Original Paper

Neurocognitive performance following acute mephedrone administration, with and without alcohol

Journal of Psychopharmacology
2016, Vol. 30(12) 1305–1312

EB de Sousa Fernandes Perna[1*], E Papaseit[2,3,4 *], C Pérez-Mañá[2,3], J Mateus[2,3], EL Theunissen[1], KPC Kuypers[1], R de la Torre[2,5,6], M Farré[2,3,4 *] and JG Ramaekers[1*]

Abstract

Recreational use of mephedrone, alone and in combination with alcohol, has increased over the past years. Pharmacological properties of mephedrone share similarities with methylenedioxymethamphetamine (MDMA), but its effect on neurocognitive function has not been well established in humans. The present study assessed the effect of mephedrone alone and after co-administration with alcohol on neurocognitive function. It was hypothesised that mephedrone would improve psychomotor performance but impair memory performance, when administered alone. Neurocognitive performance was expected to be impaired following mephedrone when combined with alcohol. Eleven participants received single doses of 200 mg mephedrone or placebo combined with 0.8 g/kg alcohol or placebo. Neurocognitive performance was assessed at baseline (T_0), at one hour (T_1) and four hours after (T_2) mephedrone administration, by means of the Divided Attention Task (DAT), Critical Tracking Task (CTT), and the Spatial Memory Test (SMT). Mephedrone intoxication impaired short-term spatial memory at T_1 and improved critical tracking performance at T_2. Mephedrone alone did not affect divided attention, but did show an interaction with alcohol on reaction time at T_2. Reaction time decreased when mephedrone was combined with alcohol as compared to alcohol alone. Alcohol intoxication impaired both short- and long-term spatial memory at T_1 and divided attention at T_1 and T_2. Critical tracking performance was not affected by alcohol intoxication. The current findings support the hypothesis that mephedrone improves psychomotor performance, impairs spatial memory and does not affect divided attention performance. Stimulatory effects of mephedrone were not sufficient to compensate for the impairing effects of alcohol on most performance parameters.

Keywords

Acute, mephedrone, spatial memory, alcohol co-administration, psychomotor performance

Introduction

A large number of new psychoactive substances (NPSs) have rapidly spread across Europe and the USA over the last decade (Corazza et al., 2013). The United Nations Office on Drugs and Crime (2014) reported that the majority of NPSs between 2008–2013 were synthetic cannabinoids (28%) and synthetic cathinones (25%), followed by phenethylamines (17%). Synthetic cathinones are marketed as 'legal highs', 'bath salts' or 'plant feeders' under labels stating 'not for human consumption'. Recreational use of these substances has gained popularity despite legal actions taken to ban their use (European Monitoring Centre for Drugs and Drug Addiction, 2015).

4-Methylmethcathinone (4-MMC) or mephedrone is a prototypical compound from the synthetic cathinone group (Deluca et al., 2012). Results from a web-based survey show that mephedrone was the sixth most commonly used drug after classical drugs such as alcohol, tobacco, cannabis, 3,4-methylenedioxymethamphetamine (MDMA) and cocaine (Winstock et al., 2011a). The Crime Survey of England and Wales indicates a previous year prevalence of 0.6% in individuals aged 16–59 years and 1.9% in individuals aged 16–24 years, which meant that mephedrone was the second most frequently used drug after MDMA/ecstasy (Home Office, 2014). This is especially striking considering the lack of knowledge about the potential harm that synthetic cathinones pose to individual health and society (Green and Nutt, 2014). Mephedrone is usually administered through oral and/or intranasal routes. Acute effects include euphoria, alertness, sweating, loss of appetite and jaw clenching (Dines et al., 2015; Winstock et al., 2011a,b). Users often report that stimulant,

[1]Department of Neuropsychology and Psychopharmacology, Maastricht University, Maastricht, the Netherlands
[2]Integrative Pharmacology & Neurosciences Systems Research Group, Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain
[3]Universitat Autonoma de Barcelona, Barcelona, Spain
[4]Hospital Universitari Germans Trias i Pujol, Clinical Pharmacology, Badalona, Spain
[5]Spanish Biomedical Research Centre in Physiopathology of Obesity and Nutrition (CIBEROBN), Santiago de Compostela, Spain
[6]Universitat Pompeu Fabra, CEXS-UPF, Barcelona, Spain

*These authors contributed equally

Corresponding author:
EB de Sousa Fernandes Perna, Department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, 6200 MD, the Netherlands.
Email: e.desousafernandes@maastrichtuniversity.nl

Table 1. Participant demographics and history of alcohol and drug use.

	Mean (SD)	Min.	Max.
Age (years)	28.2 (6.7)	22.0	39.0
BMI (kg/m²)	23.8 (2.6)	19.0	26.8
Alcohol use/day	2.4 (1.1)	1.0	4.0
Tobacco use/day (*n*=6)	3.4 (5.0)	0.0	15.0
Lifetime use of other drugs	**Current user**	**Past user**	**Total**
Mephedrone	4	0	4
MDMA	9	1	10
Cannabis	8	2	10
Cocaine	10	1	11
GHB	0	1	1
BDZ	1	1	2
Mushrooms	0	6	6
Ketamine	2	5	7
2-CB	2	3	5
Other (e.g. LSD, METH, AMP, opioids)	3	6	9

AMP: amphetamine; BDZ: benzodiazepine; 2-CB: 4-bromo-2,5-dimethoxy-phenethylamine; GHB: gamma hydroxybutyrate; LSD: lysergic acid diethylamide; MDMA: 3,4-methylenedioxymethamphetamine; METH: methamphetamine.

euphoric, and emphatic properties of mephedrone are comparable to those induced by MDMA (Carhart-Harris et al., 2011). This suggests that behavioural and cognitive effects of mephedrone may be similar to those reported with MDMA as well.

Similarly to amphetamines and MDMA, cathinones act as behavioural stimulants and promote the release of monoamine neurotransmitters, including serotonin (5-HT), dopamine (DA) and noradrenaline (NA) (Baumann et al., 2011; Simmler et al., 2013). Mephedrone non-selectively inhibits monoamine re-uptake with a higher affinity for DA compared to 5-HT transporters (López-Arnau et al., 2012). Cathinones also stimulate 5-HT_{2A} receptors with affinities that are similar to those of MDMA (López-Arnau et al., 2012). Doses between 25–75 mg of mephedrone produce a rapid but short-lasting effect when insufflated, appearing within minutes, and lasting less than an hour. Oral administration usually occurs in doses ranging between 150–250 mg and elicits an effect after 45–60 min lasting for about 1–2 h (Schifano et al., 2011). Mephedrone is often consumed in a binge-like manner, during which small doses (i.e. 0.5–1 g) are taken repeatedly over a period of approximately 8–10 h (Winstock et al., 2011a). The majority of mephedrone users frequently combine mephedrone with other drugs such as alcohol, cocaine, ecstasy, cannabis and ketamine (Deluca et al., 2012; Psychonaut WebMapping Research Group, 2009). Online surveys conducted among mephedrone users indicate simultaneous mephedrone and alcohol consumption on a regular basis (Carhart-Harris et al., 2011; O'Neill and McElrath, 2012).

Despite the prevalence of combined alcohol-mephedrone use, information on the combined effects on cognitive performance is generally lacking. To date, no experimental placebo-controlled studies have been conducted to assess the effects of mephedrone or the combination of mephedrone and alcohol on cognitive function. A naturalistic study reported on the effects of mephedrone on memory, executive functioning and psychomotor performance (Freeman et al., 2012). Cognitive function of 20 mephedrone users was assessed during intoxication and during abstinence. Results indicated that mephedrone impaired memory performance and improved psychomotor speed. Although relevant, methodological limitations such as polydrug use, unknown dose and purity of mephedrone may hamper straightforward interpretation of results. In order to prevent these limitations, the present study was conducted according to a placebo-controlled experimental design.

The present study assessed the acute effects of a single mephedrone dose on neurocognitive performance in healthy males. In addition, the cognitive effects of mephedrone were also investigated after co-administration with alcohol to see whether their separate effects are additive or synergistic. Cognitive performance was assessed by means of a Spatial Memory Test, Critical Tracking Test and a Divided Attention Test. We expected that the effects of mephedrone on neurocognitive function would be similar to those induced by MDMA (e.g. Dumont et al., 2008; Kuypers and Ramaekers, 2005, 2007; Ramaekers et al., 2009; Van Wel et al., 2011) and that neurocognitive effects of mephedrone and alcohol combined would be additive. Subsequently, it was hypothesised that mephedrone would impair memory performance, improve psychomotor performance and leave divided attention intact when administered alone and that neurocognitive performance would be generally impaired when combined with alcohol.

Methods

Participants

Twelve male participants were recruited for this study. One participant dropped out due to personal circumstances. A total of 11 non-dependent polydrug users aged between 22–39 years (mean (standard deviation (SD)) 28.2 (6.7)) completed the study. Participants' demographics are shown in Table 1.

Participants were recruited through word of mouth. All participants were informed about the study characteristics and provided written informed consent. The participants underwent a medical examination including routine laboratory tests (e.g. biochemistry, haematology, coagulation, urine, serology for hepatitis and human immunodeficiency virus (HIV)) and electrocardiogram (ECG). Participants who were eligible for participation underwent a psychiatric interview (Psychiatric Research Interview for Substance and Mental Disorders (PRISM)) to exclude the presence of major psychiatric disorders, history of abuse or drug dependence (except for nicotine dependence), and psychiatric adverse drug reactions.

Inclusion criteria included: (a) males aged between 18–45 years; (b) free from organic or psychiatric disorders; (c) good physical health; (d) body mass index (BMI) within 18.5–28 kg/m²; (e) presence of CYP2D6 genotypes resulting in the intermediate, extensive or ultrarapid metaboliser phenotypes (as determined by dextromethorphan tests in urine), and (f) consumption of one of the following substances: amphetamines, ecstasy and hallucinogenic derivatives, mephedrone or other cathinones on at least six occasions in a lifetime (two in the previous year). Only participants who were phenotypically CYP2D6 extensive metabolisers (e.g. intermediate, extensive or ultrarapid metaboliser) were included in order to reduce the potential risk of developing acute mephedrone toxicity. Exclusion criteria included: (a) addiction according to Diagnostic and Statistical Manual of Mental Disorders IV Text Revision (DSM-IV-TR) criteria; (b) presence of individual psychiatric history or schizophrenia in

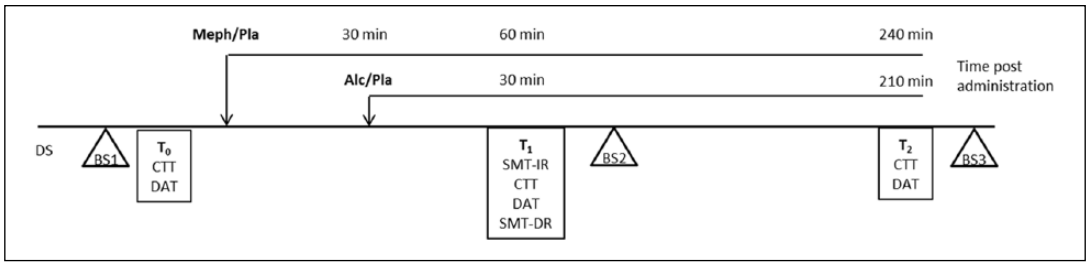

Figure 1. Schematic representation of a test day. Alc: alcohol; BS: blood sample; CTT: Critical Tracking Test; DR: delayed relocation; DS: drug screens; DAT: Divided Attention Test; IR: immediate relocation; Meph: mephedrone; Pla: placebo; SMT: Spatial Memory Test.

first-degree relatives; (c) regular intake of psychotropic medication in the month preceding the study; (d) cardiovascular, gastrointestinal, hepatic, and renal abnormalities; (e) excessive smoking (>20 cigarettes per day) or drinking (>4 units/day or 40 g/day); and (f) testing positive for hepatitis B and/or hepatitis C and/or HIV.

This study was part of a phase I clinical trial, to establish pharmacokinetic, metabolic and pharmacological effects of mephedrone and was conducted according to the code of ethics on human experimentation established by the Declaration of Helsinki (2013). Since the primary aim of this study was to evaluate safety and identify side effects, the sample size was limited to a small number of participants. The study was approved by the Research Ethical Committee of the Parc de Salut Mar (CEIC-PSMAR) in Barcelona. Mephedrone was obtained through Minister of Justice and Minister of Health. The study was carried out at the Institut Hospital del Mar d'Investigacions Mèdiques (IMIM). Participants received monetary compensation for their participation in the study.

Design and treatments

The study was conducted according to a double-blind, placebo-controlled, four-way cross-over design. The four treatment conditions consisted of: (a) mephedrone placebo + alcohol placebo, (b) mephedrone + alcohol placebo, (c) mephedrone placebo + alcohol and (d) mephedrone + alcohol. The order of treatment conditions was balanced over participants and sessions. Conditions were separated by a minimum washout period of seven days to avoid carry-over effects.

Mephedrone (200 mg) and mephedrone placebo (200 mg lactose) were administered orally in identically appearing (form and colour) capsules. Alcohol at a dose of 0.8 g/kg was served cold in the form of a combination of Absolut Vodka (Ahus, Sweden; 40% ethanol) and lemon-flavoured Fontvella water. Placebo drinks consisted solely of lemon-flavoured Fontvella water. The total volume to be taken was 350 mL during a period 15 min (117 mL every 5 min). Alcohol and alcohol placebo were similar in appearance (colour) in order to mask their content to the participants and researchers.

The mephedrone dose was selected in a series of pilot studies that included doses of 50 mg, 100 mg, 150 mg and 200 mg of mephedrone, and 100 mg MDMA (Papaseit et al., 2016). The selected dose of 200 mg was well tolerated and showed similar subjective and physiological effects to those induced by MDMA. Blood alcohol concentrations (BACs) of 0.8 g/L represent a regular dose of alcohol.

Procedures

Participants were asked to refrain from drug use at least two weeks prior to the start and during the study. Single doses of medication for symptomatic treatment (e.g. acetaminophen for headache) were allowed up to one week preceding the experimental session. Participants were not allowed to consume alcohol (48 h) or caffeine-containing beverages (24 h) before and after the onset of an experimental session and they were requested to arrive well rested. Drug and alcohol screens were carried out upon arrival at the testing facilities. Urine drug screens (Instant-View, Multipanel 10 Test Drug Screen, Alfa Scientific Designs Inc., Poway, California, USA) assessed for the presence of amphetamine, barbiturates, benzodiazepines, cocaine metabolite, methamphetamine, morphine, methadone, phencyclidine (PCP), tetrahydrocannabinol (THC) and MDMA. Study treatments were only administered after negative drug screens. Baseline BAC was measured with an alcohol breathalyser.

Mephedrone (or mephedrone placebo) administration was completed at 1 h prior to cognitive assessments. Alcohol drinks were administered 30 min after mephedrone and finished within 15 min (Figure 1). Neurocognitive performance was assessed three times, i.e. at baseline (T_0), at 1 h (T_1) and 4 h after (T_2) mephedrone administration. The DAT and CTT were administered at T_0, T_1 and T_2. The SMT was only assessed at peak drug concentrations (T_1) to prevent the occurrence of learning effects that might arise from sequential assessments. Baseline conditions served to assess differences in performance prior to placebo and drug administration. All participants received a training session before onset of the experimental sessions in order to familiarise them with tests and procedures.

Neurocognitive assessment

SMT. The SMT (derived from the object relocation test (Kessels et al., 1999; Sambeth et al., 2009)) consists of an immediate and a delayed relocation phase. The immediate relocation phase is composed of six trials in which ten black-and-white pictures (total 60 pictures) were subsequently presented at different locations on a computer screen. The participants had to remember the location of the pictures. After every trial, the pictures reappeared one by one in the middle of the screen followed by the presentation of a '1' and a '2' in different locations. The participants had to indicate on a keyboard whether each picture corresponded with either location 1 or 2 by means of button presses made with the left (key 'z') and right (key 'm') index fingers respectively. The delayed relocation phase

was completed after a 30 min delay in which the pictures reappeared in a random order in the middle of the screen after which participants had to indicate the correct picture location. The number of correct relocations in each of the six trials was summed to total immediate and delayed relocation scores and the respective reaction times were averaged accordingly. Dependent variables are the Immediate Relocation Score, mean Immediate Reaction Time, Delayed Relocation Score and mean Delayed Reaction Time. Four parallel versions of this test were balanced over test days. The duration of the task was approximately 10 min for the immediate relocation phase and 5 min for the delayed relocation phase respectively.

CCT. The CTT assesses continuous psychomotor reaction time (Jex et al., 1966), which measures the participant's ability to control a displayed error signal in a first-order compensatory tracking task. Error appears as horizontal deviation of a cursor from midpoint on a horizontal, linear scale. Compensatory joystick movements null the error by returning the cursor to the midpoint. The frequency of cursor deviations, and therefore its velocity, increases as a stochastic, linear function of time. The participants were required to make compensatory movements with a progressively higher frequency. Eventually the response frequency lags the error signal by 180°. At that point, the participant's response adds to, rather than subtracts from, the error and control is lost. The frequency at which control loss occurs is referred to as 'lambda-c' (the 'critical frequency'). The test included five trials from which the lowest and highest scores were discarded. The average of the remaining trial scores was the final dependent lambda-c score. Total task duration was approximately 1–2 min.

DAT. The DAT assesses one's ability to divide attention between two tasks performed simultaneously (Moskowitz, 1973). The primary task requires the use of a joystick to continuously null the horizontal movement of a cursor from the centre of a display. The cursor travels in both directions with irregular velocity, on the average, 50% of that which is just controllable by the particular participant. Tracking error is measured by the absolute distance (mm) between the cursor's position and the centre. Mean absolute Tracking Error and the number of Control Losses are the dependent variables of the primary task. As a secondary task, the participant monitors 24 single digits in the corners of the computer screen. The numbers change asynchronously every 5 s. The requirement is to react as rapidly as possible by lifting the foot from a pedal any time a target, the target number '2', appears. The number of Correct Detections (hits) and the mean Reaction Time to targets are dependent variables of the secondary task. Total task duration was 12 min.

Pharmacokinetics

A blood sample (BS) to determine mephedrone and alcohol concentrations in plasma was collected at three successive times during each test day (Figure 1), i.e. at baseline (BS1), 5 min after the first cognitive assessment (i.e. 1.5 h post drug; BS2) and 5 min after the second cognitive assessment (i.e. 4 h post drug; BS3). Blood samples were centrifuged immediately and the serum was subsequently frozen at –20°C until analyses for pharmacokinetic assessments.

Analysis of mephedrone concentrations in plasma

Mephedrone plasma concentrations were quantified by gas chromatography-mass spectrometry (GC-MS). A liquid-liquid extraction was performed with tert-butyl methyl ether and the silylation reagent (N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA)) was used for the derivatisation of mephedrone.

Analysis of alcohol concentrations in plasma

Alcohol plasma concentrations were determined with an enzymatic test (DRI Ethyl Alcohol Assay, Thermo Scientific) in an autoanalyser (Indiko Plus, Thermo Fisher Scientific).

Statistics

The effects of mephedrone and alcohol were analysed by means of a general linear model (GLM) repeated measures analysis of variance (ANOVA) with main factors Mephedrone (two levels: mephedrone and placebo) and Alcohol (two levels: alcohol and placebo) for the three time points separately (i.e. T_0, T_1 and T_2). If the sphericity assumption was violated, the Greenhouse-Geisser correction was used. The alpha criterion significance level was set at p=0.05. All statistical tests were conducted with SPSS version 20.1. Partial eta squared (i.e. η^2_p) values were calculated as a measure of effect size for mean differences.

Results

Eleven complete data sets entered statistical analyses. Mean (standard error (SE)) scores of cognitive performance measures at baseline, T_1 and T_2 in each treatment condition are given in Figures 2 and 3.

Baseline (T_0)

GLM analyses revealed no difference in CTT and DAT performance prior to treatments.

Performance measures at T_1 and T_2

SMT. GLM analyses revealed a main effect of Mephedrone (F1,10=4.99; p=0.050; η^2p=0.333) on Immediate Relocation Scores in the SMT (Figure 2). Participants made fewer correct relocations during mephedrone intoxication compared to placebo conditions. Delayed Relocation Scores were not affected by mephedrone. There was a main effect of Alcohol on Immediate Relocation Scores (F1,10=5.82; p=0.037; η^2_p=0.368) and Delayed Relocation Scores (F1,10=15.32; p=0.003; η^2_p=0.605). Participants made fewer correct immediate and delayed relocations during alcohol intoxication compared to placebo. There was no interaction between Mephedrone and Alcohol on any of the SMT outcome measures. Immediate or Delayed Reaction Times in the SMT were not affected by any factor.

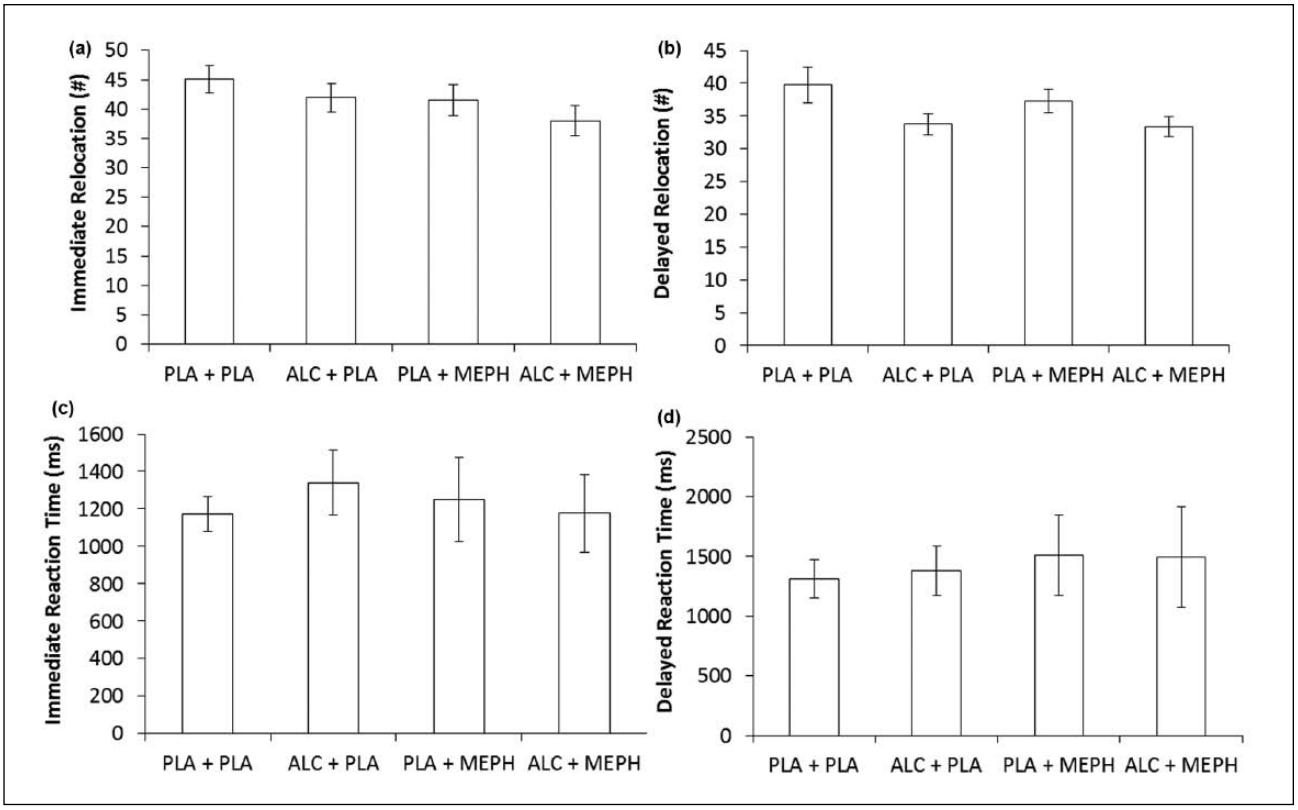

Figure 2. Mean (standard error of the mean (SEM)) (a) immediate and (b) delayed relocation scores and (c) immediate and (d) delayed reaction times in the Spatial Memory Test for the four treatment conditions at T_1. ALC: alcohol; MEPH: mephedrone; PLA: placebo.

CTT. GLM analyses revealed no main effects of Alcohol (p=0.065), Mephedrone or their interaction on lambda-c scores at T_1 (Figure 3(a)), but did show a main effect of Mephedrone (F1,10=10.78; p=0.008; η^2_p=0.519) on lambda-c scores at T_2. Participants had higher lambda-c scores during mephedrone intoxication compared to placebo. Alcohol or Alcohol×Mephedrone did not affect lambda-c scores at T_2.

DAT. Mephedrone did not affect DAT performance at T_1 (Figure 3(b)–(d)). GLM analyses revealed a main effect of Alcohol on Tracking Error (F1,10=13.69; p=0.004; η^2_p=0.578), Control Loss (F1,10=7.49; p=0.021; η^2_p=0.428) and Reaction Time (F1,10=13.93; p=0.004; η^2_p=0.582) at T_1. Alcohol increased Tracking Error and Control Loss in the primary task and Reaction Time in the secondary task of the DAT. Interactions between mephedrone and alcohol did not reach significance for any of the DAT outcome measures at T_1.

Mephedrone did not affect DAT performance at T_2. There was a main effect of Alcohol on Tracking Error (F1,10=9.84; p=0.011; η^2_p=0.496) at T_2, indicating more tracking error during alcohol intoxication. Reaction Time was significantly affected by the interaction between Mephedrone and Alcohol (F1,10=12.89; p=0.005; η^2_p=0.563). Reaction time increased when mephedrone and alcohol were given alone compared to placebo, but was decreased compared to placebo when mephedrone and alcohol were combined. Post-hoc tests revealed a significant difference (p=0.008) between alcohol and alcohol+mephedrone conditions.

Pharmacokinetics

Mean mephedrone and alcohol plasma concentrations at baseline and following treatments are shown in Table 2. Two participants showed positive mephedrone concentrations in baseline BSs during the mephedrone+placebo condition (i.e. 2.91 ng/mL) and during the mephedrone+alcohol condition (i.e. 1.80 and 0.80 ng/mL), but concentrations were very low. These participants were not excluded.

Discussion

The aim of the present study was to evaluate the effect of mephedrone alone and after co-administration with alcohol on neurocognitive function. This is the first placebo-controlled experimental study that assessed the acute effects of mephedrone on neurocognitive function. Participants were given single doses of 200 mg mephedrone or placebo combined with 0.8 g/kg alcohol or alcohol-placebo after which cognitive performance was assessed by means of the SMT, CTT and DAT. Mephedrone intoxication impaired short-term spatial memory and improved critical tracking performance. Reaction time in the DAT decreased when mephedrone was combined with alcohol as compared to alcohol alone. Alcohol intoxication impaired spatial memory and divided attention.

Mephedrone intoxication led to impairment of short-term spatial memory at T_1. This finding is in line with data from a naturalistic study in mephedrone users (Freeman et al., 2012) and is also

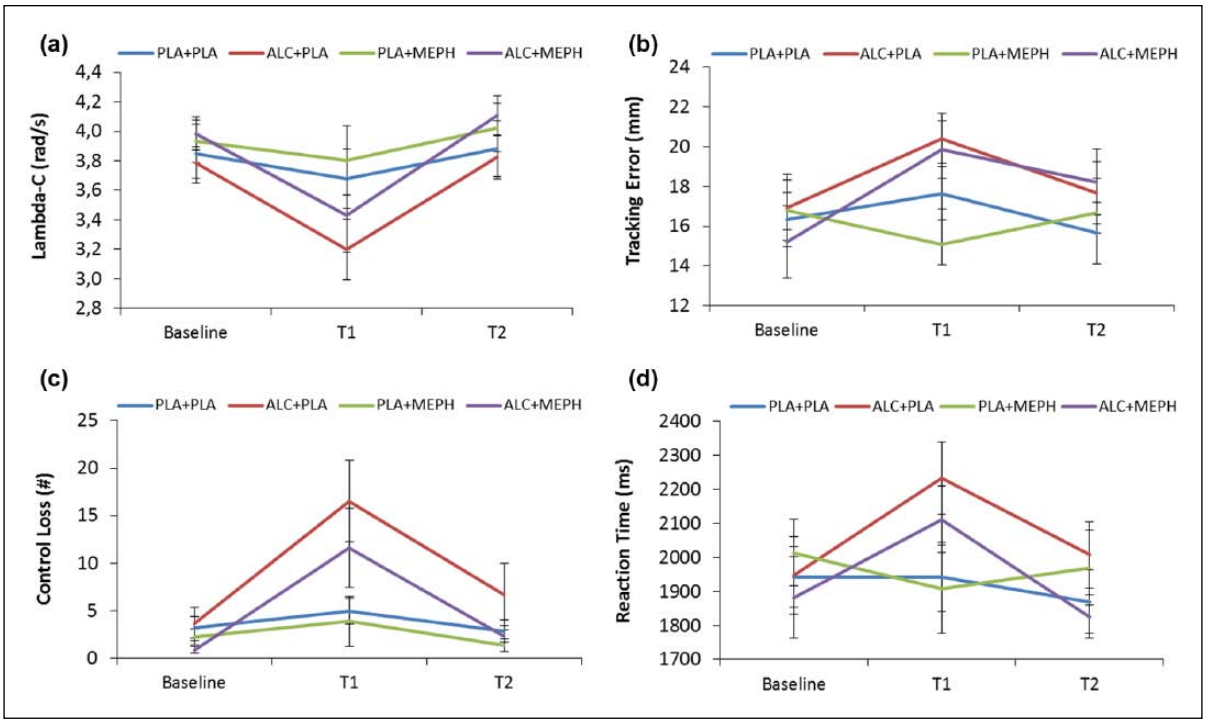

Figure 3. Mean ((standard error of the mean (SEM)) lambda-c score in the (a) Critical Tracking Test, (b) Tracking Error, (c) Control Loss and (d) Reaction Time in the Divided Attention Test for each cognitive assessment. ALC: alcohol; MEPH: mephedrone; PLA: placebo.

Table 2. Mean (standard error (SE)) concentrations of mephedrone and alcohol in plasma at the different time points (n=11).

Time	Mephedrone (ng/mL)		Alcohol (mg/dL)	
	Meph	Meph+Alc	Alc	Alc+Meph
BS 1; baseline	0.3 (0.3)	0.2 (0.2)	0.0 (0.0)	0.0 (0.0)
BS 2; 1.5 h post drug	159.7 (26.2)	159.3 (21.4)	110.7 (7.3)	93.8 (4.9)
BS 3; 4 h post drug	77.5 (14.7)	63.8 (12.1)	74.4 (2.4)	73.9 (5.1)

Alc: alcohol; BS: blood sample; Meph: mephedrone.

comparable with results of studies assessing spatial and declarative memory performance after MDMA intoxication (Dumont et al., 2008; Kuypers and Ramaekers, 2005, 2007; Ramaekers et al., 2009; Van Wel et al., 2011). Previous findings also show that the 5-HT_{2a} receptor is implicated in learning and memory processes and stimulation of 5-HT_{2a} receptors is associated with memory decrements (Meneses, 2013). Van Wel and colleagues (2011) reported that ketanserin, a 5-HT_{2a} receptor antagonist, was able to block MDMA-induced memory impairment, indicating that MDMA-induced memory impairment is caused by 5HT_{2a} stimulation. Mephedrone also stimulates the 5-HT_{2a} receptors and it's affinity for this receptor is similar to that of MDMA (López-Arnau et al., 2012). It is therefore plausible that the impairing effect of mephedrone on memory is mediated through stimulation of 5HT_{2a} receptors as well.

CTT performance improved 4 h after mephedrone intake compared to placebo, but was not affected at T_1. Stimulatory effects of mephedrone on psychomotor function have previously been reported (Freeman et al., 2012). Such fluctuations in stimulatory effects have also been reported in previous MDMA studies. Some studies indicate improvement of critical tracking performance between 1–5 h after MDMA administration (Lamers et al., 2003), while other studies reported no effects on CTT performance (De Sousa Fernandes Perna et al., 2014; Kuypers et al., 2006). Performance during the DAT in turn was not affected by mephedrone intoxication. The absence of effects of mephedrone on performance during the DAT is in line with the studies showing that similar drugs like MDMA do not affect divided attention performance (Bosker et al., 2010; De Sousa Fernandes Perna et al., 2014).

Both short- and long-term spatial memory were impaired by alcohol intoxication. Previous studies assessing acute alcohol effects generally indicate impaired memory and cognitive performance following alcohol intoxication (Oscar-Berman and Marinkovi, 2007; Peterson et al., 1990; Schweizer et al., 2006), although spatial memory was not specifically assessed in an object relocation task. In contrast to previous studies (Jongen et al., 2014; Kuypers et al., 2006; Ramaekers et al., 2011), performance during the CTT was not impaired by alcohol intoxication. Performance during both the primary and secondary tasks of the DAT, however, was affected by alcohol intoxication compared to

placebo. Participants' reaction time and the number of control losses were increased by alcohol at T_1, whereas tracking error was larger at both T_1 and T_2, which is in line with previous assessments with comparable BAC levels (e.g. 0.7–0.8 g/kg) (Jongen et al., 2014; Ramaekers et al., 2011).

There was a significant interaction between mephedrone and alcohol on reaction time during the DAT at T_2. Reaction time was significantly decreased when mephedrone and alcohol were combined compared to when alcohol was given alone. Thus, it appears that the sedating effects of alcohol on reaction time are mitigated by the stimulating effects of mephedrone when administered together. CTT performance was not affected by co-administration with alcohol. The effects of mephedrone and alcohol on spatial memory performance when administered together were additive rather than synergistic as no significant mephedrone×alcohol interaction was shown. These results are also similar to previous findings on acute effects of single and co-administration of MDMA and alcohol on memory and psychomotor performance (Dumont et al., 2008).

The current study is limited by a relatively small sample size and future studies need to be conducted in larger samples (including females) before our preliminary findings can be generalised to the user population. Moreover, it should be noted that behavioural profiles of mephedrone and MDMA may still vary due to pharmacokinetic differences between both drugs (Green et al., 2014). Mephedrone displays high brain penetration, rapid metabolism and clearance from the brain compared to MDMA. In addition, mephedrone shows greater potency at DA transporter sites and causes more DA release as compared to MDMA (Simmler et al., 2013). The release of 5-HT by mephedrone produces the entactogenic subjective effects which are similarly experienced by MDMA users. However, unlike MDMA, the capacity of mephedrone to cause a more potent release of DA and NA gives it a higher psychostimulant and abuse liability (Green et al., 2014), which may explain why it is frequently taken in a binge-like manner.

Together, the current findings suggest that mephedrone impairs spatial memory performance and improves psychomotor performance. Alcohol impaired spatial memory and divided attention also in combination with mephedrone. It can be concluded that the influence of mephedrone on neurocognitive function is comparable to that elicited by MDMA and that stimulatory effects of mephedrone are not sufficient to compensate for the impairing effects of alcohol on most performance parameters.

Acknowledgements

The authors thank Esther Menoyo, Marta Pérez, Clara Gibert, Soraya Martin, Eulalia Olesti and Joan Mestre for their assistance and valuable contribution to this study.

Declaration of conflicting interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by grants from Instituto de Salud Carlos III (ISCIII, FIS-FEDER, FIS PI11/01961), ISCIII-FEDER_Red de Trastornos Adictivos (RTA RD16/0017/0003), and The European Commission (JUST/2013/DPIP/AG/4823, EU-MADNESS project; HOME/2014/JDRU/AG/DRUG/7082, Predicting Risk of Emerging rugs with In silico and Clinical Toxicology (PREDICT). Clara Pérez-Mañá and Esther Papaseit are Rio Hortega-Juan Rodes fellowships (ISCIII, JR15/00005 and JR16/00020, respectively).

References

Baumann MH, Ayestas MA, Partilla JS, et al. (2011) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* 37: 1192–1203.

Bosker WM, Kuypers KPC, Conen S, et al. (2010) Dose-related effects of MDMA on psychomotor function and mood before, during, and after a night of sleep loss. *Psychopharmacology* 209: 69–76.

Carhart-Harris RL, King LA and Nutt DJ (2011) A web-based survey on mephedrone. *Drug Alcohol Depend* 118: 19–22.

Corazza O, Demetrovics Z, van den Brink W, et al. (2013) 'Legal highs' an inappropriate term for 'Novel Psychoactive Drugs' in drug prevention and scientific debate. *Int J Drug Policy* 24: 82–83.

De Sousa Fernandes Perna EB, Theunissen EL, Kuypers KPC, et al. (2014) Memory and mood during MDMA intoxication, with and without memantine pretreatment. *Neuropharmacology* 87: 198–205.

Deluca P, Davey Z, Corazza O, et al. (2012) Identifying emerging trends in recreational drug use; outcomes from the Psychonaut Web Mapping Project. *Prog Neuropsychopharmacol Biol Psychiatry* 39: 221–226.

Dines AM, Wood DM, Yates C, et al. (2015) Acute recreational drug and new psychoactive substance toxicity in Europe: 12 Months data collection from the European Drug Emergencies Network (Euro-DEN). *Clin Toxicol (Phila)* 53: 893–900.

Dumont GJH, Wezenberg E, Valkenberg MMGJ, et al. (2008) Acute neuropsychological effects of MDMA and ethanol (co-)administration in healthy volunteers. *Psychopharmacology* 197: 465–474.

European Monitoring Centre for Drugs and Drug Addiction (2015) *New psychoactive substances in Europe: Innovative legal responses.* Luxembourg: Publications Office of the European Union.

Freeman TP, Morgan CJA, Vaughn-Jones J, et al. (2012) Cognitive and subjective effects of mephedrone and factors influencing use of a 'new legal high'. *Addiction* 107: 792–800.

Green AR and Nutt DJ (2014) Pharmacology should be at the centre of all preclinical and clinical studies on new psychoactive substances (recreational drugs). *J Psychopharmacol* 28: 711–718.

Green AR, King MV, Shortall SE, et al. (2014) The preclinical pharmacology of mephedrone; not just MDMA by another name. *British Journal of Pharmacology* 171: 2251–2268.

Home Office (2014) *Drug Misuse: Findings from the 2013/14 Crime Survey for England and Wales*. Available at: https://www.gov.uk/government/publications/drug-misuse-findings-from-the-2013-to-2014-csew (accessed 24 May 2016).

Jex HR, McDonnell JD and Phatak AV (1966) A 'critical' tracking task for manual control research. *IEEE Transactions on Human Factors in Electronics* 4: 138–145.

Jongen S, Vuurman E, Ramaekers J, et al. (2014) Alcohol calibration of tests measuring skills related to car driving. *Psychopharmacology* 231: 2435–2447.

Kessels RP, Postma A and de Haan EH (1999) Object relocation: A program for setting up, running, and analyzing experiments on memory for object locations. *Behav Res Methods Instrum Comput* 31: 423–428.

Kuypers KPC and Ramaekers JG (2005) Transient memory impairment after acute dose of 75mg 3.4-Methylene-dioxymethamphetamine. *J Psychopharmacol* 19: 633–639.

Kuypers KPC and Ramaekers JG (2007) Acute dose of MDMA (75 mg) impairs spatial memory for location but leaves contextual processing of visuospatial information unaffected. *Psychopharmacology* 189: 557–563.

Kuypers KPC, Samyn N and Ramaekers JG (2006) MDMA and alcohol effects, combined and alone, on objective and subjective measures of actual driving performance and psychomotor function. *Psychopharmacology* 187: 467–475.

Lamers CTJ, Ramaekers JG, Muntjewerff ND, et al. (2003) Dissociable effects of a single dose of ecstasy (MDMA) on psychomotor skills and attentional performance. *J Psychopharmacol* 17: 379–387.

López-Arnau R, Martínez-Clemente J, Pubill D, et al. (2012) Neuropharmacology of three psychostimulant cathinone derivatives: Butylone, mephedrone and methylone. *British Journal of Medical Psychology* 167: 407–420.

Meneses A (2013) 5-HT systems: Emergent targets for memory formation and memory alterations. *Rev Neurosci* 24: 629–664.

Moskowitz H (1973) Laboratory studies of the effects of alcohol on some variables related to driving. *J Safety Res* 5: 185–199.

O'Neill C and McElrath K (2012) Simultaneous use of mephedrone and alcohol: A qualitative study of users' experiences. *Journal of Addiction Research & Therapy* S9: 001.

Oscar-Berman M and Marinkovi K (2007) Alcohol: Effects on neurobehavioral functions and the brain. *Neuropsychology Review* 17: 239–257.

Papaseit E, Pérez-Mañá C, Mateus J-A, et al. (2016) Human pharmacology of mephedrone in comparison to MDMA. *Neuropsychopharmacology* 41: 2704–2713.

Peterson J, Rothfleisch J, Zelazo P, et al. (1990) Acute alcohol intoxication and cognitive functioning. *J Stud Alcohol Drugs* 51: 114–22.

Psychonaut WebMapping Research Group (2009) *Mephedrone Report*. London: Institute of Psychiatry, King's College London.

Ramaekers JG, Kuypers KPC, Wingen M, et al. (2009) Involvement of inferior parietal lobules in prospective memory impairment during acute MDMA (ecstasy) intoxication: An event-related fMRI study. *Neuropsychopharmacology* 34: 1641–1648.

Ramaekers JG, Theunissen EL, De Brouwer M, et al. (2011) Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. *Psychopharmacology* 214: 391–401.

Sambeth A, Riedel WJ, Tillie DE, et al. (2009) Memory impairments in humans after acute tryptophan depletion using a novel gelatin-based protein drink. *J Psychopharmacol* 23: 56–64.

Schifano F, Albanese A, Fergus S, et al. (2011) Mephedrone (4-methylmethcathinone; 'Meow meow'): Chemical, pharmacological and clinical issues. *Psychopharmacology* 214: 593–602.

Schweizer TA, Vogel-sprott M, Danckert J, et al. (2006) Neuropsychological profile of acute alcohol intoxication during ascending and descending blood alcohol concentrations. *Neuropharmacology* 31: 1301–1309.

Simmler L, Buser T, Donzelli M, et al. (2013) Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol* 168: 458–470.

United Nations Office on Drugs and Crime (UNODC). *Global synthetic drugs assessment*. Vienna: UNODC, 2014.

Van Wel JHP, Kuypers KPC, Theunissen EL, et al. (2011) Blockade of 5-HT2 Receptor Selectively Prevents MDMA-Induced Verbal Memory Impairment. *Neuropsychopharmacology* 36: 1932–1939.

Winstock, Mitcheson LR, Deluca P, et al. (2011a) Mephedrone, new kid for the chop? *Addiction* 106: 154–61.

Winstock, Mitcheson L, Ramsey J, et al. (2011b) Mephedrone: Use, subjective effects and health risks. *Addiction* 106: 1991–1996.