI data were collapsed across prepulse intensity and average PPI was used as the main dependent measure. ED₅₀ values were calculated using nonlinear regression. Startle magnitude data were analyzed with one-factor ANOVA. Post-hoc analyses were performed using Tukey's studentized range method. The alpha level was set at 0.05. We examined the startle data for outliers (values >2 standard deviations from the mean) and removed 4 subjects from the experiment with *R*(-)-ketamine; removal of the subjects had negligible effects on PPI values.

BPM—The raw data were reduced to the X and Y coordinates of the rat in the chamber; further analyses produced specific measures of behavior (Geyer et al., 1986). Locomotor activity was quantified by the number of crossings between any of eight equal square sectors within the BPM. The number of holepokes and rearings were calculated. Analysis of the spatial structure of locomotor paths was performed by calculating a descriptive statistic, spatial *d*. The statistic *d* is based conceptually on fractal geometry and calculated using scaling arguments, as described in detail by Paulus and Geyer (1991). Changes in *d* reflect smoother (decreases in *d*) or rougher (increases in *d*) locomotor paths. Data were examined in 5- and 10-min time resolutions (locomotor activity) or 30-min time resolutions (spatial *d*, rearings, and holepokes). Data were analyzed using two-way ANOVA with treatment as between-factor and time as a repeated measure. Specific post hoc comparisons between selected groups were done using Tukey's studentized range method. The alpha level was set at 0.05.

RESULTS

Effect of MXE on exploratory and investigatory behavior

There was a main effect of MXE on crossings, a measure of locomotor activity ($F(2,21)=18.16$, $p<0.0001$), and an interaction between MXE treatment and time interval ($F(22,231)=4.72$, $p<0.0001$). As shown in Figure 1A, MXE altered locomotor activity in a dose- and interval-dependent manner: 1 mg/kg *reduced* activity during the first 15 min ($p<0.05$), whereas 10 mg/kg *increased* activity during the last 45 min of the 1-h test session ($p<0.01$). Administration of 10 mg/kg MXE increased spatial *d* (Main effect: $F(2,21)=12.77$, $p=0.0002$), reflecting a change toward less smooth locomotor paths (Fig. 1B). These changes in locomotor patterns are depicted in the representative plots in Figure 2. Examination of the plots shows that control animals and animals treated with 1 mg/kg MXE exhibited a preference for a "home" area, making periodic excursions to other parts of the chamber. By contrast, animals treated with 10 mg/kg MXE displayed abnormal, perseverative patterns of locomotion, often rotating their body in tight circles at either end of the BPM chamber. Similarly, dizocilpine (MK-801), a highly selective uncompetitive NMDA receptor antagonist, induces body axis circling in the BPM (Lehmann-Masten and Geyer, 1991). Administration of MXE reduced rearings during the first half-hour of the test session, resulting in a trend toward an interaction between drug and time ($F(2,21)=2.99$, $p<0.08$). The effect of MXE on holepokes was dependent on dose ($F(2,21)=4.92$, $p<0.02$) and time (Drug

$\times$ Time: $F(2,21)=7.03$, $p<0.005$). 10 mg/kg MXE had no effect on holepokes whereas 1 mg/kg reduced holepokes during the first half of the test session ($p<0.05$; Table 1).

Effects of phencyclidine (PCP) on exploratory and investigatory behavior

For comparative purposes, we conducted dose-response studies with PCP to assess its effects on exploratory behavior. Studies have shown that PCP produces a specific profile of effects in the BPM, including hyperactivity, reductions in investigatory behaviors, induction of perseverative locomotor patterns, and increased spatial d (at moderate and high doses) (Lehmann-Masten and Geyer, 1991; Krebs-Thompson et al., 1998). We are able to confirm those effects of PCP. The ability of PCP to alter crossings was dependent on dose (Drug effect: $F(4,51)=6.99$, $p=0.001$) and time (Drug Time: $F(44,561)=4.25$, $p<0.0001$). PCP altered locomotor activity with an inverted U-shaped dose-response function: animals treated with 2.25 mg/kg PCP were hyperactive for most of the 2-h test session, whereas animals treated with 6.75 mg/kg PCP exhibited a transient reduction in activity at the beginning of the experiment (see Fig. 3A). The effect of PCP on spatial d was only assessed during the first 60 min of the 2-h test session because in rats treated with 6.75 mg/kg PCP the level of activity at subsequent time points was often too low to calculate d . Administration of 2.25 and 6.75 mg/kg PCP significantly increased spatial d ($F(4,51)=31.32$, $p<0.0001$). 2.25 mg/kg PCP increased d from 1.37–0.01 (mean SEM) to 1.51–0.04 during the first 30-min time block ($p<0.05$; Fig. 3B) but had no effect during the second block, resulting in an interaction between dose and time ($F(4,51)=12.59$, $p<0.0001$). Figure 3C shows representative locomotor patterns in animals treated with vehicle or 2.25 mg/kg PCP. Animals treated with 2.25 mg/kg PCP displayed perseverative locomotor patterns, typically circling around in the periphery of the chamber. This observation is consistent with reports that PCP and the competitive NMDAR antagonists SDZ 220–581 and SDZ EAB-515 induce perseverative circling in rats (Lehmann-Masten and Geyer, 1991; Bakshi et al, 1999). As shown in Table 2, PCP reduced rearings ($F(4,51)=14.47$, $p<0.0001$) and holepokes ($F(4,51)=6.81$, $p=0.0002$).

Effect of methoxetamine (MXE) on PPI

MXE significantly decreased PPI ($F(3,27)=34.98$, $p<0.0001$; Fig. 4). Although there was an interaction between treatment and prepulse intensity ($F(6,54)=4.56$, $p=0.0008$), the 3 mg/kg and 10 mg/kg doses of MXE reduced PPI at all three prepulse intensities ($p<0.01$, Tukey's test). MXE reduced average PPI with an $ED_{50} = 1.89$ mg/kg (see Table 3 and Figure 4). There was a significant main effect of prepulse intensity in this experiment ($F(2,54)=34.91$, $p<0.0001$) and in all subsequent tests (data not shown). For the startle response, there was a main effect of MXE ($F(3,27)=6.20$, $p<0.003$); post hoc comparisons demonstrated that 1 mg/kg MXE produced a 3-fold increase in the magnitude of the startle response ($p<0.01$; Table 4). It is unlikely that the effect of MXE on startle played a role in the PPI disruption because the doses of MXE that affected PPI did not significantly alter the magnitude of the startle response.

Effect of phencyclidine on PPI

As reported previously (Mansbach and Geyer, 1989; Varty and Higgins, 1994), PCP significantly reduced PPI ($F(3,22)=12.19$, $p<0.0001$). Because PCP disrupted PPI at all

prepulse intensities and there was no interaction between intensity and dose ($F(6,44)=0.63$, NS), the effect of PCP on PPI is shown in Figure 5A averaged across the three trial types. PCP produced a 50% reduction of PPI at 0.88 mg/kg (Table 3). Treatment with PCP did not alter the amplitude of the startle response ($F(3,22)=1.41$, NS ; Table 4).

Effect of ketamine isomers on PPI

The effects of the stereoisomers of ketamine were assessed in two separate PPI experiments. Because of the short time-course of (–)-ketamine effects on PPI in rats (Mansbach and Geyer, 1991), the effects of *S*(+)- and *R*(–)-ketamine were assessed during the first half of the test session. Figure 5B shows the effects of the ketamine isomers on average PPI. PPI was reduced by both *S*(+)-ketamine ($F(4,49)=10.71$, $p<0.0001$) and *R*(–)-ketamine ($F(4,50)=8.06$, $p<0.0001$), but the *S*(+) isomer was more potent than the *R*(–) isomer. The ED_{50} values were 2.86 mg/kg for *S*(+)-ketamine and 6.33 mg/kg for *R*(–)-ketamine (Table 3). There was an interaction between drug and prepulse intensity for *S*(+)-ketamine ($F(8,98)=5.02$, $p<0.0001$) but not for *R*(–)-ketamine ($F(8,100)=1.39$, NS). Figure S1 shows the effects of the ketamine isomers on PPI at three prepulse intensities. *S*(+)-ketamine reduced PPI at the 71- and 77-dB prepulse intensities but not at 68-dB. The ketamine isomers had no effect on the magnitude of the startle response (*S*(+)-ketamine: $F(4,49)=0.02$, NS ; *R*(–)-ketamine: $F(4,50)=0.24$, NS ; Table 4).

Effect of NANM on PPI

Administration of NANM disrupted PPI ($F(3,36)=16.41$, $p<0.0001$), but there was no interaction between drug and prepulse intensity ($F(6,72)=0.89$, NS). As shown in Figure 5C, average PPI was reduced by 5 and 10 mg/kg NANM ($p<0.01$). NANM disrupted PPI with an $ED_{50} = 4.87$ mg/kg (Table 3). The effect of NANM on PPI at three prepulse intensities is illustrated in Figure S2. 10 mg/kg NANM reduced PPI at all three prepulse intensities ($p<0.01$, Tukey's test), whereas 5 mg/kg NANM significantly decreased PPI only at the 68- and 71-dB prepulse intensities ($p<0.01$). NANM had no effect on startle amplitude ($F(3,36)=1.66$, NS ; Table 4).

DISCUSSION

MXE reportedly produces ketamine-like effects but is more potent and longer-lasting (Corazza et al., 2013; Kjellgren and Jonsson, 2013). The present investigation demonstrates that MXE produces a dose-dependent reduction of PPI, a measure of sensorimotor gating, in rats. Other uncompetitive NMDAR antagonists that produce dissociative effects in humans, including the arylcyclohexylamines PCP and ketamine, and the benzomorphan NANM, also produced significant PPI deficits in our studies. When tested in the BPM, rats treated with MXE exhibited a PCP-like profile of effects, including locomotor hyperactivity, reductions in investigatory behavior, and perseverative patterns of locomotion. Taken together, these findings show that MXE produces profound PCP-like behavioral alterations in rats.

All of the psychotomimetic NMDAR antagonists tested thus far disrupt PPI in rats (Mansbach and Geyer, 1989, 1991; Wiley et al., 2003). Our experiments have now confirmed that NANM and MXE are also capable of disrupting PPI in rats. NANM was

originally developed as an analgesic but was later found to produce profound hallucinogenic and dysphoric effects in postoperative patients (Keats and Telford, 1964). In addition to binding to σ -1 sites and μ - and κ -opioid receptors, NANM has moderate affinity for the PCP binding site (Tam, 1985; Largent et al., 1986) and blocks the response to NMDA *in vivo* (Wong et al., 1986). Evidence indicates that most of the behavioral effects of NANM in monkeys and rodents are mediated by NMDAR blockade (Shearman and Herz, 1982; Brady et al., 1982; Balaster, 1989). NANM produces complete substitution in laboratory animals trained to discriminate PCP or MK-801 (Brady et al., 1982; Shannon et al., 1982; Sanger and Zivkovic, 1989). Likewise, the discriminative stimulus effects of NANM generalize completely to PCP and ketamine (Shearman and Herz, 1982). Together with the evidence that PPI is disrupted by dextrorphan (Wiley et al., 2003), an uncompetitive NMDAR antagonist with a morphinan structure, the current findings with NANM demonstrate that sensorimotor gating is reduced by dissociative hallucinogens from a variety of different structural classes.

Our studies also confirmed that both stereoisomers of ketamine are capable of disrupting PPI in rats, and demonstrated that *S*-(+)-ketamine disrupts PPI with 2.5-fold higher potency than *R*-(-)-ketamine (see Table 1). Another group has shown that *S*-(+)- and *R*-(-)-ketamine disrupt PPI in Sprague-Dawley rats (Littlewood et al., 2006), but the dose-dependence of the effect was not clear because they only tested the isomers at a single dose level (25 mg/kg). The observed stereoselectivity of the effect of ketamine on PPI is consistent with the fact that at equimolar doses *S*-(+)-ketamine causes more perceptual disturbances than *R*-(-)-ketamine (Oye et al., 1992; Vollenweider et al., 1997). Furthermore, *S*-(+)-ketamine binds to the PCP site with higher affinity than *R*-(-)-ketamine (Table 1; Zuckin et al., 1984; Oye et al., 1992; Ebert et al., 1997).

The pattern of effects on PPI is consistent with mediation by NMDAR blockade. As shown in Table 1, the relative potencies of MXE, PCP, NANM, and the ketamine isomers in the PPI paradigm (PCP > MXE > *S*-(+)-ketamine > NANM > *R*-(-)-ketamine) parallels their binding affinities at the PCP site in the NMDAR. MXE is less potent in rats than PCP, yet more potent than either *S*-(+)- or *R*-(-)-ketamine, which is consistent with the fact that the affinity of MXE for the PCP site is intermediate between that of PCP and ketamine (Roth et al., 2014). These results are consistent with NMDAR blockade being the primary mechanism for the modulation of sensorimotor gating by dissociative anesthetics such as PCP and ketamine.

MXE and PCP have similar effects on unconditioned motor activity. Locomotor hyperactivity is a prominent component of the behavioral syndrome induced by competitive and uncompetitive NMDAR antagonists with psychotomimetic effects (Sturgeon et al., 1979; Mattia et al., 1986; Lehmann-Masten and Geyer, 1991; Danysz et al., 1994; Bakshi et al., 1999; Imre et al., 2006; Adams et al., 2013). Importantly, although many stimulant drugs increase locomotor activity, multivariate assessments in the BPM have shown that the effects of PCP and MK-801 on unconditioned motor activity are qualitatively distinct from those produced by other stimulant classes (Lehmann-Masten and Geyer, 1991; Krebs-Thomson et al., 1998). Amphetamine and methamphetamine increase investigatory behaviors (rearings and holepokes) and stimulate locomotion throughout the test chamber without altering the

geometrical organization of movement, and therefore do not alter spatial d unless administered at high doses that provoke stereotypies (repetitive local movements) (Kelley et al., 1986; Geyer et al., 1986, 1987; Paulus and Geyer, 1991, 1992). By contrast, although rats treated with PCP are hyperactive, they also exhibit a reduction in the frequency of investigatory behaviors, increased spatial d , and perseverative patterns of locomotion. Administration of MXE mimicked most of the effects of PCP in the BPM, although their behavioral profiles show subtle differences. First, only the low dose of MXE (1 mg/kg) reduced holepokes. Second, although both PCP and MXE induced perseverative patterns of ambulation, their effects were qualitatively different. Animals treated with 2.25 mg/kg PCP tended to repetitively circle the BPM chamber whereas 10 mg/kg MXE caused the animals to rotate in tight circles, similar to the effect of MK-801 (Lehmann-Masten and Geyer, 1991). Thus, MXE produces effects on exploratory and investigatory behavior that are consistent with NMDAR blockade but the specific profile of effects is most similar to the response induced by MK-801.

In addition to the similarities between the effects of MXE and MK-801 on locomotor patterns, MXE and MK-801 also alter acoustic startle in a comparable manner. As shown in Table 2, administration of 2 mg/kg MXE produced a 196% increase in the magnitude of the acoustic startle response. The startle response is also reportedly enhanced by MK-801 in rats (Mansbach et al., 1989; Bakshi et al., 1994; Bast et al., 2000). PCP, however, does not increase the acoustic startle response (Mansbach et al., 1989; Bakshi et al., 1994). In summary, aspects of the behavioral profile produced by MXE are more reminiscent of the response to MK-801 than of PCP. Compared with PCP, MK-801 displays greater selectivity for NMDAR (Akunne et al., 1991; Maurice et al., 1991). PCP binds to a variety of sites, including dopamine and serotonin transporters, nicotinic acetylcholine receptors, voltage-gated potassium channels, and σ -1 and σ -2 sites (Bartschat and Blaustein, 1986; Chaudieu et al., 1989; Walker et al., 1990; Akunne et al., 1991, 1992; Maurice et al., 1991; Eterovic et al., 1999). These auxiliary interactions may influence the effects of PCP on startle and locomotor patterns, causing its behavioral profile to diverge from that of MK-801 and MXE.

In contrast to the present studies, where MXE induced hyperactivity, another group found MXE does not to alter locomotor activity in Sprague-Dawley rats when tested at 1.25–5 mg/kg (Botanas et al., 2015). The most likely explanation for these discrepant findings is that 5 mg/kg falls at the bottom of the MXE dose-response function and is not sufficient to provoke a robust behavioral response. Another unexpected result is that administration of a low dose of MXE (1 mg/kg) produced a transient reduction of locomotor activity. PCP can also reduce locomotor activity but the effect typically occurs at high doses as a consequence of ataxia, which can disrupt ambulation (Sturgeon et al., 1979; Krebs-Thomson et al., 1998). It has been shown, however, that very low doses of ketamine (~5–10 mg/kg) suppress locomotor activity in rats (Pálení ek et al., 2011; Trujillo et al., 2011; Botanas et al., 2015). The hypoactivity observed in rats treated with 1 mg/kg MXE may parallel the reduction of activity induced by low doses of ketamine. The mechanism underlying the hypoactivity produced by low doses of ketamine and MXE remains to be elucidated, but the effect may be caused by a transient anxiogenic response. Low doses of ketamine reportedly produce anxiogenic effects in the elevated plus maze (Silvestre et al., 1997) and the unconditioned one-way escape task (Babar et al., 2001).

In summary, we used multiple paradigms to show that MXE can mimic the behavioral effects of other uncompetitive NMDAR antagonists in rats. These studies also demonstrated that MXE has higher potency than ketamine. These results are consistent with the classification of MXE as a dissociative drug. This is not an unexpected finding because MXE is reportedly used recreationally because it produces ketamine-like effects. Based on the potency of MXE and its similarity to PCP and ketamine, it is anticipated that MXE will have significant abuse potential. Indeed, like other uncompetitive NMDAR antagonists (Moreton et al., 1977; Balaster, 1986; Marglin et al., 1989; Marquis et al., 1989; Suzuki et al., 1999), there is evidence that MXE produces rewarding effects in laboratory animals (Botanas et al., 2015). MXE also produces robust increases in DA and 5-HT release in prefrontal cortex (Fuchigami et al., 2015), effects that also occur with PCP and ketamine and are thought to underlie many of their hallucinogenic and behavioral effects. Taken together, these findings indicate that MXE is likely to produce roughly the same subjective effects and abuse potential as PCP and ketamine. Additional studies are required to fully elucidate the mechanism(s) responsible for the behavioral effects of MXE, which would help to predict the dangers associated with MXE abuse and facilitate the management of MXE intoxication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effect of methoxetamine (MXE) on activity in the BPM. (A) Effect of MXE on crossings, a measure of locomotor activity. (B) Effect of MXE on spatial d . Values represent mean $\pm$ SEM for each group during the indicated block of time. * $p < 0.05$, ** $p < 0.01$, significantly different from vehicle control.

Vehicle

1 mg/kg

10 mg/kg

Figure 2.

Plots of spatial patterns of locomotor activity showing the effect of methoxetamine (MXE) in representative animals. Plots show activity traces during the 30–60 min interval after placement of the animal in the BPM. Animals were treated with saline (*Top*), 1 mg/kg MXE (*Middle*), or 10 mg/kg MXE (*Bottom*).

Figure 3.

Effects of phencyclidine (PCP) on activity in the BPM. (A) Effect on crossings, a measure of locomotor activity. (B) Effect on spatial *d*. (C) Plots of spatial patterns of locomotor activity in representative animals. Plots show activity traces during the 30–60 min interval after placement of the animal in the BPM. Animals were treated with saline (*Left panel*) or 2.25 mg/kg PCP (*Right panel*). Values represent mean $\pm$ SEM for each group during the indicated time block. * $p < 0.05$, ** $p < 0.01$, significantly different from vehicle control.

Figure 4.

Effect of methoxetamine (MXE) on prepulse inhibition (PPI) in rats. (*Top panel*) Effect of MXE on PPI. (*Bottom panel*) Effect of MXE on PPI averaged across the three prepulse intensities. Values represent mean $\pm$ SEM for each group. * $p < 0.01$, significantly different from vehicle control.

Figure 5.

Effect of (A) phencyclidine (PCP), (B) ketamine stereoisomers, and (C) *N*-allylnormetazocine (NANM) on prepulse inhibition (PPI) averaged across the three prepulse intensities. Values represent mean $\pm$ SEM for each group. ** $p < 0.01$, significantly different from the respective vehicle control.

Table 1

Effect of methoxetamine (MXE) on investigatory behaviors.

Group	N	Rearings		Holepokes	
		0–30 min	30–60 min	0–30 min	30–60 min
Vehicle	8	41±12	9±3	89±14	28±6
1 mg/kg	8	11±4 *	13±4	41±9 *	39±15
10 mg/kg	8	1±0 **	34±31	110±13	63±13

Values represent mean ± SEM for each group.

* $p<.05$,

** $p<.01$, compared with vehicle.

Table 2

Effect of phencyclidine (PCP) on investigatory behaviors.

Group	N	Rearings		Holepokes	
		0–30 min	30–60 min	0–30 min	30–60 min
Vehicle	11	134±9	46±5	144±14	97±12
0.25 mg/kg	12	82±12 *	50±6	127±16	97±13
0.75 mg/kg	11	49±14 **	48±9	81±15 *	96±14
2.25 mg/kg	11	17±16 **	12±11 *	43±16 **	55±13
6.75 mg/kg	11	12±11 **	11±3 *	75±14 *	46±10 *

Values represent mean ± SEM for each group.

* $p<.05$,

** $p<.01$, compared with vehicle.

Table 3
Comparison of potencies in the PPI paradigm and binding affinities for the PCP site

Drug	Disruption of prepulse inhibition (PPI) ^a				PCP site binding affinity	
	ED ₅₀ (mg/kg)	95% CL	ED ₅₀ (μmol/kg)	95% CL	IC ₅₀ (μM) ^b	K _i (μM)
Phencyclidine	0.88	0.73–1.07	3.15	2.60–3.83	0.2	0.059 ^c
Methoxetamine	1.89	1.31–2.73	6.66	4.62–9.62		0.259 ^c
S-(+)-Ketamine	2.86	1.54–5.32	10.43	5.62–19.40	0.85	1.1 ^d
N-Allylnormetazocine	4.87	3.68–6.46	16.57	12.52–21.98	1.0	
R-(−)-Ketamine	6.33	3.68–10.90	23.09	13.42–39.75	3.0	3.2 ^d

^aPotencies were calculated by collapsing PPI values across the three prepulse intensities.
^bDisplacement of [³H]PCP binding (rat brain). Data from: Zukin et al., 1984.
^cDisplacement of [³H]MK-801 binding (rat brain). Data from: Roth et al., 2013.
^dDisplacement of [³H]TCP binding (guinea pig brain). Data from: Hustveit et al., 1995.

Table 4

Effect of drug treatment on startle magnitude.

	Dose	N	Startle Magnitude (mean $\pm$ SEM)
Methoxetamine (MXE)	Vehicle	8	199.0 $\pm$ 36.2
	1 mg/kg	8	589.1 $\pm$ 107.6 *
	3 mg/kg	8	263.6 $\pm$ 37.4
	10 mg/kg	7	376.3 $\pm$ 72.4
Phencyclidine (PCP)	Vehicle	7	200.8 $\pm$ 32.6
	0.8 mg/kg	7	217.5 $\pm$ 26.5
	1.0 mg/kg	6	407.2 $\pm$ 148.9
	1.2 mg/kg	6	296.1 $\pm$ 67.9
<i>S</i> -(+)-Ketamine	Vehicle	12	345.2 $\pm$ 79.7
	1.5 mg/kg	10	221.1 $\pm$ 55.3
	3 mg/kg	9	223.8 $\pm$ 20.9
	6 mg/kg	11	323.1 $\pm$ 53.3
	12 mg/kg	12	289.9 $\pm$ 50.7
<i>R</i> -(-)-Ketamine	Vehicle	10	370.9 $\pm$ 69.9
	1.5 mg/kg	9	344.9 $\pm$ 90.3
	3 mg/kg	12	372.8 $\pm$ 34.6
	6 mg/kg	12	387.2 $\pm$ 79.0
	12 mg/kg	12	310.4 $\pm$ 41.0
<i>N</i> -Allylnormetazocine (NANM)	Vehicle	10	276.5 $\pm$ 70.0
	2.5 mg/kg	10	228.0 $\pm$ 53.7
	5 mg/kg	10	167.9 $\pm$ 27.3
	10 mg/kg	10	142.0 $\pm$ 16.9

* $p < 0.01$ versus respective vehicle control.