Effect of the combination of mephedrone plus ethanol on serotonin and dopamine release in the nucleus accumbens and medial prefrontal cortex of awake rats

Raúl López-Arnau1 · Mario Buenrostro-Jáuregui2 · Jorge Camarasa1 · David Pubill1 · Elena Escubedo1

Received: 2 June 2017 / Accepted: 9 January 2018 / Published online: 18 January 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract
Cathinones, such as mephedrone (Meph), are often co-abused with alcoholic drinks. In the present study, we investigated the combined effects of Meph plus ethanol (EtOH) on neurotransmitter release in the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC). A guide canula was stereotaxically implanted into either the NAc or the mPFC of male Sprague-Dawley rats. Seven days after surgery, a microdialysis probe was inserted and rats were administered saline, EtOH (1 g/kg, i.p.), Meph (25 mg/kg, s.c.), or their combination, and dialysates were collected. Serotonin (5-HT), dopamine (DA), and their metabolites (5-HIAA, DOPAC and HVA) were determined through high-pressure liquid chromatography coupled to mass spectrometry. 5-HT and DA peaked 40 min after Meph administration (with or without EtOH co-treatment) in both areas. EtOH combined with Meph increased the 5-HT release compared with the rats receiving Meph alone (85% in NAc, 65% in mPFC), although the overall change in the area under the curve only reached statistical significance in the NAc. In mPFC, the increased release of 5-HT lasted longer in the combination than that in the Meph group. Moreover, EtOH potentiated the psychostimulant effect of Meph measured as a locomotor activity. Given that both 5-HT and DA are also related with reward and impulsivity, the observed effects point to an increased risk of abuse liability when combining Meph with EtOH compared with consuming these drugs alone.

Keywords Mephedrone · Ethanol · Cathinones · Microdialysis · Drug polyabuse · Bath salts

Introduction
Mephedrone (4-methylmethcathinone, Meph) is one of the most popular new designer drugs of the cathinones’ group. It is a β-keto-amphetamine which has powerful psychostimulant and entactogenic effects and has been distributed as bath salts or as a component of ecstasy tablets (Brunt et al. 2012).

Most recreational drug use occurs in leisure environments, where alcoholic drinks are omnipresent, so most cathinone consumers combine them with it (O’Neill and McElrath 2012), and interaction between drugs and alcohol may occur. In fact, the effects of the combination of another popular amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA), with alcohol were broadly studied by the group of Cassel, Jones, and colleagues (see Mohamed et al. 2009 for a review), showing that alcohol potentiates the hyperlocomotion and conditioning (Cassel et al. 2004; Jones et al. 2010) effects of MDMA, increases brain MDMA concentrations (Ben-Hamida et al. 2009), and attenuates its hyperpyretic effects.

Abbreviations
5-HIAA 5-Hydroxyindoleacetic acid
5-HT Serotonin
DA Dopamine
DOPAC 3,4-Dihydroxyphenylacetic acid
EtOH Alcohol/ethanol
LC-MS Liquid chromatography-mass spectrometry
MDMA 3,4-Methylenedioxymethamphetamine
Meph Mephedrone
mPFC Medial prefrontal cortex
NAc Nucleus accumbens

David Pubill
d.pubill@ub.edu

1 Department of Pharmacology, Toxicology and Therapeutic Chemistry, Pharmacology Section and Institute of Biomedicine (IBUB), Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII 27-31, 08028 Barcelona, Spain

2 Neuroscience Laboratory, Department of Psychology, Universidad Iberoamericana, Mexico City, Mexico
Studies in animals have shown that Meph stimulates the release of serotonin (5-HT), dopamine (DA), and norepinephrine and inhibits their reuptake in the CNS (Baumann et al. 2012; Kehr et al. 2011; López-Arnau et al. 2012). Ethyl alcohol (EtOH) also exerts complex effects on neurotransmitter release (see Clapp et al. 2008 and Siggins et al. 2005 for reviews) due to its ability to cross biological membranes and to interact on several molecular targets (i.e., ligand-gated ion channels such as glutamate receptors) which can lead to uncertain interactions affecting the behavioral and toxic effects when combined with Meph, such as increased hyperlocomotion and place conditioning in mice (Ciudad-Roberts et al. 2015, 2016). EtOH is capable of increasing hyperlocomotion by inhibiting GABAergic interneurons in the substantia nigra reticulata, which leads to disinhibition and increased burst firing of dopamine neurons in the nucleus accumbens (NAc), but it also increases DA release in other areas of mesocortical pathways. Indirect mechanisms involving acetylcholine in the ventral tegmental area, and more particularly the activation of nicotinic receptors, contribute to the increased DA release within the NAc. Also, activation of the opioid reward pathway has been reported (Mitchell et al. 2012). On the other hand, systemic and local (Riegert et al. 2008) EtOH increases the release of 5-HT in the striatum, suggesting the participation of local mechanisms, and an inhibitory effect of EtOH at the serotonin transporters has been reported as well (Daws et al. 2006). At the same time, 5-HT was found to potentiate the EtOH-induced activation of ventral tegmental area neurons (Brodie et al. 1995).

Other groups have investigated the effects of Meph on neurotransmitter release (Baumann et al. 2012; Golembiowska et al. 2016; Kehr et al. 2011; Wright et al. 2012), but none has studied such effects in combination with ethanol. The aim of the present study is to assess the effects of such drug combination on DA and 5-HT release in the NAc and medial prefrontal cortex (mPFC), two key areas involved in drug-induced behavior (Nestler 2001; Hammerslag et al. 2014). Also, the effects of such treatments on the psychostimulant effects will be measured.

Methods

Animals and drug

Male Sprague-Dawley rats (Charles River, Spain) weighing 250–300 g were used. They were housed two to three per cage at 22 ± 1 °C with a humidity of 50–55%, food and water ad libitum, and under a normal light/dark cycle (lights on for 12 h starting at 8:00 a.m.). After surgery, they were individually housed in order to avoid damaging of the cannula guide implant. Pure racemic Meph hydrochloride was synthesized and characterized in our laboratory as described previously (López-Arnau et al. 2012).

Microdialysis experiments

The microdialysis experiments were carried out on awake rats (n = 3–4/group) according to the protocol described by Kehr et al. (2011), with some modifications. An intracerebral guide cannula (Agntho’s, Lidingö, Sweden) was surgically implanted in rats at the NAc (2.2 mm anterior to bregma (AP), 1.6 mm lateral (L), and 6.0 mm ventral to the dura surface (V)) or mPFC (3.2 mm (AP), 0.5 mm (L), and 1.6 mm (V)). Rats were allowed at least 1 week for recovery from surgery. On the evening before an experiment, a microdialysis probe (Agntho’s, Lidingö, Sweden; 2- or 3-mm membrane length with 15,000 Da cut-off) was inserted into the guide cannula and perfused overnight with artificial cerebrospinal fluid solution (148 mM NaCl, 2.2 mM CaCl₂, 0.8 mM MgCl₂, 1.2 mM Na₂HPO₄, and 0.3 mM NaH₂PO₄) at a flow rate of 0.6 μL/min. On the next day, after a stabilization period of 2 h, microdialysis samples were collected at 20-min intervals (flow 1 μL/min). The first three samples were used for estimation of basal levels of DA, 5-HT, DOPAC, HVA, and 5-HIAA. Thereafter, saline, Meph (25 mg/kg s.c.), EtOH (1 g/kg i.p.), or both were injected to separate groups of rats, and the fractions were collected for 180 min and stored at −80 °C before analysis. At the end of the experiments, the animals were perfused with paraformaldehyde and examined for correct placement of the probe. Only the data from those rats with correct probe placements were included in the study. Of the 32 rats that underwent a successful surgical process and were tested in microdialysis experiments, 4 were excluded due to missed cannula placement.

The dose of Meph (25 mg/kg) was chosen according to a previous work showing powerful psychostimulant effects of this drug (Martínez-Clemente et al. 2013), and although it may model an acute consumption of Meph in humans (López-Arnau et al. 2015), previous microdialysis assays only studied the effect of lower doses (Baumann et al. 2012; Golembiowska et al. 2016; Kehr et al. 2011; Wright et al. 2012). The dose of EtOH (1 g/kg) is a low/moderate dose that does not produce marked behavioral effects when administered once for the first time (Imperato and Di Chiara 1986; Brabant et al. 2014), and when administered to rats weighing 250–300 g leads to a blood ethanol concentration (BEC) of around 0.6 g/L (Bloom et al. 1982). In humans, this BEC can easily be reached after a moderate recreational consumption.

LC-MS/MS determination of DA, 5-HT, and metabolites in dialysate samples

An Agilent 1290 Liquid Chromatography (LC) system equipped with an autosampler and coupled to AB Sciex
QTRAP 6500 mass spectrometer (MS) was used to quantify DA, 5-HT, and metabolites. Chromatographic separation was achieved in a Discovery HS F5 (150 mm × 4 mm, 3 μm, Sigma-Aldrich, St. Louis, MO, USA) pentafluorophenyl column thermostatted at 37 °C. The mobile phase was water (A) and methanol (B) with 0.1% of formic acid in both solvents. An increasing linear gradient (v/v) of B was used (t (min), % B), as follows, (0, 0), (0.5, 0), (5.90, 30), (6, 100), (9, 100), (9.10, 0), (10, 0) at a constant flow rate (500 μl/min). The flow was directed to waste for the first 2 min to prevent the inorganic ions of aCSF solution to enter the mass spectrometer. The microdialysate samples were refrigerated at 4 °C and 20 μL was injected, without sample pretreatment, into the LC-MS/MS system. Mass spectrometric quantification in positive ion mode was carried out using the following transitions: DA (m/z 134 → 137 and 154 → 91, collision energies (CE) of 15 and 31 V, respectively), DOPAC (m/z 123 → 77, CE of 24 V), 5-HT (m/z 177 → 160, CE of 13 V), and 5-HIAA (m/z 192 → 146, CE of 23 V). A negative ion mode was used in the analysis of HVA (m/z 181 → 122, CE of –20 V).

### Locomotor activity recording

The locomotor responses induced by Meph (25 mg/kg, s.c.), EtOH (1 g/kg, i.p.), and their combination were assessed in black Plexiglass open field arenas (l × w × h 45 × 45 × 40 cm) under low-light conditions. Two days before testing, the animals were handled for 10 min, administered saline (1 ml/kg), and placed in the arena for habituation. The test day, the rats were administered with the assigned treatment and placed in the arenas, and their horizontal traveling was video-monitored by a zenithal video camera coupled to a computer running a tracking software (Smart 3.0, Panlab, S.L.U., Barcelona, Spain) for 90 min. Both cumulative distances in 10-min blocks and total traveled distances were obtained.

### Data analysis

All data are expressed as mean ± standard error of the mean (S.E.M.). All statistical calculations were performed using InVivoStat software (http://invivostat.co.uk/). The power of the analysis was assessed for all determined monoamines, and areas resulted higher than 95% for an n value of 3–4 animals per group. The temporal evolution of monoamine levels and locomotor activities were analyzed performing a three-way ANOVA for repeated measures with between-subjects variables Meph and EtOH, and a within-subject variable “time”. Differences among AUCs of monoamine levels in dialysates and among total distances traveled were assessed through two-way ANOVA, with Meph and EtOH as variables. The α error probability was set at 0.05. Significant differences were analyzed using a multiple comparison adjustment of P values (Bonferroni and Tukey’s post hoc tests for three-way and two-way ANOVA, respectively). Graphs and calculations of AUCs were performed using GraphPad Prism 6.00 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com).

### Results

#### Effects on 5-HT and DA release

Administration of Meph alone (25 mg/kg, s.c.) produced an increase of both 5-HT and DA in the NAc (Fig. 1a, b) and the mPFC (Fig. 1c, d) which peaked 40 min after the injections and declined until around 180 min, with the exception of mPFC, where dialysate DA levels were still increased at this time point (Fig. 1d). In NAc, the release of 5-HT was much higher (around 10,000–15,000%) than that of DA (around 5000–6000%), whereas in mPFC, the increases of both 5-HT and DA where in a similar range (3000–4000%).

As can be seen in Figs. 1a, c, 2a, c, ethanol combined with Meph provoked an increase in released 5-HT compared with the rats receiving Meph alone (85% in NAc, 65% in mPFC; % of AUC higher than Meph alone), although the overall change in AUC only reached statistical significance in the NAc (P < 0.01). When analyzing the 5-HT values along time, three-way ANOVA showed a significant effect of Meph treatment and interaction between Meph and time in both NAc and mPFC. Moreover, the three-way ANOVA revealed a significant-interaction between Meph and EtOH in NAc (F1,10 = 9.37, P = 0.01, Fig. 1a). Post hoc analyses reported that ethanol potentiated the serotonergic increase induced by Meph along the time points between 20 and 120 min in NAc (Fig. 1a) and at 60 min in the mPFC (Fig. 1c). In the latter region, the significantly increased release of 5-HT with respect to saline lasted longer in the combination than in the Meph group (120 vs. 60 min).

Similarly to 5-HT, DA time-course values in NAc showed significant effects of Meph treatment and time factors (Fig. 1b). DA levels showed a trend to be potentiated by coadministration of EtOH in NAc. In fact, post hoc comparisons revealed a significant difference between Meph and Meph+EtOH groups at 60 min post-administration (3732 ± 291% release in Meph group; 5182 ± 532% in Meph+EtOH group). However, when considering the total DA released during 180 min, analysis of AUC reported an increase of 38% that did not reach statistical significance (Fig. 2b).

In mPFC, although three-way ANOVA revealed effect of time and Meph treatment on DA levels in Meph and Meph+EtOH groups; no differences were observed between them (Figs. 1d and 2c).

EtOH alone, at the dose we used, had no significant effect in both DA and 5-HT basal levels in the studied brain areas.
Effects on 5-HT and DA metabolites

The levels of 5-HIAA showed a slight and non-significant increase (by 10%) in the EtOH-treated rats (Fig. 2b, d). Conversely, 5-HIAA values were significantly decreased by 25% in the rats receiving Meph alone. The two-way ANOVA analysis revealed a significant interaction between Meph and EtOH in both NAc and mPFC, suggesting that EtOH potentiates the decrease in 5-HIAA induced by Meph (NAc $F_{1,10} = 5.57, P < 0.05$; mPFC $F_{1,10} = 3.29, P < 0.05$).

Effects on locomotor activity

Meph administration significantly increased locomotor activity with respect to saline and EtOH (Fig. 3). Moreover, when Meph was combined with EtOH, the increase in locomotion was significantly potentiated with respect to Meph alone, and three-way ANOVA revealed a significant interaction between EtOH and Meph ($F_{1,20} = 7.78; P = 0.01$). The potentiation was apparent at several time points and in the total traveled distance as well.

Discussion

Alcoholic drinks are frequently combined with the new psychostimulant substances such as Meph (Elliott and Evans 2014). Alcohol enhances the subjective effects of other drugs of abuse such as MDMA, and studies have shown that it increases its rewarding and psychostimulant effects (Ben-Hamida et al. 2009). Similarly, our group reported that alcohol increases Meph-induced conditioned place preference and psychostimulant properties in mice (Ciudad-Roberts et al. 2015).

These previous results led us to perform microdialysis studies to assess the effects of such combination on the release of DA and 5-HT in two key areas involved in drug-induced behavior, namely the NAc and mPFC. Release of DA in the NAc is a key process related with the reinforcing and rewarding
properties of a drug (Nestler 2001), and this area projects to other regions which are directly related with drug-induced behavior such as the mPFC. For instance, the mPFC is involved in the establishment of motor (Dalley et al. 2002) and amphetamine-induced (Hammerslag et al. 2014) impulsivity.

We found an increased release of neurotransmitters after administration of Meph in both studied brain areas, being the increases of 5-HT much higher than those of DA, which is in agreement with previous reports (Golembiowska et al. 2016; Keh et al. 2011; Wright et al. 2012). Nevertheless, the increase in DA (peaking around 4000%) was more than sufficient to account for the rewarding effects of the drug. The percentages of increase in neurotransmitters release are over the double than those reported by Wright et al. (2012) in rats administered with 10 mg/kg of Meph (peaks of around 1000% for DA and 2200% for 5-HT in the NAc) which indicates a clear dose-response relationship.

The overall three-way ANOVA of DA levels along time did not reveal a significant interaction between Meph and EtOH in any of the studied brain areas. However, in NAc, a tendency to potentiation of DA release was observed in the Meph + EtOH group with respect to Meph alone between 20 and 100 min post-administration. In fact, the post hoc test showed a significant difference at 60 min. This slight increase might account for increased rewarding effects. Previous experiments in mice point in this direction (Ciudad-Roberts et al. 2015). By contrast, we found a significant potentiation of Meph-induced 5-HT release by EtOH in NAc. The increase in 5-HT was much higher than that of DA, and the addition of EtOH did not change this proportionality, which contrasts with the work by Riegert et al. (2008) using superfused striatal slices and reporting that addition of EtOH shifted the MDMA-induced monoamine overflow towards higher DA release. This difference could be mainly explained by the different inhibition profile of Meph and MDMA at DA and 5-HT transporters: Meph shows IC₅₀ for DA and 5-HT uptake inhibition of the same order of magnitude, whereas MDMA shows a much higher potency inhibiting 5-HT than DA uptake (Hadlock et al. 2011). Also, the examined areas (striatum vs. NAc), doses, and the different techniques may partially contribute to such difference. In mPFC, the overall interaction between Meph and EtOH did not reach statistical significance, although the post hoc analysis revealed a significant potentiation of 5-HT release by combination with EtOH at the 60-min time point.

The effects of EtOH are multiple and complex (reviewed by Clapp et al. 2008 and Siggins et al. 2005), including
increased release of DA in the NAc. However, at the dose we used, EtOH alone had few or no significant effect on neurotransmitter levels, suggesting that the effect in animals receiving the drug combination is due to a synergistic pharmacodynamic or pharmacokinetic interaction rather than to a simple addition. A number of interactions of EtOH with other drugs have been reported. For example, Ben-Hamida et al. (2009) demonstrated that ethanol is capable of increasing the concentration of MDMA in areas with high DA transmission (striatum and frontal cortex) in a much higher proportion than in the hippocampus. Although no mechanistic description was found for this effect, we cannot rule out a similar pharmacokinetic interaction between ethanol and Meph in the brain areas we studied.

The increase in DA and 5-HT in the rats receiving Meph was accompanied by a decrease in the levels of their metabolites. This can be attributed to monoamine uptake inhibition by Meph (Baumann et al. 2012, López-Arnau et al. 2012) because the key enzyme in the metabolism of DA and 5-HT, namely monoaminoxidase (MAO), is localized inside the nerve terminal, so that monoamines have to be taken up to be metabolized. As Meph blocks reuptake, a decrease in metabolites is detected despite the increase in monoamine release. A similar effect was described by Kehr et al. (2011). This mechanism is backed by the fact that EtOH alone, which induces DA release but does not inhibit uptake, does not produce a decrease, but rather tends to increase the metabolites. Statistical analysis revealed a significant interaction between Meph and EtOH treatment with respect to 5-HIAA levels, suggesting that Meph exerts a higher blockade of the transporter in presence of EtOH. A direct effect of EtOH on serotonin transporter can be ruled out because a decrease in 5-HIAA levels should have been shown in the EtOH group (Daws et al. 2006). Therefore, this is in agreement with the possibility of a pharmacokinetic interaction beforehand mentioned, leading to increased interstitial levels of Meph that would further prevent 5-HT reuptake and metabolism. Additional studies need to be performed to corroborate this hypothesis. Similar results were described by Cassel et al. (2005) when studying the effects of MDMA combined with EtOH on monoamine levels and their metabolites.

To assess whether the observed potentiation of neurotransmitter release in the Meph+EtOH group had a measurable effect on the psychostimulant properties of Meph, we performed locomotor activity experiments in rats using the same doses and combinations that we did in the microdialysis...
assays. The results demonstrated that the combination of EtOH with Meph potentiates the psychostimulant effects of the cathinone. Moreover, the higher hyperlocomotion periods co-incide with the peaks in neurotransmitters. The observed increases in 5-HT-release could be responsible of such potentiation of locomotor activity. In fact, previous works demonstrate a role of 5-HT receptors on Meph-induced hyperlocomotion as it was reduced by administration of ketanserin (López-Arnau et al. 2012; Ciudad-Roberts et al. 2015). Moreover, elevated 5-HT release in the nPFC positively relates with motor impulsivity, which is related with drug relapse (Dalley et al. 2002; Hammerslag et al. 2014).

To sum up, in this work, we demonstrate a potentiation of the increase in monoamine release and in psychostimulant effects when combining Meph plus EtOH which might involve increased psychostimulant subjective effects and therefore increased abuse liability. Therefore, an experimental-based warning concerning the risks regarding the combined consumption of these drugs could be conveyed to the population at large.

Funding This work was supported by grants from “Plan Nacional sobre Drogas” (20121102) and from “Plan Nacional de Investigación Científica” (SAF2013-46135-P). Our group holds the quality mention from the “Generalitat de Catalunya” (2014SGR1081). None of the funding sources had further role in study design, collection, analysis, and interpretation of data, in the writing of the report and in the decision to submit the paper for publication. R. López-Arnau position was funded by an institutional program of the Universitat de Barcelona in collaboration with Obra Social de la Fundació Bancària La Caixa.

Compliance with ethical standards All animal care and experimental protocols in this study complied with the guidelines of the European Community Committee (86/609/ECC) and were approved by the Animal Ethics Committee of the University of Barcelona. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Conflict of interest The authors declare that they have no conflict of interest.

References

Baumann MH, Ayestas MA Jr, Partilla JS, Sink JR, Shulgin AT, Daley PF, Brandt SD, Rothman RB, Ruoho AE, Cozzi NV (2012) The designer methcathinone analogs, mephedrone and methyleneone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 37(5):1192–1203. https://doi.org/10.1038/npp.2011.304

Ben-Hamida S, Tracqui A, de Vasconcelos AP, Szwarc E, Lazarus C, Kelche C, Jones BC, Cassel JC (2009) Ethanol increases the distribution of MDMA to the rat brain: possible implications in the ethanol-induced potentiation of the psychostimulant effects of MDMA. Int J Neuropsychopharmacol 12(3-4):749–759. https://doi.org/10.1111/j.1476-5381.1982.tb00780.x

Brabant C, Guamieri DJ, Quertemont E (2014) Stimulant and motivational effects of alcohol: lessons from rodent and primate models. Pharmacol Biochem Behav 122:37–52. https://doi.org/10.1016/j.pbb.2014.03.006

Brodie MS, Trifunović RD, Shefner SA (1995) Serotonin potentiates ethanol-induced excitation of ventral tegmental area neurons in brain slices from three different rat strains. J Pharmacol Exp Ther 273(3):1139–1146

Brunt TM, Koeter MW, Niesink RJ, van den Brink W (2012) Linking the pharmacological content of ecstasy tablets to the subjective experiences of drug users. Psychopharmacology 220(4):751–762. https://doi.org/10.1007/s00213-011-2529-4

Cassel JC, Jeltsch H, Koenig J, Jones BC (2004) Locomotor and pyretic effects of MDMA-ethanol associations in rats. Alcohol 34(2-3):285–289. https://doi.org/10.1016/j.alecohab.2004.09.003

Cassel JC, Rieger C, Rutz S, Koenig J, Rothmaier K, Cosquer B, Lazarus C, Bärthelmer A, Jeltsch H, Jones BC, Jackisch R (2005) Ethanol, 3, 4-methylenedioxymethamphetamine (ecstasy) and their combination: long-term behavioral, neurochemical and neuropharmacological effects in the rat. Neuropsychopharmacology 30(10):1870–1882. https://doi.org/10.1038/sj.npp.1300714

Ciudad-Roberts A, Camarasa J, Ciudad CJ, Pubill D, Escudero E (2015) Alcohol enhances the psychostimulant and conditioning effects of mephedrone in adolescent mice; postulation of unique roles of D3 receptors and BDNF in place preference acquisition. Br J Pharmacol 172:4970–4984. https://doi.org/10.1111/bph.13266.

Ciudad-Roberts A, Duart-Castells L, Camarasa J, Pubill D, Escudero E (2016) The combination of ethanol with mephedrone increases the signs of neurotoxicity and impairs neurogenesis and learning in adolescent CD-1 mice. Toxicol Appl Pharmacol 293:10–20. https://doi.org/10.1016/j.taap.2015.12.019.

Clapp P, Bhave SV, Hoffman PL (2008) How adaptation of the brain to alcohol leads to dependence: a pharmacological perspective. Alcohol Res Health 31(4):310–319.

Dalley JW, Theobald DE, Eagle DM, Passetti F, Robbins TW (2002) Deficits in impulse control associated with tonically- elevated serotonergic function in rat prefrontal cortex. Neuropsychopharmacology 26(6):716–728. https://doi.org/10.1016/S0893-133X(01)00412-2.

Daws LC, Montañez S, Munn JL, Owens WA, Baganz NL, Boyce-Rustay JM, Millstein RA, Wiedholz LM, Murphy DL, Holmes A (2006) Ethanol inhibits clearance of brain serotonin by a serotonin transporter-independent mechanism. J Neurosci 26(24):6431–6438. https://doi.org/10.1523/JNEUROSCI.4050-05.2006.

Elliot S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243:55–60. https://doi.org/10.1016/j.forsciint.2014.04.017.

Golembiowska K, Jurczak A, Kaminska K, Noworyta-Sokolowska K, Gorska A (2016) Effect of some psychoactive drugs used as “legal highs” on brain neurotransmitters. Neurotox Res 29:394–407. https://doi.org/10.1007/s12640-015-9569-1.

Hadlock GC, Webb KM, McFadden LM, Chu PW, Ellis JD, Allen SC, Andrenyak DM, Vieira-Brock PL, German CL, Conrad KM, Hoonakker AJ, Gibb JW, Wilkins DG, Hanson GR, Fleckenstein AE (2011) 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designer stimulant of abuse. J Pharmacol Exp Ther 339(2):530–536. https://doi.org/10.1124/jpet.111.184119.

Hammerslag LR, Waldman AJ, Gulley JM (2014) Effects of amphetamine exposure in adolescence or young adulthood on inhibitory control in adult male and female rats. Behav Brain Res 263:22–33. https://doi.org/10.1016/j.bbr.2014.01.015.

Imperato A, Di Chiara G (1986) Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 239(1):219–228.

Jones BC, Ben-Hamida S, de Vasconcelos AP, Kelche C, Lazarus C, Jackisch R, Cassel JC (2010) Effects of ethanol and ecstasy on...
conditioned place preference in the rat. J Psychopharmacol 24(2): 275–279. https://doi.org/10.1177/0269881109102775

Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, Yoshitake T (2011) Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. Br J Pharmacol 164(8):1949–1958. https://doi.org/10.1111/j.1476-5381.2011.01499.x

López-Arnau R, Martínez-Clemente J, Pubill D, Escubedo E, Camarasa J (2012) Comparative neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylone. Br J Pharmacol 167:407–420. https://doi.org/10.1111/j.1476-5381.2012.01998.x.

López-Arnau R, Martínez-Clemente J, Rodrigo T, Pubill D, Camarasa J, Escubedo E (2015) Neuronal changes and oxidative stress in adolescent rats after repeated exposure to mephedrone. Toxicol Appl Pharmacol 286(1):27–35. https://doi.org/10.1016/j.taap.2015.03.015

Martínez-Clemente J, López-Arnau R, Carbó M, Pubill D, Camarasa J, Escubedo E (2013) Mephedrone pharmacokinetics after intravenous and oral administration in rats: relation to pharmacodynamics. Psychopharmacology 230(2):295–306. https://doi.org/10.1007/s00213-013-3108-7

Mitchell JM, O'Neil JP, Janabi M, Marks SM, Jagust WJ, Fields HL (2012) Alcohol consumption induces endogenous opioid release in the human orbitofrontal cortex and nucleus accumbens. Sci Transl Med 4(116):116ra6. https://doi.org/10.1126/scitranslmed.3002902

Mohamed WM, Ben Hamida S, de Vasconcelos AP, Cassel JC, Jones BC (2009) Interactions between 3,4-methylenedioxymethamphetamine and ethanol in humans and rodents. Neuropsychobiology 60(3-4): 188–194. https://doi.org/10.1159/000253554

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2(2):119–128. https://doi.org/10.1038/3503570

O'Neill C, McElrath K (2012) Simultaneous use of mephedrone and alcohol: a qualitative study of users’ experiences. J Addict Res Ther S9-001(02):1–5. https://doi.org/10.4172/2155-6105.S9-001

Riegert C, Wedekind F, Ben Hamida S, Rutz S, Rothmaier AK, Jones BC, Cassel JC, Jackisch R (2008) Effects of ethanol and 3,4-methylenedioxymethamphetamine (MDMA) alone or in combination on spontaneous and evoked overflow of dopamine, serotonin and acetylcholine in striatal slices of the rat brain. Int J Neuropsychopharmacol 11(06):743–763. https://doi.org/10.1017/S1461145708008481

Siggins GR, Roberto M, Nie Z (2005) The tipsy terminal: presynaptic effects of ethanol. Pharmacol Ther 107:80–98

Wright MJ Jr, Angrish D, Aarde SM, Barlow DJ, Buczynski MW, Creehan KM, Vandewater SA, Parsons LH, Houseknecht KL, Dickerson TJ, Taffe MA (2012) Effect of ambient temperature on the thermoregulatory and locomotor stimulant effects of 4-methylmethcathinone in Wistar and Sprague-Dawley rats. PLoS One 7(8):e44652. https://doi.org/10.1371/journal.pone.0044652