Neuropharmacology 141 (2018) 167–180

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Neurological, sensorimotor and cardiorespiratory alterations induced by methoxetamine, ketamine and phencyclidine in mice

Andrea Ossato[a,b,1], Sabrine Bilel[a,1], Adolfo Gregori[c], Anna Talarico[d], Claudio Trapella[d], Rosa Maria Gaudio[e], Fabio De-Giorgio[b], Franco Tagliaro[f,g], Margherita Neri[h], Liana Fattore[i], Matteo Marti[h,j,*]

[a] Department of Life Sciences and Biotechnology (SVeB), University of Ferrara, Italy
[b] Institute of Public Health, Section of Legal Medicine, Catholic University of Rome, Rome, Italy
[c] Carabinieri, Department of Scientific Investigation (RIS), 00191, Rome, Italy
[d] Department of Chemistry and Pharmaceutical Sciences, University of Ferrara, Italy
[e] Department of Medical Sciences, Section of Forensic Pathology, University of Ferrara, Italy
[f] Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Policlinico "G.B. Rossi", Verona, Italy
[g] Institute of Pharmacy and Translational Medicine, Sechenov First Moscow State Medical University, Russian Federation
[h] Department of Morphology, Surgery and Experimental Medicine, Section of Legal Medicine, University of Ferrara, Italy
[i] Institute of Neuroscience-Cagliari, National Research Council (CNR), Italy
[j] Collaborative Center for the Italian National Early Warning System, Department of Anti-Drug Policies, Presidency of the Council of Ministers, Italy

HIGHLIGHTS

- Methoxetamine (MXE) is a ketamine (KET) - like novel psychoactive substance (NPS).
- Number of MXE-induced acute toxicity are increasing at an alarming rate.
- MXE induces significant neurological, sensorimotor, cardiorespiratory alterations.
- MXE effects were qualitatively but not quantitatively similar to KET and phencyclidine.

ARTICLE INFO

Keywords:
Methoxetamine
Ketamine
Phencyclidine
Dissociative drugs
Behavioral alterations

ABSTRACT

Novel psychoactive substances are intoxicating compounds developed to mimic the effects of well-established drugs of abuse. They are not controlled by the United Nations drug convention and pose serious health concerns worldwide. Among them, the dissociative drug methoxetamine (MXE) is structurally similar to ketamine (KET) and phencyclidine (PCP) and was created to purposely mimic the psychotropic effects of its "parent" compounds. Recent animal studies show that MXE is able to stimulate the mesolimbic dopaminergic transmission and to induce KET-like discriminative and rewarding effects. In light of the renewed interest in KET and PCP analogs, we decided to deepen the investigation of MXE-induced effects by a battery of behavioral tests widely used in studies of "safety-pharmacology" for the preclinical characterization of new molecules. To this purpose, the acute effects of MXE on neurological and sensorimotor functions in mice, including visual, acoustic and tactile responses, thermal and mechanical pain, motor activity and acoustic startle reactivity were evaluated in comparisons with KET and PCP to better appreciate its specificity of action. Cardiorespiratory parameters and blood pressure were also monitored in awake and freely moving animals. Acute systemic administrations of MXE, KET and PCP (0.01–30 mg/kg i.p.) differentially alter neurological and sensorimotor functions in mice depending in a dose-dependent manner specific for each parameter examined. MXE and KET (1 and 30 mg/kg i.p.) and PCP (1 and 10 mg/kg i.p.) also affect significantly cardiorespiratory parameters, systolic and diastolic blood pressure in mice.

Abbreviations: PCP, 1-(1-phenylcyclohexyl)piperidine, phencyclidine; KET, 2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one, ketamine; MXE, 2-(ethylamino)-2-(3-methoxyphenyl)cyclohexan-1-one, methoxetamine

* Corresponding author. Department of Morphology, Surgery and Experimental Medicine, Section of Legal Medicine, University of Ferrara, Via Fossato di Mortara 17-19, 44121, Ferrara, Italy.
E-mail address: matteo.marti@unife.it (M. Marti).

[1] Authors equally contributed to the manuscript.

https://doi.org/10.1016/j.neuropharm.2018.08.017
Received 23 May 2018; Received in revised form 10 August 2018; Accepted 17 August 2018
Available online 27 August 2018
0028-3908/

1. Introduction

Seeking for psychoactive substances and exploring their potential uses is unanimously recognized as a human trait; since antiquity humans consume psychoactive plant preparations and isolate psychoactive ingredients yielding "natural" drugs. More recently, however, chemists started synthesizing new compounds that mimic the psychotropic effects of "natural" (e.g. cannabis) or "chemical" (e.g. amphetamine) compounds. The growing use of these New Psychoactive Substances (NPS) represents today a social and health concern worldwide. Synthetic cathinones and cannabinoids are most popular classes of NPS and thus receive particular attention (Weinstein et al., 2017). Synthetic opioids have also emerged recently on the recreational drug market and are causing numerous lethal intoxications (Zawilska, 2017).

Dissociative drugs are less commonly used than cathinones, cannabinoids or opioids, but numerous fatal and fatal intoxications have been reported following consumption of phencyclidine (PCP) (Bäckberg et al., 2015), ketamine (KET) (Gill and Stajíc, 2000) and dissociative drugs of new generation (Adamowicz and Zuba, 2015; Helander et al., 2015). Animal and human studies clearly indicate that these drugs alter users' mental states and behavioral performances and induce a feeling of detachment from reality, impaired sensorimotor and cardiorespiratory functions, body tremors and numbness (Kesner et al., 1981; Li and Vlisides, 2016). Due to their increasing popularity, they are cause of clinical concern (Chiappini et al., 2015; Corazza et al., 2012; Schifano et al., 2008).

Methoxetamine (2-(3-methoxyphenyl)-2-(N-ethylamino)cyclohexanone), also known as MXE or 'Special M', is an arylcyclohexylamine derivative with a chemical structure similar to that of KET and PCP (Fig. S1, Supplemental Materials), but with few modifications though to confer higher potency than PCP (Corazza et al., 2013) and longer action than KET (Morris and Wallach, 2014). Due to the increasing number of intoxications, MXE is under control in many Countries, but is not listed in the 1971 UN Convention (Zanda et al., 2016). Like KET and PCP, MXE is a dissociative anesthetic thought to act as a noncompetitive N-methyl D-aspartate (NMDA) receptor antagonist (Coppola and Mondola, 2012), able to significantly stimulate the mesolimbic dopaminergic system in rats (Mutti et al., 2016), alter monoamine metabolism in *in vitro* models (Hondebrink et al., 2017) and affect brain functions and behavior in both animals and humans (Zanda et al., 2017). Its mechanism of action in the brain and periphery started only recently being investigated (Hajkova et al., 2016; Horsley et al., 2016). Research is quite active in the field and in recent years it was shown that, in rodents, MXE possesses ketamine-like discriminative stimulus properties (Chiamulera et al., 2016), induces conditioned place preference and maintain intravenous self-administration behavior (Botanas et al., 2015). It also substitutes for ketamine in a drug self-administration substitution study (Mutti et al., 2016) and produces dissociative-like behavioral effects in rodents (Halberstadt et al., 2016).

PCP was synthesized in the 1950s and sold as intravenous anesthetic under the trade names Sernyl and Sernylan until 1967, when it was withdrawn from the market due to intensely negative hallucinogenic effects (e.g. delirium, psychosis). PCP is now listed in Schedule I of the 1971 United Nations Convention on Psychotropic Substances (UN Convention), but a number of its derivatives (e.g. 3-MeOPCE, 4-MeO-PCP) are not under International control. These PCP-type substances appeared for the first time in Europe in 2010 and are currently sold as 'research chemicals'. Regrettably, there is very limited information on the PCP analogs. Chemically related to PCP, KET was synthesized in 1962 and starting from early 1970s was marketed under the brand name Ketalar as a replacement anesthetic to PCP. Similarly to PCP, KET induces cognitive disruption and psychotic-spectrum reactions (Altura and Altura, 1984; Ellison, 1995). Recently, due to a growing concern over its use as NPS, KET is now listed in Schedule II of the 1971 UN Convention.

Given the resurging interest in KET and PCP-type substances (UNODC, 2017), we evaluate here the effects of a single exposure to MXE on neurological and sensorimotor functions by a battery of tests widely used in studies of "safety-pharmacology" for the preclinical characterization of new molecules in rodents (Hamdam et al., 2013; ICH S7A, 2001; Irwin, 1968; Porsolt et al., 2002) which we recently used to characterize the pharmacological profile of other NPS in mice (Fantinati et al., 2017; Ossato et al., 2015, 2016; Vigolo et al., 2015). Moreover, cardiorespiratory parameters and blood pressure were monitored in awake and freely moving animals with no invasive instruments and minimal handling. In all experiments, MXE was tested in parallel with KET and PCP to better appreciate the specificity of its action.

2. Materials and methods

2.1. Animals

Male ICR mice, 25–30 gr (Harlan Italy), were housed 8–10/cage under a 12:12-h light-dark cycle (light on: 6:30 a.m.) with standard room temperature (20–22 °C) and humidity (45–55%) and *ad libitum* access to food and water. Experimental protocols were in accordance with the new European Communities Council Directive of September 2010 (2010/63/EU) a revision of the Directive 86/609/EEC, and were approved by the Italian Ministry of Health (license 335/2016-PR) and the local Ethics Committee. Adequate measures were taken to minimize the number of animals used, their pain and discomfort.

2.2. Drug preparation and dose selection

Phencyclidine-HCl (PCP), ketamine-HCl (KET) and methoxetamine-HCl (MXE) (LGC Standards S.r.L., Milan, Italy) were dissolved in saline solution and administered intraperitoneally (i.p.) at a volume of 4 μl/g. Doses of MXE (0.01–30 mg/kg i.p.) were selected basing on previous preliminary study (Marti et al., 2017) and the behavioral/neurological effects reported by users (https://www.erowid.org/experiences/subs/exp_Methoxetamine_.shtml). Doses of KET (0.01–30 mg/kg i.p.) and PCP (0.01–10 mg/kg i.p.) were also chosen according to previous preclinical studies in rodents (Bonito-Oliva et al., 2016; Kolányi et al., 2016; Marti et al., 2017).

2.3. Behavioral studies

Experiments were performed between 8:30 and 2:00 p.m. and conducted in blind by trained observers working in pairs (Ossato et al., 2016). Animals' behavior was videotaped and analyzed off-line by a different trained operator. Mice were tested for multiple test as previously described (Canazza et al., 2016). To reduce the number of animals used, the behavior of mice was evaluated in 5 consecutive experimental sections carried out at different time period: 0–95 min, 120–150 min, 180–210 min, 240–270 min, 300–340 min. Each experimental section includes the following behavioral tests performed in a consecutive manner according to the following sequence: observation of main neurological changes and aggressive responses, measures of visual object responses (frontal and lateral view), acoustic response, tactile response (pinna, vibrissae and corneal reflexes) and visual placing response, determination of the mechanical (tail pinch) and thermal (tail withdrawal) acute pain and stimulated motor activity (accelerod and drag test). Between the 1st (0–95min) and the 2nd (120–150 min) section, animals recovered 25 min while, between further sections, they rest 30 min. During analysis, the period of rest between different tests was about 300 s. Each dose was tested in at least three different groups of animals (e.g. 3 + 3+2); each mouse was treated only once.

2.3.1. Major neurological changes

Stereotypies (i.e. stereotyped head movements, stereotyped biting and excessive sniffing), hyperactivity (i.e. restlessness and turning),

inadvertent falls (from a high plate) and aggressiveness (spontaneous and stimulated; Canazza et al., 2016) were monitored in mice immediately after PCP, KET and MXE (0.01–30 mg/kg, i.p.) administration. Neurological changes are expressed as frequency (percent of animals that develop symptoms) and as maximum intensity of symptoms recorded in one minute (i.e. head movements/min, rotations/min and falls/min). Animal's rotation were classified as narrow (i.e. the mouse rotates tight around its body axis) or large (i.e. the mouse moves circularly in an open space making circles of diameter of about 45–50 cm). The mouse was placed in a square area (70 × 70 cm) and narrow and large rotations were measured. The unintentional falls were measured by placing the mouse over a square plate (30 × 30 cm) raised from the ground (20 cm) and the number of times that the mouse falls down unintentionally from the plate due to the involuntary psychomotor agitation was recorded. Animal's spontaneous aggressiveness was estimated as number of bites to an object, namely a gray cloth, that approaches the front of the snout of the animal in an animal's mobility condition. Conversely, in stimulated aggressiveness the animal is manually restrained and held in a supine position. For both aggressive behavior tests, a gray cloth was placed in front of the mouse nose for 10 consecutive times (score: 0/10 not aggressive, 10/10 very aggressive).

2.3.2. Sensorimotor studies

Voluntary and involuntary sensorimotor responses resulting from different reactions to visual, acoustic and tactile stimuli were evaluated as previously described (Marti et al., 2017; Ossato et al., 2015).

2.3.2.1. Evaluation of the visual response. Visual response was verified by two behavioral tests, which evaluated the ability of the mouse to capture visual information either when the animal is stationary (visual object response) or when moving (visual placing response). The visual object response test was used to evaluate the ability of the mouse to see an object approaching from the front or the side, then inducing the animal to shift or turn the head or retreat it (Ossato et al., 2015). For the frontal visual response, a white horizontal bar was moved frontally to the mouse head and the manoeuvre was repeated 3 times. For the lateral visual response, a small dentist's mirror was moved into the mouse's field of view in a horizontal arc, until the stimulus was between the mouse's eyes. The procedure was conducted bilaterally and was repeated 3 times. The score assigned was 1 if there was a reflection in the mouse movement or 0 if not. The total value was calculated by adding the scores obtained in the frontal with that obtained in the lateral visual object response (maximum overall score 9). Evaluation of the visual object response was measured at 0, 10, 30, 60, 120, 180, 240 and 300 min post injection. The visual placing response test was performed using a tail suspension modified apparatus able to bring down the mouse towards the floor at a constant speed of 10 cm/s (Ossato et al., 2015). The downward movement of the mouse was videotaped by a camera. The analysis, frame by frame, allows to evaluate the beginning of the reaction of the mouse while it is close to the floor. When the mouse starts the reaction an electronic ruler evaluates the perpendicular distance (in millimetres) between the eyes of the mouse and the floor. Typically, untreated control mice perceive the floor and prepare to contact at a distance of about 28 ± 4.7 mm. Evaluation of the visual placing response is measured at 0, 15, 35, 70, 125, 185, 245 and 305 min post injection.

2.3.2.2. Evaluation of acoustic and tactile response. Acoustic response measures the reflex of the mouse in response to an acoustic stimulus produced behind the animal (Koch, 1999). In particular, four acoustic stimuli of different intensity and frequency were tested as already described (Ossato et al., 2015). Briefly, 1) a snap of the fingers (four snaps repeated in 1.5 s), 2) a sharp click (produced by a metal instrument; four clicks repeated in 1.5 s), 3) an acute sound (produced by an audiometer that reproduces a high-pitched sound at a frequency of around 5.0–5.1 kHz), 4) a severe sound (produced by an audiometer that reproduces a sound at a frequency of around 125–150 Hz). Each sound test was repeated 3 times, assigning as arbitrary units a value of 1 if there was a response, or 0 if not, for a total score of 3 for each sound. The acoustic total score was calculated by adding scores obtained in the four tests (overall score 12). The background noise (about 40 ± 4 dB) and the sound from the instruments were measured with a digital sound level meter. Evaluation of the acoustic response was measured at 0, 10, 30, 60, 120, 180, 240 and 300 min post injection. The tactile response in the mouse was verified through vibrissae, pinna and corneal reflex as previously described (Ossato et al., 2015) and data expressed as the sum of the three parameters. The vibrissae reflex was evaluated by touching vibrissae (right and left) with a thin hypodermic needle once for each side giving a value of 1 if there was a reflex (turning of the head to the side of touch or vibrissae movement) or 0 if not (overall score 2). The pinna reflex was assessed by touching pinnae (left and right) with a thin hypodermic needle. First the interior pinna and then the external pinna were stimulated. This test was repeated twice for each side giving a value of 1 if a reflex was present or 0 if not (overall score 4). The corneal reflex was assessed by gently touching bilaterally the cornea of the mouse with a thin hypodermic needle and evaluating the response, assigning a value of 1 if the mouse moved only the head, 2 if it only closed the eyelid, 3 if it both closed the eyelid and moved the head (overall score 6). Each tactile response was measured at 0, 10, 30, 60, 120, 180, 240 and 300 min post injection.

2.3.2.3. Evaluation of startle reactivity. Mice underwent the pre-pulse inhibition (PPI) test for measuring the acoustic startle reactivity in startle chambers (Ugo Basile, Milan, Italy) consisting of a sound-attenuated, lighted and ventilated enclosure holding a transparent non-restrictive Perspex® cage (90 × 45 × 50 mm). A loudspeaker mounted laterally the holder produced all acoustic stimuli. Peak and amplitudes of the startle response were detected by a loadcell. At the onset of the startling stimulus, 300-ms readings were recorded and the wave amplitude evoked by the movement of the animals startle response was measured. Acoustic startle test sessions included startle trials (pulse-alone) and prepulse trials (prepulse + pulse) consisting, respectively, of a 40-ms 120-dB pulse and of a 20-ms acoustic prepulse +80-ms delay and then a 40-ms 120-dB startle pulse (100-ms onset–onset). There was an average of 15 s (range: 9–21 s) between the trials. Animals were placed in the startle chambers 5 min after drug administration; the entire PPI test lasted 20 min. Each session began with a 10-min acclimation period with a 65-dB broadband white noise that remained present throughout the session. The test session contained 30 trials composed by pulse-alone and prepulse + pulse trials (with two different prepulses of 75-dB and 85-dB) presented in a pseudorandomized order. PPI responses were recorded 15 and 240 min (including the 10-min acclimation period) after drug injections and were expressed as percentage decrease in the amplitude of the startle reactivity caused by the presentation of the prepulse (% PPI). A selected range of doses of PCP (0.1 and 1 mg/kg), KET (1 and 10 mg/kg) and MXE (1 and 10 mg/kg) were tested. Lower doses of PCP (0.01 mg/kg), KET and MXE (0.01 and 0.1 mg/kg) were ineffective on PPI, whereas higher doses (PCP 10 and 30 mg/kg; KET and MXE 30 mg/kg) evoked severe psychomotor activation and neurological alterations (see present data) which prevented the proper execution of the test.

2.3.2.4. Evaluation of pain induced by mechanical and thermal stimuli. Acute mechanical nociception was evaluated using the tail pinch test (Vigolo et al., 2015). A special rigid probe connected to a digital dynamometer (ZP-50N, IMADA, Japan) was gently placed on the distal portion of the tail of the mouse (i.e. the last 1.5 cm) and a progressive pressure was applied. When the mouse flicked its tail, the pressure was stopped and the digital instrument saved the maximum peak of weight supported (g/force). A cut off (500 g/force) was set to avoid tissue damage. The test was repeated three times and the final

value was calculated as the average of the 3 scores. Acute thermal nociception was evaluated using the tail withdrawal test (Vigolo et al., 2015). The mouse was restrained in a dark plastic cylinder and half of its tail was dipped in water at 48 °C: the time elapsed from the immersion into the water to the retraction of the tail, i.e. latency (in seconds), was recorded. A cut off (15 s) was set to avoid tissue damage. Acute mechanical and thermal nociception was measured at 0, 35, 55, 90, 145, 205, 265 and 325 min post injection.

2.3.2.5. Motor activity assessment. Alterations of motor activity induced by PCP, KET and MXE were measured using the accelerod and drag tests and by analysing spontaneous locomotor activity (Ossato et al., 2016; Canazza et al., 2016). In the accelerod test animals were placed for 5 min on a rotating cylinder whose speed increased automatically in a constant manner (0–60 rotations/min). The time spent on the cylinder was measured. The accelerod test was performed at 0, 40, 60, 95, 150, 210, 270 and 330 min post injection. In the drag test the mouse was lifted by the tail, leaving the front paws on the table and dragged backward at a constant speed of about 20 cm/s for a fixed distance (100 cm). The number of steps performed by each paw was recorded by two different observers. Five to seven measurements were collected for each animal. The drag test was performed at 0, 45, 70, 105, 160, 220, 280 and 340 min post injection. Spontaneous locomotor activity was measured by using the ANY-maze video-tracking system (Ugo Basile, application version 4.99g Beta). The mouse was placed in a square plastic cage (60 × 60 cm) located in a sound- and light-attenuated room and the distance travelled (m) was analyzed every 15 min and monitored for 240 min. Four mice were placed individually in 4 separate boxes and monitored simultaneously in each experiment. To avoid olfactory cues, cages were carefully cleaned with a dilute (5%) ethanol solution and washed with water between each trial. All experiments were performed between 9:00 a.m. to 1:00 p.m.

2.4. Cardiorespiratory and blood pressure analysis

To monitor cardiorespiratory parameters in awake and freely moving animals with not invasive instruments and minimal handling, a collar equipped with a sensor was applied to detect continuously heart rate, breath rate and oxygen saturation at a frequency of 15 Hz. During the experiment the mouse was allowed to freely move in a cage (30 × 30 × 20 cm) with no access to food and water while being monitored by the sensor collar through the software MouseOx Plus (STARR Life Sciences® Corp. Oakmont, PA). In the first hour of acclimatization, a fake collar similar to the real one used in the test but with no sensor was used to minimize the potential stress during the experiment. Then, the real collar (with sensor) was replaced and baseline parameters were monitored for 60 min. Subsequently, PCP (1 and 10 mg/kg), KET or MXE (1 and 30 mg/kg) or vehicle (saline) was administered and data recorded for 5-h.

Systolic and diastolic blood pressure was measured by tail cuff plethysmography using a BP-2000 blood pressure analysis system (Visitech Systems, Apex, NC). For each session, the mouse was placed in a metal box restraint with its tail passing through the optical sensor and compression cuff and finally taped to the platform. A traditional tail-cuff occluder was placed proximal to the mouse's tail, which was then immobilized with tape in a V-shaped block between a light source

Table 1
Stereotyped head movements were observed for PCP at 10 and 30 mg/kg (37.5% and 100% of treated-mice, respectively) and for KET and MXE at 30 mg/kg (50% and 50%, respectively). PCP at 30 mg/kg induced stereotyped head movements with an intensity higher than that induced by KET and MXE (ANOVA detected a significant effect of treatment: $F_{(2,15)} = 14.43$, $p = 0.0005$). Hyperactivity, characterized by narrow rotations, was observed for PCP at 10 and 30 mg/kg (25%, and 50% of treated-mice, respectively) while for KET and MXE at 30 mg/kg (25% and 62.5% of treated-mice, respectively). MXE at 30 mg/kg induced narrow rotations with an intensity higher than that induced by KET ($F_{(2,12)} = 6.394$, $p = 0.0163$). Large rotations were observed for PCP at 1, 10 and 30 mg/kg (25%, 100% and 100% of treated-mice, respectively), for KET at 10 and 30 mg/kg (25% and 75% of treated-mice, respectively) and for MXE at 10 and 30 mg/kg (50% and 100% of treated-mice, respectively). MXE at 10 mg/kg induced large rotations with an intensity higher than that induced by KET and PCP at 10 mg/kg ($F_{(2,13)} = 93.49$, $p = 0.042$). MXE 30 mg/kg was more effective than KET at same dose ($F_{(2,21)} = 3.181$, $p = 0.0060$). Inadvertent falls from the high plate were observed for PCP at 10 and 30 mg/kg (87.5% and 100% of treated-mice, respectively) and for KET and MXE at 30 mg/kg (77% and 87.5% of treated-mice, respectively).

Compound	PCP					KET					MXE				
Doses (mg/kg)	0.01	0.1	1	10	30	0.01	0.1	1	10	30	0.01	0.1	1	10	30
Stereotyped head movements															
Frequency (%)	–	–	–	37.5	100	–	–	–	–	50	–	–	–	–	50
Max intensity (head movements/min)	–	–	–	7.0 ± 0.6	11.5 ± 0.9	–	–	–	–	5.0 ± 0.4°°°	–	–	–	–	8.0 ± 0.7°
Stereotyped biting	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Excessive sniffing	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Narrow rotation															
Frequency (%)	–	–	–	25	50	–	–	–	–	25	–	–	–	–	62.5
Max intensity (rotations/min)	–	–	–	39.5 ± 14.5	45.3 ± 4.8	–	–	–	–	27.0 ± 8.4	–	–	–	–	54.1 ± 4.4§
Large rotation															
Frequency (%)	–	–	25	100	100	–	–	–	25	75	–	–	–	50	100
Max intensity (rotations/min)	–	–	5.5 ± 2.5	7.6 ± 0.6	17.0 ± 1.6	–	–	–	7.0 ± 2.0	13.3 ± 0.8	–	–	–	15.3 ± 2.6§°°	20.6 ± 1.3§
Inadvertent falls from the high plate															
Frequency (%)	–	–	–	87.5	100	–	–	–	–	75	–	–	–	–	87.5
Max intensity (falls/min)	–	–	–	48.6 ± 2.3	59.8 ± 1.9	–	–	–	–	48.8 ± 3.7°	–	–	–	–	57.7 ± 2.1
Spontaneous aggressiveness	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stimulated aggressiveness	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Neurological effects of the systemic administration of PCP, KET and MXE (0.01-30 mg/kg i.p.) in mice. Data are expressed as percentage (i.e. frequency of animal showing neurological signs) and as absolute values (i.e. maximum intensity of neurological signs recorded in one minute). Statistical analysis was performed with one-way ANOVA followed by Tukey's test for multiple comparisons. °$p < 0.05$ and °°°$p < 0.001$ versus PCP; §$p < 0.05$ versus KET; °°$p < 0.01$.

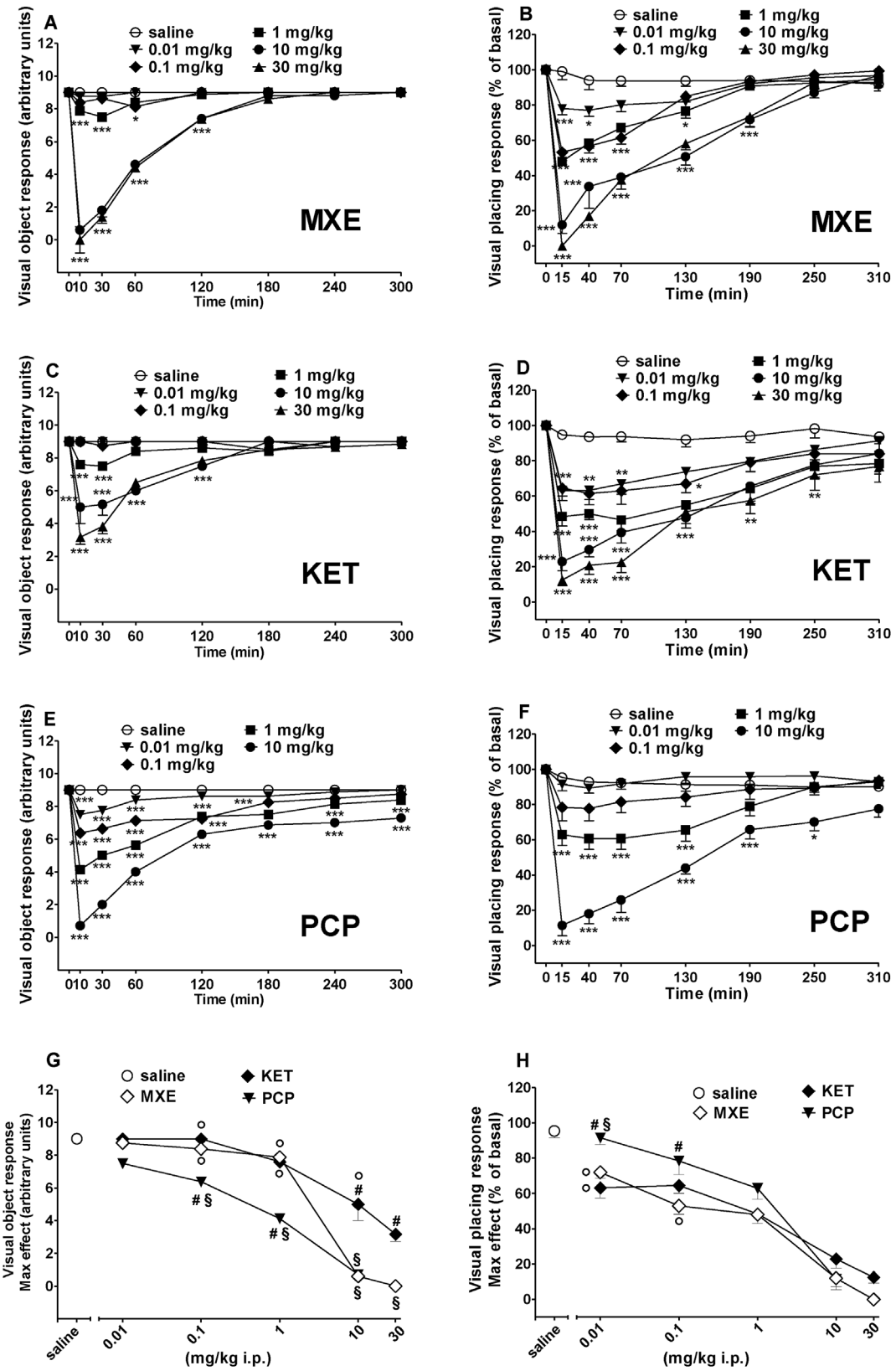

Fig. 1. Effect of MXE (0.01–30 mg/kg; A,B), KET (0.01–30 mg/kg; C-D) and PCP (0.01–10 mg/kg; *E*-F), and on the visual object (*left*) and placing response (*right*) test in the mouse and comparison of the maximum effect observed in 5 h on visual object (G) and visual placing (H) test. Data are expressed as mean ± SEM (n = 8/group). Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons for the dose-response curve of each compound at different times (A–F), while the statistical analysis of the maximum effect observed in 5 h (G,H) was performed with one-way ANOVA followed by Tukey's test for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001 versus saline; #p < 0.05 versus MXE; °p < 0.05 versus PCP; §p < 0.05 versus KET.

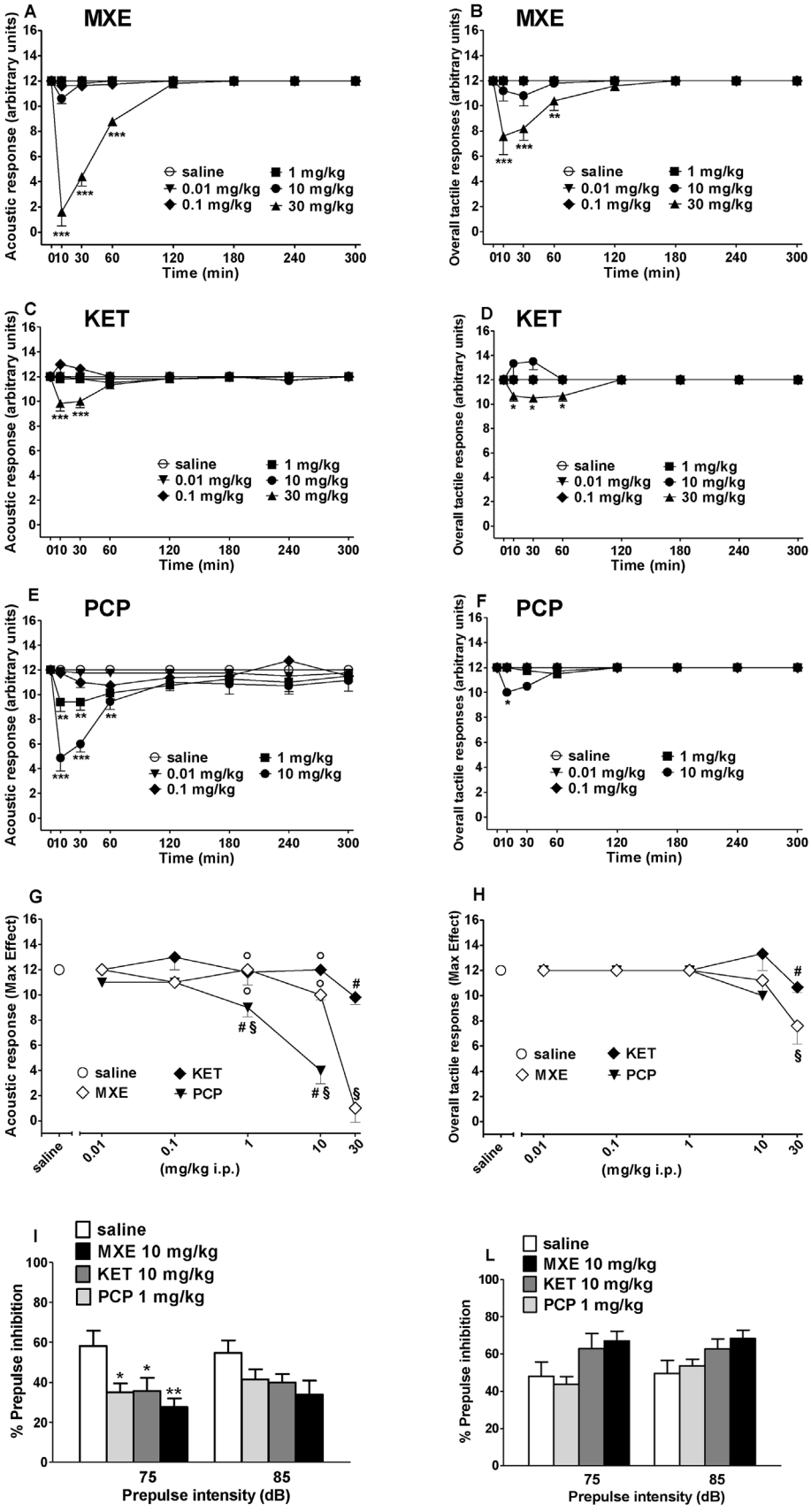

Fig. 2. *Panels A-H*: effect of MXE (0.01–30 mg/kg; A,B), KET (0.01–30 mg/kg; C,D) and PCP (0.01–10 mg/kg; E,F), and on the acoustic (*left*) and the overall tactile (*right*) response in the mouse and comparison of the maximum effect observed in 5 h on acoustic (G) and overall tactile (H) response. Data are expressed as arbitrary units and represent the mean ± SEM of 8 mice/group. Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons for the dose-response curve of each compound at different times (A–F), while the statistical analysis of the maximum effect observed in 5 h (G,H) was performed with one-way ANOVA followed by Tukey's test for multiple comparisons. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus saline. $^{\#}p < 0.05$ versus MXE; $^{\circ}p < 0.05$ versus PCP; $^{\S}p < 0.05$ versus KET. *Panels I,L*: effects of MXE and KET (10 mg/kg) and PCP (1 mg/kg), on pre-pulse inhibition (PPI) in mice. Effects on PPI are shown for the two prepulse intensities (75 and 85 dB), 15 min (A) and 4 h (B) after drug treatment. PPI was expressed as the percentage decrease in the amplitude of the startle reactivity caused by presentation of the pre-pulse (%PPI) and values represent mean ± SEM of 9 mice/group. Statistical analysis was performed with one-way ANOVA followed by Tukey's test for multiple comparisons $^{*}p < 0.05$, $^{**}p < 0.01$ versus saline.

above and a photoresistor below. On inflation, the occluder stopped blood flow through the tail, and on deflation the return of blood flow was detected by the sensor. The restraint platform was maintained at 37 °C. Before experimental sections, mice were acclimated to restraint and tail-cuff inflation for 5–7 days. On the test day, 10 measurements were made as basal blood pressure. At the tenth analysis, the software was paused and mice were injected with PCP (1 and 10 mg/kg), KET or MXE (1 and 30 mg/kg) or vehicle; animals were then repositioned in the restraints and 60 measurements were acquired.

2.5. Statistical analysis

Neurological changes are expressed as percentage of animals that developed symptoms (frequency %) and absolute values that indicate the maximum intensity of symptoms recorded in one minute, i.e. head movements/min, rotations/min and falls/min. In sensorimotor response experiments, data are expressed as arbitrary units (visual objects response, acoustic response and tactile responses) or percentage of baseline (visual placing response). The amount of PPI was calculated as a percentage score for each prepulse + pulse trial type: % PPI = 100 − {[(startle response for prepulse + pulse trial)/(startle response for pulse-alone trial)] × 100}. Startle magnitude was calculated as the average response to all of the pulse-alone trials. Antinociception (tail withdrawal and tail pinch tests) is expressed as percent of maximal possible effect {EMax% = [(test - control latency)/(cut off time - control)] × 100}, while motor activity data are expressed as absolute values (metres) for distance travelled and as percentage of basal values in the drag and accelerod test. Changes in heart rate, breath rate and SpO_2 saturation, expressed as heart beat × minute (bpm), breath rates × minute (brpm) and % Oxygen blood saturation, respectively, are expressed as percentage of basal values. Changes in systolic and diastolic blood pressure are expressed as absolute values (mm/Hg). Data were analyzed by repeated measures ANOVA followed by post-hoc Tukey's test for multiple comparison, where appropriated. Statistical significance was set at $p < 0.05$. Effects of different concentrations of each substance over time were analyzed by two-way ANOVA followed by Bonferroni's test for multiple comparisons. PPI data and the total average effects induced by treatments were analyzed by one-way ANOVA followed by Tukey's test for multiple comparisons. All analyses were performed using GraphPad Prism software (GraphPad Prism, USA).

3. Results

3.1. Behavioral studies

3.1.1. Major neurological changes

Significant neurological alterations were observed in mice following systemic administration of high doses (30 mg/kg) of drugs. Yet, PCP was active at lower doses than KET and MXE in inducing stereotyped head movements, hyperactivity (narrow and large rotations) and inadvertent falls from the high plate. None of the substances caused spontaneous or stimulated aggressive behavior in mice (Table 1).

3.1.2. Sensorimotor studies

3.1.2.1. Evaluation of the visual response. All drugs affected both the visual object (Fig. 1, panels A,C,E) and the visual placing (Fig. 1, panels B,D,F) response in mice over the 5-h observation in a significant ($p < 0.0001$) and dose-dependent manner. MXE reduced the visual object response in mice at 1, 10 and 30 mg/kg and the effect of the 2 highest doses persisted up to 120 min [(Fig. 1A); ANOVA, main effect of treatment ($F_{(5,336)} = 756.4$), time ($F_{(7,336)} = 547.2$) and time × treatment interaction ($F_{(35,336)} = 151.6$)]. MXE also reduced the visual placing response and the effect of the highest dose (30 mg/kg) persisted up to 190 min [(Fig. 1B); ANOVA, main effect of treatment ($F_{(5,336)} = 112.4$), time ($F_{(7,336)} = 151.8$) and time × treatment interaction ($F_{(35,336)} = 14.34$)]. KET reduced the visual object response in mice at 1, 10 and 30 mg/kg and the effect of the 2 highest doses persisted up to 120 min [(Fig. 1C); ANOVA, main effect of treatment ($F_{(5,336)} = 140.6$), time ($F_{(7,336)} = 82.83$) and time × treatment interaction ($F_{(35,336)} = 25.01$)]. KET also reduced the visual placing response, with the lowest (0.01 mg/kg) and the highest doses (30 mg/kg) tested inducing significant ($p < 0.0001$) effects up to 70 and 250 min, respectively [(Fig. 1D); ANOVA, main effect of treatment ($F_{(5,336)} = 61.99$), time ($F_{(7,336)} = 59.47$) and time × treatment interaction ($F_{(35,336)} = 3.885$)]. PCP (0.01–10 mg/kg) dose-dependently reduced the visual object response in mice with the effect of high doses persisting up to 5-h (Fig. 1E); ANOVA, main effect of treatment ($F_{(4,280)} = 429.491$), time ($F_{(7,280)} = 200.212$) and time × treatment interaction ($F_{(28,280)} = 34.250$; post-hoc Tukey's, $p < 0.0001$). Similarly, PCP significantly ($p < 0.0001$) reduced also the visual placing response and at 10 mg/kg the effect persisted up to 250 min [(Fig. 1F); ANOVA, main effect of treatment ($F_{(4,280)} = 123.1$), time ($F_{(7,280)} = 34.22$) and time × treatment interaction ($F_{(28,280)} = 8.267$)]. The comparison of the maximum effects caused by the three compounds highlights that PCP was more potent than KET and MXE in reducing visual object response and that MXE at 10 and 30 mg/kg was more effective than KET (Fig. 1G; main effect of treatment: $F_{(14,119)} = 137$, $p < 0.0001$). On the contrary, KET and MXE were more potent than PCP in reducing visual placing response (Fig. 1H; main effect of treatment: $F_{(14,119)} = 41.56$, $p < 0.0001$).

3.1.2.2. Evaluation of acoustic and tactile response. As shown in Fig. 2, saline injection did not change acoustic (panels A,C,E) and overall tactile (panels B,D,F) response in mice over the 5-h observation. MXE significantly ($p < 0.0001$) reduced the acoustic response in mice only at the highest dose (Fig. 2A) but its effect was more rapid and robust than those caused by KET (Fig. 2C) and persisted up to 60 min (ANOVA, main effect of treatment [($F_{(5,336)} = 183.4$), time ($F_{(7,336)} = 74.01$) and time × treatment interaction ($F_{(35,336)} = 54.50$)]. Similarly, MXE reduced the tactile response in mice only at the highest dose tested (Fig. 2B) but its effect was more rapid and robust than that caused by KET (Fig. 2D) and PCP (Fig. 2F) and persisted up to 60 min [(ANOVA, main effect of treatment ($F_{(5,336)} = 19.78$), time ($F_{(7,336)} = 8.197$) and time × treatment interaction ($F_{(35,336)} = 5.158$)]. KET transiently and modestly, but significantly ($p < 0.0001$), reduced both the acoustic [(Fig. 2C); ANOVA, main effect of treatment ($F_{(5,336)} = 9.710$), time ($F_{(7,336)} = 1.247$) and time × treatment interaction ($F_{(35,336)} = 3.138$)] and the tactile [(Fig. 2D); ANOVA, main effect of treatment ($F_{(5,336)} = 11.18$), time ($F_{(7,336)} = 0.6585$) and time × treatment interaction ($F_{(35,336)} = 3.260$)] response only at the highest dose tested (30 mg/kg). PCP transiently reduced the acoustic response at 1 and 10 mg/kg (Fig. 2E); ANOVA main effect of treatment ($F_{(4,280)} = 36.56$), time ($F_{(7,280)} = 12.36$) and time × treatment interaction ($F_{(28,280)} = 5.812$); post-hoc Tukey's, $p < 0.0001$) and mildly reduced the tactile response at the highest dose tested (Fig. 2F); ANOVA, main effect of treatment ($F_{(4,280)} = 12.344$), time ($F_{(7,280)} = 1.812$) and time × treatment interaction ($F_{(28,280)} = 3.8953$); post-hoc Tukey's, $p < 0.01$). The comparison of the maximum effects caused by the three compounds highlights that PCP was more potent than KET and MXE in reducing acoustic response and that MXE at 30 mg/kg was more effective than KET (Fig. 2G; main effect of treatment: $F_{(14,119)} = 29.45$, $p < 0.0001$). Furthermore, MXE 30 mg/kg was more effective than KET in reducing tactile response (Fig. 2H; main effect of treatment: $F_{(14,119)} = 2.648$, $p = 0.0024$).

3.1.2.3. Evaluation of startle reactivity. Saline injection did not change startle and PPI response in mice and the effect was similar in naïve untreated animals (see Table S1, Supplemental Materials). MXE and KET (10 mg/kg) and PCP (1 mg/kg) inhibited PPI in mice at 15-min [(Fig. 2I); significant effect at 75 dB ($F_{(3,35)} = 5.083$, $p = 0.0054$) but no effect at 85 dB ($F_{(3,35)} = 2.335$, $p = 0.0924$)] but not at 4-h (Fig. 2L).

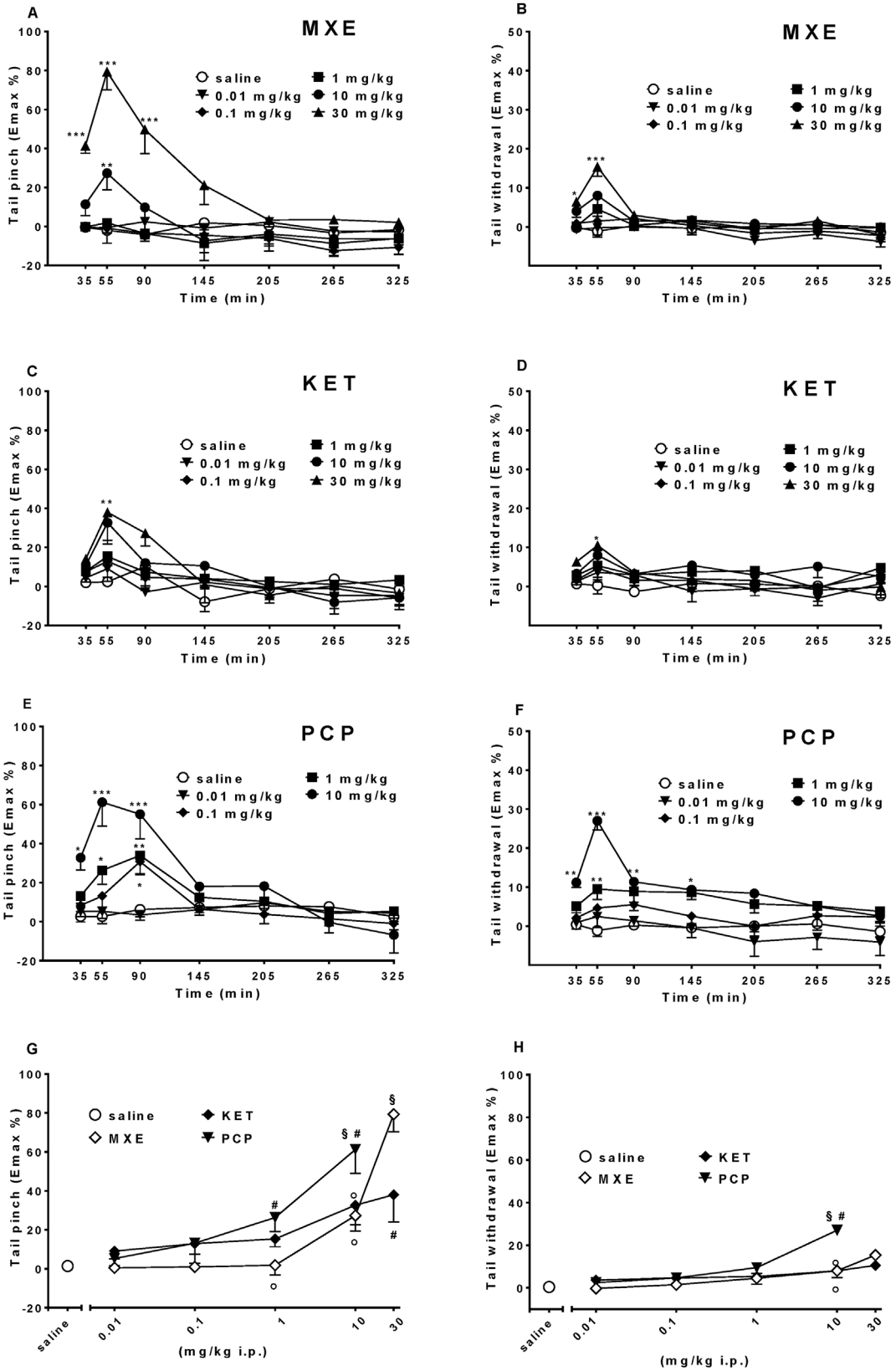

Fig. 3. Effect of MXE (0.01–30 mg/kg i.p.; A,B), KET (0.01–30 mg/kg i.p.; C,D) and PCP (0.01–10 mg/kg i.p.; E,F) on the tail pinch (*left*) and tail withdrawal (*right*) test in the mouse and comparison of the maximum effect observed in 5 h on mechanical (G) and thermal (H) analgesia. Data are expressed as percentage of maximum effect and represent the mean ± SEM of 8 mice/group. Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons for the dose-response curve of each compound at different times (A–F), while the statistical analysis of the maximum effect observed in 5 h (G,H) was performed with one-way ANOVA followed by Tukey's test for multiple comparisons. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus saline; $^{\#}p < 0.05$ versus MXE; $^{\circ}p < 0.05$ versus PCP; $^{\S}p < 0.05$ versus KET.

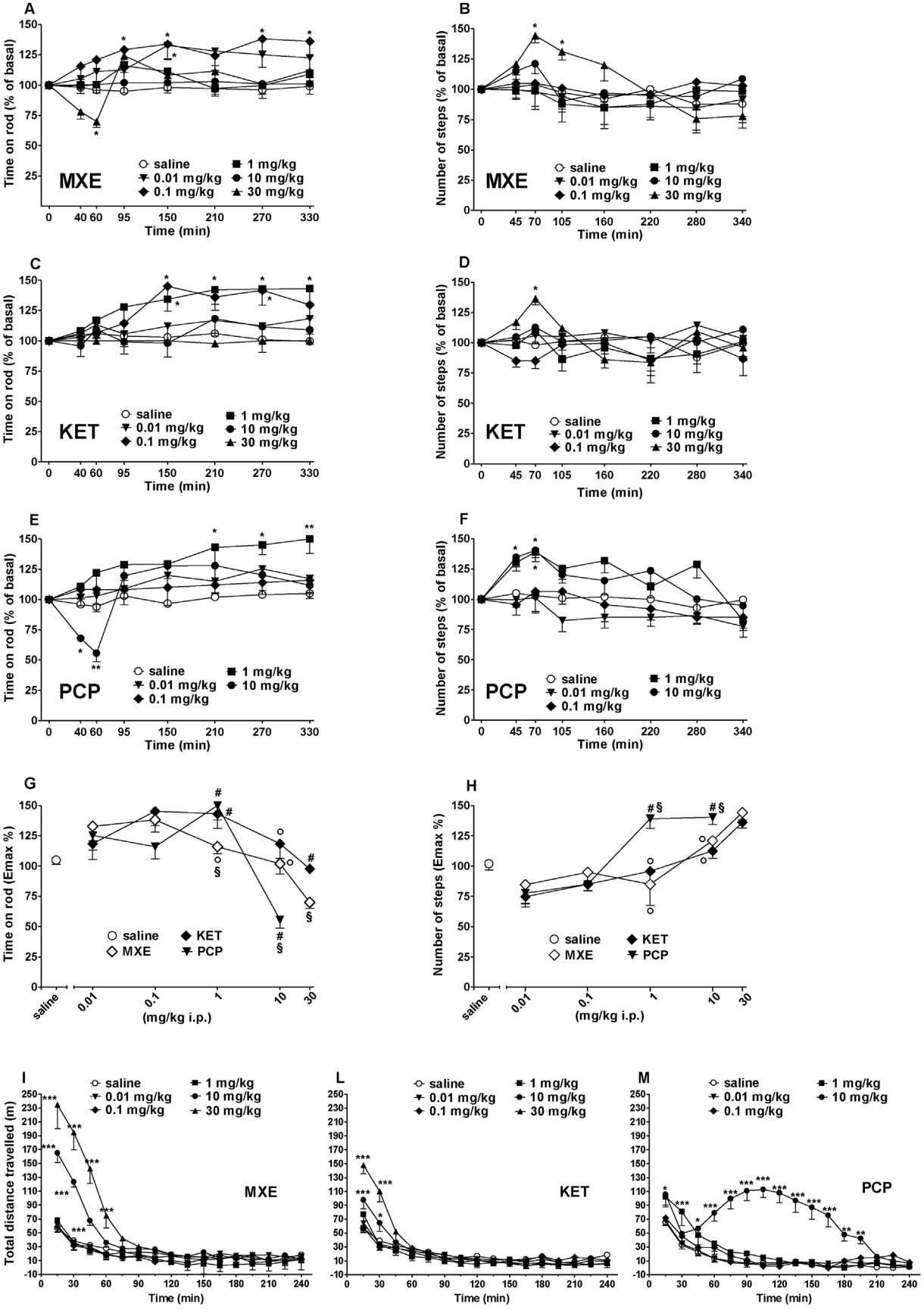

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Fig. 4. *Panels A-H*: effect of MXE (0.01–30 mg/kg; A,B), KET (0.01–30 mg/kg; C,D) and PCP (0.01–10 mg/kg; E,F), on the accelerod (*left*) and drag test (*right*) in the mouse and comparison of the maximum effect observed in 5 h on accelerod (G) and drag test (H). Data are expressed as percentage of baseline and represent the mean ± SEM of 8 mice/group. Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons for the dose-response curve of each compound at different times (A–F), while the statistical analysis of the maximum effect observed in 5 h (G,H) was performed with one-way ANOVA followed by Tukey's test for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001 versus saline; #p < 0.05 versus MXE; °p < 0.05 versus PCP; §p < 0.05 versus KET. *Panels I-M*: effect of MXE and KET (0.01–30 mg/kg) and PCP (0.01–10 mg/kg) on the total distance travelled. Data are expressed as meters travelled and represent the mean ± SEM of 10 mice/group. Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001 versus saline.

Lower doses of MXE (1 mg/kg), KET (1 mg/kg) and PCP (0.1 mg/kg) did not change PPI response in mice (see Table S1, Supplemental Materials). Startle Amplitude was not affected by administration of PCP (0.1 and 1 mg/kg i.p.), KET and MXE (1 and 10 mg/kg i.p.) both at 15 min and 4-h (see Table S1, Supplemental Materials).

3.1.2.4. Evaluation of pain induced by mechanical and thermal stimuli. All drugs differently affected mechanical (Fig. 3A,C,E) and thermal (Fig. 3B,D,F) pain threshold in mice over the 5-h observation. MXE significantly ($p < 0.0001$) and dose-dependently increased the threshold to acute mechanical [(Fig. 3A); ANOVA, main effect of treatment ($F_{(5,294)} = 36.24$), time ($F_{(6,294)} = 13.15$) and time × treatment interaction ($F_{(30,294)} = 4.301$)] and thermal [(Fig. 3B); ANOVA, main effect of treatment ($F_{(5,294)} = 7.913$), time ($F_{(6,294)} = 7.270$) and time × treatment interaction ($F_{(30,294)} = 1.842$)] pain stimuli in a more rapid and robust way than KET (Fig. 3C and D). In fact, only at 30 mg/kg KET transiently and modestly increased the threshold to acute mechanical [(Fig. 3C); ANOVA, main effect of treatment ($F_{(5,294)} = 3.096$), time ($F_{(6,294)} = 10.52$) and time × treatment interaction ($F_{(30,294)} = 1.383$)] and thermal [(Fig. 3D; ANOVA, main effect of treatment ($F_{(5,294)} = 6.480$), time ($F_{(6,294)} = 3.771$) and time × treatment interaction ($F_{(30.,294)} = 0.6208$)] pain stimuli. PCP significantly ($p < 0.0001$) increased the threshold to acute mechanical [(Fig. 3E); ANOVA, main effect of treatment ($F_{(4,245)} = 15.41$), time ($F_{(6,245)} = 12.53$) and time × treatment interaction ($F_{(24,245)} = 3.936$)] and thermal [(Fig. 3F); ANOVA, main effect of treatment ($F_{(4,245)} = 30.95$), time ($F_{(6,245)} = 5.428$) and time × treatment interaction ($F_{(24,245)} = 1.342$)] pain stimuli in a dose-dependent manner. The comparison of the maximum effects caused by the three compounds highlights that PCP 10 mg/kg was more effective than MXE and KET 10 mg/kg and that MXE 30 mg/kg was more active than KET in increasing the threshold to both the acute mechanical (Fig. 3G; effect of treatment: $F_{(14,119)} = 9.579$, $p < 0.0001$) and thermal (Fig. 3F; effect of treatment $F_{(14,119)} = 5.720$, $p < 0.0001$) pain stimuli.

3.1.2.5. Motor activity assessment. As shown in Fig. 4, saline injection did not change motor activity in the accelerod (panels A,C,E) and drag (panels B,D,F) test in mice over the 5-h observation. At 0.01 mg/kg, MXE transiently facilitated the motor performance of mice in both test (Fig. 4A and B). However, similarly to PCP, at the highest dose tested (30 mg/kg) MXE transiently inhibited the motor performance on the accelerod (Fig. 4A; ANOVA, main effect of treatment ($F_{(5,336)} = 8.991$), time ($F_{(7,336)} = 6.056$) and time × treatment interaction ($F_{(35,336)} = 1.405$); post-hoc Tukey's, $p < 0.0001$), and increased the number of steps performed with the front paws in the drag test (Fig. 4B; ANOVA, main effect of treatment ($F_{(5,336)} = 2.813$), time ($F_{(7,336)} = 2.992$) and time × treatment interaction ($F_{(35,336)} = 1.152$); post-hoc Tukey's, $p < 0.005$). KET modestly and dose-dependently facilitated the motor performance on the accelerod (Fig. 4C; ANOVA, main effect of treatment ($F_{(5,336)} = 11.17$), time ($F_{(7,336)} = 3.711$) and time × treatment interaction ($F_{(35,336)} = 0.9387$); post-hoc Tukey's, $p < 0.0001$) and at 30 mg/kg transiently increased the number of steps performed with the front paws in the drag test (Fig. 4D; ANOVA, main effect of treatment ($F_{(5,336)} = 2.415$), time ($F_{(7,336)} = 0.9157$) and time × treatment interaction ($F_{(35.336)} = 0.9392$); post-hoc Tukey's, $p < 0.05$). PCP long facilitated at 1 mg/kg and transiently inhibited at 10 mg/kg the motor performance on the accelerod (Fig. 4E; ANOVA, main effect of treatment ($F_{(4,280)} = 9.946$), time ($F_{(7,280)} = 6.317$) and time × treatment interaction ($F_{(28,80)} = 1.573$); post-hoc Tukey's, $p < 0.0001$) and significantly ($p < 0.0001$) increased the number of steps performed with the front paws of the mice at 1 and 10 mg/kg [(Fig. 4F); ANOVA, main effect of treatment ($F_{(4,280)} = 16.30$), time ($F_{(7,280)} = 5.415$) and time × treatment interaction ($F_{(28,280)} = 1.241$)]. The comparison of the maximum effects caused by the three compounds highlights that PCP 10 mg/kg was more potent than MXE and KET in inhibiting the motor performance on the accelerod while MXE (0.01 and 0.1 mg/kg) was more active than KET (0.1 and 1 mg/kg) in facilitating motor activity on the accelerod (Fig. 4G; effect of treatment: $F_{(14,119)} = 7.323$, $p < 0.0001$). Moreover, PCP was more potent than MXE and KET in facilitating the drag motor performance of mice (Fig. 4H; effect of treatment: $F_{(14,119)} = 7.358$, $p < 0.0001$).

All three compounds also facilitated the spontaneous locomotor activity in mice (Fig. 4, panels I–M). MEX increased in a significant ($p < 0.005$) and dose-dependent manner the spontaneous locomotion in mice at 10 and 30 mg/kg and the effect persisted up to 60 min [(Fig. 4I); ANOVA, main effect of treatment ($F_{(2,336)} = 1.514$), time ($F_{(15,336)} = 20.02$) and time × treatment interaction ($F_{(30,336)} = 0.4208$)]. Similarly, KET induced hypermotility at 10 and 30 mg/kg but the effect lasted for 30 min only [(Fig. 4L; ANOVA, main effect of treatment ($F_{(2,336)} = 1.514$), time ($F_{(15,336)} = 20.02$) and time × treatment interaction ($F_{(30,336)} = 0.4208$, $p = 0.9972$); post-hoc Tukey's, $p < 0.005$)]. Notably, MXE was more effective than KET in affecting spontaneous locomotor activity in mice. The stimulating motor effect of PCP was transient at 1 mg/kg and long lasting at 10 mg/kg (Fig. 4M, ANOVA, main effect of treatment ($F_{(2,336)} = 6.082$), time ($F_{(15,336)} = 33.34$) and time × treatment interaction ($F_{(30,336)} = 0.6311$); post-hoc Tukey's, $p < 0.005$).

3.2. Cardiorespiratory analysis

As shown in Fig. 5, basal heart rate (640 ± 12 bpm) was decreased by: (i) MEX 30 mg/kg [(panel A); ~40% reduction at 15-min; significant ($p < 0.0001$) effect of treatment ($F_{(2,1512)} = 201.0$), time ($F_{(71,1512)} = 5.207$) and time × treatment interaction ($F_{(142,1512)} = 4.676$)]; (ii) KET 30 mg/kg [(panel B); ~35% reduction at 10-min; significant ($p < 0.0001$) effect of treatment ($F_{(2,1512)} = 68.21$), time ($F_{(71,1512)} = 1.984$) and time × treatment interaction ($F_{(142,1512)} = 2.818$)]; (iii) PCP 10 mg/kg [(panel C); ~30% reduction at 20-min; significant ($p < 0.0001$) effect of treatment: ($F_{(2,1512)} = 63.12$), time ($F_{(71,1512)} = 2.081$) and time × treatment interaction ($F_{(142,1512)} = 1.918$)]. The inhibition of heart rate was transient and lasted about 95-min for MXE and PCP and 60-min for KET before returning to baseline values.

Basal breath rate activity (120 ± 11 brpm) was decreased by (i) MEX 30 mg/kg [(panel D); ~35% reduction at 30-min, significant ($p < 0.0001$) effect of treatment ($F_{(2,1512)} = 120.5$), time ($F_{(71,1512)} = 2.573$) and time × treatment interaction ($F_{(142,1512)} = 2.297$)]; (ii) KET 30 mg/kg [(panel E); ~30% reduction at 10-min, significant ($p < 0.0001$) effect of treatment

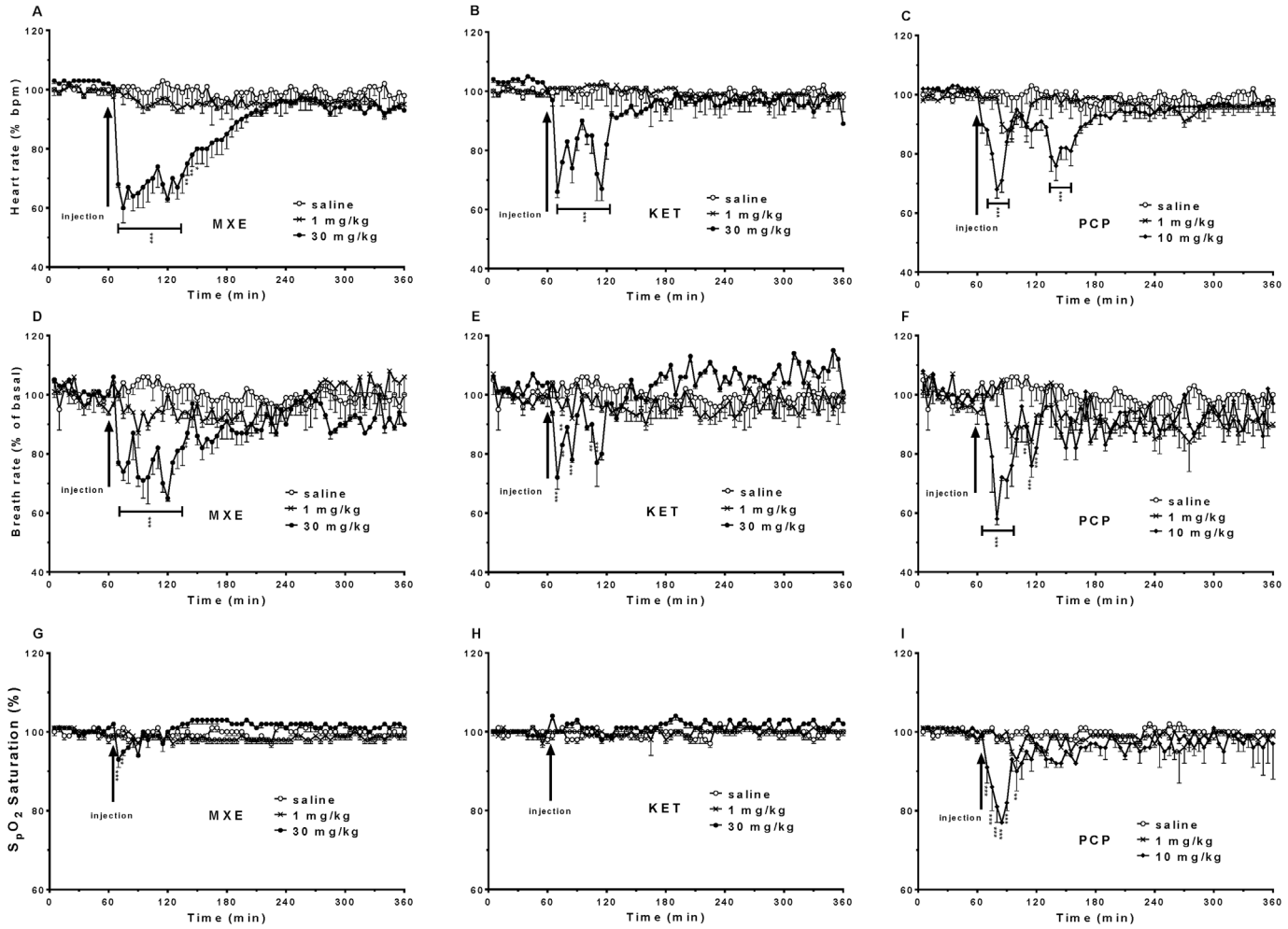

Fig. 5. Effect of MXE (1 and 30 mg/kg; *left*), KET (1 and 30 mg/kg; *middle*) and PCP (1 and 10 mg/kg; *right*) on the heart rate (A–C), the breath rate (D–F) and the oxygen arterial saturation (G–I). Data are expressed as percentage of basal value (heart and breath rate) and as percentage of oxygen blood saturation (% SpO_2 saturation) and represent the mean ± SEM of 4 mice/group. Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons for the dose response curve of each compound at different times.

($F_{(2,1512)} = 31.57$), time ($F_{(71,1512)} = 1.883$) and time × treatment interaction ($F_{(142,1512)} = 2.784$)]; (iii) by PCP 10 mg/kg [(panel F); ~42% reduction at 20-min; significant ($p < 0.0001$) effect of treatment ($F_{(2,1512)} = 84.17$), time ($F_{(71,1512)} = 1.997$) and time × treatment interaction ($F_{(142,1512)} = 1.972$)]. The inhibition of breath rate was transient and lasted about 70-min for MXE, 55-min for KET and 60-min for PCP before returning to baseline values.

Basal SpO_2 saturation (99.2 ± 1.4%) was transiently decreased by MXE 30 mg/kg [(panel G); ~7% reduction at 10-min; significant ($p < 0.0001$) effect of treatment ($F_{(2,1512)} = 110$), time ($F_{(71,1512)} = 2.682$) and time × treatment interaction ($F_{(142,1512)} = 2.730$)] and PCP 10 mg/kg [(panel I); ~23% reduction at 25-min; significant ($p < 0.0001$) effect of treatment ($F_{(2,1512)} = 71.74$), time ($F_{(71,1512)} = 3.089$) and time × treatment interaction ($F_{(142,1512)} = 2.327$)], while KET was ineffective (panel H).

3.3. Blood pressure analysis

As shown in Fig. 6, basal systolic pressure (104 ± 5 mm/Hg) was increased by (i) MEX 30 mg/kg [(panel A); significant ($p < 0.0001$) effect of treatment ($F_{(2,1470)} = 146.8$), time ($F_{(69,1470)} = 3.002$) and time × treatment interaction ($F_{(138,1470)} = 1.799$)]; (ii) KET 30 mg/kg [(panel B; significant ($p < 0.0001$) effect of treatment ($F_{(2,1470)} = 724.8$), time ($F_{69,1470)} = 4.778$) and time × treatment interaction ($F_{(138,1470)} = 2.775$)]; PCP 10 mg/kg [(panel C); significant ($p < 0.0001$) effect of treatment: ($F_{(2,1470)} = 1295$), time ($F_{(69,1470)} = 7.741$) and time × treatment interaction ($F_{(138,1470)} = 5.168$)]. The increase of systolic blood pressure was prompt and persisted up to 60-min after drugs injection.

Basal diastolic pressure (68 ± 5 mm/Hg) was increased by (i) MEX 30 mg/kg [(panel D); significant ($p < 0.0001$) effect of treatment ($F_{(2,1470)} = 39.12$), time ($F_{(69,1470)} = 6.956$) and time × treatment interaction ($F_{(138,1470)} = 4.399$)]; (ii) KET 30 mg/kg [(panel E); significant ($p < 0.0001$) effect of treatment ($F_{(2,1470)} = 133.2$), time ($F_{(69,1470)} = 5.317$) and time × treatment interaction ($F_{(138,1470)} = 2.626$)]; (iii) PCP 10 mg/kg [(panel F); significant ($p < 0.0001$) effect of treatment: ($F_{(2,1470)} = 33.78$), time ($F_{(69,1470)} = 5.112$) and time × treatment interaction ($F_{(138,1470)} = 3.018$)]. The increase of diastolic blood pressure was prompt for MXE (96.2 ± 9.1 mm/Hg at 13-min after drug injection), while delayed for PCP (107 ± 10.6 mm/Hg at 30-min) and KET (103.5 ± 5 mm/Hg at 37-min). The effect induced by KET and MXE persisted up to the end of experimental section while that of PCP was transient (48-min).

4. Discussion

This study provides the first direct comparison of the in vivo effects of MXE with the two parental compounds, PCP and KET. Similarly to other newly emerged arylcyclohexylamine, MXE shares with KET and

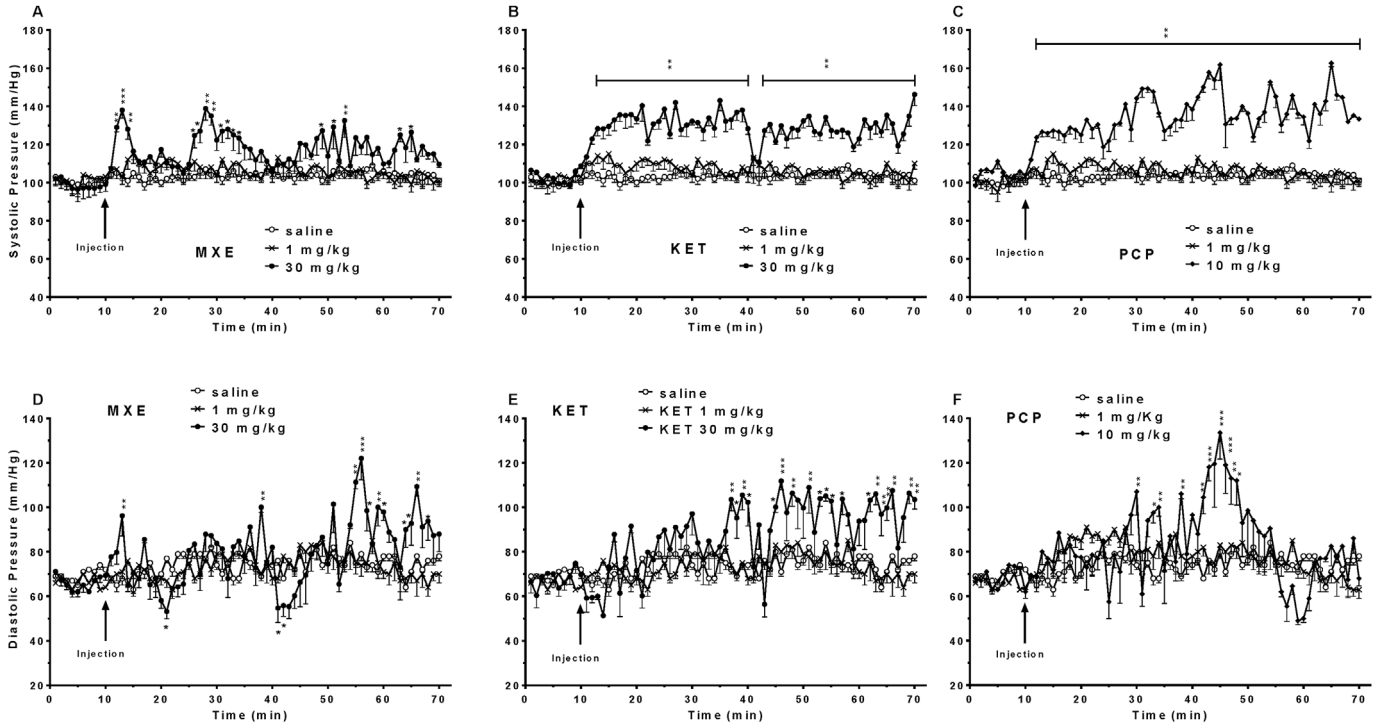

Fig. 6. Effect of MXE (1 and 30 mg/kg; *left*), KET (1 and 30 mg/kg; *middle*) and PCP (1 and 10 mg/kg; *right*) on the systolic (L–N) and diastolic (O–Q) blood pressure in mice. Data are expressed as absolute values (mm/Hg) and represent the mean ± SEM of 8 mice/group. Statistical analysis was performed by two-way ANOVA followed by the Bonferroni's test for multiple comparisons for the dose response curve of each compound at different times. *p < 0.05, **p < 0.01, ***p < 0.001 versus saline.

PCP structural features but also a number of pharmacological effects (Zanda et al., 2016). Use of MXE by humans has been recently associated to acute neurological (Elian and Hackett, 2014; Fassette and Martinez, 2016) and cerebellar toxicity (Shields et al., 2012), including motor incoordination and psychomotor agitation (Craig and Loeffler, 2014). Here we show significant alterations induced in mice by acute systemic administration of different doses of MXE on neurological, sensorimotor and cardiorespiratory parameters, with only partial overlapping with those induced by the same doses of KET or PCP. Interestingly, although PCP was behaviorally active at lower doses than KET and MXE, some effects induced by MXE resulted to be the most intense or frequent, e.g. rotations. These MXE-induced neurological alterations are in line with the effects of high doses of MXE reported by users in drug fora or described in clinical case reports of intoxications (Wood et al., 2012; Craig and Loeffler, 2014; Zawilska, 2014) and likely contribute to the severe general impairment observed in subjects driving under the influence of the drug (Elian and Hackett, 2014; Fassette and Martinez, 2016). In line with our findings, aggressiveness or violent behavior in MXE users has been reported in one young men only (Łukasik-Głebocka et al., 2013), and KET and PCP were shown not to induce aggressive behavior in animals (Tyler and Miczek, 1982; Takahashi et al., 1984).

We showed for the first time significant alterations induced by MXE on sensory function processing, which resemble those observed in intoxicated patients or reported by MXE users (Kjellgren and Jonsson, 2013) and persisted for hours when MXE was administered at high doses. The direct comparison of the three drugs revealed that MXE was less potent than PCP but more potent than KET in reducing visual object response, and more potent than PCP in reducing visual placing response. Notably, MXE reduced the acoustic response in mice only at the highest dose tested (30 mg/kg), but its effect was stronger and lasted longer than that caused by KET. A common feature of dissociative drugs like PCP, KET and other NMDA receptor antagonists is the disruption of the prepulse inhibition (PPI) of the acoustic startle reflex (Mansbach, 1991), an operational measure of the sensory gating (or filtering), a process through which a subject filters irrelevant information from an external milieu. Impairment in PPI is a hallmark of schizophrenia (Javitt and Zukin, 1991) and MXE is known to induce psychosis and dissociative-like states in humans (de Jong et al., 2014). In the present study, a transient disruption of the prepulse inhibition was observed 15-min following MXE, KET and PCP administration, but not after 4-h. Once again, the effect of PCP on sensorimotor gating was evident at doses lower than MXE and KET, the two latter drugs showing similar efficacy in disrupting PPI in mice. Psychotomimetic drugs like PCP and KET are well known to disrupt PPI in rodents (Curzon and Decker, 1998; Geyer et al., 2001) and, more recently, MXE was found to disrupt PPI in rats (Halberstadt et al., 2016). In agreement with the latter, we found that low doses of MXE did not affect PPI while higher doses significantly attenuated PPI in mice, which confirms the ability of MXE to induce sensory disturbances and information processing deficits that may underlie its dissociative/psychotic effects in humans. However, present observations add the novel information that drug effects were no more evident after 4-h from administration, thus revealing the transient nature of drug-induced disruption of PPI. A decreased PPI has been consistently associated to neuropsychiatric disorders like schizophrenia (Powell et al., 2009) that are linked, among others, to a hypofunctionality of NMDA receptors (Lau and Zukin, 2007). Notably, the rank order of potency showed by the three compounds in our PPI paradigm (PCP > MXE > KET) parallels their affinity for the PCP binding site on the NMDA receptor (Roth et al., 2013; Halberstadt et al., 2016).

Consistent with the hypothesis that NMDA receptors are crucial for pain perception in humans (Hewitt, 2003) and that drugs blocking NMDA receptors may represent important pharmacological tools to treat some forms of pain (Schug and Goddard, 2014), we show that MXE dose-dependently increased the threshold to acute mechanical and thermal pain stimulus in mice in a way more effective (mechanical pain) or similar (thermal pain) to KET but less effective than PCP,

which confirms its ability to increase the thermal nociceptive threshold as recently reported in rats (Zanda et al., 2017).

When looking at motor skills and performance after drugs administration we found that, although with distinct temporal patterns, all drugs showed a transient facilitating motor effect in the drag test, while in the accelerod test MXE and PCP, but not KET, exhibited a biphasic action, with low doses typically facilitating and higher doses inhibiting motor performance. Intriguingly, MXE resulted more potent than KET. The overall facilitating motor effects of the three drugs were confirmed by the increased total distance travelled by treated mice with respect to control (saline-treated) mice, with KET and MEX showing a dose-dependent effect and PCP showing the most enduring effect. Notably, MXE was once again more effective than KET in facilitating locomotion in mice. KET (10 and 30 mg/kg) and PCP (10 mg/kg) were previous reported to induce transient hypermotility in rats (Castagné et al., 2012; Castellani and Adams, 1981). However, contrary to our finding of a maximal effect of MXE (30 mg/kg and, to lesser extent, 10 mg/kg) on the distance travelled by mice, Berquist and colleagues have recently reported no significant effect of MXE (1–30 mg/kg) on motor activity (Berquist et al., 2017). Such an incongruity could be ascribed to (i) the different strain of animals (CD1 vs NIH Swiss mice, respectively), (ii) the different experimental procedure (in the previous study involving anesthesia for surgical implantation of a radiotelemetry probe) or (iii) the acute testing in drug-naïve animals (our study) vs repeated testing after repeated drug treatment. On the other hand, our findings are in line with MXE (10 mg/kg)-induced hypermotility described by Halberstadt et al. (2016) and Horsley et al. (2016) and suggest that like PCP (Mouri et al., 2007), KET (Imre et al., 2006) and other NMDA receptor antagonists (Danysz et al., 1994), MXE can induce locomotion stimulation in rodents, which is commonly considered a preclinical index of the psychotomimetic effects of these drugs.

In 2012, Paul Dargan and colleagues described a case series of patients that presented to hospitals catatonic and with tachycardia and hypertension (Wood et al., 2012). Few years later, sympathomimetic effects were reported in MXE users, among which were tachycardia, hypertension and respiratory depression (Imbert et al., 2014; Zawilska, 2014; Adamowicz and Zuba, 2015). Cardiorespiratory alterations have been long documented following administration of KET (Lippmann et al., 1983), PCP (Matsuzaki et al., 1984) and other NMDA receptor antagonists (Abrahams et al., 1993). Here we demonstrated that all drugs differentially, but significantly, altered the cardiorespiratory parameters measured when administered to mice at high doses. As expected, PCP (10 mg/kg) was the most potent in reducing both the basal heart and breath rate; yet, the inhibitory effect on heart rate was longer for MXE and PCP with respect to KET, while that on the breath rate was longer-lasting for MXE than for PCP and KET. Different were also the effects on SpO_2 saturation, which was transiently decreased by PCP (10 mg/kg) and, to a lesser extent, by MXE (30 mg/kg) but unaffected by KET administration. The increased basal systolic and diastolic pressure observed after administration of PCP (10 mg/kg), KET (30 mg/kg) and MXE (30 mg/kg) is in line with clinical case reports describing intoxicated patients with hypertension and tachycardia following use of MXE (Łukasik-Głebocka et al., 2013; Thornton et al., 2017), KET (Kalsi et al., 2011), PCP (Akmal et al., 1981), methoxylated-PCP analogs (Bäckberg et al., 2015) or other novel psychoactive substances (Hondebrink et al., 2015). Findings that MXE may induce potent and long-lasting alterations in cardiorespiratory functions have important clinical implications while managing intoxication cases presenting at hospitals or at emergency units (Wiergowski et al., 2014).

5. Conclusions

This study provides the first direct, systematic comparison of the effects of the NPS methoxetamine (MXE), ketamine (KET) and phencyclidine (PCP) in a battery of behavioral tests widely validated in studies of “safety-pharmacology” for the preclinical characterization of new psychoactive drugs in rodents. We show that acute administration of MXE induces in vivo effects qualitatively similar to PCP and KET on neurological and sensorimotor responses and cardiorespiratory functions in mice. Yet, quantitative differences were noted, with PCP typically producing more robust effects than MXE and KET, and MXE producing more long-lasting effects that the other two compounds. Altogether, our findings clearly indicate the need for more research in the field of dissociative drugs and for more information about the consequences of their use to make social and health professionals aware of their acute intoxicating effects.

Funding and disclosure

This research has been funded by the Drug Policies Department; Presidency of the Council of Ministers, Italy (project NS-Drugs to M. Marti) by local funds from the University of Ferrara (FAR 2016 and FAR 2017 to M. Marti), by funds from the ICS Maugeri, Pavia, Italy (NPS project to C. Locatelli) and by FIRB 2012 from the Italian Ministry of the University (Grant no. RBFR12LDOW to F. De-Giorgio; Institute of Public Health, *Section of Legal Medicine,* Catholic University of Rome, Italy). The authors declare no conflict of interest disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuropharm.2018.08.017.

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