frontiers in Psychiatry

ORIGINAL RESEARCH
published: 17 November 2017
doi: 10.3389/fpsyt.2017.00232

Pharmacokinetic, Ambulatory, and Hyperthermic Effects of 3,4-Methylenedioxy-*N*-Methylcathinone (Methylone) in Rats

*Kristýna Štefková[1], Monika Židková[2], Rachel R. Horsley[1], Nikola Pinterová[1,3], Klára Šíchová[1], Libor Uttl[1,4], Marie Balíková[2], Hynek Danda[1,3], Martin Kuchař[1,5] and Tomáš Páleníček[1,3]**

[1] Department of Experimental Neurobiology, National Institute of Mental Health, Klecany, Czechia, [2] First Faculty of Medicine, Institute of Forensic Medicine and Toxicology, Charles University and General University Hospital in Prague, Prague, Czechia, [3] Third Faculty of Medicine, Charles University in Prague, Prague, Czechia, [4] Department of Physiology, Faculty of Science, Charles University, Prague, Czechia, [5] Forensic Laboratory of Biologically Active Compounds, Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czechia

OPEN ACCESS

Edited by:
Liana Fattore, Consiglio Nazionale Delle Ricerche (CNR), Italy

Reviewed by:
Mariaelvina Sala, Università degli Studi di Milano, Italy
Steven J. Simmons, Temple University, United States

***Correspondence:**
Tomáš Páleníček
tomas.palenicek@nudz.cz

Specialty section:
This article was submitted to Addictive Disorders, a section of the journal Frontiers in Psychiatry

Received: *15 September 2017*
Accepted: *31 October 2017*
Published: *17 November 2017*

Citation:
Štefková K, Židková M, Horsley RR, Pinterová N, Šíchová K, Uttl L, Balíková M, Danda H, Kuchař M and Páleníček T (2017) Pharmacokinetic, Ambulatory, and Hyperthermic Effects of 3,4-Methylenedioxy-N-Methylcathinone (Methylone) in Rats. Front. Psychiatry 8:232. doi: 10.3389/fpsyt.2017.00232

Methylone (3,4-methylenedioxy-*N*-methylcathinone) is a synthetic cathinone analog of the recreational drug ecstasy. Although it is marketed to recreational users as relatively safe, fatalities due to hyperthermia, serotonin syndrome, and multi-organ system failure have been reported. Since psychopharmacological data remain scarce, we have focused our research on pharmacokinetics, and on a detailed evaluation of temporal effects of methylone and its metabolite nor-methylone on behavior and body temperature in rats. Methylone [5, 10, 20, and 40 mg/kg subcutaneously (s.c.)] and nor-methylone (10 mg/kg s.c.) were used in adolescent male Wistar rats across three behavioral/physiological procedures and in two temporal windows from administration (15 and 60 min) in order to test: locomotor effects in the open field, sensorimotor gating in the test of prepulse inhibition (PPI), and effects on rectal temperature in individually and group-housed rats. Serum and brain pharmacokinetics after 10 mg/kg s.c. over 8 h were analyzed using liquid chromatography mass spectrometry. Serum and brain levels of methylone and nor-methylone peaked at 30 min after administration, both drugs readily penetrated the brain with serum: brain ratio 1:7.97. Methylone dose-dependently increased overall locomotion. It also decrease the amount of time spent in the center of open field arena in dose 20 mg/kg and additionally this dose induced stereotyped circling around the arena walls. The maximum of effects corresponded to the peak of its brain concentrations. Nor-methylone had approximately the same behavioral potency. Methylone also has weak potency to disturb PPI. Behavioral testing was not performed with 40 mg/kg, because it was surprisingly lethal to some animals. Methylone 10 and 20 mg/kg s.c. induced hyperthermic reaction which was more pronounced in group-housed condition relative to individually housed rats. To conclude, methylone increased exploration and/or decreased anxiety in the open field arena and with nor-methylone had short duration of action with effects typical for mixed indirect dopamine–serotonin agonists such as 3,4-metyhlenedioxymethamphetamine

(MDMA) or amphetamine. Given the fact that the toxicity was even higher than the known for MDMA and that it can cause hyperthermia it possess a threat to users with the risk for serotonin syndrome especially when used in crowded conditions.

Keywords: methylone, bk-3,4-metyhlenedioxymethamphetamine, nor-methylone, novel psychoactive substances, cathinones, behavior, pharmacokinetics, metabolites

INTRODUCTION

Methylone (3,4-methylenedioxy-*N*-methylcathinone, also known as MDMC, bk-MDMA, M1) belongs to the group of new psychoactive substances called synthetic cathinones often also termed as β-keto amphetamines or the new generation of designer phenethylamines (1). This β-keto analog of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") was first synthetized in 1996 as an antidepressant and anti-Parkinsonian agent (2) but was never used for therapeutic purposes; instead, it gained popularity as a recreational "legal high" owing to its MDMA-cocaine-like effects (3). Methylone users describe their subjective experience as feeling stimulated, with a great need to socialize, spiritual, and empathic connection. Methylone first appeared in 2004 on the illicit drug market (in the Netherlands) and quickly became commonly available and easily obtainable (4), leading to extensive abuse worldwide (5). European Monitoring Center for Drugs and Drug Addiction and Europol have monitored methylone since 2005 (6) and starting in 2011, methylone was reclassified as Schedule I under the Controlled Substance Act in the Unites States (7). In the United Kingdom, methylone has been illegal since 2010. There have been a number of reports of methylone toxicity and even fatal overdoses have been registered. The causes of death include hyperthermia where body temperature elevated up to 41.7°C as a core symptom of serotonin syndrome, metabolic acidosis, and multi-organ system failure (8, 9). Even though the popularity of methylone among users as well as its availability on the gray/black market is widespread, the relevant scientific data are still relatively scarce, and there are no published data on behavioral effects of nor-methylone. Therefore, we investigated effects of these substances on behavior, pharmacokinetics, and body temperature.

Methylone only differs from MDMA by the presence of a ketone at the benzylic position. Based on their structural similarity, and, in turn, similar mechanism of action, comparable effects on behavior, and neurochemistry could be postulated (10). *In vitro* neuropharmacological studies in rat's brain, synaptosomes reported methylone as a non-selective inhibitor of the dopamine, norepinephrine, and serotonin transporters (DAT, NET, and SERT, respectively). Further, methylone *via* blocking re-uptake evokes high-releasing activity of all monoamines (dopamine, norepinephrine, and serotonin) (10, 11). The ratio of DAT:SERT inhibition is 3.3 which suggests that methylone has a high-abuse potential similar to cocaine (DAT:SERT ratio 3.1) (3, 12) as has been also confirmed in behavioral tests (13, 14). On the other hand, in discrimination studies, methylone substituted for MDMA indicating a similar profile (and subjective effects) to this serotonergic compound (15). Thus far, behavioral research has shown that methylone increases locomotor activity, an effect which was inhibited by both dopamine (D2) or serotonin (5-HT_{2A}) receptor antagonists (16–19). Repeated administration of methylone, similar to acute effects of other MDMA-like compounds, induced hyperthermia in rats: an effect typically mediated by serotonin and typically associated with acute toxicity of sertonergic drugs (20–22). On the other hand, simultaneous study of Javadi-Paydar et al. (19) showed no change in body temperature in rats treated by methylone. Methylone is the subject of extensive metabolism in the liver at cytochrome P450 (isoenzymes CYP2D6, CYP1A2, CYP2B6, and CYP2C19) with major primary metabolite nor-methylone, which are subsequently excreted to urine unchanged or in their conjugated forms (23, 24). Other metabolites (dihydroxymethcathinone, *N*-hydroxy-methylone, and dihydro-methylone) were also detected and to date no information about the biological activity of these have been published (23).

Our primary intention was to describe in detail temporal profile of methylone's effects in behavioral tests alongside pharmacokinetics and the effects on body temperature to evaluate its eventual serotonergic toxicity related to hyperthermia. An added value of our study was in the evaluation of the effects of nor-methylone as the primary metabolite in the same series of behavioral tasks. Finally, since we have performed series of experiments in our laboratory with related cathinones this allows us to make indirect comparisons between those (21, 25–27).

In the behavioral study, to test its effects on locomotion, exploratory activity, anxiety, and stereoytypy the open field test was used, further on effects on sensorimotor gating indicative of its psychomimetic potential were tested in the test of prepulse inhibition of acoustic startle response (PPI ASR) (28). To cover the peak effects as well as possible late onset of changes in these tests, we tested both paradigms in two temporal windows (15 and 60 min) after drug administration. To link the behavioral data to serum and brain levels of methylone, the samples for pharmacokinetics were collected from animals involved in behavioral experiments. According to its structural and pharmacological similarity with MDMA and cocaine, we hypothesized that it will have similar behavioral profile (stimulatory and disruptive) but shorter duration of action. Finally, as it has been shown previously for other compounds, environmental factors, especially an effect of "individually/group-housed" rats (e.g., people dancing in a crowded clubs) significantly increase the risk of hyperthermic reaction, we also tested the effect of isolation and aggregation on hyperthermic effects of methylone.

MATERIALS AND METHODS

Animals

All animals were male Wistar rats (Hannover breed, obtained from Konárovice, Czech Republic) weighing 200–250 g and aged

8 weeks at the start of testing. Rats were housed two per cage under controlled temperature (22 ± 2°C) and humidity (30–70%) with food pellets and water freely available. Lights were on from 6:00 to 18:00 h and all experiments were carried out between 7:00 and 13:00 h, except the temperature study where the test lasted until 17:00 h. Animals were allowed 7–10 days to habituate to laboratory conditions before being used in experiments, during which they were weighed twice and handled four times. All behavioral experiments were conducted in the same standard conditions (temperature and humidity) as in the animal housing facility. Each experimental group for behavioral and temperature studies included 10 animals and each animal was tested only once with the exception that (to reduce animal use) rats from behavioral experiments were used for subsequent pharmacokinetic sampling [for pharmacokinetic studies, $n = 8$ (for methylone) and $n = 5$ (for nor-methylone) per experimental group]. All procedures were conducted in accordance with the principles of laboratory animal care of the National Committee for the Care and Use of Laboratory Animals (Czech Republic), and according to Guidelines of the European Union (86/609/EU). The protocol was approved by the National Committee for the Care and Use of Laboratory Animals (Czech Republic) under the number: MEYSCR-27527/2012-31.

Drugs and Chemicals

3,4-Methylenedioxy-*N*-methylcathinone (methylone) was purchased *via* the internet and subsequently purified and converted to a hydrochloride (HCl) by Alfarma s.r.o. (Czech Republic). The resulting methylone was certified to be of 99.18% purity (analyzed by infrared spectroscopy) and also served as a reference standard for pharmacokinetic analyses using liquid chromatography. Nor-methylone was synthesized in the Forensic Laboratory of Biologically Active Substances (University of Chemistry and Technology Prague, Czech Republic) in a purity of 99.18%. Internal standards for quantitative liquid chromatography/mass spectrometry (LC/MS) assays were deuterated MDA-D2. HCl with the purity 99.7% (Lipomed, Inc., Switzerland). Reference standards for confirmation of metabolites by LC/HRMS (high-resolution mass spectrometry) and gas chromatography/mass spectrometry were synthesized with purity within 97.5–89.3% (Institute of Chemical Technology, Department of Organic Chemistry, Prague). β-Glucuronidase type HP-2 from Helix Pomatia, EC 3.2.1.31 (184,973 U/ml) was purchased from Sigma-Aldrich, Prague. Extraction columns Bond Elut Certify 50 mg/3 ml were supplied by Labio s.r.o., Olomouc. Other chemicals used for laboratory purposes were of analytical grade purity. Methylone was stored in dry and dark place and dissolved in physiological saline (0.9% NaCl) immediately before experiments.

Dosage

The methylone doses used in the present study were estimated according to the reported usage by humans and according to our previous studies with entactogens MDMA, para-methoxymethamphetamine (PMMA), and 2C-B (4-bromo-2,5-dimethoxyphenylethylamine). Doses were selected to range from those that: (1) at the lower end are close to those used by humans, to (2) higher doses that might produce significant acute non-lethal toxicity, and (3) with respect to our previous analogous experiments with MDMA, PMMA, and 2C-B (21, 29–31). The treatment range for methylone was set to be 5, 10, 20, and 40 mg/kg, nor-methylone at 10 mg/kg for behavioral experiments. In behavioral experiments (open field, PPI ASR) nor-methylone was tested only in 15 min testing onset. Doses for pharmacokinetic [10 mg/kg subcutaneously (s.c.)] and temperature experiments (10 and 20 mg/kg s.c.) were selected according to the inherent sensitivity of the analytical LC/HRMS procedure utilized and according to the effectiveness in behavioral tasks (effects body temperature). For the pharmacokinetic study, a single bolus of methylone 10 mg/kg s.c. was administered, subsequently animals were decapitated after 30, 60, 120, 240, or 480 min. Additional pharmacokinetic data with the same design were also obtained for nor-methylone 10 mg/kg s.c. Separated sera and whole brains were kept at −20°C until the toxicological analyses. Both drugs were dissolved in a volume of 2 ml/kg and administered s.c. (for comparability with previous studies) in all cases.

Pharmacokinetic Analyses

Determination of Methylone and Nor-Methylone Levels in Serum and Brain Sample Using LC/HRMS

Serum Pre-Treatment

0.2 ml of rat serum was fortified with the internal standard mephedrone-D7 and nor-mephedrone-D7 in methanolic solution (in an amount with respect to the level of methylone and nor-methylone in assayed samples) and 0.5 ml of a 0.1 M phosphate buffer (pH 6) in a labeled tube.

Brain Pre-Treatment

250 mg of brain was homogenized with 5 ml methanol and the internal standard methylone-D3 (in an amount with respect to the methylone levels in samples). The specimen was then ultrasonicated for 20 min and after supernatant separation by centrifugation, the supernatant was transferred into a clean labeled tube and evaporated to dryness. The residue was reconstituted in a 0.1 M phosphate buffer (pH 6). Solid phase extraction of methylone in pre-treated samples: a pre-treated sample of serum or brain with the buffer and internal standard was loaded onto a Bond Elut Certify cartridge previously conditioned with 0.5 ml of a 0.1 M phosphate buffer (pH 6). After application of a pre-treated sample, the cartridge was washed with 0.5 ml of distilled water, 0.5 ml of 0.1 M HCl, and 0.5 ml of CH_3OH/H_2O (1/1, v/v) and then dried by air for 5 min. The analyses were eluted three times with 0.5 ml of a freshly prepared mixture of dichloromethane/2-propanol/ammonium hydroxide (25%), 80/20/4, v/v/v. The eluate was gently evaporated to dryness under a stream of air at 40°C and then dissolved into mobile phase for LC/HRMS analysis.

Determination of 4-Hydroxy-3-Methoxymethcathinone Metabolite in Serum

4-hydroxy-3-methoxymethcathinone (4-OH-3-MeO-MC) was identified in rat serum samples according its exact mass. The calculated $[M + H^+]$ m/z for 4-OH-3-MeO-MC ($C_{11}H_{16}NO_3$)

was 210.1125. Any peak at the same m/z was found in blank rat sera.

LC/HRMS Conditions

The analyses were performed using Dionex Ultimate 3000 UHPLC coupled to an Exactive Plus-Orbitrap MS (ThermoFisher Scientific, Bremen, Germany) equipped with an HESI-II source. The chromatographic analyses of serum and tissue samples were performed using a Kinetex PFP 100 A (50 mm × 2.1 mm, 2.6 mm) and Security Guard Cartridge PFP 4 mm × 2.0 mm (Phenomenex) with a flow rate of 400 ml/min, gradient elution with 10 mM ammonium formate in 0.1% of formic acid as the mobile phase B. Gradient 0 min 5%, 4 min 45% B, and 5–6 min hold at 95%. The MS conditions were: full MS in a scan range of 50–500 m/z with positive electrospray ionization, resolution of 70,000 FWHM (scan speed 3 Hz), spray voltage of 3 kV, and ion transfer capillary temperature of 320°C.

Behavioral Procedures

Open Field

Open field testing was conducted in a temperature 22 ± 2°C, sound-proof, and evenly lit chamber with low levels of light intensity. The open field apparatus comprised a black square plastic open field arena (68 cm × 68 cm) with walls (30 cm high). At the beginning of each test, the rat was placed individually into the center of arena 15 or 60 min after drug administration and allowed to move about the arena freely for 30 min. The apparatus was cleaned with 50% ethanolic solution to avoid odors after each test. Behavioral activity was registered by an automatic video tracking system (EthoVision Color Pro v. 3.1.1, Noldus, the Netherlands).

Dependent variables were (i) total locomotor activity over 30 min, (ii) locomotor activity in 5 min intervals, (iii) time spent in the center of the arena and (T_{center}), and (iv) thigmotaxis (i.e., likelihood of appearance in the periphery). For evaluation of time spent in the center and thigmotaxis the arena was virtually divided into 5 × 5 grid of identical square zones with 16 being located on the periphery and 9 centrally. Time spent in the center of the arena is the sum of time spent in the nine central zones ($T_{center} = \Sigma time_{1-9}$). Thigmotaxis indicates probability of appearances in peripheral zones (f; the total number of appearances of the animal in each zones) and is calculated as $\Sigma f_{pertpheral\ zones}$ divided by $\Sigma f_{all\ zones}$.

PPI of ASR

Prepulse inhibition took place in startle chambers (SR-LAB, San Diego Instruments, CA, USA), each containing sound-proof and evenly lit enclosure, high-frequency loudspeaker (produced background noise at 75 dB and all acoustic stimuli), and Plexiglas stabilimeter (8.7 cm inner diameter). A piezoelectric accelerometer detected amplitudes of the startle responses which were digitized for subsequent analysis. 15 or 60 min prior to test rats were administered with methylone, nor-methylone, or vehicle. The experimental design was according to previous studies (21, 27, 29) and consisted of acclimatization and two sessions.

Acclimatization performed 2 days before test, drug-free rats were habituated in 5 min session with five presentations of pulse alone stimuli (115 dB/20 ms) over background white noise (75 dB). Startle data were not recorded for acclimatization.

The test started with a habituation period lasting 5 min in the startle chamber in which a 75 dB background white noise was continuously presented. The PPI test followed with 72 trials in all with an inter-trial interval (ITI) of 4–20 s (mean ITI: 12.27 s). Six 125 dB/40 ms duration pulse alone trials were then delivered to establish baseline ASR. Following this, 60 trials of the following were presented in a pseudorandom order: (A) pulse alone: 40 ms 125 dB; (B) prepulse-pulse: 20 ms 83 or 91 dB prepulse, a variable (30, 60, or 120 ms) inter-stimulus interval (mean 70 ms), then 40 ms 125 dB pulse; and (C) 60 ms no stimulus. Finally, six pulse alone trials were delivered. Habituation was calculated by the percentage reduction in ASR from the initial six baseline trials, to the final six trials. The PPI was calculated as [100 − (mean response for the prepulse − pulse trial/startle response for the single pulse trials) × 100].

Rectal Temperature

Rats were divided into two groups: rats housed individually and five animals per cage. These two conditions compared isolated and group-housed conditions and their interaction of drug on body temperature. Rectal temperature was measured using a digital thermometer; every temperature measurement lasted 10 s and rat was momentarily immobilized in a Plexiglas tube. The first measurements were taken every hour at 7:00 until 9:00 h and were taken under drug-free conditions. Methylone or vehicle was administered at 9:00 h and temperature was recorded every half hour until 11:00 h. Thereafter, temperature was recorded at hourly intervals until 17:00 h.

Design and Statistical Analysis

All statistical analyses were conducted using IBM SPSS version 22. For the open field, PPI, and temperature analyses, factorial designs for later analysis with analysis of variance (ANOVA) were used.

Significant main effects and interactions ANOVAs were followed with pairwise comparisons using independent t-tests. For repeated measures ANOVAs, where Mauchly's test of sphericity was significant (and Mauchley's $W < 0.75$), Greenhouse–Geisser corrected statistics are reported. For independent t-tests, where Levene's test for equality of variances was significant, statistics corrected for unequal variances are given $p < 0.05$ (two tailed) was considered the minimal criterion for statistical significance. For multiple comparisons, t-tests were used with Bonferroni correction. Nor-methylone was not included in ANOVA analyses (only one time of administration was tested, 15 min) and data were analyzed using additional independent t-test.

RESULTS

Pharmacokinetics of Methylone and Nor-Methylone in Serum and Brain Tissue

For methylone, maximum brain and serum concentration were attained within 30 min after the drug administration. The influx into the brain was not delayed and the concentration

of methylone in brain was approximately five times higher than serum levels throughout the experiment (serum:brain ratio during the peak was 1:4.54; **Figure 1A**). Serum levels of nor-methylone were also quantified after methylone administration; they peaked 30 min later than methylone (1 h after administration) and reached about 20% of methylone levels (350 ng/ml). The second most abundant metabolite identified in the serum was 4-OH-MeO-MC (4-hydroxy-3-methoxymethcathinone) (**Figures 1C,D**), quantification was not possible because of lack of reference standard at the time of analysis. 4-OH-MeO-MC peaked quickly at 30 min after administration of methylone, the peak had bigger area under the curve and then quickly disappeared and nor-methylone became the most abundant at later time points evaluated. After nor-methylone administration, the maximum serum concentrations were reached between 30 min and 1 h, the brain peak appeared at 30 min; the maximum levels were approximately one half when compared with methylone and serum:brain ratio during the peak was 1:7.97 (**Figure 1B**).

Acute Toxicity

Rats, treated with methylone 40 mg/kg, were tested only in 15 min testing onset in PPI because after 2 h after administration seven rats no longer produced much behavior only lying on the floor. After 5 h rats began moving around the home cage again, however, mortality occurred within 24 h after injection in six rats. In open field testing, only one rat died within 24 h after administration of methylone (40 mg/kg). Behavioral testing at 60 min after administration was not performed since 40 mg/kg was lethal to some animals.

Locomotor Activity in the Open Field

Trajectory length was evaluated using $4 \times 2 \times 6$ mixed factorial ANOVAs with drug treatment (methylone at 5, 10, or 20 mg/kg versus vehicle) and time of administration (15 and 60 min) as independent factor, and blocks (5 min interval) as a repeated measures factor. Mauchly's test of sphericity was significant and Greenhause–Geisser correction are presented for repeated measures, Mauchly's $W(14) = 0.21$, $p < 0.001$. Degrees of freedom were rounded to whole number for presentational purposes.

Analyses produced significant main effects of drug treatment [$F_{(3,71)} = 22.43$, $p < 0.001$], time of administration [$F_{(1,71)} = 50.68$, $p < 0.001$], and blocks [$F_{(3,211)} = 188.43$, $p < 0.001$]. In addition, there was a significant time of administration × drug treatment interaction [$F_{(3,71)} = 8.37$, $p < 0.001$] and a significant time of administration × blocks interaction [$F_{(3,211)} = 6.81$, $p < 0.001$] no other interactions were observed (**Figure 2**).

Since no interaction between drug treatment × blocks was observed further pairwise comparisons using independent *t*-tests were used to explore the significant on total trajectory

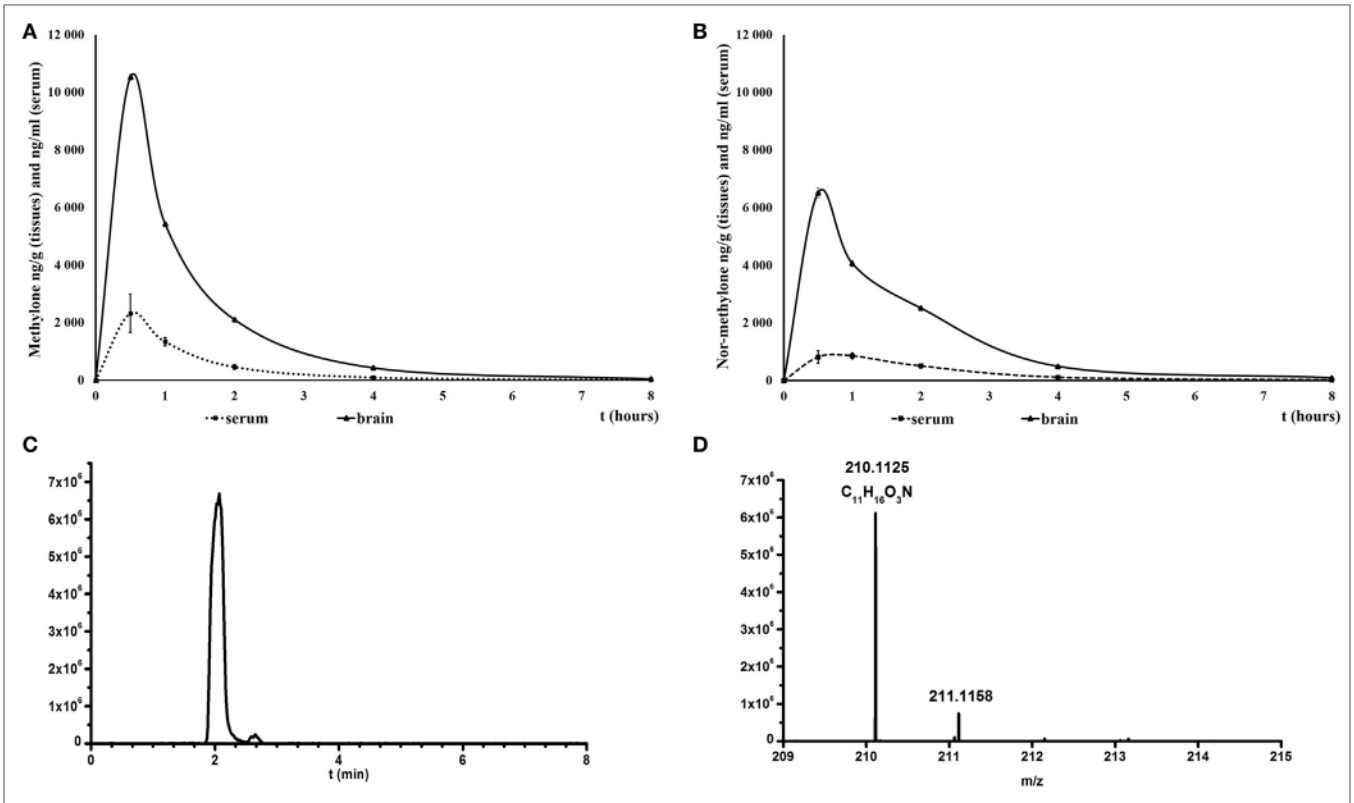


FIGURE 1 | Mean concentrations of methylone **(A)** and nor-methylone **(B)** in serum (nanogram per milliliter) and brain (nanogram per gram) over 8 h after subcutaneously administration of methylone 10 mg/kg and nor-methylone 10 mg/kg, respectively. Symbols represent means and vertical bars SEMs. Second panel represents extracted ion chromatogram of 4-OH-3-MeOH-MC taken at *m/z* 210.1125 in rat serum **(C)** and the measured [M + H⁺] *m/z* in full spectrum **(D)**.

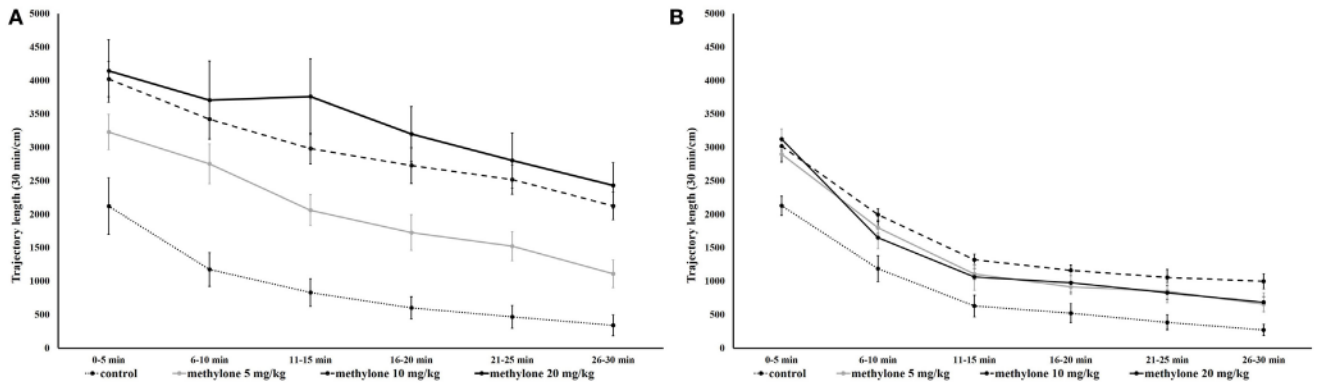


FIGURE 2 | Mean traveled distance within 5-min interval 15 min **(A)** and 60 min **(B)** after administration of methylone (5, 10, and 20 mg/kg). Symbols represent means and vertical bars SEMs.

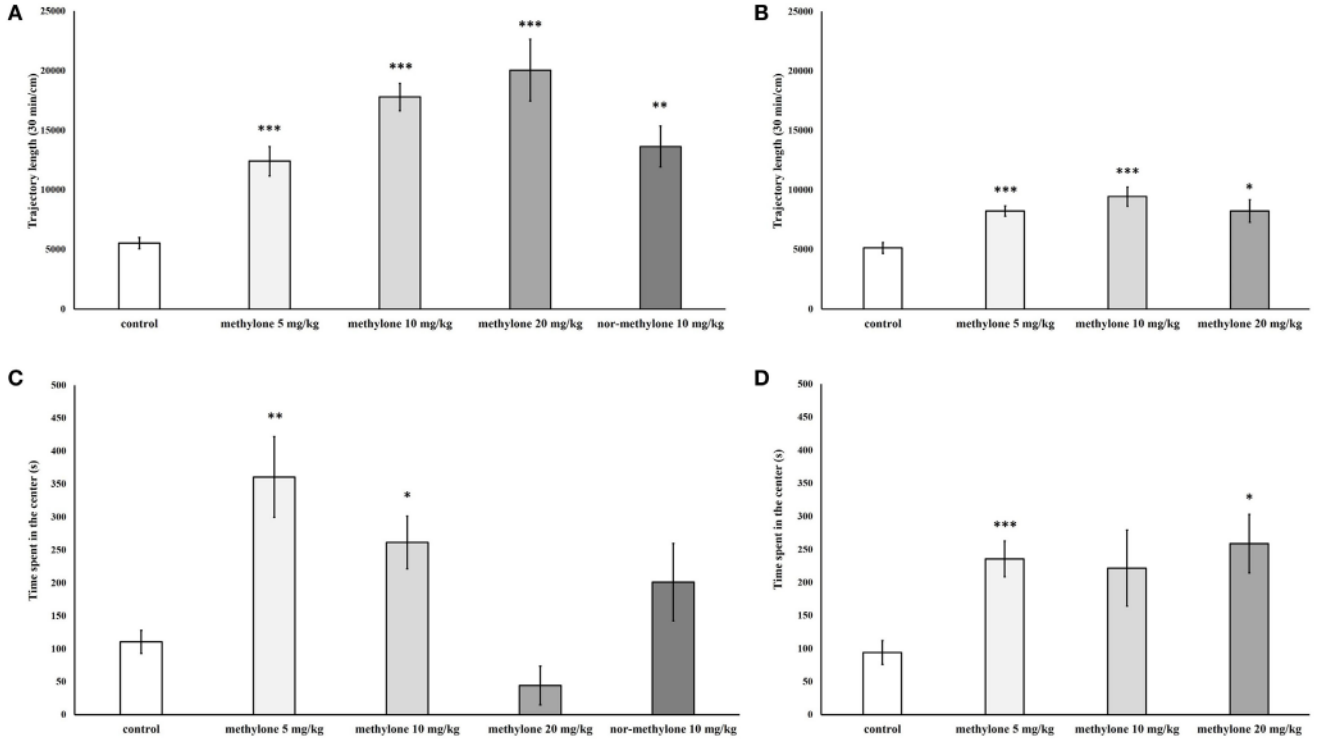


FIGURE 3 | Total locomotion measured 15 min **(A)** and 60 min **(B)** after methylone (5, 10, and 20 mg/kg) and nor-methylone (10 mg/kg) administration. Second panel represents mean T_{center} measured 15 min **(C)** and 60 min **(D)** after methylone (5, 10, and 20 mg/kg) and nor-methylone (10 mg/kg) administration. Columns represent means and vertical bars SEMs. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

length; these revealed that compared with vehicle all three doses of methylone significantly increased locomotion at 15 min [minimum $t(13) = 5.17$, $p < 0.001$; **Figure 3A**] as well as at 60 min [minimum $t(12) = 2.99$, $p < 0.05$; **Figure 3B**]. The increase at 60 min was much less pronounced.

Nor-methylone 10 mg/kg compared with vehicle significantly increased total locomotion at 15 min after administration [$t(18) = 4.57$, $p < 0.01$], by contrast, compared nor-methylone to methylone 10 mg/kg there was no significant difference (**Figure 3A**).

Thigmotaxis and Time Spent in the Center Part of the Apparatus

Thigmotaxis and T_{center} of arena were each analyzed with 4 × 2 ANOVAs with drug treatment and time of administration as independent factors.

T_{center}

There was only a significant main effect of drug treatment [$F_{(3, 71)} = 9.82$, $p < 0.001$] and a significant interaction of time of administration × drug treatment [$F_{(3, 71)} = 6.37$, $p < 0.001$].

Independent *t*-tests showed that methylone at all doses and both times of administration, except 20 mg/kg 15 min and 10 mg/kg at 60 min, significantly increased T_{center} [minimum $t(11) = 3.44$, $p < 0.05$]. Nor-methylone had no effect on T_{center} (**Figures 3C,D**).

Thigmotaxis

The main effect of time of administration [$F_{(1, 71)} = 22.15$, $p < 0.001$], drug treatment [$F_{(3, 71)} = 7.38$, $p < 0.001$] as well as their interaction [$F_{(3, 71)} = 14.89$, $p < 0.001$] were significant.

Methylone 20 mg/kg at 15 min before measurement significantly increased thigmotaxis [$t(18) = 7.93$, $p < 0.001$]. In contrast, at 60 min 5 and 20 mg/kg decreased it [minimum $t(18) = 2.68$, $p < 0.05$], while nor-methylone had no effect on this parameter (**Table 1**).

Prepulse Inhibition

Habituation, ASR, and PPI data were each analyzed with 4 × 2 independent ANOVAs with drug treatment (methylone at 5, 10, and 20 mg/kg versus vehicle) and time of administration (15 and 60 min) as independent factors.

Habituation data showed a significant main effect of time of administration on habituation [$F_{(1, 72)} = 8.17$, $p < 0.01$] but no interaction was observed. Independent *t*-tests revealed that methylone compared with vehicle did not affect habituation at any of the doses tested (**Table 2**).

Acoustic startle response data showed a significant main effect of drug treatment [$F_{(3, 72)} = 2.83$, $p < 0.05$] and again no interaction was detected. Independent *t*-tests revealed that methylone compared with vehicle did not affect ASR at any of the doses tested (**Table 2**).

Independent ANOVA showed a significant main effect of drug treatment on PPI [$F_{(3, 72)} = 2.88$, $p < 0.05$], no other interactions were observed. Subsequent independent *t*-test revealed a trend to decrease for 20 mg/kg at 15 min, compared with control, $t(18) = 1.91$, $p = 0.1$ (one-tailed). Nor-methylone did not differ from vehicle (**Figure 4**).

The Effect of Methylone on Body Temperature

The effect of methylone on body temperature was analyzed using 3 × 2 × 13 mixed factorial ANOVAs with drug treatment

TABLE 1 | Mean thigmotaxis measured 15 and 60 min after methylone (5, 10, and 20 mg/kg) and nor-methylone (10 mg/kg) administration.

		Drug treatment				
Measure	**Admin time**	**Vehicle**	**5 mg/kg**	**10 mg/kg**	**20 mg/kg**	**Nor-methylone**
Thigmotaxis	15 min	0.82 (0.01)	0.78 (0.02)	0.83 (0.02)	0.97 (0.02)	0.84 (0.04)
	60 min	0.81 (0.01)	0.75 (0.02)	0.82 (0.03)	0.74 (0.02)	xxx

Numbers represent means and in brackets are shown SEMs.

TABLE 2 | The effect of methylone (5, 10, and 20 mg/kg) and nor-methylone (10 mg/kg) on acoustic startle response (ASR) and habituation.

		Drug treatment				
Measure	**Admin time**	**Vehicle**	**5 mg/kg**	**10 mg/kg**	**20 mg/kg**	**Nor-methylone**
ASR (*arbitrary units*)	15 min	183.4 (60.1)	79.9 (12)	237.2 (36)	188.5 (23.4)	172 (33)
	60 min	157.5 (36.2)	125 (26.5)	157.2 (34.5)	145 (17.2)	xxx
Percentage habituation	15 min	40.4 (10.9)	25.7 (13.4)	19.4 (9)	35.7 (8)	35.6 (7.1)
	60 min	67.1 (6.1)	50.8 (8.4)	43.3 (8.6)	47.2 (6.9)	xxx

Numbers represent means and in brackets are shown SEMs.

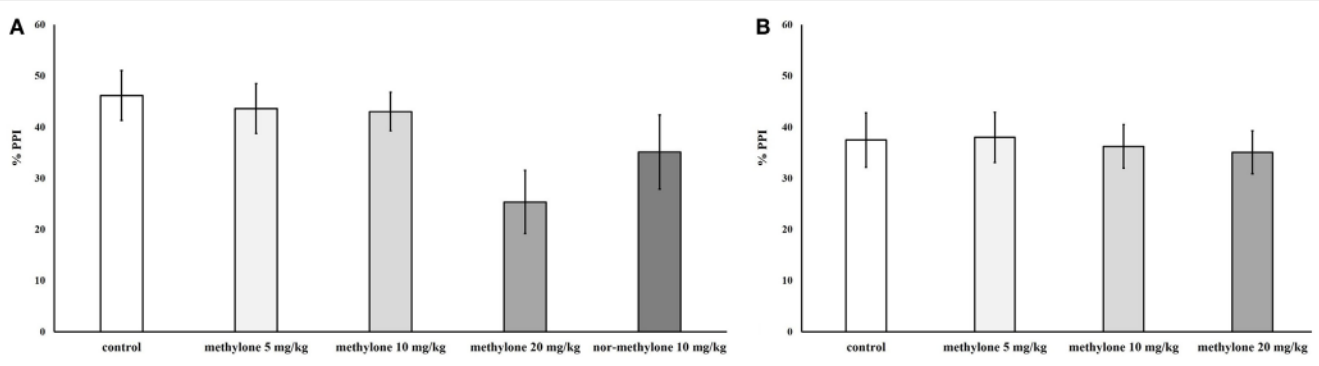


FIGURE 4 | Mean percentage prepulse inhibition of methylone (5, 10, and 20 mg/kg) and nor-methylone (10 mg/kg) 15 min **(A)** and 60 min **(B)** after administration. Columns represent means and vertical bars SEMs.

(methylone at 10 and 20 mg/kg versus vehicle) and home-cage conditions (individually and group-housed rats) as independent factors, and time as a repeated measures factor. Mauchly's test of sphericity was significant and Greenhouse–Geisser correction are presented for repeated measures, Mauchly's $W(44) = 0.05$, $p < 0.001$. Although temperature data before drug administration were significantly different from vehicle, these data were averaged for individual treatment and subtracted from temperature data after drug administration.

Temperature data showed a significant main effect of drug treatment [$F_{(2, 54)} = 5.29$, $p < 0.05$], home-cage conditions [$F_{(1, 54)} = 4.41$, $p < 0.05$], and time [$F_{(5, 289)} = 161.58$, $p < 0.001$]. The interaction of drug treatment × time [$F_{(11, 289)} = 6.87$, $p < 0.001$] and the three-way interaction of drug treatment × time × home-cage conditions [$F_{(11, 289)} = 4.3$, $p < 0.001$] were significant.

Independent *t*-tests revealed that under individually conditions, methylone significantly increased body temperature half an hour (9.30 h) after administration for both doses (10 and 20 mg/kg), an effect that was maintained until 13.00 for 10 mg/kg groups, minimum $t(18) = 2.15$, $p < 0.05$, and to 14.00 in the case of 20 mg/kg, minimum $t(18) = 2.07$, $p = 0.05$, **Figure 5A**.

In rats housed under group-housed condition, the temperature started to increase at 30 min after methylone administration after each of the doses. Methylone 10 mg/kg significantly increased body temperature from 9.30 to 10.30 h, minimum $t(18) = 2.6$, $p < 0.05$. At 20 mg/kg dose, temperature maintained elevated until 11.00, minimum $t(18) = 2.46$, $p < 0.05$, **Figure 5B**.

DISCUSSION

The main findings were as follows: methylone (i) had fast pharmacokinetics with a peak at 30 min, readily crossed the blood–brain barrier and reached levels approximately five times higher in the brain tissue (compared with serum); the major metabolite nor-methylone peaked in the brain at 30 min after methylone administration; (ii) showed marked stimulant effects at 15 min after administration which significantly diminished when tested 1 h after administration; (iii) methylone has relatively weak potency to disrupt PPI; and (iv) methylone significantly increased rectal temperature in individually as well as group-housed rats. When nor-methylone was administered alone, even though it reached approximately 1/2 and 1/3 of the serum and brain levels compared with methylone, it had comparable stimulant potency to methylone.

Pharmacokinetics

Compared with our data, Elmore et al. (32) found the peak serum of methylone levels even earlier at 15 min using the same route of administration. Interestingly, Lopez-Arnau et al. (33) found maximum plasma levels at 30 min after oral administration of methylone in rats which is indicative of a very fast gastrointestinal absorption. Additionally, only our experiments indicate a very fast and effective crossing of blood–brain barrier as methylone levels in the brain were more than five times those in serum. The incorporation of methylone into the brain may be associated with high lipophilicity, as we have already suggested for other compounds, e.g., PMMA or MDAI (5,6-methylenedioxy-2-aminoindane) (21, 27). Similarly, as methylone, its metabolite nor-methylone showed similar serum:brain ratio. The other important and major metabolites 4-OH-MeO-MC (4-hydroxy-3-methoxymethcathinone) (1, 34) were also detected in serum and brain. The rapid decrease of its dominance in the analytical spectrum at 60 min may be related to its fast conjugation, which would explain its lower plasmatic concentrations compared with nor-methylone. Even though we did not perform enzymatic hydrolysis, we might assume that the rapid decrease of its levels might be related to its fast conjugation with glucuronic and/or sulfuric acid that is typical for fenolic metabolites (35). Compared with MDMA where peak MDMA concentrations are achieved within 1 h after subcutaneous or oral administration both methylone and its metabolite nor-methylone showed a more rapid kinetic profile (36, 37) which is in line with the reported shorter duration of effects in humans (and might lead to more frequent re-dosing by users).

Acute Toxicity

According to our knowledge, there is no evidence about determination of lethal methylone dose in animals. In our

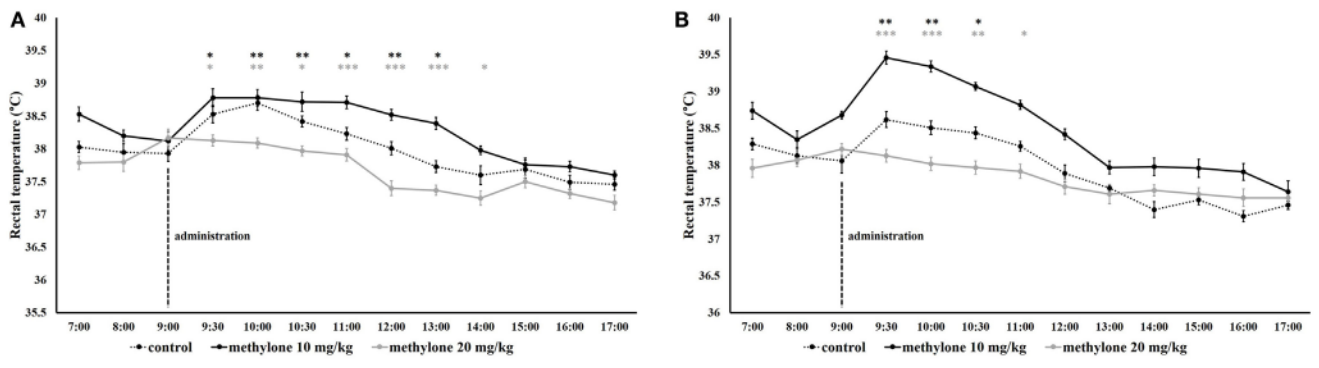


FIGURE 5 | The effect of methylone on rectal temperature in individually **(A)** and group-housed **(B)** rats. Vertical lines represented administration of methylone (10 and 20 mg/kg or vehicle). Symbols represent means and vertical bars SEMs. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$, gray asterisks refer to methylone (10 mg/kg) versus vehicle comparison, black asterisk methylone (20 mg/kg) versus vehicle comparison.

study, we obtained unexpected findings on the lethal effects of the highest dose of methylone (40 mg/kg) in the rats. The symptoms observed in this case (i.e., hyperventilation, seizures) were similar to symptoms detected in MDAI (27) and may be associated with serotonin syndrome, mainly hyperthermia which is one of the core symptom caused by 5-HT release (38).

Open Field

In accordance with its kinetic profile, the overall locomotor stimulatory activity was more pronounced 15 min after administration and was also comparable with other studies in rats (16, 19) and mice (17, 18). In mice, after methylone 30 mg/kg, locomotor activity was lower compared with 10 mg/kg (18) indicative of an inverted U shaped curve of locomotor effects. This inverted U shaped locomotor curve is also typical for most of the stimulants and characteristically linked to an increase in stereotyped behavior (e.g., circling) (21, 39). It is well established that the stimulatory versus hallucinogenic potency of cathinones and other related compounds is related to their DAT:SERT inhibition ratio. As stated above, methylone has been reported to have similar DAT:SERT inhibition ratio to cocaine (3, 12), and in contrast to other related cathinones, e.g., mephedrone, naphyrone, and methylenedioxypyrovalerone (MDPV) methylone has lower selectivity over DAT making it less stimulatory (3, 40). As reported in comparable behavioral studies of our currently submitted manuscripts, its stimulatory potency is slightly lower compared with mephedrone and much less potent compared with MDPV (unpublished observations Horsley et al. and Sichova et al.).

According to the temporal and spatial patterns in locomotor activity, methylone disrupted habituation, increased exploration, and stimulated activity at lower doses, however, high doses induced stereotyped behavior. In this respect, methylone behaves in a very similar manner to other stimulants and entactogens tested in identical (or near-identical) paradigms in our laboratory (21, 31).

Prepulse Inhibition

Methylone has a relatively weak potency to disturb sensorimotor processing. In line with this, our recent experiments with mephedrone, nor-mephedrone, and MDPV showed comparable weak or negative effects on PPI in rats (unpublished observations Horsley et al. and Sichova et al.). Interestingly amphetamine, which is approximately 10 times more potent in disrupting PPI in rodents, in humans also failed to have disruptive effect on PPI (41). However, this might be related to the fact that it was used in much lower dose (0.45 mg/kg) in humans compared with rodents (typically 1–4 mg/kg). On the contrary drugs affecting mainly SERT, e.g., MDMA, PMMA, or MDAI seem to have much stronger ability to disrupt sensorimotor gating in animals as well as in humans (26, 31, 42). Since PPI is typically used as a model of psychotomimetic potential in animals with translational validity, we may conclude that methylone has only mild psychotomimetic effects. Apart from PPI, and similarly like with the open field, the habituation to startle was attenuated during the peak of methylone effect (i.e., in 15 min time of administration). This can be theoretically also related to the overall stimulatory effect or to anxiety, since with the highest dose also the decreased time spent in the center was present.

Temperature

As expected and in accordance with previous studies with methylone (22, 43, 44), MDMA (45, 46) as well as our comparable studies with phenethylamine PMMA, and aminoindane MDAI (21, 27) the hyperthermic reaction was more pronounced in group-housed condition where it increased up to 1.5°C. The temperature increase was rapid and was not associated with visible perspiration as has been described with PMMA and MDAI (21, 27). In animals housed separately, the increase in temperature lasted for a 1 h longer compared with animals housed in groups. This is surprising since the opposite would be expected. This might be explained by accelerated metabolism due to higher increase in body temperature (cca 1°C) in animals housed in groups. Serotonergic drugs have more pronounced hyperthermic effects compared with drugs with dopaminergic actions. Since serotonin is a critical neuromodulator involved in the thermoregulation, with 5-HT2A receptors being a key mechanism responsible for hyperthermia (47). It is therefore very probable that this is also the case for methylone where the 5-HT2A receptor is stimulated *via* indirect mechanisms related to the increased serotonergic tone (44). On the other hand, in study of Javadi-Paydar et al. (19) was shown that mean body temperature did not vary more than 0.5°C from baseline temperature after methylone application. These findings of different (negative) results could be caused by methodological differences, where they measured temperature using radiotelemetry with lower doses of methylone than us.

Also in some of the cathinones, e.g., mephedrone have been also reported to induce hypothermia in rats but not mice (43, 48, 49). This effect is typically stimulated by activity at 5-HT1A receptors, and sometimes drugs that induce serotonin release might have biphasic effects on temperature or bidirectional depending on pharmacodynamics and the stimulation of these receptors (50).

Since here rats in group-housed conditions exhibited greater elevations in temperature (than under individually housed rats), this provides more support for the idea that environmental conditions that are crowded and/or hot (e.g., people dancing in a crowded clubs) increase the risk of hyperthermia and acute toxicity associated with methylone (51).

CONCLUSION

Methylone and its primary metabolite, nor-methylone induced behavioral, and temperature changes that are comparable with MDMA and other related stimulants, however, our results indicate it has a weaker capacity to disrupt PPI than MDMA and other stimulants. Since we have observed lethal toxicity in our study and that several deaths have been also associated with methylone in humans, its toxicity should not be underestimated,

especially when hyperthermic reaction appears in a crowded environments.

ETHICS STATEMENT

All procedures were conducted in accordance with the principles of laboratory animal care of the National Committee for the Care and Use of Laboratory Animals (Czech Republic), and according to Guidelines of the European Union (86/609/EU). The protocol was approved by the National Committee for the Care and Use of Laboratory Animals (Czech Republic) under the number: MEYSCR-27527/2012-31.

AUTHOR CONTRIBUTIONS

All authors made a significant contribution to this study, read, revised, and gave final approval for the current version of the work to be published.

FUNDING

This work was supported by projects VI20172020056, VG20122015075, MH CZ—DRO (NIMH-CZ, 00023752), ED 2.1.00/03.0078, LO1611 from the MEYS CR under the NPU I program, and grant PROGRES Q35 SVV260388.

REFERENCES

1. Prosser JM, Nelson LS. The toxicology of bath salts: a review of synthetic cathinones. *J Med Toxicol* (2012) 8:33–42. doi:10.1007/s13181-011-0193-z
2. Jacob P, Shulgin AT, Inventors; Neurobiological Technologies, Inc., *Preparation of Novel N-Substituted-2-Amino-3',4'-Methylenedioxy Propiophenone As Anti-Depressant and Anti-Parkinsonism Agents*. US Patent WO9639133 (1996).
3. Liechti M. Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. *Swiss Med Wkly* (2015) 145:w14043. doi:10.4414/smw.2015.14043
4. Bossong MG, Van Dijk JP, Niesink RJM. Methylone and mCPP, two new drugs of abuse? *Addict Biol* (2005) 10:321–3. doi:10.1080/13556210500350794
5. Spiller HA, Ryan ML, Weston RG, Jansen J. Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. *Clin Toxicol* (2011) 49:499–505. doi:10.3109/15563650.2011.590812
6. EMCDDA. *Annual Report: The State of the Drugs Problem in Europe*. Lisbon (2006).
7. Drug Enforcement Administration, Department of Justice. Schedules of controlled substances: temporary placement of three synthetic cathinones in Schedule I. Final order. *Fed Regist* (2011) 76:65371–75.
8. Pearson JM, Hargraves TL, Hair LS, Massucci CJ, Frazee CC, Garg U, et al. Three fatal intoxications due to methylone. *J Anal Toxicol* (2012) 36:444–51. doi:10.1093/jat/bks043
9. Warrick BJ, Wilson J, Hedge M, Freeman S, Leonard K, Aaron C. Lethal serotonin syndrome after methylone and butylone ingestion. *J Med Toxicol* (2012) 8:65–8. doi:10.1007/s13181-011-0199-6
10. Cozzi NV, Sievert MK, Shulgin AT, Jacob P, Ruoho AE. Inhibition of plasma membrane monoamine transporters by beta-ketoamphetamines. *Eur J Pharmacol* (1999) 381:63–9. doi:10.1016/S0014-2999(99)00538-5
11. Nagai F, Nonaka R, Kamimura KSH. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *Eur J Pharmacol* (2007) 559:132–7. doi:10.1016/j.ejphar.2006.11.075
12. Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, et al. Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol* (2013) 168:458–70. doi:10.1111/j.1476-5381.2012.02145.x
13. Miyazawa M, Kojima T, Nakaji S. Behavioral and rewarding effects of methylone, an analog of MDMA in mice. *Hirosaki Med J* (2011) 62:56–71.
14. Watterson LR, Hood L, Sewalia K, Tomek SE, Yahn S, Johnson CT, et al. The reinforcing and rewarding effects of methylone, a synthetic cathinone commonly found in "bath salts". *J Addict Res Ther* (2012) (Suppl 9):18. doi:10.4172/2155-6105.S9-002
15. DalCason TA, Young R, Glennon RA. Cathinone: an investigation of several N-alkyl and methylenedioxy-substituted analogs. *Pharmacol Biochem Behav* (1997) 58:1109–16. doi:10.1016/S0091-3057(97)00323-7
16. Lopez-Arnau R, Martinez-Clemente J, Pubill D, Escubedo E, Camarasa J. Comparative neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylone. *Br J Pharmacol* (2012) 167:407–20. doi:10.1111/j.1476-5381.2012.01998.x
17. Marusich JA, Grant KR, Blough BE, Wiley JL. Effects of synthetic cathinones contained in "bath salts" on motor behavior and a functional observational battery in mice. *Neurotoxicology* (2012) 33:1305–13. doi:10.1016/j.neuro.2012.08.003
18. Gatch MB, Taylor CM, Forster MJ. Locomotor stimulant and discriminative stimulus effects of 'bath salt' cathinones. *Behav Pharmacol* (2013) 24:437–47. doi:10.1097/FBP.0b013e328364166d
19. Javadi-Paydar M, Nguyen JD, Vandewater SA, Dickerson TJ, Taffe MA. Locomotor and reinforcing effects of pentedrone, pentylone and methylone. *Neuropharmacology* (2017):1–8. doi:10.1016/j.neuropharm.2017.09.002
20. Malberg JE, Seiden LS. Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J Neurosci* (1998) 18:5086–94.
21. Palenicek T, Balikova M, Rohanova M, Novak T, Horacek J, Fujakova M, et al. Behavioral, hyperthermic and pharmacokinetic profile of para-methoxymethamphetamine (PMMA) in rats. *Pharmacol Biochem Behav* (2011) 98:130–9. doi:10.1016/j.pbb.2010.12.011
22. Baumann MH, Ayestas MA, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al. The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* (2012) 37:1192–203. doi:10.1038/npp.2011.304
23. Pedersen AJ, Petersen TH, Linnet K. In vitro metabolism and pharmacokinetic studies on methylone. *Drug Metab Dispos* (2013) 41:1247–55. doi:10.1124/dmd.112.050880
24. Zidkova M, Linhart I, Balikova M, Himl M, Dvorackova V, Lhotkova E, et al. Identification of three new phase II metabolites of a designer drug methylone formed in rats by N-demethylation followed by conjugation with dicarboxylic acids. *Xenobiotica* (2017):1–8. doi:10.1080/00498254.2017.1349964
25. Palenicek T, Hlinak Z, Bubenikova-Valesova V, Votava M, Horacek J. An analysis of spontaneous behavior following acute MDMA treatment in male and female rats. *Neuro Endocrinol Lett* (2007) 28:781–8.
26. Horsley RR, Lhotkova E, Hajkova K, Jurasek B, Kuchar M, Palenicek T. Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue—behavioural, pharmacokinetic and metabolic studies in the Wistar rat. *Brain Res Bull* (2016) 126:102–10. doi:10.1016/j.brainresbull.2016.05.002
27. Palenicek T, Lhotkova E, Zidkova M, Balikova M, Kuchar M, Himl M, et al. Emerging toxicity of 5,6-methylenedioxy-2-aminoindane (MDAI): pharmacokinetics, behaviour, thermoregulation and LD50 in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 69:49–59. doi:10.1016/j.pnpbp.2016.04.004
28. Swerdlow NR, Braff DL, Geyer MA. Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. *Behav Pharmacol* (2000) 11:185–204. doi:10.1097/00008877-200006000-00002
29. Bubenikova V, Votava M, Horacek J, Palenicek T. Relation of sex and estrous phase to deficits in prepulse inhibition of the startle response induced by ecstasy (MDMA). *Behav Pharmacol* (2005) 16:127–30. doi:10.1097/00008877-200503000-00009
30. Palenicek T, Votava M, Bubenikova V, Horacek J. Increased sensitivity to the acute effects of MDMA ("ecstasy") in female rats. *Physiol Behav* (2005) 86:546–53. doi:10.1016/j.physbeh.2005.08.043

31. Palenicek T, Fujakova M, Brunovsky M, Horacek J, Gorman I, Balikova M, et al. Behavioral, neurochemical and pharmaco-EEG profiles of the psychedelic drug 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in rats. *Psychopharmacology* (2013) 225:75–93. doi:10.1007/s00213-012-2797-7
32. Elmore JS, Dillon-Carter O, Partilla JS, Ellefsen KN, Concheiro M, Suzuki M, et al. Pharmacokinetic profiles and pharmacodynamic effects for methylone and its metabolites in rats. *Neuropsychopharmacology* (2017) 42(3):649–60. doi:10.1038/npp.2016.213
33. Lopez-Arnau R, Martinez-Clemente J, Carbo ML, Pubill D, Escubedo E, Camarasa J. An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as "bath salts". *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 45:64–72. doi:10.1016/j.pnpbp.2013.04.007
34. Kamata HT, Shima N, Zaitsu K, Kamata T, Miki A, Nishikawa M, et al. Metabolism of the recently encountered designer drug, methylone, in humans and rats. *Xenobiotica* (2006) 36:709–23. doi:10.1080/00498250600780191
35. Ellefsen KN, Concheiro M, Suzuki M, Rice KC, Elmore JS, Baumann MH, et al. Quantification of methylone and metabolites in rat and human plasma by liquid chromatography-tandem mass spectrometry. *Foren Toxicol* (2015) 33:202–12. doi:10.1007/s11419-015-0263-z
36. Baumann MH, Zolkowska D, Kim I, Scheidweiler KB, Rothman RB, Huestis MA. Effects of dose and route of administration on pharmacokinetics of (±)-3,4-methylenedioxymethamphetamine in the rat. *Drug Metab Dispos* (2009) 37:2163–70. doi:10.1124/dmd.109.028506
37. Concheiro M, Baumann MH, Scheidweiler KB, Rothman RB, Marrone GF, Huestis MA. Nonlinear pharmacokinetics of (±)3,4-methylenedioxymethamphetamine (MDMA) and its pharmacodynamic consequences in the rat. *Drug Metab Dispos* (2014) 42:119–25. doi:10.1124/dmd.113.053678
38. Boyer EW, Shannon M. The serotonin syndrome. *N Engl J Med* (2005) 352:1112–20. doi:10.1056/NEJMra041867
39. McNamara RK, Logue A, Stanford K, Xu M, Zhang J, Richtand NM. Dose-response analysis of locomotor activity and stereotypy in dopamine D3 receptor mutant mice following acute amphetamine. *Synapse* (2006) 60:399–405. doi:10.1002/syn.20315
40. Gregg RA, Rawls SM. Behavioral pharmacology of designer cathinones: a review of the preclinical literature. *Life Sci* (2014) 97:27–30. doi:10.1016/j.lfs.2013.10.033
41. Chitty K, Albrecht MA, Graham K, Kerr C, Lee JWY, Iyyalol R, et al. Dexamphetamine effects on prepulse inhibition (PPI) and startle in healthy volunteers. *Psychopharmacology* (2014) 231:2327–37. doi:10.1007/s00213-013-3395-z
42. Vollenweider FX, Remensberger S, Hell D, Geyer MA. Opposite effects of 3,4-methylenedioxymethamphetamine (MDMA) on sensorimotor gating in rats versus healthy humans. *Psychopharmacology* (1999) 143:365–72. doi:10.1007/s002130050960
43. Den Hollander B, Rozov S, Linden AM, Uusi-Oukari M, Ojanpera I, Korpi ER. Long-term cognitive and neurochemical effects of "bath salt" designer drugs methylone and mephedrone. *Pharmacol Biochem Behav* (2013) 103:501–9. doi:10.1016/j.pbb.2012.10.006
44. Piao YS, Hall FS, Moriya Y, Ito M, Ohara A, Kikura-Hanajiri R, et al. Methylone-induced hyperthermia and lethal toxicity: role of the dopamine and serotonin transporters. *Behav Pharmacol* (2015) 26:345–52. doi:10.1097/FBP.0000000000000135
45. Green AR, O'Shea E, Colado MI. A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. *Eur J Pharmacol* (2004) 500:3–13. doi:10.1016/j.ejphar.2004.07.006
46. Jaehne EJ, Salem A, Irvine RJ. Effects of 3,4-methylenedioxymethamphetamine and related amphetamines on autonomic and behavioral thermoregulation. *Pharmacol Biochem Behav* (2005) 81:485–96. doi:10.1016/j.pbb.2005.04.005
47. Herin DV, Liu SJ, Urich T, Rice KC, Cunningham KA. Role of the serotonin 5-HT2A receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine. *Psychopharmacology* (2005) 178:505–13. doi:10.1007/s00213-004-2030-4
48. Shortall SE, Green AR, Swift KM, Fone KCF, King MV. Differential effects of cathinone compounds and MDMA on body temperature in the rat, and pharmacological characterization of mephedrone-induced hypothermia. *Br J Pharmacol* (2013) 168:966–77. doi:10.1111/j.1476-5381.2012.02236.x
49. Shortall SE, Green AR, Fone KC, King MV. Caffeine alters the behavioural and body temperature responses to mephedrone without causing long-term neurotoxicity in rats. *J Psychopharmacol* (2016) 30:698–706. doi:10.1177/0269881116650408
50. Aguirre N, Ballaz S, Lasheras B, Del Rio J. MDMA ('ecstasy') enhances 5-HT1A receptor density and 8-OH-DPAT-induced hypothermia: blockade by drugs preventing 5-hydroxytryptamine depletion. *Eur J Pharmacol* (1998) 346:181–8. doi:10.1016/S0014-2999(98)00062-4
51. Brown PL, Kiyatkin EA. Brain hyperthermia induced by MDMA ('ecstasy'): modulation by environmental conditions. *Eur J Neurosci* (2004) 20:51–8. doi:10.1111/j.0953-816X.2004.03453.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 10 January 2018
doi: 10.3389/fpsyt.2017.00306

Mephedrone (4-Methylmethcathinone): Acute Behavioral Effects, Hyperthermic, and Pharmacokinetic Profile in Rats

*Klára Šíchová[1], Nikola Pinterová[1,2], Monika Židková[3], Rachel R. Horsley[1], Eva Lhotková[1], Kristýna Štefková[1], Čestmír Vejmola[1,2], Libor Uttl[1,4], Marie Balíková[3], Martin Kuchař[1,5] and Tomáš Páleníček[1,2]**

[1] Department of Experimental Neurobiology, National Institute of Mental Health, Klecany, Czech Republic, [2] Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic, [3] Institute of Forensic Medicine and Toxicology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic, [4] Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic, [5] Forensic Laboratory of Biologically Active Compounds, Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czech Republic

OPEN ACCESS

Edited by:
Liana Fattore,
Consiglio Nazionale Delle Ricerche (CNR), Italy

Reviewed by:
Carla Cannizzaro,
Università degli Studi di Palermo, Italy
Ruben David Baler,
National Institutes of Health (NIH), United States
Marco Diana,
University of Sassari, Italy

***Correspondence:**
Tomáš Páleníček
tomas.palenicek@nudz.cz

Specialty section:
This article was submitted to Addictive Disorders, a section of the journal Frontiers in Psychiatry

Received: 26 September 2017
Accepted: 20 December 2017
Published: 10 January 2018

Citation:
Šíchová K, Pinterová N, Židková M, Horsley RR, Lhotková E, Štefková K, Vejmola Č, Uttl L, Balíková M, Kuchař M and Páleníček T (2018) Mephedrone (4-Methylmethcathinone): Acute Behavioral Effects, Hyperthermic, and Pharmacokinetic Profile in Rats. Front. Psychiatry 8:306. doi: 10.3389/fpsyt.2017.00306

Mephedrone (MEPH) is a synthetic cathinone derivative with effects that mimic MDMA and/or cocaine. Our study in male Wistar rats provides detailed investigations of MEPH's and its primary metabolite nor-mephedrone's (nor-MEPH) pharmacokinetics and bio-distribution to four different substrates (serum, brain, lungs, and liver), as well as comparative analysis of their effects on locomotion [open field test (OFT)] and sensorimotor gating [prepulse inhibition of acoustic startle reaction (PPI ASR)]. Furthermore, in order to mimic the crowded condition where MEPH is typically taken (e.g., clubs), the acute effect of MEPH on thermoregulation in singly- and group-housed rats was evaluated. Pharmacokinetics of MEPH and nor-MEPH after MEPH (5 mg/kg, sc.) were analyzed over 8 h using liquid chromatography with mass spectrometry. MEPH (2.5, 5, or 20 mg/kg, sc.) and nor-MEPH (5 mg/kg, sc.) were administered 5 or 40 min before the behavioral testing in the OFT and PPI ASR; locomotion and its spatial distribution, ASR, habituation and PPI itself were quantified. The effect of MEPH on rectal temperature was measured after 5 and 20 mg/kg, sc. Both MEPH and nor-MEPH were detected in all substrates, with the highest levels detected in lungs. Mean brain: serum ratios were 1:1.19 (MEPH) and 1:1.91 (nor-MEPH), maximum concentrations were observed at 30 min; at 2 and 4 h after administration, nor-MEPH concentrations were higher compared to the parent drug. While neither of the drugs disrupted PPI, both increased locomotion and affected its spatial distribution. The effects of MEPH were dose dependent, rapid, and short-lasting, and the intensity of locomotor stimulant effects was comparable between MEPH and nor-MEPH. Despite the disappearance of behavioral effects within 40 min after administration, MEPH induced rectal temperature elevations that persisted for 3 h even in singly housed rats. To conclude, we observed a robust, short-lasting, and most likely synergistic stimulatory effect of both drugs which corresponded to brain pharmacokinetics. The dissociation between the duration of behavioral and hyperthermic effects is indicative of the possible contribution

of nor-MEPH or other biologically active metabolites. This temporal dissociation may be related to the risk of prolonged somatic toxicity when stimulatory effects are no longer present.

Keywords: mephedrone, 4-methylmethcathinone, nor-mephedrone, pharmacokinetics, open field, prepulse inhibition, thermoregulation, Wistar rat

INTRODUCTION

Mephedrone (4-methylmethcathinone, 4-MMC; MEPH, hereafter), a synthetic derivative of cathinone was first synthetized in 1929 with the aim of developing this compound for therapeutic purposes (1). At the turn of the twenty-first century MEPH was rediscovered by recreational users (as a so-called "new psychoactive substance": NPS) and owing to its psychoactive effects, it became widely used as party drug known under the street name "meow meow" (2, 3). Based on users' reports, MEPH's effects are very similar to amphetamine, to 3,4-methylenedioxymethamphetamine (MDMA) and to cocaine, or their combination (4–6). MEPH's effects are rapid and of relatively short duration depending on the administration route (intranasal: ~30 min, oral: ~2–3 h) (7, 8), resulting in a tendency for recreational users to re-dose, as is the case with cocaine (9, 10). Prolonged and/or poly-drug use [including "slamming"—intravenous injection of MEPH combined with other drugs (11)] may be associated with adverse psychological (e.g., paranoia, depression, panic attacks), cardiovascular, or renal effects (12, 13). Furthermore, at least 90 deaths have been documented where MEPH alone (or its combination with other psychoactive compounds) was implicated (14–17). In 2010, MEPH was classified as a controlled substance in some European countries, and 2 years later in the USA (7). Despite its ban, it has remained a popular recreational drug to this day (18, 19).

Mephedrone acts as non-selective monoamine uptake inhibitor and releaser with dopamine transporter: serotonin transporter (DAT: SERT) inhibition ratio being 1.4, which led authors to label MEPH as mixed MDMA-cocaine-like compound (20, 21). However, while MEPH's uptake of dopamine (DA) is roughly equivalent to that of serotonin (5-HT), it is (such as MDMA or cathinone) several times more potent at nor-epinephrine transporter (NET) with NET: DAT ratio being approximately 13 (20). MEPH is also active on vesicular monoamine transporters 2, where its activity is approximately 10 times less potent than MDMA (22). Partly contrasting the transporter studies, according to *in vivo* microdialysis studies in nucleus accumbens (NAcc), MEPH had approximately twofold greater effect on 5-HT than DA release (23, 24). Furthermore, MEPH also has some activity at serotonin 5-HT_{2A}, noradrenaline $\alpha_{1,2}$ and trace amine associated receptor ($TAAR_1$). Affinity for DAT together with its high blood–brain barrier permeability (twofold greater than amphetamine and MDMA) (20) and direct effects on DA in NAcc make MEPH a compound with high addictive potential, which is confirmed by users (10, 20, 25, 26) and by animal studies (27–29). Its strong affinity for NET then might be indicative of cardiovascular toxicity (7).

Mayer et al. (30), using *in vitro* assays, showed that the phase I metabolites 4-methylcathinone (nor-mephedrone (nor-MEPH) hereafter), 4-hydroxytolylmephedrone (4-OH-MEPH) and dihydromephedrone also have measureable activity at DAT, NET, and SERT, although of these, only nor-MEPH and 4-OH-MEPH at a range meaningful for behavioral tests. Therefore, bioactive metabolites can also contribute to MEPH's effects. However, this was previously confirmed only for nor-MEPH, which displayed *in vivo* behavioral stimulatory activity (30).

In rodent models, MEPH administration leads to dose-dependent increases in locomotion [reviewed in Ref. (7)]. The intensity and duration of these changes is comparable to those observed after the same dose of MDMA, but lesser than amphetamine's effects (23, 24). MEPH's effect on sensorimotor gating has only been evaluated in a chronic administration paradigm by Shortall et al. (31); in order to mimic weekend type recreational use of drugs, they administered MEPH (1, 4, or 10 mg/kg) twice a week on two consecutive days for 3 weeks and tested prepulse inhibition of acoustic startle reaction [PPI ASR; a behavioral operationalization of sensorimotor gating (32)]; 30 min (min) after the final injection; this yielded no disruptive effect. On the other hand, related drugs, such as MDMA, amphetamine, cocaine, also cathinone itself, and methylone, have shown some disruptive effects in this paradigm (33–39). No information currently exists on MEPH's acute effect nor the effects of its metabolites on PPI.

Studies of MEPH effects on thermoregulation are inconsistent in their results; both hyperthermic (Sprague-Dawley rats (24, 27)) and hypothermic (40) responses have been documented. Alteration of body temperature is an effect that is dose- and environment-dependent in the case of MDMA and related compounds [e.g., Ref. (38, 39, 41, 42)]. In two of our previous studies, we have found that serotonergic compounds, along with severe hyperthermia, can induce profound sweating, particularly when rats are housed in cages in groups (38, 41). Group-housing mimics the crowded conditions in clubs where drugs, such as MDMA and MEPH are typically used. It is generally known that the hyperthermia associated with the use of these compounds is one of the key preceding conditions of neurotoxicity as well as of acute somatic toxicity related to serotonin syndrome (43). Therefore detailed examination of dose-related interactions with environmental conditions (such as crowding) is necessary in order to elucidate inconsistencies in MEPH's effects on thermoregulation.

Our main intention was to enrich current knowledge of MEPH by detailed description of the temporal characteristics of its behavioral effects in relation to its pharmacokinetics and bio-distribution and to investigate effects of its major active metabolite nor-MEPH. To describe the temporal profile of behavioral changes, two testing-onsets (5 or 40 min after drug administration) were used to register both peak and prolonged drug effects. Stimulatory locomotor effects, exploration and/or anxiogenic/anxiolytic potential were tested in the open field test (OFT) and the effects on sensorimotor gating were measured in

PPI ASR. Alongside this, pharmacokinetic profile of MEPH and nor-MEPH in brain and serum, and their bio-distribution to liver and lungs were established, over 8 h. To evaluate MEPH's effects on thermoregulation under crowded and isolated environmental conditions, rectal temperatures were measured over 8 h in groups of five rats versus rats housed alone.

MATERIALS AND METHODS

Animals

Male outbred Wistar rats (VELAZ, Czech Republic) weighing approximately 180–250 g were housed in pairs under controlled conditions (light/dark arrangement: 12/12 hours, temperature: 22 ± 2°C, humidity: 30–70%) with *ad libitum* water and standard diet. In each study, rats acclimatized to the laboratory facility for seven days, with tests performed in the seven days following. Therefore, testing/sampling occurred when rats were approximately 10–11 weeks old (adult) and they were in the laboratory for approximately 10–14 days in total. During the acclimatization period, rats were handled four times and weighed twice. Experiments and measurements were conducted in the light phase of the cycle (between 07:00 and 15:00 h). Experimental groups consisted of 10 individuals, each rat was tested only once, with the exception that to reduce the number of animals used, rats treated by MEPH/nor-MEPH in behavioral studies were subsequently used for pharmacokinetic sampling. Hence, only eight additional rats were needed (for 30 min post-drug administration samples).

Drugs and Chemicals

Mephedrone was purchased *via* the internet and subsequently purified and converted to MEPH hydrochloride by Alfarma s.r.o. (Czech Republic). The resulting MEPH was certified to be of 99.18% purity (analyzed by infrared spectroscopy) and also served as a reference standard for pharmacokinetic analyses using liquid chromatography. Nor-MEPH was synthesized at the Department of Organic Chemistry, Faculty of Chemical Technology (University of Chemistry and Technology Prague, Czech Republic) at a purity of 99.18%. Internal standards MEPH-D7.HCl and nor-MEPH-D7.HCl for quantitative liquid chromatography/mass spectrometry (LC/MS) assays were synthesized at the Department of Organic Chemistry, Faculty of Chemical Technology (University of Chemistry and Technology Prague, Czech Republic). Extraction columns (Bond Elut Certify 50 mg/3 ml) were supplied by Labicom s.r.o., Olomouc. Other chemicals used for laboratory purposes were of analytical grade purity. MEPH was stored in dry and dark place and dissolved in physiological saline (0.9% NaCl) immediately before experiments.

Dosage

The doses for subcutaneous (sc.) administration were estimated with respect to the amounts usually used by humans, reported potency/affinity at transporters and based on our previous studies with related compounds especially MDMA, MDAI, and related ring-substituted cathinone methylone (35, 38, 39, 44, 45). Furthermore, we set these doses with the intention to mimic the dosage comparable to human use and intermediate—high dose with expected strong acute effect, but non-lethal toxicity. Finally, the doses were also adequately adjusted for interspecies differences according the formula suggested by Reagan-Shaw et al. (46). All substances were dissolved in vehicle (0.9% physiological saline) at a volume of 2 ml/kg administered sc. (for comparability with our previous studies). Rats used for pharmacokinetic sampling were treated by MEPH 5 mg/kg. MEPH 5 or 20 mg/kg was used in the temperature monitoring study, and MEPH 2.5, 5, or 20 mg/kg and nor-MEPH 5 mg/kg were used in behavioral tests. As vehicle controls (VEH) animals were treated with an equivalent volume of 0.9% physiological saline.

Pharmacokinetics

For pharmacokinetics, rats were administered MEPH (5 mg/kg sc.) and subsequently decapitated after 30, 60, 120, 240, or 480 min (n = 8/experimental group). Sera, brain, liver, and lung tissues were collected and stored at −20°C until analysis.

Determination of MEPH and Nor-MEPH Levels in Serum and Tissue Samples Using LC/HRMS

Serum Pretreatment

0.2 ml of rat serum was fortified with the internal standard MEPH-D7 and nor-MEPH-D7 in methanolic solution (in an amount with respect to the levels of MEPH/nor-MEPH in assayed samples) and 0.5 ml of a 0.1 M phosphate buffer (pH 6) in a labeled tube.

Tissue Pretreatment

250 mg of tissue (brain, lung, liver) was homogenized with 5 ml methanol and the internal standard MEPH-D7 and nor-MEPH-D7 (in an amount with respect to the MEPH/nor-MEPH levels in samples). Each specimen was then ultrasonicated for 20 min and after supernatant separation by centrifugation, the supernatant was transferred into a clean labeled tube and evaporated to dryness. The residue was reconstituted in 0.1 M phosphate buffer (pH 6). For solid-phase extraction (SPE) of MEPH/nor-MEPH, a pretreated sample of serum or tissue, along with the buffer and internal standard, was loaded onto a Bond Elut Certify cartridge previously conditioned with 0.5 ml of 0.1 M phosphate buffer (pH 6). After application of each pretreated sample, the cartridge was washed with 0.5 ml of distilled water, 0.5 ml of 0.1 M HCl and 0.5 ml of CH_3OH/H_2O (1/1, v/v) and then air-dried for 5 min. The analytes were eluted three times with 0.5 ml of a freshly prepared mixture of dichloromethane/2-propanol/ammonium hydroxide (25%), 80/20/4, v/v/v. The eluate was gently evaporated to dryness under a stream of air at 40°C and then dissolved into mobile phase for LC/HRMS analysis.

LC/HRMS Conditions

The analyses were performed using Dionex Ultimate 3000 UHPLC coupled to an Exactive Plus-Orbitrap MS (ThermoFisher Scientific, Bremen, Germany) equipped with a HESI-II source. The chromatographic analyses of the serum and tissue samples were performed using a Kinetex PFP 100 A (50 × 2.1 mm, 2.6 mm) and Security Guard Cartridge PFP 4 × 2.0 mm (Phenomenex) with a flow rate of 400 ml/min, and gradient elution with 10 mM

ammonium formate in 0.1% of formic acid as the mobile phase B. Gradient 0 min 5%, 4 min 45% B, 5–6 min held at 95%. The MS conditions were as follows: full MS in scan range of 50–500 *m/z* with positive electrospray ionization, resolution of 70000 FWHM (full width at half-maximum, scan speed 3 Hz), spray voltage of 3 kV, and an ion transfer capillary temperature of 320°C.

Behavior: Open Field and PPI

Open Field

The OFT was performed in accordance with our previous studies (38, 47). An empty black square arena (68 cm × 68 cm × 30 cm) was used, which was virtually divided into a 5 × 5 grid of identical squares; 16 squares were located near the arena walls (comprising the peripheral zone), and 9 squares were situated centrally (comprising the central zone). Rats were placed individually into the center of the arena 5 or 40 min after the drug administration (testing-onset) and their behavior was recorded for 30 min (nor-MEPH-treated rats were tested at the 5 min testing-onset only). The software EthoVision Color Pro v. 3.1.1 (Noldus, Netherlands) was used to capture the raw data used in the calculation of the following dependent variables: trajectory length (cm; corrected for deviations of <3 cm) and its temporal dynamics in 5 min intervals; thigmotaxis ($\sum f_{\text{peripheral zones}}/\sum f_{\text{all zones}}$, where f = frequency of appearance in the zone) reflects the probability of appearance in the peripheral zone; T_{center} reflects time spent centrally ($\sum \text{time}_{\text{centralzones}}$).

Prepulse Inhibition

Prepulse inhibition was evaluated in two identical startle chambers (SR-LAB, San Diego Instruments, CA, USA) each consisting of a sound-proof, evenly lit, ventilated enclosure with a Plexiglas stabilimeter (8.7 cm inner diameter). The experimental design was adopted from our previous studies [e.g., Ref. (38, 41, 47)]. Briefly, 2 days before testing, rats were acclimatized to the startle chamber with a drug-free 5 min pre-training procedure consisting of 5 pulse alone stimuli (115 dB/20 ms) presented over background white noise (75 dB). Startle data were not recorded for acclimatization. On the test day, the testing session was initiated 5 or 40 min after drug administration (only 5 min for nor-MEPH). The test session consisted of 72 trials in total with an inter-trial interval (ITI) of 4–20 s (mean ITI: 12.27 s). After 5 min exposure to a continuous 75 dB background white noise, six 125 dB/40 ms duration pulse alone trials were delivered to establish baseline ASR (for later calculation of habituation). Following this, 60 trials of the following were presented in a pseudorandom order: (A) pulse alone: 40 ms/125 dB; (B) prepulse–pulse: 20 ms/83 dB or 20 ms/91 dB prepulse with a variable (30, 60, or 120 ms) inter-stimulus interval (ISI: mean = 70 ms), then 40 ms/125 dB pulse; (C) 60 ms no stimulus. Finally, six pulse alone trials were delivered. Habituation was expressed as the percentage reduction in ASR from the initial six baseline trials, to the final six trials. PPI was calculated as follows: [100 − (mean prepulse − pulse trials/mean pulse alone trials) × 100]. Mean ASR was obtained from pulse alone trials. All measures were derived from the average of the area under the curve in arbitrary units (AVG). Animals with a mean ASR (AVG) response lower than 10 were excluded from analyses as non-responders.

Body Temperature

To evaluate the possible interactive effect of drugs and environmental conditions, we measured rectal temperatures in rats housed singly or in groups of five per cage. In total, 13 measurements were conducted as follows: three drug-free hourly measurements (07:00–09:00 h) followed by administration of (MEPH 5 or 20 mg/kg or VEH) at 09:00 h, then four 30 min measurements (09:30–11:00 h), and finally six hourly measurements (12:00–17:00 h). A digital thermometer was used; each rat was briefly (max. 10 s) immobilized in a Plexiglas tube during the procedure. Rats were kept under controlled laboratory conditions (temperature: 22 ± 2°C, humidity: 30–70%) in the experimental room throughout the study (which was where all temperature measurements were taken).

Statistics

All statistical analyses were performed using the data analysis software system STATISTICA version 9.1. [StatSoft, Inc. (2010)]. Tests used a default alpha set at $p = 0.05$, two tailed. Behavioral and thermoregulation studies used factorial designs; therefore, analysis of variance (ANOVA) or analysis of covariance (ANCOVA) were used. Where these yielded significant main effects involving a factor with >2 levels or significant interactions, pair-wise *post hoc* comparisons were conducted using Newman–Keuls tests.

Behavioral Data (OFT and PPI)

Open field test spatial distribution (thigmotaxis and T_{center}) and PPI parameters (habituation, ASR, and PPI) were each analyzed using a 2 × 4 factorial ANOVA with testing-onset (5 or 40 min) and drug treatment (VEH or MEPH 2.5, 5, and 20 mg/kg sc.) as between subjects factors. In the case of significant main effects on ASR or habituation, the significant factor was included as a covariate in subsequent analysis of PPI data (using ANCOVA). The temporal pattern of locomotor activity in the OFT (trajectory length in 5 min blocks) was analyzed using a 2 × 4 × 6 mixed factorial ANOVA with testing-onset and drug treatment as between subjects factors, and time blocks (6 × 5 min) as a within-subjects factor.

Additional analyses to compare the potency of nor-MEPH to MEPH were analyzed using one-way ANOVA with five drug treatment levels (VEH or nor-MEPH 5 mg/kg or MEPH 2.5, 5, and 20 mg/kg sc.) as a between-subjects factor. For the OFT, the temporal pattern of locomotor activity was analyzed using a 5 × 6 mixed factorial ANOVA with drug treatment as a between subjects factor and 5 min time blocks as a within subjects factor. Only data from the 5 min testing-onset were used in this analysis (because data for the 40 min testing-onset were not available for all drug treatments).

Body Temperature

Data were analyzed using 3 × 2 × 13 mixed factorial design with drug treatment (VEH or MEPH 5 or 20 mg/kg) and home-cage

condition (singly- or group housed) as between subjects factors and time (13 measurements) as a within subjects factor.

RESULTS

Pharmacokinetics

The maximum mean MEPH serum concentration (826.2 ng/ml) was attained within 30 min. Influx into the brain was not evidently delayed compared to serum; maximum mean concentration in the brain tissue (767 ng/g) was also attained by 30 min after the dose. MEPH robustly accumulated in lung: concentration at 30 min was 1,044.5 ng/g, exceeding concentrations in sera, brain, and liver. Four hours after administration, the levels in sera and all tissues were almost undetectable (**Figure 1A**).

The maximum mean nor-MEPH (metabolized from MEPH *in vivo*; recall that nor-MEPH itself was not administered in pharmacokinetic studies) serum concentration of 351.9 ng/ml was attained within 1 h of treatment. The maximum mean concentration in the brain (197.1 ng/g) was also evident at 30 min. Nor-MEPH accumulated in lung tissue with a maximum mean concentration of 382.9 ng/g observed at 30 min. Six hours after administration, nor-MEPH was only slightly above the level of detection in all tissues and plasma (**Figure 1B**).

Mean brain: serum ratio was 1:1.19 for MEPH and 1:1.91 for nor-MEPH throughout the whole temporal observation.

Behavior

Open Field Test

Analysis of locomotion revealed a main effect of drug treatment [$F(3, 72) = 24.754$, $p < 0.001$], testing-onset [$F(1, 72) = 72.042$, $p < 0.001$] as well as blocks [$F(5, 360) = 101.67$, $p < 0.001$]. All interactions were significant, including the three-way drug × testing-onset × blocks interaction [minimum $F(15, 360) = 2.979$, $p < 0.001$]. The three-way interaction was explored further; at the 5 min testing-onset, while the normal pattern of locomotor habituation (i.e., a progressive decrease in activity over the session) was evident in all groups, *post hoc* tests showed that all MEPH-treated rats were hyperactive (compared to VEH) across the six time blocks ($p < 0.001$) (**Figure 2A**). At the 40 min testing-onset, elevated activity was no longer present ($p > 0.05$), although rats still showed normal locomotor habituation (**Figure 2B**). Additional analysis of total locomotion including nor-MEPH (5 min testing-onset) confirmed a significant main effect of drug treatment [$F(4, 45) = 27.699$, $p < 0.001$], blocks [$F(5, 225) = 50.171$, $p < 0.001$], and their interaction [$F(20, 225) = 3.350$, $p < 0.001$]. *Post hoc* tests showed that nor-MEPH 5 mg/kg rats displayed elevated activity (compared to VEH) across all six time blocks ($p < 0.001$) (**Figure 2A**). For typical trajectory patterns induced by the treatments see **Figure 2C**.

The effects of drug treatment, testing-onset, and their interaction were each significant for both T_{center} [minimum $F(3, 72) = 5.385$, $p < 0.01$] and for thigmotaxis [minimum $F(3, 72) = 6.792$, $p < 0.001$]. Additional one-way ANOVA analyses with nor-MEPH confirmed an effect of drug treatment on T_{center} [$F(4, 45) = 26.845$, $p < 0.001$] and thigmotaxis [$F(4, 45) = 48.704$, $p < 0.001$]. *Post hoc* tests showed that the 5-min testing-onset, MEPH 2.5 and 5 mg/kg-treated rats spent more time in the center ($p < 0.001$) compared to VEH. Thigmotaxis was reduced after MEPH 5 mg/kg and nor-MEPH 5 mg/kg ($p < 0.001$), and increased after MEPH 20 mg/kg ($p < 0.001$) (**Figures 3A,B**). No such significant effects were observed at the 40 min testing-onset (data not shown). Finally, MEPH 5 mg/kg treated rats spent more time in the center ($p < 0.001$) and exhibited lower thigmotaxis ($p < 0.001$) at the 5 min compared to 40 min testing-onset; this pattern was absent in the rest of the groups (data not shown).

Prepulse Inhibition

Acoustic startle reaction was not affected by drug treatment or testing-onset, or their interaction [maximum $F(1, 72) = 3.322$, $p > 0.05$; see **Table 1**]. Analysis of habituation data revealed a main effect of drug treatment [$F(3, 72) = 3.345$, $p < 0.05$]; *post hoc* tests revealed reduced habituation in MEPH 2.5 mg/kg rats compared to VEH ($p < 0.05$); the other MEPH doses did not differ from VEH. There was also a significant main effect of testing-onset [$F(1, 72) = 6.405$, $p < 0.05$] manifested as

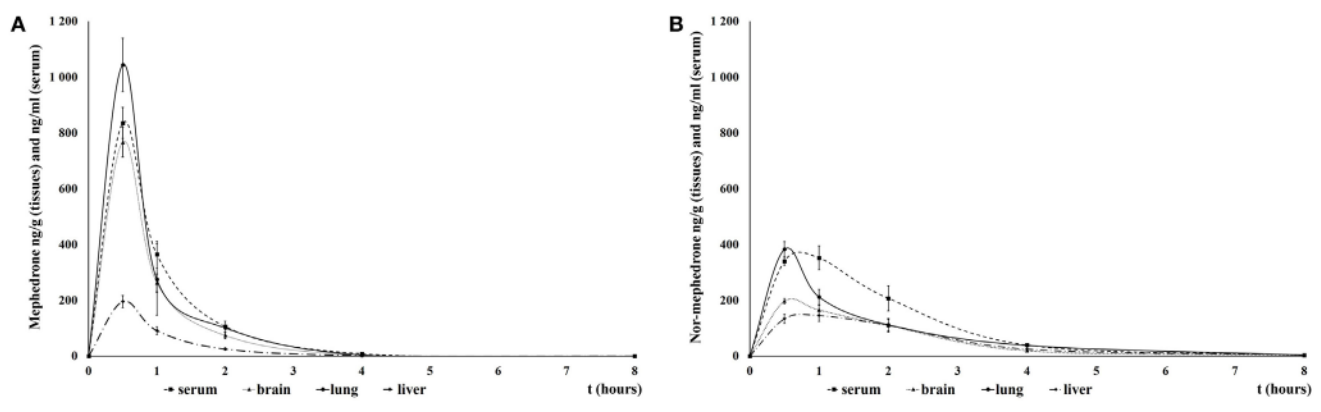


FIGURE 1 | Mean mephedrone (MEPH) **(A)** and its metabolite nor-mephedrone **(B)** levels in serum, brain, lungs, and liver over 6 h after application of MEPH 5 mg/kg sc. Error bars display ±1 SEM.

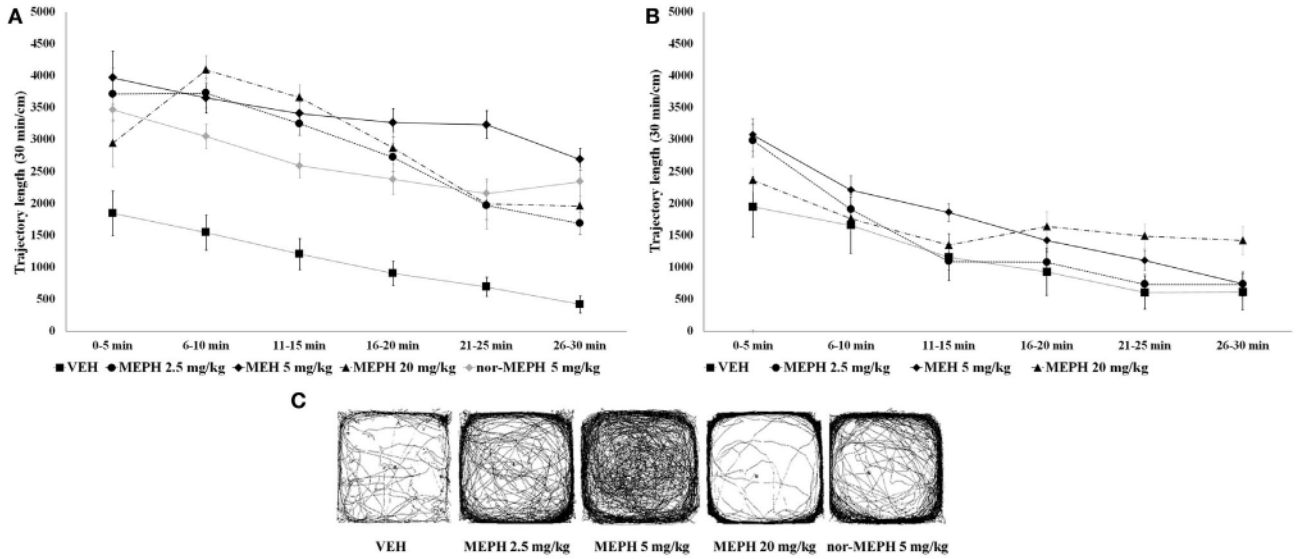


FIGURE 2 | Open field test (OFT): mean trajectory length (divided into 5-min blocks) by testing-onsets [5 and 40 min; **(A)** and **(B)**, respectively] and drug treatments [vehicle controls (VEH), mephedrone (MEPH) 2.5, 5, and 20 mg/kg and nor-mephedrone (nor-MEPH 5 mg/kg)]. Compared to VEH, significant hyperactivity ($p < 0.001$ for all drug groups and in all time blocks) was present at the 5-min testing-onset **(A)**, however the treatment effects were no longer significant at the 40 min testing-onset **(B)**. Error bars display ±1 SEM. Picture inserts below **(C)** show typical trajectory patterns induced by the treatment in animals with 5-min testing-onset.

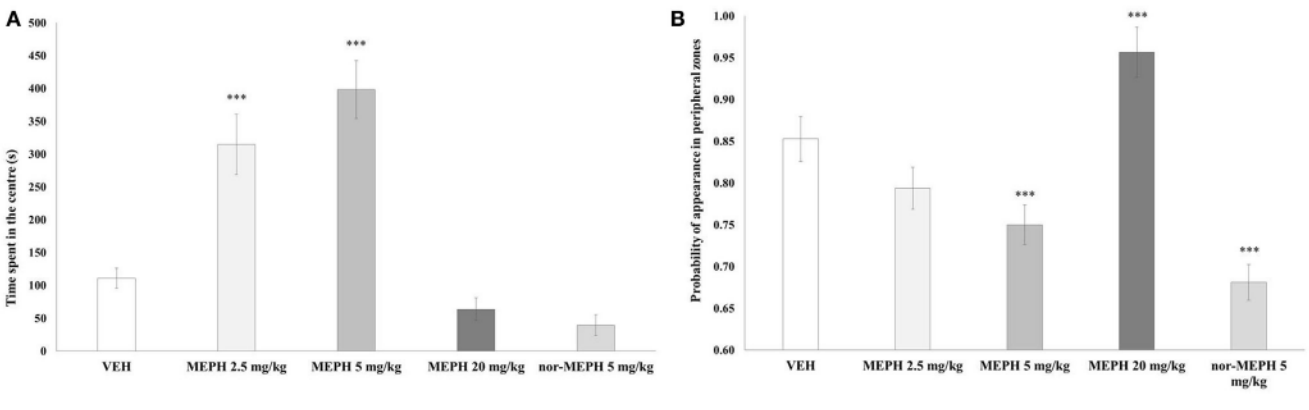


FIGURE 3 | Mean time spent in the arena center [T_{center}, **(A)**] and mean probability of appearance in peripheral zones [thigmotaxis, **(B)**] after vehicle controls (VEH), mephedrone (MEPH) 2.5, 5, and 20 mg/kg, and nor-mephedrone (nor-MEPH) 5 mg/kg administered at the 5-min testing-onset. MEPH 2.5 and 5 mg/kg-treated rats spent significantly more time in the central zones compared to VEH, and thigmotaxis was decreased by MEPH 5 mg/kg and nor-MEPH 5 mg/kg, and increased by MEPH 20 mg/kg. Error bars display ±1 SEM. ***$p < 0.001$ compared to VEH.

reduced habituation at the 5 min testing-onset compared to 40 min. The drug treatment × testing-onset interaction was not significant.

Since there were significant effects of drug treatment and testing-onset on habituation, it was included as a covariate in PPI analyses. PPI was not affected by the drug treatment or testing-onset, while their interaction was significant [F (3, 71) = 3.483, $p < 0.05$]. At the 40 min testing-onset, means suggested some disruption of PPI (MEPH 5 and 20 mg/kg); however, *post hoc* tests comparisons showed that differences from VEH were only marginal ($p = 0.062$, $p = 0.081$, respectively). There were no clear differences in means (eye-balling the data) at 5 min that seemed likely to account for the significant interaction; since a further one-way ANOVA was planned to explore effects of MEPH (alongside nor-MEPH) on PPI, further *post-hoc* tests on the 5 min testing-onset data were not conducted at this time. This additional one-way ANOVA showed no significant effect of treatment (MEPH or nor-MEPH) on PPI at the 5 min testing-onset [F (4, 45) = 0.696, $p > 0.05$]; therefore, the marginal effects at 40 min must explain the previous interaction. Similarly, there was no effect of MEPH

TABLE 1 | Mean values of acoustic startle reaction (ASR) amplitude and percentage of prepulse inhibition (PPI) after vehicle controls (VEH), mephedrone (MEPH), and nor-mephedrone (nor-MEPH) by testing-onsets (5 and 40 min).

Measure	Testing-onsets (min)	Drug treatment				
		VEH	MEPH 2.5 mg/kg	MEPH 5 mg/kg	MEPH 20 mg/kg	nor-MEPH 5 mg/kg
ASR	5	104.5 (14.3)	117.5 (17.4)	155.5 (32.7)	110.5 (14.2)	72.1 (11.5)
	40	137.2 (20.0)	140.8 (26.4)	144.6 (22.3)	173.9 (24.8)	–
% PPI	5	36.8 (5.4)	32.8 (5.6)	31.1 (6.2)	31.3 (4.1)	30.2 (6.5)
	40	41.3 (3.7)	41.1 (2.1)	25.1 (7.5)	28.4 (3.3)	–

Numbers represent means and SEMs are shown in brackets. Differences between testing-onsets and drug treatments were non-significant.

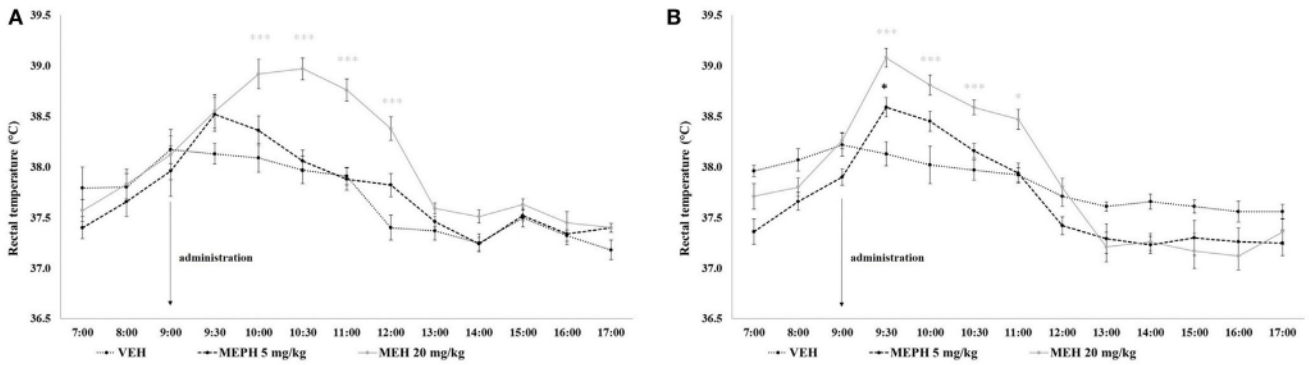


FIGURE 4 | Mean rectal temperature (°C) over 10 h after vehicle control (VEH), and mephedrone (MEPH) 5 and 20 mg/kg treatments for rats housed singly **(A)** or in groups of five **(B)**. Substances were administered at 09:00 h. Temperatures of rats treated by 5 mg/kg did not differ from VEH, except for the short-term elevation in the first 30 min after the administration in group-housed rats. The increase induced by 20 kg/kg was maintained from 10:00 to 12:00 h in singly housed rats and from 09:30 to 11:00 h in group-housed rats. Error bars display ±1 SEM. *$p < 0.05$, ***$p < 0.001$ compared to VEH.

or nor-MEPH on ASR [$F(4, 45) = 2.454$, $p > 0.05$] or habituation [$F(4, 45) = 1.912$, $p > 0.05$] at the 5-min testing-onset.

Body Temperature

Rectal temperature was significantly affected by drug treatment [$F(2, 54) = 9.409$, $p < 0.001$] and time [$F(12, 648) = 124.560$, $p < 0.001$] but not home-cage condition [$F(1, 54) = 0.127$, $p > 0.05$]. All interactions were significant including the three-way drug treatment × time × home-cage interaction [minimum $F(12, 648) = 2.406$, $p < 0.010$]. *Post-hoc* tests revealed no significant differences between MEPH 5 mg/kg and VEH groups, except the elevation (~0.5°C) which occurred in the first 30 min after administration in group-housed rats ($p < 0.05$). Compared to VEH, MEPH 20 mg/kg induced modest elevation (~0.4°C) in singly-housed rats that appeared in the first 30 min after administration; however, it became statistically significant 30 min later and the effect was maintained for the next 2 h (~1°C; minimum $p < 0.001$). In group-housed rats, the elevation became significant within first 30 min and remained increased for next 2 h (~1°C; minimum $p < 0.001$)—**Figure 4**.

DISCUSSION

Mephedrone quickly peaked in the serum and was rapidly incorporated into all tissues, with lungs showing the highest concentrations and liver the lowest. MEPH was almost undetectable in serum and tissue by 4 h after its administration. Nor-MEPH had a similar profile; however the concentrations of nor-MEPH decreased more gradually in comparison to the parent drug (with MEPH, a steep decrement occurred immediately after the peak). Therefore, compared to MEPH, the elimination of nor-MEPH was slightly delayed. Acute administration of both compounds resulted in dose-dependent stimulatory effects, disrupted habituation, and altered the spatial distribution of locomotor behavior in the open field; however, there was no significant effect on PPI. MEPH induced dose- and environment-dependent increases in rectal temperature (of up to ~1°C) in both group-housed rats (as expected), but also in singly housed rats, where temperature remained elevated for 3 h after administration of the highest MEPH dose.

Pharmacokinetics

In their study with iv. administration, Aarde et al. (29) showed that MEPH peaked in the brain within 2 min; since the most pronounced locomotor effects in our study were present within 5–10 min of administration, it is likely that the peak concentration in serum also occurred earlier than suggested by our pharmacokinetic study (where the first measurement was at 30 min after the sc. administration). As expected, we detected the highest serum levels of both compounds in our dataset slightly

earlier compared to oral administration, where MEPH peaked in serum within 45 min–1.5 h after administration (48). The speed of crossing the blood–brain barrier by MEPH implied by our current results was consistent with Aarde et al. (29); as shown by others (20), MEPH easily crosses blood–brain barrier and, thus, influx into brain and lung tissues is most likely due to its lipophilic profile. This finding is also consistent with the pharmacokinetics of another ring-substituted cathinone, methylone (39) as well as with the phenethylamines 2C-B and PMMA, aminoindanes such as MDAI where highest tissue concentrations were detected in lungs and brains (41, 49, 50). Not surprisingly, since nor-MEPH is not the only one major metabolite, it reached lower overall serum and tissue levels than the parent drug and the slope of its elimination was less steep, resulting in higher serum and brain concentrations compared to MEPH 3 h after its administration. One possible explanation could be the slightly higher polarity of nor-MEPH leading to slower crossing of the blood–brain barrier (30) and, theoretically, nor-MEPH may, therefore, be responsible for some delayed or prolonged effects of MEPH.

Behavioral Effects: Open Field and PPI

In line with pharmacokinetics, locomotor stimulant effects declined quickly, so MEPH and nor-MEPH lacked any significant stimulatory effects 40 min after administration. The rapid action of MEPH observed here is in line with other rodent studies (23, 28, 30) and reports from human users (10). Since MEPH and nor-MEPH have both been shown to act on DAT (23, 30), it is most likely the underlying cause of these effects (51). MEPH and nor-MEPH seemed to be behaviorally equipotent. The fact that the effects lasted a very short time (due to fast kinetics) may increase the likelihood of re-dosing by humans and, together with its strongly reinforcing effects (shown in self-administration studies), indicates highly addictive characteristics (10).

Spatial characteristics of the trajectory after MEPH showed bi-directional effects dependent on the dose used. While increased exploration of the central zones following lower doses might imply decreased anxiety, increased thigmotaxis following the highest dose could suggest the opposite (52, 53). Compared to our findings, studies measuring anxiety using the elevated plus-maze (EPM) revealed contradictory results including either increased anxiety after acute treatment with low doses [0.25–10 mg/kg (54)], or no effect after sub-chronic MEPH treatment with very high doses (30 mg/kg twice a day) (55, 56). Direct comparison of anxiety measures in the OFT versus EPM, however, may be difficult. While some authors report a good comparability (57) others have questioned this (58). In our study, spatial trajectory characteristics may be also affected by other mechanisms, such as increased stereotyped behaviors (e.g., circling the perimeter of the arena) such as was also observed in our previous studies with other related compounds (38, 41, 47).

In accordance with previous research (31), we did not see any significant effect of acute MEPH or nor-MEPH on PPI. When our data are compared with similar data sets from phenethylamines, cathinones and aminoindanes performed in our laboratory, it is evident that that the more serotonergic the drug is [e.g., according to their DAT: SERT inhibition ratios (20)], the more pronounced the disruptive effect on PPI. While MDMA, PMMA, and MDAI significantly disrupted PPI at the lowest doses used (35, 38, 41), which have mild-to-moderate stimulatory effects and do not induce stereotyped circling in the OFT, amphetamine and MDPV was effective only at the highest dose used where stereotyped behaviors were also evident [(37); unpublished observation Horsley et al.]. MEPH has also shown some activity at 5-HT_{2A} receptor (20), however, it is not clear whether it acts as agonist or antagonist. In relation to this, disruption of PPI is typically seen after administration of various 5-HT_{2A} agonists, serotonergic hallucinogens, such as LSD, mescaline, psilocybin, 2C-B or DOI, etc., and it is known that antagonists at this receptor can reinstate normal PPI (37, 59–63). Similarly, MDMA-induced PPI deficits in rats can be also normalized by 5-HT2A antagonists (64, 65), therefore suggesting a role for this receptor subtype in PPI; if MEPH acts as an antagonist at 5-HT2A receptors, this might theoretically be protective against psychomimesis.

Temperature

The hypothesis that MEPH, such as other cathinones (7), has a potency to alter thermoregulation was supported by evidence in our study. It is in line with reports of recreational users suffering from adverse effects related to altered peripheral thermoregulation, such as cold-blue fingers, hot flushes, and/or intensive sweating (9, 26). Likewise comparable preclinical studies [for review, see Green et al. (7)], we observed significant hyperthermia in both singly housed as well as group-housed rats under normal room temperature (22 ± 2°C). In contrast to our expectations, the temperature increase was almost identical (~1°C) in both groups but had slightly longer duration in singly housed rats. A possible explanation might be the faster onset of the temperature increase in the group-housed animals, where aggregation of animals in one cage would increase the microclimate temperature and in turn increase the speed of metabolism. The persistence of the temperature increase (3 h in singly housed rats), surprisingly, did not correspond with the rapid pharmacokinetic and locomotor profile of MEPH. Therefore additional factors, such as other active metabolite/s, may contribute to this prolonged effect and may indicate a potential for prolonged somatic drug toxicity, as in the case of toxic MDMA metabolites (66). In general, thermoregulation is mainly affected by drugs that primarily target serotonergic system [e.g., MDMA, PMMA, or MDAI (38, 41, 67)]. Dopaminergic stimulants may also increase body temperature (by increasing the behavioral activity), but effects are not as robust as with serotonergics (7). Direct comparisons of MEPH with other related cathinones, methylone 20 mg/kg sc., and MDPV 2 mg/kg sc. tested in our laboratory shows that the temperature increase was similar [(39); unpublished observation Horsley et al.]. This is of interest since the stimulant activity relative to the potency of the drug (DAT inhibition) should be approximately the same; however, the inhibition of SERT is much lower compared to DAT, and in the case of the lower MPDV dose would be approximately five times less effective (inhibiting SERT) than with MEPH or methylone (20). Taken together with the fact that the temperature increase was more prolonged in singly- than in group-housed rats and that it did not exceed 40°C, we suggest that increases in the overall behavioral activity relevant to dopaminergic stimulation are responsible for the hyperthermia observed. However,

against this interpretation, locomotor activation disappeared within 40 min of administration which is not consistent with the prolonged temperature increases. Further experiments will be needed in order to explain these discrepancies.

CONCLUSION

To conclude, both MEPH and nor-MEPH had rapid kinetics with accumulation in lungs and behaved as short-acting, potent stimulants with low capacity to disrupt sensorimotor gating. Dissociation between the duration of behavioral and hyperthermic effects may be due to the presence of another active metabolite with slower pharmacokinetic profile and may be indicative of prolonged risk of somatic toxicity even though acute stimulant-like effects have already worn off.

ETHICS STATEMENT

All procedures were conducted in accordance with the principles of laboratory animal care of the National Committee for the Care and Use of Laboratory Animals (Czech Republic), and according to Guidelines of the European Union (86/609/EU). The protocol was approved by the National Committee for the Care and Use of Laboratory Animals (Czech Republic) under the number: MEYSCR-27527/2012-31.

AUTHOR CONTRIBUTIONS

All authors made a substantial contribution to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. All authors were involved in drafting the work or revising it critically for important intellectual contents. All authors gave final approval for the current version of the work to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This work was supported by projects VI20172020056, VG20122015075, PROGRES Q35, SVV260388, MH CZ—DRO (NIMH-CZ, 00023752), ED 2.1.00/03.0078 and grant LO1611 from the MEYS CR under the NPU I program.

REFERENCES

1. Kelly BC. Legally tripping: a qualitative profile of salvia divinorum use among young adults. *J Psychoactive Drugs* (2011) 43:46–54. doi:10.1080/02791072.2011.566500
2. Hill SL, Thomas SHL. Clinical toxicology of newer recreational drugs (vol 49, pg 705, 2011). *Clin Toxicol* (2011) 49:880–880. doi:10.3109/15563650.2011.615318
3. Iversen L, Gibbons S, Treble R, Setola V, Huang X-P, Roth BL. Neurochemical profiles of some novel psychoactive substances. *Eur J Pharmacol* (2013) 700:147–51. doi:10.1016/j.ejphar.2012.12.006
4. Carhart-Harris RL, King LA, Nutt DJ. A web-based survey on mephedrone. *Drug Alcohol Depend* (2011) 118:19–22. doi:10.1016/j.drugalcdep.2011.02.011
5. Brunt T, Koeter M, Niesink R, Van Den Brink W. Linking the pharmacological content of ecstasy tablets to the subjective experiences of drug users. *Psychopharmacology* (2012) 220:751–62. doi:10.1007/s00213-011-2529-4
6. Varner KJ, Daigle K, Weed PF, Lewis PB, Mahne SE, Sankaranarayanan A, et al. Comparison of the behavioral and cardiovascular effects of mephedrone with other drugs of abuse in rats. *Psychopharmacology (Berl)* (2013) 225:675–85. doi:10.1007/s00213-012-2855-1
7. Green AR, King MV, Shortall SE, Fone KC. The preclinical pharmacology of mephedrone; not just MDMA by another name. *Br J Pharmacol* (2014) 171:2251–68. doi:10.1111/bph.12628
8. Karch SB. Cathinone neurotoxicity ("The "3Ms"). *Curr Neuropharmacol* (2015) 13:21–5. doi:10.2174/1570159X13666141210225009
9. Schifano F, Albanese A, Fergus S, Stair JL, Deluca P, Corazza O, et al. Mephedrone (4-methylmethcathinone; 'meow meow'): chemical, pharmacological and clinical issues. *Psychopharmacology (Berl)* (2011) 214:593–602. doi:10.1007/s00213-010-2070-x
10. Jones L, Reed P, Parrott A. Mephedrone and 3,4-methylenedioxy-methamphetamine: comparative psychobiological effects as reported by recreational polydrug users. *J Psychopharmacol* (2016) 30:1313–20. doi:10.1177/0269881116653106
11. Papaseit E, Moltó J, Muga R, Torrens M, De La Torre R, Farré M. Clinical pharmacology of the synthetic cathinone mephedrone. In: Baumann MH, Glennon RA, Wiley JL, editors. *Neuropharmacology of New Psychoactive Substances (NPS): The Science Behind the Headlines*. Cham: Springer International Publishing (2017). p. 313–31.
12. EMCDDA, Europol. *Europol–EMCDDA Joint Report on a New Psychoactive Substance: 4-Methylmethcathinone (Mephedrone)*. Lisbon: EMCDDA and Europol (2010).
13. Hope VD, Cullen KJ, Smith J, Jessop L, Parry J, Ncube F. Is the recent emergence of mephedrone injecting in the United Kingdom associated with elevated risk behaviours and blood borne virus infection? *Euro Surveill* (2016) 21:25–33. doi:10.2807/1560-7917.ES.2016.21.19.30225
14. Dickson AJ, Vorce SP, Levine B, Past MR. Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin. *J Anal Toxicol* (2010) 34:162–8. doi:10.1093/jat/34.3.162
15. Schifano F, Corkery J, Ghodse AH. Suspected and confirmed fatalities associated with mephedrone (4-methylmethcathinone, "meow meow") in the United Kingdom. *J Clin Psychopharmacol* (2012) 32:710–4. doi:10.1097/JCP.0b013e318266c70c
16. Loi B, Corkery JM, Claridge H, Goodair C, Chiappini S, Gimeno Clemente C, et al. Deaths of individuals aged 16-24 years in the UK after using mephedrone. *Hum Psychopharmacol* (2015) 30:225–32. doi:10.1002/hup.2423
17. Hockenhull J, Murphy KG, Paterson S. Mephedrone use is increasing in London. *Lancet* (2016) 387:1719–20. doi:10.1016/S0140-6736(16)30258-6
18. Wood DM, Dargan PI. Mephedrone (4-methylmethcathinone): what is new in our understanding of its use and toxicity. *Prog Neuropsychopharmacol Biol Psychiatry* (2012) 39:227–33. doi:10.1016/j.pnpbp.2012.04.020
19. Assi S, Gulyamova N, Kneller P, Osselton D. The effects and toxicity of cathinones from the users' perspectives: a qualitative study. *Hum Psychopharmacol* (2017) 32:7. doi:10.1002/hup.2610
20. Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, et al. Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol* (2013) 168:458–70. doi:10.1111/j.1476-5381.2012.02145.x
21. Liechti M. Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. *Swiss Med Wkly* (2015) 145:w14043. doi:10.4414/smw.2015.14043
22. Pifl C, Reither H, Hornykiewicz O. The profile of mephedrone on human monoamine transporters differs from 3,4-methylenedioxymethamphetamine primarily by lower potency at the vesicular monoamine transporter. *Eur J Pharmacol* (2015) 755:119–26. doi:10.1016/j.ejphar.2015.03.004
23. Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, et al. Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of

awake rats. *Br J Pharmacol* (2011) 164:1949–58. doi:10.1111/j.1476-5381.2011.01499.x
24. Baumann MH, Ayestas MA Jr, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al. The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* (2012) 37:1192–203. doi:10.1038/npp.2011.304
25. Dargan PI, Albert S, Wood DM. Mephedrone use and associated adverse effects in school and college/university students before the UK legislation change. *QJM* (2010) 103:875–9. doi:10.1093/qjmed/hcq134
26. Winstock A, Mitcheson L, Ramsey J, Davies S, Puchnarewicz M, Marsden J. Mephedrone: use, subjective effects and health risks. *Addiction* (2011) 106:1991–6. doi:10.1111/j.1360-0443.2011.03502.x
27. Hadlock GC, Webb KM, Mcfadden LM, Chu PW, Ellis JD, Allen SC, et al. 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designer stimulant of abuse. *J Pharmacol Exp Ther* (2011) 339:530–6. doi:10.1124/jpet.111.184119
28. Lisek R, Xu W, Yuvasheva E, Chiu YT, Reitz AB, Liu-Chen LY, et al. Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation. *Drug Alcohol Depend* (2012) 126:257–62. doi:10.1016/j.drugalcdep.2012.04.021
29. Aarde SM, Angrish D, Barlow DJ, Wright MJ Jr, Vandewater SA, Creehan KM, et al. Mephedrone (4-methylmethcathinone) supports intravenous self-administration in Sprague-Dawley and Wistar rats. *Addict Biol* (2013) 18:786–99. doi:10.1111/adb.12038
30. Mayer FP, Wimmer L, Dillon-Carter O, Partilla JS, Burchardt NV, Mihovilovic MD, et al. Phase I metabolites of mephedrone display biological activity as substrates at monoamine transporters. *Br J Pharmacol* (2016) 173:2657–68. doi:10.1111/bph.13547
31. Shortall SE, Macerola AE, Swaby RT, Jayson R, Korsah C, Pillidge KE, et al. Behavioural and neurochemical comparison of chronic intermittent cathinone, mephedrone and MDMA administration to the rat. *Eur Neuropsychopharmacol* (2013) 23:1085–95. doi:10.1016/j.euroneuro.2012.09.005
32. Geyer MA, Swerdlow NR. Measurement of startle response, prepulse inhibition, and habituation. *Current Protocols in Neuroscience*. John Wiley & Sons, Inc. (2001). doi:10.1002/0471142301.ns0807s03
33. Martinez ZA, Ellison GD, Geyer MA, Swerdlow NR. Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. *Psychopharmacology (Berl)* (1999) 142:253–60. doi:10.1007/s002130050887
34. Banjaw MY, Fendt M, Schmidt WJ. Clozapine attenuates the locomotor sensitisation and the prepulse inhibition deficit induced by a repeated oral administration of *Catha edulis* extract and cathinone in rats. *Behav Brain Res* (2005) 160:365–73. doi:10.1016/j.bbr.2005.01.002
35. Bubenikova V, Votava M, Horacek J, Palenicek T. Relation of sex and estrous phase to deficits in prepulse inhibition of the startle response induced by ecstasy (MDMA). *Behav Pharmacol* (2005) 16:127–30. doi:10.1097/00008877-200503000-00009
36. Horrillo R, Gonzalez-Periz A, Martinez-Clemente M, Lopez-Parra M, Ferre N, Titos E, et al. 5-Lipoxygenase activating protein signals adipose tissue inflammation and lipid dysfunction in experimental obesity. *J Immunol* (2010) 184:3978–87. doi:10.4049/jimmunol.0901355
37. Palenicek T, Fujakova M, Brunovsky M, Horacek J, Gorman I, Balikova M, et al. Behavioral, neurochemical and pharmaco-EEG profiles of the psychedelic drug 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in rats. *Psychopharmacology* (2013) 225:75–93. doi:10.1007/s00213-012-2797-7
38. Palenicek T, Lhotkova E, Zidkova M, Balikova M, Kuchar M, Himl M, et al. Emerging toxicity of 5,6-methylenedioxy-2-aminoindane (MDAI): pharmacokinetics, behaviour, thermoregulation and LD50 in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 69:49–59. doi:10.1016/j.pnpbp.2016.04.004
39. Štefková K, Židková M, Horsley RR, Pinterová N, Šíchová K, Uttl L, et al. Pharmacokinetic, ambulatory, and hyperthermic effects of 3,4-methylenedioxy-N-methylcathinone (methylone) in rats. *Front Psychiatry* (2017) 8:232. doi:10.3389/fpsyt.2017.00232
40. Shortall SE, Spicer CH, Ebling FJ, Green AR, Fone KC, King MV. Contribution of serotonin and dopamine to changes in core body temperature and locomotor activity in rats following repeated administration of mephedrone. *Addict Biol* (2015) 21:1127–39. doi:10.1111/adb.12283
41. Palenicek T, Balikova M, Rohanova M, Novak T, Horacek J, Fujakova M, et al. Behavioral, hyperthermic and pharmacokinetic profile of para-methoxymethamphetamine (PMMA) in rats. *Pharmacol Biochem Behav* (2011) 98:130–9. doi:10.1016/j.pbb.2010.12.011
42. Parrott AC. MDMA and temperature: a review of the thermal effects of 'ecstasy' in humans. *Drug Alcohol Depend* (2012) 121:1–9. doi:10.1016/j.drugalcdep.2011.08.012
43. Halpern P, Moskovich J, Avrahami B, Bentur Y, Soffer D, Peleg K. Morbidity associated with MDMA (ecstasy) abuse: a survey of emergency department admissions. *Hum Exp Toxicol* (2011) 30:259–66. doi:10.1177/0960327110370984
44. Palenicek T, Votava M, Bubenikova V, Horacek J. Increased sensitivity to the acute effects of MDMA ("ecstasy") in female rats. *Physiol Behav* (2005) 86:546–53. doi:10.1016/j.physbeh.2005.08.043
45. Palenicek T, Hlinak Z, Bubenikova-Valesova V, Votava M, Horacek J. An analysis of spontaneous behavior following acute MDMA treatment in male and female rats. *Neuro Endocrinol Lett* (2007) 28:781–8.
46. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* (2008) 22:659–61. doi:10.1096/fj.07-9574LSF
47. Horsley RR, Lhotkova E, Hajkova K, Jurasek B, Kuchar M, Palenicek T. Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue – behavioural, pharmacokinetic and metabolic studies in the Wistar rat. *Brain Res Bull* (2016) 126:102–10. doi:10.1016/j.brainresbull.2016.05.002
48. Martinez-Clemente J, Lopez-Arnau R, Carbo M, Pubill D, Camarasa J, Escubedo E. Mephedrone pharmacokinetics after intravenous and oral administration in rats: relation to pharmacodynamics. *Psychopharmacology (Berl)* (2013) 229:295–306. doi:10.1007/s00213-013-3108-7
49. Rohanova M, Palenicek T, Balikova M. Disposition of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and its metabolite 4-bromo-2-hydroxy-5-methoxyphenethylamine in rats after subcutaneous administration. *Toxicol Lett* (2008) 178:29–36. doi:10.1016/j.toxlet.2008.01.017
50. Hajkova K, Jurasek B, Sykora D, Palenicek T, Miksatkova P, Kuchar M. Salting-out-assisted liquid-liquid extraction as a suitable approach for determination of methoxetamine in large sets of tissue samples. *Anal Bioanal Chem* (2016) 408:1171–81. doi:10.1007/s00216-015-9221-1
51. Beninger RJ. The role of dopamine in locomotor activity and learning. *Brain Res* (1983) 287:173–96. doi:10.1016/0165-0173(83)90038-3
52. Archer J. Tests for emotionality in rats and mice: a review. *Anim Behav* (1973) 21:205–35. doi:10.1016/S0003-3472(73)80065-X
53. Walsh RN, Cummins RA. The open-field test: a critical review. *Psychol Bull* (1976) 83:482–504. doi:10.1037/0033-2909.83.3.482
54. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Kurzepa J, Biala G. Mephedrone and nicotine: oxidative stress and behavioral interactions in animal models. *Neurochem Res* (2015) 40:1083–93. doi:10.1007/s11064-015-1566-5
55. Den Hollander B, Rozov S, Linden AM, Uusi-Oukari M, Ojanpera I, Korpi ER. Long-term cognitive and neurochemical effects of "bath salt" designer drugs methylone and mephedrone. *Pharmacol Biochem Behav* (2013) 103:501–9. doi:10.1016/j.pbb.2012.10.006
56. Motbey CP, Clemens KJ, Apetz N, Winstock AR, Ramsey J, Li KM, et al. High levels of intravenous mephedrone (4-methylmethcathinone) self-administration in rats: neural consequences and comparison with methamphetamine. *J Psychopharmacol* (2013) 27:823–36. doi:10.1177/0269881113490325
57. Cannizzaro C, Plescia F, Gagliano M, Cannizzaro G, Mantia G, Labarbera M, et al. Perinatal exposure to 5-metoxytryptamine, behavioural-stress reactivity and functional response of 5-HT1A receptors in the adolescent rat. *Behav Brain Res* (2008) 186:98–106. doi:10.1016/j.bbr.2007.07.036
58. Lalonde R, Strazielle C. Relations between open-field, elevated plus-maze, and emergence tests in C57BL/6J and BALB/c mice injected with GABA- and 5HT-anxiolytic agents. *Fundam Clin Pharmacol* (2010) 24:365–76. doi:10.1111/j.1472-8206.2009.00772.x
59. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* (2001) 156:117–54. doi:10.1007/s002130100811
60. Palenicek T, Balikova M, Bubenikova-Valesova V, Horacek J. Mescaline effects on rat behavior and its time profile in serum and brain tissue after a single subcutaneous dose. *Psychopharmacology (Berl)* (2008) 196:51–62. doi:10.1007/s00213-007-0926-5

61. Palenicek T, Hlinak Z, Bubenikova-Valesova V, Novak T, Horacek J. Sex differences in the effects of N,N-diethyllysergamide (LSD) on behavioural activity and prepulse inhibition. *Prog Neuropsychopharmacol Biol Psychiatry* (2010) 34:588–96. doi:10.1016/j.pnpbp.2010.02.008
62. Nichols DE. Psychedelics. *Pharmacol Rev* (2016) 68:264–355. doi:10.1124/pr.115.011478
63. Tyls F, Palenicek T, Kaderabek L, Lipski M, Kubesova A, Horacek J. Sex differences and serotonergic mechanisms in the behavioural effects of psilocin. *Behav Pharmacol* (2016) 27:309–20. doi:10.1097/FBP.0000000000000198
64. Kehne JH, Padich RA, Mccloskey TC, Taylor VL, Schmidt CJ. 5-HT modulation of auditory and visual sensorimotor gating: I. Effects of 5-HT releasers on sound and light prepulse inhibition in Wistar rats. *Psychopharmacology (Berl)* (1996) 124:95–106. doi:10.1007/BF02245609
65. Padich RA, Mccloskey TC, Kehne JH. 5-HT modulation of auditory and visual sensorimotor gating: II. Effects of the 5-HT2A antagonist MDL 100,907 on disruption of sound and light prepulse inhibition produced by 5-HT agonists in Wistar rats. *Psychopharmacology (Berl)* (1996) 124:107–16. doi:10.1007/BF02245610
66. Kalant H. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs. *Can Med Assoc J* (2001) 165:917–28.
67. Green AR, O'shea E, Saadat KS, Elliott JM, Colado MI. Studies on the effect of MDMA ('ecstasy') on the body temperature of rats housed at different ambient room temperatures. *Br J Pharmacol* (2005) 146:306–12. doi:10.1038/sj.bjp.0706318

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 17 November 2017
doi: 10.3389/fpsyt.2017.00236

Synthetic Aminoindanes: A Summary of Existing Knowledge

*Nikola Pinterova[1,2], Rachel R. Horsley[1] and Tomas Palenicek[1,2]**

[1] National Institute of Mental Health, Klecany, Czechia, [2] 3rd Medical Faculty, Charles University, Prague, Czechia

Objectives: Aminoindanes ("bath salts," a class of novel psychoactive substances, NPSs) increased rapidly in popularity on the recreational drug market, particularly after mephedrone and other synthetic cathinones were banned in the UK in 2010. Novel aminoindanes continue to emerge, but relatively little is known about their effects and risks. Their history, chemistry, pharmacology, behavioral effects, pharmacokinetics, and toxicity are reviewed in this paper.

Methods: Scientific literature was searched on ISI Web of Knowledge: Web of Science (WoS) during June and July 2017, using English language terms: aminoindanes such as 5,6-methylenedioxy-2-aminoindane (MDAI), 5-iodo-2-aminoindane (5-IAI), 2-aminoindane (2-AI), 5,6-methylenedioxy-*N*-methyl-2-aminoindane (MDMAI), and 5-methoxy-6-methyl-2-aminoindane (MMAI). WoS was selected as it searches several databases simultaneously and has quality criteria for inclusion. For typical use and effects, Erowid, PsychonautWiki, Bluelight, and Drugs-Forum were searched; for legal status and epidemiology, the European Information System and Database on New Drugs (EDND) was used.

Results: Aminoindanes were first synthesized for medical use, e.g., as anti-Parkinsonian drugs and later as a potential compound facilitating psychotherapy; however, they are now widely substituted for ecstasy. Their mechanisms of action (primarily *via* serotonin) mean that they may pose a significant risk of serotonin syndrome at high doses or when combined with other drugs. Fatally toxic effects have been observed both in the laboratory in animal studies and in clinic, where deaths related with aminoindanes have been reported.

Conclusion: Greater knowledge about aminoindanes is urgently required to decrease risks of fatal intoxication, and appropriate legislation is needed to protect public health without impeding research.

Keywords: aminoindanes, 5,6-methylenedioxy-2-aminoindane, 5-iodo-2-aminoindane, 2-aminoindane, 5,6-methylenedioxy-*N*-methyl-2-aminoindane, 5-methoxy-6-methyl-2-aminoindane

OPEN ACCESS

Edited by:
Liana Fattore, Consiglio Nazionale Delle Ricerche (CNR), Italy

Reviewed by:
Oussama Kebir, Institut national de la santé et de la recherche médicale, France
John Martin Corkery, University of Hertfordshire, United Kingdom

***Correspondence:**
Tomas Palenicek
tomas.palenicek@nudz.cz

Specialty section:
This article was submitted to Addictive Disorders, a section of the journal Frontiers in Psychiatry

Received: *14 September 2017*
Accepted: *31 October 2017*
Published: *17 November 2017*

Citation:
Pinterova N, Horsley RR and Palenicek T (2017) Synthetic Aminoindanes: A Summary of Existing Knowledge. Front. Psychiatry 8:236. doi: 10.3389/fpsyt.2017.00236

INTRODUCTION

During the past decade, there has been a dramatic increase in the number and variety of novel psychoactive substances (NPSs) available on the illicit and gray drug markets (particularly *via* the Internet and "dark web"). In 2014, the number of NPSs boomed with 101 new compounds detected. In 2016, approximately one new NPS per week was identified, and the European Monitoring Centre

for Drugs and Drug Addiction (EMCDDA) was monitoring more than 620 NPSs (1). One of the first NPSs that became widely used recreationally was the cathinone derivative mephedrone (4-MMC, 4-methylmethcathinone), marketed at the time as a "legal" substitute for ecstasy (MDMA, 3,4-methylenedioxymethamphetamine) and cocaine, sharing effects of both (2). Mephedrone and other cathinones such as methylone (βk-MDMA, 3,4-methylenedioxy-*N*-methcathinone) and butylone (βk-MBDB, β-keto-*N*-methylbenzodioxolylbutanamine) were initially sold as, e.g., "bath salts" or "plant food," labeled "not for human consumption." Mephedrone became very popular due to its low price, high purity, and "legality" and, in the UK, it rapidly became as widespread as cocaine (3). In 2009 and 2010, the UK government placed piperazine derivatives, mephedrone, and other related cathinones under legal control (4), which resulted in their immediate replacement with new structural analogs and with a new class of NPSs: synthetic aminoindanes. One of the first was 5,6-methylenedioxy-2-aminoindane (MDAI), which claimed to be a "legal," non-neurotoxic analog of MDMA, with strong empathogenic and weaker stimulatory effects (5). Aminoindanes such as MDAI, 5,6-methylenedioxy-*N*-methyl-2-aminoindane (MDMAI), 5-iodo-2-aminoindane (5-IAI), 2-aminoindane (2-AI), 5-methoxy-6-methyl-2-aminoindane (MMAI), and 5-methoxy-2-aminoindane (MEAI) represent a relatively new generation of NPS. Cases of acute toxicity, including fatal poisoning, have been reported with their use (6). Only minimal reliable information on aminoindanes exists at present and, owing to their increasing popularity, the present brief review is timely. The paper summarizes the history of their creation, therapeutic potential in medical research and subsequent discovery by recreational drug users, their pharmacology, behavioral effects, pharmacokinetics, and toxicity.

METHOD

ISI Web of Knowledge: Web of Science (WoS) was searched during June and July 2017. WoS was selected because it simultaneously searches other databases such as PubMed and ScienceDirect, and includes quality criteria for inclusion (e.g., peer review). Keywords (used separately and in combination) were as follows: aminoindane, MDAI, 5,6-methylenedioxy-2-aminoindane, 5-IAI, 5-Iodo-2-aminoindane, 2-aminoindane, MDMAI, 5,6-Methylenedioxy-N-methyl-2-aminoindane, MMAI, 5-Methoxy-6-methyl-2-aminoindane. Full empirical/review articles containing relevant information about aminoindanes written in the English language were included; no date limits applied (**Figure 1**). When suitable articles were found, citation searches were also conducted. For subjective effects, typical use and doses, Erowid,[1] PsychonautWiki,[2] Bluelight,[3] and Drugs-Forum[4] (Internet discussion fora and wikis) were searched using the same search terms as above. Information about legal status and availability of aminoindanes in the European Union (EU), the EDND was consulted *via* the senior author, Dr. Palenicek, through the "Working group: Early warning system on new drugs," National Monitoring Centre for Drugs and Addiction, Czech Republic.

RESULTS

Chemistry

2-aminoindane is an amphetamine (AMPH) analog with a rigid conformation due to a bridge between the α-carbon and the aromatic ring (8). In the 1990s, Nichols et al. synthesized cyclic analogs of 3,4-methylenedioxyamphetamine (MDA), MDMA, 3-Methoxy-4-methylamphetamine (MMA), and *p*-iodoamphetamine (PIA) containing the 2-AI compound. Their procedures for synthesizing aminoindanes were well described (9–13). NPSs synthesized from the substances listed above are MDAI, MDMAI, MMAI, and 5-IAI (**Table 1**); all of these are psychoactive and their presence on the market has been confirmed in confiscated samples of "legal highs" (14). The EU Early Warning System[5] and the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory (EWA) on New Psychoactive Substances[6] have reported additional novel substances with an aminoindane structure, such as NM-2AI (*N*-methyl-2-aminoindane), 1-AI (1-aminoindane), and a fenfluramine analog ETAI (*N*-ethyl-5-trifluoromethyl-2-aminoindane); however, there is currently no scientific information available about these compounds.

Origins of Aminoindanes in Pharmacological Research

Owing to an amino group, aminoindanes are potentially vasoactive and bronchodilatory, which was the main focus for their initial development (15, 16). Since the chemical structure of aminoindanes is similar to that of AMPHs (owing to the presence of the phenethylamine skeleton), there was a strong assumption that aminoindanes would have the same bronchodilatory effect as ephedrine. Therefore, Levin et al. (17) evaluated the bronchodilatory and toxic effects of 2-AI and its N-substituted derivatives in the rat. 2-AI hydrochloride given intravenously showed less toxicity than AMPH hydrochloride, and 2-AI derivatives were more effective bronchodilators as compared with L-ephedrine. Aminoindanes have also been studied for their analgesic potency (comparable to morphine sulfate)—potency to increase blood pressure, respiration, and spinal reflexes (18, 19).

Based on Kier's receptor mapping technique (20)—a drug discovery method where the distance between oxotremorines's heteroatoms and dopamine's heteroatoms in reported conformations is similar—Martin et al. (21) designed and synthesized a series of aminoindanes with the intention to invent an anti-Parkinsonian drug. Although none of the resulting substances antagonized Parkinsonian-like symptoms (in a model of oxotremorine-induced tremors) nor showed any dopaminergic properties in mice, some of the molecules

[1]https://www.erowid.org/.
[2]https://psychonautwiki.org/.
[3]http://www.bluelight.org/.
[4]https://drugs-forum.com/.
[5]http://www.emcdda.europa.eu/themes/new-drugs/early-warning.
[6]https://www.unodc.org/LSS/Home/NPS.

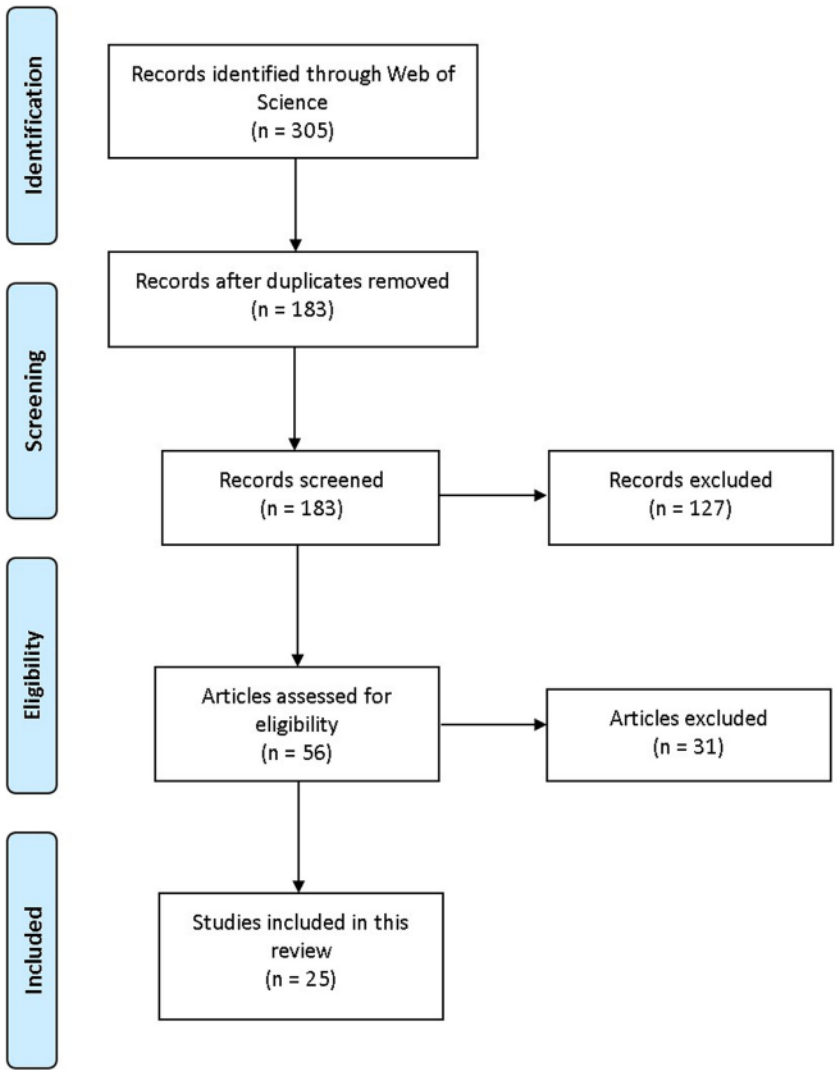


FIGURE 1 | PRISMA flowchart visualization of the search and selection process. Adapted from Moher et al. (7).

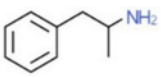
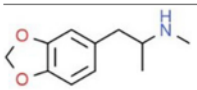
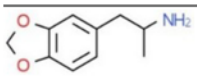
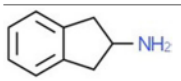
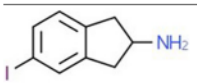
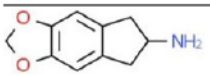
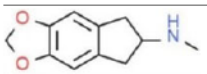
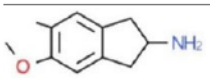
showed monoamine oxidase (MAO) inhibition and analgesic activity. Therefore, they investigated molecules with higher MAO-inhibiting potential *in vivo* and identified a candidate molecule N-methyl-5-methoxy-1-indanamine in mice. The authors concluded that the size of amine substituent and position of methoxyl substitution are most important for their biological activity (22).

Kalir et al. (23) examined the inhibitory action of substances containing aminoindanes on brain mitochondrial MAO type A and B, to ascertain MAO B inhibitors' anti-Parkinsonian potential. Two irreversible, selective-type MAO B inhibitors were identified: AGN-1133 (*N*-methyl-*N*-2-propynyl-1-indanamine hydrochloride) and AGN-1135 (*N*-propargyl-1R-aminoindane). AGN-1135 showed greater selectivity *in vitro* and *in vivo*, with no central nervous system, cardiovascular, or sympathomimetic effects and was eventually patented as a Parkinson's disease treatment (US patent no. 5457133A; US patent no. 5387612A; US patent no. 5453446A), known as rasagiline (24). The key difference between rasagiline and its analog selegiline is that rasagiline's major metabolite is aminoindane, whereas selegiline metabolizes to L-amphetamine and L-methamphetamine (24, 25). Therefore, no AMPH-like adverse effects are seen after rasagiline.

Aminoindanes–A Unique Drug Class with Entactogenic Properties

Contemporary research has focused on the psychoactive effects of substituted 2-AIs (9–13, 26–31). In their earlier work, Nichols et al. (32) proposed a new class of therapeutic psychoactive substances "entactogens," which were neither hallucinogens nor psychostimulants; instead, they facilitated communication and introspection, and were argued to be valuable agents in psychotherapy and potentially powerful tools for understanding the neurochemistry of emotion (27). To begin with, entactogens included MDMA, MDA, and 3,4-methylenedioxy-*N*-ethylamphetamine

TABLE 1 | Chemical structures and names, International Union of Pure and Applied Chemistry (IUPAC) names of amphetamine, MDMA, MDA, 2-aminoindane, and its derivatives with psychoactive effects.

Structure	Name	Chemical name	IUPAC name
	Amphetamine, AMPH, Speed	Alpha-methylphenethylamine	l-phenylpropan-2-amine
	MDMA, Ecstasy, Molly, X, XTC	3,4-methylenedioxymethamphetamine	l-(1,3-benzodioxol-5-yl)-*N*-methylpropan-2-amine
	MDA	3,4-methylenedioxyamphetamine	l-(1,3-benzodioxol-5-yl)propan-2-amine
	2-AI	2-aminoindane	2,3-dihydro-1H-inden-2-amine
	5-IAI	5-Iodo-2-aminoindane	5-iodo-2,3-dihydro-1H-inden-2-amine
	MDAI	5,6-methylenedioxy-2-aminoindane	6,7-dihydro-5H-cyclopenta[f][1,3]benzodioxol-6-amine
	MDMAI	5,6-methylenedioxy-2-methylaminoindane	N-methyl-6,7-dihydro-5H-cyclopenta[f][1,3] benzodioxol-6-amine
	MMAI	5-methoxy-6-methyl-2-aminoindane	5-methoxy-6-methyl-2,3-dihydro-1H-inden-2-amine

(MDEA), and later, related novel compounds such as 1,3-benzodioxolyl-*N*-methylbutanamine (MBDB) and MDAI were synthesized. Since MDMA and its analogs (MDA, MDEA) had been widely abused by recreational drug users and serotonergic neurotoxicity was identified, Nichols et al. refocused on the preparation of non-neurotoxic analogs of MDMA. The result was the description, for the first time, of these novel "entactogenic" compounds (27, 32).

Nichols et al. (9) described effects of MDAI on catecholamines and serotonin (5-HT), measured metabolite levels, and determined the affinity (K_D) and number of binding sites (B_{max}) for 5-HT transporter (SERT) (in rat brain cortical resp. hippocampal homogenates) measured 1 week after subcutaneous (s.c.) administration of 40 mg/kg MDAI. After 1 week of recovery, there were no significant changes in levels of any of the measured neurotransmitters or SERT compared with controls; by contrast, significant reductions in the neurotransmitter levels and SERT were induced by MDMA. No changes in K_D and B_{max} were observed, indicating no detectable 5-HT neurotoxicity or 5-HT terminal degeneration. However, drug discrimination experiments with MDMA-trained rats showed that MDAI fully substitutes for MDMA and that MDAI and MDMAI were observed to completely substitute for another MDMA-like drug MBDB (10). It was concluded that both drugs have MDMA-like behavioral pharmacology but without lasting 5-HT neurotoxicity following an acute, very high dose. However, the effects of chronic administration of MDAI (most drugs, whether for medical or recreational purposes are taken on multiple occasions) were not investigated until much later, and research on the chronic effects of aminoindanes, including MDAI, is still lacking.

A study on *in vitro* monoamine reuptake inhibition (using rats' synaptosomes) identified MDAI as a highly potent inhibitor of 5-HT and dopamine (DA) reuptake rather than causing non-vesicular DA release. 5-IAI and MMAI were subsequently evaluated, both of them increased non-vesicular release of 5-HT, DA, and norepinephrine (NE), but MMAI had 100- and 50-fold selectivity for 5-HT over DA and NE uptake inhibition, indicating that it is a very selective serotonergic releaser (28). In the monoamine reuptake transporter inhibition test performed on HEK 293 (human embryonic kidney 293) cells, MDAI's ability to preferentially inhibit the NE transporter (NET) and SERT over the DA transporter (DAT) was confirmed, with an approximately twofold lower potency compared with MDMA. The other aminoindane tested, 5-IAI, showed a similar pattern/ratio of inhibitory action at NET/SERT/DAT. 2-AI selectively inhibited just NET, and for SERT and DAT it has low potency. Apart from inhibitory actions on transporter molecules, aminoindanes have been shown to cause transporter-mediated release (reverse transport) of monoamines: MDAI released 5-HT and NE, 5-IAI released 5-HT and DA, and 2-AI released NE and DA (33).

The pharmacokinetics of MDAI in Wistar rats have been described in our recently published paper (34). Tissue samples were collected after a single bolus of MDAI (10 mg/kg, s.c.) at intervals of 30, 60, 120, 240, and 480 min after administration. Separated sera, whole brains, livers, and lungs were analyzed. MDAI showed fast and high influx into the brain; the drug

was accumulated in lungs where the concentration exceeded the concentration in the brain by approximately 30% (~30 vs. 18 μg/g, respectively) indicating its high-lipid solubility (34). When compared with s.c. MDMA in Sprague-Dawley rats (35), the kinetic profile of MDAI is much faster and its storage profile is similar to PMMA or 2C-B (36, 37). These results can be associated with potential selective MDAI neurotoxicity, exacerbated by combination with other drugs (6).

Subjective Effects and Acute Behavioral Studies

Very little is known about acute behavioral effects of aminoindanes in animal studies. We described acute behavior in Wistar rats after MDAI administration. Three different s.c. doses of MDAI (5, 10, 20, and 40 mg/kg) administered (at two testing onsets 15 respectively 60 min) prior to open field test (OFT) and prepulse inhibition test (PPI) were examined to evaluate effects on locomotor activity and sensorimotor gating. At all doses used, MDAI showed a disruptive effect on sensorimotor gating and, most evidently, at testing onset 15 min. The same disruptive effect on PPI can be seen after MDMA, AMPH or other psychoactive drugs (37), and it is related to changes in sensory filtering of information due to manipulation with DA and 5-HT levels in brain (38). These changes may alter information processing and induce a schizophrenic state (39). MDAI increased trajectory length in a dose-related manner, but not dramatically. MDAI has short-acting, slightly stimulatory and anxiolytic effects (34). In another animal model, in Swiss-Webster mice, Gatch et al. (40) examined the effect of MDAI [1, 3, 10, and 30 mg/kg; administered intraperitoneally (i.p.)] on locomotor activity. Lower MDAI doses produced a rapid onset of locomotor depression and at higher doses, a slower onset of locomotor stimulation was observed, but it was longer lasting. These findings suggest that although MDAI affects DA and stimulation, this is not a strong effect. This can lead users to combine MDAI with other drugs with stimulatory potency.

Since no clinical trial has yet been performed in humans with recreational aminoindanes, information about subjective effects and health risks comes from subjective personal experiences shared on drug website platforms, wikis, and discussion fora. Based on users' reports on PsychonautWiki, Erowid, Drugs-Forum, and Bluelight, MDAI and 5-IAI effects are mainly euphoria, empathy, stimulation (not the case with MDAI), and cognitive enhancement. The adverse effects described by users include dehydration, increased perspiration, anxiety, depression, panic attacks, and tachycardia. Several routes of administration have been reported from insufflation, oral ingestion to rectal application. The latter has the fastest onset of effects. Smoking and injecting have not been described (6, 41, 42). The onset of subjective psychoactive effects is reported to be around 30 min and their peak varies from 45 min up to 3 h after being taken orally. The wide time-window for peak effects after oral use could be caused by different product purities (6, 43): administration routes and factors influencing absorption (e.g., with oral consumption, food in the digestive tract). Users' "recommended" dose for a mild MDAI effect is 100–150 mg, for 2-AI it is 10–20 mg orally (43, 44). The doses of 5-IAI in trip reports are approximately 100 mg orally for a mild effect (45).

Toxicity and Health Risks

Palenicek et al. (34) examined an acute toxicity including median lethal dose (LD_{50}). The highest dose of MDAI (40 mg/kg) showed 50% greater locomotion activity compared with 20 mg/kg during the onset of its action; however, animals rapidly began to hyperventilate and showed signs of serotonin syndrome (intense perspiration, copious salivation, and seizures). In total, 100% of the rats died within 15 min of administration. This was unexpected, since Nichols et al. (9) had previously used this dose and route, and did not report adverse effects or fatalities. While for s.c. administration the LD_{50} was 28.3 mg/kg and i.v. 35 mg/kg, for oral administration all rats survived 40 mg/kg (34). The autopsy and histologic evaluation of tissues of deceased animals confirmed serotonin syndrome as a causal factor in death, with disseminated intravascular coagulopathy and brain edema implicated. Gatch et al. (40) tested MDAI at 100 mg/kg, with a similar outcome to Palenicek et al. (34): this dose was lethal for all mice. Experiments on thermoregulation clearly showed that MDAI dramatically increased body temperature accompanied by profound perspiration, particularly when administered to rats housed in groups. This, along with the other findings from this study, suggests a potentially higher risk of serotonergic toxicity when the drug is used by humans in settings such as clubs or rave/dance parties, where ambient temperatures are increased due to crowding.

Since recreational users take these ecstasy-like drugs frequently in the environment of rave/dance parties for euphoric and entactogenic effects but also to enhance their abilities to dance for long periods, many users desire stimulatory effects. However, in the case of aminoindanes, where primary activity is on the 5-HT system, stimulation is limited. This often leads users to consume aminoindanes in larger doses (to increase the DA release) or in drug cocktails with stimulants such as AMPH, cocaine, or MDMA to potentiate the stimulatory properties of the drug. In these combinations, when 5-HT-ergic substances potentiate DA-ergic substances, an unexpected neurotoxicity and cardiotoxicity may occur (6, 31). Tormey and Moore (46) reported a steady increase in deaths in Ireland from 9 in 2004 to 47 in 2009 from the drug category that includes NPSs (but also includes substances such as solvents); by contrast, their data for cocaine, stimulants, and hallucinogen deaths suggest a peak in 2007, followed by a decline (which would be accounted for if "classic" drugs were being replaced by NPSs). MDAI has been related to renal failure, acute respiratory distress syndrome, hepatic failure, and increased risk of primary pulmonary hypertension or valvular heart disease (47). Furthermore, MDAI-related deaths have been reported: a 17-year-old woman died of cardiac arrest with postmortem toxicological tests detecting MDAI at a concentration of 26.3 mg/L and an ethanol concentration of 14 mg/dL. No other drugs or metabolites were detected. In the other two deaths (men aged 35 and 28), the postmortem toxicology showed MDAI along with AMPHs, MDMA, lignocaine, etc., and ethanol (6). A 27-year-old man was successfully resuscitated by paramedics but died in hospital the following

day, with edema of the brain and lungs, aspiration pneumonia, blood-congested internal organs (and MDAI concentrations of 38 μg/L in peripheral blood and 1800 μg/L in urine 6 h before death) (48). Two 5-IAI and 2-AI fatalities were reported between 2010 and 2012, with one case each (49).

Legal Status

At the time of writing, only a few aminoindanes are controlled in some parts of the EU. 2-AI is controlled in Croatia, Denmark, Estonia, Finland, Hungary, Lithuania, Poland, and Portugal. MDAI is controlled in Cyprus, Czech Republic, Denmark, Estonia, Finland, Hungary, Italy, Lithuania, Portugal, and Sweden. 5-IAI is controlled in Finland, Hungary, Lithuania, and Portugal. For instance, the UK has not specifically restricted aminoindanes yet (4).

CONCLUSION

Although there are some existing studies focusing on MDAI, more research should be performed on the behavioral effects and toxicity of this substance. As we have shown in this review, fatal intoxications connected with MDAI have been reported and animal studies provided evidence of its potentially deadly toxicity due to serotonin syndrome. Furthermore, there is lack of information about toxicity, pharmacokinetics, and behavioral effects of the other aminoindanes. An important issue is also the legal status of these substances, since just a few EU countries control aminoindanes. This may increase the probability of their recreational use and, in turn, the incidence of acute toxicity.

AUTHOR CONTRIBUTIONS

NP contributed to the writing of the paper, searched for relevant literature, and composed the main idea for the study. RH and TP discussed the content of manuscript, added comments and critical feedback, and contributed to the final version of the paper.

FUNDING

This work was supported by the MEYS CR under the NPU I program (grant number LO1611) and 260388/SVV/2017.

REFERENCES

1. EMCDDA. *European Drug Report 2017: Trends and Developments* (2017). Luxembourg: Publications Office of the European Union.
2. Brunt TM, Poortman A, Niesink RJM, van den Brink W. Instability of the ecstasy market and a new kid on the block: mephedrone. *J Psychopharmacol* (2011) 25(11):1543–7. doi:10.1177/0269881110378370
3. Winstock A, Mitcheson L, Ramsey J, Davies S, Puchnarewicz M, Marsden J. Mephedrone: use, subjective effects and health risks. *Addiction* (2011) 106(11):1991–6. doi:10.1111/j.1360-0443.2011.03502.x
4. EDND. *European Database on New Drugs* (2017). Available from: https://edna.emcdda.europa.eu
5. Leach B. *New Drug to Replace Mephedrone as 'Legal High'*. The Telegraph (2010). Available from: http://www.telegraph.co.uk/news/uknews/law-and-order/7602664/New-drug-to-replace-mephedrone-as-legal-high.html
6. Corkery JM, Elliott S, Schifano F, Corazza O, Ghodse AH. MDAI (5,6-methylenedioxy-2-aminoindane; 6,7-dihydro-5H-cyclopenta[f][1,3] benzodioxol-6-amine; "sparkle"; "mindy") toxicity: a brief overview and update. *Hum Psychopharmacol Clin Exp* (2013) 28(4):345–55. doi:10.1002/hup.2298
7. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* (2009) 62(10):1006–12. doi:10.1016/j.jclinepi.2009.06.005
8. Fuller RW, Baker JC, Molloy BB. Biological disposition of rigid analogs of amphetamine. *J Pharm Sci* (1977) 66(2):271–2. doi:10.1002/jps.2600660235
9. Nichols DE, Brewster WK, Johnson MP, Oberlender R, Riggs RM. Nonneurotoxic tetralin and indan analogs of 3,4-(methylenedioxy) amphetamine (MDA). *J Med Chem* (1990) 33(2):703–10. doi:10.1021/jm00164a037
10. Oberlender R, Nichols DE. (+)-N-methyl-1-(1,3-benzodioxol-5-Yl)-2-butanamine as a discriminative stimulus in studies of 3,4-methylenedioxymethamphetamine-like behavioral activity. *J Pharmacol Exp Ther* (1990) 255(3):1098–106.
11. Johnson MP, Frescas SP, Oberlender R, Nichols DE. Synthesis and pharmacological examination of 1-(3-methoxy-4-methylphenyl)-2-aminopropane and 5-methoxy-6-methyl-2-aminoindan—similarities to 3,4-(methylenedioxy) methamphetamine (MDMA). *J Med Chem* (1991) 34(5):1662–8. doi:10.1021/jm00109a020
12. Nichols DE, Johnson MP, Oberlender R. 5-Iodo-2-aminoindan, a nonneurotoxic analogue of p-iodoamphetamine. *Pharmacol Biochem Behav* (1991) 38(1):135–9. doi:10.1016/0091-3057(91)90601-W
13. Maronalewicka D, Nichols DE. Behavioral-effects of the highly selective serotonin releasing agent 5-methoxy-6-methyl-2-aminoindan. *Eur J Pharmacol* (1994) 258(1–2):1–13. doi:10.1016/0014-2999(94)90051-5
14. Smith JP, Sutcliffe OB, Banks CE. An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). *Analyst* (2015) 140(15):4932–48. doi:10.1039/c5an00797f
15. Shelton RS. *Secondary Beta Phenyl Propyl Amines and Pharmaceutical Compositions Thereof*. U.S. Patent No 2,298,630. Washington, DC: U.S. Patent and Trademark Office (1942).
16. Woodruff EH. *Amino Compound*. U.S. Patent No 2,293,874. Washington, DC: U.S. Patent and Trademark Office (1942).
17. Levin N, Graham BE, Kolloff HG. Physiologically active indanamines1. *J Org Chem* (1944) 09(4):380–91. doi:10.1021/jo01186a010
18. Witkin LB, Heubner CF, Galdi F, O'Keefe E, Spitaletta P, Plummer AJ. Pharmacology of 2-amino-indane hydrochloride (Su-8629): a potent non-narcotic analgesic. *J Pharmacol Exp Ther* (1961) 133(3):400–8.
19. Solomons E, Sam J. 2-Aminoindans of pharmacological interest. *J Med Chem* (1973) 16(12):1330–3. doi:10.1021/jm00270a004
20. Kier LB. The prediction of molecular conformation as a biologically significant property. *Pure Appl Chem* (1973) 35(4):509. doi:10.1351/pac197335040509
21. Martin YC, Jarboe CH, Krause RA, Lynn KR, Dunnigan D, Holland JB. Potential anti-Parkinson drugs designed by receptor mapping. *J Med Chem* (1973) 16(2):147–50. doi:10.1021/jm00260a014
22. Martin YC, Holland JB, Jarboe CH, Plotnikoff N. Discriminant analysis of the relation between physical properties and the inhibition of monoamine oxidase by aminotetralins and aminoindans. *J Med Chem* (1974) 17(4):409–13. doi:10.1021/jm00250a008
23. Kalir A, Sabbagh A, Youdim MBH. Selective acetylenic suicide and reversible inhibitors of monoamine-oxidase type-A and type-B. *Br J Pharmacol* (1981) 73(1):55–64. doi:10.1111/j.1476-5381.1981.tb16771.x
24. Youdim MBH, Gross A, Finberg JPM. Rasagiline [N-propargyl-1R(+)-aminoindan], a selective and potent inhibitor of mitochondrial monoamine oxidase B. *Br J Pharmacol* (2001) 132(2):500–6. doi:10.1038/sj.bjp.0703826
25. Knudsen Gerber DS. Selegiline and rasagiline: twins or distant cousins? *Consult Pharm* (2011) 26(1):48–51. doi:10.4140/TCP.n.2011.48
26. Nichols DE, Oberlender R, Burris K, Hoffman AJ, Johnson MP. Studies of dioxole ring substituted 3,4-methylenedioxyamphetamine (MDA) analogs. *Pharmacol Biochem Behav* (1989) 34(3):571–6. doi:10.1016/0091-3057(89)90560-1
27. Nichols DE, Oberlender R. Structure-activity-relationships of mdma and related-compounds—a new class of psychoactive-drugs. *Ann N Y Acad Sci* (1990) 600:613–25. doi:10.1111/j.1749-6632.1990.tb16914.x

28. Johnson MP, Conarty PF, Nichols DE. [H-3] monoamine releasing and uptake inhibition properties of 3,4-methylenedioxymethamphetamine and para-chloroamphetamine analogs. *Eur J Pharmacol* (1991) 200(1):9–16. doi:10.1016/0014-2999(91)90659-E
29. Johnson MP, Huang X, Nichols DE. Serotonin neurotoxicity in rats after combined treatment with a dopaminergic agent followed by a nonneurotoxic 3,4-methylenedioxymethamphetamine (MDMA) analog. *Pharmacol Biochem Behav* (1991) 40(4):915–22. doi:10.1016/0091-3057(91)90106-C
30. Oberlender R, Nichols DE. Structural variation and (+)-amphetamine-like discriminative stimulus properties. *Pharmacol Biochem Behav* (1991) 38(3):581–6. doi:10.1016/0091-3057(91)90017-V
31. Monte AP, Maronalewicka D, Cozzi NV, Nichols DE. Synthesis and pharmacological examination of benzofuran, indan, and tetralin analogs of 3,4-(methylenedioxy)amphetamine. *J Med Chem* (1993) 36(23):3700–6. doi:10.1021/jm00075a027
32. Nichols DE, Hoffman AJ, Oberlender RA, Jacob P, Shulgin AT. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: representatives of a novel therapeutic class. *J Med Chem* (1986) 29(10):2009–15. doi:10.1021/jm00160a035
33. Simmler LD, Rickli A, Schramm Y, Hoener MC, Liechti ME. Pharmacological profiles of aminoindanes, piperazines, and pipradrol derivatives. *Biochem Pharmacol* (2014) 88(2):237–44. doi:10.1016/j.bcp.2014.01.024
34. Palenicek T, Lhotkova E, Zidkova M, Balikova M, Kuchar M, Himl M, et al. Emerging toxicity of 5,6-methylenedioxy-2-aminoindane (MDAI): pharmacokinetics, behaviour, thermoregulation and LD50 in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 69:49–59. doi:10.1016/j.pnpbp.2016.04.004
35. Fitzgerald RL, Blanke RV, Poklis A. Stereoselective pharmacokinetics of 3,4-methylenedioxymethamphetamine in the rat. *Chirality* (1990) 2(4):241–8. doi:10.1002/chir.530020409
36. Palenicek T, Balikova M, Rohanova M, Novak T, Horacek J, Fujakova M, et al. Behavioral, hyperthermic and pharmacokinetic profile of para-methoxymethamphetamine (PMMA) in rats. *Pharmacol Biochem Behav* (2011) 98(1):130–9. doi:10.1016/j.pbb.2010.12.011
37. Palenicek T, Fujakova M, Brunovsky M, Horacek J, Gorman I, Balikova M, et al. Behavioral, neurochemical and pharmaco-EEG profiles of the psychedelic drug 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in rats. *Psychopharmacology* (2013) 225(1):75–93. doi:10.1007/s00213-012-2797-7
38. Palenicek T, Hlinak Z, Bubenikova-Valesova V, Novak T, Horacek J. Sex differences in the effects of N,N-diethyllysergamide (LSD) on behavioural activity and prepulse inhibition. *Prog Neuropsychopharmacol Biol Psychiatry* (2010) 34(4):588–96. doi:10.1016/j.pnpbp.2010.02.008
39. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* (2001) 156(2–3): 117–54. doi:10.1007/s002130100811
40. Gatch MB, Dolan SB, Forster MJ. Locomotor, discriminative stimulus, and place conditioning effects of MDAI in rodents. *Behav Pharmacol* (2016) 27(6):497–505. doi:10.1097/Fbp.0000000000000237
41. Coppola M. Is the 5-iodo-2-aminoindan (5-IAI) the new MDMA? *J Addict Res Ther* (2012) 03(04)1–3. doi:10.4172/2155-6105.1000134
42. Coppola M, Mondola R. 5-Iodo-2-aminoindan (5-IAI): chemistry, pharmacology, and toxicology of a research chemical producing MDMA-like effects. *Toxicol Lett* (2013) 218(1):24–9. doi:10.1016/j.toxlet.2013.01.008
43. PsychonautWiki. *MDAI/Summary* (2016). Available from: https://psychonautwiki.org/wiki/MDAI/Summary
44. PsychonautWiki. *2-Aminoindane* (2016). Available from: https://psychonautwiki.org/wiki/2-Aminoindane
45. Drugs-Forum. *5-IAI (5-Iodo-2-Aminoindane) Trip Reports* (2016). Available from: https://drugs-forum.com/forum/showthread.php?t=126985
46. Tormey WP, Moore T. Poisonings and clinical toxicology: a template for Ireland. *Ir J Med Sci* (2013) 182(1):17–23. doi:10.1007/s11845-012-0828-3
47. Gallagher CT, Assi S, Stair JL, Fergus S, Corazza O, Corkery JM, et al. 5,6-methylenedioxy-2-aminoindane: from laboratory curiosity to 'legal high'. *Hum Psychopharmacol Clin Exp* (2012) 27(2):106–12. doi:10.1002/hup.1255
48. Staeheli SN, Boxler MI, Oestreich A, Marti M, Gascho D, Bolliger SA, et al. Postmortem distribution and redistribution of MDAI and 2-MAPB in blood and alternative matrices. *Forensic Sci Int* (2017) 279:83–7. doi:10.1016/j.forsciint.2017.08.007
49. Elliott S, Evans J. A 3-year review of new psychoactive substances in casework. *Forensic Sci Int* (2014) 243:55–60. doi:10.1016/j.forsciint.2014.04.017

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 November 2019 | Revised: 11 March 2020 | Accepted: 30 March 2020

DOI: 10.1111/adb.12906

ORIGINAL ARTICLE

Addiction Biology SSA WILEY

Naphyrone (naphthylpyrovalerone): Pharmacokinetics, behavioural effects and thermoregulation in Wistar rats

Nikola Pinterova-Leca[1,3] | Rachel R. Horsley[1] | Hynek Danda[1,3] | Monika Žídková[2] | Eva Lhotková[1] | Klára Šíchová[1] | Kristýna Štefková[1] | Marie Balíková[2] | Martin Kuchař[1,4] | Tomáš Páleníček[1,3]

[1]Department of Experimental Neurobiology, National Institute of Mental Health, Klecany, Czech Republic

[2]Institute of Forensic Medicine and Toxicology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

[3]Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

[4]Forensic Laboratory of Biologically Active Compounds, Department of Chemistry of Natural Compounds, University of Chemistry and Technology in Prague, Prague, Czech Republic

Correspondence
Tomáš Páleníček, National Institute of Mental Health, Topolová 748, Klecany 250 67, Czech Republic.
Email: tomas.palenicek@nudz.cz

Funding information
European Regional Development Fund, Grant/Award Number: ED 2.1.00/03.0078; Grantová Agentura České Republiky, Grant/Award Number: 20-25349S; Ministerstvo Školství, Mládeže a Tělovýchovy, Grant/Award Number: LO1611 under the NPU I programme.; Ministerstvo Vnitra České Republiky, Grant/Award Number: VI20172020056; Ministerstvo Zdravotnictví Ceské Republiky, Grant/Award Numbers: 17-31852A, MH CZ-DRO (NIMH-CZ, 00023752); Univerzita Karlova v Praze, Grant/Award Numbers: GA UK 882217, PROGRES Q35; MEYS CR, Grant/Award Number: LO1611; ED, Grant/Award Number: 2.1.00/03.0078; MH CZ—DRO, Grant/Award Number: NIMH-CZ, 00023752; AZV, Grant/Award Number: 17-31852A; GACR, Grant/Award Number: 20-25349S; GAUK, Grant/Award Numbers: PROGRES Q35, VI20172020056

Abstract

Naphthylpyrovalerone (naphyrone) is a pyrovalerone cathinone that potently inhibits monoamine transporters and provides stimulatory-entactogenic effects. Little is known about the safety of naphyrone or its effects in vivo, and more research is needed to acquire knowledge about its fundamental effects on physiology and behaviour. Our objective was to investigate naphyrone's pharmacokinetics, acute toxicity, hyperthermic potential and stimulatory and psychotomimetic properties in vivo in male Wistar rats. Pharmacokinetics after 1 mg/kg subcutaneous (sc.) naphyrone were measured over 6 h in serum, the brain, liver and lungs. Rectal temperature (degree Celsius) was measured over 10 h in group—versus individually housed rats after 20 mg/kg sc. In the behavioural experiments, 5, 10 or 20 mg/kg of naphyrone was administered 15 or 60 min prior to testing. Stimulation was assessed in the open field, and sensorimotor processing in a prepulse inhibition (PPI) task. Peak concentrations of naphyrone in serum and tissue were reached at 30 min, with a long-lasting elevation in the brain/serum ratio, consistent with observations of lasting hyperlocomotion in the open field and modest increases in body temperature. Administration of 20 mg/kg transiently enhanced PPI. Naphyrone crosses the blood–brain barrier rapidly and is eliminated slowly, and its long-lasting effects correspond to its pharmacokinetics. No specific signs of acute toxicity were observed; therefore, clinical care and harm-reduction guidance should be in line with that available for other stimulants and cathinones.

KEYWORDS

locomotion, naphyrone, novel psychoactive substance, pharmacokinetics, prepulse inhibition, thermoregulation

1 | INTRODUCTION

Naphthylpyrovalerone (1-naftalen-2-yl-2-pyrrolidin-1-ylpentan-1-on), more commonly known as naphyrone, is a pyrrolidine-containing pyrovalerone cathinone that is still readily available to buy for 'recreational' use, despite being banned in many countries.[1,2] According to users, subjective effects tend to be manifested at lower doses of naphyrone (20–35 mg) than for amphetamine (30–65 mg) and the popular drug ecstasy (3,4-methylenedioxymethamphetamine [MDMA]) (75–125 mg) but higher than for the related pyrovalerone cathinone methylenedioxypyrovalerone (MDPV) (8–15 mg).[3] The subjective effects of naphyrone are typical of novel psychoactive substances (NPSs) with a mixed profile, involving feelings of empathy, elevated mood/euphoria and physical and mental stimulation, which develop within minutes and last for 6 to 12 h.[3]

Naphyrone acts as a triple monoamine inhibitor, inhibiting the norepinephrine transporter (NET), then the dopamine transporter (DAT) as well as potently inhibiting the serotonin transporter (SERT), albeit to a lesser extent: all $IC_{50}\mu M$ values < 1.[4–7] In HEK 293 cells, naphyrone's DAT/SERT inhibition ratio is 2.0 (DAT IC_{50} = 0.47 μM, SERT IC_{50} = 0.96 μM). By comparison, the ratio for ecstasy (MDMA) in HEK cells is 0.08, cocaine is 3.1[7] and one of the most potent stimulant-like pyrovalerone analogue MDPV's is reported to be as high as 100 to 300.[7,8] Other well-known cathinones such as mephedrone and methylone act as substrate releasers (as well as inhibitors) at transporter sites; however, naphyrone, like MDPV, does not induce a transporter-mediated release.[7] Taken together, naphyrone's general pharmacological profile suggests cocaine-like characteristics, with stimulatory effects more prominent, which may predict high abuse potential.[9,10]

In the present study, concentrations of naphyrone in serum as well as in brain, liver and lung tissue were measured over 6 h. Based on its known high lipophilicity,[4] rapid transition of naphyrone concentrations from serum to brain was expected indicative of a rapid penetration of the blood–brain barrier,[7] as well as drug accumulation in the liver and particularly in the lungs, as with MDPV.[11]

Naphyrone, like other cathinones, can result in poisoning and overdose manifested as sweating, hyperthermia, arterial constriction with cold extremities, tachycardia, hypertension, restlessness/insomnia, anxiety, hallucinations and seizures.[12–14] A case of acute sympathomimetic toxidrome has been described.[13] Environmental crowding and hotter ambient temperatures can exacerbate drug-induced hyperthermia, which has been demonstrated in cathinones, including MDPV, which modestly increases brain and body temperature in rodents and under conditions of social interaction and high ambient temperatures.[15–17] In the present study, the effect of 20 mg/kg of naphyrone on thermoregulation was tested with the prediction that naphyrone would result in elevated body temperature, particularly in group-housed rats.

Locomotor stimulation in rodents after pyrovalerone[18,19] and MDPV has been reported.[20–22] However, there is only one preclinical behavioural study of naphyrone. This shows time- and dose-dependent increases in locomotion in mice after 3, 10, 30 or 100 mg/kg intraperitoneally (ip.).[23] At the time of our experiments, these results had not been published yet, and there are no data for a rat model. We tested the locomotor stimulatory effects of naphyrone (5, 10 or 20 mg/kg subcutaneously [sc.]) in the open field.

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is a behavioural measure of sensorimotor processing.[24] PPI disruption often reflects the psychotomimetic properties of drugs and is a behavioural endophenotype of schizophrenia.[25] In humans, naphyrone use can result in psychotic symptoms such as paranoia and hallucinations.[13] Naphyrone's effect on PPI has never been tested; however, because MDPV moderately disrupted PPI in rats,[11] we can expect naphyrone to behave similarly.

The present experiments were conducted as part of a larger study series that investigated the effects of NPSs across a standard battery of tests to simplify between NPS comparisons. Controlled preclinical experiments continue to be irreplaceable with regard to obtaining translationally relevant knowledge about effects in intact organisms that can help in harm reduction and clinical care in humans. We present in vivo data to further the understanding of the acute pharmacokinetic, behavioural and thermoregulatory effects of naphyrone.

Taken together, the main aim of this study was to provide a detailed evaluation of the pharmacokinetic, stimulatory, psychotomimetic and thermoregulatory effects of acute naphyrone in Wistar rats.

2 | MATERIALS AND METHODS

2.1 | Design

To capture naphyrone's temporal effects, two behavioural testing onsets were used, 15 and 60 min post-drug administration. Pharmacokinetics were measured over 8 h and thermoregulatory effects over 10 h.

2.2 | Animals

Male outbred Wistar rats (Velaz, Czech Republic: CZ) were housed in pairs in a 12/12 h light/dark cycle with ad libitum water and a pellet diet. Temperature (22 ± 2°C) and humidity (30–70%) were controlled. The rats acclimatised to the laboratory for 7 to 10 days (they were weighed twice and handled at least four times) before testing. Tests were performed between 7:00 and 17:00 h. At the start of the tests, the rats weighed between 180 and 250 g. The principles of the National Committee for the Care and Use of Laboratory Animals, CZ, and European Union guidelines (86/609/EU) were adhered to. Ethical approval was given by the National Committee for the Care and Use of Laboratory Animals, CZ (reference: MEYSCR-27527/2012–3). Across the open field (N = 80), PPI (N = 80) and thermoregulation (N = 40), altogether, 200 rats were used. Rats from the behavioural experiments were also used for pharmacokinetic sampling (N = 40), requiring eight additional rats for sampling at

30 min. Therefore, 208 naïve rats were used in total. In all of the experimental designs, n = 10 per group, except for the pharmacokinetic study where n = 8.

2.3 | Drugs and chemicals

1-Naftalen-2-yl-2-pyrrolidin-1-ylpentan-1-on (naphyrone) was purchased via the Internet, purified and converted to a hydrochloride (HCl) by Alfarma s.r.o. (CZ). The resulting naphyrone was certified as being 99.88% pure and also served as a reference standard for pharmacokinetic analyses. The internal standard for quantitative liquid chromatography/mass spectrometry (LC/MS) assays was deuterated with naphyrone-D8.HCl with 99.7% purity (Alsachim, France). Extraction columns (Bond Elut Certify 50 mg/3 ml) were supplied by Labio (CZ).

For the behavioural, pharmacokinetic and thermoregulatory experiments, naphyrone was dissolved in 0.9% physiological saline (VEH) and administered sc. at a volume of 2 ml/kg. Control animals were administered an equivalent volume of VEH sc. In the behavioural experiments, 5, 10 and 20 mg/kg were tested. All of the selected doses were based on our previous studies on NPS such as mephedrone, methylone, MDAI and MDMA[26–29] and were also estimated according to the dosages reported by users.[3] About 10 mg/kg was selected for the pharmacokinetic study and 20 mg/kg for thermoregulation. The lower doses (5 and 10 mg/kg) were intended to produce clear behavioural effects without any signs of toxicity, as they are within a range already shown to produce locomotor effects.[23] The higher dose (20 mg/kg) was chosen to simulate overdose in human use, with stereotypy and/or some acute toxic effects possible.

2.4 | Pharmacokinetics

Rats were administered 10 mg/kg of naphyrone sc. and decapitated after 30, 60, 120, 240 or 480 min (n = 8 per time point), whereupon serum, the brain, liver and lungs were taken and frozen at −20°C (until the toxicological analyses). Median maximum concentrations of naphyrone in sera and tissues were calculated as nanograms per millilitre or nanogram per gram. The brain/serum ratio was calculated as the median brain concentration/median serum concentration per sampling time point.

2.4.1 | Determination of naphyrone levels in serum and tissue samples using LC/HRMS

To prepare serum samples for analysis, 0.2 ml of rat serum was fortified with the internal standard (naphyrone-D8 in methanolic solution in an amount with respect to the levels of naphyrone in assayed samples) and 0.5 ml of 0.1M phosphate buffer (pH 6). In the case of tissue sample preparation, 250 mg of tissue (brain, lung and liver) was homogenised with 5 ml of methanol and the internal standard (naphyrone-D8 in an amount with respect to the naphyrone levels in the tissue samples). Each specimen was ultrasonicated for 20 min, and the supernatant (separated by centrifugation) was evaporated to dryness and then reconstituted in a 0.1M phosphate buffer (pH 6).

For the solid-phase extraction (SPE) of naphyrone, a pretreated sample plus a buffer and the internal standard, was loaded onto a Bond Elut Certify cartridge (preconditioned with 0.5 ml of 0.1M phosphate buffer, pH 6). The cartridge was washed between samples with 0.5 ml of distilled water, 0.5 ml of 0.1M HCl and 0.5 ml of CH_3OH/H_2O (1/1, v/v) and then air-dried for 5 min. The analytes were eluted three times with 0.5-ml dichloromethane/2-propanol/ammonium hydroxide (25%), 80/20/4, v/v/v. The eluate was gently evaporated to dryness under a stream of air (40°C) and then dissolved into a mobile phase for LC/high-resolution mass spectrometry (HRMS).

2.4.2 | LC/HRMS conditions

LC/HRMS used a Dionex Ultimate 3000 UHPLC coupled to an Exactive Plus-Orbitrap Mass Spectrometer (ThermoFisher Scientific, Bremen, Germany) equipped with a HESI-II source. The chromatographic analyses used a Kinetex PFP 100 A (50 × 2.1 mm, 2.6 mm) and Security Guard Cartridge PFP 4 × 2.0 mm (Phenomenex) with a flow rate of 400 ml/min. Gradient elution was with a 10mM ammonium formate in 0.1% of formic acid as mobile phase B. Gradient at 0 min = 5%, 4 min = 45% B, and 5–6 min = 95% (held). The MS conditions were full MS at scan range 50–500 m/z, positive electrospray ionisation, resolution of 70 000 (full width at half maximum [FWHM], 3-Hz scan speed), 3 kV spray voltage and an ion transfer capillary temperature of 320°C.

2.5 | Behavioural procedures

2.5.1 | Open field

At 15 or 60 min post administration of 5, 10 and 20 mg/kg of naphyrone or VEH, the rats were placed individually into the centre of the open field (a 68 × 68 × 30 cm square black plastic arena), and their behaviour was video recorded for 30 min. Ethovision Colour-Pro Version 3.1.1 (Noldus, Netherlands) was used for behavioural capture and preprocessing. During the preprocessing, the arena was divided into 5 × 5 identical square zones with 16 located around the periphery and nine centrally in order to derive thigmotaxis and T_{centre} values. Trajectory length (centimetre, corrected for 3 cm deviations), thigmotaxis (measured as $\Sigma f_{peripheral\ zones}/\Sigma f_{all\ zones}$, where f = frequency of line crossings) and T_{centre} (calculated as Σ_{time} in the central zones) were measured. The procedures were the same as those published previously.[26,28–31]

2.5.2 | Prepulse inhibition

Two days before the tests, the rats were acclimatised to the startle chamber (SR-LAB, San Diego Instruments, CA, USA) with a drug-free 5 min pre-exposure to five pulse alone stimuli (115 dB/20 ms) over 75 dB of continuous white noise. On the test day, 15 or 60 min prior to testing, the rats were administered 5, 10 or 20 mg/kg naphyrone or VEH, placed into the startle chamber and acclimatised for 5 min to a continuous 75 dB of white noise. They were then presented with six 125 dB/40 ms duration pulse alone trials, followed by 60 pseudorandomised trials of the following: (a) pulse alone, 40 ms of 125 dB; (b) prepulse–pulse, 20 ms of a 83 or 91 dB prepulse, a variable (30, 60 or 120 ms) interstimulus interval (ISI: mean 70 ms), then 40 ms of a 125 dB pulse; and (c) 60 ms of no stimulus. Finally, six pulse alone trials were delivered. There were 72 trials in total with intertrial intervals (ITIs) of 4–20 s (mean ITI = 12.27 s). All of the measures were derived from average startle amplitudes (AVG) and were as follows: percentage habituation (percentage reduction in ASR from six baseline trials to the final six trials), ASR (mean ASR was derived from pulse alone trials) and percentage PPI {calculated as: [100 – (mean prepulse–pulse trials/mean pulse alone trials) × 100]}.

2.6 | Thermoregulation

Under controlled laboratory conditions (as described previously), thermoregulation was observed over 10 h (13 time points) in group-housed (five animals per home cage) and individually-housed (one animal per home cage) rats. For each observation, the rats were briefly (10 s) immobilised in a Plexiglas tube, and their rectal temperature was measured with a digital thermometer. The first measurements (drug-free) were hourly (7:00–9:00 h), at 9:00 h naphyrone (20 mg/kg) or VEH was administered, then measurements were every 30 min until 11:00 h, after which they were measured hourly until 17.00 h.

2.7 | Statistical analysis

Statistical analyses of the behavioural and thermoregulation data were conducted using IBM SPSS Version 22. Default alpha was $p = .05$, two tailed. For open field, PPI and temperature data, factorial analysis of variance (ANOVA) was used. The open field and PPI experiments used a 4 × 3 factorial design with drug treatment (5, 10 and 20 mg/kg or vehicle: VEH) and testing onset (15 or 60 min) as independent factors. For the open field, trajectory length in 6 × 5 min time bins was included as a repeated measures factor. The thermoregulation study used a 4 × 2 × 13 mixed factorial design with drug treatment (5, 10 and 20 mg/kg naphyrone or VEH) and home-cage condition (group or individually caged) as independent factors and measurement time points (13) as a repeated measures factor. Planned pairwise comparisons (to follow up on any significant main effects and interactions) used independent *t* tests. In order to limit inflation of type 1 errors, the number of comparisons was restricted to those necessary to compare naphyrone with VEH. Where Mauchly's test (repeated measures ANOVA) or Levene's test were significant, corrected statistics are presented (degrees of freedom are rounded to the nearest whole number).

3 | RESULTS

3.1 | Pharmacokinetics

A maximum median serum concentration of 269 ng/ml of naphyrone was attained 30 min after a 10 mg/kg sc. bolus dose. The serum levels remained steady for the next 2 h and then declined modestly for the remainder of the observation. Influx into brain tissue was not delayed, a maximum median concentration of 1737 ng/kg was reached at 30 min and decreased steadily over the 8 h measurement period. The brain/serum ratio was 6.5 at 30 min and was varied from 3.4 (at 2 h) and 5.5 (at 6 h) through the whole temporal observation with an increase to 12.8 four hours after dosage. Naphyrone reached a maximum concentration of 3,025 mg/kg in the lungs at one hour after drug administration (double the brain levels, despite being slightly delayed by 30 min compared with the influx into the brain). Likewise, the maximum in the liver was reached at 1 h (425 ng/kg), which was only slightly higher than the serum concentrations (Figure 1).

3.2 | Locomotor activity in the open field

3.2.1 | Trajectory length

Trajectory length data showed significant main effects of the drug treatment [$F_{(3,\ 72)} = 23.65$, $p < .001$], testing onset [$F_{(1,\ 72)} = 4.09$, $p < .05$] and blocks [$F_{(3,\ 236)} = 297.7$, $p < .001$]. There was a significant drug treatment *x* blocks interaction [$F_{(10,\ 236)} = 6.06$, $p < .001$] and a significant testing-onset *x* blocks interaction [$F_{(3,\ 236)} = 6.71$, $p < .001$] (see Figure 2A,B). Pairwise comparisons using independent *t* tests showed that at the 15 min testing-onset (Figure ure 2A) naphyrone-treated rats (all doses) showed significantly higher locomotor activity in blocks two to six compared with VEH, minimum $t_{(11)} = 2.24$, $p < .05$. Figure ure 2C shows examples of locomotion in naphyrone- and VEH-treated rats tracked over the 30 min testing session (15 min testing-onset group). The tracking patterns suggest that at 20 mg/kg, stereotypy rats tend to repeatedly circle the periphery, with less activity in the central zone. At the 60 min testing-onset (Figure ure 2B), activity was higher in all six blocks for all naphyrone doses compared with VEH, minimum $t_{(18)} = 2.21$, $p < .05$.

3.2.2 | T_{centre} and Thigmotaxis

T_{centre} data showed a significant main effect of the drug treatment [$F_{(3,\ 72)} = 13.22$, $p < .001$] but no main effect of

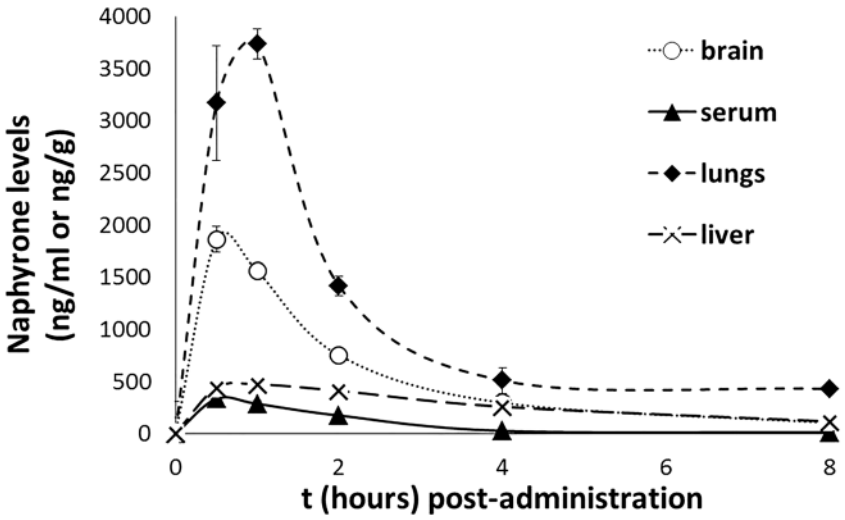


FIGURE 1 Median naphyrone concentrations (c[ng/mL or ng/g]) in serum, the brain, liver and lungs after 10 mg/kg of naphyrone sc. Measurements were made over 6 h at 30, 60, 120, 240 or 480 min post administration

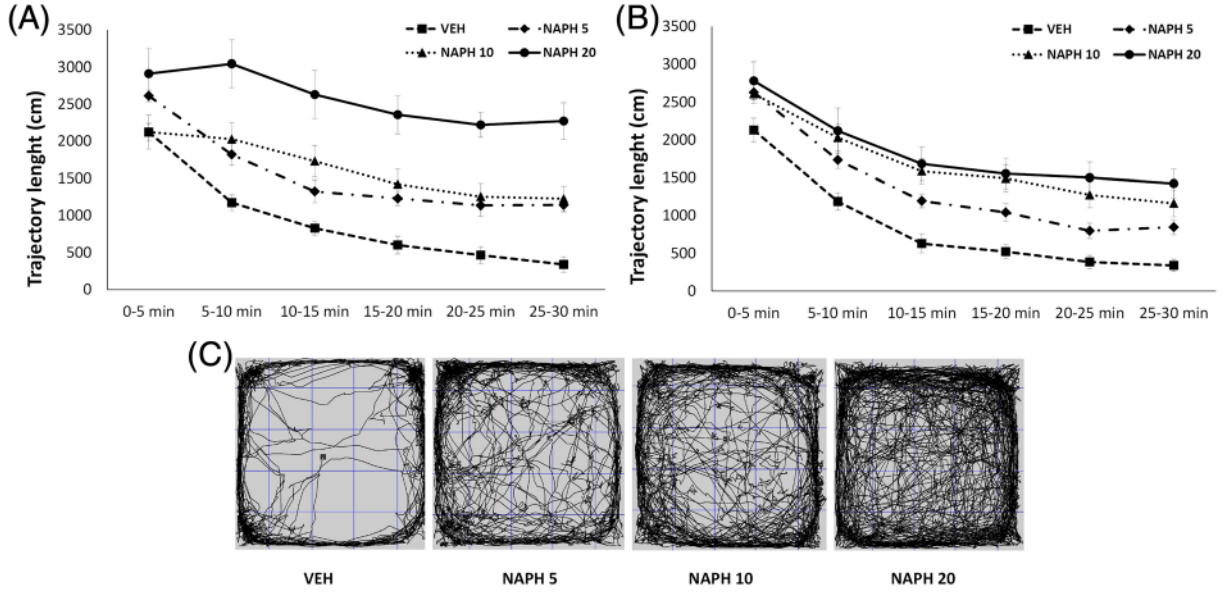


FIGURE 2 Mean trajectory length (centimetre) over 30 min (shown as 6 × 5-min time blocks) after sc. naphyrone (5, 10 or 20 mg/kg) versus vehicle. Measurements commenced at 15 min (A) or 60 min (B) post administration. Error bars represent standard error of the means (S.E.M.s) (C) shows typical trajectory patterns in the 15-min testing-onset drug groups. NAPH 5, 10, 20 = naphyrone 5, 10, 20 mg/kg [Colour figure can be viewed at wileyonlinelibrary.com]

testing onset or testing-onset *x* drug treatment interaction. Independent *t* tests showed that naphyrone (at all doses, irrespective of testing onset) increased time spent in the centre compared with VEH, minimum $t_{(25)}$ = 5.03, p < .001 (Figure 3A). Thigmotaxis data showed significant main effects of the drug treatment [$F_{(3,\ 72)}$ = 7.86, p < .001] and testing onset [$F_{(1,\ 72)}$ = 4.63, p < .05] but no testing-onset *x* drug treatment interaction. The main effect of testing onset reflected a modest reduction in thigmotaxis at the 60-min testing onset compared with the 15-min testing onset. Independent *t* tests showed that all doses of naphyrone decreased the probability of appearance in peripheral zones, compared with VEH, minimum $t_{(38)}$ = 2.03, p < .05 (Figure 3B).

3.3 | Prepulse inhibition

Habituation and ASR showed no significant main effects or interactions (see Table 1). PPI data showed no main effects, but the drug treatment *x* testing-onset interaction was significant [$F_{(3,\ 70)}$ = 3.10, p < .05]. Independent *t* tests showed that 20-mg/kg naphyrone modestly increased PPI, compared with VEH, but only at the 15-min testing onset, $t_{(18)}$ = 1.80, p < .05 (Figure 4A,B).

3.4 | Rectal temperature

Statistical analysis revealed significant main effects of the drug treatment [$F_{(1,\ 36)}$ = 49.50, p < .001] and time [$F_{(6.34,\ 432)}$ = 58.33, p < .001]

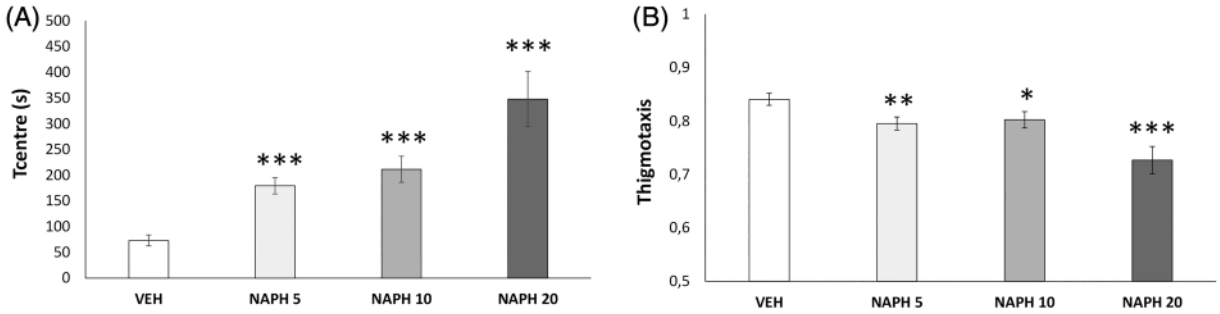


FIGURE 3 Mean total time (seconds) spent in the centre (A) and mean total thigmotaxis (probability of appearance in peripheral zone) (B) over 30 min in the open field. Data are shown by drug treatment condition (5, 10 or 20 mg/kg naphyrone versus vehicle). Because there was no significant effect of testing onset, the data are collapsed across the 15 min and 60 min testing onset groups. Error bars represent standard error of the means (S.E.M.s). $^{***}p < .001$, $^{**}p < .01$ and $^{*}p < .05$ compared with VEH. NAPH 5, 10, 20 = naphyrone 5, 10, 20 mg/kg

TABLE 1 Effect of naphyrone (5, 10 and 20 mg/kg) on acoustic startle response (ASR) in arbitrary units and habituation (testing onset 15 or 60 min). The numbers represent means and standard error of the means (S.E.M.s) are in brackets

Drug treatment					
Measure	**Testing onset**	**Vehicle**	**5 mg/kg**	**10 mg/kg**	**20 mg/kg**
ASR (AVG)	15 min	183.4 (60.1)	117.1 (22.2)	73.0 (18.6)	163.1 (35.3)
	60 min	157.5 (36.2)	98.3 (14.7)	179.6 (44.5)	180.4 (33.8)
Percentage habituation	15 min	40.4 (10.9)	56.2 (6.9)	56.4 (6.8)	34.4 (13.2)
	60 min	67.1 (6.1)	38.3 (12.9)	50.8 (8.6)	30.2 (14.8)

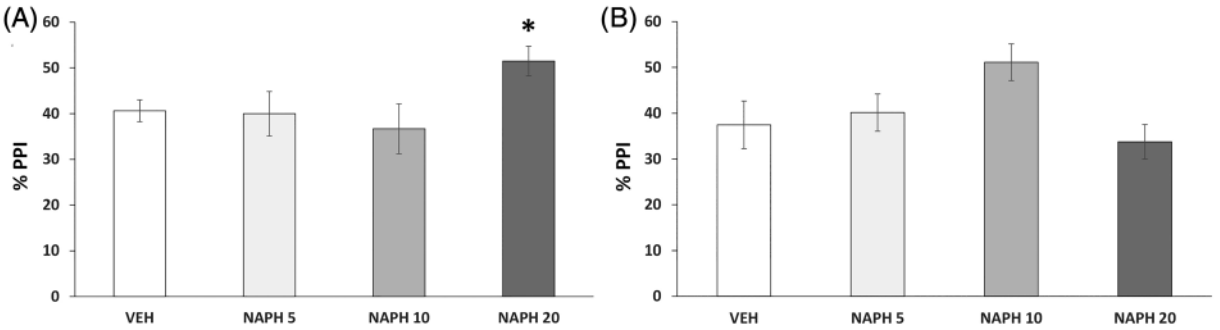


FIGURE 4 Mean prepulse inhibition (PPI) (%) after sc. naphyrone (5, 10 or 20 mg/kg) versus vehicle. PPI measurements commenced at 15 min (A) or 60 min (B) post administration. Error bars represent standard error of the means (S.E.M.s). $^{*}p < .05$ compared with VEH. NAPH 5, 10, 20 = naphyrone 5, 10, 20 mg/kg

and a significant drug treatment *x* time interaction [$F_{(6.34,\ 432)} = 19.42$, $p < .001$] (Figure 5A,B. Independent *t* tests revealed the significant effect of naphyrone 20 mg/kg (irrespective of home-cage condition) on rectal temperature, shown as raised rectal temperature lasting for 4 h from time of administration, minimum $t_{(38)} = 3.42$, $p = .001$. There was also a significant main effect of home-cage condition [$F_{(1,\ 36)} = 7.58$, $p < .01$], manifested as higher temperature in group-housed rats, $M = 38.04$ (standard error of the mean [SEM] = 0.04), compared with individually housed rats, $M = 37.88$ (SEM = 0.04).

4 | DISCUSSION

Naphyrone had fast pharmacokinetics within 30 min after application. The highest concentrations were measured in lung tissue followed by brain tissue. Acute administration of naphyrone showed a dose-dependent stimulant effect at both testing onsets, mainly at 15-min testing onset, and affect the distribution of locomotor behaviour in the open field. Only the highest naphyrone dose at 15 min testing onset altered PPI. The effect of naphyrone on rectal temperature was modest but long lasting.

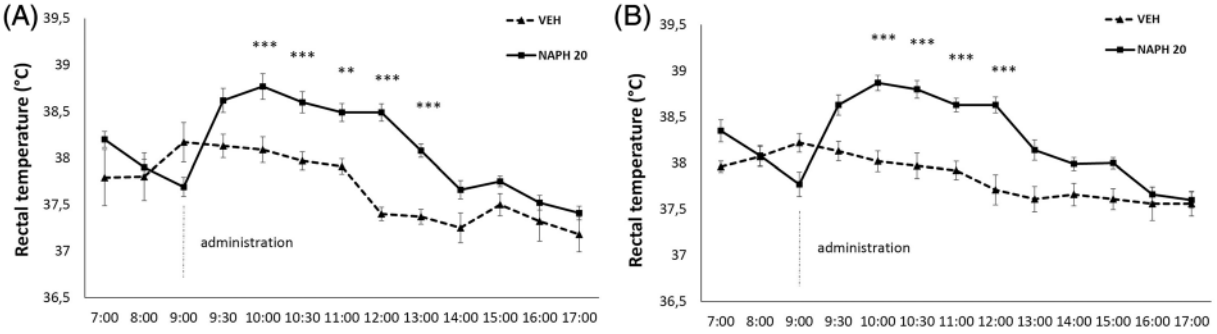


FIGURE 5 Mean rectal temperature (degree Celsius) in individually-housed (A) versus group-housed (B) rats after sc. naphyrone (20 mg/kg) versus vehicle. Temperature measurements were made hourly throughout the 10 h observation period, except for the 2 h period post administration where they were taken at 30 min intervals. Error bars represent standard error of the means (S.E.M.s). $^{***}p < .001$, $^{**}p < .01$ compared with VEH; NAPH 20 = naphyrone 20 mg/kg

The pharmacokinetic findings indicate fast absorption/distribution and a relatively long half-life of naphyrone in serum and brain, which is consistent with the reported rapid onset and long duration of its stimulatory effects in rodents and humans.[6,13] Consistent with naphyrone's high lipophilicity (log*P* = 4.88), influx into the brain was fast, and the peak brain/serum ratio of 6.5 was high. Naphyrone's elimination from other tissues was much slower than from serum, which was indicated by a brain/serum ratio of more than 12, 4 h after administration. Accumulation in lung tissue is characteristic of parenterally administered cationic compounds with a lipophilic profile, partly as a consequence of the relatively large volume of blood flowing to the lungs, which transports substances there where they can become trapped.[32]

Long-lasting, dose-dependent increases in locomotor activity were observed at both testing onsets, consistent with concentrations of naphyrone in the brain. However, in absolute terms, the stimulatory effect on locomotion was not powerful. Given that naphyrone's inhibition of DAT is potent at lower concentrations than cocaine, this is surprising. Pyrrolidine-containing synthetic cathinone derivatives have a characteristic inverted 'U' dose-response relationship with locomotor behaviour; therefore, higher doses can result in reduced, rather than increased activity.[23,33–35] This would not appear to account for the findings here because we found increasing stimulation with each higher dose tested, suggesting a dose-response on the rising slope of the inverted 'U'. Alternatively, because naphyrone is a nonselective monoamine transporter inhibitor, its powerful inhibition of SERT may have attenuated DA-induced locomotor effects.[36] A similar mechanism has been described for MDMA, when at lower doses hyperlocomotion is primarily induced by the serotonergic system and with increasing dose an effect of the dopaminergic system is manifested.[27] Rats usually spend most time in the periphery of the open field (next to the arena walls), and less in the centre, which is mildly aversive due to its brighter and more open qualities. The spatial characteristics of activity demonstrated dose-dependent increases in time in the centre, which was not a result of general locomotor activation because thigmotaxis also decreased. We did not observe obvious stimulant-typical stereotypies[22,37,38] that would account for the data. Therefore, naphyrone clearly induces general locomotor stimulation, not as potent as mephedrone[28] but more stimulatory than methylone.[29] Increased time in the centre and decreased thigmotaxis with a dose-dependent manner suggest increased exploration and decreased anxiety, like MDMA.[27] In contrast, MDPV increases thigmotaxis and stereotypes emerge due to its dopaminergic properties.[11]

Stimulants and cathinones, including MDPV, can disrupt PPI but usually only at higher doses.[11,29,39–41] Like MDPV,[11] only the highest dose of naphyrone affected PPI and only transiently so (at the 15-min testing onset), when brain and serum concentrations were highest. On the contrary to MDPV and other cathinones, naphyrone increased PPI. A possible explanation for the observed effect can be the fact that low doses of stimulants are associated with a cognition enhancing effect,[42] and robust effects on sensorimotor gating may only be present at higher doses. It is possible that because naphyrone affects mainly the serotonin system, it attenuates PPI via serotonergic receptors.[43] Although serotonergic drugs (MDMA and MDAI) disrupt PPI in rodents,[26,44] Liechti, Geyer, Hell and Vollenweider[45] showed an enhancing effect of MDMA on PPI in their study on human volunteers. Thus, specific-species differences can be involved, and more information about the underlying mechanism is needed.

Naphyrone modestly elevated rectal temperatures by approximately 0.5°C to 1°C, commencing at 30 min, peaking at 60 min and lasting for several hours (consistent with the time course for pharmacokinetics). Despite the fact that naphyrone is more serotonergic than MDPV,[5,7,9] its hyperthermic effect appears to be less powerful. This is surprising because 5-HT is the crucial neuromodulator controlling thermoregulation and where MDPV has almost no activity. An explanation may be the increase in body temperature secondary to the huge physical hyperactivity elicited by MDPV but not by naphyrone. Compared with MDPV, naphyrone has less potency for NET and DAT inhibition,[7] which may explain its milder effects. Para-methoxymethamphetamine (PMMA) and MDMA also result in profound hyperthermia (under group-housing conditions) mediated by 5-HT2A receptors.[46] It is, therefore, somewhat surprising that we did not observe more powerful effects on body temperature, particularly given the relatively high dose that was tested. We did

not observe obvious indications of toxicity (at the doses tested): no gross motor effects, stereotypes, sweating or other signs of physical distress.

Nevertheless, the possible adverse effects of long-term, chronic and binge use of naphyrone (or the effects of poly-drug use involving naphyrone) on psychological and physical health are as yet unknown,[35,47] and more studies about molecular toxicological mechanisms are needed.[48] Despite naphyrone's pharmacological characteristics with respect to its DAT/SERT ratio, surprisingly, we found only mild hyperthermia and enduring but relatively mild stimulation (with enhanced exploration). These modest effects are consistent with the fact that naphyrone never became as popular as its predecessor, mephedrone, or as widely used as other NPSs that have mixed stimulatory-entactogenic effects; it would appear that naphyrone somehow lacks the profile suitable for a 'party' drug. Understanding the pharmacological characteristics associated with NPSs that fail to become popular (as well as those that are widely used) may help to more accurately predict which emerging NPSs represent a potential public health issue.

ACKNOWLEDGEMENTS

This work was supported by projects GAUK 882217 (Charles University Grant Agency), VI20172020056 (Ministry of the Interior of the Czech Republic), PROGRES Q35 (Charles University in Prague), GACR No.: 20-25349S (Czech Science Foundation), AZV 17-31852A (Czech Health Research Council), MH CZ—DRO (NIMH-CZ, 00023752; Ministry of Health of the Czech Republic), and ED 2.1.00/03.0078 (European Regional Development Fund), and grant LO1611 under the NPU I programme (Ministry of Education, Youth and Sports).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Nikola Pinterova-Leca https://orcid.org/0000-0001-6242-4413
Rachel R. Horsley https://orcid.org/0000-0003-2536-2465
Monika Žídková https://orcid.org/0000-0001-7891-277X
Marie Balíková https://orcid.org/0000-0001-6334-1177
Tomáš Páleníček https://orcid.org/0000-0002-3109-9539

REFERENCES

1. Brandt SD, Freeman S, Sumnall HR, Measham F, Cole J. Analysis of NRG 'legal highs' in the UK: identification and formation of novel cathinones. *Drug Test Anal*. 2011;3(9):569-575.
2. EMCDDA. *European Drug Report 2017: Trends and Developments*. Luxembourg: Publications Office of the European Union; 2017.
3. TripSit. Factsheets. 2017; http://drugs.tripsit.me/. Accessed 24/05, 2018.
4. Eshleman AJ, Wolfrum KM, Hatfield MG, Johnson RA, Murphy KV, Janowsky A. Substituted methcathinones differ in transporter and receptor interactions. *Biochem Pharmacol*. 2013;85(12):1803-1815.
5. Iversen L, Gibbons S, Treble R, Setola V, Huang XP, Roth BL. Neurochemical profiles of some novel psychoactive substances. *Eur J Pharmacol*. 2013;700(1-3):147-151.
6. Meltzer PC, Butler D, Deschamps JR, Madras BK. 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (Pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. *J Med Chem*. 2006;49:1420-1432.
7. Simmler LD, Buser TA, Donzelli M, et al. Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol*. 2013;168(2):458-470.
8. Liechti M. Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. *Swiss Med Wkly*. 2015;145:1-12.
9. Rickli A, Hoener MC, Liechti ME. Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. *Eur Neuropsychopharmacol*. 2015;25(3):365-376.
10. Gannon BM, Fantegrossi WE. Cocaine-like discriminative stimulus effects of mephedrone and naphyrone in mice. *J Drug Alcohol Res*. 2016;5:1-5.
11. Horsley RR, Lhotkova E, Hajkova K, et al. Behavioural, pharmacokinetic, metabolic, and hyperthermic profile of 3,4-methylenedioxypyrovalerone (MDPV) in the Wistar rat. *Front Psych*. 2018;9(144):1-14.
12. Assi S, Gulyamova N, Kneller P, Osselton D. The effects and toxicity of cathinones from the users' perspectives: a qualitative study. *Hum Psychopharmacol*. 2017;32(3):e2610:1-7.
13. Derungs A, Schietzel S, Meyer MR, Maurer HH, Krahenbuhl S, Liechti ME. Sympathomimetic toxicity in a case of analytically confirmed recreational use of naphyrone (naphthylpyrovalerone). *Clin Toxicol (Phila)*. 2011;49:691-693.
14. Jebadurai J, Schifano F, Deluca P. Recreational use of 1-(2-naphthyl)-2-(1-pyrrolidinyl)-1-pentanone hydrochloride (NRG-1), 6-(2-aminopropyl) benzofuran (Benzofury/6-APB) and NRG-2 with review of available evidence-based literature. *Human Psychopharmacol-Clin Exp*. 2013;28(4):356-364.
15. Fantegrossi WE, Gannon BM, Zimmerman SM, Rice KC. In vivo effects of abused 'bath salt' constituent 3,4-methylenedioxypyrovalerone (MDPV) in mice: drug discrimination, thermoregulation, and locomotor activity. *Neuropsychopharmacology*. 2013;38(4):563-573.
16. Gannon BM, Galindo KI, Rice KC, Collins GT. Individual differences in the relative reinforcing effects of 3,4-methylenedioxypyrovalerone under fixed and progressive ratio schedules of reinforcement in rats. *J Pharmacol Exp Ther*. 2017;361(1):181-189.
17. Kiyatkin EA, Ren SE. MDMA, methylone, and MDPV: drug-induced brain hyperthermia and its modulation by activity state and environment. *Curr Top Behav Neurosci*. 2017;32:183-207.
18. Stille G, Ackermann H, Eichenberger H, Lauener H. Vergleiehende pharmakologische untersuching eines sentralem stimulans 1-p-tolyl-1-oxo-2-pyrrolidino-n-pentan-HCl. *Arzneimittle-Forsch*. 1963;13:871-877.
19. Vaugeois J-M, Bonnet J-J, Duterte-Boucher D, Costentin J. In vivo occupancy of the striatal dopamine uptake complex by various inhibitors does not predict their effects on locomotion. *Eur J Pharmacol*. 1993;230:195-201.
20. Anizan S, Concheiro M, Lehner KR, et al. Linear pharmacokinetics of 3,4-methylenedioxypyrovalerone (MDPV) and its metabolites in the rat: relationship to pharmacodynamic effects. *Addict Biol*. 2016;21(2):339-347.
21. Moreno-Rius J, Pubill Sánchez D, Escubedo Rafa E, Camarasa Garcia J, Miquel M. Locomotor activating effects and addiction-like

features of MDPV as assessed in preclinical studies: a review. *Agora de Salut*. 2017;4:239-246.
22. Novellas J, Lopez-Arnau R, Carbo ML, Pubill D, Camarasa J, Escubedo E. Concentrations of MDPV in rat striatum correlate with the psychostimulant effect. *J Psychopharmacol*. 2015;29(11):1209-1218.
23. Gatch MB, Taylor CM, Forster MJ. Locomotor stimulant and discriminative stimulus effects of 'bath salt' cathinones. *Behav Pharmacol*. 2013;24(5-6):437-447.
24. Swerdlow NR, Geyer MA. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull*. 1998;24(2):285-301.
25. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl)*. 2001;156(2-3):117-154.
26. Palenicek T, Lhotkova E, Zidkova M, et al. Emerging toxicity of 5,6-methylenedioxy-2-aminoindane (MDAI): pharmacokinetics, behaviour, thermoregulation and LD50 in rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 2016;69:49-59.
27. Palenicek T, Votava M, Bubenikova V, Horacek J. Increased sensitivity to the acute effects of MDMA ("ecstasy") in female rats. *Physiol Behav*. 2005;86(4):546-553.
28. Sichova K, Pinterova N, Zidkova M, et al. Mephedrone (4-methylmethcathinone): acute behavioral effects, hyperthermic, and pharmacokinetic profile in rats. *Front Psych*. 2017;8(306):1-11.
29. Štefková K, Židková M, Horsley RR, et al. Pharmacokinetic, ambulatory, and hyperthermic effects of 3,4-methylenedioxy-N-methylcathinone (methylone) in rats. *Front Psych*. 2017;8(232):1-11.
30. Horsley RR, Lhotkova E, Hajkova K, Jurasek B, Kuchar M, Palenicek T. Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue-behavioural, pharmacokinetic and metabolic studies in the Wistar rat. *Brain Res Bull*. 2016;126(Pt 1):102-110.
31. Uttl L, Szczurowska E, Hajkova K, et al. Behavioral and pharmacokinetic profile of indole-derived synthetic cannabinoids JWH-073 and JWH-210 as compared to the phytocannabinoid delta(9)-THC in rats. *Front Neurosci*. 2018;12(703):1-10.
32. Upton RN, Doolette DJ. Kinetic aspects of drug disposition in the lungs. *Clin Exp Pharmacol Physiol*. 1999;26(5-6):381-391.
33. Aarde SM, Huang PK, Creehan KM, Dickerson TJ, Taffe MA. The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: self-administration and locomotor activity in rats. *Neuropharmacology*. 2013;71:130-140.
34. Baumann MH, Partilla JS, Lehner KR, et al. Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. *Neuropsychopharmacology*. 2013;38(4):552-562.
35. Huang PK, Aarde SM, Angrish D, Houseknecht KL, Dickerson TJ, Taffe MA. Contrasting effects of d-methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxypyrovalerone, and 4-methylmethcathinone on wheel activity in rats. *Drug Alcohol Depend*. 2012;126(1-2):168-175.
36. Baumann MH, Bukhari MO, Lehner KR, et al. Neuropharmacology of 3,4-methylenedioxypyrovalerone (MDPV), its metabolites, and related analogs. *Curr Top Behav Neurosci*. 2017;32:93-117.
37. Anizan S, Ellefsen K, Concheiro M, et al. 3,4-Methylenedioxypyrovalerone (MDPV) and metabolites quantification in human and rat plasma by liquid chromatography-high resolution mass spectrometry. *Anal Chim Acta*. 2014;827:54-63.
38. Kuczenski R, Segal D, Aizenstein M. Amphetamine, cocaine, and fencamfamine: relationship between locomotor and stereotypy response profiles and caudate and accumbens dopamine dynamics. *J Neurosci*. 1991;11(9):2703-2712.
39. Hadamitzky M, Markou A, Kuczenski R. Extended access to methamphetamine self-administration affects sensorimotor gating in rats. *Behav Brain Res*. 2011;217(2):386-390.
40. Shortall SE, Macerola AE, Swaby RT, et al. Behavioural and neurochemical comparison of chronic intermittent cathinone, mephedrone and MDMA administration to the rat. *Eur Neuropsychopharmacol*. 2013;23(9):1085-1095.
41. Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF, Braff DL. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacology (Berl)*. 1990;100:413-416.
42. Spencer RC, Devilbiss DM, Berridge CW. The cognition-enhancing effects of psychostimulants involve direct action in the prefrontal cortex. *Biol Psychiatry*. 2015;77(11):940-950.
43. Dulawa SC, Scearce-Levie KA, Hen R, Geyer MA. Serotonin releasers increase prepulse inhibition in serotonin 1B knockout mice. *Psychopharmacology (Berl)*. 2000;149(3):306-312.
44. Bubenikova V, Votava M, Horacek J, Palenicek T. Relation of sex and estrous phase to deficits in prepulse inhibition of the startle response induced by ecstasy (MDMA). *Behav Pharmacol*. 2005;16(2):127-130.
45. Liechti ME, Geyer MA, Hell D, Vollenweider FX. Effects of MDMA (ecstasy) on prepulse inhibition and habituation of startle in humans after pretreatment with citalopram, haloperidol, or ketanserin. *Neuropsychopharmacology*. 2001;24(3):240-252.
46. Palenicek T, Balikova M, Rohanova M, et al. Behavioral, hyperthermic and pharmacokinetic profile of para-methoxymethamphetamine (PMMA) in rats. *Pharmacol Biochem Behav*. 2011;98(1):130-139.
47. Roth BL. Drugs and valvular heart disease. *New Engl J Med*. 2007;356(1):6-9.
48. Zhou X, Luethi D, Sanvee GM, Bouitbir J, Liechti ME, Krahenbuhl S. Molecular toxicological mechanisms of synthetic cathinones on C2C12 myoblasts. *Int J Mol Sci*. 2019;20(7):1-12.

How to cite this article: Pinterova-Leca N, Horsley RR, Danda H, et al. Naphyrone (naphthylpyrovalerone): Pharmacokinetics, behavioural effects and thermoregulation in Wistar rats. *Addiction Biology*. 2020;e12906. https://doi.org/10.1111/adb.12906

Zaostřeno 1

Národní monitorovací středisko pro drogy a závislosti

únor 2020

NOVÉ PSYCHOAKTIVNÍ SUBSTANCE

Nové psychoaktivní substance (NPS) jsou skupinou látek se širokým spektrem účinků. „Nové" jsou především z hlediska svého výskytu na ilegálním trhu, jejich struktura může být známa již poměrně dlouhou dobu. Jedná se o látky, které napodobují účinky klasických drog, jejich odlišná chemická strukturu jim však často zajišťuje dočasný únik před legislativní kontrolou. V následujícím textu bude čtenářům představena většina hlavních skupin NPS, avšak výčet není vyčerpávající. U každé ze zmíněných skupin je popsán mechanismus působení, akutní účinky na organismus včetně rizik a toxicity a stručný popis vybraných zástupců.

Úvod

Nové psychoaktivní substance (NPS, dříve nazývané nové syntetické drogy), známé také pod názvy „designer drugs" či „legal highs", se v poslední době stále častěji objevují na drogové scéně. Skupina těchto látek se vyznačuje širokým spektrem účinků, které mohou být tlumivé, stimulační, entaktogenní, ale také halucinogenní (Corazza et al. 2013; Johnson, Johnson, a Portier 2013). Hlavními důvody, proč se NPS na trhu vyskytují je, že: 1) se snaží napodobit účinky tradičních drog (např. extáze, heroinu, marihuany či pervitinu) a zároveň díky své nové struktuře, nejsou zahrnuty na seznamu kontrolovaných omamných a psychotropních látek (OPL) a 2) že prekursory pro jejich výrobu nejsou monitorovány a jsou snadno dostupné. To ve svém důsledku vede k tomu, že výrobci i distributoři se tak mohou snadno vyhnout legislativním postihům. Typickým znakem NPS tudíž je, že neustále dochází k modifikacím jejich struktury, na trhu se tak tedy objevují stále další nové substance (van Amsterdam, Nutt, a van den Brink 2013).

Mezi jedny z historicky prvních NPS se řadí látky odvozené od fenylethylaminu. Patří sem náhražky drogy extáze, např. N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamin (MBDB), či 4-bromo-2,5-dimethoxyfenethylamin (2C-B) (Giroud et al. 1998; Schifano 2005), dále náhražky tradičních amfetaminových stimulantů (halogenové amfetaminy 4-fluoroamfetamin (4-FA), 3-fluorometamfetamin (3-FMA) apod.) a potentní psychedelika 2,5-dimethoxy-4-bromoamfetamin (DOB) (Rose, Poklis, a Poklis 2013), Bromo-DragonFLY apod. Mezi těmito látkami si některé vysloužily punc jako velmi nebezpečné, jedná se zejména o para-methoxymetamfetamin (PMMA) a para-methoxyamfetamin (PMA), 4-methylthioamfetamin (4-MTA) které mají účinky podobné MDMA, ovšem s pomalejším nástupem účinků. Uživatelé tak mohou mít tendenci užít více dávek naráz, což může mít fatální následky (Johansen et al. 2003; Lin, Liu, a Yin 2007; Refstad 2003). Dnes se mezi jedny z toxických substancí řadí také N-benzyl methoxy (NBOMe) deriváty fenylethylaminových halucinogenů (např. 25B-NBOMe což je derivát 2C-B), které se díky této strukturální modifikaci staly extrémně potentními.

Obsah

Syntetické katinony jsou strukturálně blízké fenylethylaminům a amfetaminům, mají entaktogenní a stimulační účinky. Od nich se liší tím, že mají v molekule charakteristickou keto skupinu na postranním řetězci, hned na prvním uhlíku vedle aromatického jádra, v tzv. beta pozici (odtud ß-keto- nebo bk- deriváty). Prvními známými byly mefedron (droga známá jako mňau-mňau, či 4-MMC), methylon (jinak také bk-MDMA) a butylon (bk-MBDB). Do dnešní doby se na trhu vyskytly již stovky různých analog katinonů.

Náhražkami stimulantů a drogy extáze jsou také syntetické piperaziny, které jsou většinou méně účinné, avšak zároveň i méně toxické. Nejvíce vyskytujícími se a potažmo i zneužívanými piperaziny byly historicky 3-trifluoromethylfenylpiperazin (TFMPP), benzylpiperazin (BZP) a m-chlorofenylpiperazin (mCPP)(Arbo, Bastos, a Carmo 2012; Bockaert et al. 1987; de Boer et al. 2001).

Relativně mladou skupinou látek jsou aminoindany, které jsou strukturálně blízké amfetaminům a mají především entaktogenní účinky, tzn. zvýšenou hladinu empatie, usnadnění komunikace a pocit sounáležitosti s okolím. Prvním aminoindanem uvedeným na trh byl 2-aminoindan (2-AI). Většina ostatních látek z této skupiny jsou jeho analogy, jedná se například o 5-iodo-2-aminoindan (5-IAI), 5,6-methylendioxy-2-aminoindan (MDAI) a 6-methylendioxy-N-metyl-2-aminoindan (MDMAI) (Sainsbury et al. 2011).

Další velkou skupinou NPS jsou látky odvozené od tryptaminu, které mají povětšinou halucinogenní /psychedelické účinky. Mezi tradiční přírodní zástupce tryptaminů patří psilocybin, dimethyltryptamin (DMT) a 5-methoxy-dimethyltryptamin (5-MeO-DMT) (Schifano et al., 2015), mezi NPS se pak objevila jejich analoga jako např. 4-acetoxy-N,N-dimethyltryptamin (4-AcO-DMT), N,N-diisopropyltryptamin (DiPT), *N,N*-diallyl-5-methoxytryptamin (5-MeO-DALT) a celá řada dalších (Arunotayanun et al. 2013; Van Hout a Hearne 2017).

Psychedelicky působící disociativní anestetika jsou odvozena především od arylcyklohexylaminů ketaminu a fencyklidinu (PCP). Typickými příklady z této skupiny jsou aktuálně methoxetamin ((RS)-2-(ethylamino)-2-(3-methoxyfenyl)cyklohexanon; MXE), deschlorketamin (2-methylamino-2-fenylcyklohexanon; DXE) (Bryner et al. 2006; Frison et al. 2016; Tortella, Pellicano, a Bowery 1989) a methoxy deriváty fencyklidnu, např. 3-Methoxyfencyklidin (3-MeO-PCP) (Roth et al. 2013).

Početně nejrozmanitější skupinu NPS dosud pokrývají tzv. syntetické kanabinoidy. Ty se na trhu hojně vyskytují přimíchané a napuštěné do směsí nejrůznějších bylin, prodávají se pak typicky pod obchodním názvem „Spice" jako legální náhražka kanabisu/konopí. Díky tomu jsou mezi uživateli v některých zemích velice oblíbené (Vandrey et al. 2012; Seely et al. 2012). Na rozdíl od klasických kanabinoidů se ovšem může jednat i o velice nebezpečné substance.

OBRÁZEK 1
Heroin, fentanyl a carfentanil

Převzato z: https://reason.com/2018/07/20/thank-drug-warriors-for-the-escalating-d/

Chemicky jde o velmi nesourodou skupinu látek, tradičně sem patří především naftoylindoly, fenylacetylindoly a cyklohexylfenoly (např. JWH-018, CP-47 apod.) (ElSohly et al. 2014, Koller et al. 2014). Nověji pak mnoho dalších, mezi nimiž jsou některé substance (např. 5F-PB-22, MDMB-CHMICA a MDMB-FUBINACA) zodpovědné za řadu úmrtí.

Syntetické opioidy jsou alternativy ke klasickým opioidům, jejich nebezpečí často tkví především v jejich velmi vysoké potenci (účinné dávky jsou v jednotkách miligramů) díky čemu je veliké riziko předávkování s úmrtími, dále jsou samozřejmě vysoce návykové. Nejrozšířenějšími látkami z této skupiny jsou v současnosti substance odvozené od fentanylu (Armenian et al. 2018). ×

Syntetické opioidy

> Fenomén nových syntetických opioidů (NSO) je v posledních letech na vzestupu, hlavním důvodem je jejich snadná dostupnost na internetu, cena a v neposlední řadě také to, že nejsou prokazatelné pomocí standardních drogových testů.

Mnoho z těchto látek bylo vyvíjeno v 70. letech jako potenciální náhrada morfinu, další opět vznikají v současnosti prakticky na měsíční bázi a to i jen nepatrnou modifikací chemické struktury léčiv nebo známých drog, ve velkém procentu případů se jedná o deriváty opioidu používaného ve zdravotnictví – fentanylu (Prekupec, Mansky, a Baumann 2017).

Opioidy figurují velmi výrazně mezi smrtelnými případy předávkováním, zvláště v USA se v současnosti setkáváme se vzrůstajícími počty úmrtí (Rudd et al. 2016; Prekupec, Mansky, a Baumann 2017). Tato situace se patrně ještě zhoršuje jejich výskytem v padělaných lécích a v produktech prodávaných jako heroin (Amlani et al. 2015; Armenian et al. 2017). Toto často nevědomé požití obvykle vede k předávkování, NSO jsou totiž často daleko silnější než klasičtí zástupci opioidů (Baumann et al. 2017). Oficiální čísla úmrtí jsou navíc patrně mnohem nižší než reálná, protože mnoho laboratoří standardně neprovádí testy na tyto drogy a to buď z důvodu neznalosti či čistě pragmaticky, protož nemají vybavení a metody pro jejich detekci.

Jedním z prvních NSO na trhu byl tzv. Krypton, směs O-desmethyltramadolu, aktivního metabolitu tramadolu, a extraktu z kratomu (Mitragyna speciosa), rostliny z jihovýchodní Asie. Kratom obsahuje mnoho látek, mezi nimi alkaloid mitragynin s účinkem na opioidní receptory. S touto směsí je oficiálně spojeno také několik případů úmrtí (Kronstrand et al. 2011).

Protože mnoho současných NSO lze dohledat ve starých vědeckých článcích, užívajících nejednotné a zastaralé postupy, které jsou nyní nahrazované novějšími metodami, o jejich podrobném fyziologickém působení na lidský organismus je jen málo exaktních informací (Baumann et al. 2017). Přestože tyto látky mají znatelně odlišnou chemickou strukturu od přírodních opioidů odvozených od morfinu, jako klasické opioidy i NSO nejčastěji působí vysoce selektivně na μ opioidní receptory v centrální a periferní nervové soustavě a v gastrointestinálním traktu. Přes tento typ receptoru jsou mediovány hlavní účinky opioidů jako je analgezie a euforické účinky, kvůli kterým jsou zneužívány. Bývá trendem vzít si několik dávek po sobě („re-dosing“) ke zvětšení nebo prodloužení účinku drogy (Papsun et al. 2016). S rychlým rozvojem tolerance a vysokým adiktivním potenciálem si nové syntetické formy opioidů nezadají s nebezpečností klasických opioidů. Předávkování je asociované se ztrátou vědomí, zpomalením srdeční frekvence, cyanózou a miózou. Dalšími projevy jsou zácpa, svědění, nevolnost, zvracení a plicní edémy. Smrt je obvykle zapříčiněna útlumem dechového centra (Prekupec, Mansky, a Baumann 2017).

Působení NSO může být zvráceno podáním naloxonu, standardního kompetitivního antagonisty, schopného postupnou titrací vyvázat všechny molekuly opioidů z vazebných míst receptorů. Ovšem vzhledem k jejich potenci se doporučuje začít s větší dávkou oproti klasickým opioidům. Může se podat mnoha způsoby, nejčastěji intravenózně, může se však podat i intramuskulárně, intranasálně, subkutánně, endotracheálně, inhalačně i sublinguálně (Kim a Nelson 2015).

Carfentanil

Jedná se o opioid, který se používá k anestézii velkých zvířat, nebo radioaktivně značený k zobrazování opioidních receptorů in vivo. Látka se nevyskytovala samostatně, byla v řadě případů distribuována přimíchána do fentanylu a byla od roku 2009 příčinou řady úmrtí z předávkování nejen v USA, ale i v Evropském prostoru (Swanson et al. 2017; O'Donnell et al. 2018). Ví se, že řada čínských firem jej začala produkovat do té doby, než byl v roce 2017 zařazen na tamní seznam omamných a psychotropních látek. Jedná se o látku, která je zhruba 10000x potentnější, nežli morfin (George et al. 2010), je tedy účinná v řádu mikrogramů (srovnatelně jako například LSD), takže se uvažovalo i o jejím využití jako bojové látky, a to i v rámci teroristických útoků. Použita takto byla i v nechvalně známém teroristickém útoku v Moskevském divadle, kde zemřelo 125 lidí, jež se nadýchalo nějakého aerosolu. Pozdější analýzy britských služeb prokázaly u tří britských občanů, jež byli přítomni útoku, carfentanil a ramifentanyl v moči a na oblečení (Wax, Becker, a Curry 2003). Historicky byly také v Kanadě zadrženy tonery do tiskáren Hewlet Packard, jež byly naplněné carfentanilem, obsahovaly zhruba 50 milionů smrtících dávek. Dodnes se nikdo neodvážil jednoznačně odhalit, k jakým účelům se měl carfentanil použít. V roce 2017 byly hlášeny v ČR 2 případy fatálních intoxikací, v obou případech se však jednalo o syntetické opioidy – v jednom případě o methoxyacetylfentanyl a v druhém o carfentanil.

U-47700

Písmeno „U“ tohoto nefentanylového benzamidu se vztahuje k firmě Upjohn, která tuto látku v 70. letech prvně syntetizovala. Na drogové scéně se mu přezdívá „U4“ nebo „pink“, protože nečistoty vznikající při jeho syntéze zapříčiňují jeho narůžovělou barvu (Prekupec, Mansky, a Baumann 2017). Je velmi podobný droze AH-7921, oproti ní má však výraznější afinitu k μ receptorům. Z animálních studií jsou prokázány jeho typicky opioidní účinky (Mohr et al. 2016). Informace rekreačních uživatelů z internetových diskuzí popisují jeho účinky jako silně euforizující, ale díky krátkému biologickému poločasu také krátkého trvání, čímž podněcují opakované užití (Elliott, Brandt, a Smith 2016; Schneir et al. 2017). U-47700 je spojen s několika recentními případy úmrtí, nejméně 46 případů bylo zaznamenáno v USA (Elliott, Brandt, a Smith 2016; Prekupec, Mansky, a Baumann 2017).

AH-7921

AH-7921 je cyklohexylamin, může se prodávat také pod jménem doxylam. Název pochází od firmy Allen and Hanburys.

Bolest je v animálních testech schopen tlumit s podobnou silou jako morfin (Vorce et al. 2014). Ve větší míře se distribuuje v Evropě, Japonsku a USA. Za poslední roky je droga také zodpovědná již za několik úmrtí (Kronstrand et al. 2014; Vorce et al. 2014; Karinen et al. 2014).

MT-45

MT-45, jinak také „IC-6", je derivátem piperazinu. Po internetu se často prodává ve formě dihydrochloridové soli nebo bývá často smíchaný s jinými drogami (Papsun et al. 2016). Diskuze na online fórech popisují pomalý nástup účinku, což by mohlo vést k nebezpečí předávkování kvůli opětovnému užití před dosažením maximálního efektu (Helander, Bäckberg, a Beck 2014).

Tato droga má poměrně specifické vedlejší účinky, zejména dlouhodobou ototoxicitu, ztrátu vlasů, dermatitidu nebo hluboké bezvědomí, jsou u něj popsány také disociační účinky (Helander, Bäckberg, a Beck 2014). Působí jen malou miózu, což je problémem při jeho klinické identifikaci v případech předávkování. Zvláštní farmakologii této drogy by mohl vysvětlit významný agonismus s ∂ a κ opioidními receptory. V USA je její výskyt poměrně sporadický se dvěma nahlášenými případy předávkování, oproti tomu na evropském kontinentu způsobila minimálně několik desítek úmrtí (Papsun et al. 2016; Siddiqi et al. 2015).

Furanylfentanyl

Tomuto derivátu fentanylu s přítomností furanového kruhu s přezdívkou „Fu-F" se od roku 2013 v USA prokázalo nejméně 128 zapříčiněných úmrtí (Prekupec, Mansky, a Baumann 2017). Bývá také namixován ve várkách heroinu. Přestože nejsou dostupné žádné plnohodnotné informace o jeho působení, očekává se podobný farmakologický efekt jako u fentanylu (Mohr et al. 2016).

Butyrylfentanyl

Butyrylfentanyl je derivátem fentanylu s methylovou skupinou navíc. Ve vědecké literatuře je prvně zmiňován v 80. letech. Je velmi účinným analgetikem, oproti morfinu je přibližně 7× silnější (Baumann et al. 2017). Od roku 2014 je velmi progresivně se rozmáhajícím se NSO. S touto látkou bylo spojeno již minimálně několik desítek úmrtí (Prekupec, Mansky, a Baumann 2017).

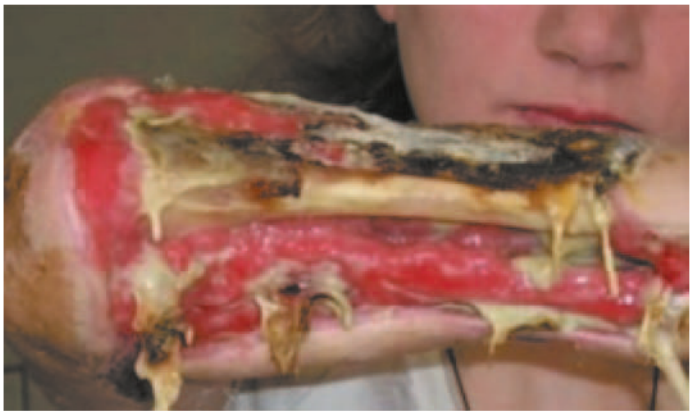
Acetylfentanyl

Acetylfentanyl, jinak také des-methylfentanyl, je dalším významným analogem fentanylu. Od června 2013 do prosince 2015 bylo oficiálně zaznamenáno 582 případů s užitím acetyl fentanylu, převážně u vyšetřování úmrtí předávkováním (Mohr et al. 2016).

Krokodil

Mediálně dobře známým případem je krokodil, který především v Rusku a některých dalších východoevropských státech způsoboval svým uživatelům hrozivě vypadající rány v nejhorších případech obnažení kosti – viz Obr. 2 (Grund, Latypov, a Harris 2013). Jeho hlavní složkou je syntetický opioid desomorfin, ovšem vyráběný zjednodušeně, pouze jednokrokově v nelegálních domácích laboratořích, z léčiv obsahujících kodein. Kromě kodeinu se však při výrobě používá i jod, červený fosfor, získaným nejčastěji ze škrtátek krabiček od sirek, ředidla na barvy a další látky. Při výrobě tak vzniká řada vedlejších produktů, které nejsou z výsledné směsi nijak odstraněny a při dlouhodobém užívání zapříčiňují nechvalně známé vedlejších účinky – rozsáhlé nekrotické změny (Grund, Latypov, a Harris 2013; Alves et al. 2015). Kromě toho má výsledná substance velice nízké pH, nezřídka nižší než 3.

OBRÁZEK 2
Ruka ženy závislé na droze krokodil



Převzato z http://westernslopelabs.com/krokodil-desomorphine/

V okolí aplikace látky dochází k rohovatění kůže, později se tyto zrohovatělé části odlupují a dochází k odhalení tkáně. Rány se postupně zvětšují, v nejtěžších případech může dojít až k úplnému obnažení kosti. K úmrtí takto zasaženého jedince většinou dojde následkem infekce, která do otevřených ran vniká velice snadno (Grund, Latypov, a Harris 2013). ×

Fenylethylaminy

> Fenylethylamin je látka, která je obsažena v řadě rostlin, ale také je přirozeně produkována septem limbického sytému, kde vzniká dekarboxylací fenylalaninu. Většina látek odvozených od fenyletylaminu vykazuje psychotropní aktivitu, avšak spektrum jejich účinku může zahrnovat působení stimulační, entaktogenní, anorektické, antidepresivní a bronchodilatační. Do skupiny fenylethylaminů je řazeno více než 300 různých látek.

Mezi fenylethylaminy patří látky stimulační jako je amfetamin, metamfetamin (pervitin) a jejich deriváty, entaktogeny jako je MDMA a její analogy a syntetické náhražky (3,4-methylendioxyamfetamin (MDA), 3,4-methylendioxy-N-ethylamfetamin (MDEA), *para*-methoxymetamfetamin (PMMA), *para*-methoxyamfetamin (PMA), 4-Methylthioamfetamin (4-MTA)), tzv. 2C´s, jejichž účinky jsou na pomezí entaktogenů a halucinogenů (4-brom-2,5-dimethoxyfenylethylamin (2C-B), 4-Ethyl-2,5-dimethoxyfenelethylamin (2C-E), 2,5-dimethoxy-4-ethylthiofenylethylamin (2C-T-2) a i čistě halucinogenní látky jako je typický halucinogen mezkalin a syntetické halucinogeny (2,5-dimethoxy-4-bromoamfetamin (DOB), 2,5-dimethoxy-4-methylamfetamin (DOM), 2,5-dimethoxy-4-iodoamfetamin (DOI), trimethoxyamfetaminy (TMA) apod.). Zatímco entaktogenně působící fenylethylaminy typicky blokují aktivitu transportérů pro monoaminy serotonin, dopamin a noradrenalin, díky čemuž zvyšují jejich vyplavování, halucinogenně působící 2C´s a další látky z této skupiny typicky stimulují serotoninové receptory, zejména typ 5-HT2A, jenž je hlavním mechanismem, jímž vyvolávají halucinogenní účinky analogicky jako je tomu v případě LSD. O řadě z nich jsme psali v předchozích publikacích (Zaostřeno na drogy 4/2004, Zaostřeno na drogy 4/2010). Některé z těchto látek jsou velmi potentní a toxické. ×

Halogenované deriváty amfetaminu a metamfetaminu

3-FA (3-fluoroamfetamin)
Je stimulant, který působí velice podobně jako metamfetamin. Kromě stimulačního efektu je často popisován i mírné entaktogenní působení. Obvyklá dávka 30 až 50 mg, doba trvání účinku 4 až 6 hodin (Yanini et al. 2018).

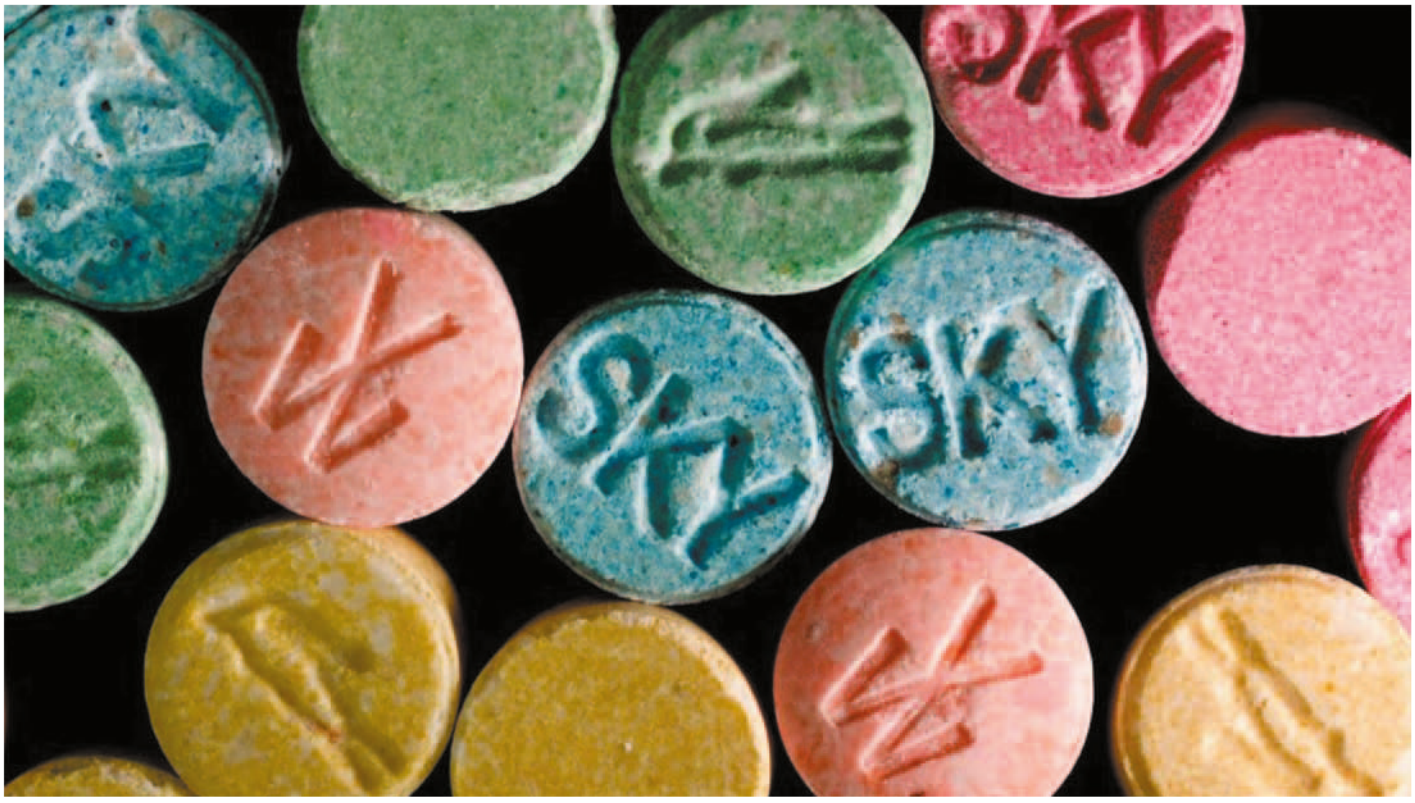
4-FA (4-fluoroamfetamin, PAL-303, Flux)
Jeho účinky jsou popisovány jako kombinace stimulačního a entaktogenního působení, mezi uživateli je proto poměrně oblíbený (Linsen et al. 2015). Prodává se především po internetu, velmi rozšířený a oblíbený je i na černém trhu v Nizozemí. Obvyklá dávka 100 až 130 mg, doba trvání účinku 5 až 8 hodin. V roce 2019 byla publikována klinická studie, která testovala účinky 4-FA na 12 dobrovolnících. Klinická studie na dobrovolnících popisovala po perorálním užití dávky 100 mg velmi mírné psychedelické účinky, spíše byly přítomny depersonalizační a derealizační účiny srovnatelné s MDMA. Přestože peak účinků (stejně jako peak koncentrace v séru) se dostavuje zhruba za hodinu po podání, v séru byl 4-FA detekovatelný ještě za 12 hodin po podání (Kuypers et al. 2019).

4-FMA (4-fluorometamfetamin)
Byl poprvé zachycen v roce 2006 v Japonsku. Účinek je opět stimulační i entaktogenní (Rösner et al. 2005). Běžná dávka 50 až 75 mg. Doba působení 4 až 8 hodin.

Analogy/náhražky MDMA

Patří sem zejména jejích blízké analogy MDA, MDEA, MBDB, jež mají velmi podobné účinky, MDA je pouze více dopaminergně působící, MDEA naopak více serotonergně. Účinky jsou entaktogenní, podobné MDMA, tedy hlavně euforie, pocity štěstí, lásky, sounáležitosti s okolím, mechanismem je pak zvýšené vyplavení monoaminů na synapsi, zejména cestou interakce s transportéry pro monoaminy (hlavně serotoninu, méně pak dopaminu a noradrenalinu). Obě látky jsou vesměs i podobně toxické, pokud jsou užívány samostatně tak je jejich akutní toxicita relativně nízká. Největším rizikem akutním je serotoninový syndrom s přehřátím, jeho riziko je zvyšováno okolní teplotou prostředí, excesivní námahou (např. tanec na párty) a nedostatečným příjmem mineralizovaných tekutin (velké ztráty profuzním pocením). Z dlouhodobého

OBRÁZEK 3
Tablety obsahující PMA, které připomínají extázi



Převzato z: http://www.bbc.co.uk/newsbeat/article/23618912/fresh-fears-over-pma-being-used-in-ecstasy-pills

hlediska je lze považovat za látky, které vykazují serotonergní toxicitu na synaptických zakončeních. Dále sem patří Para-metoxyamfetamin (PMA) a *Para*-methoxy-*N*-methyl-amfetamin (PMMA).

PMA (*Para*-methoxyamfetamin)
Je látka vyráběná synteticky, avšak zjistilo se, že ji ve stopovém množství obsahují i některé rostliny. Poprvé se objevuje na začátku 70. let. Často je prodáván jako extáze, účinky jsou téměř totožné, avšak výroba PMA je značně levnější. Látce se mezi uživateli přezdívá smrt (death), protože po užití větší dávky, případně v kombinaci s dalšími látkami může být smrtelně nebezpečný. Má opožděný nástup účinku (až 2 hodiny) a může vyvolávat serotoninový syndrom, zejména díky tomu, že působí jako inhibitor monoaminoxidázy (IMAO) a že díky opožděnému nástupu účinku jsou uživatelé často přesvědčeni, že droga, kterou užili, je slabá nebo neúčinná (Refstad 2003). PMA je proto zodpovědná za celou řadu úmrtí (Kraner et al. 2001; Felgate et al. 1998; Martin 2001). Obvyklá dávka 40–60 mg, doba trvání účinků je velice krátká.

PMMA (*Para*-methoxy-*N*-methylamfetamin)
Je látka velice podobná PMA, ale dle uživatelů má slabší účinky, stejně jako předchozí látka má vlastní IMAO aktivitu a opožděný účinek, je proto také srovnatelně nebezpečná a zodpovědná za řadu úmrtí (Becker et al. 2003; Nicol et al. 2015; Lin, Liu, a Yin 2007). Obvyklá dávka okolo 100 mg, doba trvání účinků je velice krátká stejně jako u PMA.

2C´s
2C-B (4-brom-2,5-dimethoxyfenylethylamin) na trhu byl tento fenylethylamin nejčastěji prodáván pod názvy Bromo, Nexus či Venus, jedná se o nejpopulárnějšího zástupce z rodiny 2C´s. Poprvé byl syntetizován Alexandrem Shulginem roku 1974. Shulgin sám ho později zařadil do skupiny „magical half-dozen", která označuje 6 dle Shulgina nejvýznamnějších fenylethylaminů. Při své práci ho využívali někteří psychiatři a psychologové, když se však v 80. letech rozšířil mezi rekreační uživatele, došlo k jeho přidání na seznam zakázaných látek. Při nižších dávkách působí stimulačně a entaktogenně při vyšších je to jednoznačné psychedelikum (Dean et al. 2013; Giroud et al. 1998). Obvyklá dávka 15 až 25 mg, doba trvání účinků 4 až 6 hodin. Jeho nejbližší analogy jsou 2C-I, 2C-C kde je atom bromu nahrazen iodem nebo chlorem.

2C-E (Aquarust) byl poprvé stejně jako mnoho dalších látek z 2C rodiny poprvé syntetizován Alexandrem Shulginem. Jde o halucinogenní látku s dobou působení přibližně 6 až 10 hodin (Sacks et al. 2012). Velkou zajímavostí týkající se této látky je mediálně exponovaný případ z roku 2015, kdy na kongresu homeopatiků v Hamburku došlo k hromadnému předávkování 29 účastníků akce. Internetový deník idnes psal: „Pacienty ve věku od 24 do 56 let lékaři hospitalizovali s halucinacemi, dýchacími problémy, bušícím srdcem a křečemi, některé z nich ve vážném stavu." Běžná dávka 10 až 15 mg, doba působení 4 až 8 hodin.

2C-T-7 (2,5-dimethoxy-4-propylthiofenylethylamin) známý také jako „Blue Mystic" či „Beautiful" je další látka zařazena do elitního výběru „magical half-dozen", účinek opět na pomezí entaktogenů a psychedelik/halucinogenů. Této látce jsou přisuzována 3 úmrtí ve Spojených státech amerických, buď po intranasálním požití větší dávky případně po kombinaci s dalšími látkami (de Boer a Bosman 2004; Schifano et al. 2005) Obvyklá dávka 15 až 25 mg, doba trvání účinků 6 až 10 hodin.

2C-T-2 (2,5-dimethoxy-4-ethylthiofenylethylamin) je poslední látka z 2C´s skupiny zařazená Shulginem mezi „magical half-dozen", známá také pod názvem Rosy. Účinky je velice podobný 2C-T-7 (Dean et al. 2013). Obvyklá dávka 10 až 20 mg, doba působení 6 až 10 hodin. ×

Fenylethylaminové halucinogeny

TMA skupina zahrnuje 6 izomerů trimethoxyamfetaminu, známé jsou pod zkratkou TMA s čísly 1 až 6 (TMA-1 často označován pouze jako TMA). Jedná se o syntetické látky s psychedelickými účinky. Jednotlivá analoga se od sebe liší pouze umístěním methoxy skupiny na benzenovém jádře. Jak strukturálně, tak účinky jsou velice podobné mezkalinu. TMA byl poprvé syntetizován roku 1947, jeho účinky byly později zdokumentovány ve výše zmíněné knize PIHKAL. Nejpotentnější a zároveň nejrozšířenější látkou na je TMA-2 (běžná dávka 20 až 40 mg, doba působení 8 až 12 hodin), dále se lze na černém trhu setkat s TMA a TMA-6, s ostatními látkami se na trhu nesetkáváme vůbec nebo pouze sporadicky (Zaitsu et al. 2008; Shulgin, Bunnell, a Sargent 1961).

DMA skupina neboli dimethoxyamfetaminy jsou psychedelicky či stimulačně působící látky. Stejně jako v případě trimethoxyamfetaminů jde o 6 izomerů, které se liší pozicí methoxy skupiny na benzenovém jádře. Jednotlivé látky (2,3-DMA; 2,4-DMA; 2,5-DMA; 2,6-DMA; 3,4-DMA a 3,5-DMA) se však mohou lišit svým účinkem 2,4-DMA má účinek spíše stimulační podobný amfetaminu, zatímco 3,4-DMA je co do účinku spíše podobný mezkalinu (Shaler a Padden 1972).

Zásadní jsou vysoce potentní halucinogenní fenylethylaminy typu DOB, DOM, DOI apod., jež účinkují v řádech jednotek miligramů (je tedy snadné se předávkovat) a jejichž účinky nastupují často velmi pomalu a trvají i několik desítek hodin. Z těchto látek se nejvíce v posledních letech vyskytovaly DOI a DOC. DOB a DOM se vyskytovali již na přelomu tisíciletí a DOM dokonce v 70. letech minulého století (DOM byl nazýván jako STP - serenity tranquility peace). Hlavním mechanismem účinku je agonismus na 5-HT2A receptorech. Jejich toxicita spočívá jednak s jejich vysokou potencí a relativně malým bezpečným efektivním oknem z hlediska dávky (u DOB jsou účinné dávky kolem 1-2 mg a 10 mg už může být smrtelná), pravděpodobně analogicky jako u PMMA a PMA i v aktivitě IMAO a současně i v opožděném nástupu účinku a tím riziku předávkování. Tyto látky se díky své potenci mohou vyskytovat i v papírových tripech. ×

FLY varianty halucinogenních a entaktogenních fenylethylaminů

> Tyto varianty fenylethylaminů mají na benzenovém jádře navěšeny furanové heterocykly, takže strukturně to vypadá, jako by jim dávaly křídla. První zástupce z této skupiny byl syntetizovaný v laboratoři prof. Davida NIcholse na základě grafické podobnosi struktury s tělem vážky, byl pojmenován Bromo-DragonFLY, následovala pak další analoga.

Bromo-DragonFLY je potentní halucinogen, který se chová jako neselektivní agonista 5-HT2 receptorů – jeho afinita pro 5-HT2B i pro 5-HT2c receptory je vyšší než pro 5-HT2A receptory. Dále funguje také jako inhibitor monoaminoxidázy A (MAO-A inhibitor). Pro tuto látku je typický extrémně dlouhý čas působení. Běžná dávka Bromo-dragonFLY je 300 až 500 µg, doba účinku 1 až 4 dny. Díky své vysoké potentnosti může snadno dojít k fatálnímu předávkování. Historicky se tak stalo při záměně s látkou 2C-B-FLY, která je strukturálně podobná, avšak více než 20x méně potentní (Corazza et al. 2011). Bromo-dragonFLY byl distribuován přes internet v pytlíčcích, které byly chybně označeny jako 2C-B-FLY což vedlo k předávkování řady lidí.

2C-B-FLY vykazuje jak psychedelický tak entaktogenní účinky, které běžně přetrvávají 6 až 8 hodin. Jeho jméno bylo značně démonizováno po výše zmíněné aféře s předchozí substancí. V posledních letech se znovu objevuje na trhu (Rickli et al. 2015). Běžná dávka 10 až 20 mg, doba účinku 7 až 12 hodin.

2C-B-BUTTERFLY
Látka byla syntetizována poprvé roku 1999, zaznamenána je vyšší selektivita pro 5-HT2C než pro 5-HT2A. Účinky jsou podobné jako 2C-B-FLY (Whiteside 2002). ×

Tryptaminy

> Samotný tryptamin je monoaminový alkaloid, který je strukturou velice podobný tryptofanu. Ve stopovém množství se vyskytuje také v mozku savců a jednou z hypotéz je, že zde plní funkci neuromodulátoru či neurotransmiteru. Deriváty tryptaminu jsou skupinou látek s psychotropními, u většiny tryptaminů halucinogenními účinky. Alexander Shulgin popisuje desítky těchto substancí ve své knize TIHKAL. Nejznámějšími tryptaminy jsou v přírodě se vyskytující psilocin (resp. jeho fosfátový ester psilocybin) a dimethyltriptamin (DMT, obsažena v jihoamerickém nápoji Ayahuasca) a syntetický N,N-diethylamid kyseliny lysergové (LSD).

5-MeO-DMT (5-metoxy-*N,N*-dimetyltryptamin)
je halucinogen s extrémně krátkým účinkem (nejčastěji se udává 15 až 30 minut), který se v přírodě nachází ve velkém množství rostlin (např. *Anadenanthera peregrina*) a přinejmenším u jednoho druhu ropuch (Bufo alvarius). Často je považována jeden z nejsilnějších halucinogenů/psychedelik vůbec. Při perorálním podání je látka bez koaplikace inhibitorů monoaminoxidázy neúčinná, nejčastěji se proto volí inhalační podání (kouřením). Běžná dávka čisté syntetické substance je 6 až 12 mg, často se však užívá ve formě extraktu z výše zmíněné žáby, a to opět kouřením (Stoff et al. 1978; Riga et al. 2014). Látka je i součástí přírodních šňupacích přípravků z Amazonie vyrobených z rostliny *Anadenanthera peregrina*. S 5-Meo-DMT byla zaznamenána úmrtí, ovšem vždy se jednalo o kombinace s IMAO, případně dalšími substancemi, po samotné látce úmrtí popsána dosud nebyla.

4-AcO-DMT (4-Acethoxy-*N,N*-dimethyltryptamin, psilacetin)
Je látka, která je strukturálně velice podobná psilocybinu, a její účinky jsou popisovány téměř identicky. Charakteristické jsou iluze geometrických tvarů, zkreslení barev, zpomalené vnímání času, introspekce a spojení s vesmírem. Nejčastější dávkování je v rozmezí 15 až 25 mg., doba účinku přibližně 4 až 7 hodin. 4-AcO-DMT může být patrně stejně jako psilocybin metabolizován na psilocin. Poprvé byl syntetizován roku 1963 Albertem Hoffmannem a Franzem Troxlerem (Nichols 1999).

5-MeO-MiPT (5-methoxy-N-methyl-N-isopropyltryptamin, moxy)
Je podobný více rozšířenému 5-MeO-DiPT, působí vysoce stimulačně a stejně jako tak i u něj jsou popisovány taktilní projevy, zejména zesílené vnímaní dotyků, avšak bez nežádoucích vedlejších účinků. Uživateli je popisováno zvýšení libida a díky zesílenému vjemu dotyků i zvýšená prožívání sexuální rozkoše, má tedy výrazní afrodiziakální účinky (Rickli et al. 2016; Shimizu et al. 2007). Poprvé byl syntetizován roku 1985 Davidem Repkem a Alexanderem Shulginem. Obvyklá dávka 7 až 15 mg, doba působení 5 až 8 hodin.

Bufotenin (5-OH-DMT), tento alkaloid je řazen mezi takzvané bufotoxiny, tyto látky získaly své jméno díky přítomnosti u ropuch rodu Bufo. Vykytují se však také v celé řadě rostlin (například ve dřevině *Anadenanthera peregrina* pocházející z Jižní Ameriky) a hub. Využití bufoteninu pro jeho halucinogenní účinky má dlouhou tradici (Lyttle, Goldstein, a Gartz 1996). Běžná dávka 20 až 40 mg. Doba účinku 15 až 90 minut. ×

Syntetické piperaziny

> Strukturálním základem těchto sloučenin je piperazin, který však sám o sobě není psychoaktivní. Látky odvozené od piperazinu využívány v hojné míře v humánní medicíně jako antihistaminika, antidepresiva a antipsychotika. Syntetické piperaziny byly na přelomu tisíciletí, v době kdy byl nedostatek prekurzorů na syntézu MDMA, často detekovány jako příměsi v tabletách extáze. Někdy byly také nalezeny v „koupelových solích", tedy produktech, které v této době obsahovaly primárně katinony. Nezřídka byly prodejci označovány jako přírodní produkty, avšak jejich původ je čistě syntetický. Na trh se dostávají jako tablety, kapsle nebo v práškové či kapalné formě. Zajímavostí je, že například v Austrálii byly po určitou dobu některé syntetické piperaziny prodávány přímo na benzinových pumpách jako legální stimulanty.

Mechanismus účinku
Piperaziny se nejčastěji dělí na 2 skupiny – benzylpiperaziny (BZP, MBZP a DBZP) a fenylpiperaziny (TFMPP, MeOPP). Benzylpiperaziny uvolňují z presynaptického zakončení dopamin a noradrenalin a fungují jako inhibitory zpětného vychytávání monoaminů (Smith, Sutcliffe, & Banks, 2015; Wikström, Holmgren, & Ahlner, 2004). Naproti tomu fenylpiperaziny působí především na serotoninový systém, jednak jako inhibitory zpětného vychytávání, ale i jeho přímím uvolňováním. Na dopaminergní a noradrenergní systém působí také, avšak v mnohem menší míře (Nelson et al., 2014).

Zdravotní rizika a toxicita
Příznaky akutní toxicity jsou hyponatrémie, prodloužení QT intervalu EKG záznamu (které je spojeno se zvýšeným rizikem arytmie) a serotoninový syndrom zejména v kombinaci s jinými látkami. Samotné piperaziny nejsou pravděpodobně samy přímo zodpovědné za jakýkoli případ úmrtí, pokud se jejich přítomnost v těle prokázala, vždy v kombinaci s některými dalšími látkami, zejména MDMA či amfetaminy (Smith et al., 2015; Wikström et al., 2004).)

Akutní účinky
U benzylpiperazinů převažují především stimulační účinky, fenylpiperaziny jsou oproti nim více entaktogenní a při užití vyšší dávky mohou působit dokonce halucinogenně.
Ve vyšších dávkách mohou syntetické piperaziny způsobovat nepříjemné reakce jako jsou zmatení, paranoia, nespavost, úzkost, třes, pocení, bolesti hlavy, nauzea a palpitace (Nelson et al., 2014). ×

Syntetické kanabinoidy

> Syntetické kanabinoidy jsou látky, které jsou svým farmakologickým profilem podobné s přírodními kanabinoidy, v některých případech mají podobnou i svou chemickou strukturu. Psychoaktivní účinky těch co se vyskytují na trhu, jsou většinou velmi podobné delta-9-tetrahydrocannabinolu (THC), který je hlavním zdrojem psychoaktivních účinků konopných produktů. Některé mají však i účinky podobné kanabidiolu (CBD). V řadě případů se primárně jednalo o látky designovány ke studiu endokanabinoidního systému a pro eventuální terapeutická využití, dnes jsou nicméně hojně zneužívány jako „levné a legální" varianty marihuany, často v podobě homogenizovaných směsí s rostlinným nosičem (směs nejrůznější sušených bylin) označovaných jako „Spice" (Barratt, Cakic, a Lenton 2013). Nutno podotknout, že otázka legality se v čase mění, a řada z těchto látek je nyní již na seznamu kontrolovaných substancí. Chemicky jsou rozděleny do 4 skupin: (i) nejpočetnější skupinu tvoří deriváty indol 3-karbonylu; (ii) 3-karbonylové

OBRÁZEK 4
Balení směsi obsahující syntetické kanabinoidy

Převzato z: https://www.homeless.org.uk/connect/blogs/2018/jul/19/spice-mamba-kronic-synthetics-many-names-one-problem

deriváty pyrrolu a indazolu, (iii) 3-karbonilamodové (iv) a 3-karbonylesterové deriváty indolu příp. indazolu (Seely et al. 2012; Spaderna, Addy, a D'Souza 2013; Castaneto et al. 2014). Některé skupiny syntetických kanabinoidů jsou pojmenovány podle společnosti, která nese jejich patent, některé také podle vědců, kteří sloučeninu syntetizovali. Například skupina JWH je pojmenovaná podle iniciálů Johna Williama Huffmana, který působí jako profesor organické chemie na univerzitě v Jižní Karolíně.

Mechanismus účinku
Farmakologicky působí především jako agonisté kanabinoidních receptorů. Ve srovnání s přírodními kanabinoidy tyto NPS mají často větší afinitu i aktivitu na CB1 a/nebo i CB2 receptorech, a dalších součástech endokanabinoidního systému, např. transportér pro anandamid či FAAH (fatty amino acid hydroxylase, enzym jenž degraduje endogenní kanabinoidy). Vysoké afinitě mají leckdy mohutnější účinek ve srovnání s přírodními kanabinoidy. Navíc vzhledem k faktu, že jsou defacto neprozkoumány, nelze vyloučit, že mohou pravděpodobně bohatě interagovat i s jinými receptorovými systémy, což může být i podkladem závažnější toxicity u některých z nich.

Zdravotní rizika a toxicita
Kromě klasických účinků intoxikace kanabinoidy srovnatelnými s THC se u intoxikovaného člověka mohou vyskytnout závratě, ospalost, deprese, podráždění, nevolnost, zvracení, často se také objevují poruchy paměti, halucinace, bludy a agresivita (van Amsterdam, Brunt, a van den Brink 2015; Castaneto et al. 2014; Gurney et al. 2014). U syntetických kanabinoidů bylo také zaznamenáno několik smrtelných případů předávkování (Berry-Cabán et al. 2013; Trecki, Gerona, a Schwartz 2015). Jedním z důvodů, proč dochází k předávkování, je i fakt, že pokoutně vyráběné směsi Spice nejsou dobře homogenizovány. To vede k situaci, kdy i například v jednom jointu mohou být části, kde je koncentrace kanabinoidy výrazně vyšší než v jiných, může se tak stát, že se při kouření otráví pouze jedna osoba, zatímco ostatní jsou intoxikovaní pouze mírně. Jelikož syntetické kanabinoidy mají odlišnou chemickou strukturu než přírodní kanabinoidy, používané testy na přítomnost THC a jiných kanabinoidů přítomných v marihuaně zde selhávají, proto detekce syntetických kanabinoidů u intoxikovaných pacientů může být problematická (Namera et al. 2015; Seely et al. 2012). I v Čechách jsme se setkali s fatálními intoxikacemi, byť se nejednalo o působení pouze těchto látek ale o kombinaci s alkoholem. 11. září 2018 v Ostravě dochází k úmrtí muže (32 let), který požil syntetický kanabinoid spolu s větším množstvím alkoholu. Druhý, o 4 roky starší, muž, který spolu se zemřelým látky konzumoval, byl hospitalizován ve vážném stavu.

Adiktivní potenciál
Na rozdíl od přírodních, některé ze syntetických kanabinoidů mají vysoký adiktivní potenciál, po přerušení jejich užívání se objevují nejen psychické ale i tělesné (somatické) odvykací symptomy. Mezi nejzávažnější abstinenční projevy patří záchvaty, tachykardie, dyspnea, bolest na hrudi, palpitace, závažné úzkosti, bolesti hlavy, nespavost, nauzea případně zvracení, ztráta chuti (Cooper 2016; Uttl et al. 2018).

5F-MDMB-PINACA
Tento syntetický kanabinoid byl v roce 2017 NPS s vůbec nejvyšším celkovým zachyceným množstvím v ČR (5,4 kg) (Mravčík et al. 2018). Poprvé byl identifikován roku 2014 v postmortem vzorcích odebraných ze zemřelé osoby v Japonsku po předávkování neznámou substancí. Následné testování odhalilo přítomnost této látky u 10 dalších zemřelých osob (Hasegawa, Wurita, Minakata, Gonmori, Yamagishi, et al. 2015).

MDMB-FUBINACA
V roce 2014 došlo v Rusku během 2 týdnů k více než 600 otravám v 15 případech smrtelných. Další smrtelné případy byly zaznamenány na území Běloruska (Gamage et al. 2018).

MDMB-CHMICA
Opět se jedná o vysoce potentního agonistu CB1 receptorů. Nejzávažnějšími vedlejšími účinky jsou metabolická a respiratorní acidóza, záchvaty, ztráta vědomí, případně kóma. V Evropě má na svědomí přinejmenším 29 lidských životů, nejvíce ve Velké Británii a Švédsku (Adamowicz 2016; Westin et al. 2015).

5F-PB-22
Látka je zodpovědná minimálně za 5 smrtelných případů na území USA. Někteří uživatelé hlásí vedlejší účinky jako například nevolnost popřípadě i zvracení, úzkostné stavy, zhoršenou koordinaci a zmatení, záchvatovité stavy (Behonick et al. 2014).

JWH-018 (1-pentyl-3-(1-naftoyl)indol)
Látka získala jméno podle chemika John W. Huffmana, který ji poprvé syntetizoval. Jeho afinita k CB1 receptoru je přibližně 5x vyšší, než u THC. Obvyklá dávka 2 až 3 mg, doba trvání účinků 1 až 2 hodiny. JWH-018 je zodpovědné za přinejmenším dva případy mozkové mrtvice u jinak zdravých jedinců (Every-Palmer 2011; Shanks, Dahn, a Terrell 2012).

Některé studie uvádějí, že halogenované analogy syntetických kanabinoidů mají stejné či velice podobné účinky, ale mají méně vedlejších efektů. U některých látek však podle všeho vede halogenace k výrazném zvýšení toxicity dané látky (Vigolo et al. 2015).

Například v souvislosti s užitím MDMB-FUBINACA, MDMB--CHMICA, 5F-MDMB-PINACA bylo zaznamenáno 45 závažných nežádoucích účinků, 18 úmrtí a 27 nefatálních intoxikací.

Mezi nejčastěji zachycené syntetické kanabinoidy v r. 2015 patřily ADB-FUBINACA, AB-CHIMINACA, UR-144, 5F-AKB48, ADB-CHMINACA. ×

Aminoindany

> Jak již bylo zmíněno výše v textu, aminoindany jsou relativně nová skupina vyskytující se na poli NPS. První charakteristiky této skupiny látek můžeme vystopovat ve 40. letech 20. století, kdy se zkoumal jejich bronchodilatační a vasoaktivní účinek (Levin, Graham, a Kolloff 1944), v 60. létech pak i potenciální analgetické účinky (Solomons a Sam 1973). Zásadním okamžikem byl ovšem až výzkum Dr. Nicholse zabývající se psychoaktivními efekty aminoindanů (Nichols et al. 1990; Johnson et al. 1991; Johnson, Conarty, a Nichols 1991; Marona-Lewicka a Nichols 1994). Spolu se zavedením nové třídy drog – entaktogeny/empatogeny[1] (Nichols et al. 1986) (typickou drogou této skupiny je např. MDMA neboli extáze), začal Dr. Nichols a jeho tým rozvíjet výzkum látek s kýženým entaktogenním účinkem. Jeho vizí bylo vyvinout látku, která nebude mít neurotoxické účinky jako MDMA a bude využívána pro facilitaci psychoterapie (Nichols a Oberlender 1990).

[1] Látky, které nespadají svými účinky ani do stimulantů, ani do halucinogenů. Podstatou jejich účinku je facilitovat komunikaci, navozují empatii a hlubší emoční prožitky

Po chemické stránce jsou aminoindany rigidní analogy amfetaminu díky vazbě alfa uhlíku na aromatický kruh (Fuller, Baker, a Molloy 1977). Nejznámějšími aminoindany mezi uživateli jsou 5,6-methylendioxy-2-aminoindan (MDAI), 5,6-methylendioxy-N-methyl-2-aminoindan (MDMAI), 5-iodo-2-aminoindan (5-IAI), 2-aminoindan (2-AI), 5-methoxy-6-methyl-2-aminoindan (MMAI) a 5-methoxy-2-aminoindan (MEAI).

Mechanismus účinku

Hlavním místem působení aminoindanů je serotonergní (5-HT) systém. Ve studiích zaměřených obecně na monoaminový systém se MDAI ukázalo jako vysoce potentní inhibitor zpětného vychytávání právě 5-HT spíše než dopaminu (DA) či nonvesikulárního výlevu DA. Látky jako 5-IAI a MMAI pak naopak nonvesikulární výlev 5-HT, dále DA a noradrenalinu (NE) zvyšují. MMAI je navíc 100x selektivnější pro inhibici zpětného vychytávání 5-HT než DA (Johnson, Conarty, a Nichols 1991). Akorát u 2-AI byla prokázána zejména inhibice NE transportérů oproti 5-HT a DA transportérům. Neméně důležitým faktem je i to, že aminoindany způsobují reversní výlev monoaminů přes transportéry zpětného vychytávání (Simmler et al. 2014).

Akutní účinky

Vzhledem k tomu, že žádné klinické studie zaměřené na aminoindany neexistují, zdrojem informací o akutních účincích jsou diskuzní fóra a webové platformy uživatelů (Drugs-Forum[2], Erowid[3], PsychonautWiki[4] aj.). Zde uživatelé sdílejí své subjektivní zážitky, nežádoucí efekty, doporučené dávky apod. Mezi hlavní účinky MDAI a 5-IAI jsou řazeny euforie, empatie, stimulace (není případ MDAI) a zlepšená kognice. Účinky MEAI jsou připodobňovány alkoholu – jako středně euforické, až na absenci kocoviny následující den (Shimshoni et al. 2017). Jako nežádoucí účinky jsou uváděny dehydratace, zvýšená perspirace, úzkost, deprese, panické záchvaty a tachykardie. Způsoby podání jsou různé, nejčastěji se ovšem setkáme s orálním podáním a tzv. šňupáním. Kouření aminoindanů či jejich injekční aplikace zatím nebyly nikde zmíněny. Nástup požadovaných účinku po orálním podání je zpravidla okolo 30 min s vrcholem mezi 45 min – 3 h (tento široký rozptyl je dán vnějšími okolnostmi, jako je čistota látky nebo obsah jídla v žaludku) (Corkery et al. 2013). Uživateli doporučené dávky pro střední efekt jsou pro MDAI 100–150 mg, 2-AI 10–20 mg, 5-IAI 100 mg a MEAI 70–140 mg, vše pro orální podání (Shimshoni et al. 2017).

Zdravotní rizika a toxicita

Pro účinky podobné extázi jsou aminoindany často užívány na tanečních party a večírcích. Neočekává se ovšem pouze entaktogenní účinek, ale také stimulace kvůli tanci. Stimulační efekt aminoindanů je nicméně minimální, jelikož cílovým systémem je systém 5-HT, nikoliv DA (Nichols et al. 1990; Johnson, Conarty, a Nichols 1991). To často vede ke konzumaci aminoindanů ve větších dávkách (kvůli navýšení vyplavování DA) nebo ke kombinaci s jinými stimulanty jako je amfetamin, kokain nebo MDMA. Kombinování s jinými drogami může vést k nečekané neurotoxicitě a kardiotoxicitě a je hodnoceno jako vysoce rizikové (Corkery et al. 2013; Monte et al. 1993).

Intoxikace MDAI je spojována s ledvinovým selháním, syndromem akutní respirační tísně, jaterním selháním a zvýšeným rizikem výskytu primárná plicní hypertenze či chlopenními vadami (Gallagher et al. 2012). V animálním modelu MDAI výrazně zvyšovalo tělesnou teplotu zvířat a ve vysokých dávkách (40 mg/kg) se ukázalo vysoce 5-HT toxické. Pitva uhynulých zvířat prokázala serotoninový syndrom, mozkový edém a diseminovanou intravaskulární koagulopatii (Páleníček et al. 2016). Ve spojitosti s MDAI bylo hlášeno i několik úmrtí, avšak v toxikologických testech se vždy prokázaly i jiné drogy (Corkery et al. 2013; Staeheli et al. 2017). 5-IAI a 2-AI bylo též spojeno s několika úmrtími mezi lety 2010–2012 (Elliott a Evans 2014). ×

Katinony

> Katinon je látka, které se přirozeně vyskytuje v listech katy jedlé (Catha edulis), keře vyskytujícího se v oblastech západní Afriky a na Arabském poloostrově (Brenneisen et al. 1990). Žvýkání listů katy hraje důležitou roli v tamějších kulturních a sociálních tradicích již po dlouhá staletí. Po identifikaci katinonu jakožto hlavní psychoaktivní složky bylo postupně syntetizováno mnoho derivátů této látky, s účinky stimulačními až entaktogenními. Některé syntetické deriváty byly původně určeny k terapeutickým účelům, ale díky svým euforickým účinkům začaly být záhy zneužívány (Valente et al. 2014) a dodnes se objevilo obrovské množství nových látek, které nikdy neměly jiné využití nežli jako NPS.

Nejčastěji se na ilegálním trhu katinony vyskytují v práškové či krystalické formě, mají typicky bílou barvu, někdy však mohou být zbarveny i do žluta či do hněda a jsou prakticky bez zápachu (Zawilska a Wojcieszak 2013). K velkému rozvoji jejich prodeje došlo stejně jako u ostatních NPS především díky jejich dočasně legálnímu statusu a také díky snižující se kvalitě drog běžně dostupných na černém trhu. Prvními z katinonů, které se na trhu objevili již v 90. letech, byly methylon a butylon, velmi záhy se pak na scéně objevil mefedron (4-methylmetakatinon, 4-MMC, MCAT), často také nazývaný jako droga „mňau, mňau). Po jeho zákazu následovaly blízké analogy, např. 4- a 3-methylethkatinon (4-MEC, 3 MEC), halogenova-

OBRÁZEK 5
„Koupelová sůl"

Převzato z: https://drugfreeva.org/sink-or-swim/drug-facts/street-drugs/bath-salts/#gallery-image/3

[2] https://drugs-forum.com/
[3] https://www.erowid.org/
[4] https://psychonautwiki.org

né deriváty a řada dalších. V poslední době se setkáváme s velkým rozšířením pyrrolidinofenonových katinonů alpha-PVP a MDPV, jež mají velmi výrazný návykový potenciál, odvykací stavy jsou provázeny delirantními příznaky a byly popsány i případy kanibalismu. V USA se vyskytují pod názvem „Flakka".

Akutní účinky
Syntetické katinony mohou v závislosti na konkrétní látce navozovat účinky stimulační a entaktogenické. Typické jsou u stimulačních katinonů pocity velkého množství energie, nepotřeba spánku, zvýšení pozornosti. U těch s entaktogenním účinkem pak i pocity empatie a otevřenosti, případně mohou zvyšovat libido. Na druhou stranu však mohou vyvolávat nepříjemné stavy, zejména paranoidní a úzkostné prožitky, ale i podrážděnost, neklid, agresi, zmatení, halucinace, neschopnost prožívání emoce. Mezi somatické účinky patří kardiovaskulární (tachykardie, arteriální hypertenze, palpitace, dyspnoe, bolest na hrudi, vazokonstrikce), metabolické (hyponatrémie, hypokalémie a acidóza), neurologické sympatomimetické (bolest hlavy, záchvaty, třes, mydriáza, parestezie), gastrointestinální (pocit na zvracení, zvracení, bolest břicha) a i některé další (myoklonus, zvýšení tělesné teploty (hypertermie), pocení, skřípání zubů (bruxismus)) (Murphy et al. 2013; Ross, Watson, a Goldberger 2011; Spiller et al. 2011; Wood et al. 2010).

Zdravotní rizika a toxicita
Syntetické katinony mohou působit toxicky: akutně somaticky – infarkt myokardu, serotoninový syndrom, hypertermie. Například u oblíbeného mefedronu byla popsána také akutní myokarditida (zánět srdečního svalu), přisuzovaná přímému toxickému působení na srdeční svalové buňky, případně skrze účinek na imunitní systém (Nicholson, Quinn, a Dodd 2010). Při chronickém užívání mohou působit hepatotoxicky, neurotoxicky, způsobovat multiorgánová selhání, metabolické acidózy; z hlediska psychiatrických účinků pak mohou indukovat deprese, závislost, panické reakce, toxické psychózy. Toxicita může být ovlivněna i způsobem podání látky přičemž největšímu nebezpečí se uživatelé vystavují při intravenózní aplikaci (Maurer et al. 2004).

Mechanismus účinku
Katinony zvyšují synaptickou hladinu monoaminů, především serotoninu, dopaminu a noradrenalinu. Zvýšení hladiny těchto neurotransmiterů je způsobeno blokádou resp. dokonce převrácením funkce transportérových systémů, které jsou zodpovědné za zpětné vychytávání výše uvedených neurotransmiterů ze synaptické štěrbiny (Cozzi et al. 1999). Jejich selektivita pro jednotlivé transportérové systémy – dopaminový (DAT), serotoninový (SERT) a norepinefrinový (NET) se mezi odlišnými substancemi značně liší (Simmler et al. 2013). Dalším mechanismem vedoucím k navýšení hladiny monoaminů, kterým některé katinony působí, je zvýšené vyplavování z presynaptického zakončení neuronu. Zvýšená hladina neurotransmiterů v synaptické štěrbině vede ke zvýšení stimulace postsynaptických receptorů jak v mozku, tak i na periferii (Osorio-Olivares et al. 2004).

Obecně syntetické katinony přecházejí přes hematoencefalickou bariéru hůře než amfetaminy a to především díky ß-keto skupině, která značně zvyšuje jejich polaritu (Coppola a Mondola, 2012, (Krikorian 1984), proto je pro vyvolání účinku srovnatelného s amfetaminy často nutno užít vyšší dávku (Krikorian 1984). Výjimku z tohoto pravidla však tvoří pyrrolidinové deriváty (Gibbons a Zloh 2010), mezi které patří například výše zmíněné MDPV a nafyron, ale také další látky jako například 3',4'-methylenedioxy-α-pyrrolidinopropiofenon (MDPPP) (Springer, Fritschi, a Maurer 2003).

Návykový potenciál
Schopnost vyvolat závislost u pokusných zvířat již byla u některých ze substancí jednoznačně potvrzena v testech podmíněné preference místa[5] či v self-administračních experimentech[6] – například u mefedronu, metylonu, nafyronu a MDPV (Karlsson et al. 2014).

Odvykací stavy
Odvykací stavy po katinonech se příliš neliší od jiných stimulantů, zahrnují jak fyzické (pocení, třes, únava, zvýšený tep a tlak, bolest hlavy, průjem), tak psychické (bažení po droze, úzkost, deprese, paranoia, spánkové problémy) příznaky, které se liší v závislosti na užívané látce (Winstock et al. 2011; Coppola a Mondola 2012). Odvykací stavy byly pozorovány zejména při dlouhodobém užívání MDPV a α-PVP.

Zneužívané substance spadající do skupiny syntetických katinonů
V současné době je známo již více než 100 substancí, které se řadí mezi syntetické katinony. Úkolem následujícího přehledu proto rozhodně není podat vyčerpávající seznam všech jednotlivých látek, ale seznámit čtenáře s významnějšími z nich.

Mefedron (4-methylmethkatinon, 4-MMC), MCAT, mef či mňau-mňau, je prvním syntetickým katinonem, který dosáhl v Evropě masového rozšíření. Na trh pronikl okolo roku 2006 ve Velké Británii. Jeho účinky jsou jak stimulační, tak i entaktogenní (Winstock et al. 2011; Winstock et al. 2011). Nejpříbuznějšími katinony, které ho začaly záhy nahrazovat, byly látky 4-methylethkatinon (4-MEC), 3-MEC, 3-FMC a efedron. V posledních letech se nejhojnějšího rozšíření dočkal především pentedron, který má účinky víceméně podobné mefedronu. Obvyklá dávka 45 až 80 mg, doba trvání účinků 3 až 6 hodin.

Efedron (α-methylamino-propiofenon, methkatinon), stimulant typicky vyráběný z efedrinu, je zajímavý mj. i díky tomu, že po delší době injekčního podávání, především v některých východoevropských zemích, způsoboval obtíže nápadně připomínající Parkinsonovu chorobu. Později bylo zjištěno, že tyto nepříznivé účinky jsou vyvolané oxidem manganičitým, který vzniká při výrobě z katalyzátoru oxidoredukčních reakcí a díky nedokonalému čištění zůstává ve finálním produktu jako nečistota (Sikk et al. 2011).

Metylon jeho účinky jsou především stimulační, avšak i zde se částečně projevuje entaktogenní působení (Štefková et al. 2017). Obvyklá dávka 150 až 225 mg, doba trvání účinků 2 až 4 hodiny.

MDPV (3,4-methylendioxypyrovaleron, v Česku nejčastěji pod názvem Funky) i tento stimulant je díky přítomnosti pyrrolidinového kruhu, stejně jako nafyron, více lipofilní než ostatní katinony. Tato látka má pravděpodobně na svědomí několik případů úmrtí (Coppola a Mondola 2012), chová se jako typický stimulant s mírnými psychomimetickými účinky

[5] Při testu podmíněné preference místa je podání drogy asociováno s přítomností v jednom ze dvou kompartmentů aparatury (ten musí být zvířetem spolehlivě rozlišitelný), druhý kompartment je asociován s podáním vehikula, které nemá žádný účinek. Pokud po určité době podmiňování zvíře ve zvýšené míře vyhledává kompartment asociovaný s podáním drogy i bez její přítomnosti, lze usoudit, že podávaná látka vyvolává závislost.

[6] Self-administrace je formou operantního podmiňování, kdy zvíře po stlačení páčky obdrží nejčastěji intravenózně či intracerebrálně dávku drogy. V experimentu se sleduje zda, a případně kolikrát, je zvíře ochotno stlačovat páčku pro obdržení své dávky drogy.

(Horsley et al., 2018). Obvyklá dávka 8 až 14 mg, doba trvání účinků 2 až 7 hodin.

α-PVP, substance častěji známá pod svým pouličním názvem flakka, dostala od médií přezdívku zombie drug. Oblíbenou se stala díky velice příznivé ceně. Euforické účinky po jejím požití se však mohou velice snadno vystupňovat a osoba pod vlivem drogy může prožívat děsivé bludy, paranoidní psychózu, či extrémní agitaci (Crespi 2016). Zajímavostí je, že některé případy úmrtí po užití drogy tak nejsou přímo fyziologickým následkem jejího působení, ale jsou způsobeny další osobou, která je konfrontována s výrazně agresivním chováním jedince (European Monitoring Centre for Drugs and Drug Addiction a Europol 2015). Dnes se kromě této látky setkáváme s dalšími analogy, například α-PHP a α-PBP. Obvyklá dávka 5 až 15 mg, doba trvání účinků 30 až 60 minut.

Nafyron, nazýván NRG-1, O-2482, případně Energy 1, se objevil na trhu spolu s dalšími syntetickými katinony brzy po přidání mefedronu na seznam zakázaných látek (Schifano et al. 2011). Tato stimulační droga je přibližně tisíckrát lipofilnější než jí příbuzné MDMA a mefedron (Meltzer et al. 2006). Vysoká lipofilita zajišťuje vyšší prostupnost látky hematoencefalickou bariérou. ×

Disociativní anestetika (disociativa)

> Mezi disociativní anestetika, jež jsou primárně antagonisté N-methyl-D-aspartátových (NMDA) glutamátových receptorů, patří především arylcyklohexylaminy (ketamin, fencyklidin (PCP) a jejich analoga), morfinany (dextrametorfan, dextrorfan) a diarylethylaminy (difenidin (DPD), metoxfenidin (MXP), efenidin (EPE).

Akutní účinky

Mezi uživateli jsou vyhledávané pro své halucinogenní/psychedelické účinky, charakteristické je pro ně zkreslení smyslového vnímání. Popisovány jsou příznaky derealizace, senzorické deprivace, disociace z tělesné schránky, euforie, pocity klidu a míru, zvýšená empatie a potřeba sociálních interakcí, pocity hlubšího porozumění vlastního já, separace od vnějšího světa. Nepříjemné a ohrožující reakce po požití arylcyklohexylaminů zahrnují úzkost, paranoidní a psychotické reakce, zmatenost, dezorientaci, problémy se spánkem, tachykardii, hypertenzi, nauzeu případně i zvracení, záchvaty, katatonii, ataxii a nystagmus (Horsley et al. 2016; Shields et al. 2012; Corazza et al. 2012).

Arylcyklohexylaminy

Metoxetamin (3-MeO-2'-Oxo-PCE, MXE, Mexxy) byl poprvé syntetizován v roce 2010 za účelem prodeje na černém trhu jako náhrada za ketamin, která je šetrná k močovému měchýři. Byl speciálně syntetizován tak, aby byl účinnější při nižších koncentracích než ketamin a v těle tak nedocházelo k hromadění urotoxických metabolitů (Morris a Wallach 2014). Tato jeho charakteristika je však vysoce sporná, jelikož jsou publikovány studie, které toto tvrzení vyvracejí (Dargan et al. 2014). Metoxetamin má účinky velice podobné ketaminu, tzn. zlepšení nálady s disociativními a halucinogenními prvky při nižších dávkách, při vyšších změněné stavy vědomí. Na rozdíl od ketaminu, který působí po krátkou dobu, účinky metoxetaminu mohou přetrvávat po několik hodin (Corazza et al. 2012). Po požití metoxetaminu byl již zaznamenán větší počet fatálních intoxikací (Adamowicz a Zuba 2015; Wikström et al. 2013). Obvyklá dávka 25 až 45 mg, doba trvání účinků 4 až 6 hodin.

Deschlorketamin je nejbližším analogem ketaminu, jedinou změnou oproti jeho struktuře je absence chloridové skupiny na aromatickém jádru. Tato látka byla původně patentována jako léčivo vhodné k léčbě bakteriálních, houbových, virových a protozoálních infekcí a také jako imunomodulátor s imunosupresivními účinky (Jurásek et al. 2018) . Obvyklá dávka 20 až 30 mg, doba trvání účinků 4 až 6 hodin.

3-Methoxyfencyklidin (3-MeO-PCP, případně pouze 3-MeO) se váže na NMDA receptory s vyšší afinitou než PCP i než 2- a 4- MeO-PCP. Subjektivní účinky jsou popisovány jako výrazně více stimulující než je tomu například u ketaminu či metoxetaminu. Dle uživatelských reportů látka vyvolává ve vyšší míře, než je tomu u ostatních disociativních anestetik, manické stavy, bludy a psychotické stavy. Jeho adiktivní potenciál je vyšší než u většiny ostatních arylcyklohexylaminů, uživateli je popsána fyzická závislost s nepříjemnými odvykacími stavy. Jde o látku poměrně nebezpečnou, je jí připisováno několik smrtelných intoxikací (Johansson et al. 2017; Bakota et al. 2016). Obvyklá dávka 8 až 15 mg, doba trvání účinků 4 až 8 hodin.

Morfinany

Dextrometorfan a dextrorfan

Dextrometorfan se běžně využívá jako účinná látka v mnoha volně prodejných antitusicích. Řadí se mezi syntetické opioidy, avšak při užití vyšší dávky jeho účinek odpovídá disociativům. Dochází k tomu proto, že v těle je metabolizován na dextrorfan, který působí jako antagonista NMDA receptorů (Martinak et al. 2017). Obvyklá disociativní dávka dextrometorfanu je 200 až 400 mg, doba trvání účinků 8 až 12 hodin.

Diarylethylaminy

Difenidin (1,2-DEP, DPD) byl poprvé syntetizován roku 1924, na trhu se však objevil až v roce 2013, kdy byly v UK arylcyklohexylaminy zařazeny na seznam zakázaných látek. V následujícím roce se v Japonsku látka začala prodávat ve směsi spolu se syntetickými kanabinoidy, tato kombinace měla na svědomí minimálně jeden případ smrtelného předávkování (Hasegawa, Wurita, Minakata, Gonmori, Nozawa, et al. 2015). Při nižší dávce vyvolává difenidin euforické pocity připomínající účinek efedrinu. Obvyklá dávka 85 až 110 mg, doba trvání účinků 2 až 5 hodin.

Methoxfenidin (2-MeO-Diphenidin, MXP) je poprvé zmíněn roku 1989 v patentu, kde se počítá s jeho testováním jako potenciální léčbou při neurotoxických poškozeních. Na černém trhu se objevuje ve stejnou dobu jako difenidin. Obvyklá dávka 75 až 120 mg, doba trvání účinků 6 až 8 hodin (Hofer et al. 2014; Wallach et al. 2016).

Efenidin (NEDPA, EPE) se objevuje na trhu spolu s výše zmíněnými diarylethylaminy. Uvádí se, že nástup účinku je mnohem rychlejší, pokud je přijímán inhalačně nikoli perorálně (Kang et al. 2017). ×

Literatura

Adamowicz, Piotr. 2016. „Fatal Intoxication with Synthetic Cannabinoid MDMB-CHMICA". *Forensic Science International* 261 (duben): e5–10. https://doi.org/10.1016/j.forsciint.2016.02.024.

Adamowicz, Piotr, a Dariusz Zuba. 2015. „Fatal Intoxication with Methoxetamine". *Journal of Forensic Sciences* 60 (leden): S264–68. https://doi.org/10.1111/1556-4029.12594.

Alves, Emanuele Amorim, Jean-Paul Cornelis Grund, Carlos Manuel Afonso, Annibal Duarte Pereira Netto, Félix Carvalho, a Ricardo Jorge Dinis-Oliveira. 2015. „The Harmful Chemistry behind Krokodil (Desomorphine) Synthesis and Mechanisms of Toxicity". *Forensic Science International* 249 (duben): 207–13. https://doi.org/10.1016/j.forsciint.2015.02.001.

Amlani, Ashraf, Geoff McKee, Noren Khamis, Geetha Raghukumar, Erica Tsang, a Jane A. Buxton. 2015. „Why the FUSS (Fentanyl Urine Screen Study)? A cross-sectional survey to characterize an emerging threat to people who use drugs in British Columbia, Canada". *Harm Reduction Journal* 12 (1): 1–7. https://doi.org/10.1186/s12954-015-0088-4.

Amsterdam, J. van, D. Nutt, a W. van den Brink. 2013. „Generic Legislation of New Psychoactive Drugs". *Journal of Psychopharmacology* 27 (3): 317–24. https://doi.org/10.1177/0269881112474525.

Amsterdam, Jan van, Tibor Brunt, a Wim van den Brink. 2015. „The Adverse Health Effects of Synthetic Cannabinoids with Emphasis on Psychosis-like Effects". *Journal of Psychopharmacology 29* (3): 254–63. https://doi.org/10.1177/0269881114565142.

Arbo, M.D., M.L. Bastos, a H.F. Carmo. 2012. „Piperazine Compounds as Drugs of Abuse". *Drug and Alcohol Dependence* 122 (3): 174–85. https://doi.org/10.1016/j.drugalcdep.2011.10.007.

Armenian, Patil, Alexander Olson, Andres Anaya, Alicia Kurtz, Rawnica Ruegner, a Roy R. Gerona. 2017. „Fentanyl and a Novel Synthetic Opioid U-47700 Masquerading as Street "Norco" in Central California: A Case Report". *Annals of Emergency Medicine* 69 (1): 87–90. https://doi.org/10.1016/j.annemergmed.2016.06.014.

Armenian, Patil, Kathy T. Vo, Jill Barr-Walker, a Kara L. Lynch. 2018. „Fentanyl, Fentanyl Analogs and Novel Synthetic Opioids: A Comprehensive Review". *Neuropharmacology* 134 (květen): 121–32. https://doi.org/10.1016/j.neuropharm.2017.10.016.

Arunotayanun, Warunya, Jeffrey W. Dalley, Xi-Ping Huang, Vincent Setola, Ric Treble, Leslie Iversen, Bryan L. Roth, a Simon Gibbons. 2013. „An Analysis of the Synthetic Tryptamines AMT and 5-MeO-DALT: Emerging 'Novel Psychoactive Drugs'". *Bioorganic & Medicinal Chemistry Letters* 23 (11): 3411–15. https://doi.org/10.1016/j.bmcl.2013.03.066.

Bakota, Erica, Crystal Arndt, Amelia A. Romoser, a Stephen K. Wilson. 2016. „Fatal Intoxication Involving 3-MeO-PCP: A Case Report and Validated Method". *Journal of Analytical Toxicology* 40 (7): 504–10. https://doi.org/10.1093/jat/bkw056.

Barratt, Monica J., Vince Cakic, a Simon Lenton. 2013. „Patterns of Synthetic Cannabinoid Use in Australia: Synthetic Cannabinoids in Australia". *Drug and Alcohol Review* 32 (2): 141–46. https://doi.org/10.1111/j.1465-3362.2012.00519.x.

Baumann, Michael H., Susruta Majumdar, Valerie Le Rouzic, Amanda Hunkele, Rajendra Uprety, Xi Ping Huang, Jin Xu, Bryan L. Roth, Ying Xian Pan, a Gavril W. Pasternak. 2017. „Pharmacological characterization of novel synthetic opioids (NSO) found in the recreational drug marketplace". *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2017.08.016.

Becker, Jürgen, Peter Neis, Jörg Röhrich, a Siegfried Zörntlein. 2003. „A Fatal Paramethoxymethamphetamine Intoxication". *Legal Medicine* 5 (březen): S138–41. https://doi.org/10.1016/S1344-6223(02)00096-2.

Behonick, George, Kevin G. Shanks, Dennis J. Firchau, Gagan Mathur, Charles F. Lynch, Marcus Nashelsky, David J. Jaskierny, a Chady Meroueh. 2014. „Four Postmortem Case Reports with Quantitative Detection of the Synthetic Cannabinoid, 5F-PB-22". *Journal of Analytical Toxicology* 38 (8): 559–62. https://doi.org/10.1093/jat/bku048.

Berry-Cabán, Cristóbal S., Juliana Ee, Victoria Ingram, Carlos E. Berry, a Eugene H. Kim. 2013. „Synthetic Cannabinoid Overdose in a 20-Year-Old Male US Soldier". *Substance Abuse* 34 (1): 70–72. https://doi.org/10.1080/08897077.2012.677754.

Bockaert, Joël, Aline Dumuis, Rochdi Bouhelal, Michèle Sebben, a Robert N. Cory. 1987. „Piperazine Derivatives Including the Putative Anxiolytic Drugs, Buspirone and Ipsapirone, Are Agonists at 5-HT1A Receptors Negatively Coupled with Adenylate Cyclase in Hippocampal Neurons". *Naunyn-Schmiedeberg's Archives of Pharmacology* 335 (5). https://doi.org/10.1007/BF00169129.

Boer, Douwe de, a Ingrid Bosman. 2004. „A new trend in drugs-of-abuse; the 2C-series of phenethylamine designer drugs". *Pharmacy World and Science* 26 (2): 110–13. https://doi.org/10.1023/B:PHAR.0000018600.03664.36.

Boer, Douwe de, Ingrid J Bosman, Elöd Hidvégi, Carmo Manzoni, András A Benkö, Lourenço J.A.L dos Reys, a Robert A.A Maes. 2001. „Piperazine-like Compounds: A New Group of Designer Drugs-of-Abuse on the European Market". *Forensic Science International* 121 (1–2): 47–56. https://doi.org/10.1016/S0379-0738(01)00452-2.

Bryner, Jodi K., Uerica K. Wang, Jenny W. Hui, Merilin Bedodo, Conan MacDougall, a Ilene B. Anderson. 2006. „Dextromethorphan Abuse in Adolescence: An Increasing Trend: 1999–2004". *Archives of Pediatrics & Adolescent Medicine* 160 (12): 1217. https://doi.org/10.1001/archpedi.160.12.1217.

Castaneto, Marisol S., David A. Gorelick, Nathalie A. Desrosiers, Rebecca L. Hartman, Sandrine Pirard, a Marilyn A. Huestis. 2014. „Synthetic Cannabinoids: Epidemiology, Pharmacodynamics, and Clinical Implications". *Drug and Alcohol Dependence* 144 (listopad): 12–41. https://doi.org/10.1016/j.drugalcdep.2014.08.005.

Cooper, Ziva D. 2016. „Adverse Effects of Synthetic Cannabinoids: Management of Acute Toxicity and Withdrawal". *Current Psychiatry Reports* 18 (5). https://doi.org/10.1007/s11920-016-0694-1.

Coppola, M., a R. Mondola. 2012. „3,4-Methylenedioxypyrovalerone (MDPV): Chemistry, Pharmacology and Toxicology of a

New Designer Drug of Abuse Marketed Online". *Toxicology Letters* 208 (1): 12–15. https://doi.org/10.1016/j.toxlet.2011.10.002.

Corazza, Ornella, Zsolt Demetrovics, Wim van den Brink, a Fabrizio Schifano. 2013. „'Legal Highs' an Inappropriate Term for 'Novel Psychoactive Drugs' in Drug Prevention and Scientific Debate". *International Journal of Drug Policy* 24 (1): 82–83. https://doi.org/10.1016/j.drugpo.2012.06.005.

Corazza, Ornella, Fabrizio Schifano, Magi Farre, Paolo Deluca, Zoe Davey, Colin Drummond, Marta Torrens, et al. 2011. „Designer Drugs on the Internet: A Phenomenon Out-of--Control? The Emergence of Hallucinogenic Drug Bromo--Dragonfly". *Current Clinical Pharmacology* 6 (2): 125–29. https://doi.org/10.2174/157488411796151129.

Corazza, Ornella, Fabrizio Schifano, Pierluigi Simonato, Suzanne Fergus, Sulaf Assi, Jacqueline Stair, John Corkery, et al. 2012. „Phenomenon of New Drugs on the Internet: The Case of Ketamine Derivative Methoxetamine: NEW DRUGS ON THE INTERNET". *Human Psychopharmacology: Clinical and Experimental* 27 (2): 145–49. https://doi.org/10.1002/hup.1242.

Corkery, John M, Simon Elliott, Fabrizio Schifano, Ornella Corazza, a A Hamid Ghodse. 2013. „MDAI (5,6-Methylenedioxy--2-Aminoindane; 6,7-Dihydro-5H-Cyclopenta[f][1,3]Benzo-dioxol-6-Amine; 'Sparkle'; 'Mindy') Toxicity: A Brief Overview and Update: MDAI TOXICITY: BRIEF REVIEW AND UPDATE". *Human Psychopharmacology: Clinical and Experimental* 28 (4): 345–55. https://doi.org/10.1002/hup.2298.

Cozzi, Nicholas V, Michael K Sievert, Alexander T Shulgin, Peyton Jacob, a Arnold E Ruoho. 1999. „Inhibition of Plasma Membrane Monoamine Transporters by ß-Ketoamphetamines". *European Journal of Pharmacology* 381 (1): 63–69. https://doi.org/10.1016/S0014-2999(99)00538-5.

Crespi, Craig. 2016. „Flakka-Induced Prolonged Psychosis". *Case Reports in Psychiatry* 2016: 1–2. https://doi.org/10.1155/2016/3460849.

Dargan, P. I., H. C. Tang, W. Liang, D. M. Wood, a D. T. Yew. 2014. „Three Months of Methoxetamine Administration Is Associated with Significant Bladder and Renal Toxicity in Mice". *Clinical Toxicology* 52 (3): 176–80. https://doi.org/10.3109/15563650.2014.892605.

Dean, Be Vang, Samuel J. Stellpflug, Aaron M. Burnett, a Kristin M. Engebretsen. 2013. „2C or Not 2C: Phenethylamine Designer Drug Review". *Journal of Medical Toxicology* 9 (2): 172–78. https://doi.org/10.1007/s13181-013-0295-x.

Elliott, Simon, a Julie Evans. 2014. „A 3-Year Review of New Psychoactive Substances in Casework". *Forensic Science International* 243 (říjen): 55–60. https://doi.org/10.1016/j.forsciint.2014.04.017.

Elliott, Simon P., Simon D. Brandt, a Christopher Smith. 2016. „The first reported fatality associated with the synthetic opioid 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenz-amide (U-47700) and implications for forensic analysis". Drug *Testing and Analysis* 8 (8): 875–79. https://doi.org/10.1002/dta.1984.

ElSohly, Mahmoud A., Waseem Gul, Amira S. Wanas, a Mohamed M. Radwan. 2014. „Synthetic Cannabinoids: Analysis and Metabolites". *Life Sciences* 97 (1): 78–90. https://doi.org/10.1016/j.lfs.2013.12.212.

European Monitoring Centre for Drugs and Drug Addiction, a Europol. 2015. *EMCDA-Europol Joint Report on a New Psychoactive Substance: 1-Phenyl-2-(1-Pyrrolidinyl)-1-Pentanone (-PVP) : In Accordordance with Article 5 of Council Decision 2005/387/JHA on the Information Exchange, Risk Assessment and Control of New Psychoactive Substances*. Lisbon: EMCDDA.

Every-Palmer, Susanna. 2011. „Synthetic Cannabinoid JWH-018 and Psychosis: An Explorative Study". *Drug and Alcohol Dependence* 117 (2–3): 152–57. https://doi.org/10.1016/j.drugalcdep.2011.01.012.

Felgate, Heather E., Peter D. Felgate, Ross A. James, D. Noel Sims, a Dominic C. Vozzo. 1998. „Recent Paramethoxyamphe-tamine Deaths". *Journal of Analytical Toxicology* 22 (2): 169–72. https://doi.org/10.1093/jat/22.2.169.

Frison, Giampietro, Luca Zamengo, Flavio Zancanaro, Francesco Tisato, a Pietro Traldi. 2016. „Characterization of the Designer Drug Deschloroketamine (2-Methylamino-2--Phenylcyclohexanone) by Gas Chromatography/Mass Spectrometry, Liquid Chromatography/High-Resolution Mass Spectrometry, Multistage Mass Spectrometry, and Nuclear Magnetic Resonance: Characterization of the Designer Drug Deschloroketamine". *Rapid Communications in Mass Spectrometry* 30 (1): 151–60. https://doi.org/10.1002/rcm.7425.

Fuller, Ray W., John C. Baker, a Bryan B. Molloy. 1977. „Biological Disposition of Rigid Analogs of Amphetamine". *Journal of Pharmaceutical Sciences* 66 (2): 271–72. https://doi.org/10.1002/jps.2600660235.

Gallagher, Cathal T., Sulaf Assi, Jacqueline L. Stair, Suzanne Fergus, Ornella Corazza, John M. Corkery, a Fabrizio Schifano. 2012. „5,6-Methylenedioxy-2-Aminoindane: From Laboratory Curiosity to 'Legal High': MDAI: FROM LABORATORY CURIOSITY TO 'LEGAL HIGH' ". Human Psychopharmacology: *Clinical and Experimental* 27 (2): 106–12. https://doi.org/10.1002/hup.1255.

Gamage, Thomas F., Charlotte E. Farquhar, Timothy W. Lefever, Julie A. Marusich, Richard C. Kevin, Iain S. McGregor, Jenny L. Wiley, a Brian F. Thomas. 2018. „Molecular and Behavioral Pharmacological Characterization of Abused Synthetic Cannabinoids MMB- and MDMB-FUBINACA, MN-18, NNEI, CUMYL-PICA, and 5-Fluoro-CUMYL-PICA". *Journal of Pharmacology and Experimental Therapeutics* 365 (2): 437–46. https://doi.org/10.1124/jpet.117.246983.

George, Antony V., Jenny J. Lu, Matthew V. Pisano, Jessica Metz, a Timothy B. Erickson. 2010. „Carfentanil—an Ultra Potent Opioid". *The American Journal of Emergency Medicine* 28 (4): 530–32. https://doi.org/10.1016/j.ajem.2010.03.003.

Giroud, C., M. Augsburger, L. Rivier, P. Mangin, F. Sadeghipour, E. Varesio, J. L. Veuthey, a P. Kamalaprija. 1998. „2C-B: A New Psychoactive Phenylethylamine Recently Discovered in Ecstasy Tablets Sold on the Swiss Black Market". *Journal of Analytical Toxicology* 22 (5): 345–54. https://doi.org/10.1093/jat/22.5.345.

Grund, Jean-Paul C., Alisher Latypov, a Magdalena Harris. 2013. „Breaking Worse: The Emergence of Krokodil and Excessive Injuries among People Who Inject Drugs in Eurasia". *International Journal of Drug Policy* 24 (4): 265–74. https://doi.org/10.1016/j.drugpo.2013.04.007.

Gurney, S. M. R., K. S. Scott, S. L. Kacinko, B. C. Presley,

a B. K. Logan. 2014. „Pharmacology, Toxicology, and Adverse Effects of Synthetic Cannabinoid Drugs". *Forensic Science Review* 26 (1): 53–78.

Hasegawa, Koutaro, Amin Wurita, Kayoko Minakata, Kunio Gonmori, Hideki Nozawa, Itaru Yamagishi, Kanako Watanabe, a Osamu Suzuki. 2015. „Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms". *Forensic Toxicology* 33 (1): 45–53. https://doi.org/10.1007/s11419-014-0245-6.

Hasegawa, Koutaro, Amin Wurita, Kayoko Minakata, Kunio Gonmori, Itaru Yamagishi, Hideki Nozawa, Kanako Watanabe, a Osamu Suzuki. 2015. „Identification and Quantitation of 5-Fluoro-ADB, One of the Most Dangerous Synthetic Cannabinoids, in the Stomach Contents and Solid Tissues of a Human Cadaver and in Some Herbal Products". *Forensic Toxicology* 33 (1): 112–21. https://doi.org/10.1007/s11419-014-0259-0.

Helander, A., M. Bäckberg, a O. Beck. 2014. „MT-45, a new psychoactive substance associated with hearing loss and unconsciousness". *Clinical Toxicology* 52 (8): 901–4. https://doi.org/10.3109/15563650.2014.943908.

Hofer, Katharina E., Colette Degrandi, Daniel M. Müller, Ursina Zürrer-Härdi, Schirin Wahl, Christine Rauber-Lüthy, a Alessandro Ceschi. 2014. „Acute Toxicity Associated with the Recreational Use of the Novel Dissociative Psychoactive Substance Methoxphenidine". *Clinical Toxicology* 52 (10): 1288–91. https://doi.org/10.3109/15563650.2014.974264.

Horsley, Rachel R., Eva Lhotkova, Katerina Hajkova, Bronislav Jurasek, Martin Kuchar, a Tomas Palenicek. 2016. „Detailed Pharmacological Evaluation of Methoxetamine (MXE), a Novel Psychoactive Ketamine Analogue—Behavioural, Pharmacokinetic and Metabolic Studies in the Wistar Rat". *Brain Research Bulletin* 126 (září): 102–10. https://doi.org/10.1016/j.brainresbull.2016.05.002.

Johansen, S. S., A. Carsten Hansen, I. B. Muller, J. B. Lundemose, a M.-B. Franzmann. 2003. „Three Fatal Cases of PMA and PMMA Poisoning in Denmark". *Journal of Analytical Toxicology* 27 (4): 253–56. https://doi.org/10.1093/jat/27.4.253.

Johansson, Anna, Daniel Lindstedt, Markus Roman, Gunilla Thelander, Elisabet I. Nielsen, Ulrica Lennborn, Håkan Sandler, et al. 2017. „A Non-Fatal Intoxication and Seven Deaths Involving the Dissociative Drug 3-MeO-PCP". *Forensic Science International* 275 (červen): 76–82. https://doi.org/10.1016/j.forsciint.2017.02.034.

Johnson, Lucas A., Rebecca L. Johnson, a Ray-Bernard Portier. 2013. „Current "Legal Highs" ". The Journal of Emergency Medicine 44 (6): 1108–15. https://doi.org/10.1016/j.jemermed.2012.09.147.

Johnson, Michael P., Stewart P. Frescas, Robert Oberlender, a David E. Nichols. 1991. „Synthesis and Pharmacological Examination of 1-(3-Methoxy-4-Methylphenyl)-2-Aminopropane and 5-Methoxy-6-Methyl-2-Aminoindan: Similarities to 3,4-(Methylenedioxy)Methamphetamine (MDMA)". *Journal of Medicinal Chemistry* 34 (5): 1662–68. https://doi.org/10.1021/jm00109a020.

Jurásek, Bronislav, František Králík, Silvie Rimpelová, Jan Čejka, Vladimír Setnička, Tomáš Ruml, Martin Kuchař, a Michal Kohout. 2018. „Synthesis, Absolute Configuration and in Vitro Cytotoxicity of Deschloroketamine Enantiomers: Rediscovered and Abused Dissociative Anaesthetic". *New Journal of Chemistry* 42 (24): 19360–68. https://doi.org/10.1039/C8NJ03107J.

Kang, Heather, Pojeong Park, Zuner A. Bortolotto, Simon D. Brandt, Tristan Colestock, Jason Wallach, Graham L. Collingridge, a David Lodge. 2017. „Ephenidine: A New Psychoactive Agent with Ketamine-like NMDA Receptor Antagonist Properties". *Neuropharmacology* 112 (leden): 144–49. https://doi.org/10.1016/j.neuropharm.2016.08.004.

Karinen, Ritva, Silja Skogstad Tuv, Sidsel Rogde, Mariana Dadalto Peres, Unni Johansen, Joachim Frost, Vigdis Vindenes, a Åse Marit Leere Øiestad. 2014. „Lethal Poisonings with AH-7921 in Combination with Other Substances". *Forensic Science International* 244 (listopad): e21–24. https://doi.org/10.1016/j.forsciint.2014.08.013.

Karlsson, Louise, Mikael Andersson, Robert Kronstrand, a Fredrik C. Kugelberg. 2014. „Mephedrone, Methylone and 3,4-Methylenedioxypyrovalerone (MDPV) Induce Conditioned Place Preference in Mice". *Basic & Clinical Pharmacology & Toxicology* 115 (5): 411–16. https://doi.org/10.1111/bcpt.12253.

Kim, Hong K, a Lewis S Nelson. 2015. „Reducing the harm of opioid overdose with the safe use of naloxone: a pharmacologic review". *Expert Opinion on Drug Safety* 14 (7): 1137–46. https://doi.org/10.1517/14740338.2015.1037274.

Koller, Verena J., Volker Auwärter, Tamara Grummt, Bjoern Moosmann, Miroslav Mišík, a Siegfried Knasmüller. 2014. „Investigation of the in Vitro Toxicological Properties of the Synthetic Cannabimimetic Drug CP-47,497-C8". *Toxicology and Applied Pharmacology* 277 (2): 164–71. https://doi.org/10.1016/j.taap.2014.03.014.

Kraner, J. C., D. J. McCoy, M. A. Evans, L. E. Evans, a B. J. Sweeney. 2001. „Fatalities Caused by the MDMA-Related Drug Paramethoxyamphetamine (PMA)". Journal of Analytical Toxicology 25 (7): 645–48. https://doi.org/10.1093/jat/25.7.645.
Krikorian, Abraham D. 1984. „Kat and Its Use: An Historical Perspective". *Journal of Ethnopharmacology* 12 (2): 115–78. https://doi.org/10.1016/0378-8741(84)90047-3.

Kronstrand, R., G. Thelander, D. Lindstedt, M. Roman, a F. C. Kugelberg. 2014. „Fatal Intoxications Associated with the Designer Opioid AH-7921". *Journal of Analytical Toxicology* 38 (8): 599–604. https://doi.org/10.1093/jat/bku057.

Kronstrand, Robert, Markus Roman, Gunilla Thelander, a Anders Eriksson. 2011. „Unintentional fatal intoxications with mitragynine and o-desmethyltramadol from the herbal blend krypton". *Journal of Analytical Toxicology* 35 (4): 242–47. https://doi.org/10.1093/anatox/35.4.242.

Kuypers, K. P. C., E. B. De Sousa Fernandes Perna, E. L Theunissen, S. W. Toennes, N. L. Mason, N. R. P. W. Hutten, a J. G. Ramaekers. 2019. „A First-in-Man Study with 4-Fluoroamphetamine Demonstrates It Produces a Mild Psychedelic State". *Journal of Psychoactive Drugs*, leden, 1–11. https://doi.org/10.1080/02791072.2019.1569286.

Levin, Nathan, Boyd E. Graham, a H. G. Kolloff. 1944. „PHYSIOLOGICALLY ACTIVE INDANAMINES 1". *The Journal of Organic Chemistry* 09 (4): 380–91. https://doi.org/10.1021/jo01186a010.

Lin, D.-L., H.-C. Liu, a H.-L. Yin. 2007. „Recent Paramethoxymethamphetamine (PMMA) Deaths in Taiwan". *Journal of Analytical Toxicology* 31 (2): 109–13. https://doi.org/10.1093/jat/31.2.109.

Linsen, Felix, Raoul P. J. Koning, Margriet van Laar, Raymond J. M. Niesink, Maarten W. Koeter, a Tibor M. Brunt. 2015. „4-Fluoroamphetamine in the Netherlands: More than a One-Night Stand: 4-Fluoroamphetamine in the Netherlands". *Addiction* 110 (7): 1138–43. https://doi.org/10.1111/add.12932.

Lyttle, Thomas, David Goldstein, a Jochen Gartz. 1996. „Bufo Toads and Bufotenine: Fact and Fiction Surrounding an Alleged Psychedelic". *Journal of Psychoactive Drugs* 28 (3): 267–90.

Martin, T. L. 2001. „Three Cases of Fatal Paramethoxyamphetamine Overdose". *Journal of Analytical Toxicology* 25 (7): 649–51. https://doi.org/10.1093/jat/25.7.649.

Martinak, Bridgette, Ramy A. Bolis, Jeffrey Ryne Black, Rachel E. Fargason, a Badari Birur. 2017. „Dextromethorphan in Cough Syrup: The Poor Man's Psychosis". *Psychopharmacology Bulletin* 47 (4): 59–63.

Meltzer, P. C., D. Butler, J. R. Deschamps, a B. K. Madras. 2006. „1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (Pyrovalerone) analogues: a promising class of monoamine uptake inhibitors". *J Med Chem* 49 (4): 1420–32. https://doi.org/10.1021/jm050797a.

Mohr, Amanda L.A., Melissa Friscia, Donna Papsun, Sherri L. Kacinko, David Buzby, a Barry K. Logan. 2016. „Analysis of novel synthetic opioids U-47700, U-50488 and furanyl fentanyl by LC-MS/MS in postmortem casework". *Journal of Analytical Toxicology* 40 (9): 709–17. https://doi.org/10.1093/jat/bkw086.

Monte, Aaron P., Danuta Marona-Lewicka, Nicholas V. Cozzi, a David E. Nichols. 1993. „Synthesis and Pharmacological Examination of Benzofuran, Indan, and Tetralin Analogs of 3,4-(Methylenedioxy)Amphetamine". *Journal of Medicinal Chemistry* 36 (23): 3700–3706. https://doi.org/10.1021/jm00075a027.

Morris, Hamilton, a Jason Wallach. 2014. „From PCP to MXE: A Comprehensive Review of the Non-Medical Use of Dissociative Drugs: PCP to MXE". *Drug Testing and Analysis* 6 (7–8): 614–32. https://doi.org/10.1002/dta.1620.

Murphy, Christine M., Anna R. Dulaney, Michael C. Beuhler, a Sherri Kacinko. 2013. „ "Bath Salts" and "Plant Food" Products: The Experience of One Regional US Poison Center". *Journal of Medical Toxicology* 9 (1): 42–48. https://doi.org/10.1007/s13181-012-0243-1.

Namera, Akira, Maho Kawamura, Akihiro Nakamoto, Takeshi Saito, a Masataka Nagao. 2015. „Comprehensive Review of the Detection Methods for Synthetic Cannabinoids and Cathinones". *Forensic Toxicology* 33 (2): 175–94. https://doi.org/10.1007/s11419-015-0270-0.

Nicol, J. J. E., M. C. Yarema, G. R. Jones, W. Martz, R. A. Purssell, J. C. MacDonald, I. Wishart, M. Durigon, D. Tzemis, a J. A. Buxton. 2015. „Deaths from Exposure to Parathoxymethamphetamine in Alberta and British Columbia, Canada: A Case Series". *CMAJ Open* 3 (1): E83–90. https://doi.org/10.9778/cmajo.20140070.

Nichols, David E. 1999. „Improvements to the Synthesis of Psilocybin and a Facile Method for Preparing the O-Acetyl Prodrug of Psilocin". *Synthesis* 1999 (06): 935–38. https://doi.org/10.1055/s-1999-3490.

Nichols, David E., William K. Brewster, Michael P. Johnson, Robert Oberlender, a Robert M. Riggs. 1990. „Nonneurotoxic Tetralin and Indan Analogs of 3,4-(Methylenedioxy)Amphetamine (MDA)". *Journal of Medicinal Chemistry* 33 (2): 703–10. https://doi.org/10.1021/jm00164a037.

Nichols, David E., Andrew J. Hoffman, Robert A. Oberlender, Peyton Jacob, a Alexander T. Shulgin. 1986. „Derivatives of 1-(1,3-Benzodioxol-5-Yl)-2-Butanamine: Representatives of a Novel Therapeutic Class". *Journal of Medicinal Chemistry* 29 (10): 2009–15. https://doi.org/10.1021/jm00160a035.

Nichols, David E., a Robert Oberlender. 1990. „Structure-Activity Relationships of MDMA and Related Compounds: A New Class of Psychoactive Drugs?" *Annals of the New York Academy of Sciences* 600 (1 The Neurophar): 613–23. https://doi.org/10.1111/j.1749-6632.1990.tb16914.x.

Nicholson, P. J., M. J. Quinn, a J. D. Dodd. 2010. „Headshop Heartache: Acute Mephedrone ‚meow´ Myocarditis". *Heart* 96 (24): 2051–52. https://doi.org/10.1136/hrt.2010.209338.

O'Donnell, Julie, R. Matthew Gladden, Christine L. Mattson, a Mbabazi Kariisa. 2018. „Notes from the Field: Overdose Deaths with Carfentanil and Other Fentanyl Analogs Detected — 10 States, July 2016–June 2017". *MMWR. Morbidity and Mortality Weekly Report* 67 (27): 767–68. https://doi.org/10.15585/mmwr.mm6727a4.

Osorio-Olivares, Mauricio, Marcos Caroli Rezende, Silvia Sepúlveda-Boza, Bruce K Cassels, a Angélica Fierro. 2004. „MAO Inhibition by Arylisopropylamines: The Effect of Oxygen Substituents at the ß-Position". *Bioorganic & Medicinal Chemistry* 12 (15): 4055–66. https://doi.org/10.1016/j.bmc.2004.05.033.

Papsun, Donna, Alison Krywanczyk, James C. Vose, Elizabeth A. Bundock, a Barry K. Logan. 2016. „Analysis of MT-45, a novel synthetic opioid, in human whole blood by LC-MS-MS and its identification in a drug-related death". *Journal of Analytical Toxicology* 40 (4): 313–17. https://doi.org/10.1093/jat/bkw012.

Prekupec, Matthew P., Peter A. Mansky, a Michael H. Baumann. 2017. „Misuse of Novel Synthetic Opioids: A Deadly New Trend". *Journal of Addiction Medicine* 11 (4): 256–65. https://doi.org/10.1097/ADM.0000000000000324.

Refstad, S. 2003. „Paramethoxyamphetamine (PMA) Poisoning; a ‚party Drug´ with Lethal Effects". Acta Anaesthesiologica Scandinavica 47 (10): 1298–99. https://doi.org/10.1046/j.1399-6576.2003.00245.x.

Rickli, Anna, Simone Kopf, Marius C Hoener, a Matthias E. Liechti. 2015. „Pharmacological Profile of Novel Psychoactive Benzofurans: Novel Psychoactive Benzofurans". *British Journal of Pharmacology* 172 (13): 3412–25. https://doi.org/10.1111/bph.13128.

Rickli, Anna, Olivier D. Moning, Marius C. Hoener, a Matthias E. Liechti. 2016. „Receptor Interaction Profiles of Novel Psychoactive Tryptamines Compared with Classic Hallucinogens". *European Neuropsychopharmacology* 26 (8): 1327–37. https://doi.org/10.1016/j.euroneuro.2016.05.001.

Riga, Maurizio S., Guadalupe Soria, Raúl Tudela, Francesc Artigas, a Pau Celada. 2014. „The Natural Hallucinogen 5-MeO-DMT, Component of Ayahuasca, Disrupts Cortical Function in Rats: Reversal by Antipsychotic Drugs". *The International Journal of Neuropsychopharmacology* 17 (08): 1269–82. https://doi.org/10.1017/S1461145714000261.

Rose, S. Rutherfoord, Justin L. Poklis, a Alphonse Poklis. 2013. „A Case of 25I-NBOMe (25-I) Intoxication: A New Potent 5-HT2A Agonist Designer Drug". *Clinical Toxicology* 51 (3): 174–77. https://doi.org/10.3109/15563650.2013.772191.

Rösner, Peter, Bernd Quednow, Ulrich Girreser, a Thomas Junge. 2005. „Isomeric Fluoro-Methoxy-Phenylalkylamines: A New Series of Controlled-Substance Analogues (Designer Drugs)". *Forensic Science International* 148 (2–3): 143–56. https://doi.org/10.1016/j.forsciint.2004.05.003.

Ross, Edward A., Mary Watson, a Bruce Goldberger. 2011. „"Bath Salts" Intoxication". *New England Journal of Medicine* 365 (10): 967–68. https://doi.org/10.1056/NEJMc1107097.

Roth, Bryan L., Simon Gibbons, Warunya Arunotayanun, Xi-Ping Huang, Vincent Setola, Ric Treble, a Les Iversen. 2013. „The Ketamine Analogue Methoxetamine and 3- and 4-Methoxy Analogues of Phencyclidine Are High Affinity and Selective Ligands for the Glutamate NMDA Receptor". Editoval Kenji Hashimoto. *PLoS ONE* 8 (3): e59334. https://doi.org/10.1371/journal.pone.0059334.

Rudd, R A, N Aleshire, J E Zibbell, a R Matthew Gladden. 2016. „Increases in drug and opioid overdose deaths — United States, 2000–2014". *Morbidity and Mortality Weekly Report* 64 (50–51): 1378–82. https://doi.org/10.1111/ajt.13776.

Sacks, Justin, M. Jordan Ray, Sue Williams, a Michael J. Opatowsky. 2012. „Fatal Toxic Leukoencephalopathy Secondary to Overdose of a New Psychoactive Designer Drug 2C-E ("Europa")". *Baylor University Medical Center Proceedings* 25 (4): 374–76. https://doi.org/10.1080/08998280.2012.11928883.

Sainsbury, P.D., A.T. Kicman, R.P. Archer, L.A. King, a R.A. Braithwaite. 2011. „Aminoindanes-the next Wave of 'Legal Highs'?" *Drug Testing and Analysis* 3 (7–8): 479–82. https://doi.org/10.1002/dta.318.

Seely, Kathryn A., Jeff Lapoint, Jeffery H. Moran, a Liana Fattore. 2012. „Spice Drugs Are More than Harmless Herbal Blends: A Review of the Pharmacology and Toxicology of Synthetic Cannabinoids". *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 39 (2): 234–43. https://doi.org/10.1016/j.pnpbp.2012.04.017.

Shaler, Robert C., a John J. Padden. 1972. „Identification of Hallucinogens in Illicit Seizures I: 2,5-Dimethoxyamphetamine". *Journal of Pharmaceutical Sciences* 61 (11): 1851–55. https://doi.org/10.1002/jps.2600611142.

Shanks, K. G., T. Dahn, a A. R. Terrell. 2012. „Detection of JWH-018 and JWH-073 by UPLC-MS-MS in Postmortem Whole Blood Casework". *Journal of Analytical Toxicology* 36 (3): 145–52. https://doi.org/10.1093/jat/bks013.

Shields, Jennifer E., Paul I. Dargan, David M. Wood, Malgorzata Puchnarewicz, Susannah Davies, a W. Stephen Waring. 2012. „Methoxetamine Associated Reversible Cerebellar Toxicity: Three Cases with Analytical Confirmation". *Clinical Toxicology* 50 (5): 438–40. https://doi.org/10.3109/15563650.2012.683437.

Shimizu, Eiji, Hiroyuki Watanabe, Takashi Kojima, Hiroko Hagiwara, Mihisa Fujisaki, Ryosuke Miyatake, Kenji Hashimoto, a Masaomi Iyo. 2007. „Combined Intoxication with Methylone and 5-MeO-MIPT". *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 31 (1): 288–91. https://doi.org/10.1016/j.pnpbp.2006.06.012.

Shimshoni, Jakob A., Ilan Winkler, Nir Edery, Ezekiel Golan, René van Wettum, a David Nutt. 2017. „Toxicological Evaluation of 5-Methoxy-2-Aminoindane (MEAI): Binge Mitigating Agent in Development". *Toxicology and Applied Pharmacology* 319 (březen): 59–68. https://doi.org/10.1016/j.taap.2017.01.018.

Shulgin, Alexander T., Sterling Bunnell, a Thornton Sargent. 1961. „The Psychotomimetic Properties of 3,4,5-Trimethoxyamphetamine". *Nature* 189 (4769): 1011–12. https://doi.org/10.1038/1891011a0.

Schifano, Fabrizio, Paolo Deluca, Lisa Agosti, Giovanni Martinotti, John M. Corkery, a The Psychonaut 2002 Research Group. 2005. „New Trends in the Cyber and Street Market of Recreational Drugs? The Case of 2C-T-7 ('Blue Mystic')". *Journal of Psychopharmacology* 19 (6): 675–79. https://doi.org/10.1177/0269881105056660.

Schifano, Fabrizio, Laura Orsolini, G. Duccio Papanti, a John M. Corkery. 2015. „Novel Psychoactive Substances of Interest for Psychiatry". *World Psychiatry* 14 (1): 15–26. https://doi.org/10.1002/wps.20174.

Schneir, Aaron, Imir G. Metushi, Christian Sloane, David J. Benaron, a Robert L. Fitzgerald. 2017. „Near death from a novel synthetic opioid labeled U-47700: emergence of a new opioid class". *Clinical Toxicology* 55 (1): 51–54. https://doi.org/10.1080/15563650.2016.1209764.

Siddiqi, Sindhu, Charlotte Verney, Paul Dargan, a David M. Wood. 2015. „Understanding the availability, prevalence of use, desired effects, acute toxicity and dependence potential of the novel opioid MT-45". *Clinical Toxicology* 53 (1): 54–59. https://doi.org/10.3109/15563650.2014.983239.

Sikk, Katrin, Sulev Haldre, Sten-Magnus Aquilonius, a Pille Taba. 2011. „Manganese-Induced Parkinsonism Due to Ephedrone Abuse". *Parkinson's Disease* 2011: 1–8. https://doi.org/10.4061/2011/865319.

Simmler, Ld, Ta Buser, M Donzelli, Y Schramm, L-H Dieu, J. Huwyler, S Chaboz, Mc Hoener, a Me Liechti. 2013. „Pharmacological Characterization of Designer Cathinones in Vitro: Pharmacology of Cathinones". *British Journal of Pharmacology* 168 (2): 458–70. https://doi.org/10.1111/j.1476-5381.2012.02145.x.

Simmler, Linda D., Anna Rickli, York Schramm, Marius C. Hoener, a Matthias E. Liechti. 2014. „Pharmacological Profiles of Aminoindanes, Piperazines, and Pipradrol Derivatives". *Biochemical Pharmacology* 88 (2): 237–44. https://doi.org/10.1016/j.bcp.2014.01.024.

Solomons, Everett, a Joseph Sam. 1973. „2-Aminoindans of Pharmacological Interest". *Journal of Medicinal Chemistry* 16 (12): 1330–33. https://doi.org/10.1021/jm00270a004.

Spaderna, Max, Peter H. Addy, a Deepak Cyril D'Souza. 2013. „Spicing Things up: Synthetic Cannabinoids". *Psychopharmacology* 228 (4): 525–40. https://doi.org/10.1007/s00213-013-3188-4.

Spiller, Henry A., Mark L. Ryan, Robert G. Weston, a Joanne Jansen. 2011. „Clinical Experience with and Analytical Confirmation of "Bath Salts" and "Legal Highs" (Synthetic Cathinones) in the United States". *Clinical Toxicology* 49 (6): 499–505. https://doi.org/10.3109/15563650.2011.590812.

Springer, Dietmar, Giselher Fritschi, a Hans H. Maurer. 2003. „Metabolism and Toxicological Detection of the New Designer Drug 3',4'-Methylenedioxy-α-Pyrrolidinopropiophenone Studied in Urine Using Gas Chromatography–Mass Spectrometry". *Journal of Chromatography B* 793 (2): 377–88. https://doi.org/10.1016/S1570-0232(03)00350-7.

Staeheli, Sandra N., Martina I. Boxler, Andrea Oestreich, Michelle Marti, Dominic Gascho, Stephan A. Bolliger, Thomas Kraemer, a Andrea E. Steuer. 2017. „Postmortem Distribution and Redistribution of MDAI and 2-MAPB in Blood and Alternative Matrices". *Forensic Science International* 279 (říjen): 83–87. https://doi.org/10.1016/j.forsciint.2017.08.007.

Stoff, D.M., D.A. GoreLick, T. Bozewicz, W.H. Bridger, J.C. Gillin, a R.J. Wyatt. 1978. „The Indole Hallucinogens, N,N-Dimethyltryptamine (DMT) and 5-Methoxy-N,N-Dimethyltryptamine (5-MeO-DMT), Have Different Effects from Mescaline on Rat Shuttlebox Avoidance". *Neuropharmacology* 17 (12): 1035–40. https://doi.org/10.1016/0028-3908(78)90030-8.

Swanson, Dina M., Laura S. Hair, Selly R. Strauch Rivers, Brianna C. Smyth, Sara C. Brogan, Alexis D. Ventoso, Samantha L. Vaccaro, a Julia M. Pearson. 2017. „Fatalities Involving Carfentanil and Furanyl Fentanyl: Two Case Reports". *Journal of Analytical Toxicology* 41 (6): 498–502. https://doi.org/10.1093/jat/bkx037.

Štefková, Kristýna, Monika Židková, Rachel R. Horsley, Nikola Pinterová, Klára Šíchová, Libor Uttl, Marie Balíková, Hynek Danda, Martin Kuchař, a Tomáš Páleníček. 2017. „Pharmacokinetic, Ambulatory, and Hyperthermic Effects of 3,4-Methylenedioxy-N-Methylcathinone (Methylone) in Rats". *Frontiers in Psychiatry* 8 (listopad). https://doi.org/10.3389/fpsyt.2017.00232.

Tortella, Frank C., Mario Pellicano, a Norman G. Bowery. 1989. „Dextromethorphan and Neuromodulation: Old Drug Coughs up New Activities". *Trends in Pharmacological Sciences* 10 (12): 501–7. https://doi.org/10.1016/0165-6147(89)90050-3.

Trecki, Jordan, Roy R. Gerona, a Michael D. Schwartz. 2015. „Synthetic Cannabinoid–Related Illnesses and Deaths". *New England Journal of Medicine* 373 (2): 103–7. https://doi.org/10.1056/NEJMp1505328.

Uttl, Libor, Ewa Szczurowska, Kateřina Hájková, Rachel R. Horsley, Kristýna Štefková, Tomáš Hložek, Klára Šíchová, et al. 2018. „Behavioral and Pharmacokinetic Profile of Indole-Derived Synthetic Cannabinoids JWH-073 and JWH-210 as Compared to the Phytocannabinoid Δ9-THC in Rats". *Frontiers in Neuroscience* 12 (říjen). https://doi.org/10.3389/fnins.2018.00703.

Valente, Maria João, Paula Guedes de Pinho, Maria de Lourdes Bastos, Félix Carvalho, a Márcia Carvalho. 2014. „Khat and Synthetic Cathinones: A Review". *Archives of Toxicology* 88 (1): 15–45. https://doi.org/10.1007/s00204-013-1163-9.

Van Hout, Marie Claire, a Evelyn Hearne. 2017. „New Psycho active Substances (NPS) on Cryptomarket Fora: An Exploratory Study of Characteristics of Forum Activity between NPS Buyers and Vendors". *International Journal of Drug Policy* 40 (únor): 102–10. https://doi.org/10.1016/j.drugpo.2016.11.007.

Vandrey, Ryan, Kelly E. Dunn, Jeannie A. Fry, a Elizabeth R. Girling. 2012. „A Survey Study to Characterize Use of Spice Products (Synthetic Cannabinoids)". *Drug and Alcohol Dependence* 120 (1–3): 238–41. https://doi.org/10.1016/j.drugalcdep.2011.07.011.

Vigolo, A., A. Ossato, C. Trapella, F. Vincenzi, C. Rimondo, C. Seri, K. Varani, G. Serpelloni, a M. Marti. 2015. „Novel Halogenated Derivates of JWH-018: Behavioral and Binding Studies in Mice". *Neuropharmacology* 95 (srpen): 68–82. https://doi.org/10.1016/j.neuropharm.2015.02.008.

Vorce, Shawn P., Jessica L. Knittel, Justin M. Holler, Joseph Magluilo, Barry Levine, Philip Berran, a Thomas Z. Bosy. 2014. „A Fatality Involving Ah-7921". *Journal of Analytical Toxicology* 38 (4): 226–30. https://doi.org/10.1093/jat/bku011.

Wallach, Jason, Heather Kang, Tristan Colestock, Hamilton Morris, Zuner A. Bortolotto, Graham L. Collingridge, David Lodge, Adam L. Halberstadt, Simon D. Brandt, a Adeboye Adejare. 2016. „Pharmacological Investigations of the Dissociative 'Legal Highs' Diphenidine, Methoxphenidine and Analogues". Editoval Joohyung Lee. *PLOS ONE* 11 (6): e0157021. https://doi.org/10.1371/journal.pone.0157021.

Wax, Paul M., Charles E. Becker, a Steven C. Curry. 2003. „Unexpected "Gas" Casualties in Moscow: A Medical Toxicology Perspective". *Annals of Emergency Medicine* 41 (5): 700–705. https://doi.org/10.1067/mem.2003.148.

Westin, Andreas Austgulen, Joachim Frost, Wenche Rødseth Brede, Per Ole M. Gundersen, Steinar Einvik, Harald Aarset, a Lars Slørdal. 2015. „Sudden Cardiac Death Following Use of the Synthetic Cannabinoid MDMB-CHMICA". *Journal of Analytical Toxicology*, září, bkv110. https://doi.org/10.1093/jat/bkv110.

Whiteside, M. 2002. „Substituted hexahydrobenzodipyrans as 5-HT2A/2C receptor probes". *Bioorganic & Medicinal Chemistry* 10 (10): 3301–6. https://doi.org/10.1016/S0968-0896(02)00209-2.

Wikström, Maria, Gunilla Thelander, Maria Dahlgren, a Robert Kronstrand. 2013. „An Accidental Fatal Intoxication with Methoxetamine". *Journal of Analytical Toxicology* 37 (1): 43–46. https://doi.org/10.1093/jat/bks086.

Winstock, Adam, Luke Mitcheson, John Ramsey, Susannah Davies, Malgorzata Puchnarewicz, a John Marsden. 2011. „Mephedrone: Use, Subjective Effects and Health Risks: Mephedrone and Health Risks". *Addiction* 106 (11): 1991–96. https://doi.org/10.1111/j.1360-0443.2011.03502.x.

Winstock, Adam R., Luke R. Mitcheson, Paolo Deluca, Zoe Davey, Ornella Corazza, a Fabrizio Schifano. 2011. „Mephedrone, New Kid for the Chop?: Mephedrone and Legal Highs". *Addiction* 106 (1): 154–61. https://doi.org/10.1111/j.1360-0443.2010.03130.x.

Wood, David M., Susannah Davies, Shaun L. Greene, Jenny Button, David W. Holt, John Ramsey, a Paul I. Dargan. 2010. „Case Series of Individuals with Analytically Confirmed Acute Mephedrone Toxicity". *Clinical Toxicology* 48 (9): 924–27. https://doi.org/10.3109/15563650.2010.531021.

Yanini, Ángela, Sergio Armenta, Francesc A. Esteve-Turrillas, Nieves Galipienso, a Miguel de la Guardia. 2018. „Identification and characterization of the new psychoactive substance 3-fluoroethamphetamine in seized material". *Forensic Toxicology* 36 (2): 404–14. https://doi.org/10.1007/s11419-018-0416-y.

Zaitsu, Kei, Munehiro Katagi, Hiroe Kamata, Tooru Kamata, Noriaki Shima, Akihiro Miki, Tatsunori Iwamura, a Hitoshi Tsuchihashi. 2008. „Discrimination and Identification of the Six Aromatic Positional Isomers of Trimethoxyamphetamine (TMA) by Gas Chromatography-Mass Spectrometry (GC-MS)". *Journal of Mass Spectrometry* 43 (4): 528–34. https://doi.org/10.1002/jms.1347.

Zawilska, Jolanta B., a Jakub Wojcieszak. 2013. „Designer Cathinones—An Emerging Class of Novel Recreational Drugs". *Forensic Science International* 231 (1–3): 42–53. https://doi.org/10.1016/j.forsciint.2013.04.015.

Tato práce je podpořena projekty: VI20172020056, GAČR 18-16218S, MZCR –DRO (NUDZ-CZ, 00023752), grant LO1611 od MŠMT pod programem NPU I, PROGRES Q35 a SVV UK 260388/SVV/2019.

Doporučená citace

Danda, H., Leca, N., Štefková, K., Syrová, K., Páleníček, T. 2020. Nové psychoaktivní substance. Zaostřeno 6 (1), 1–20.

Národní monitorovací středisko pro drogy a závislosti

OZNÁMENÍ / ODKAZY

Informace o drogové situaci a o situaci v oblasti hazardního hraní v ČR https://www.drogy-info.cz/.

Veškeré publikace vydané Národním monitorovacím střediskem pro drogy a závislosti, včetně všech čísel Zaostřeno, jsou v elektronické podobě ke stažení na https://www.drogy-info.cz/publikace/.
Případné objednávky tištěných publikací zasílejte na e-mail: grygarova.marketa@vlada.cz.

Mapa pomoci
https://www.drogy-info.cz/mapa-pomoci/?.
– změny kontaktních údajů zasílejte na e-mail: grygarova.marketa@vlada.cz.

Kalendář akcí
https://www.drogy-info.cz/kalendar-akci/ – informace o vzdělávacích akcích a seminářích v adiktologii nebo zajímavých pro obor adiktologie, které je možno v kalendáři zveřejnit, zasílejte na e-mail: grygarova.marketa@vlada.cz.

Reedice projektu „Když musíš, tak musíš“
DVD s 6 dokumentárními filmy o drogách – objednávky zasílejte na e-mail: grygarova.marketa@vlada.cz.

Aplikace UniData a PrevData k evidenci klientů a intervencí v drogových službách včetně uživatelské podpory
http://www.drogovesluzby.cz/.

Internetová stránka Evropského monitorovacího centra pro drogy a drogovou závislost (EMCDDA):
http://www.emcdda.europa.eu/.

Portál dobré praxe EMCDDA:
http://www.emcdda.europa.eu/best-practice.

Evropská zpráva o drogách EMCDDA
http://www.emcdda.europa.eu/edr2017.

Národní stránky na podporu odvykání kouření
https://www.koureni-zabiji.cz/.
Národní linka pro odvykání kouření 800 35 00 00.

Národní stránky pro snížení rizik hazardního hraní
https://www.hazardni-hrani.cz/.

Národní stránky pro podporu omezení konzumace alkoholu: www.alkohol-skodi.cz/.

Národní linka pro odvykání kouření, alkoholu, hazardnímu hraní a nelegálním drogám **800 35 00 00.**

Zaostřeno

ISSN 2336-8241

Vydává > **Úřad vlády České republiky**
nábřeží E. Beneše 4, 118 01 Praha 1, IČO 00006599

Adresa redakce > E. Beneše 4, 118 01 Praha 1, tel. 224 002 111
Odpovědný redaktor > Lucie Grolmusová
Autoři tohoto čísla > Hynek Danda, Nikola Leca, Kristýna Štefková, Kateřina Syrová, Tomáš Páleníček
Editor > Kateřina Grohmannová

Toto číslo vyšlo 19. 2. 2020
Vychází nejméně 6× ročně.
Evidenční číslo Ministerstva kultury ČR: E 14088.

ISSN 2336-8241

Abuse potential of novel psychoactive substance naphyrone - conditioned place preference in Wistar rats.

Hynek Danda[1,2]*, Nikola Pinterová-Leca[1,2], Klára Šíchová[1], Kristýna Štefková[1], Kateřina Syrová[1,2], Lucie Olejníková[1], Markéta Končická[1], Vladimír Mazoch[1], Tomáš Páleníček[1,2]

[1] National Institute of Mental Health, Klecany

[2] Third Faculty of Medicine – Charles University, Prague

* Corresponding author

• Abstract

Naphyrone is cathinone analogue that is still available to buy for recreational use despite being banned in many countries. Little is known about its safety and abusive potential. Our study is designed to evaluate abuse potential of naphyrone in CPP test using animal model (male Wistar rats) and compare its effect with an active comparator – methamphetamine. Wistar rats received subcutaneous injections of naphyrone (5, 10 and 20 mg/kg) or methamphetamine (1.5 mg/kg) prior to conditioning sessions in a conditioned place preference (CPP) paradigm. The drug was paired for 4 conditioning sessions with one of the two main compartments of a 3 compartment place preference apparatus. After the conditioning phase animals were tested for place preference by determining the change in proportion of time spent in the drug-paired compartment during a 15 min test session. Naphyrone 5 mg/kg and 20 mg/kg alongside with methamphetamine significantly increased time spent in the drug-paired compartment.

• Introduction

Novel Psychoactive Substance (NPS) is a term that refers to substances which mimic the effects of established illicit drugs, but at least for a while they are not controlled by legislation. NPSs are usually sold as research chemicals, bath salts or plant fertilizers. There is no assurance that content is the same as advertised (Brenneisen, Fisch et al. 1990, Corazza, Schifano et al. 2011, Rosenbaum, Carreiro et al. 2012).

Synthetic cathinones are chemically related to monoamine alkaloid cathinone, the primary psychoactive compound of khat plant *(Catha edulis)* (Brenneisen, Fisch et al. 1990). Khat chewing is a social custom in the Horn of Africa and in Arabian Peninsula for more than a

thousand years, it causes excitement and euphoria (Valente, Guedes de Pinho et al. 2014). Structurally and pharmacologically they resemble amphetamine and 3,4-methylenedioxymethamphetamine (MDMA). Synthetic cathinones are mostly less lipophilic than amphetamine and methamphetamine, it means they have a lower ability to cross the blood-brain barrier (BBB). Naphyrone and some other cathinones carry pyrolidione circle which is highly lipophilic structure, so the molecules that contains it permeate the BBB much easier (Meltzer et al., 2006). Synthetic cathinones pose risk for public health for more reasons - possible Interaction between synthetic cathinones and prescription drugs may influence therapeutical efficacy of the drug and also increase drug toxicity (Contrucci, Brunt et al. 2020).

Few months after mephedrone was listed as controlled substance, the press was already reporting on the new drug - naphyrone (Brandt, Wootton et al. 2010). Naphyrone acts as a non-selective reuptake inhibitor, it inhibits dopamine (DAT), norephinephrine (NET) and serotonine (SERT) transporters. For this reason, together with substances with similar effects, it is called triple reuptake inhibitor. All three transporters are inhibited with approximately same selectivity. *In Vitro* DAT/SERT ratio is 2,0 (1,5-2,7), it resambles other cathinones – methylone, mephedrone, buthylone and ethylone and also cocaine (Simmler, Buser et al. 2013). *In Vitro* studies suggest that naphyrone inhibits DAT, NET and SERT with potency approximately 10x higher than cocaine does (Meltzer, Butler et al. 2006). Naphyrone has fast pharmacokinetics within 30 min after application. The highest concentrations is found in lung tissue followed by brain tissue. Acute administration of naphyrone shows a dose dependent stimulant effect (Pinterova-Leca, Horsley et al. 2020).

- **Materials and methods**

  - **Animals**

All animals were male outbred Wistar rats acquired from VELAZ (Prague, Czech Republic) weighing varied between 200-275 g at the start of testing. Rats were housed two per cage under controlled humidity (30 - 70%) and temperature (22±2°C) with unlimited access to food pellets and water. Lights were on from 6:00 h to 18:00 h and experiments were carried out between 7:00 h and 16:00 h. Animals were habituated at least 7 days to laboratory conditions before being used in experiments; during this period they were weighed at least

twice and handled four times. Experiments were conducted under the same standard temperature and humidity conditions as in the animal housing facility. Experimental groups included 11 to 13 animals.

All procedures were conducted in accordance with the principles of laboratory animal care of the National Committee for the Care and Use of Laboratory Animals (Czech Republic), and according to Guidelines of the European Union (86/609/EU). The protocol was approved by the National Committee for the Care and Use of Laboratory Animals (Czech Republic).

- **Dosage**

The naphyrone doses used in the present study were selected in line with our current study (Pinterova-Leca, Horsley et al. 2020) focusing on acute effects and pharmacokinetic and in accordance with user reports on potency and also based on its affinity to monoamine transporters (Liechti 2015). Naphyrone was dissolved in saline solution (0.9% NaCl) at doses 5, 10 and 20 mg/kg. In a volume of 2 ml/kg and administered subcutaneously (s.c.) in all cases. On days without active drug, animals were treated with an equivalent volume of saline solution (VEH). As an active comparator, methamphetamine in a dose of 1,5 mg/kg was used. This dose was selected since several previous studies found doses ranging from 1 to 3 mg/kg producing CPP (Shimosato and Ohkuma 2000).

- **Conditioned place preference test (CPP)**

Apparatus and software

Three-compartment CPP apparatus was used, which consisted of two equally-sized conditioning compartments (45x50 cm) and one neutral compartment (35x20 cm). In order to distinguish the two conditioning compartments, visual differentiation were used: I) different wall patterns - one of the conditioning compartments had striped walls, the second had checkered walls; II) shape alternations of the space - three-sided plastic prisms placed in different corners in each compartment and III) different floor patterns were used for tactile differentiation – one floor was striped, the other with round-shaped holes. The neutral compartment had transparent walls and floor. Each compartment was separated from the other by a guillotine-style door that were operated manually by the experimenter. Time spent in each compartment was registered and pre-processed by an automatic video tracking system (EthoVision XT v. 11.5, Noldus, Netherlands).

The CPP experiment consisted of four phases: (I) habituation; (II) pre-conditioning; (III) conditioning; (IV) post-conditioning. During habituation (day 1), rats received vehicle immediately before being placed individually into the neutral compartment, the doors were opened and they were allowed to explore freely the whole CPP apparatus for 15 min. On day 2, a pre-conditioning test was performed to determine baseline chamber preference. The rats received vehicle immediately before placement in the neutral compartment followed by 15 min exposure to the entire CPP apparatus, as in habituation. The rats which spent more than 80 % of total time (i.e.12 min or more) in one chamber were considered to have an initial bias for one chamber and were eliminated from experiment (Berry et al., 2012). Conditioning started the day after pre-conditioning and was conducted for eight days (days 3 – 10). We used an unbiased design which means regardless of initial preference rats were pseudo-randomly distributed to drug treatment groups, and drug treatment was counterbalanced across compartments. In the conditioning session, the guillotine doors were closed and after the drug injection rats were immediately placed in the paired compartment for 30 min. Naphyrone/methamphetamine and VEH were paired with a specific compartment on alternate days for a total of eight sessions (four with naphyrone/methamphetamine-paired and four with VEH-paired compartment). The final phase of experiment was post-conditioning test (day 11), all rats received vehicle injection and were let to explore the CPP area for 15 min in exactly the same manner as on the pre-conditioning test. Preference score was calculated by dividing the time spent in drug-paired compartment by the time spent in both conditioning compartments (i.e. without neutral zone) and multiplied by 100.

- **Statistics**

For CPP data, the time spent in the drug-paired compartment was analyzed using two-way repeated-measure analysis of variance (two-way mixed ANOVA) with one between variable – group, with 4 levels (naphyrone 5 mg/kg, naphyrone 10 mg/kg, naphyrone 20 mg/kg and methamphetamine 1,5 mg/kg) – and one within variable – time, with 2 levels (pre-test and post-conditioning test). Subsequently, Bonferroni's multiple comparisons post–hoc tests were calculated.

- **Results**

Results of the naphyrone and methamphetamine CPP in animals receiving the three different doses of naphyrone and one dose of methamphetamine are presented in Fig. 1. The two-way repeated-measure ANOVA for the time spent in the drug-paired compartment revealed an effect of the within variable - time [F (1, 46) = 31,78), p <0,0001]; naphyrone 5 mg/kg, n = 13/group; naphyrone 10 mg/kg, n = 13/group; naphyrone 20 mg/kg, n = 13/group and methamphetamine 1,5 mg/kg, n = 11/group). Subsequently, Bonferroni's multiple comparisons post–hoc tests were calculated. These comparisons revealed significant increase of CPP score in methamphetamine 1,5 mg/kg (p=0,0305), naphyrone 5mg/kg, (p=0,0352) and naphyrone 20 mg (p=0,0070] between the pre-test and post-conditioning test, results for naphyrone 10 mg/kg were slightly above the significance level (p=0,0752)

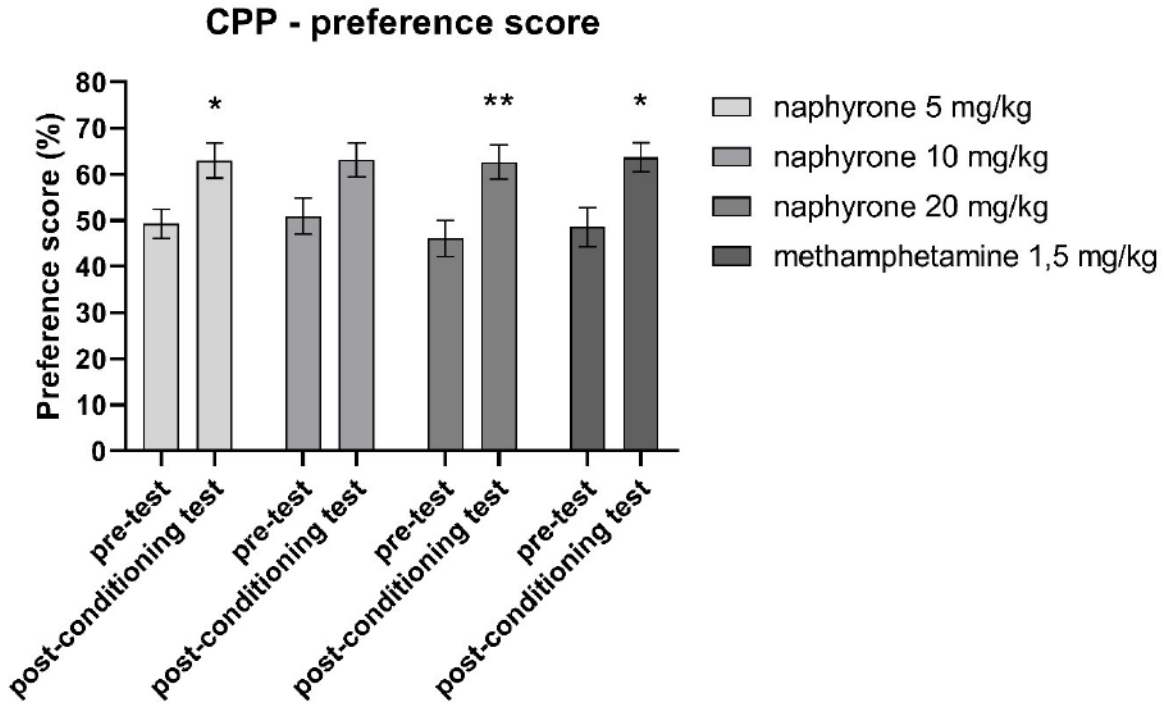


Fig. 1. The effects of naphyrone and methamphetamine on the conditioned place preference test in Wistar rats. Bars represent the mean ± SEM of the CPP score. $n = 11$ for methamphetamine group, 13 for naphyrone groups. Significant within-group changes from pre-test to post-conditioning test are denoted by asterisk (*$p < 0.05$, **$p < 0.01$)

- **Discussion**

Our study was designed to evaluate abuse potential of naphyrone in CPP test using animal model (male Wistar rats) and compare its effect with an active comparator - methamphetamine. As shown before, doses of 5 mg/kg and 20 mg/kg significantly increased CPP score (i.e. percentage of time spent in drug paired compartment), dose of 10 mg/kg was slightly above the significance level (p=0,05977). This results suggest naphyrone's ability to produce rewarding effect and can thereby be a risk of abuse even in recreational context. Other substances with strong dopaminergic effect have been acknowledged to produce conditioned place preference. Other cathinones produce reward and reinforcement as well - methylone, mephedrone and MDPV produced conditioned place preference (Lisek, Xu et al. 2012, Karlsson, Andersson et al. 2014) and mephedrone and MDPV were also self-administered by rodents (Aarde, Huang et al. 2013, Creehan, Vandewater et al. 2015) . *α*-PBP and *α*-PVP produced conditioned place preference, but 4'-MePPP failed to produce significant effects. The dose-effect curves for *α*-PBP and *α*-PVP were both inverted *U*-shaped functions, with the low and high doses not producing conditioned place preference (Gatch, Dolan et al. 2015)

- **Conflict of interest**

No conflict declared.

- **Acknowledgements**

This work was supported by projects GAUK 882217, VI20172020056, GACR no.: 18-16218S and 20-25349S, AZV 17-31852A, MH CZ—DRO (NIMH-CZ, 00023752), grant LO1611 from the MEYS CR under the NPU I program, PROGRES Q35 and 260533/SVV/2020.

- **References**

Aarde, S. M., P. K. Huang, K. M. Creehan, T. J. Dickerson and M. A. Taffe (2013). "The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: Self-administration and locomotor activity in rats." Neuropharmacology **71**: 130-140.

Brandt, S. D., R. C. R. Wootton, G. De Paoli and S. Freeman (2010). "The Naphyrone Story: The Alpha or Beta-naphthyl Isomer?" Drug Testing and Analysis **2**(9-10): 496-502.

Brenneisen, R., H. U. Fisch, U. Koelbing, S. Geisshusler and P. Kalix (1990). "Amphetamine-Like Effects in Humans of the Khat Alkaloid Cathinone." British Journal of Clinical Pharmacology **30**(6): 825-828.

Contrucci, R. R., T. M. Brunt, F. Inan, E. J. F. Franssen and L. Hondebrink (2020). "Synthetic Cathinones and Their Potential Interactions with Prescription Drugs." Therapeutic Drug Monitoring **42**(1): 75-82.

Corazza, O., F. Schifano, M. Farre, P. Deluca, Z. Davey, M. Torrens, Z. Demetrovics, L. Di Furia, L. Flesland, H. Siemann, A. Skutle, P. Van Der Kreeft and N. Scherbaum (2011). "Designer drugs on the internet: a phenomenon out-of-control? the emergence of hallucinogenic drug Bromo-Dragonfly." Curr Clin Pharmacol **6**(2): 125-129.

Creehan, K. M., S. A. Vandewater and M. A. Taffe (2015). "Intravenous self-administration of mephedrone, methylone and MDMA in female rats." Neuropharmacology **92**: 90-97.

Gatch, M. B., S. B. Dolan and M. J. Forster (2015). "Comparative Behavioral Pharmacology of Three Pyrrolidine-Containing Synthetic Cathinone Derivatives." Journal of Pharmacology and Experimental Therapeutics **354**(2): 103-110.

Karlsson, L., M. Andersson, R. Kronstrand and F. C. Kugelberg (2014). "Mephedrone, methylone and 3,4-methylenedioxypyrovalerone (MDPV) induce conditioned place preference in mice." Basic Clin Pharmacol Toxicol **115**(5): 411-416.

Liechti, M. (2015). "Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling." Swiss Med Wkly **145**: w14043.

Lisek, R., W. Xu, E. Yuvasheva, Y. T. Chiu, A. B. Reitz, L. Y. Liu-Chen and S. M. Rawls (2012). "Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation." Drug Alcohol Depend **126**(1-2): 257-262.

Meltzer, P. C., D. Butler, J. R. Deschamps and B. K. Madras (2006). "1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (Pyrovalerone) analogues: a promising class of monoamine uptake inhibitors." J Med Chem **49**(4): 1420-1432.

Pinterova-Leca, N., R. R. Horsley, H. Danda, M. Aidkova, E. Lhotkova, K. Sichova, K. Stefkova, M. Balikova, M. Kuchar and T. Palenicek (2020). "Naphyrone (naphthylpyrovalerone): Pharmacokinetics, behavioural effects and thermoregulation in Wistar rats." Addiction Biology.

Rosenbaum, C. D., S. P. Carreiro and K. M. Babu (2012). "Here today, gone tomorrow...and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, Salvia divinorum, methoxetamine, and piperazines." J Med Toxicol **8**(1): 15-32.

Shimosato, K. and S. Ohkuma (2000). "Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine." Pharmacol Biochem Behav **66**(2): 285-292.
Simmler, L. D., T. A. Buser, M. Donzelli, Y. Schramm, L. H. Dieu, J. Huwyler, S. Chaboz, M. C. Hoener and M. E. Liechti (2013). "Pharmacological characterization of designer cathinones in vitro." Br J Pharmacol **168**(2): 458-470.

Valente, M. J., P. Guedes de Pinho, M. de Lourdes Bastos, F. Carvalho and M. Carvalho (2014). "Khat and synthetic cathinones: a review." Arch Toxicol **88**(1): 15-45.

Pharmacokinetic, pharmacodynamic, and behavioural studies of dissociative anaesthetic deschloroketamine (DCK) in Wistar rats.

Kristýna Štefková-Mazochová[1*], Hynek Danda[1,2], Wim Dehaen[1,7], Bronislav Jurásek[1,3], Klára Šíchová[1], Nikola Pinterová-Leca[1,2], Vladimír Mazoch[1], Barbora Hrčka Krausová[4], Bohdan Kysilov[4], Tereza Smejkalová[4], Ladislav Vyklický[4], Michal Kohout[5], Kateřina Hájková[3,6], Daniel Svozil[7,8], Rachel R. Horsley[1], Martin Kuchař[1,3*], Tomáš Páleníček[1,2*]

[1] Department of Experimental Neurobiology, National Institute of Mental Health, Topolová 748, Klecany, 250 67, Czech Republic

[2] 3rd Faculty of Medicine, Charles University, Ruská 87, Prague 10, 100 00, Czech Republic

[3] Forensic Laboratory of Biologically Active Compounds, Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Technická 5, Prague 6, 166 28, Czech Republic

[4] Department of Cellular Neurophysiology, Institute of Physiology, CAS., Vídeňská 1083, Prague 4, 142 20, Czech Republic

[5] Department of Organic Chemistry, University of Chemistry and Technology, Technická 5, Prague 6, 166 28, Czech Republic

[6] Department of Analytical Chemistry, University of Chemistry and Technology, Technická 5, Prague 6, 166 28, Czech Republic

[7] CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Department of Informatics and Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology, Technická 5, Prague 6, 166 28, Czech Republic

[8] CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics, CAS, Vídeňská 1083, Prague 4, 142 20, Czech Republic

***Correspondence:**

- *Kristýna Štefková-Mazochová*
  Tel. (+420) 283 088 212
  E-mail: kristyna.stefkova@nudz.cz
- *Tomáš Páleníček*
  Tel. (+420) 283 088 454
  E-mail: tomas.palenicek@nudz.cz
- *Martin Kuchař*
  Tel. (+420) 283 088 212
  E-mail: martin.kuchar@vscht.cz

Abstract

Deschloroketamine (DCK), a structural analogue of ketamine, is recently present on illicit drug market as a recreational drug with modestly longer duration of effects. Despite the fact being used widely by recreational users, no systematic research on its effects *in vivo* has been performed.

In order to characterize its acute effects and addictive potential series of behavioural tests (open field test, prepulse inhibition of acoustic startle reaction (PPI), condition place preference (CPP)) have been performed along with pharmacokinetics and activity at human *N*-methyl-*D*-aspartate (NMDA) receptors. In addition, effects of the *S*-DCK and *R*-DCK enantiomers were also studied.

DCK rapidly crosses blood brain barrier, with maximum brain levels achieved 30 min and remaining high at 2 hrs after administration. Its antagonist activity at NMDA receptors is comparable to ketamine with *S*-DCK being more potent. DCK in all doses (5, 10 and 30 mg/kg s.c.) had robust stimulatory effects on locomotion including the highest dose used, induced some stereotyped like behaviour and robustly disrupted PPI. Locomotor stimulant effects tended to disappear more quickly than disruptive effects on PPI. *S*-DCK had more pronounced stimulatory properties then its counterpart; however the potency in disrupting PPI was comparable in both enantiomers. Interestingly, the *R*-DCK seems to penetrate into the brain more readily. DCK also exhibited dose independent rewarding properties in the CPP test.

In conclusion, DCK shows similar behavioural and addictive profile and pharmacodynamics to ketamine, with *S*-DCK being in general more active. It has slightly slower pharmacokinetic profile than ketamine, which is consistent with its reported longer duration of effects.

1 Introduction

Deschloroketamine (2-methylamino-2-phenylcyclohexanone, DCK, Fig. 1a) also known as DXE, or 2'-Oxo-PCM and O-PCM, is an analogue of ketamine (Fig. 1b), functioning as a dissociative anaesthetic. DCK is categorised as an arylcyclohexylamine, together with phencyclidine (PCP, Fig. 1c) and methoxetamine (MXE, Fig. 1d) [1]. Based on the structure-activity relationship for ketamine, its most probable activity is an antagonism of *N*-methyl-*D*-aspartate (NMDA) receptors [2]. DCK was originally patented for treating bacterial, fungal, viral, and protozoan infections, and for immunomodulation, and was believed to have a wide therapeutic spectrum [3]. DCK has a recent history of human use as a "designer drug" easily obtainable *via* the Internet and is used recreationally for its dissociative and hallucinogenic effects. The effects on humans are described only in user reports published on drug related servers and chat rooms (e.g. PsychonautWiki, Erowid, Bluelight) and comprise of a sense of subjective separation (dissociation) from the physical body and the external world, internal hallucinations, as well as euphoria, enhanced mood and empathy. Reported adverse effects included nausea, tachycardia, immobility, confusion, amnesia, and respiratory distress. According to users, the oral dose of DCK for recreational purposes is 10 – 50 mg with a duration of four to six hours. Since the dose range reported to be used by humans is slightly lower than with ketamine and MXE (indicating that it is more potent), we expected a similar trend for DCK in behavioural experiments in rats.

Both previously tested drugs, ketamine and MXE, as well as other NMDA receptor antagonists have shown dose-dependent effects on locomotion with a characteristic inverted U-shaped curve (an increase followed by a decrease) and potently disrupted prepulse inhibition (PPI) [4-6]. However, no data about pharmacological properties, metabolism, or behavioural effects of DCK either in animals or in humans have been published so far. Hence, our primary objective was to describe in detail the pharmacology, behavioural pharmacology/effects and pharmacokinetics of DCK. As the two *R*-(−) and *S*-(+) ketamine enantiomers have a different potency, pharmacology, and pharmacokinetics [7-11], we also focused on a comparison of the two DCK enantiomers. In behavioural experiments, we focused on locomotor activity, exploration in the open field test (OFT), and on the effects on sensorimotor gating in the test of PPI of the acoustic startle response (ASR), which is indicative of psychotomimetic-like potential [12].

Evidence supports the addictive potential of ketamine [13] and MXE [14]; therefore, we also determined the rewarding or aversive effects of DCK in a conditioned place preference (CPP) task.

Furthermore, we also evaluated the effect of DCK enantiomers on NMDA receptor function and on network activity in primary hippocampal cultures, with a direct comparison to ketamine.

*****Figure 1*****

2 Materials and methods

2.1 Drugs and chemicals

Deschloroketamine hydrochloride was synthesised in the Forensic Laboratory of Biologically Active Substances (University of Chemistry and Technology, Prague, Czech Republic) and characterised by ^{1}H and ^{13}C NMR spectroscopy [15]. DCK also served as a reference standard for pharmacokinetic analyses using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). DCK was subjected to chiral separation using a chiral amylose-based column. The enantiopurity of both enantiomers was verified (>99 % for both enantiomers) by the LC-UV system in the analytical mode. The absolute configuration was determined using a combination of *ab initio* calculation with circular dichroism spectra comparison and single crystal data [15].

Nordeschloroketamine (norDCK, Fig. 2a), *trans*-dihydrodeschloroketamine (*trans*-dihydroDCK, Fig. 2b), *cis*-dihydronordeschloroketamine (*cis*-dihydronorDCK, Fig. 2c), *trans*-dihydronordeschloroketamine (*trans*-dihydronorDCK, Fig. 2d), and *trans*-dihydrodeschloroketamine-*d4* (*trans*-dihydroDCK-*d4*, Fig. 2e) were synthesised in the Forensic Laboratory of Biologically Active Substances (University of Chemistry and Technology Prague, Czech Republic). All of the prepared standards were stabilised in the form of hydrochloride salt and characterised by HRMS, ^{1}H and ^{13}C NMR spectroscopy. The internal standard for quantitative LC/MS assays was deuterated *trans*-dihydroDCK-*d4* [16].

Methanol, acetonitrile and formic acid (all LC-MS grade) were purchased from Sigma-Aldrich (Czech Republic). Ultrapure water (18.2 $M\Omega cm^{-1}$) was produced by a PureLab Ultra system (Elga, UK). Sodium sulphate and ammonium bicarbonate were obtained from Sigma-

Aldrich and sodium chloride from Lach-ner (Czech Republic). Homogenisation beads, SiLibeads type ZY 0.4-0.6 mm (Ginzel, Czech Republic), facilitated tissue homogenisation.

Figure 2

2.2 Measurement of the activity at NMDA receptors

Cell cultures

Human embryonic kidney 293 (HEK293) cells were plated at a density of 10^5 cells/cm^2 and cultured in Opti-MEM® I (Thermo Fischer Scientific, Waltham, USA) supplemented with 5% foetal bovine serum (FTS; PAN Biotech, Aidenbach, Germany) at 37°C in 5% CO_2. After 24 hrs, the HEK293 cells were transiently transfected with cDNAs encoding the human wild-type hGluN1-1a and hGluN2A or hGluN2B subunits and green fluorescent protein (GFP) using Matra-A Reagent (IBA, Goettingen, Germany). Equal amounts (200 μg) of cDNAs encoding for hGluN1, hGluN2A or hGluN2B subunits, and GFP were used. After trypsinisation, the transfected cells were resuspended in Opti-MEM® I containing 1% FTS supplemented with 20 mM $MgCl_2$, 3 mM kynurenic acid, and 1 mM *D,L*-2-amino-5-phosphonopentanoic acid to prevent excitotoxicity.

Primary hippocampal neurons.

Hippocampi were dissected from newborn (P0-P1) male Wistar rat pups. Cells were dissociated in papain, washed and plated in a Neurobasal A medium supplemented with B27 (Gibco) at a density of 50,000 cells/cm^2 on top of a confluent layer of cortical astrocytes grown on collagen/poly-*D*-lysine-covered glass coverslips.

Electrophysiology

Whole-cell voltage-clamp recordings of the HEK293 cells were made 24–48 hrs after transfection with a patch-clamp amplifier (Axopatch 200B; Axon Instruments. Inc., Foster City, CA) after compensation of capacitance and serial resistance (<10 MΩ) by 80-90%. Unless otherwise stated, the recordings were made at a holding potential of -60 mV. Whole-cell current responses were low-pass filtered at 2 kHz, digitally sampled at 5 kHz, and analysed using pClamp software version 10.7 (Molecular Devices). Borosilicate glass patch pipettes (3–5 MΩ) were filled with an intracellular solution (ICS) containing (in mM): 120 gluconic acid, 10 BAPTA, 10 HEPES, 15 CsCl, 1 $CaCl_2$, 3 $MgCl_2$, and 2 ATP-Mg salt (pH-adjusted to 7.2 with CsOH). The extracellular solution (ECS) contained (in mM): 160 NaCl,

2.5 KCl, 10 glucose, 10 HEPES, 0.7 $CaCl_2$, and 0.2 EDTA (pH-adjusted to 7.3 with NaOH). A microprocessor-controlled multibarrel fast-perfusion system (with a time constant for solution exchange of ~10 ms) was used for the application of test and control solutions [17]. Action potentials were recorded from cultured hippocampal neurons at DIV 11-13 in the current-clamp mode with ICS containing (in mM): 125 gluconic acid, 15 KCl, 5 EGTA, 10 HEPES, 0.5 $CaCl_2$, 2 ATP-Mg salt, 0.3 GTP-Na salt, and 10 creatine phosphate (pH adjusted to 7.2 with KOH). ECS contained (in mM): 160 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, 2 $CaCl_2$, 1 $MgCl_2$, and 0.03 glycine (pH-adjusted to 7.3 with NaOH). Compound stock solutions were prepared fresh before each experiment and measurements were performed at room temperature (21–25°C) [18].

Data analysis of measurement of the activity at NMDA receptors

The concentration-response relationship for the relative inhibitory effect (I) of compounds at recombinant human NMDA receptors was determined by fitting the normalised data to the following logistic equation:

$$I = 1/(1 + ([\text{Compound}]/IC_{50})^{h}) \qquad \textit{Equation 1}$$

where [Compound] is the compound concentration, IC_{50} is the concentration producing half-maximal inhibition, and h is the apparent Hill coefficient. The calculations were made assuming full inhibition at the saturating compound concentration and statistical comparisons were performed for $logIC_{50}$ and $logHill$ values.

The I–V relationship for the inhibitory effect of the compounds was evaluated by fitting the data to the following Boltzmann function equation:

$$I(V) = a.g_0(V - V_{rev})/(a + [\text{Compound}]e^{bV}) \qquad \textit{Equation 2}$$

where g_0 is the estimated conductance of the NMDA receptor whole-cell response in the absence of the compound, V_{rev} is the reversal potential of NMDA receptor current, and a, b are parameters with the following interpretation:

$$a = K_d e^{bV} \qquad \textit{Equation 3}$$

where K_d represents the apparent dissociation constant for a compound binding to the NMDA receptor at a given membrane potential (V) and:

$$b = \delta F/(RT) \qquad \textit{Equation 4}$$

where δ indicates the apparent electrical distance of the compound binding site from the outside of the membrane, and F, R and T have their standard thermodynamic meanings [19]. The currents were recorded at different holding potentials changing in 20-mV steps within the range of -80 to +40 mV (values of the holding potential were corrected for the liquid junction potential of -14 mV).

The data are presented as a mean ± SEM with n corresponding to the number of independent measurements. Statistical comparisons among treatment groups were performed using either Student's t-test or One-way ANOVA followed by all pairwise Student-Newman-Keuls or Bonfferoni post-hoc tests ($p < 0.05$ or $p < 0.001$ were used to determine the significance).

2.3 Pharmacokinetic analyses

For the pharmacokinetic study, the animals were decapitated after 0.5, 1, 2, 4, 8 or 24 hrs after a single bolus of racemic DCK, and 1, 2 or 4 hrs after a single bolus of either enantiomer. Serum was obtained by enabling the blood to clot in a refrigerator for 1 hr. The clotted blood was centrifuged for 10 min at 1500g, 10°C, and the serum was then collected. Concentration levels of DCK and its metabolites in the sera and dissected brain tissue were calculated as ng/ml or ng/g, respectively.

Determination of DCK and its metabolite levels in serum and brain samples using LC-MS/MS

Serum and brain tissue samples were collected and stored at -20°C. These samples were analysed using optimised and validated LC/MS methods, according to the 2001 FDA Guidance, and Standard Practices for Method Validation in Forensic Toxicology (Scientific Working Group for Forensic Toxicology; SWGTOX) [16]. DCK, *trans*-dihydroDCK, norDCK, *cis*-dihydronorDCK, and *trans*-dihydronorDCK were quantified using an external matrix-matched calibration and deuterated internal standard *trans*-dihydroDCK-d_4.

Serum sample preparation consisted in protein precipitation and was performed as follows: 1) 800 µl of 0.1% solution of formic acid in acetonitrile (v/v) was cooled for 10 min at -20°C; 2) 200 µl of serum was added to the cooled solution and immediately mixed in a Bullet Blender

Storm homogeniser (Next Advance, USA) for 5 min; 3) centrifugation for 10 min (14,000 RPM) at 5°C; 4) evaporation of 800 µl of supernatant to dryness (Centrivap Concentrator); and 5) reconstitution with 0.1% formic acid in water/acetonitrile, 80/20 (v/v).

Brain tissue sample preparation followed the protocol published in [16]. Briefly: 1) 100 mg of brain tissue with 200 mg of ZY SiLibeads was homogenised in a BulletBlender homogeniser (5 min) with 200 µl of 10 mmol/l /mM NH_4HCO_3 and 100 µl of acetonitrile; 2) the mixture was cooled (10 min, -20°C); 3) 300 µl of fresh acetonitrile was added and the mixture was homogenised in the BulletBlender homogeniser (5 min); 4) the mixture was subsequently shaken with 20 mg of NaCl and 100 mg of Na_2SO_4 and again mixed in the BulletBlender homogeniser for 5 min and then centrifuged (5 min at 18,700 RPM/ *g*); 5) 250 µl of supernatant was evaporated to dryness (Centrivap Concentrator); and 6) subsequently reconstituted in 10 mM ammonium acetate in methanol/water, 10/90 (v/v).

LC/MS conditions

The analyses were performed using an Infinity 1290 – 6460 Triple Quad LC/MS (Agilent Technologies, USA) equipped with an Agilent Jet Stream electrospray ionisation (ESI) source. The chromatographic separation of serum and tissue samples was performed using a ZORBAX Eclipse Plus C18 (50 × 2.1 mm, 1.8 µm) with a pre-column at flow rate of 0.3 ml/min, and gradient elution with 10 mM ammonium acetate (A) and 10 mM ammonium acetate in methanol (B). The gradient profile was: 0-0.5 min, from 90% A to 70% A; 0.5-5 min, from 70% A to 65% A; 5-5.5 min, from 65% A to 0 % A; 5.5-6 min hold at 0% A; 6-6.8 min back to 90% A; and equilibration to 9 min. Data were acquired in a positive ESI mode by a multiple reaction monitoring (MRM) method using MassHunter software (Agilent Technologies, USA).

Pharmacokinetic measurement

The pharmacokinetic measurements, the concentrations in a function of time [t], were fitted to a scaled gamma distribution function:

$$[\boldsymbol{conct}](\boldsymbol{t}) = \boldsymbol{a} \times \boldsymbol{t}^{\boldsymbol{c-1}} \times \boldsymbol{e}^{-\boldsymbol{b}\times\boldsymbol{t}} \qquad \textit{Equation 5}$$

With parameters a, b, c > 0. As the distribution is not normalised (we are interested in absolute values in the present study), the parameter *a* is the amplitude constant. The parameter *b* is the rate parameter and the parameter *c* is the shape parameter. This allows for a flexible range of distribution patterns and a good estimation of half-life [20]. The fitting and plotting of the data were performed using gnuplot [www.gnuplot.info].

2.4 Behavioural experiments

Animals

All animals were male outbred Wistar rats acquired from VELAZ (Prague, Czech Republic) weighing between 200-275 g at the beginning of testing. The rats were housed two *per* cage under a controlled temperature (22 ± 2°C) and humidity (30 - 70%) with food pellets and water *ad libitum*. Lights were on from 6:00 to 18:00 and all of the experiments were performed between 7:00 and 13:00, except CPP, where testing lasted until 16:00. The animals were habituated 7–10 days to laboratory conditions before being used in the experiments; during this period they were weighed at least twice and handled four times. All of the behavioural experiments were conducted under the same standard temperature and humidity conditions as in the animal housing facility. Each experimental group for behavioural testing included 10 animals, except CPP, where 12 animals *per* group were used. Each animal was tested only once. After completion of behavioural testing the rats were subsequently used for pharmacokinetic sampling, i.e. collection of brain and serum, $n = 7$ *per* time point.
All of the procedures were conducted in accordance with the principles of laboratory animal care of the National Committee for the Care and Use of Laboratory Animals (Czech Republic), and according to Guidelines of the European Union (86/609/EU). The protocol was approved by the National Committee for the Care and Use of Laboratory Animals (Czech Republic) under the number: 69371/2015-MZE-17214.

Dosage

The DCK doses used in the present study were selected according to our previous studies with ketamine and MXE [4, 5] and adjusted in accordance with user reports on potency. For the behavioural experiments, the treatment range for racemic DCK was set to 5, 10 and 30 mg/kg, and for *R*- and *S*-enantiomers we used 10 mg/kg. The amount of 10 mg/kg of DCK, *S*-DCK,

and *R*-DCK was also used in the pharmacokinetic sampling. The drugs were dissolved in physiological saline (0.9% NaCl) in a volume of 2 ml/kg and administered subcutaneously (s.c., as a single bolus, for comparability with previous studies) in all cases. Vehicle (VEH) control animals were treated with an equivalent volume of physiological saline.

Study design

In order to cover a wider temporal window of drug effects and to exclude the influence of habituation on the testing procedures, the rats were tested independently in two temporal administration schemes. The first scheme consisted of groups of animals that were injected with the tested substances and/or VEH s.c. 15 min before testing, and the second scheme consisted of different groups treated 60 min before testing.

Open field test (OFT)

OFT was conducted in a sound-proof and evenly-lit chamber with low levels of light intensity. The OFT apparatus comprised a black square plastic arena (80 x 80 cm) with walls (40 cm high). At the beginning of each test, each rat was placed individually into the centre of the arena and allowed to move freely within the arena for 30 min. The apparatus was cleaned with 50% ethanol solution after each test. Raw behavioural data were registered and pre-processed using automatic video tracking software (EthoVision XT v. 11.5, Noldus, Netherlands).

For the evaluation of the time spent in the centre (T_{centre}) and thigmotaxis (i.e., likelihood of appearance in the periphery), the arena was virtually divided into a 5 x 5 grid of identical square zones with 16 being located on the periphery and nine centrally. Time spent in the centre of the arena is the sum of time spent in the nine central zones ($T_{centre}=\sum time_{1\text{-}9}$). Thigmotaxis indicates the probability of appearances in the peripheral zones (*f*; the total number of appearances of the animal in each zone) and is calculated as:

$$f = \sum f_{peripheral\ zones} / \sum f_{all\ zones} \qquad \textit{Equation 6}$$

Prepulse inhibition (PPI) of acoustic startle response (ASR)

PPI took place in startle chambers (SR-LAB, San Diego Instruments, California, USA), each containing a soundproof and evenly-lit enclosure, a high-frequency loudspeaker (producing a background noise at 75 dB and all acoustic stimuli), and Plexiglas stabilimeter (8.7 cm inner diameter). A piezoelectric accelerometer detected amplitudes of the ASR, which were

digitised for subsequent analysis. Two days before the behavioural testing, drug-free rats were habituated to the startle boxes in/for a 5 min session with five presentations of a pulse-alone stimulus (125 dB/40 ms) over background white noise (75 dB). ASR data from this habituation session were not included in the further analyses.

The PPI measurement itself was designed in accordance with our previous studies [21-25]. On the test day, the PPI test started with an acclimatisation period lasting 5 min in the startle chamber in which a 75 dB background white noise was continuously presented. The PPI test followed with 72 trials with an inter-trial interval (ITI) of 15–30 s (mean ITI: 22.5 s). Six 125 dB/40 ms-duration pulse-alone trials were then delivered to establish baseline ASR. Following this, 60 trials of the following scheme were presented in a pseudorandom order: (A) pulse alone: 40 ms 125 dB; (B) prepulse alone: 20 ms 83 dB or 91 dB; (C) prepulse-pulse: 20 ms 83 dB or 91 dB prepulse, a variable (30, 60 or 120 ms) inter-stimulus interval (ISI, mean ISI: 70 ms), then 40 ms 125 dB pulse; (D) 60 ms no stimulus. Finally, six pulse-alone trials were delivered. Habituation was calculated by the percentage reduction in ASR from the initial six baseline trials, to the final six trials. The PPI was calculated as:

$$PPI = (100 - \left(\frac{\textbf{mean prepulse−pulse trials}}{\textbf{mean pulse alone trials}}\right) * 100 \qquad \textit{Equation 7}$$

Conditioned place preference (CPP)

A three-compartment plastic CPP chamber, which consisted of two equally-sized conditioning compartments (45 × 50 cm) and a neutral compartment in between (35 × 20 cm), was used. One of the conditioning compartments had striped walls, the other had chequered walls, and the floors were made from black plastic each with a different pattern (stripes and circles). The space was deformed by plastic boxes that were inserted into each corner of each conditioning compartment. The neutral compartment had transparent walls and floor. Each compartment was separated from the other one by a guillotine-style door and each door was operated manually by the experimenter. Time spent in each compartment was registered and pre-processed by an automatic video tracking system (EthoVision XT v. 11.5, Noldus, Netherlands).

The CPP experiment lasted 11 days and consisted of four phases: (i) habituation; (ii) pre-conditioning; (iii) conditioning; (iv) post-conditioning. During habituation and pre-conditioning (days 1 and 2), the rats received VEH immediately before being placed individually into the neutral compartment. The doors were opened and they were allowed to

freely explore all three chambers of the CPP apparatus for 15 min. In the pre-conditioning, a baseline chamber preference was determined. Rats that spent over 80% of the total time in one of the chambers were considered to have an initial bias and were removed from the experiment [26]. Conditioning was conducted for eight subsequent days (days 3-10). We used an unbiased design, which means that regardless of the initial preference, the rats were pseudorandomly distributed into the drug treatment groups, and drug treatment was counterbalanced across the compartments. In the conditioning session, the doors were closed and immediately after the drug injection, the rats were confined in the paired compartment for 30 min. DCK and VEH were paired with a specific compartment on alternate days for a total of eight sessions (four with DCK-paired and four with VEH-paired compartment). The last phase of the experiment was post-conditioning (day 11), when all of the rats received a VEH injection and a 15-min test was performed in the same way as the pre-conditioning baseline test. The preference score was calculated as:

$$Preference\ score = \frac{\text{time spent in each compartment}}{\text{total time spent in both compartments}} \qquad \textit{Equation 8}$$

Design and statistical analysis of behavioural experiments

All of the statistical behavioural analyses were conducted using IBM SPSS version 22. For the behavioural tests, factorial designs were constructed and analysed with an ANOVA test. ANOVAs with significant main effects and interactions were followed with pairwise comparisons using independent *t*-tests. In the case of ANOVAs for repeated measurements where Mauchly's test of sphericity was significant, Greenhouse-Geisser (Greenhouse-Geisser estimate of sphericity (ε) < 0.75 or Huynh-Feldt (ε) > 0.75) correction(s) is/are reported. Degrees of freedom were rounded to whole numbers for presentation purposes. For independent *t*-tests where Levene's test for equality of variances was significant, statistics corrected for unequal variances are given. The value of $p < 0.05$ (two-tailed) was considered the minimal criterion for statistical significance. Enantiomers of DCK were not included in the ANOVAs (only one time of administration was tested, 15 min) and data were analysed using additional independent *t*-tests.

Locomotor activity in the OFT was evaluated using 4 × 2 × 6 mixed factorial ANOVAs with drug treatment (DCK at 5, 10, or 30 mg/kg versus VEH) and testing onset (15 or 60 min) as independent factors, and blocks (5-min intervals) as a repeated measurement factor. The spatial distribution of behaviour in the OFT (thigmotaxis and T_{centre}) and PPI parameters

(habituation and ASR) were each analysed with 4 × 2 independent ANOVAs with drug treatment (DCK at 5, 10, or 30 mg/kg versus VEH) and testing onset (15 or 60 min) as independent factors. In the case of significant main effects on habituation or ASR, the significant factor was used as a covariate in subsequent analysis of PPI (analysed with analysis of covariance (ANCOVA)). Data from CPP were analysed using 4 x 2 mixed factorial ANOVAs with drug treatment (DCK at 5, 10, or 30 mg/kg) as an independent factor and conditioning (pre/post-conditioning) as a repeated measurement factor.

3 Results

3.1 Activity at NMDA receptors

Recombinant NMDA receptors

Since ketamine acts as a voltage-dependent inhibitor of NMDA receptors [27] we decided to analyse the effect of *R*-DCK and *S*-DCK on recombinant hGluN1/hGluN2A and hGluN1/hGluN2B receptors expressed in HEK293 cells. Our results (Fig. 3a-d) indicate that the inhibitory effect of both *R*-DCK and *S*-DCK at human NMDA receptors is concentration-dependent. Concentration-response analysis showed that the *S*-DCK analogue is a more potent inhibitor of hGluN1/hGluN2B receptors than *R*-DCK analogue ($p = 0.03$; One-way ANOVA; Fig. 3d; Table 1). The results further indicate that the inhibitory effect of *S*-DCK is subunit dependent, showing a higher apparent affinity for hGluN1/hGluN2B over hGluN1/hGluN2A receptors ($p = 0.03$; Student's *t*-test). In accordance with previous measurements on rat GluN1/GluN2A-B receptors [28], the effect of ketamine on hGluN1/hGluN2A-B receptors showed a weak subunit selectivity ($p = 0.07$; Student's t-test). Similar to ketamine, the inhibitory effect of both *R*-DCK and *S*-DCK at human NMDA receptors is voltage-dependent (Fig. 3e-h). The analysis of the *I-V* relationship indicates that in the case of hGluN1/hGluN2A receptors (Fig. 3g; Table 2), *S*-DCK binds deeper within the ion channel than *R*-DCK ($p = 0.02$; One-way ANOVA). However, for hGluN1/hGluN2B receptors (Fig. 3h; Table 2), the apparent electrical distance of binding was not significantly different among the compounds ($p = 0.63$; One-way ANOVA).

*****Figure 3*****

*****Table1*****

Table2

Native NMDA receptors

Given the inhibitory activity of ketamine and the ketamine analogues at the NMDA receptor, we decided to examine the influence of these compounds on network activity using current-clamp recording in primary hippocampal cultures. We focused on the more potent ketamine analogue *S*-DCK in these experiments. The extracellular solution contained physiological concentrations of Ca^{2+} (2mM), Mg^{2+} (1mM) and glycine (30μM). As shown in Fig. 4, both ketamine and *S*-DCK at 0.3 μM consistently and robustly decreased action potential (AP) frequency; ketamine to 29 ± 10 % of control values ($p < 0.01$; Student's *t*-test) and *S*-DCK to 41 ± 9 % of control values ($p < 0.01$; Student's *t*-test). There was no difference in the degrees of AP frequency inhibition between the two compounds ($p = 0.45$; Student's *t*-test). For both drugs, the inhibition of network activity was only partially reversible in 2-4 min of washing, possibly because the solution exchange around the more distant cells in the network is relatively slow.

Figure 4

3.2 Pharmacokinetics of DCK in serum and brain tissue

After the administration of racemic DCK (10 mg/kg), the measured concentration peaked in the serum (593.1 ng/ml) and the brain (3329 ng/g) at 30 min and then rapidly decreased, with a half-life of ~0.45 and ~0.52 hr, respectively. The maximum brain levels were approximately 5-fold higher than in the serum (Fig. 5a,c).

Of the two enantiomers, *R*-DCK reached a higher maximal concentration in brain tissue, while the *S*-enantiomer penetrated less readily. At 1 hr, *S*-DCK had a serum concentration of 579.7 ng/ml, whereas *R*-DCK had a serum concentration of 468.1 ng/ml (Fig. 5b). This difference was statistically significant (Student's *t*-test, $p = 0.04$). In brain tissue, the concentration at 1 hr was 1801 ng/g for the *S*-enantiomer and 2164 ng/g for the *R*-enantiomer (Fig. 5d). Although the difference is suggestive, it is not statistically significant (Student's *t*-test, $p = 0.09$). Half-lives in the serum were ~0.41 hr for *R*-DCK and ~0.48 hr for *S*-DCK, and half-lives in brain tissue were ~ 0.46 hr for *R*-DCK and ~0.47 hr for *S*-DCK. The difference between the half-lives of the enantiomers were not statistically significant either in brain tissue or in the serum (Student's *t*-test, $p = 0.40$ and $p = 0.13$, respectively) (Fig. 5b,d).

Metabolites

Following the administration of racemic DCK (10 mg/kg), the major metabolite, norDCK (Fig. 6 compound A), was observed in high concentrations comparable to the parent substance with the maximum concentration of 477.3 ng/ml in the serum at 2 hrs, and 641.5 ng/g in the brain at 1 hr after administration (Fig. 5a,c).

When individual enantiomers were administered, the maximum serum concentrations were 705.3 ng/ml at 1 hr for *S*-norDCK and 845.2 ng/ml at 1 hr for *R*-norDCK (Fig. 5d). This difference was statistically significant (Student's *t*-test, $p = 0.03$). In brain tissue, the maximum concentration was 502.2 ng/g for *S*-norDCK at 1 hr and 944.2 ng/g for *R*-norDCK at 1 hr (Fig. 5d). This difference was statistically significant (Student's *t*-test, $p < 0.001$). The metabolites investigated (*trans*-dihydroDCK and dihydronorDCK) were present in lower quantities (see Supplementary material).

*****Figure 5*****

*****Figure 6*****

3.3 Behavioural experiments

Locomotor activity in the OFT

Mauchly's test of sphericity was significant (Mauchly's $W(14) = 0.18$, $p < 0.001$) and Greenhouse-Geisser corrections are presented for repeated measurements.

Analysis of locomotor activity revealed a significant main effect of drug treatment ($F_{(3, 72)} = 8.72$, $p < 0.001$), testing onset ($F_{(1, 72)} = 20.06$, $p < 0.001$) as well as blocks ($F_{(3, 210)} = 133.10$, $p < 0.001$). All of the interactions were significant, including the three-way drug × testing onset × blocks interaction (minimum, $F_{(9, 210)} = 8.77$, $p < 0.001$). In all of the tested groups, rats showed a normal pattern of locomotor habituation (a progressive decrease of locomotor activity over the session) except: **(i)** Rats treated with *S*-DCK at the 15-min testing onset displayed an apparent U-shape curve of locomotor activity (Fig. 7a). During the second and third intervals, locomotor activity slightly decreased compared to the initial interval; however, the trajectory in the first interval was not significantly longer than the trajectory in the second and third intervals (maximum, $t(18) = 1.49$, $p = 0.15$). From the third to the sixth interval, the locomotion started to progressively increase and the trajectory in the last interval was

437 significantly longer compared to the third interval (t (18) = 2.14, p = 0.05). (**ii**) In rats
438 administered with 30 mg/kg of racemic DCK at the 60-min testing onset, the activity was
439 stable over the course of the six blocks, which is shown as significantly lower activity in
440 block 1 (t (18) = 2.21, p = 0.04) and significantly higher activity in blocks 4 - 6 (minimum, t
441 (18) = 3.49, p = 0.001) compared to the control (Fig. 7b).
442 For the 15-min testing onset, all of the DCK-treated rats showed significantly elevated
443 locomotor activity in each block compared to the VEH-treated rats (minimum, t (18) = 2.51, p
444 = 0.03), except 10 mg/kg of DCK in block 2 (t (18) = 1.46, p = 0.16). *R*-DCK was statistically
445 indistinguishable from VEH in blocks 2, 4 - 6 (maximum, t (18) = 1.79, p = 0.1), marginally
446 increased in block 1 (t (10) = 1.96, p = 0.08) and significantly increased in block 3 (t (18) =
447 2.17, p = 0.04). *S*-DCK significantly increased locomotor activity in blocks 2 – 6 (minimum, t
448 (18) = 3.75, p = 0.001) and marginally in block 1 (t (10) =2.12, p = 0.06). Both enantiomers
449 showed significantly different activity levels at the end of test session compared to 10 mg/kg
450 of racemic DCK; *S*-DCK elevated locomotor activity in block 6 (t (18) =2.70, p = 0.02),
451 whereas *R*-DCK decreased locomotor activity in blocks 5 and 6 (minimum, t (18) =2.12, p =
452 0.05) (Fig 7a).
453 For the 60-min testing onset, 5 mg/kg DCK slightly reduced locomotor activity compared to
454 the vehicle, and significantly in blocks 1, 3, and 6 (minimum, t (18) = 2.38, p = 0.03).
455 Differences between 10 mg/kg DCK and the vehicle treated rats were non-significant for all
456 blocks (maximum, t (18) = 1.87, p = 0.08) (Fig. 7b).
457 The main effects of the drug treatment and the interaction of time of administration × drug
458 treatment were each significant for both T_{centre} (minimum, $F_{(3, 79)} = 5.67$, p = 0.001) and for
459 thigmotaxis (minimum, $F_{(3, 79)} = 3.42$, p = 0.02). Independent t-tests showed that for the 15-
460 min testing onset, 5 mg/kg DCK-treated rats spent significantly less time in the centre
461 compared to the vehicle (t (11) = 4.62, p = 0.001) (Fig. 7c). For the 60-min testing onset, all
462 of the doses of DCK differed significantly from the vehicle (minimum, t (18) = 1.56, p =
463 0.05), although the direction differed: 5 and 10 mg/kg treated rats spent less time in central
464 zones (minimum, t (18) = 5.60, p = 0.001), whereas 30 mg/kg treated rats spent more time in
465 the centre (t (18) = 1.56, p = 0.05) (Fig. 7d). Thigmotaxis was increased after 5 and 10 mg/kg
466 of DCK for both times of administration (minimum, t (18) = 1.93, p = 0.02) (Fig. 7e,f).
467 *R*-DCK (10 mg/kg) significantly decreased T_{centre} compared to the vehicle and 10 mg/kg DCK
468 (minimum, t (9) = 2.88, p = 0.02), whereas *S*-DCK had no effect on this parameter. Both
469 enantiomers (10 mg/kg) significantly increased thigmotaxis at the 15-min testing onset when

compared to the vehicle (minimum, t (18) = 3.23, p = 0.01). Comparison of the both enantiomers (10 mg/kg) to DCK 10 mg/kg showed a significant increase in thigmotaxis only in *R*-DCK (t (11) = 2.70, p = 0.02) (Fig. 7c,e).

Prepulse inhibition test

Habituation data showed a significant main effect of testing onset and drug treatment, (minimum, $F_{(3;\ 77)} = 9.27$, p = 0.001) but no interaction was observed. Independent t-test revealed reduced habituation both at the 60-min testing onset compared to 15-min (t (76) = 2.83, p = 0.01) and at all of the tested doses and for both enantiomers compared to the vehicle (minimum, t (17) = 2.35, p = 0.03). Baseline acoustic startle response was not affected by drug treatment, testing onset, or their interaction, (maximum, $F_{(3;\ 77)} = 1.59$, p = 0.2) (see Table 3 for full results). The analysis of PPI data showed a significant main effect of drug treatment on PPI ($F_{(5;\ 52)} = 8.36$, $p < 0.001$) manifested as reduced PPI at all doses compared to the vehicle (minimum, t (17) = 3.29, p = 0.001). The main effect of testing onset and drug treatment × testing onset interaction was not significant. Both enantiomers (10 mg/kg) compared with the vehicle significantly reduced PPI at the 15-min testing onset (minimum, t (18) = 3.85, p = 0.001). When enantiomers were compared to DCK 10 mg/kg there were no significant differences (Fig. 7g,h).

*****Table 3*****

Conditioned place preference test

The main effect of pre/post-condition was significant ($F_{(1,\ 31)} = 12.81$, $p < 0.001$), which is indicative of an overall increased place preference induced by DCK treatment, but this was seen irrespective of the dose and the dose × pre/post-condition interaction, maximum ($F_{(1,\ 31)} = 2.48$, p = 0.1).

*****Figure 7*****

4 Discussion

The main findings of the present study were as follows: **(i)** DCK had fast pharmacokinetics with maximal brain and serum levels at 30 min after administration, the maximum brain concentrations were approximately 5-fold higher than in the serum **(ii)** *S*-DCK was a more potent inhibitor of hGluN1/hGluN2B receptors than *R*-DCK; however, the latter more readily

499 penetrated the BBB and more rapidly metabolised to *R*-norDCK. (**iii**) DCK showed a
500 pronounced stimulant effect at 15 min after administration, which diminished when tested 1 h
501 after administration; (**iv**) DCK has a strong potency to disrupt PPI; (**v**) in the CPP test, DCK
502 demonstrated rewarding properties.

503 **4.1 Activity at NMDA receptors**

504 Just like ketamine or any other dissociative anaesthetic, both DCK enantiomers inhibited the
505 activity of NMDA receptors. NMDA receptor inhibition is thought to be mainly responsible
506 for the anaesthetic effect of ketamine [29]. The IC_{50} values for ketamine and DCK
507 enantiomers were of a similar range (see Table 1 for full results/detailed analysis). A
508 difference in activity was observed between the *R* and *S* enantiomers, were the *S* enantiomer
509 was more potent at hGluN1/hGluN2B receptors. Some degree of preference was observed for
510 hGluN1/hGluN2B versus hGluN1/hGluN2A receptors in the case of *S*-DCK – a difference
511 that is also observed for ketamine. Furthermore, the measured IC_{50} values for ketamine are
512 consistent with those reported earlier using FLIPR/Ca^{2+} assay, where a similar preference for
513 hGluN1/hGluN2B over hGluN1/hGluN2A receptors was also observed [30]. When
514 comparing K_d values between enantiomers, the apparent binding affinity of the *S* enantiomer
515 for hGluN1/hGluN2B receptors was slightly increased, following the trend seen for the IC_{50}
516 values. Moreover, the effect of ketamine and *S*-DCK on network activity in cultured neurons
517 are consistent with potent inhibition of native NMDA receptors, in accordance with the results
518 observed for recombinant NMDA receptors expressed in HEK293 cells. These results
519 generally support the differences between the two enantiomers observed in the behavioural
520 experiments.

521 **4.2 Pharmacokinetics**

522 Compared to ketamine, a very similar relative distribution between brain tissue and serum
523 concentration was observed for racemic DCK at peak (brain/serum concentration ratio at 30
524 min of 5.61 versus 5.11 for ketamine) [31]. This suggests good BBB penetration of DCK,
525 owing to its lipophilic nature. A comparison of enantiomers revealed that 1 h after
526 administration (where the peak levels are not to be expected) the *R* enantiomer had a higher
527 brain/serum concentration ratio than the *S* enantiomer (4.68 versus 3.11, respectively) which

is the opposite trend observed for the respective ketamine enantiomers (*R* 4.77 and *S* 5.54) [31]. For comparison the racemic DCK at 1 h after administration had brain/serum ratio 5.45. The primary metabolite, norDCK, rapidly reached its maximum concentration and had a half-life of 1.89 hrs in the serum and 1.01 hrs in brain tissue, which suggests this metabolite is more persistent than DCK (0.45 hr for serum, 0.52 hr for brain tissue). The relative concentration in brain tissue compared to the serum was significantly lower than in the parent compound (1.34 versus 5.61), which may be explained by the lower lipophilicity of the *N*-demethylated compound. When the two enantiomers of norDCK were compared, the brain/serum ratio of *R*-norDCK was found to be significantly higher than that of *S*-norDCK (1.12 versus 0.71), following the same trend observed in *R*-DCK versus *S*-DCK. Interestingly, some of the minor metabolites showed higher brain/serum concentration ratios than the parent compound: 7.28 for *trans*-dihydroDCK, 6.7 for *cis*-dihydronorDCK, and 7.09 for *trans*-dihydronorDCK.

4.3 Behavioural experiments

Locomotor activity in the OFT

The overall stimulatory effects of DCK (at all of the tested doses) were more pronounced at the 15-min testing onset, which corresponds to the highest brain concentrations of the drug. *S*-DCK has almost double the potency of *R*-DCK. The stimulatory effects of 5 and 10 mg/kg diminished rapidly over time and were almost absent 60 min after administration; however, at 30 mg/kg they still persisted after one hour, which may be indicative of the fact that the drug saturated its metabolic pathways. Overall, all of the used doses appeared sub-anaesthetic, which is in line with comparable studies with ketamine in rats and mice [4, 32], where doses of up to 150 mg/kg still had a stimulatory effect. Congruently, *S*-ketamine shows more pronounced stimulatory effects than the *R*-enantiomer [33]. On the other hand, when comparing 30 mg/kg of DCK to the same dose of racemic ketamine, the total distance travelled after DCK treatment was almost two times higher [4], suggesting the stimulatory effects are either more potent than ketamine or there is less stereotypy after DCK (as this may decrease the overall locomotor activity, see below). DCK also seems to have a similar potency to the MXE tested in our previous experiments [5]. Although we did not perform measurements of DCK activity at the dopamine transporter (DAT), the locomotor stimulation is typically dependent on increased activity of the dopaminergic system. Based on the

559 knowledge of ketamine pharmacology and its direct effects on DAT, it is likely that DCK also
560 shares this mechanism.
561 All of the forms of racemic DCK as well as the *R* and *S* enantiomers increased thigmotaxis
562 and congruently decreased the time spent in the central zones of the arena. The effect was
563 most pronounced for the lower doses of DCK and for the *R*-enantiomer. With the highest
564 dose, the spatial characteristics of the movement were almost normalised, with regards to the
565 fact that it was still under the state with hyperlocomotion. With lower doses, the animals were
566 technically leaning around the walls, which may be a matter of increased anxiety from open
567 spaces with decreased exploration and/or stereotyped running. On the contrary, behavioural
568 patterns such as stereotyped circling that may contribute to an increased probability of
569 appearance in the centre of the arena were observed in the animals treated with the highest
570 DCK dose. Interestingly, the *R*-enantiomer seemed to be more anxiogenic-like and at the
571 same time it showed less potent stimulatory locomotor effects.

572 *Prepulse inhibition test*

573 In line with the effects of other dissociative anaesthetics [4, 5, 34-36] racemic DCK as well as
574 both of its enantiomers very potently disrupted sensorimotor gating at both testing onsets.
575 Similarly to locomotor activity, habituation to the startle was severely disrupted at all of the
576 doses used. Interestingly, in contrast to the relatively rapid disappearance of the stimulatory
577 locomotor effects, the disruption of sensorimotor gating persisted for at least one hour,
578 suggesting longer duration/persistence of psychotomimetic effects. As stimulatory effects are
579 typically related to enhanced dopaminergic activity and PPI deficits are linked to NMDA
580 blockade (as dopaminergic drugs induce only mild deficits in PPI), we may speculate about
581 the possible temporal dissociation between the DCK activity on dopaminergic
582 neurotransmission and activity at NMDA receptors.

583 *Conditioned place preference test*

584 Our findings are in line with the majority of studies with other dissociatives / noncompetitive
585 NMDA antagonists. Apart from phencyclidine (PCP), drugs like ketamine [37], MK-801 [38]
586 and MXE [14] induce place preference and show some abuse potential. Conversely, PCP
587 causes place aversion in rats [39], possibly via the involvement of serotonin 5-HT_{2A} and
588 dopamine-D_1 receptor dependent mechanisms [40, 41]. Therefore, based on our results, DCK
589 definitely has an addictive potential. If we accept the already discussed similarity with

590 ketamine and its putative effect on dopaminergic neurotransmission, we may also link the
591 activity to mesolimbic dopamine as the underlying mechanism.

592 **4.4 Comparison of the *R*- and *S*-enantiomers**

593 Our results with DCK are pretty much comparable to what we know about ketamine. Firstly,
594 *S*-DCK and *S*-ketamine are more potent antagonists at NMDA receptors than the respective *R*-
595 enantiomers. *S*-ketamine has more pronounced psychotomimetic effects in humans and is
596 more active at the dopamine transporter (by about 8 times) than *R*-ketamine. This, in turn,
597 may fit with the more pronounced locomotor stimulatory effects and reports of more
598 pleasurable effects by humans. In line with this, the *S*-DCK used in our setup has obviously
599 more pronounced stimulatory effects and induces less anxiogenic-like effects in the open
600 field. In contrast to what is known about the pharmacokinetics and duration of effects, where
601 *R*-ketamine is reported to have a longer duration [42], we did not see any dramatic differences
602 in the case of *R*- and *S*-DCK. Unfortunately, due to the low quantity of the two enantiomers
603 available, we ran the CPP only with the racemic DCK. Therefore, we may finally only
604 speculate about the more pronounced rewarding effects of the *S*-DCK variant, which would fit
605 into the overall model, especially when further experiments are needed regarding the potency
606 and activity at the DAT.

607 **4.5 DCK and its possible clinical use**

608 Although DCK is primarily abused as a recreational drug, it is important to note that due to its
609 similarity with ketamine it may also share its antidepressant effects. With ketamine, a single
610 subanaesthetic dose can produce a rapid, robust, and long-lasting anti-depressant response and
611 adequate findings have also been seen in pre-clinical [43-45] and clinical studies [46-50].
612 Both ketamine and *S*-ketamine are becoming widely used in clinical practice worldwide, and
613 *S*-ketamine already been officially approved for intranasal application.
614 Interestingly, despite the fact that the *S*-ketamine has already been approved for the treatment
615 of depression in humans, data from animal studies in rodents show a better efficacy of *R*-
616 ketamine as an antidepressant [10, 51, 52]. In light of this, it is very likely that DCK as an
617 analogue of ketamine may exhibit similar antidepressant effects. This promising hypothesis,
618 however, remains to be confirmed by future studies and the rewarding properties and potential
619 DCK abuse have to be considered. Nevertheless, one other issue concerning the use of DCK

as a potential antidepressant remains. One of the disadvantages of repeated use of ketamine is the fact that it causes toxic inflammation of the bladder – cystitis. And this toxic effect has been also described for the frequently abused analogue – MXE [53]. Based on the website https://psychonautwiki.org, "DCK seems to exhibit almost identical bladder and urinary tract problems to those found within ketamine but to a lesser extent. This is suspected to be because DCK is more potent than ketamine, meaning that less of the drug needs to be consumed to produce analogous effects". Our data confirm the hypothesis that DCK is slightly more potent, whereby decreasing the relative risk of this side effect.

5 Conclusion

DCK shows a pharmacological profile very similar to ketamine. It is slightly more potent and has longer duration of effects and has a rewarding potential. The *S*-DCK is more active than the *R*-DCK. With respect to the current knowledge about ketamine as a rapid onset antidepressant, DCK may be another promising candidate.

Funding

This work was supported by projects VI20172020056, GACR No. 18-16218S, 20-179458, and 20-25349S, AZV 17-31852A, MH CZ—DRO (NIMH-CZ, 00023752), grant LO1611 from the Ministry of Education, Youth and Sports of the Czech Republic under the NPU I programme, PROGRES Q35, GAUK 882217, 260533/SVV/2020, Czech Neuropsychopharmacology society and the Ministry of Education, Youth and Sports of the Czech Republic - project number LM2018130, ERDF/ESF project: PharmaBrain (No. CZ.02.1.01/0.0/0.0/16_025/0007444); and by RVO 61388963 and 68378050-KAV-NPUI.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions:

All of the authors made a significant contribution to this study, read, revised, and gave final approval for the current version of the work to be published.

Kristýna Štefková-Mazochová - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Hynek Danda - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Wim Dehaen - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Bronislav Jurásek - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Klára Šíchová - Drafting the work or revising it critically for important intellectual content.

Nikola Pinterová-Leca - Drafting the work or revising it critically for important intellectual content.

Vladimír Mazoch - Drafting the work or revising it critically for important intellectual content.

Barbora Hrčka Krausová - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Bohdan Kysilov - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Tereza Smejkalová - Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Ladislav Vyklický - Final approval of the version to be published.

Michal Kohout - Final approval of the version to be published.

Kateřina Hájková - Drafting the work or revising it critically for important intellectual content**.**

Daniel Svozil - Final approval of the version to be published.

Rachel R. Horsley - Final approval of the version to be published.

Martin Kuchař - Final approval of the version to be published.

Tomáš Páleníček - Drafting the work or revising it critically for important intellectual content, final approval of the version to be published.

References

[1] G. Frison, L. Zamengo, F. Zancanaro, F. Tisato, P. Traldi, Characterization of the designer drug deschloroketamine (2-methylamino-2-phenylcyclohexanone) by gas chromatography/mass spectrometry, liquid chromatography/high-resolution mass spectrometry, multistage mass spectrometry, and nuclear magnetic resonance, Rapid Commun Mass Sp 30(1) (2016) 151-160.

[2] D. Martin, D. Lodge, Ketamine Acts as a Non-Competitive N-Methyl-D-Aspartate Antagonist on Frog Spinal-Cord Invitro, Neuropharmacology 24(10) (1985) 999-1003.

[3] D. Preiss, A. Tatar, U.S. Patent No. US5811464, Washington, DC: U. S. Patent and Trademark Office, (1998).

[4] T. Palenicek, M. Fujakova, M. Brunovsky, M. Balikova, J. Horacek, I. Gorman, F. Tyls, B. Tislerova, P. Sos, V. Bubenikova-Valesova, C. Hoschl, V. Krajca, Electroencephalographic Spectral and Coherence Analysis of Ketamine in Rats: Correlation with Behavioral Effects and Pharmacokinetics, Neuropsychobiology 63(4) (2011) 202-218.

[5] R.R. Horsley, E. Lhotkova, K. Hajkova, B. Jurasek, M. Kuchar, T. Palenicek, Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue-Behavioural, pharmacokinetic and metabolic studies in the Wistar rat, Brain Res Bull 126 (2016) 102-110.

[6] V. Bubenikova, M. Votava, J. Horacek, T. Palenicek, C. Dockery, The effect of zotepine, risperidone, clozapine and olanzapine on MK-801-disrupted sensorimotor gating, Pharmacol Biochem Be 80(4) (2005) 591-596.

[7] P.F. White, J. Ham, W.L. Way, A.J. Trevor, Pharmacology of Ketamine Isomers in Surgical Patients, Anesthesiology 52(3) (1980) 231-239.

[8] J. Schuttler, D.R. Stanski, P.F. White, A.J. Trevor, Y. Horai, D. Verotta, L.B. Sheiner, Pharmacodynamic Modeling of the Eeg Effects of Ketamine and Its Enantiomers in Man, J Pharmacokinet Biop 15(3) (1987) 241-253.

[9] H. Ihmsen, G. Geisslinger, J. Schuttler, Stereoselective pharmacokinetics of ketamine: R(-)-ketamine inhibits the elimination of S(+)-ketamine, Clin Pharmacol Ther 70(5) (2001) 431-438.

[10] K. Fukumoto, H. Toki, M. Iijima, T. Hashihayata, J. Yamaguchi, K. Hashimoto, S. Chaki, Antidepressant Potential of (R)-Ketamine in Rodent Models: Comparison with (S)-Ketamine (vol 361, pg 9, 2017), J Pharmacol Exp Ther 362(1) (2017) 1-1.

[11] J. Muller, S. Pentyala, J. Dilger, S. Pentyala, Ketamine enantiomers in the rapid and sustained antidepressant effects, Ther Adv Psychopharm 6(3) (2016) 185-192.

[12] N.R. Swerdlow, D.L. Braff, M.A. Geyer, Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon, Behav Pharmacol 11(3-4) (2000) 185-204.

[13] Y. Liu, D.Y. Lin, B.L. Wu, W.H. Zhou, Ketamine abuse potential and use disorder, Brain Res Bull 126 (2016) 68-73.

[14] C.J. Botanas, J.B. de la Pena, I.J. Dela Pena, R. Tampus, R. Yoon, H.J. Kim, Y.S. Lee, C.G. Jang, J.H. Cheong, Methoxetamine, a ketamine derivative, produced Conditioned place preference and was self-administered by rats: Evidence of its abuse potential, Pharmacol Biochem Be 133 (2015) 31-36.

[15] B. Jurasek, F. Kralik, S. Rimpelova, J. Cejka, V. Setnicka, T. Ruml, M. Kuchar, M. Kohout, Synthesis, absolute configuration and in vitro cytotoxicity of deschloroketamine enantiomers: rediscovered and abused dissociative anaesthetic, New J Chem 42(24) (2018) 19360-19368.

[16] K. Hajkova, B. Jurasek, J. Cejka, K. Stefkova, T. Palenicek, D. Sykora, M. Kuchar, Synthesis and identification of deschloroketamine metabolites in rats' urine and a quantification method for deschloroketamine and metabolites in rats' serum and brain tissue using liquid chromatography tandem mass spectrometry, Drug Test Anal 12(3) (2020) 343-360.

[17] V. Vyklicky, B. Krausova, J. Cerny, M. Ladislav, T. Smejkalova, B. Kysilov, M. Korinek, S. Danacikova, M. Horak, H. Chodounska, E. Kudova, L. Vyklicky, Surface Expression, Function, and Pharmacology of Disease-Associated Mutations in the Membrane Domain of the Human GluN2B Subunit, Front Mol Neurosci 11 (2018).

[18] S.K. Adla, B. Slavikova, H. Chodounska, V. Vyklicky, M. Ladislav, P. Hubalkova, B. Krausova, T. Smejkalova, M. Nekardova, M. Smidkova, L. Monincova, R. Soucek, L. Vyklicky, E. Kudova, Strong Inhibitory Effect, Low Cytotoxicity and High Plasma Stability of Steroidal Inhibitors of N-Methyl-D-Aspartate Receptors With C-3 Amide Structural Motif, Front Pharmacol 9 (2018).

[19] G. Abdrachmanova, J. Teisinger, L. Vyklicky, Axotomy-induced changes in the properties of NMDA receptor channels in rat spinal cord motoneurons, J Physiol-London 538(1) (2002) 53-63.

[20] M. Rohan, A. Fairweather, N. Grainger, Using gamma distribution to determine half-life of rotenone, applied in freshwater, Sci Total Environ 527 (2015) 246-251.

[21] K. Stefkova, M. Zidkova, R.R. Horsley, N. Pinterova, K. Sichova, L. Uttl, M. Balikova, H. Danda, M. Kuchar, T. Palenicek, Pharmacokinetic, Ambulatory, and Hyperthermic Effects of 3,4-Methylenedioxy-N-Methylcathinone (Methylone) in Rats, Front Psychiatry 8 (2017).

[22] K. Sichova, N. Pinterova, M. Zidkova, R.R. Horsley, E. Lhotkova, K. Stefkova, C. Vejmola, L. Uttl, M. Balikova, M. Kuchar, T. Palenicek, Mephedrone (4-Methylmethcathinone): acute Behavioral effects, hyperthermic, and Pharmacokinetic Profile in rats, Front Psychiatry 8 (2018).

[23] T. Palenicek, M. Balikova, M. Rohanova, T. Novak, J. Horacek, M. Fujakova, C. Hoschl, Behavioral, hyperthermic and pharmacokinetic profile of para-methoxymethamphetamine (PMMA) in rats, Pharmacol Biochem Be 98(1) (2011) 130-139.

[24] L. Uttl, E. Szczurowska, K. Hajkova, R.R. Horsley, K. Stefkova, T. Hlozek, K. Sichova, M. Balikova, M. Kuchar, V. Micale, T. Palenicek, Behavioral and Pharmacokinetic Profile of Indole-Derived Synthetic Cannabinoids JWH-073 and JWH-210 as Compared to the Phytocannabinoid Delta(9)-THC in Rats, Front Neurosci-Switz 12 (2018).

[25] N. Pinterova-Leca, R.R. Horsley, H. Danda, M. Aidkova, E. Lhotkova, K. Sichova, K. Stefkova, M. Balikova, M. Kuchar, T. Palenicek, Naphyrone (naphthylpyrovalerone): Pharmacokinetics, behavioural effects and thermoregulation in Wistar rats, Addict Biol (2020).

[26] J.N. Berry, N.M. Neugebauer, M.T. Bardo, Reinstatement of methamphetamine conditioned place preference in nicotine-sensitized rats, Behav Brain Res 235(2) (2012) 158-165.

[27] J.F. Macdonald, Z. Miljkovic, P. Pennefather, Use-Dependent Block of Excitatory Amino-Acid Currents in Cultured Neurons by Ketamine, J Neurophysiol 58(2) (1987) 251-266.

[28] S.E. Kotermanski, J.W. Johnson, Mg2+ Imparts NMDA Receptor Subtype Selectivity to the Alzheimer's Drug Memantine, J Neurosci 29(9) (2009) 2774-2779.

[29] J. Sleigh, M. Harvey, L. Voss, B. Denny, Ketamine - More mechanisms of action than just NMDA blockade, Trends Anaesth Crit 4(2-3) (2014) 76-81.

[30] P. Zarantonello, E. Bettini, A. Paio, C. Simoncelli, S. Terreni, F. Cardullo, Novel analogues of ketamine and phencyclidine as NMDA receptor antagonists, Bioorg Med Chem Lett 21(7) (2011) 2059-2063.

[31] R. Moaddel, D.A. Luckenbaugh, Y. Xie, A. Villasenor, N.E. Brutsche, R. Machado-Vieira, A. Ramamoorthy, M.P. Lorenzo, A. Garcia, M. Bernier, M.C. Torjman, C. Barbas, C.A. Zarate, I.W. Wainer, D-serine plasma concentration is a potential biomarker of (R,S)-ketamine antidepressant response in subjects with treatment-resistant depression, Psychopharmacology 232(2) (2015) 399-409.

[32] M. Irifune, T. Shimizu, M. Nomoto, Ketamine-Induced Hyperlocomotion Associated with Alteration of Presynaptic Components of Dopamine Neurons in the Nucleus-Accumbens of Mice, Pharmacol Biochem Be 40(2) (1991) 399-407.

[33] L. Chang, K. Zhang, Y. Pu, Y. Qu, S.M. Wang, Z. Xiong, Q. Ren, C. Dong, Y. Fujita, K. Hashimoto, Comparison of antidepressant and side effects in mice after intranasal administration of (R,S)-ketamine, (R)-ketamine, and (S)-ketamine, Pharmacol Biochem Behav 181 (2019) 53-59.

[34] M. Fujakova, T. Palenicek, M. Brunovsky, I. Gorman, F. Tyls, A. Kubesova, D. Ripova, V. Krajca, J. Horacek, The effect of ((-)-2-oxa-4-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY379268), an mGlu2/3 receptor agonist, on EEG power spectra and coherence in ketamine model of psychosis, Pharmacol Biochem Be 122 (2014) 212-221.

[35] C. Johansson, D.M. Jackson, L. Svensson, The Atypical Antipsychotic, Remoxipride, Blocks Phencyclidine-Induced Disruption of Prepulse Inhibition in the Rat, Psychopharmacology 116(4) (1994) 437-442.

[36] N.M.W.J. de Bruin, B.A. Ellenbroek, A.R. Cools, A.M.L. Coenen, E.L.J.M. van Luijtelaar, Differential effects of ketamine on gating of auditory evoked potentials and prepulse inhibition in rats, Psychopharmacology 142(1) (1999) 9-17.

[37] T. Suzuki, H. Kato, T. Aoki, M. Tsuda, M. Narita, M. Misawa, Effects of the non-competitive NMDA receptor antagonist ketamine on morphine-induced place preference in mice, Life Sci 67(4) (2000) 383-389.

[38] R.T. Layer, F.G. Kaddis, L.J. Wallace, The Nmda Receptor Antagonist Mk-801 Elicits Conditioned Place Preference in Rats, Pharmacol Biochem Be 44(1) (1993) 245-247.

[39] K. Kitaichi, Y. Noda, T. Hasegawa, H. Furukawa, T. Nabeshima, Acute phencyclidine induces aversion, but repeated phencyclidine induces preference in the place conditioning test in rats, Eur J Pharmacol 318(1) (1996) 7-9.

[40] K. Kitaichi, Y. Noda, Y. Miyamoto, A. Numaguchi, H. Osawa, T. Hasegawa, H. Furukawa, T. Nabeshima, Involvement of the serotonergic neuronal system in phencyclidine-induced place aversion in rats, Behav Brain Res 103(1) (1999) 105-111.

[41] E. Acquas, E. Carboni, P. Leone, G. Dichiara, Sch 23390 Blocks Drug-Conditioned Place-Preference and Place-Aversion - Anhedonia (Lack of Reward) or Apathy (Lack of Motivation) after Dopamine-Receptor Blockade, Psychopharmacology 99(2) (1989) 151-155.

[42] N.D. Iadarola, M.J. Niciu, E.M. Richards, J.L. Vande Voort, E.D. Ballard, N.B. Lundin, A.C. Nugent, R. Machado-Vieira, C.A. Zarate, Ketamine and other N-methyl-D-aspartate receptor antagonists in the treatment of depression: a perspective review, Ther Adv Chronic Dis 6(3) (2015) 97-114.

[43] N.X. Li, B. Lee, R.J. Liu, M. Banasr, J.M. Dwyer, M. Iwata, X.Y. Li, G. Aghajanian, R.S. Duman, mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists, Science 329(5994) (2010) 959-964.

[44] A.E. Autry, M. Adachi, E. Nosyreva, E.S. Na, M.F. Los, P.F. Cheng, E.T. Kavalali, L.M. Monteggia, NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses, Nature 475(7354) (2011) 91-U109.

[45] P. Zanos, S.M. Thompson, R.S. Duman, C.A. Zarate, T.D. Gould, Convergent Mechanisms Underlying Rapid Antidepressant Action, Cns Drugs 32(3) (2018) 197-227.

[46] R.M. Berman, A. Cappiello, A. Anand, D.A. Oren, G.R. Heninger, D.S. Charney, J.H. Krystal, Antidepressant effects of ketamine in depressed patients, Biol Psychiat 47(4) (2000) 351-354.

[47] K.A.B. Lapidus, C.F. Levitch, A.M. Perez, J.W. Brallier, M.K. Parides, L. Soleimani, A. Feder, D.V. Iosifescu, D.S. Charney, J.W. Murrough, A Randomized Controlled Trial of Intranasal Ketamine in Major Depressive Disorder, Biol Psychiat 76(12) (2014) 970-976.

[48] J.W. Murrough, D.V. Iosifescu, L. Chang, R.K. Al Jurdi, C. Green, A. Perez, S.Z. Iqbal, S. Pillemer, A. Foulkes, S. Mathew, Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site, randomised controlled trial, Eur Neuropsychopharm 23 (2013) S411-S412.

[49] C.A. Zarate, J.B. Singh, P.J. Carlson, N.E. Brutsche, R. Ameli, D.A. Luckenbaugh, D.S. Charney, H.K. Manji, A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression, Arch Gen Psychiat 63(8) (2006) 856-864.

[50] P. Sos, M. Klirova, T. Novak, B. Kohutova, J. Horacek, T. Palenicek, Relationship of ketamine's antidepressant and psychotomimetic effects in unipolar depression, Neuroendocrinol Lett 34(4) (2013) 287-293.

[51] C. Yang, Y. Shirayama, J.C. Zhang, Q. Ren, W. Yao, M. Ma, C. Dong, K. Hashimoto, R-ketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects, Transl Psychiat 5 (2015).

[52] J.C. Zhang, S.X. Li, K. Hashimoto, R(-)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine, Pharmacol Biochem Be 116 (2014) 137-141.

[53] P.I. Dargan, H.C. Tang, W. Liang, D.M. Wood, D.T. Yew, Three months of methoxetamine administration is associated with significant bladder and renal toxicity in mice, Clin Toxicol 52(3) (2014) 176-180.

Figures

Figure 1: Structures of DCK (A), ketamine (B), PCP (C) and MXE (D).

Figure 2: Structures of prepared metabolites: norDCK (A), *trans*-dihydroDCK (B), *cis*-dihydronorDCK (C), *trans*-dihydronorDCK (D) and *trans*-dihydroDCK-*d4* (E).

Figure 3. The effect of ketamine and *R*/*S*-DCK on recombinant human NMDA receptors. A-B, Examples of traces obtained from HEK293 cells expressing either hGluN1/hGluN2A (A) or hGluN1/hGluN2B (B) receptors. The receptors were activated by 1 mM glutamate and 30 µM glycine and the degree of inhibition was measured in the presence of ketamine, *R*-DCK, and *S*-DCK at a concentration range of 0.1 - 100 µM (grey bars). C-D, Concentration-response curves for the effect of compounds at hGluN1/hGluN2A (C) and hGluN1/hGluN2B (D) receptors. The fitted parameters are summarised in Table 1. Data points are mean ± SEM. E-F, Representative current responses from HEK293 cells transfected with hGluN1/hGluN2A (E) or hGluN1/hGluN2B (F) receptors to 1 mM glutamate and 30 µM glycine recorded at -80 and +40 mV. The compounds were applied at a concentration of 3 µM in the case of hGluN1/hGluN2A receptors and 1 µM in the case of hGluN1/hGluN2B receptors. G-H, *I-V* plots of normalised current amplitudes of hGluN1/hGluN2A (G) or hGluN1/hGluN2B (H) receptors recorded in the presence (filled circle and arrow down symbols) or absence (open circle and arrow up symbols) of the compounds. Control responses were fitted by a linear equation and normalised to apparent whole-cell conductance. The fitted parameters are summarised in Table 2. Data points are mean ± SEM.

Figure 4. Effect of ketamine and *S*-DCK on network activity in primary hippocampal cultures. A,C, Example of current-clamp traces showing a reduction in AP firing in the presence of 0.3 µM ketamine (A) or *S*-DCK (C). B,D, Plots of normalised AP frequency before and during ketamine (B) or *S*-DCK (D) application. The small empty circles show data from individual cells, and the large filled circles show mean ± SEM.

Figure 5: Concentrations of DCK and norDCK over 24 hrs (A, C) and concentrations of *R*-DCK, *S*-DCK, *R*-norDCK, and *S*-norDCK over 4 hrs (B, D) in serum (ng/ml) and brain tissue (ng/g) after s.c. administration of DCK (10 mg/kg). The symbols represent the mean values and the vertical bars SEMs.

Figure 6: Proposed metabolism pathway. Metabolite structures of norDCK (A), *trans*-dihydroDCK (B), *cis*-dihydronorDCK (C), *trans*-dihydronorDCK (D) and *trans*-dihydroDCK-d_4 (E).

Figure 7: The first panel represents the effect of DCK and both its enantiomers on locomotor activity in the OFT in 5-min intervals, 15 min (A) or 60 min (B) after drug administration. The second panel represents their effect on time spent in the centre of the arena with examples of characteristic trajectories at 15 min (C) and 60 min (D) after drug administration. The third panel shows mean thigmotaxis measured 15 min (E) and 60 min (F) after administration. The bottom panel shows mean percentage PPI, 15 min (G) and 60 min (H) after drug administration. The symbols/columns represent the mean values and the vertical bars SEMs. Asterisk represent a significant difference from vehicle.

Tables

Table 1: The parameters obtained from the concentration-response relationship for ketamine, *S*-DCK and *R*-DCK at recombinant hGluN1/hGluN2A and hGluN1/hGluN2B receptors expressed in HEK293 cells.

	hGluN1/hGluN2A			hGluN1/hGluN2B		
	IC50 (μM)	*h*	*n*	*IC50* (μM)	*h*	*n*
R-DCK	1.4 ± 0.2	1.12 ± 0.04	9	1.2 ± 0.2	0.99 ± 0.08	6
S-DCK	1.1 ± 0.2	1.30 ± 0.12	7	0.6 ± 0.1	1.07 ± 0.12	7
Ketamine	1.7 ± 0.3	1.07 ± 0.09	9	0.8 ± 0.2	1.20 ± 0.10	5

Footnote: The *IC50* values and Hill coefficients (*h*) were obtained by fitting the data to the Eq.2. The values are mean ± SEM with *n* corresponding to the number of independent measurements.

Table 2: The parameters obtained from the *I-V* relationship for ketamine, *S*-DCK and *R*-DCK at recombinant hGluN1/hGluN2A and hGluN1/hGluN2B receptors expressed in HEK293 cells.

	hGluN1/hGluN2A			hGluN1/hGluN2B		
	Kd (μM)	δ	*n*	*Kd* (μM)	δ	*n*
R-DCK	24.4 ± 11.2	0.44 ± 0.03	4	10.9 ± 1.7	0.58 ± 0.02	5
S-DCK	21.1 ± 4.9	0.67 ± 0.02	4	3.4 ± 0.9	0.53 ± 0.05	3
Ketamine	20.8 ± 10.0	0.65 ± 0.10	3	7.8 ± 2.1	0.56 ± 0.05	5

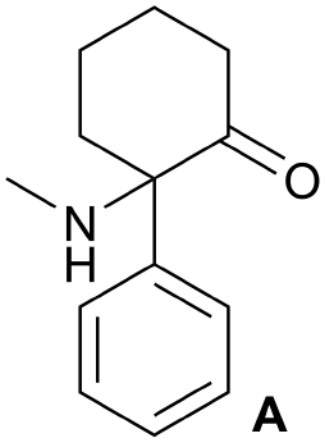
Footnote: The values of *Kd* and δ were obtained by fitting the current responses measured in the presence of compounds to the Eq.3-5 and normalising the data for individual cells to the estimated whole-cell conductance (g_0). The values are mean ± SEM with *n* corresponding to the number of independent measurements.

884 **Table 3:** The effect of DCK on acoustic startle response (ASR) and habituation.

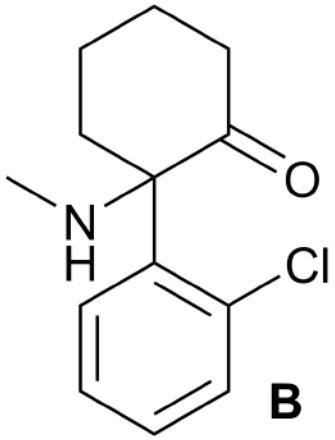
		Drug treatment					
Measure	**Testing onsets**	Vehicle	5 mg/kg	10 mg/kg	30 mg/kg	*S*-DCK (10 mg/kg)	*R*-DCK (10 mg/kg)
ASR *(arbitrary units)*	15 min	159.2(±41.8)	127.3(±15.4)	168.2(±22.8)	157.9(±8.3)	161.4(±12.7)	122.5(±12.9)
	60 min	206.5(±44.4)	126(±28.6)	129.7(±18.9)	115.3(±18.3)	N/A	N/A
Percentage habituation	15 min	65.1(±7.1)	5.5(±13.1)	28.2(±9.1)	24.2(±7.1)	11.0(±12.2)	20.25 (±7.0)
	60 min	37.9(±9.7)	-2(±13.6)	10.3(±6.9)	-13.2(±13.9)	N/A	N/A

885 Footnote: The numbers represent the mean values, and the numbers in parentheses indicate
886 ±SEM.

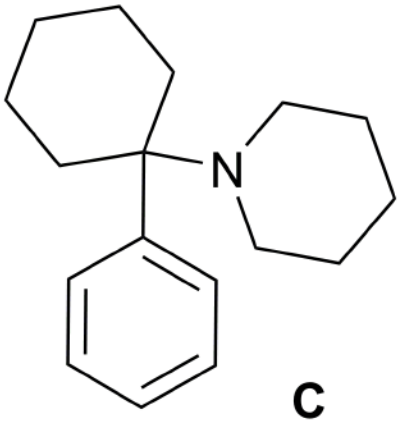
887



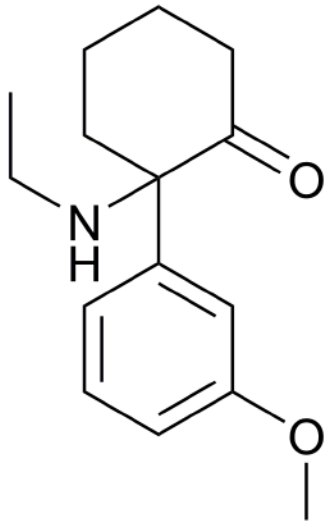
A



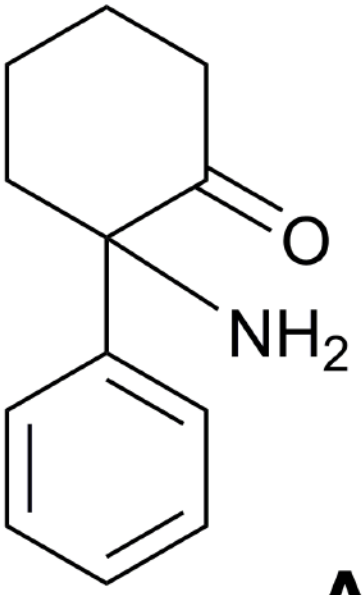
B



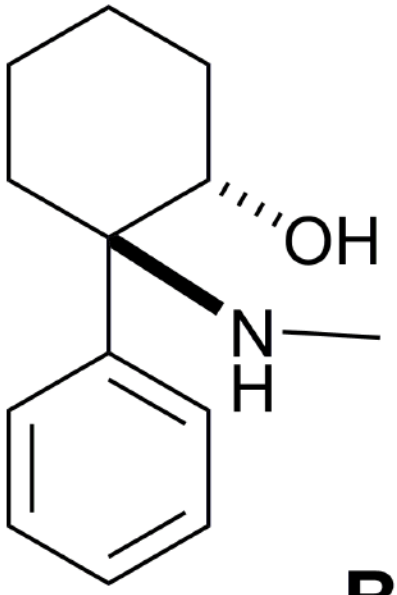
C



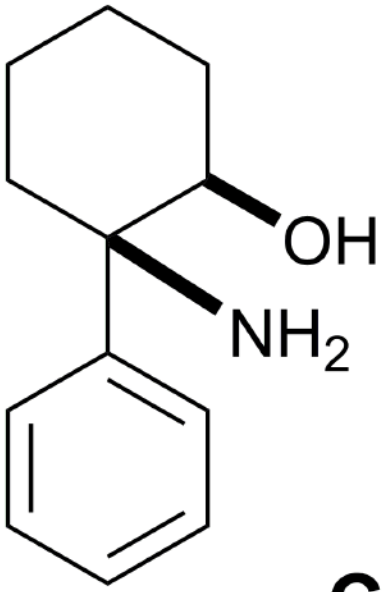
D



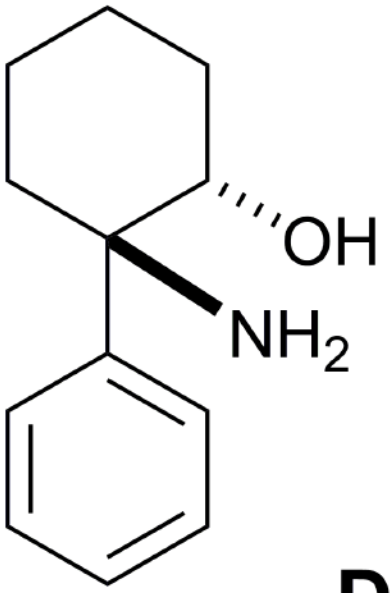
A



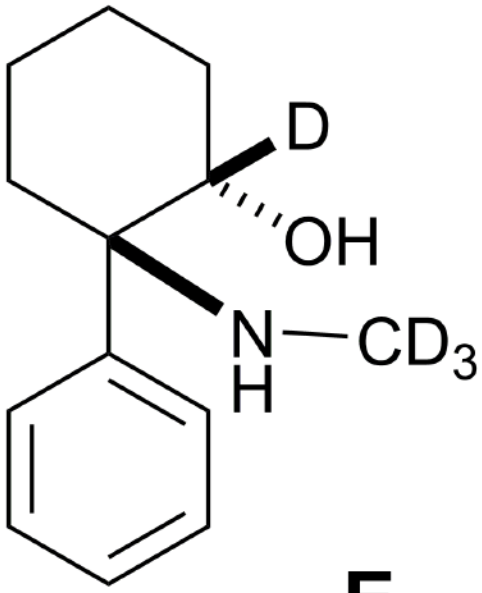
B



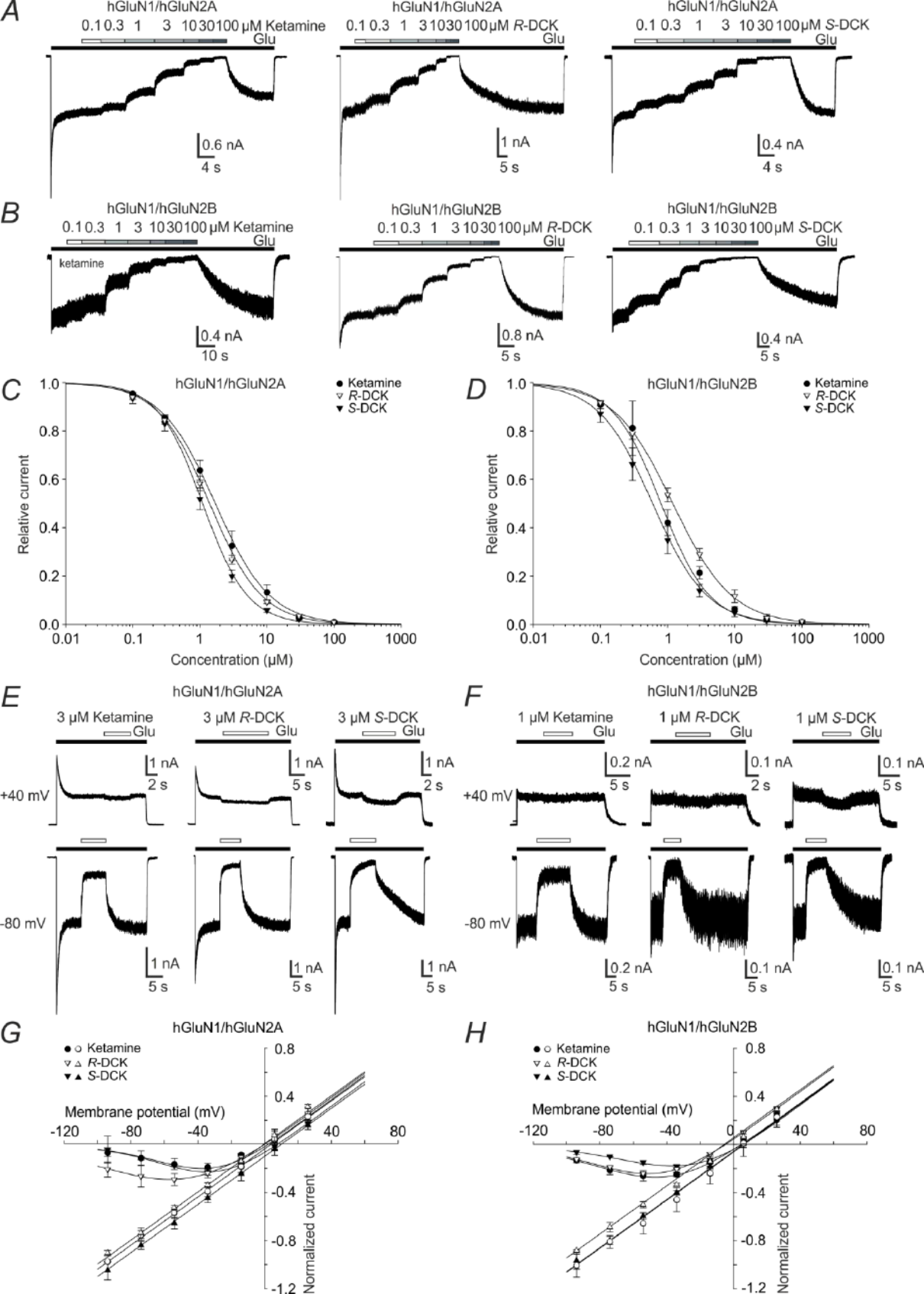
C

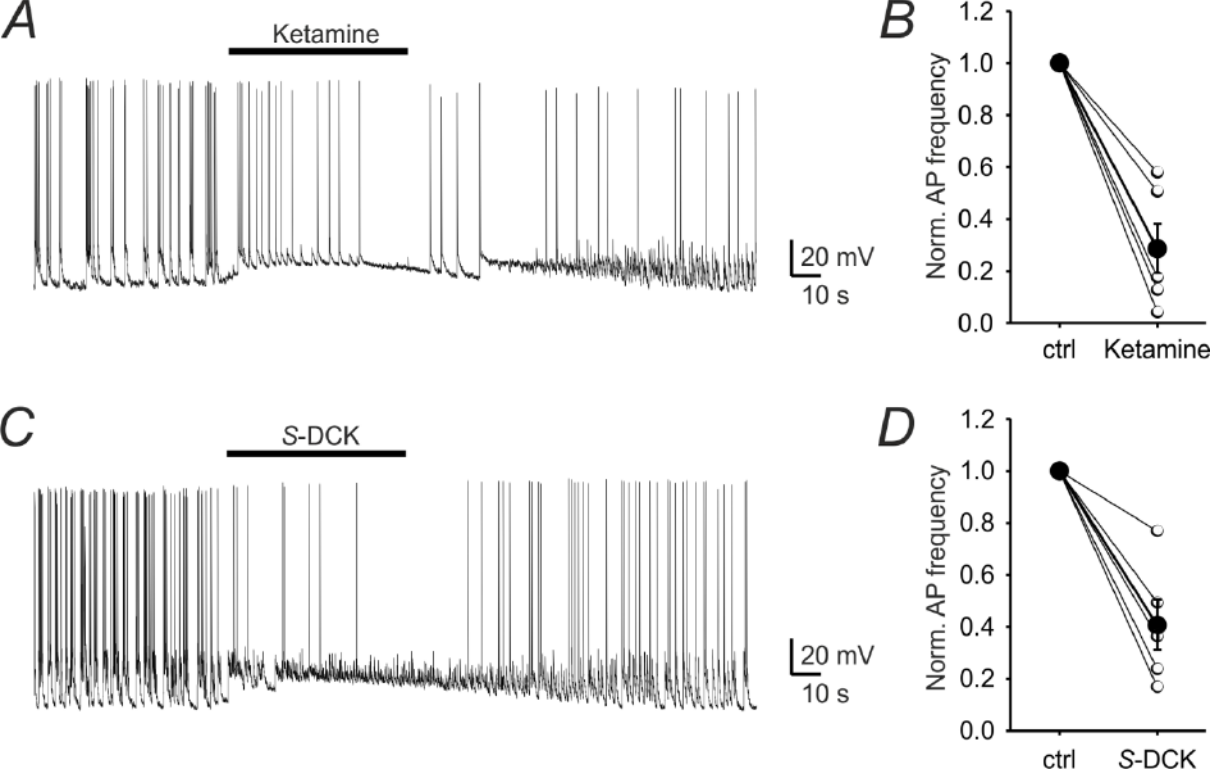


D

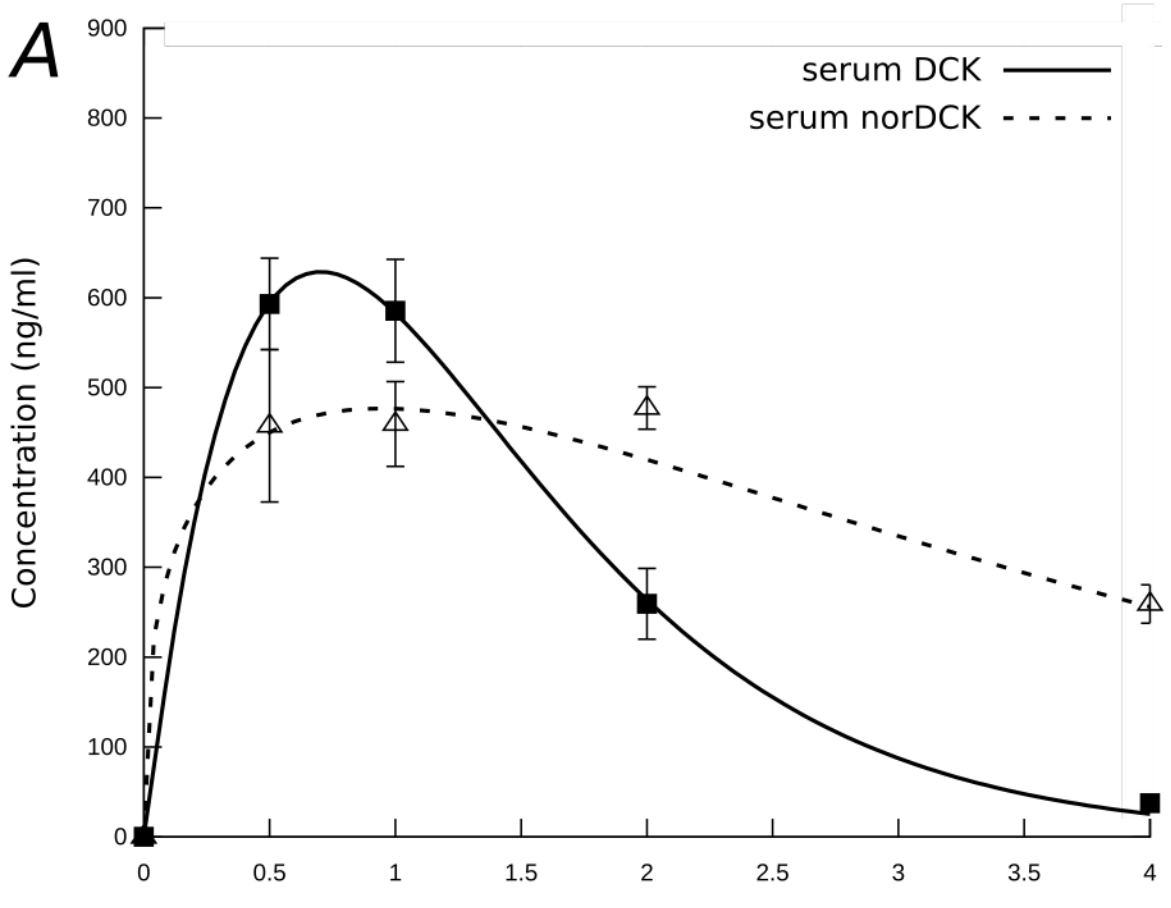


E

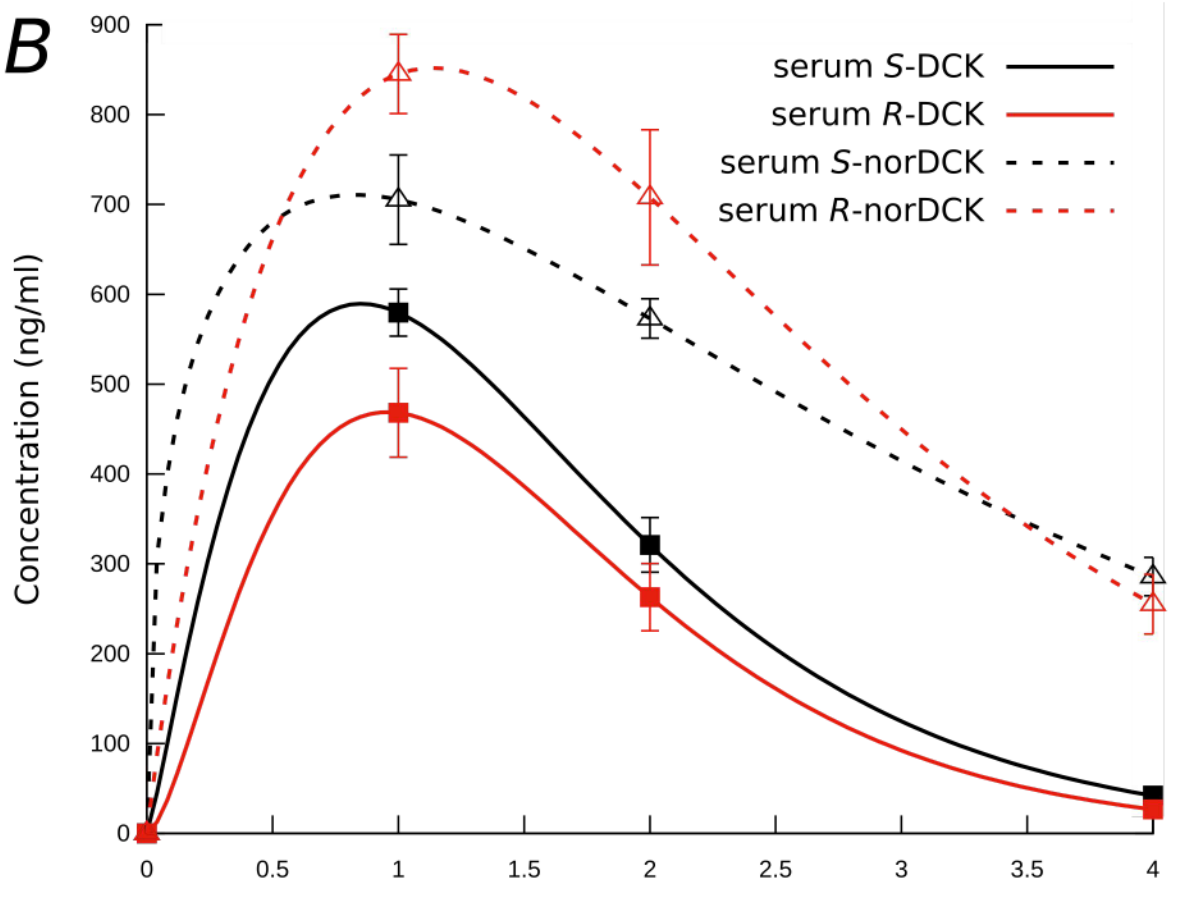

A
hGluN1/hGluN2A
0.1 0.3 1 3 10 30 100 μM Ketamine
Glu
0.6 nA
4 s
hGluN1/hGluN2A
0.1 0.3 1 3 10 30 100 μM R-DCK
Glu
1 nA
5 s
hGluN1/hGluN2A
0.1 0.3 1 3 10 30 100 μM S-DCK
Glu
0.4 nA
4 s
B
hGluN1/hGluN2B
0.1 0.3 1 3 10 30 100 μM Ketamine
Glu
ketamine
0.4 nA
10 s
hGluN1/hGluN2B
0.1 0.3 1 3 10 30 100 μM R-DCK
Glu
0.8 nA
5 s
hGluN1/hGluN2B
0.1 0.3 1 3 10 30 100 μM S-DCK
Glu
0.4 nA
5 s
C
hGluN1/hGluN2A
Ketamine
R-DCK
S-DCK
Relative current
Concentration (μM)
D
hGluN1/hGluN2B
Ketamine
R-DCK
S-DCK
Relative current
Concentration (μM)
E
hGluN1/hGluN2A
3 μM Ketamine
3 μM R-DCK
3 μM S-DCK
Glu
+40 mV
-80 mV
1 nA
2 s
5 s
F
hGluN1/hGluN2B
1 μM Ketamine
1 μM R-DCK
1 μM S-DCK
Glu
+40 mV
-80 mV
0.2 nA
0.1 nA
2 s
5 s
G
hGluN1/hGluN2A
Ketamine
R-DCK
S-DCK
Membrane potential (mV)
Normalized current
H
hGluN1/hGluN2B
Ketamine
R-DCK
S-DCK
Membrane potential (mV)
Normalized current


A
Ketamine
20 mV
10 s
B
Norm. AP frequency
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ctrl
Ketamine
C
S-DCK
20 mV
10 s
D
Norm. AP frequency
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ctrl
S-DCK

A


serum DCK
serum norDCK
Concentration (ng/ml)
0
100
200
300
400
500
600
700
800
900
0
0.5
1
1.5
2
2.5
3
3.5
4

B

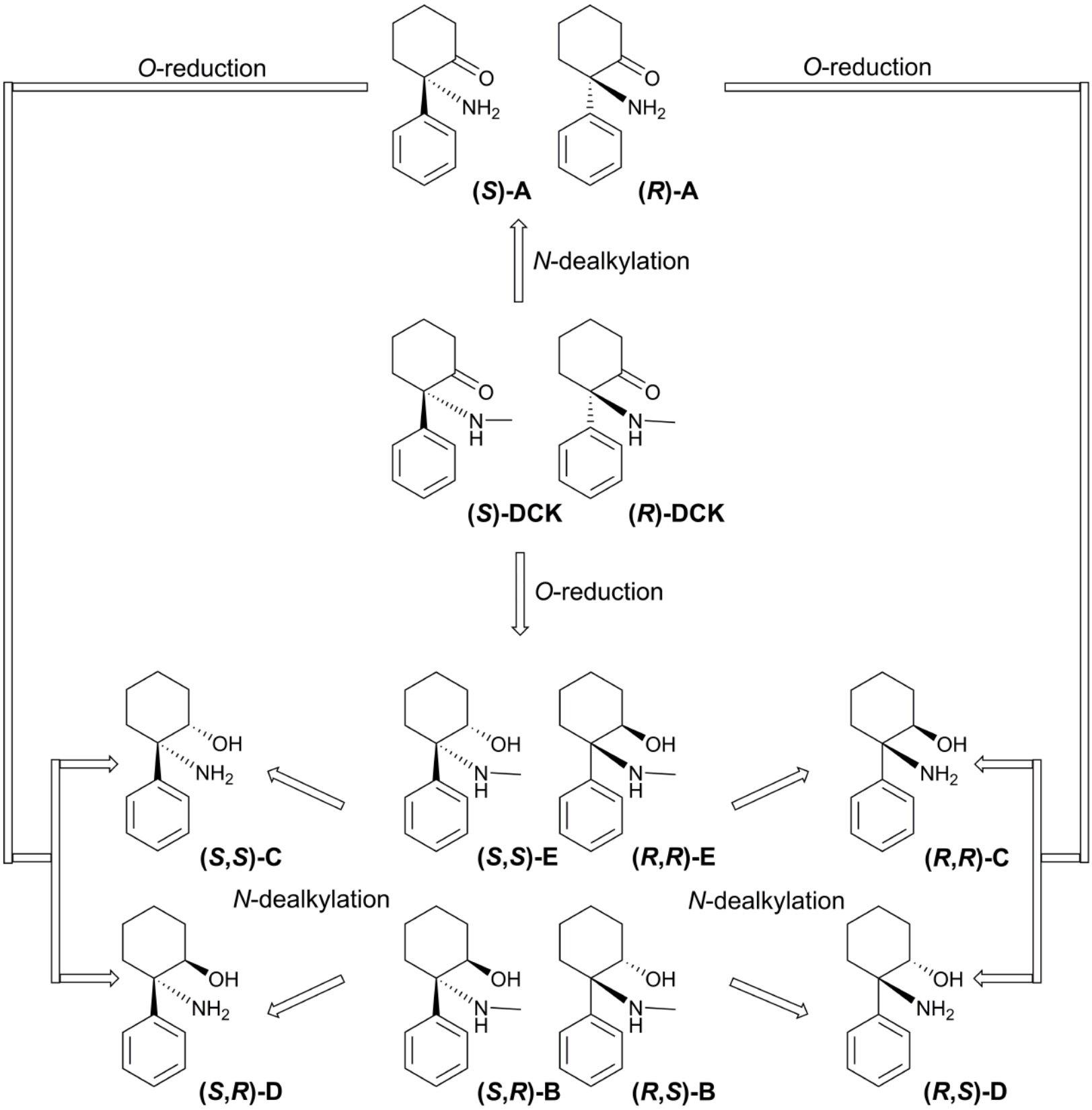

serum S-DCK
serum R-DCK
serum S-norDCK
serum R-norDCK
Concentration (ng/ml)
0
100
200
300
400
500
600
700
800
900
0
0.5
1
1.5
2
2.5
3
3.5
4

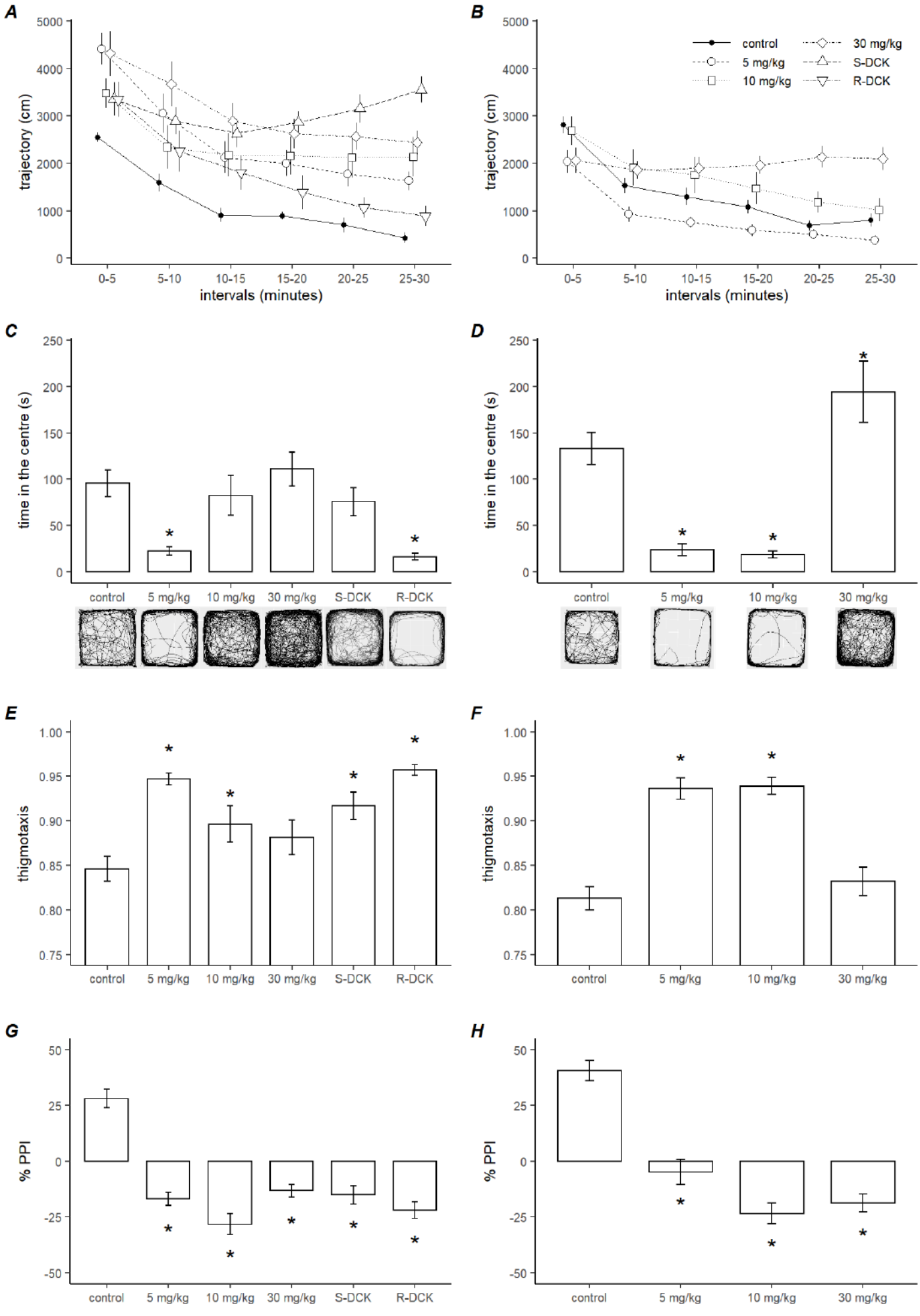
C

brain DCK
brain norDCK
Concentration (ng/g)
0
500
1000
1500
2000
2500
3000
3500
4000
0
0.5
1
1.5
2
2.5
3
3.5
4
Time (hr)

D

Time (hr)
brain S-DCK
brain R-DCK
brain S-norDCK
brain R-nor DCK
Concentration (ng/g)
0
500
1000
1500
2000
2500
3000
3500
4000
0
0.5
1
1.5
2
2.5
3
3.5
4
Time (hr)


O-reduction
O-reduction
(S)-A
(R)-A
N-dealkylation
(S)-DCK
(R)-DCK
O-reduction
(S,S)-C
(S,S)-E
(R,R)-E
(R,R)-C
N-dealkylation
N-dealkylation
(S,R)-D
(S,R)-B
(R,S)-B
(R,S)-D


A
B
C
D
E
F
G
H
trajectory (cm)
intervals (minutes)
0-5
5-10
10-15
15-20
20-25
25-30
control
5 mg/kg
10 mg/kg
30 mg/kg
S-DCK
R-DCK
time in the centre (s)
thigmotaxis
% PPI