

Exposed

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Exposed: Interactions between acute drug experiences and affective cues

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Exposed: Interactions between acute drug
experiences and affective cues

DISSERTATION

To obtain the degree of doctor at Maastricht University,
on the authority of Rector Magnificus, Prof dr. Rianne M. Letschert,
in accordance with the decision of the Board of Deans,
to be defended in public on Thursday November 17th at 12.00 hours.

by

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“Yesterday I was clever, so I wanted to change the world. Today I am
wise, so I am changing myself.”

- *Rumi*

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Chapter 1

General introduction

Exposure to drugs of abuse

Humans are natural reward seekers engaging regularly in activities which elicit feelings of pleasure such as eating delicious food, experiencing romantic and sexual pleasure, listening to music or dancing and singing. Generating feelings of well-being is a consequence of natural release of chemical messengers, such as dopamine and endorphins, throughout the brain and body. These pleasurable feelings help build memories that teach us whether the activities we engage in should be repeated in the future. Drugs of abuse mimic the effects of natural chemical messengers.

A great number of psychoactive drugs with stimulatory, depressant/sedative, empathogenic and psychedelic properties are taken for pleasure. Around 2 billion individuals worldwide have been estimated to consume alcohol on a regular basis (World Health Organization, 2004), with the highest consumption levels found in the European Union (EU) and in the United States of America (USA) (World Health Organization, 2014). Over 80 million adults in the EU are predicted to have been exposed to illegal drugs at least once in their lives, translating to about one in every four Europeans (EMCDDA, 2015b). The most frequently used illicit drug is cannabis (78.9 million), while the estimates reported for the lifetime use of cocaine (15.6 million) and MDMA (12.3 million) are lower. Mephedrone, a psychoactive substance which has emerged on the illicit drug market about 10 years ago, is estimated to have been used by 0.5 million adults in the United Kingdom (Home Office Statistical Bulletin, 2011). Both legal and illegal drugs of abuse exert their reinforcing effects by either directly or indirectly activating the brain's reward circuit. The mesolimbic reward circuitry is thought to govern the rewarding properties of drugs of abuse and includes dopaminergic projections from the ventral tegmental area (VTA) through the nucleus accumbens (NAcc) extending further to limbic structures including the amygdala, hippocampus, anterior cingulate and (pre)frontal cortex areas (Anton, 1999; Heinz et al., 2005; Yacubian and Büchel, 2009).

Besides the activation of the mesolimbic reward circuitry, drugs can also affect brain functioning and impair neurocognitive performance (e.g. learning, memory, attention, executive function, affective processing and psychomotor performance) both

acutely (during intoxication) and non-acutely (after the acute effects wear off) (Crane et al., 2013; Miller et al., 2015; Spronk et al., 2013). Recreational drug use can develop quickly into a continued compulsive drug using habit or drug-dependence, which could impair or disrupt brain function beyond repair (Everitt and Robbins, 2013; Kessler et al., 2007). Acute drug exposure is associated with transient disruptions in neurocognitive performance. Repeated or chronic drug exposure however can trigger neuroadaptations that distort executive function, increase the brain's sensitivity to drugs and drug-related cues in the environment, while reducing the sensitivity to non-drug rewards and making the brain more susceptible to anxiety and stress (Everitt et al., 2008; Volkow and Morales, 2015). Excessive drug consumption is therefore not without consequences and can be very harmful for both the individual and society (EMCDDA, 2015b; World Health Organization, 2014).

Drug exposure alters interpretation of affective cues

The influence of drug exposure on neurocognitive performance cannot be interpreted without taking the context and environment into account. Affective cues can influence internal states, such as cognition, affect and arousal. Drug intoxication following acute and chronic drug exposure can alter the interpretation and appraisal of affective cues. The notion that social contexts promote drug taking (i.e., initiation, maintenance and relapse) and influence the magnitude of the drug effects is supported by naturalistic and experimental studies (Doty and de Wit, 1995; Kelly et al., 1994; Kirkpatrick and De Wit, 2013; Miller et al., 2015). Likewise, affective cues, such as drug marketing, emotional face expressions and aggressive stimuli may alter the neurocognitive response to drug intoxication.

Public advertisement of drugs in the media includes portrayals of drug use in movies, magazines or during music events. Exposure to pictorial representations of drug-related cues induces craving, physiological and affective reactions (Hogarth and Duka, 2006), which can lead to positive expectancies and attitudes towards drug use and increase the intention to consume (Dal Cin et al., 2008; Martin et al., 2002). Drug

marketing cues can become motivationally salient once reinforced by acute drug intoxication (Belin et al., 2009), although the reward sensitivity of marketing cues during intoxication can be altered by chronic drug exposure. With chronic drug exposure, the reinforcing properties of drugs are not only a result of acute drug effects but also a consequence of the learned responses and the increased saliency of drug-related events gained by the individual through experience. Heavy drug users can therefore have a different neurocognitive response to alcohol and drug marketing cues during drug intoxication (Anderson et al., 2009; British Medical Association, 2009; Lovato et al., 2003a) compared to when sober.

Drugs exposure, especially alcohol (Beck and Heinz, 2013), can further lead to maladaptive behaviors, including aggression and anti-social behavior (Lammers et al., 2014; Smith et al., 2010). Aggression-facilitating factors such as provocation, frustration and presence of aggressive stimuli can in turn influence internal states and have a stronger effect on people when they are intoxicated with drugs (Anderson and Bushman, 2002; Hoaken and Stewart, 2003). This poses an immediate threat to the individual and to others who become victims of intoxicated aggression.

Previous studies suggest that both acute and chronic drug exposure can interfere with the processing of emotional stimuli, such as the interpretation of emotions in face expressions (Attwood et al., 2009; Miller et al., 2015). Acute drug exposure may boost social interaction by increasing the desire to socialize (i.e., increased verbal behavior and social bonding) (Sayette et al., 2012), but prolonged chronic exposure can impair or bias social interaction in a negative way (i.e., impaired recognition of fearful/angry face expression) leading to diminished social cognition (Miller et al., 2015).

Exposure to multiple drugs

Drug users often consume more than one kind of drug at the time or within the same drug episode (Mohamed et al., 2011). Drugs are usually combined to counter some of the negative effects and/or to potentiate the positive effects (Mohamed et al., 2011). Co-administration of drugs is especially common in experienced drug users, who often also

ingest other drugs, such as amphetamines, alcohol, cannabis, cocaine, ketamine, benzodiazepines or hallucinogens (Cole and Sumnall, 2003; Scholey et al., 2004). Mephedrone emerged later on the illicit drug market as a substitute for MDMA. The majority of mephedrone users have reported concurrent use of alcohol on a regular basis (Moore et al., 2013; O’Neill and McElrath, 2012). Since the effects of mephedrone by itself are not well-known in humans, mixing it with alcohol or other drugs could considerably increase the harms and lead to fatal outcomes. For example, alcohol when administered with MDMA can increase MDMA concentrations in blood plasma (Hamida et al., 2009), whereas mephedrone can enhance neurotoxicity caused by MDMA and other amphetamines when combined (Angoa-Pérez et al., 2013). This shows that the presence of one drug can alter the function of another drug when administered together. Therefore, examining the underlying neuropharmacological mechanisms during multiple drug exposure is crucial to better understand their effects on neurocognitive performance.

Aims of this dissertation

Several experimental studies were conducted in order to gain more insight into the acute effects of single and simultaneous drug exposure and the interaction with affective cues. The studies included a broad range of drugs of abuse: alcohol, cannabis, cocaine, MDMA and mephedrone. The main goal of the present dissertation was to investigate whether:

- 1) brain activity and neurocognitive performance change during drug intoxication following exposure to drug marketing cues, emotional cues and aggression cues
- 2) the exposure to one drug potentiate or block the effects induced by another drug

Methods and Outline

The experimental studies discussed in this dissertation were all conducted according to a double-blind placebo-controlled within-subject design. All participants were healthy and had prior experience with the investigated drugs. Neuro-cognitive performance and subjective effects were measured at peak drug plasma concentrations (T_{max}).

The effects of alcohol and cannabis on the brain reward circuitry and on implicit drug attitudes were examined during exposure to alcohol and cannabis marketing clips by means of a pharmaco-functional magnetic resonance imaging (fMRI) paradigm (**Chapter 2**). A second study investigated the effects of alcohol and cannabis on subjective aggression and implicit aggressive attitudes following exposure to aggression cues (**Chapter 3**). The effects of cannabis and cocaine on brain activity/amygdala reactivity following exposure to affective cues were assessed by means of a pharmaco-fMRI paradigm during which face expressions from different emotional categories were presented (**Chapter 4**). In order to gain more insight into the underlying neuro-pharmacological mechanisms through which drugs effects are induced, interactions between drugs when combined was investigated (**Chapters 5 and 6**). Several cognitive tasks were used to examine whether the impairing effects of MDMA on neurocognitive performance can be blocked when co-administered with memantine, an NMDA and alpha-7 nicotinic acetylcholine (ACh) receptor antagonist (**Chapter 5**). Another study assessed the acute effect of mephedrone alone and after co-administration with alcohol on neurocognitive function (**Chapter 6**). Subjective effects, such as subjective intoxication and mood, following drug treatments were measured by means of a visual analogue scale (VAS) and the profile of mood states (POMS) respectively (**Chapters 2, 3 and 5**). Finally, **chapter 7** discusses and integrates the main findings of the studies illustrated in the experimental chapters of this dissertation. Clinical implications and recommendations for future research are provided.

Chapter 2

Brain reactivity to alcohol and cannabis marketing during sobriety and intoxication

De Sousa Fernandes Perna, E.B., Theunissen, E.L., Kuypers, K.P., Evers, E.A., Stiers, P., Toennes, S.W., Witteman, J., Dalen, W. and Ramaekers, J.G., 2016. Brain reactivity to alcohol and cannabis marketing during sobriety and intoxication. *Addiction biology*.

Abstract

Drugs of abuse stimulate striatal dopamine release and activate reward pathways. This study examined the impact of alcohol and cannabis marketing on the reward circuit in alcohol and cannabis users while sober and intoxicated. It was predicted that alcohol and cannabis marketing would increase striatal activation when sober and that reward sensitivity would be less during alcohol and cannabis intoxication. Heavy alcohol (N=20) and regular cannabis users (N=21) participated in a mixed factorial study involving administration of alcohol and placebo in the alcohol group and cannabis and placebo in the cannabis group. Non-drug users (N=20) served as between group reference. Brain activation after exposure to alcohol and cannabis marketing movies was measured using fMRI and compared between groups while sober and compared to placebo while intoxicated. Implicit alcohol and cannabis cognitions were assessed by means of the Single-Category Implicit Association Test (SC-IAT). Alcohol and cannabis marketing significantly increased striatal BOLD activation across all groups while sober. Striatal activation however decreased during intoxication with alcohol and cannabis. Implicit associations with cannabis marketing cues were significantly more positive in alcohol and cannabis users as compared to non-drug using controls. Public advertising of alcohol or cannabis use elicits striatal activation in the brain's reward circuit. Reduction of marketing would reduce brain exposure to reward cues that motivate substance use. Conversely, elevated dopamine levels protect against the reinforcing potential of marketing.

Introduction

Alcohol and cannabis are the most widely used drugs in the western world. It is estimated that around 2 billion individuals consume alcohol worldwide (World Health Organization, 2004). People typically drink alcohol and smoke cannabis to induce euphoria or reduce anxiety. Both drugs facilitate the release of tonic dopamine in reward and motivation circuits in the brain (Anton, 1999; Bossong et al., 2009; Gilman et al., 2008; Heinz et al., 2005; Yacubian and Büchel, 2009) that accounts for the pleasurable effects of drugs. The hedonic response is often a motive for people to repeat drug use (Franken et al., 2005).

Drug-associated cues have also been shown to stimulate dopamine release (Berger et al., 1996; Koob and Volkow, 2010) and activate the reward circuit of abstinent drug users (Cousijn et al., 2013; Filbey et al., 2009; Goudriaan et al., 2010; Vollstädt-Klein et al., 2010). This suggests that drug-related cues may trigger the reward system to a similar extent as do drugs. Consequently, motivations to use alcohol or drugs may increase due to marketing exposure to drug-related cues such as alcohol and drug advertisements. Earlier studies on soft drink brands have shown that brand knowledge influences expressed behavioural preferences and measured brain responses (McClure et al., 2004). Likewise, cue-elicited reactivity to alcohol and cannabis has been shown to activate reward pathways in the brain associated with the neuropathology of addiction (Filbey et al., 2009; Tapert et al., 2003). Research on alcohol and tobacco marketing has shown that marketing can significantly increase consumption patterns (Anderson et al., 2009; Cassisi et al., 1998; Smith and Foxcroft, 2009; Tye et al., 1987).

While longitudinal studies consistently show that alcohol and tobacco marketing negatively affect adolescents' drinking and smoking behaviour (Anderson et al., 2009; Lovato et al., 2003b), no research has examined the impact of marketing on brain activity during intoxication. One might expect that the reinforcing properties of marketing cues and actual drug or alcohol use add up to increase the hedonic response. However, current knowledge on the dopaminergic response within the reward system would predict that reinforcing properties of drug and alcohol marketing may actually diminish during drug

and alcohol intoxication. Reinforcing stimuli have previously been shown to cause burst firing of midbrain dopamine neurons that leads to a temporary, phasic release of dopamine in the striatum (Schultz, 2007). The striatal response or reward sensitivity to such phasic dopaminergic innervations has been posed to vary with the availability of tonic dopamine in the same area (Cools and D'Esposito, 2011). Reward sensitivity is high when tonic dopamine is low and vice versa. This implies that a phasic response to marketing may decrease in the presence of elevated tonic dopamine levels induced by alcohol (Gilman et al., 2008) and cannabis (Bossong et al., 2009).

The aim of the present study was to assess the impact of alcohol and cannabis marketing on the brain's reward circuit. It was predicted that alcohol and cannabis marketing would increase brain activation in the striatum when sober. In addition, the study aimed to obtain direct evidence for the predicted impact of tonic dopamine (cannabis and alcohol intoxication) on the reward-related phasic dopamine effects (cannabis and alcohol marketing) in a pharmacofMRI paradigm. It was predicted that brain networks that are activated after alcohol/cannabis marketing exposure are similar during sobriety and intoxication, but that reinforcement of the striatum after marketing exposure will be less during intoxication as compared to when sober. An implicit association task was used to register implicit cognitions towards alcohol and cannabis marketing cues during intoxication and while sober.

Methods

Participants

The present study included a group of heavy alcohol users, a group of regular cannabis users and a control group. Heavy alcohol use was defined as using on average 21 to 50 alcoholic drinks a week for males or 15 to 35 alcoholic drinks a week for females during the last year (Cassisi et al., 1998). Experimental use of cannabis in the alcohol group was allowed only if it occurred more than a year ago. Regular cannabis use was defined as having used cannabis at least three times a week but no more than 10 times a week,

during the previous year (Ramaekers et al., 2009). Alcohol use between 1-14 units/week was allowed in the cannabis group. Controls were defined as not currently using cannabis or other drugs; experimental use of cannabis was allowed if it occurred more than a year ago and incidental alcohol use was permitted (1-7 units/week for women and 1-14 units of alcohol/week for men).

Inclusion criteria included: (i) age 18–40 years (ii) free from psychotropic medication; (iii) good physical health and, (iv) body mass index within 18.5 –28 kg/m². Exclusion criteria included: (i) addiction according to DSM-IV criteria, (ii) presence or history of psychiatric or neurological disorder as assessed by a medical questionnaire (iii) pregnancy (iv) cardiovascular abnormalities, (v) excessive smoking (>15 cigarettes per day) and (vi) hypertension.

Five participants from the alcohol group and two participants from the cannabis group dropped out due to personal circumstances and one participant from the cannabis group failed to complete the fMRI session during placebo, but otherwise completed both behavioural sessions. The dropouts were replaced but the behavioural data of the participant with incomplete fMRI session was also added to the final data set. The final dataset therefore consisted of 61 participants spread among the alcohol and control group (N=20 each) and the cannabis group (N=21). Participants (35 male, 26 female) were aged between 18 and 28 (mean (SD) 22.5 (2.3) years). Participants underwent a general medical examination including routine laboratory tests and provided a written informed consent. The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008) and approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University (Dutch Trial Register: trial number: NTR3428). A summary of participant demographics and drug use history is given in Table 1.

Table 1 Participant demographics and history of alcohol and drug use. LSD = Lysergic Acid Diethylamide

	Mean (SD)	Min.	Max.
Age (years)	22.5 (2.3)	18	28
Weight (kg)	67.9 (10.7)	50	92
Alcohol group (N=20; 10♂, 10♀)			
# of alcohol units/week	24 (7.7)	15	50
Cannabis group (N=21; 15♂, 6♀)			
Frequency of cannabis use /week	4.8 (1.9)	3	7
# of alcohol units/week	4.9 (4.7)	0	14
Control group (N=20; 10♂, 10♀)			
# of alcohol units/week	5.3 (3.5)	1	14
Lifetime use of other drugs	Alcohol Group	Cannabis Group	Control Group
Ecstasy	8	10	2
Amphetamine	2	5	1
Cocaine	1	5	0
LSD	0	3	0
Mushrooms	2	11	0
Other (e.g., truffles, ketamine)	3	8	0

Design and treatments

Groups of heavy alcohol and regular cannabis users participated in a double-blind, placebo-controlled, mixed factorial study involving two experimental conditions consisting of alcohol and placebo in the alcohol group and cannabis and placebo in the cannabis group. The order of treatment conditions was balanced over participants and sessions. Conditions were separated by a minimum washout period of 7 days to avoid carry-over effects. An age-matched control group of non-drug users was added that received no treatment but the testing day was similar on all other aspects. The alcohol and cannabis group received treatment prior to the fMRI session (T_1) and a second dose prior to the implicit association task (T_2).

Alcohol (96%v/v) was mixed with orange juice to a total volume of 250 mL. Alcohol doses were individually calibrated using the formula of Watson et al. (Watson et al., 1981) to achieve a blood alcohol concentration (BAC) of 0.8 g/L. Males received between 52-68 mL and females received between 39-48 mL of alcohol depending on their weight. Participants' BAC was monitored frequently (every 15-20 min approximately) with an alcohol breathalyzer (Dräger Alcotest® 6510) and was kept constant by administering maintenance drinks. Maintenance (booster) doses were administered during before behavioural testing. Each participant received a booster dose, the volume depended on their BAC level at the end of the scanning session.

The cannabis group received a total of 300 µg THC/kg bodyweight, divided in two successive doses of 200 µg and 100 µg THC/kg bodyweight (booster dose) with an interval of approximately one hour. THC was administered using a Volcano vaporizer produced by Storz-Bickel, Germany (<http://www.storz-bickel.com>). Hot air would pass through the filling chamber holding the cannabis (containing 12% THC), which caused the THC or placebo to vaporize and blend with the air. The THC molecules or the placebo (vapor) was trapped in a valve balloon. For inhalation, the valve of the balloon was put to participants' lips and they were instructed to inhale deeply.

Procedures

Participants were asked to refrain from drug use at least a week prior to the start and during the study. Participants were not allowed to use alcohol on the day before an experimental session and were requested to arrive at experimental sessions well rested. Drug and alcohol screens were carried out upon arrival at our testing facilities. Urine drug screens assessed for the presence of benzodiazepines, opiates, cocaine, marijuana, MDMA and (meth)amphetamine. Women were also tested for pregnancy. Study treatments were only administered after negative drug screens, except for marijuana in the cannabis group, and negative pregnancy tests.

Brain activity was measured by means of functional magnetic resonance imaging (fMRI) during a one hour session. Cannabis (or cannabis placebo) and alcohol (or alcohol placebo) administration was completed at 15 and 30 min prior to scanning (T_1). The scanning session was followed by a 45 min break in which a booster dose was administered. Implicit association was measured by means of implicit association tests between at 15 and 30 minutes after completion of cannabis (placebo) or alcohol (placebo) booster administration (T_2). All participants received a training session before the onset of the experimental sessions in order to familiarize them with tests and procedures. Blood samples and breath tests were taken at baseline (T_0) and prior to scanning (T_1) and the SC-IAT (T_2).

fMRI Marketing Exposure Task

Brain activity was assessed during a marketing-exposure task using a block design. In this task, marketing clips were randomly presented on a computer screen in blocks of 30s. The clips consisted of three categories, i.e., alcohol marketing clips (10x), cannabis-related clips (10x) and neutral clips (10x). Total task duration was approximately 33 min. Alcohol clips were mainly non-local advertisement of beers, wines and other alcoholic beverages that were not readily available in the Netherlands and were spoken in foreign languages (e.g. Polish, Spanish or English) that did not correspond to the participants' native language (Dutch). This was done to ensure that participants were not reacting to the specific alcohol brand, but to the alcohol itself. Cannabis clips included advertisement for cannabis paraphernalia and a selection of short film fragments where portrayal of cannabis use and marketing practices at cannabis selling points were displayed. The neutral clips consisted of local and non-local advertisement of non-drug-related stimuli (e.g. advertisement for cameras, water, hearing aid etc.).

fMRI images were acquired with a Siemens 3T head-only scanner (MAGNETOM Allegra, Siemens Medical Systems, Erlangen, Germany). During the cue exposure task whole brain functional volumes were acquired using gradient-echo echo-planar imaging (GE-EPI, TR= 2000 ms, TE= 30 ms; FA= 90°; FOV 224mm; matrix size= 64 x 64; voxel size=

3.5 x 3.5 x 3.5 mm). The T1-weighted anatomical scan was acquired using a 3D MPRAGE (magnetization-prepared rapid gradient echo; TR= 9,7 ms; TE= 4 ms; flip angle=12°; matrix=256×256; voxel size=1×1×1 mm³).

Data preprocessing and analysis were conducted using SPM8 (Wellcome Trust Center for Neuroimaging, London, UK). The first two volumes were removed from each fMRI data set to allow for magnetic equilibration. Firstly, framewise displacement (FD) calculations were carried out to quantify head displacement within and across runs (Power et al., 2012). In total, two participants in the alcohol group, one participant in the cannabis group and one participant in the control group were excluded from further processing due to excessive movement (in > 20% of the volumes). In addition, motion parameters in the alcohol and cannabis group were then compared to check for motion differences between placebo and active drug/alcohol conditions. These analyses indicated no difference between sessions for the most susceptible motion parameters (Mayer et al., 2007; Yoo et al., 2005).

Thereafter the following preprocessing steps were carried out: (1) realignment, (2) slice time correction, (3) individual anatomical data sets were normalized to standard 3-D MNI space (voxel size was resampled to 2×2×2 mm), and (4) spatial smoothing was applied with a FWHM 6 mm Gaussian kernel.

Single Category Implicit Association Test

Implicit cognition was assessed by means of the Single Category Implicit Association Test (SC-IAT), which measures the strength of evaluative associations (positive vs. negative) with a single attitude object (alcohol or cannabis marketing pictures). During the first block of 24 trials (target discrimination), only the target concepts were presented and participants had to respond using the corresponding keys (i.e. press left button for positive words, and the right button for negative words). In the second block (compatible block) of 72 trials, positive words and drug marketing cues were categorized on the left key, and negative words were categorized on the right key. In the third block

(incompatible block) of 72 trials, negative words and drug marketing cues were categorized on the right key, and positive words were categorized on the left key. The rationale behind this task is that if participants have a positive evaluation for alcohol or cannabis rather than a negative evaluation, they should be quicker to respond when alcohol/cannabis marketing + positive words (compatible block) share the same response key compared to the incompatible block, where alcohol/marketing clips + negative words share the same response key. Target words and marketing cues were presented at random order within each block. Blocks 2 and 3 were counterbalanced across treatments conditions. Data from the 1st block (practice block) was discarded. Non responses and responses faster than 350 ms were eliminated and error responses were replaced with the block mean plus an error penalty of 400 ms. Participants who exceeded an error rate of 20% were excluded. The dependent variable was the D-score (Greenwald et al., 2003; Karpinski and Steinman, 2006), which was calculated by subtracting the mean reaction time (RT) of correct responses in the compatible block from the mean RT of correct responses in the incompatible block, divided by the standard deviation (SD) of all correct responses within the compatible and incompatible block. D-scores were log transformed ($\ln(D\text{-score}+1)$) before entering statistical analysis.

Pharmacokinetic Measures

In the cannabis group, blood samples to determine cannabinoid concentrations (THC and metabolites OH-THC and THC-COOH) were collected at 3 successive times during each test day, i.e. at baseline (T_0) and 0.5 h (T_1), 1.5 h (T_2) after the first dose. The blood samples (8mL) were centrifuged immediately; serum was transferred into a tube and was stored at -20°C . Cannabinoid concentrations were determined by the Institute of Forensic Toxicology, University of Frankfurt, using solid phase extraction and gas chromatography with mass spectrometric detection with a limit of quantification of 1.0 ng/mL. In the alcohol group, BAC levels were measured throughout the test day with the breathalyzer.

Statistics

fMRI data

Two generalized linear model (GLM) full factorial models were built to calculate marketing cue-related BOLD activations during sobriety (i.e. placebo/no treatment) and how these were affected by cannabis or alcohol intoxication. For both models, contrast images from the individual GLM analysis (first level) were used as input for the second level GLMs. Individual analysis consisted of contrast images of cannabis marketing movies vs neutral marketing movies (cannabis marketing; contrast [1 -1]) and alcohol marketing movies vs neutral marketing movies (alcohol marketing; contrast [1 -1]). All individual GLM models included the 6 realignment parameters as regressors.

The first GLM full factorial model focused on BOLD activation during cannabis marketing and alcohol marketing across the three groups while being sober. The model included the factors Group (3 levels: cannabis group on placebo, alcohol group on placebo and controls) and Marketing Cue (cannabis marketing and alcohol marketing).

The second GLM full factorial model was designed to assess the influence of cannabis and alcohol intoxication on brain activation during marketing exposure. The model consisted of the following factors: Group (2 levels: cannabis group and alcohol group); Treatment (2 levels: placebo and cannabis/alcohol) and Marketing Cue (2 levels: cannabis marketing and alcohol marketing).

For these two models, whole brain analyses were performed to explore general effects of marketing and treatments. ROI analyses were conducted in order to specifically test our hypotheses that marketing cues as well as drug intoxication would affect striatal activations within the brain reward network. A striatal ROI was built with the WFU PickAtlas (Maldjian et al., 2003, 2004; Tzourio-Mazoyer et al., 2002) by combining the bilateral putamen, caudate and globus pallidus. Results were considered significant when p_{FWE} -corrected at cluster level < 0.05 .

Subsequently, we quantified mean percent (%) BOLD signal change in (combined) striatal areas that showed significant brain activation following marketing exposure during sobriety (GLM1) and intoxication (GLM2). Functional striatal masks were created with Marsbar. Mean % BOLD signal change was quantified for all marketing clips (cannabis, alcohol and neutral) in each group and in each treatment condition using the SPM toolbox rfxplot (Gläscher, 2009). % BOLD signal change was analyzed in SPSS following the same outline as previous GLM models, with the exception that Marketing cue consisted of three levels (cannabis, alcohol and neutral movies).

Implicit cognition

The dependent parameter of the SC-IAT (i.e. D-score) was analyzed by means of a GLM univariate ANOVA with a main factor Group (3 levels: alcohol group on placebo, cannabis group on placebo and control). These were followed by simple group contrast relative to the controls. The effects of the factors Alcohol treatment (2 levels: alcohol and placebo) and Cannabis treatment (2 levels: cannabis and placebo) cues were assessed in repeated measures GLMs in the alcohol and cannabis group respectively. 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 20.0.

Results

Whole brain analyses

Figure 1 shows mean increments in BOLD activation following exposure to cannabis and alcohol marketing cues (vs neutral) collapsed over the three groups while sober and mean decrements in BOLD response to marketing (collapsed over alcohol and marketing movies) while under the influence of cannabis or alcohol.

GLM1 analyses revealed a main effect of Group on BOLD response in the left hippocampus and right precuneus and a main effect of Marketing Cue in parietal,

temporal and frontal brain regions. Overall, exposure to marketing cues increased BOLD activations across these brain regions in the three groups, and more so during alcohol marketing movies. Significant brain clusters associated with main effects of Group and Marketing cue are given in Table 2.

GLM2 analyses revealed a main effect of Group on BOLD response in the cuneus, rolandic operculum, brainstem, insula, amygdala, cerebellum and temporal and frontal clusters. A main effect of Marketing Cue on BOLD response was found in the postcentral cluster, cingulum, temporal, parietal, frontal and occipital cortex. A main effect of Treatment on BOLD response in the right supplementary motor area was observed indicating reduction of marketing induced BOLD activation. Significant brain clusters associated with main effects of Group, Treatment and Marketing cue are given in Table 2.

ROI analyses striatum

GLM1 revealed a main effect of Group on BOLD response in the left pallidum during marketing exposure across all groups. The factor Marketing cue did not differentially affect BOLD response in the striatum. The GLM2 analysis revealed a main effect of Group on BOLD response in the right caudate. The factor treatment caused an overall decrease in the BOLD response in the bilateral pallidum and right caudate. The factor Marketing cue did not differentially affect BOLD response in the striatum. Significant brain clusters associated with main effects of Group, Treatment and Marketing cues for the GLM analyses are given in Table 3.

Table 2 Brain areas showing changes in BOLD activation during marketing exposure while being sober (GLM1) and while intoxicated (GLM2). BA = Brodmann Area; p* = Familywise Error Corrected <.05; MNI =Montreal Neurological Institute

	BA	Number of voxels	Peak MNI coordinates	F	p*
WHOLE BRAIN ANALYSES (GLM 1)					
Group					
			-20, -20, --		
Left hippocampus	30	185	26	17.68	0.007
Right precuneus	7,31	915	0, -50, 20	14.71	0.050
Marketing cue					
Right superior parietal cluster	7	2027	32, -48, 66	38.81	0.000
Left middle temporal cluster	37	1253	-54, -68, 4	35.61	0.001
Right inferior temporal cluster	37	1190	50, -64, -6	33.90	0.002
Left interior parietal cluster	40	1202	-36, -38, 52	30.46	0.006
Right inferior frontal cluster	44	397	54, 10, 26	28.21	0.014
WHOLE BRAIN ANALYSES (GLM2)					
Group					
Left cuneus		5658	-10, -72, 26	46.57	0.000
Right superior temporal cluster	42	432	62, -44, 20	33.87	0.001
Right rolandic operculum	48	898	50, -24, -20	27.83	0.012
Brainstem		130	0, -12, -26	27.09	0.015
Right insula	48	317	36, 20, 30	26.38	0.020
			-56, -32, --		
Left middle temporal cluster	21	245	12	25.55	0.027
Left amygdala		38	-16, 4, -14	24.76	0.037
Left medial superior frontal cluster	10	230	-2, 60, 34	24.72	0.038
			-16, -42, --		
Left cerebellum	30	109	14	24.02	0.049
Treatment					
Right supplementary motor area	6	39977	14, 8, 54	41.61	0.000
Marketing cue					
Right inferior temporal cluster	37	1703	56, -64, 4	47.25	0.000
Left middle occipital cluster	19	1336	-46, -76, 4	46.87	0.000
Right superior parietal cluster	2	1607	36, -44, 62	39.08	0.000

	BA	Number of voxels	Peak MNI coordinates	F	p*
Left middle temporal cluster	22	417	-60, -20, 2	30.44	0.004
Left postcentral cluster	40	976	-38, -34, 44	30.14	0.005
Right superior temporal cluster	21	372	60, -2, -6	28.69	0.008
Right inferior frontal cluster	44	458	54, 12, 24	28.00	0.011
Right middle cingulum	24	117	2, 26, 36	24.80	0.036

% Signal change striatum

GLM1 revealed a main effect of Marketing cue ($F[2,52] = 12,8; p < .001$). Simple contrast indicated that cannabis marketing cues ($p < .001$) and alcohol marketing cues ($p < .001$) increased BOLD activation in the striatum, relative to neutral marketing cues (Figure 2). The factors Group and Group x Marketing cue did not reach significance.

GLM2 revealed main effects of Treatment ($F[1,35] = 4,18; p = .048$) and Marketing cue ($F[2,34] = 14,6; p < .001$). Treatment with alcohol and cannabis generally reduced BOLD activation in the striatum relative to placebo ($p = .048$) whereas cannabis ($p = 0.014$) and alcohol ($p < .001$) marketing cues generally increased BOLD activation, relative to neutral cues. The interactions between Treatment, Group and Marketing cue did not reach significance.

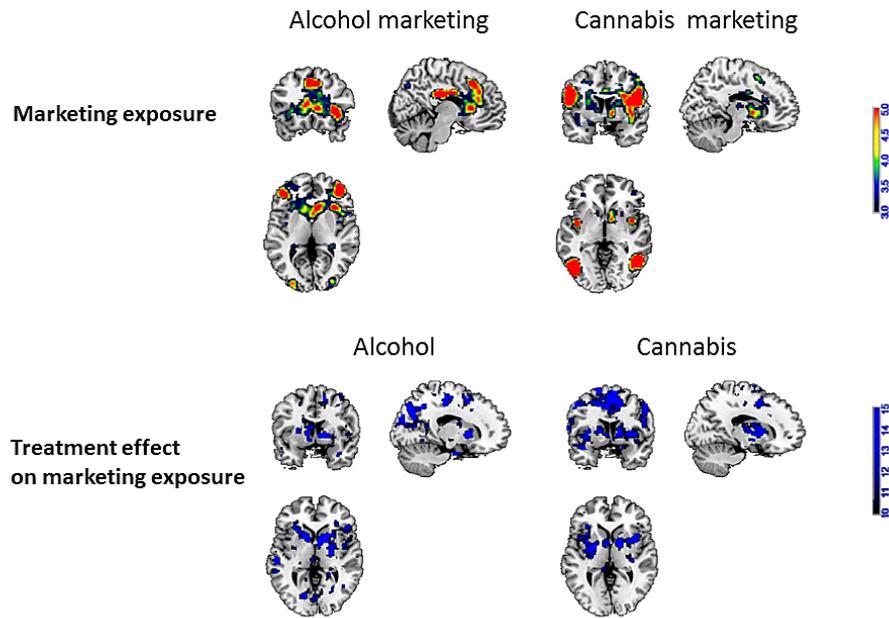


Figure 1 The upper panel shows BOLD activation (red) following cannabis and alcohol marketing exposure (versus neutral) collapsed over all three groups while sober. The lower panel shows how alcohol and cannabis intoxication deactivates (blue) the BOLD response to marketing exposure (collapsed across alcohol and cannabis marketing cues) relative to placebo.

Table 3 Striatal areas showing changes in BOLD activation during marketing exposure while being sober (GLM1) and while intoxicated (GLM2). BA = Brodmann Area; p^* = Familywise Error Corrected $<.05$; MNI =Montreal Neurological Institute

	Number of voxels	Peak MNI coordinates	F	P^*
ROI analysis (GLM1)				
Group				
Left pallidum	22	-24, -8, -6	13.22	0.006
ROI analysis (GLM2)				
Group				
Right caudate	32	18, 8, 22	16.87	0.035
Treatment				
Right pallidum	1672	16, 6, 2	39.45	0.000
Left pallidum	1533	-26, -6, -2	33.25	0.000
Right caudate	6	20, -24, 20	16.76	0.037

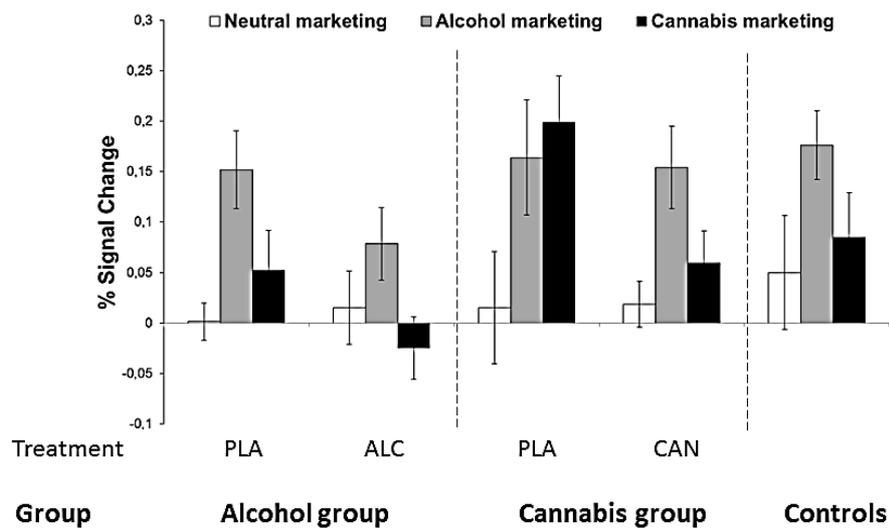


Figure 2 Mean (SE) percent signal change in the striatum separately for each group, treatment condition and marketing cue (PLA = Placebo; ALC = Alcohol; CAN = Cannabis).

Implicit cognition

Overall, implicit associations (SC-IAT) following exposure to cannabis cues significantly differed between groups during sobriety ($F[2,58] = 4.16; p = .021$). Simple groups contrast revealed that D- scores following cannabis cues were more positive in the cannabis ($p = .012$) and alcohol group ($p = .020$) relative to controls. Overall, implicit associations with alcohol cues did not differ between groups. Simple contrasts revealed that associations with alcohol cues tended to be higher in the alcohol group as compared to the group of controls ($p = .058$).

During intoxication with alcohol and cannabis, mean D-scores were less relative to placebo but failed to reach statistical significance. Mean D-scores obtained in the cannabis group, alcohol group and controls following alcohol and cannabis cues are shown in Figure 3.

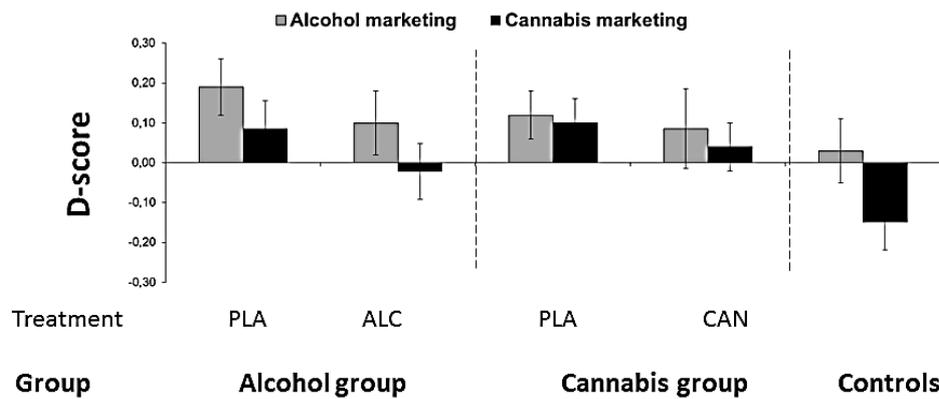


Figure 3 Mean (SE) D-Scores following alcohol and cannabis marketing cues in each group and each treatment condition (PLA = Placebo; ALC = Alcohol; CAN = Cannabis).

Pharmacokinetics

Mean (SE) alcohol concentrations in breath and cannabinoid concentrations in serum for the alcohol and cannabis treatment conditions are shown in Table 4.

Table 4 Mean (SE) concentrations of THC and metabolites in serum in the cannabis group and blood alcohol concentrations (BAC) levels in the alcohol group, at the different time points. THC = Tetrahydrocannabinol; THC-OH = 11-Hydroxy-THC; THC-COOH = 11-nor-9-Carboxy-THC; BAC = Blood Alcohol Concentration

	Cannabis group			Alcohol group
	THC [$\mu\text{g/L}$]	THC-OH [$\mu\text{g/L}$]	THC-COOH [$\mu\text{g/L}$]	BAC [g/L]
Baseline (T_0)	1.24 (.45)	.44 (.28)	15.89 (1.36)	.00 (.00)
Prior to scanning (T_1)	46.48 (1.59)	3.93 (.26)	27.66 (0.84)	.76 (.03)
Prior to SC-IAT (T_2)	24.17 (1.46)	3.16 (.28)	27.34 (1.02)	.79 (.02)

Discussion

Whole brain analysis generally revealed increased BOLD activation during marketing exposure in a large number of cortical networks and in all groups while being sober. ROI analysis of the striatal region furthermore indicated a strong increase in BOLD activation in the pallidum. These results are consistent with those from studies that reported widespread brain activations in reward, motivation and memory circuits in drug users compared to non-drug users after exposure to drug-cues (e.g., (Cousijn et al., 2013; Goldstein et al., 2009; Janes et al., 2010; McClernon et al., 2005; Myrick et al., 2004; Smolka et al., 2006; Zijlstra et al., 2009)). Activation of striatal and cortical networks following exposure to marketing movies of cannabis and alcohol use strongly suggests that such marketing can trigger similar brain responses that have also been observed during drug use or drug craving (Martín-Santos et al., 2010; Volkow et al., 2006; Wong et al., 2006).

Whole brain and striatal ROI analysis revealed decrements in BOLD activation in the right supplementary motor area and the striatum during intoxication with alcohol or cannabis. Overall, cannabis and alcohol marketing movies significantly increased % signal

changes in the striatum relative to neutral marketing movies during placebo treatments. Administration of alcohol and cannabis significantly decreased % signal change. Together, these results indicate that alcohol and cannabis marketing movies can stimulate striatal parts of the human reward system when drug users are not under the influence of drugs or alcohol and the reinforcing effects of marketing movies are reduced during alcohol or cannabis intoxication. The present data seem to fit well with our hypothesis that the phasic dopaminergic response (i.e., reward sensitivity) to marketing cues decreases when tonic dopamine levels in the striatum are high.

Performance during the cannabis SC-IAT differed significantly between the alcohol and cannabis users and controls while sober. The control group had negative bias scores, which contrasted with positive bias scores of the alcohol and cannabis group indicating positive implicit association for cannabis-related stimuli in cannabis and alcohol users. Performance during the alcohol SC-IAT tended to differ between the 3 groups during sobriety. Alcohol and cannabis users did display higher alcohol bias scores during placebo as compared to controls, but these differences just failed to reach significance in the alcohol group ($p=0.058$). In general, these results are in line with previous reviews indicating that a positive, implicit attitude towards drug-related cues is a characteristic of alcohol and substance users (Field et al., 2010; Field and Cox, 2008). Performance during the alcohol and cannabis SC-IAT decreased during cannabis and alcohol intoxication, but failed to reach significance. This strongly suggests that implicit attitudes towards cannabis and alcohol marketing cues do not change during acute intoxication, even when the actual experience or expectancy of brain 'reward' during marketing exposure decreases.

The main strengths include the placebo controlled administration of cannabis and alcohol to assess brain reactivity to marketing exposure of alcohol and cannabis. It should be noted however that participants were always exposed to the same set of marketing clips on the first or second day of treatment, even though the order of clips was randomised. This may have mitigated some of the marketing effects due to practice or

repeated exposure. If present however, such practice effects were equally balanced between placebo and active treatment sessions.

Policy and clinical implications are twofold. The present data confirm that public advertising of alcohol or cannabis use elicits striatal activation in the brain's reward circuit that are similar to those seen after primary rewards such as liquids, drugs and food. Alcohol and cannabis marketing thus increases reward sensitivity for these substances and increases motivation for actual use. A reduction of alcohol and drug marketing would diminish its impact, particularly in regular alcohol and cannabis users, by reducing brain exposure to reward cues that motivate and prepare for alcohol or drug use. Conversely, the present dataset also demonstrates that high tonic levels of dopamine protect against the reinforcing potential of alcohol and cannabis marketing. This suggests that prescription drugs that increase tonic dopamine levels, such as methylphenidate, may be of prophylactic value to alcohol and cannabis abusers to defy alcohol and cannabis marketing exposure in our society.

Chapter 3

Subjective aggression during alcohol and cannabis intoxication before and after aggression exposure

De Sousa Fernandes Perna, E.B., Theunissen, E.L., Kuypers, K.P.C., Toennes, S.W. and Ramaekers, J.G., 2016. Subjective aggression during alcohol and cannabis intoxication before and after aggression exposure. *Psychopharmacology*, 233(18), pp.3331-3340.

Abstract

Alcohol and cannabis use have been implicated in aggression. Alcohol consumption is known to facilitate aggression, whereas a causal link between cannabis and aggression has not been clearly demonstrated. This study investigated the acute effects of alcohol and cannabis on subjective aggression in alcohol and cannabis users respectively, following aggression exposure. Drug-free controls served as a reference. It was hypothesized that aggression exposure would increase subjective aggression in alcohol users during alcohol intoxication, whereas it was expected to decrease subjective aggression in cannabis users during cannabis intoxication. Heavy alcohol (n=20) and regular cannabis users (n=21), and controls (n=20) were included in a mixed factorial study. Alcohol and cannabis users received single doses of alcohol and placebo or cannabis and placebo respectively. Subjective aggression was assessed before and after aggression exposure consisting of administrations of the Point-Subtraction Aggression Paradigm (PSAP) and the Single-Category Implicit Association Test (SC-IAT). Testosterone and cortisol levels in response to alcohol/cannabis treatment and aggression exposure were recorded as secondary outcome measures. Subjective aggression significantly increased following aggression exposure in all groups while being sober. Alcohol intoxication increased subjective aggression whereas cannabis decreased the subjective aggression following aggression exposure. Aggressive responses during the PSAP increased following alcohol and decreased following cannabis relative to placebo. Changes in aggressive feeling or response were not correlated to the neuroendocrine response to treatments. It is concluded that alcohol facilitates feelings of aggression whereas cannabis diminishes aggressive feelings in heavy alcohol and regular cannabis users respectively.

Introduction

Alcohol and cannabis are among the most frequently used drugs worldwide (EMCDDA, 2012). The elicitation of aggressive behaviour following alcohol consumption, also called 'intoxicated aggression', has been frequently reported on a global scale (Murdoch et al., 1990). Cannabis intoxication, however, does not typically lead to aggression in most individuals (Hoaken and Stewart, 2003), but it might increase or facilitate aggression in certain subgroups (i.e. violent offenders, clinical population) (Cherek et al., 1993). However not everybody who uses alcohol or cannabis engages in aggressive behaviours (Heinz et al., 2001; Kopak et al., 2014; Lammers et al., 2014). A clear relationship between alcohol, drugs and intoxicated aggression is neither linear nor invariant. Some drugs can facilitate aggressive behaviour through their direct pharmacological effects during intoxication, through neurotoxic effects caused by chronic drug use over time or through withdrawal effects during abstinence (Hoaken and Stewart, 2003).

The relation between alcohol consumption and aggression has been well established. Experimental studies on aggression have demonstrated that acute doses of alcohol facilitate aggressive behaviour in a dose-related manner as assessed by vocal recordings and questionnaires (Bushman and Cooper, 1990; Ito et al., 1996). Studies using laboratory-based measures of aggression have generally found that aggression was higher in participants who were intoxicated compared to those who received no alcohol (for a review see Giancola and Chermack, 1998). Longitudinal and observational studies suggest that acute episodes of heavy alcohol consumption are more strongly related to aggressive behaviour than chronic alcohol consumption (Chermack and Blow, 2002; Fals-Stewart, 2003). This indicates that alcohol-induced aggression is more likely to occur in users who are consuming excessively within a given drinking episode (Heinz et al., 2011), although it is only a minority of people who become aggressive when under the influence of alcohol (Beck and Heinz, 2013).

The relation between cannabis use and aggression has also been investigated in studies with animals and with humans. In animals, studies have shown a decrease in

aggressive behaviour of rodents and primates following cannabis administration (Miczek, 1978; Miczek and Barry, 1977). In humans, experimental findings on acute effects of cannabis on aggression are mixed (Moore and Stuart, 2005; Taylor et al., 1976). Some studies indicate that cannabis intoxication is associated with the elicitation of aggression (Cherek et al., 1993; Howard and Menkes, 2007). However, interpretation of these findings is difficult as they are based on relatively small sample sizes (Cherek et al., 1993; Howard and Menkes, 2007) or only included male participants with self-reported anti-social tendencies (Cherek et al., 1993). Moreover, dose and route of administration differed considerably between studies. Cannabis effects on aggression are exerted in a dose-dependent manner. Low doses (0.1 mg/kg) of tetrahydrocannabinol (THC) slightly increased the willingness of participants to increase shock intensity given to opponents, were moderate to high doses (0.25 mg/kg - 0.4 mg/kg) decreased aggressive response during a laboratory-based aggression study (Myerscough and Taylor, 1985). In the latter study however, participants were randomly assigned to one of the three dose conditions without a placebo condition or control group, making it difficult to assess whether the effect was pharmacological, contextual or due to individual differences. One study, monitored aggression in long-term heavy cannabis users (Kouri et al., 1999) and reported increased aggressive responses relative to controls when tested 3 and 7 days into abstinence.

Aggressive behaviour is modulated by neuroendocrine mechanisms and it is suggested that changes in cortisol and testosterone are predictive of changes in aggression (Brown et al., 2008; Brown and Dobs, 2002; Terburg et al., 2009). Fluctuations in cortisol levels can affect the relationship between testosterone and the expression of aggression (Popma et al., 2007). It is unclear whether hormones could play a mediating role in the relationship between drugs and aggression. Previous studies report significant changes in testosterone and cortisol levels following acute alcohol and cannabis administration (Brown and Dobs, 2002; Frias et al., 2002; Lovallo et al., 2000; Mendelson et al., 1977; Sarkola et al., 2001; Thayer et al., 2006; Välimäki et al., 1990; Ylikahri et al., 1974). Suppression of male testosterone levels has been reported after short-term heavy

drinking (Sarkola and Eriksson, 2003), and a reduction in cortisol reactivity was found in heavy drinkers compared to light drinkers after a high (0.8 g/kg) alcohol dose (King et al., 2006). Studies on the effects of cannabis show decreased male testosterone levels after both acute and chronic cannabis use (Kolodny et al., 1974, 1976) and elevated cortisol levels in occasional smokers (Cone et al., 1986), these findings were not corroborated by subsequent studies however (Block et al., 1991; Mendelson et al., 1974; Schaefer et al., 1975).

While aggression is defined objectively as any type of behaviour aimed at harming another living being who is motivated to avoid such a behavioural act (Baron, 1977), aggression in humans could also be operationalized on a subjective level as the increase in aggressive inclination that is triggered by an aversive/aggressive stimulus or event underlying emotional-cognitive state. The present study investigated the acute effects of alcohol and cannabis on subjective aggression following aggression exposure in heavy alcohol and regular cannabis users respectively. Subjective aggression was directly measured by means of a Visual Analogue Scale (VAS) that allowed participants to rate their feeling of aggression on a linear scale ranging from “not aggressive at all” to “very aggressive”. Previous studies (Bond and Lader, 1974; Cleare and Bond, 1995) that have used rating scales of subjective aggression in human drug studies demonstrated that subjective feelings of aggression and hostility are positively correlated to behavioural acts or measures of aggression. The relevance of subjective aggression therefore lies in the notion that it may predict or coincide with behavioural acts of aggression. Aggression exposure consisted of two tasks developed to evoke and measure aggressive responses: i.e. the Single Category Implicit Association Test (SC-IAT) and the Point-Subtraction Aggression Paradigm (PSAP). Subjective aggression occurring in response to some perceived provocation can be categorized as subjective affective/reactive aggression (Anderson and Bushman, 2002). A control group served as between group reference in order to compare aggressive responses of alcohol and cannabis users with non-drug users. Subjective aggression in alcohol and cannabis users was compared with controls while sober and compared to placebo while intoxicated. It was expected that aggression

exposure would increase subjective aggression in alcohol users during alcohol intoxication and decrease subjective aggression in cannabis users during cannabis intoxication. It was further expected that subjective aggression in all 3 groups would increase after aggression exposure in all groups when sober. Neuroendocrine measures of cortisol and testosterone in response to alcohol/cannabis treatment and after aggression exposure were recorded as additional, secondary outcome measures.

Methods

Participants

The present study included a group of heavy alcohol and regular cannabis users, and a control group. Heavy alcohol use was defined as using on average 21 to 50 alcoholic drinks a week for males or 15 to 35 alcoholic drinks a week for females during the last year (Cassisi et al., 1998). Regular cannabis use was defined as having used cannabis at least 3 times a week but no more than 10 times a week, during the previous year (Ramaekers et al., 2009).

Experimental use of cannabis in the alcohol group was allowed only if it occurred more than a year ago. Alcohol use between 1-14 units/week (for both males and females) was allowed in the cannabis group. Controls were defined as not currently using cannabis or other drugs; experimental use of cannabis was allowed if it occurred more than a year ago and incidental alcohol use was permitted (1-7 units/week for women and 1-14 units of alcohol/week for men). Inclusion criteria were: (i) age 18–40 years, (ii) free from psychotropic medication, (iii) good physical health and, (iv) body mass index within 18.5–28 kg/m². Exclusion criteria included: (i) history of drug abuse as assessed by drug urine screens and questionnaires, (ii) presence or history of psychiatric or neurological disorder as assessed by a medical questionnaire, (iii) pregnancy, (iv) cardiovascular abnormalities as assessed by 12-lead ECG, (v) excessive smoking (> 15 cigarettes per day) and (vi) hypertension.

Five participants from the alcohol group and 2 participants from the cannabis group dropped out due to personal circumstances. The dropouts were replaced and the final dataset consisted of 61 participants, i.e. 20 participants in the alcohol and control group and 21 participants in the cannabis group. Participants (35 male, 26 female) were aged between 18 and 28 (mean (SD) 22.5 (2.3) years) (Table 1). Participants' age in the alcohol and cannabis group were matched with controls. The participants underwent a general medical examination including routine laboratory tests, provided a written informed consent and filled out a questionnaire on history of substance use. This study was part of a larger experiment and was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Fortaleza (2013) and approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University (*Dutch Trial Register: trial number: NTR3428*). A permit from the Dutch drug enforcement administration was acquired for obtaining, storing, and administering cannabis. Participants received monetary compensation for their participation in the study.

Design and treatments

Participants in the alcohol and cannabis group participated in a double-blind, placebo-controlled, within-subject study involving two experimental conditions consisting of placebo and alcohol or cannabis treatment for the alcohol and cannabis group respectively. An age matched control group of non-drug users was added that received no treatment.

Alcohol (ethyl alcohol 96%) was mixed with orange juice to a total volume of 500 mL, divided into two beverages (250 mL each). Alcohol doses were individually calibrated using the formula of Watson et al. (Watson et al., 1981) to achieve a total blood alcohol concentration (BAC) of 0.8 g/L, and was kept constant at 0.8 g/L by means of booster doses with an interval of approximately 1 hour. The alcohol placebo consisted of 500 mL orange juice, divided into two beverages, which contained a small amount of alcohol (3mL) to provide an alcohol scent when consuming the beverage. Participants' BAC was

measured at baseline (T_0), before aggression exposure (T_1) and after aggression exposure (T_2) by means of a breathalyser (Figure 1).

Table 1 Participant demographics and history of alcohol and drug use. LSD = Lysergic Acid Diethylamide

	Mean (SD)	Min.	Max.
Age (years) all groups	22.5 (2.3)	18	28
Age alcohol group	22.7 (2.4)	19	28
Age cannabis group	21.9 (2.2)	18	26
Age control group	22.9 (2.3)	19	27
Weight (kg)	67.9 (10.7)	50	92
Alcohol group (N=20; 10 men, 10 women)			
# of alcohol units/week	24 (7.7)	15	50
Cannabis group (N=21; 15 men, 6 women)			
Frequency of cannabis use/week	4.8 (1.9)	3	7
# of alcohol units/week	4.9 (4.7)	0	14
Control group (N=20; 10 men, 10 women)			
# of alcohol units/week	5.3 (3.5)	1	14
Lifetime use of other drugs	Alcohol Group	Cannabis Group	Control Group
Ecstasy	8	10	2
Amphetamine	2	5	1
Cocaine	1	5	0
LSD	0	3	0
Mushrooms	2	11	0
Other (e.g., truffles, ketamine)	3	8	0

The cannabis group received in total 300 µg THC/kg bodyweight, divided over two successive doses of 200 µg and 100 µg THC/kg bodyweight with an interval of approximately 1 hour. THC or placebo was administered using a Volcano vaporizer produced by Storz-Bickel, Germany (<http://www.storz-bickel.com>). Hot air would pass through the filling chamber holding the cannabis (containing 12% THC), which caused the THC or placebo to vaporize and blend with the air. The THC molecules or the placebo (vapor) was trapped in a valve balloon. The valve of the balloon was put against participants' lips and they were instructed to inhale deeply for about 10 seconds and then exhale. The volume of the balloon was inhaled in 7 to 10 subsequent breaths and the balloon had to be emptied within 10 min.

Procedures

Participants were asked to refrain from drug use at least one week prior to the start and during the study. Participants were not allowed to use alcohol, tobacco or caffeinated beverages on the day before an experimental session and were requested to arrive at experimental sessions well-rested. Drug and alcohol screens were carried out upon arrival at our testing facilities. Urine drug screens assessed for the presence of benzodiazepines, opiates, cocaine, marijuana, MDMA and (meth)amphetamine. Women were also tested for pregnancy. Study treatments were only administered after negative pregnancy tests and negative drug screens, except for marijuana in the cannabis group. For a detailed schematic representation of a test day see Figure 1.

The experimental session included an aggression exposure block, which consisted of the administration of the SC-IAT and the PSAP. Subjective aggression was measured before and after aggression exposure. Alcohol (or alcohol placebo) or cannabis (or cannabis placebo) administration was completed at 30 and 15 min prior to aggression exposure, with placebo conditions serving as reference. Conditions were separated by a minimum washout period of 7 days to avoid carry-over effects. The control group received no treatment but the test day was similar on all other aspects. All participants received a

training session before onset of the experimental sessions in order to familiarize them with tests, procedures and in using the vaporizer.

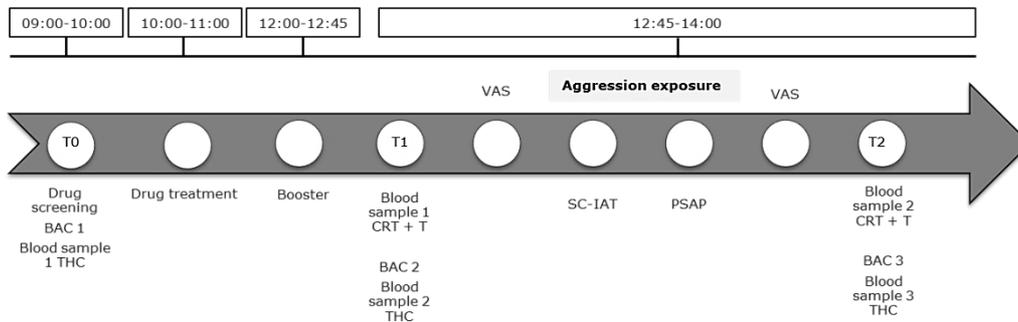


Figure 1 Schematic representation of an experimental session (BAC = Blood Alcohol Concentration; THC = Tetrahydrocannabinol; CRT = Cortisol; T = Testosterone; VAS = Visual Analogue Scale; SC-IAT = Single Category Implicit Association Test; PSAP = Point Subtraction Aggression Paradigm).

Assessment of aggression

Subjective aggression

Subjective aggression was measured using a 100 mm VAS with ‘not aggressive at all’ at one end and ‘very aggressive’ at the other end of the line. Participants had to rate how aggressive they felt at two different time points (i.e. before and after the aggression exposure block). The first time point was aimed to assess the acute effects of alcohol and cannabis treatment on subjective aggression in the alcohol and cannabis group respectively. The second assessment point followed after a period of aggression manipulation, in which participants were exposed to aggressive stimuli during two laboratory tasks (SC-IAT and PSAP) in order to provoke aggression in the participants. The second time point was aimed to assess both the effects of alcohol and cannabis treatment and the effects of the provocation on subjective aggression.

Aggression exposure

1) The Single Category Implicit Association Test

The SC-IAT measures the strength of individuals' affective evaluative associations (positive vs. negative) with a single attitude object (Greenwald et al., 2003; Karpinski and Steinman, 2006). In this task, positive and negative words were coupled with an aggressive stimulus. Aggression stimuli were static pictures displaying aggressive acts carried out by other individuals, e.g. violent protests, restraintment with a weapon. Acts of aggression were both physical (i.e., punching, kicking) and verbal (i.e. provocation in traffic-related aggression). The task was divided into a practice block and 2 test blocks (Table 2). During the practice block (target discrimination), only the target concepts were presented, and participants had to respond using the corresponding keys (i.e. press right button for positive words and the left button for negative words or vice versa). In the first block (compatible block), positive words and aggressive stimuli were categorized on the right key, and negative words were categorized on the left key. In the second block (incompatible block), negative words and aggressive stimuli were categorized on the left key, and positive words were categorized on the right key. A correct response was defined when a participant would react to a positive/negative word or an aggressive stimulus by pressing the corresponding key. The two blocks were counterbalanced across treatments conditions.

The rationale behind this task is that when participants have a positive association with aggressive behaviour rather than a negative association, they are quicker to respond when aggression cues are paired up with positive words compared to blocks where aggression cues and negative words are paired up. The dependent variable was the D-score, which was calculated by subtracting the mean reaction time (RT) of correct responses in the compatible block from the mean RT of correct responses in the incompatible block, divided by the standard deviation (SD) of all correct responses within the compatible and incompatible block.

Table 2. Schematic representation of the Single Category Implicit Association Test.

Block	Task	Left key	Right key
1	Target discrimination	Negative words	Positive words
2	Compatible	Negative words	Positive words + aggression cues
3	Incompatible	Negative words + aggression cues	Positive words

2) The Point-Subtraction Aggression Paradigm

The PSAP is a free-operant measure of human aggression (Cherek, 1981). It is a computer-based task where participants are paired up with a fictitious (unbeknownst to the participant) counterpart and could earn money by pressing buttons. A counter indicating the net value of money earned was shown on the screen. Three response buttons (labelled A, B or C) were presented to the participants on a row across a response panel: a monetary-reinforced option (A), an aggressive option (B) and an escape option (C). By pressing button A 100 consecutive times, 15 cents was added to the participants' counter. By pressing button B 10 times, 15 cents was subtracted from the counterpart at no gain to the participant. Button C, the escape option, had to be pressed 10 times and temporarily protected the participants' money from being subtracted by the fictitious counterpart. Participants were provoked at random intervals throughout the session by having 15 cents subtracted from their counter, which was ostensibly ascribed to the counterpart.

Participants were told that their counterpart was sitting at a different location in the same building. The participants were instructed to earn as much money as possible and had 20 minutes to complete the task. Participants could freely decide which buttons to press throughout the task and were aware that pressing button C would protect their money for a period of time. Aggression was not mentioned in the instructions. In reality, a computer program controlled all points subtracted by the fictitious counterpart. The dependent variable was the number of times the B button (aggressive responses) had been pressed.

Neuroendocrine measures

Testosterone and cortisol levels were collected as neuroendocrine measures in response to alcohol and cannabis treatment for the alcohol and cannabis group respectively, and in response to aggression exposure for all 3 groups. Blood samples (8mL) to determine cortisol and testosterone concentrations were collected from the participants before (T_1) and after aggression exposure (T_2) (Figure 1). The blood samples were centrifuged immediately and sent away for analysis after each test day. Concentrations were determined by means of the Cobas assay (Roche Diagnostics Limited, West Sussex, UK). The quantification limit for testosterone and cortisol were 0.087 nmol/L and 0.500 nmol/L respectively.

Pharmacokinetic measures

In the cannabis group, blood samples (8mL) to determine cannabinoid concentrations (THC and metabolites OH-THC and THC-COOH) were collected at 3 successive times during each test day, i.e. at baseline (T_0) before aggression exposure (T_1) and after aggression exposure (T_2) (Figure 1). These blood samples were centrifuged immediately; serum was transferred into a tube and was stored at -20°C . Cannabinoid concentrations were determined by the Institute of Forensic Toxicology, University of Frankfurt, using solid phase extraction and gas chromatography with mass spectrometric detection with a limit of quantification of 1.0 ng/mL.

Statistics

Two generalized linear models (GLM) were used to analyze differences in subjective aggression and neuroendocrine measures between the 3 groups during abstinence (GLM1) and to test how these measures were affected by acute cannabis and/or alcohol intoxication following aggression exposure compared to placebo (GLM2). VAS data for subjective aggression were log-transformed to obtain a normal distribution.

GLM1 included Group (3 levels: alcohol group when sober, cannabis group when sober and controls) as the between subject factor and Aggression exposure (2 levels:

before and after aggression exposure) as the within-subject factor. These were followed by simple group contrasts with the control group as reference.

GLM2 included Group (2 levels: alcohol and cannabis users) as the between-subject factor and Treatment (2 levels: placebo and alcohol/cannabis) and Aggression exposure (2 levels: before and after aggression exposure) as the within-subjects factors.

The same approach in GLM 1 and 2 was followed for SC-IAT and PSAP with the exclusion of the factor Aggression exposure. In case the sphericity assumption was violated, the Greenhouse-Geisser correction was used. The alpha criterion significance level was set at $p = 0.05$.

Spearman correlations were used to investigate associations between neuroendocrine measures, subjective aggression and performance in the PSAP and SC-IAT when sober and when intoxicated. All statistical tests were conducted with SPSS version 21.

Results

Missing data

A total of 20 complete datasets for the alcohol and control group and 21 datasets for the cannabis group entered the analyses for the SC-IAT. Due to technical failures, complete data sets were missing for the PSAP (4 participants) and aggression VAS (2 participants) PSAP data of one participant was excluded from analysis due to extreme values. Due to difficulties during blood sample collection, testosterone and cortisol samples from 14 participants could not be collected during both experimental sessions (see Table 3).

Table 3. Mean (SE) concentrations of testosterone and cortisol during placebo conditions for all 3 groups and drug conditions for the alcohol and cannabis group at the different time points. PLA = Placebo, ALC = Alcohol, THC = Cannabis, CRT = Cortisol; T = Testosterone

Group	Alcohol		Cannabis		Alcohol		Cannabis		Control	Control
	T		T		CRT		CRT		T	CRT
Conditions	PLA [nmol/L]	ALC [nmol/L]	PLA [nmol/L]	THC [nmol/L]	PLA [nmol/L]	ALC [nmol/L]	PLA [nmol/L]	THC [nmol/L]	(no treatment)	
Before aggression measures (12:45)	8.79 (2.00)	8.81 (1.98)	14.12 (2.00)	14.48 (1.98)	545.51 (51.69)	477.00 (56.08)	361.39 (53.29)	490.59 (57.80)	9.06 (2.12)	463.86 (52.25)
<i>N</i>	<i>N</i> = 18	<i>N</i> = 19	<i>N</i> = 18	<i>N</i> = 17	<i>N</i> = 18	<i>N</i> = 20	<i>N</i> = 18	<i>N</i> = 17	<i>N</i> = 17	<i>N</i> = 17
After aggression measures (14:00)	8.83 (2.03)	9.01 (2.08)	14.76 (2.03)	13.93 (2.08)	456.69 (44.82)	461.57 (53.52)	304.64 (46.20)	385.36 (55.17)	9.03 (2.17)	465.39 (54.32)
<i>N</i>	<i>N</i> = 17	<i>N</i> = 19	<i>N</i> = 18	<i>N</i> = 18	<i>N</i> = 17	<i>N</i> = 20	<i>N</i> = 18	<i>N</i> = 18	<i>N</i> = 16	<i>N</i> = 16

Measures of aggression

1) GLM 1: comparisons across groups while sober

GLM analyses revealed a main effect of Aggression exposure on subjective aggression ($F_{2,58} = 28.31$; $p = .000$) when sober. Subjective ratings across groups were higher after aggression exposure compared to before (Figure 2). There was no effect of Group or interaction with Aggression exposure when sober.

There was no main difference in aggressive responses between groups during the PSAP when sober (Figure 3). Escape response rates did differ across the 3 groups during sobriety ($F_{2,53} = 4.17$; $p = .021$). Simple contrast revealed a difference in escape response rates between the control group and alcohol group ($p = .006$), but not between the cannabis group and controls ($p = .189$). Escape response rates in the alcohol group was

lower compared to controls. A summary of mean (SE) monetary, aggressive and escape rates are given in Table 4.

Table 4. Mean (SE) number of monetary and escape responses in the Point Subtraction Aggression Paradigm for each group and treatment condition. One monetary response equals 100 button (A) presses. One aggressive/ escape response equals 10 button B/ C presses. PLA = Placebo; TREAT = Treatment

	Monetary responses (A) PLA	Monetary responses (A) TREAT	Aggressive responses (B) PLA	Aggressive responses (B) TREAT	Escape responses (C) PLA	Escape responses (C) TREAT
Alcohol group	200.94 (22.74)	221.44 (24.13)	18.95 (4.37)	24.98 (5.07)	17.34 (4.19)	17.80 (4.94)
Cannabis group	231.36 (21.51)	240.82 (22.82)	30.85 (4.14)	25.32 (4.80)	26.84 (3.96)	26.20 (4.67)
Control group	205.83 (80.34)	N/A	32.20 (4.42)	N/A	34.53 (19.34)	N/A

Mean D-scores (SE) during the SC-IAT were negative and did not differ between groups when sober (i.e., alcohol (-0.124 (.07)), cannabis (-0.118(.04)), controls (-0.195(.07))).

GLM analyses revealed no main effects of Group or Aggression exposure nor the interaction between Group and Aggression on testosterone levels (Figure 2). Analyses revealed a main effect of Aggression exposure on cortisol levels ($F_{2,46} = 6.62$; $p = .013$) when sober. Cortisol levels across groups were lower after aggression exposure compared to before. Cortisol levels in the control group before and after exposure actually did not differ significantly from each other (difference score 1,53 nmol/L.) There was no main effect of Group ($F_{2,46} = 3.09$; $p = .055$) or interaction with Aggression exposure on cortisol levels when sober.

There were no significant correlations between neuroendocrine measures, subjective aggression and performance in the PSAP and SC-IAT in the 3 groups when sober.

2) GLM 2: comparisons between treatments and placebo

GLM analyses revealed a main effect of Aggression exposure ($F_{1,37} = 17.051$; $p = .000$) and Group ($F_{1,37} = 4.19$; $p = .048$) on subjective aggression. Subjective aggression was generally higher after aggression exposure compared to before, but differed between the alcohol and cannabis group. Subjective aggression in the alcohol group was overall higher compared to the cannabis group. Analysis revealed a significant interaction between Treatment and Group ($F_{1,37} = 7.08$; $p = .011$) on subjective aggression. Subjective aggression in the alcohol group was higher in the alcohol compared to placebo condition, whereas subjective aggression in the cannabis group was lower in the cannabis conditions compared to placebo (Figure 2). There was no effect of Treatment or interaction with Aggression exposure.

There was no effect of Treatment or Group on D-scores during the SC-IAT. Mean D-scores (SE) following alcohol (-0.160 (.07)) and cannabis (-0.229 (.06)) treatment did not differ from placebo conditions or between groups.

GLM analyses revealed no main effects, but a significant interaction between Treatment and Group ($F_{1,34} = 6.16$; $p = .018$) on aggressive responses in the PSAP. Aggressive responses in the alcohol group were higher in the alcohol compared to placebo condition, whereas aggressive responses in the cannabis group were lower in the cannabis conditions compared to placebo (Figure 3). Both monetary response and escape rates (i.e., A responses and C responses) during alcohol or cannabis intoxication did not differ from placebo conditions or between these two groups.

GLM analyses revealed no main effects of Treatment, Aggression exposure or Group, but a significant 3-way interaction of Treatment*Aggression exposure*Group on testosterone ($F_{1,30} = 4.92$; $p = .034$) and cortisol levels ($F_{1,31} = 6.32$; $p = .017$) (Figure 2). The

alcohol group did not show a change in testosterone levels after alcohol treatment following aggression exposure compared to placebo. The cannabis group on the other hand showed a decrease in testosterone levels after cannabis treatment, particularly after aggression exposure. Participants in the alcohol group had a small decrease in cortisol levels after alcohol treatment whereas participants in the cannabis group showed an increase in cortisol levels after cannabis treatment, particularly prior to aggression, compared to placebo.

During alcohol and cannabis intoxication, subjective ratings following aggression exposure positively correlated with respectively aggressive responses ($r_s(13) = .637$; $p = .011$) and escape responses ($r_s(18) = .491$; $p = .028$) in the PSAP.

There were no significant correlations between neuroendocrine measures and performance during the PSAP and SC-IAT in the alcohol and cannabis group during intoxication.

Subjective aggression during alcohol and cannabis intoxication

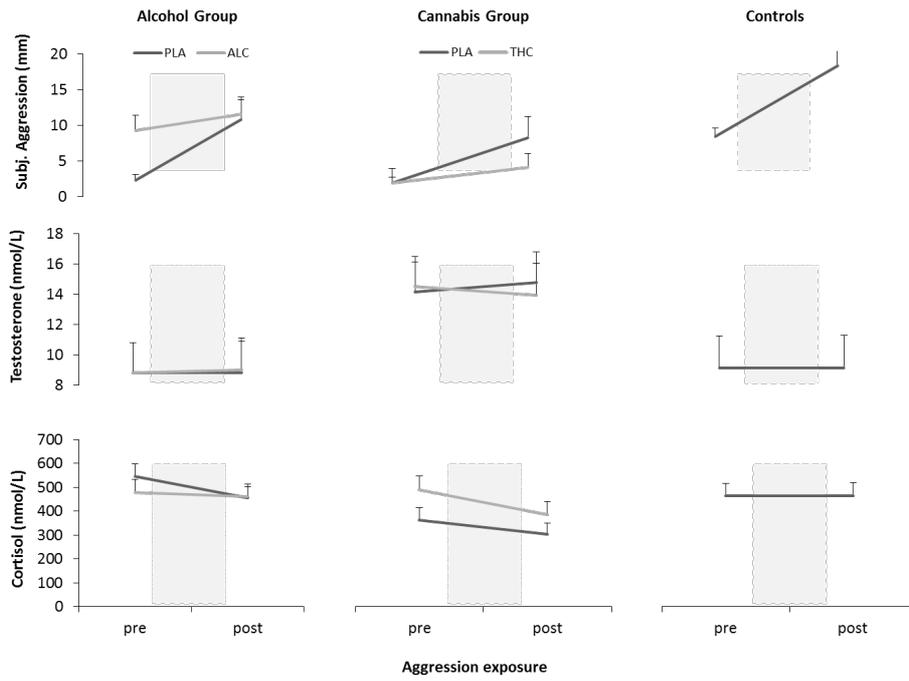


Figure 2 Mean (SE) subjective aggressive ratings (upper panel), testosterone concentrations (middle panel), cortisol concentrations (lower panel) before and after aggression exposure for each group and treatment condition (PLA = Placebo; ALC = Alcohol; THC = Cannabis).

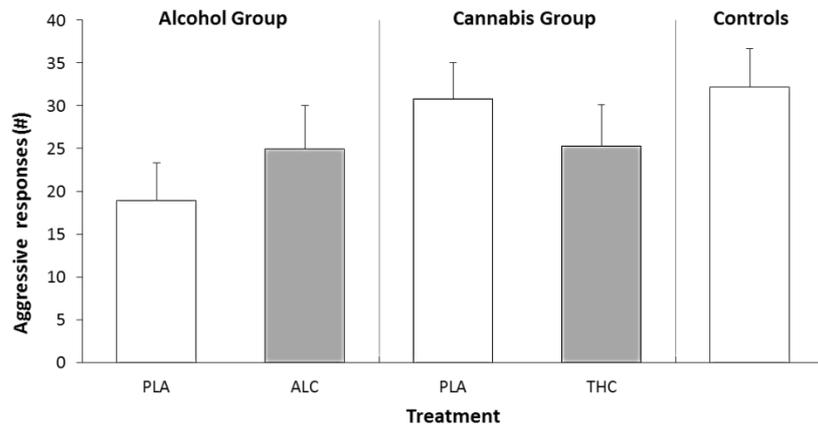


Figure 3 Mean (SE) number of aggressive responses* in the Point Subtraction Aggression Paradigm for each group and treatment condition. *The number of aggressive responses (B) was equal to 10 button presses (PLA = Placebo; ALC = Alcohol; THC = Cannabis).

Pharmacokinetic measures

Mean alcohol concentrations in blood and cannabinoid concentrations in serum for the alcohol and cannabis treatment conditions from the participants are shown in Table 5.

Table 5 Mean (SE) concentrations of THC and metabolites in serum in the cannabis group and blood alcohol concentrations levels in the alcohol group at the different time points. THC = Tetrahydrocannabinol; THC-OH = 11-Hydroxy-THC; THC-COOH = 11-nor-9-Carboxy-THC; BAC = Blood Alcohol Concentration

	THC [µg/L]	THC-OH [µg/L]	THC-COOH [µg/L]	BAC [g/L]
Baseline	1.07(.40)	.36(.23)	16.84(1.32)	.00 (.00)
Before aggression measures (12:45)	46.48 (1.59)	3.93 (.26)	27.66 (0.84)	.79 (.02)
After aggression measures (14:00)	24.17 (1.46)	3.16 (.28)	27.34 (1.02)	.60 (.03)

Discussion

The aim of the present study was to assess the acute effects of alcohol and cannabis on subjective aggression in heavy alcohol and regular cannabis users after aggression exposure. Alcohol users received alcohol or placebo and cannabis users were given cannabis or placebo. A group of non-drug users served as controls. Neuroendocrine measure of testosterone and cortisol were collected as additional outcome measures in response to acute alcohol and cannabis intoxication and after aggression exposure.

Subjective aggression after aggression exposure was increased across all 3 groups but did not differ between groups when sober, indicating that the aggression manipulation was successful. Aggressive responses of sober alcohol and cannabis users after aggression exposure did not differ from controls during the PSAP. All groups had equal negative D-scores in the SC-IAT when sober, indicating that all 3 groups had a negative implicit association with aggression. Testosterone levels did not change after aggression exposure or differ between groups. Cortisol levels, on the other hand, decreased to similar degrees in all groups after aggression exposure. Taken together, we

did not record any difference in subjective aggression and aggressive responses between sober alcohol and cannabis users and drug-free controls.

Subjective experience of aggression exposure was modified by treatments as compared to placebo, as indicated by significant Group x Treatment interactions. Alcohol intoxication increased subjective aggression in the alcohol group. The cannabis group in contrast experienced a reduction in subjective aggression during cannabis intoxication. Although alcohol intoxication increased subjective feelings of aggression in heavy alcohol users, the general increment was relatively mild. Yet, the direction of the change suggests that alcohol users might feel more aggressive with higher alcohol doses. Likewise, alcohol increased the number of aggressive responses in the PSAP in the alcohol group, whereas cannabis reduced the number of aggressive responses in the cannabis group. These interactions between Treatment and Group point to opposing effects of alcohol and cannabis on aggression. These findings are generally in line with previous studies that showed alcohol induced aggression at higher doses. A study conducted among healthy male and female social drinkers showed that moderate (0.4 g/kg) to high (0.8 g/kg) alcohol doses do not increase aggression (Gowin et al., 2010), while others showed a dose-related increase in aggression for both genders at the 0.75 g/kg and 1.0 g/kg alcohol doses (Duke et al., 2011). The cannabis group received a moderate to high cannabis dose which diminished aggressive responses during intoxication, which is in line with previous findings (Myerscough and Taylor, 1985). Subjective measures of aggressive feelings in the alcohol group were positively correlated to performance in the PSAP during intoxication, i.e., aggressive responses increased in the PSAP with increased feelings of aggression, which was not observed in the cannabis group. This indicates that subjective feelings of aggression in heavy alcohol users coincide with behavioural outcome measures of aggression.

Neuroendocrine responses to alcohol and cannabis were very minimal. We observed no main effects of Treatment, Group and Aggression exposure, but their 3-way interactions reached significance. These indicated that cannabis reduced testosterone

levels following aggression exposure whereas alcohol did not. In addition, there were indications that cannabis increased cortisol levels whereas alcohol decreased cortisol, particularly prior to aggression exposure. Attenuated cortisol response in regular alcohol users following a high alcohol dose have been reported before (Cone et al., 1986; King et al., 2006). During sobriety, cortisol levels in the alcohol and cannabis group were decreased after aggression exposure, whereas no changes in testosterone levels were observed. It has been suggested that the elicitation of aggression is related to fluctuations in testosterone and cortisol or their ratio (Popma et al., 2007). More specifically, heightened levels of testosterone are not enough to elicit aggression as sensitivity to punishment and fear are still inhibiting behaviour in the presence of high cortisol levels. When high testosterone is combined with low cortisol levels, aggression is not inhibited and could lead to the expression of aggressive behaviours (Terburg et al., 2009). The decrease in cortisol in the alcohol and cannabis group could also be attributed to the passing of time, but this decrease was not seen in controls. Cortisol levels in the control group before and after exposure actually did not significantly differ from each other (difference score 1.53 nmol/L). Furthermore, a previous study was conducted that analyzed circadian cortisol levels in a group of healthy participants (N=33) to define the parameters of physiological cortisol secretion (Debono et al., 2009). All participants had undergone detailed, 24-h, 20-min, cortisol profiling and serum cortisol levels between 13 – 14 o' clock were approximately between 244.55- 199.50 nmol/L respectively, indicating a decrease of 45.05 nmol/L in 60 min. In the current study, serum cortisol level decreases in the alcohol (88.81 nmol/L) and cannabis group (56.75 nmol/L) were larger compared to the results of Debono et al. (2009) suggesting that the decrease in cortisol levels after aggression exposure was not exclusively due to the passing of time. In the current study, changes in neuroendocrine measures and alcohol- or cannabis-induced aggression did not significantly correlate, which suggests that both phenomena are unrelated. Future research however in larger samples of both males and females is needed to investigate the relation between intoxicated aggression and associated changes in testosterone and cortisol levels in more detail. A further limitation of the current study is that it did not

assess the effect of higher doses of alcohol and cannabis on aggression. As a final limitation we note that the sample sizes of the current groups may have been too low to detect all potential but small effects of cannabis and alcohol on behavioural measures of aggression.

The results in the present study support the hypothesis that acute alcohol intoxication increases feelings of aggression and that acute cannabis intoxication reduces feelings of aggression following aggression exposure. Future studies examining the drug-aggression relationship should investigate additional variables, such as consumption patterns of alcohol and drug use, different alcohol and/or drug doses, combined with neuroendocrine measures associated with aggression. A multi-causal approach might be more effective in identifying healthy individuals who are particularly at risk of engaging in intoxicated aggression following exposure to aggression.

Chapter 4

Amygdala reactivity to affective stimuli during cannabis and cocaine intoxication: a pilot fMRI study

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Abstract

Acute cannabis and cocaine intoxication are associated with impaired emotional processing in drug users. The aim of the present study was to assess the acute effect of cannabis and cocaine on amygdala activation following exposure to affective facial stimuli. It was expected that amygdala reactivity to affective stimuli decrease during cannabis and cocaine intoxication. Regular drug users (N=12), participated in a double-blind, placebo controlled, three-way crossover study. Participants received cannabis, cocaine HCl and placebo, after which brain activity was measured by means of an amygdala reactivity fMRI paradigm. Correlations between brain activity and reaction time during task performance were additionally investigated. Results did not reveal any significant amygdala activation during task performance, but increased activity in occipital and temporal brain regions was found following exposure to affective stimuli. Acute cannabis and cocaine intoxication did not affect amygdala activity. Significant positive and negative correlations between BOLD signals and reaction times were revealed in the right inferior occipital, left inferior frontal operculum, right middle temporal, right superior temporal areas and left amygdala following exposure to threat-related faces during cannabis intoxication. These preliminary findings suggest that amygdala reactivity in regular drug users is not attenuated by cannabis or cocaine intoxication during exposure to affective facial stimuli.

Introduction

The ability to engage in social interaction and adequately perceive the intentions and dispositions of others through emotional face expressions is essential in guiding human social behavior. There are indications that illicit drugs such as cocaine and cannabis can alter the evaluation and/or processing of motivational and emotional stimuli (Ersche et al., 2011; Gruber et al., 2009). Both drugs are well known for their euphorogenic effects through the activation of the reward system, yet are quite different in their cognitive and social effects, such as increased alertness (cocaine) and decreased attention (cannabis) (Crane et al., 2013; Spronk et al., 2013).

Previous studies on social cognition indicate that both cannabis and cocaine exposure is associated with deficits in emotional perception and affective processing. Behavioral studies indicate reduced sensitivity to negative emotions in abstinent regular cannabis users (Somaini et al., 2012), as well as in intoxicated recreational users (Ballard et al., 2012, 2013; Hindocha et al., 2015). Neuroimaging research examining the brain areas associated with emotional processing report disruptions in limbic and paralimbic networks, although the direction of the cannabis induced-effects are mixed. Studies in abstinent chronic users show reduced threat-related amygdala reactivity during affective processing (Cornelius et al., 2010; Gruber et al., 2009). Studies examining acute cannabis exposure in recreational users on the other hand have shown both increased (Bhattacharyya et al., 2010) and reduced amygdala reactivity (Phan et al., 2009) or no amygdala engagement (Fusar-Poli et al., 2009) in response to threat-related affective stimuli during intoxication. Taken together, these findings suggest that exposure to cannabis may decrease/attenuate or modulate amygdala reactivity consequently leading to impaired emotional processing.

In regular abstinent cocaine users, impaired fear recognition (Kemmis et al., 2007; Morgan and Marshall, 2013) and diminished emotional engagement in social interaction (Preller, Herdener, et al., 2014; Preller, Hulka, et al., 2014) was shown. Another study investigating the acute effects of cocaine during emotional perception found impaired recognition of negative affective stimuli in recreational cocaine users during intoxication

(Kuypers et al., 2015). Neuroimaging studies in current/regular cocaine users found both hypoactivity during a reward processing tasks (Patel et al., 2013) and hyperactivity during an emotional face-matching task (Crunelle et al., 2015) in the amygdala during abstinence. However, amygdala reactivity was assessed by means of two different tasks. Patel et al. (2013) used a task that included various reward processing items (e.g., reward and loss prospect, anticipation and outcome), while the study of Crunelle et al. (2015) focussed more on emotional processing without reward processing items, which could explain the differences in results. Other studies in regular cocaine users report deactivation in the amygdala during intoxication (Kufahl et al., 2005), although this study was not specifically aimed at probing amygdala responses to affective stimuli. Thus, the acute pharmacological effects of cocaine on amygdala reactivity in response to affective stimuli in cocaine users remain elusive.

The amygdala is a critical structure in the limbic neurocircuitry implicated in affect-related processes (e.g. social interaction). Disruption of emotional processes through enhanced or decreased amygdala reactivity as a result of excessive or chronic drug intake can have detrimental consequences for both the individual and society (Frith, 2009). Understanding differences in amygdala responsivity during social-emotional processing is paramount in order to evaluate drug-induced alterations in the context of a pharmacologic challenge.

The aim of the current study was to assess the effects of acute cannabis and cocaine intoxication on amygdala reactivity following exposure to affective stimuli. Based on previous studies it was hypothesised that amygdala reactivity to emotional face expressions decrease during cannabis and cocaine intoxication.

Methods

Participants

A total of 12 regular cannabis and cocaine users (8 male, 4 female), mean age 21.76 years ($SD = 1.23$) were included. Participants were recruited through advertisements and word-of-mouth. Inclusion criteria were: age 18-40 years; regular cannabis use: ≥ 2 times/week; cocaine use > 5 times in the previous year, free from psychotropic medication, good physical health, normal weight (BMI 18-28), and written informed consent. Exclusion criteria were: dependence on cocaine according to DSM-IV criteria, presence or history of psychiatric or neurological disorder as assessed during a clinical interview, pregnancy or lactation, cardiovascular abnormalities as measured by ECG, hypertension and excessive drinking (> 20 units per week) or smoking (> 20 cigarettes per day). All participants had normal or corrected-to-normal vision, were free from psychiatric or neurological abnormalities, did not use medication that could influence cognitive functioning and were screened for MRI contra-indications.

This study was part of a larger trial on the association between drug use and impulse control (Dutch Trial Register: trial number: NTR2127) conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008) and was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University.

Design and treatments

The study was conducted according to a double-blind, placebo-controlled, 3-way crossover design. Treatments consisted of placebo, 450 μg THC/kg bodyweight (divided in two successive doses) and 300 mg cocaine HCl. Cannabis or placebo was administered using a Volcano vaporizer produced by Storz-Bickel, Germany (<http://www.storz-bickel.com>). Hot air would pass through the filling chamber holding the cannabis (containing 11% THC), which caused the THC or placebo to vaporize and blend with the air. The THC molecules or the placebo (vapor) was trapped in a valve balloon. For inhalation, the valve of the balloon was put to participants' lips and they were instructed to inhale

deeply. Cocaine HCl or placebo was administered in an opaque white capsule. The order of treatment conditions was balanced over participants and sessions. Conditions were separated by a minimum washout period of 7 days to avoid carry-over effects.

Procedure

Drug and alcohol screens were carried out upon arrival at our testing facilities. Women were also tested for pregnancy. Urine drug screens assessed for the presence of benzodiazepines, opiates, cocaine, marijuana, MDMA and (meth)amphetamine. Treatments were only administered after negative drug screens (except cannabis) and negative pregnancy tests.

Participants received a capsule containing either 300 mg of cocaine HCl or placebo orally (T_1). Cannabis or placebo administration followed 45 min after capsule administration (T_2). Between T_1 and T_2 , participants were allowed to read a book or watch television. A test-battery was conducted between 15-60 minutes following T_2 (see Van Wel et al., 2013). This was followed by a booster inhalation of 150 μg THC/kg bodyweight or placebo (T_3), after which brain activity was measured by means of functional magnetic resonance imaging (fMRI). All participants received a training session before the onset of the experimental sessions in order to familiarize them with tests and procedures.

Amygdala reactivity task

A variant of the amygdala reactivity paradigm as developed by Hariri et al. (2002) was used. The amygdala reactivity task (ART) is an fMRI paradigm in which a set of three stimuli is presented simultaneously. Participants were required to match one of the two choice-items (bottom) with the target (top) by pressing a button. A total of nine stimuli blocks consisting of six images or shapes were presented. Participants viewed four face expression blocks (e.g., angry, fear, surprise or neutral) which were interleaved with five control blocks of geometrical shapes (e.g. ovals and circles). Each image in the face block was balanced for gender and target-affect and participants selected which face was identical to the target. Task performance measures were reaction times (RT) to stimuli blocks from correct-response trials.

fMRI images were acquired with a Siemens 3T head-only scanner (MAGNETOM Allegra, Siemens Medical Systems, Erlangen, Germany). During the task, whole brain functional volumes were acquired using gradient-echo echo-planar imaging (GE-EPI, TR= 2000 ms, TE= 30 ms; FA= 90°; FOV 224mm; matrix size= 64 x 64; voxel size= 3.5 x 3.5 x 3.5 mm). The T1-weighted anatomical scan was acquired using a 3D MPRAGE (magnetization-prepared rapid gradient echo; TR= 9,7 ms; TE= 4 ms; flip angle=12°; matrix=256×256; voxel size=1×1×1 mm³).

Data preprocessing and analysis were conducted using SPM8 (Wellcome Trust Center for Neuroimaging, London, UK). The first two volumes were removed from each fMRI data set to allow for magnetic equilibration. Thereafter the following preprocessing steps were carried out: (1) realignment, (2) slice time correction, (3) individual anatomical data sets were normalized to standard 3-D MNI space (voxel size was resampled to 2×2×2 mm), and (4) spatial smoothing was applied with a FWHM 4 mm Gaussian kernel.

Statistics

fMRI data

To acquire a general overview of task-related BOLD activation for each condition, brain activity during task performance was determined during placebo and treatment conditions separately. To assess task performance during placebo, individual contrast images (first-level analyses) were first calculated by contrasting faces against shapes (blocks) for each emotion category (e.g., angry, fear and surprise) to determine affect-specificity and also against neutral faces to control for activation related to face processing of non-emotional expression. In addition, all faces across emotion categories were grouped and contrasted with shapes in order to maximize the likelihood to elicit a response in the amygdala to examine treatment effects (Sutherland et al., 2013). To identify regions showing task-related changes in activity, individual contrast images were used as input for a group-level one-sample *t*-test (second level analysis). The six realignment parameters were modelled as regressors of no interest.

To specify the effects of treatment, a GLM full factorial model with factor Treatment (three levels: placebo, cannabis and cocaine) was conducted. Reaction time was modelled as a covariate. Correlations between brain activity and reaction times across treatment conditions were calculated to investigate the association between drug-related changes in BOLD activation and changes in task performance.

Whole brain analyses were performed to explore general effects of task performance. ROI analyses were conducted in order to specifically test our hypotheses that exposure to facial expressions as well as drug intoxication would affect amygdala activation. An amygdala ROI was built with the WFU PickAtlas (Maldjian et al., 2003, 2004; Tzourio-Mazoyer et al., 2002) by combining the left and right amygdala. Results were considered significant when P_{FWE} -corrected at cluster level < 0.05 .

The behavioral parameter of the ART (i.e. reaction time) was analyzed with a repeated measures GLM ANOVA with factor Treatment (3 levels: placebo, cannabis and cocaine) as a within-subject factor. If the sphericity assumption was violated, the Greenhouse-Geisser correction was used. In case of significant main effect of Treatment, separate drug-placebo contrasts were conducted. The alpha criterion significance level was set at 0.05. All statistical tests were conducted with SPSS version 21.0.

Results

A total of eleven complete fMRI datasets entered the analyses. One participant dropped out of the study due to failure to perform the fMRI session after cannabis administration. Behavioral data from one participant was not complete due to failure to perform the task during cocaine treatment.

fMRI data analyses

Whole brain analyses

Elevation in brain activation during placebo was revealed following exposure to separate and combined facial expressions compared to shapes (Table 1). Significant increments in BOLD signal were found in the right inferior occipital and right lingual lobe following

exposure to angry faces compared to shapes. Significant BOLD increments were found in the right inferior occipital, left cerebellum, right lingual and in the calcarine lobe following exposure to combined facial expressions compared to shapes.

There was no effect of Treatment on brain activity following exposure to faces (combined or separate) compared to shapes or neutral faces.

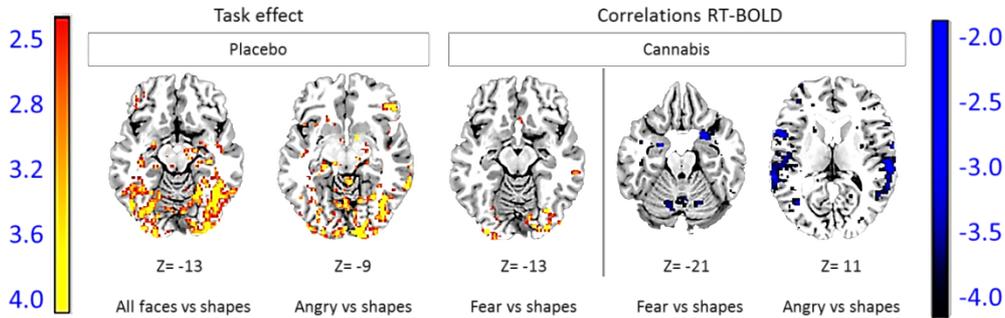


Figure 1 The left panel shows BOLD activation (red) following facial exposure (angry and combined) compared to shapes while sober. The right panel shows correlations between reaction time and BOLD activation (red) in response to fearful face and BOLD deactivation (blue) in response to angry face exposure compared to shapes.

ROI analyses amygdala

ROI analyses did not reveal any task or treatment effects following exposure to faces during placebo and treatment conditions.

Table 1 Brain areas showing changes in BOLD activation following exposure to facial stimuli compared to shapes while being sober. BA = Brodmann Area; p^* = Familywise Error Corrected $<.05$; MNI =Montreal Neurological Institute

WHOLE BRAIN ANALYSES	BA	Number of voxels	Peak MNI coordinates	T	p^*
Faces vs shapes					
Right inferior occipital	37	146	41, -60, -13	10.45	0.001
Left cerebellum	18	99	-20, -80, -17	7.33	0.009
Right lingual	18	197	17, -94, -9	7.02	0.000
Calcarine	17	210	1, -96, -1	6.97	0.000
Angry vs shapes					
Right inferior occipital	37	60	41, -62, -11	12.62	0.014
Right lingual	18	53	15, -94, -9	9.98	0.027

Behavioral data

There was a significant effect of Treatment [$F(2,18) = 5.17$; $p = .017$] on reaction time following exposure to facial expressions (combined) and shapes. Simple contrasts revealed that cocaine decreased RT to faces [$p = .023$] and to shapes [$p = .024$]. Separate analyses of face categories revealed a significant effect of Treatment on reaction times following exposure to angry faces [$F(2,18) = 5.38$; $p = .015$] and shapes [$F(2,20) = 8.00$; $p = .003$]. Simple contrasts revealed that cocaine decreased RT to angry faces [$p = .048$] and to shapes [$p = .011$] compared to placebo.

Correlations brain activity and reaction time

Whole brain analyses

Analyses yielded significant correlations between BOLD signals and reaction times following exposure to both fearful and angry faces compared to shapes (Table 2). During placebo conditions, positive correlations were found between activity in the left paracentral, left postcentral, left inferior parietal, right postcentral, right supramarginal, right angular, right cuneus, right precentral lobe and response latency to fearful faces. During cannabis conditions, a positive correlation was found between activity the right inferior occipital lobe and reaction time to fearful faces, whereas negative correlations

were found between activity in the left frontal inferior operculum, right middle temporal and right superior temporal lobe and reaction time to angry faces. There were no correlations between reaction time and brain activation during cocaine conditions.

ROI analyses amygdala

A significant negative correlation between BOLD signal and reaction time during cannabis conditions was found in the left amygdala following exposure to fearful faces.

Table 2 Correlations between BOLD signals and reaction times across treatment conditions. PLA= Placebo; THC = Tetrahydrocannabinol; BA = Brodmann Area; p* = Familywise Error Corrected <.05; MNI =Montreal Neurological Institute

WHOLE BRAIN ANALYSES	BA	Number of voxels	Peak MNI coordinates	T	p*
Fear vs shapes					
PLA					
Left paracentral		106	-10, -32, 51	6.28	0.002
Right supramarginal	42	96	55, -46, 25	5.73	0.004
Right postcentral	3	151	57, -14, 45	5.18	0.000
Left inferior parietal	40	66	-40, -38, 43	5.02	0.032
Left postcentral	3	71	-56, -22, 53	4.99	0.022
Right angular	39	65	45, -54, 37	4.91	0.034
Right cuneus	19	74	15, -84, 43	4.84	0.018
Right precentral	4	73	39, -24, 57	4.78	0.019
THC					
Right inferior occipital	37	66	31, -90, -13	5.96	0.032
ROI ANALYSES					
Left amygdala		6	-22, -4, -21	4.23	0.042
Angry vs shapes					
THC					
Left frontal inferior operculum	48	80	-52, 8, 11	5.96	0.008
Right middle temporal	21	111	65, -22, -7	5.92	0.001
Right superior temporal	22	89	61, -26, 11	5.23	0.004

Discussion

This study examined the effects of acute cannabis and cocaine administration on amygdala reactivity following exposure to affective stimuli in regular drug users. Participants received 450 µg THC/kg bodyweight, 300 mg of cocaine HCl and placebo, after which brain activity was measured by means of an amygdala reactivity fMRI paradigm. Results show increased activity in occipital and temporal brain regions following exposure to affective stimuli during placebo conditions. There was no significant amygdala activation during task performance in placebo conditions nor did cannabis and cocaine intoxication affect amygdala activity.

Increased activation in the inferior occipital and lingual gyrus was found during task performance in the placebo condition when viewing angry faces and when all facial categories were grouped. These findings are in line with previous studies reporting changes in brain activity within the same areas during emotional processing (Bhattacharyya et al., 2010; Fusar-Poli et al., 2009; Phan et al., 2002; Sutherland et al., 2013). The occipital face area (OFA) (Gauthier et al., 2000; Puce et al., 1996), a region within the inferior occipital gyrus, has been identified as a spatially and functionally distinct face-selective area that preferentially processes parts of the face (e.g., eyes, nose and mouth) (Liu et al., 2009; Nichols et al., 2010; Pitcher et al., 2007). Initial structural face description in the OFA is first required prior to processing of more complex facial components in higher specialized cortical face regions, such as the amygdala (Haxby et al., 2000; Pitcher et al., 2011). The amygdala is involved in biasing cognition depending on the social-emotional significance of perceived stimuli (Adolphs, 2008). Deficits in emotional face processing can be seen in individuals with autism spectrum disorder (ASD) (Harms et al., 2010; Philip et al., 2010). Previous studies investigating emotional face processing in individuals with ASD report hyperactivation in subcortical and fronto-temporal brain regions and hypoactivation in the hypothalamus and amygdala in individuals with ASD in contrast to typically developed individuals (Aoki et al., 2015; Baron-Cohen et al., 1999), suggesting atypical activity in emotion-processing circuitry. Although speculative, activation of the OFA following facial expressions in the absence of amygdala activation in

the current study could be suggestive of a lack of interaction among these structures in regular drug users. It has been argued that the amygdala may be one element from a larger network that is responsible for processing biological relevance in a more general manner, such as the modulation of attention by emotional stimuli (Brosh et al., 2008). It could therefore be that the facial stimuli in the current study were not perceived as being salient/biologically relevant (non-threatening) enough to significantly engage the amygdala (Whalen, 2007).

Results did not show any significant changes in amygdala activity following exposure to facial expressions during task performance in the placebo condition. This is in contrast to previous studies which have used different paradigms to assess emotional processing, and which included more emotional stimuli block trials (3 of each target expression) (Phan et al., 2009) or a larger stimulus set consisting of more images (96) presented during several functional runs (8) (Fitzgerald et al., 2006) and could therefore elicit a more reliable amygdala response. Differences in amygdala activation could also be related to the intensity of facial expressions (e.g. mild vs. intensely fearful face) as significant amygdala activation was only found following exposure to intensely fearful compared to mildly fearful (Fusar-Poli et al., 2009) and neutral faces (Bhattacharyya et al., 2010). The task used in the current study included a total of six facial expression stimuli per emotional block, which did not vary in intensity, and was only administered one time per session. Given this low number of facial stimuli and categorisation per expression-type, it could be that the paradigm in the current study was not optimal to probe neither affect-related nor affect-specific amygdala responses.

Cannabis and cocaine administration did not have any significant effect on BOLD signal in the amygdala or other brain regions. Regarding cannabis effects, this finding is in line with the results of Fusar-Poli et al. (2009), but in contrast to previous studies that reported decrements in amygdala BOLD activation during cannabis intoxication (Kufahl et al., 2005; Patel et al., 2013; Phan et al., 2009). This discrepancy may be related to differences in methodology across these studies. For instance, Phan et al. (2008) recruited

recreational drug users and orally administered a low THC dose (7.5 mg), while the effects of a higher oral dose (10 mg) in similar test populations did not elicit such a response in other studies (Bhattacharyya et al., 2010; Fusar-Poli et al., 2009). Participants in the current study were regular drugs users (i.e. primarily cannabis) and received a considerably higher THC dose (30 mg) by means of inhalation. The dose-related differences across these studies and the current one could be related to the mood-altering effects of THC. Subjective response to THC intoxication may vary considerably depending on individual differences and lifetime cannabis exposure (Gonzalez, 2007). Acute intoxication in experienced users is mainly associated with feelings of euphoria, relaxation and depersonalization, while psychotic and anxiety phenomena can also occur, although particularly in inexperienced users at higher doses (Crippa et al., 2009). Furthermore, regular cannabis use is associated with alterations in brain function and structures, particularly in the frontal-limbic neurocircuitry, which might persist during abstinence (Batalla et al., 2013; Martín-Santos et al., 2010). Results from a study conducted in chronic cannabis users during a facial affect paradigm did not show amygdala activation following exposure to masked facial stimuli and displayed more diffuse brain activations in other areas compared to controls (Gruber et al., 2009). This is consistent with previous reports indicating that chronic cannabis exposure and administration is associated with attenuated brain activity in task-activated regions or activation of compensatory brain regions (Batalla et al., 2013; Quickfall and Crockford, 2006). These findings suggest alterations in affective processing in regular cannabis users, even when the stimuli are presented below a conscious awareness level (e.g. Gruber et al., 2009).

Regarding the effects of cocaine, intravenous cocaine administration in the study of Kufahl et al. (2005) during a non-emotional task may have elicited a different neural response as compared to oral cocaine administration in the current study while participants (who used cocaine recreationally) actively engaged in an emotional face processing. Studies examining amygdala reactivity during emotional processing in recreational cocaine users during intoxication are generally lacking (Miller et al., 2015) and results can be confounded by polydrug use (Fernández-Serrano et al., 2010). For example,

Crunelle et al. (2015) allowed consumption of other drugs (with the exception of heroin) and participants were required to not use drugs (with the exception of nicotine) at least 10 hours prior to testing. However, drug screens were not performed and potential pharmacologic effects of other drugs on brain activity could therefore not be excluded. Chronic cocaine exposure is further associated with functional and neuroadaptive changes in fronto-striatal/limbic brain structures (Ersche et al., 2011; Preller, Herdener, et al., 2014; Verdejo-Garcia et al., 2014). In addition, and poor emotion recognition was predicted by the impact of quantity of cocaine use (Fernández-Serrano et al., 2010) and dysfunctional amygdala reactivity and reduced connectivity with the prefrontal cortex was associated with both the onset and duration of cocaine use (Crunelle et al., 2015). Future research employing placebo-controlled experimental designs could address the discrepancy in findings by assessing the acute effect of cocaine during emotional processing in both recreational and regular cocaine users (ideally) without concurrent drug use.

Both positive and negative correlations between BOLD signals and reaction times were revealed following exposure to threat-related faces specifically. Increased activation in temporal, parietal and frontal brain regions was associated with increased reaction time to fearful faces during placebo conditions. During cannabis intoxication, increased reaction time was associated with increased activation in the right inferior occipital cluster following fearful faces and decreased activation in frontal and temporal regions following angry faces. ROI analyses revealed a negative correlation between reaction time and activity in the left amygdala following fearful faces. Increased reaction time to fearful faces was associated with decreased BOLD activity in the amygdala, which is consistent with a previous report showing an association between high levels of cannabis use and a lower level of amygdala reactivity in response to angry and fearful face expressions (Cornelius et al., 2010).

Despite the fact that acute cannabis and cocaine administration did not affect amygdala reactivity or task performance, behavioral performance was affected by cocaine administration relative to placebo. Reaction time following cocaine intoxication was

decreased for facial stimuli, particularly for angry faces. However, this was also the case in response to geometrical shapes, suggesting a general decrease in reaction time probably due to cocaine-induced increase in psychomotor speed (Spronk et al., 2013). The absence of a cannabis-induced decrease in reaction time confirms previous findings (Fusar-Poli et al., 2009; Phan et al., 2009), indicating that, despite differences in neural response, the behavioral response to emotional stimuli is unaffected during cannabis intoxication.

The results reported in this paper however cannot be generalized to population level due to its modest sample size. Secondly, a non-cannabis using control group was not included to compare individuals with and without a history of cannabis use in order to investigate changes in brain function associated with chronic cannabis exposure. Future studies involving larger samples need yet to be conducted to confirm or counter the results of this study. These preliminary findings suggest that amygdala reactivity in regular cannabis users is not attenuated by cannabis or cocaine intoxication during exposure to affective facial stimuli.

Chapter 5

Memory and mood during MDMA intoxication, with and without memantine pretreatment

De Sousa Fernandes Perna, E.B., Theunissen, E.L., Kuypers, K.P.C., Heckman, P., De La Torre, R., Farre, M. and Ramaekers, J.G., 2014. Memory and mood during MDMA intoxication, with and without memantine pretreatment. *Neuropharmacology*, 87, pp.198-205.

Abstract

Previous studies have shown that single doses of MDMA can affect mood and impair memory in humans. The neuropharmacological mechanisms involved in MDMA-induced memory impairment are not clear. Memantine, an NMDA and alpha 7 nicotinic acetylcholine (ACh) receptor antagonist, was able to reverse MDMA-induced memory impairment in rats. This study investigated whether treatment with memantine can prevent MDMA-induced memory impairment in humans. 15 participants participated in a double-blind, placebo controlled, within-subject design. Participants received both pre-treatment (placebo/memantine 20 mg) (T₁) and treatment (placebo/MDMA 75 mg) (T₂) on separate test days. T₁ preceded T₂ by 120 minutes. Memory function was assessed 90 minutes after T₂ by means of a visual verbal learning task, a prospective memory task, the Sternberg memory test and the abstract visual pattern learning task. Profile of Mood State (POMS) and psychomotor performance were also assessed to control whether MDMA and memantine interactions would selectively pertain to memory or transfer to other domains as well. MDMA significantly impaired performance in the visual verbal learning task and abstract visual pattern learning task. Pre-treatment with memantine did not prevent MDMA-induced memory impairment in these two tasks. Both positive (vigor, arousal, elation) and negative effects (anxiety) were increased by MDMA. The responses were not altered by pretreatment with memantine which had no effect on memory or mood when given alone. These preliminary results suggest that memantine does not reverse MDMA-induced memory impairment and mood in humans.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is the primary psychoactive component of the drug ecstasy that is known to elevate mood and enhance auditory and visual perception (Baylen and Rosenberg, 2006; Kuypers et al., 2013a; van Wel et al., 2012). Chronic use of MDMA has also been linked to impairments of cognitive functions (Gouzoulis-Mayfrank and Daumann, 2009). Learning and memory deficits as assessed with a large range of neuropsychological tests have been frequently described in abstinent ecstasy users (Cole and Sumnall, 2003; Curran and Travill, 1997; Morgan, 2000; Wareing et al., 2000). There have also been several reports of impaired cognitive functioning in current ecstasy users. Meta-analytic reviews revealed that current ecstasy users are significantly impaired compared to non-using control subjects on tasks measuring memory, attention, executive function and psychomotor performance (Kalechstein et al., 2007; Zakzanis et al., 2007). A decrease in verbal memory performance is the most often reported effect associated with ecstasy/MDMA use, which appear to be more distinct in heavy users compared to light users (Gouzoulis-Mayfrank and Daumann, 2009).

To date, it is not fully clear through which underlying neuropharmacological mechanisms the effects of MDMA on memory are exerted. The majority of ecstasy studies have been conducted in abstinent users with a history of polydrug use, which hinders causal attribution of memory deficits to a single drug. Placebo-controlled studies on the other hand may serve as well-controlled models to directly investigate the neuropharmacology of memory impairment during MDMA intoxication (Kuypers and Ramaekers, 2005a, 2007; Johannes G Ramaekers et al., 2009). Kuypers and Ramaekers (2007, 2005) treated 18 recreational MDMA users with single doses of MDMA 75 mg and assessed memory performance by means of memory tests during intoxication (i.e. 1,5-2 h post drug) and during withdrawal phase (i.e. 25,5-26 h post drug). Memory performance was impaired after a single dose of MDMA when MDMA concentrations were maximal, but no residual memory impairment was evident after a 24h withdrawal phase, when blood MDMA concentrations were low. These data also implied that MDMA-induced

memory impairment actually occurred during the intoxication phase when 5-HT brain levels are high as compared to the withdrawal or abstinence phase when 5-HT levels are low (Green et al., 2003; Schmidt and Kehne, 1990). This finding contrasted with the prevailing notion that MDMA-induced cognitive impairment is associated with a general decrease in 5-HT availability in long-term users (Bolla et al., 1998; Morgan, 1999).

Subsequent studies revealed that MDMA-induced memory impairment may in part be exerted via 5-HT₂ receptor stimulation (van Wel et al., 2011). In the latter study, single doses of MDMA combined with placebo, ketanserin (a 5-HT_{2A} receptor antagonist) or pindolol (a mixed β -adrenergic/5-HT₁ receptor antagonist) were administered to recreational MDMA users who subsequently performed verbal, spatial and prospective memory tasks. Performance was impaired in all memory tasks after a single dose of MDMA. The effects of MDMA on verbal memory however were selectively blocked by ketanserin indicating a role for 5-HT_{2A} receptors. The failure of ketanserin to block impairments on other memory tasks also indicated additional mechanisms may underlie MDMA induced memory impairments as well.

More attempts to reverse or block MDMA-induced neurotoxicity have been made by other researchers (Chipana et al., 2006; Chipana, Camarasa, et al., 2008; Chipana, Torres, et al., 2008). Chipana et al.(2008a) found that memantine, a low-affinity non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (Danysz et al., 2000; Frankiewicz and Parsons, 1999; Shimono et al., 2002), was able to reverse MDMA-induced neurotoxicity in rodents. The same research group also reported a therapeutic effect of memantine in preventing MDMA-induced cognitive impairment in rats (Camarasa et al., 2008). They examined the effects of MDMA and memantine on non-spatial and spatial learning in rats by means of Novel Object Recognition and Morris water maze tasks. The animals were divided into four experimental groups and were administered 2 doses (separated by 7 h) of MDMA, memantine, memantine + MDMA or saline treatments for 4 consecutive days. Rats in the memantine + MDMA condition were able to discriminate between familiar and novel objects compared to rats in the MDMA condition. In addition,

rats treated with MDMA showed impaired learning in the Morris Water Maze, and this impairment was not present in the memantine + MDMA treated rats. The authors hypothesized that memantine reversed MDMA impairment through blockade of alpha-7 nicotinic receptors, which is confirmed by previous research which demonstrated that clinically relevant concentrations of memantine blocked alpha-7 nicotinic receptors in a non-competitive manner more potently than NMDA receptors (Aracava et al., 2005). MDMA has affinity for, and interacts with nicotinic acetylcholine (ACh) receptors. Binding of amphetamines to nicotine receptors enhances their activation, leading to activation of calcium-dependent pathways involved in neurotoxicity (Garcia-Ratés et al., 2007). By blocking the alpha-7 nicotinic receptors receptors, memantine may offer neuroprotection against serotonergic neurotoxicity induced by MDMA.

In humans, memantine has been found to improve learning and memory in patients suffering from moderate to severe Alzheimer's disease (AD). In AD, there is an excessive glutamate release causing neuronal excitotoxicity. Memantine blocks the NMDA ion channel, decreasing the activity of the glutamate receptor by reducing the tonic, but not synaptic, NMDA receptor activity and thereby improving memory in AD patients (Rammes et al., 2008; Reisberg et al., 2003). To date, no study has examined the potential of memantine to overcome the memory impairment caused by MDMA in humans.

The present study was designed to investigate whether memantine is able to prevent memory impairment subsequent to MDMA intake. In addition, the effect of MDMA and memantine on psychomotor performance and mood will also be investigated to see whether memantine would affect subjective mood ratings after MDMA intoxication. Recreational MDMA users performed a number of memory tasks after receiving single doses of MDMA in a double blind, placebo-controlled study. It was hypothesized that (1) an acute dose of MDMA will affect mood and produce impairment on behavioural measures of learning and memory; (2) MDMA-induced memory impairment will be reversed by pre-treatment with memantine.

Methods

Participants

21 participants were medically screened, five dropped out due to personal circumstances and one participant was excluded due to failure to comply with study procedures. A total of 15 healthy MDMA-users (11 male, 4 female), aged between 20 and 28 (mean (SD) 22.2 (1.9) y) completed the study. Lifetime use of MDMA varied between participants ranging from light (≤ 15 occasions, as determined by Kuypers et al. (2011)) in 13 participants to moderate use (16–40 occasions) in two participants. Overall, participants reported to have taken MDMA 3 to 40 times in their lifetime (mean 18.8 times). Participant's demographics are shown in Table 1.

Participants were recruited through advertisements placed on a university website, university newspapers and by word of mouth. Participants underwent a general medical examination including routine laboratory tests, provide a written informed consent and a history on substance use behaviour including MDMA. Inclusion criteria included: (i) age 18–40 years (ii) free from psychotropic medication; (iii) good physical health and, (iv) BMI within 18.5 –28 kg/m². Exclusion criteria included: (i) addiction according to DSM-IV criteria, (ii) presence or history of psychiatric or neurological disorder as assessed by a medical questionnaire (iii) pregnancy (iv) cardiovascular abnormalities, (v) excessive smoking (> 15 cigarettes per day) or drinking (> 20 standard units of alcohol per week) and (vi) hypertension). This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008) and approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University. A permit from the Dutch drug enforcement administration was acquired for obtaining, storing, and administering MDMA. Participants received monetary compensation for their participation in the study.

Table 1 Participant demographics and history of drug use. MDMA = 3,4-methylenedioxymethamphetamine; LSD = Lysergic Acid Diethylamide

	Mean	SD	Min	Max
Age (years)	22.2	1.9	20	28
Weight (kg)	69.7	9.1	53	85
Frequency of MDMA use	18.8	12.2	3	40
Number of Pills (previous year)	11.5	11.5	0	48
Frequency of MDMA use (previous 3 months)	1.7	1.3	0	5
Frequency of MDMA use (previous year)	6.7	6.0	1	24
Occasional use of other drugs (no. participants)				
Alcohol	(15)			
Marijuana	(15)			
Amphetamine	(4)			
Cocaine	(8)			
LSD	(1)			
Mushrooms	(9)			
Other	(6)			

Design, treatments and procedures

Participants participated in a double-blind, placebo-controlled, within-subject design involving four experimental conditions consisting of pre-treatment (T_1) and treatment (T_2). T_1 preceded T_2 by 120 min. T_1 - T_2 combinations were: placebo– placebo, memantine 20 mg–placebo, placebo–MDMA 75 mg, memantine 20 mg–MDMA 75 mg. Conditions were separated by a minimum washout period of 7 days to avoid carry-over effects. The selected 75 mg dose of MDMA falls within the normal range of recreational use (Gouzoulis-Mayfrank and Daumann, 2009), and has been consistently shown to produce robust subjective mood changes and memory impairment in healthy volunteers in a number of studies conducted previously (Kuypers and Ramaekers, 2007, 2005; Kuypers et

al., 2006; Ramaekers et al., 2009). The therapeutic dose of memantine ranges from 5 to 20 mg and has an absolute bioavailability of approximately 100%. T_{\max} of memantine is between 3 and 8 hours. There is no indication that food influences its absorption.

The order of treatment conditions was balanced over participants and sessions. All participants received a training session before onset of the experimental sessions in order to familiarize them with tests and procedures. Performance was assessed by means of a number of memory and psychomotor tasks and subjective mood assessments between 1.5–2.75 h after T_2 (at T_{\max} of MDMA). Psychomotor tasks and subjective measures of mood were included to control whether potential T_1 and T_2 interactions would selectively pertain to memory domains or transfer to other neuropsychological domains as well. In addition, blood pressure/heart rate was assessed as a safety measure. A summary of procedures in a testing day is given in Table 2.

Participants were asked to refrain from drug use at least a week prior to the start and during the study. Participants were not allowed to use alcohol on the day before an experimental session and were requested to arrive at experimental sessions well rested. Drug and alcohol screens were carried out upon arrival at our testing facilities. Urine drug screens assessed for the presence of benzodiazepines, opiates, cocaine, marijuana, MDMA, and (meth)amphetamine. Women were also tested for pregnancy. Study treatments were only administered, if participants tested negative for drugs, alcohol and pregnancy.

Table 2 Schematic representation of a testing day in 4 treatment conditions. During pre-treatment (T₁) participants received either placebo or memantine 20mg. Treatment (T₂) consisted of either placebo or MDMA 75mg. VAS = Visual Analogue Scale; POMS = Profile of Mood States; MDMA = 3,4 methylenedioxymethamphetamine

08:30	08:50 T ₁	11:00 T ₂	12:20-12:35	12:35-13:45
Arrival, Drug screening & Sleep Scale	Pretreatment (Placebo/ memantine)	Treatment (Placebo/ MDMA)	VAS 1, POMS, Vital-signs measurement & blood sample collection	Behavioural tasks & VAS 2

Behavioural assessment

Memory performance

Tests with demonstrated sensitivity (Kuypers and Ramaekers, 2005; Kuypers et al., 2006; Ramaekers et al., 2009; van Wel et al., 2011) to the impairing effects of MDMA on memory were included in the test battery.

The visual verbal learning task (VVLТ) is a modified version of the Rey Auditory Verbal Learning Test (Rey, 1964). 30 Dutch mono-syllabic meaningful nouns (18) and adjectives (12) are consecutively presented on a computer screen. Participants have to recall verbally as many words as possible (immediate recall) at the end of the list presentation. This procedure is repeated 3 times; Immediate Recall Scores are summed to comprise the Total Immediate Recall Score. After a 30-minute delay Participants are asked to recall as many of the previously learnt words as possible (= delayed recall) (Klaassen et al., 2002). Hereafter, participants are given a delayed recognition task containing 15 new words and 15 words of the previously shown list. Participants' task is to indicate whether the presented word is a new one or one from the original list. Dependent variables are the Total Immediate Recall Score, the Delayed Recall Score, the Delayed Recognition Score

and Reaction Time. Four different lists of words are used for the different test days, which had been matched for abstraction.

The prospective memory task (PMT) is a newly developed paradigm (Ramaekers et al., 2009) to examine prospective memory performance in an event-based memory task. The foreground task consists of 240 successive presentations of a letter (A or B) in the centre of a computer screen. Participants are required to respond to each letter as quickly as possible by pressing one of two response buttons. One button is pressed to indicate that the letter 'A' appeared and the other to indicate the letter 'B'. Both letters are presented equally often. Participants are informed about the trial number by means of a trial counter that is always present in the top left corner of the screen. In addition, participants are presented at irregular times with a future trial number in the right top corner of the display. Participants are instructed to remember this future trial number and withhold from responding to the foreground task during the actual occurrence of the future trial. The memory set of trial numbers is dynamic and contains up to three future trial numbers. A novel future trial number replaces a trial number in the memory set, whenever the actual trial number matches a future trial number in the set. Trials during which participants are expected to respond are classified as Go trials. Trials during which participants are instructed to withhold a response are classified as No-Go trials (prospective memory trials). Time between presentation of a future trial number and the actual occurrence of the trial (i.e., memory delays) varies between 30, 60 and 90 sec, and is equally divided over all No-Go trials. In total, the prospective memory task consists of 216 Go trials and 24 No-Go trials. Dependent variables are the number of Correct Prospective Memory Recalls (i.e., number of correct response inhibitions) in the No-Go trials and Reaction Time (Go trials).

The Sternberg memory test (SMT) assesses speed of encoding (Sternberg, 1966). In the memory scanning test participants are briefly shown a set of unrelated letters and are told to memorise them. This is called the "memory set". Then 3 sets consisting of a series of 90 letters each are displayed on a computer screen. The participants' task is to

respond to each letter as rapidly as possible by pressing either a 'YES' or 'NO' button to indicate whether or not each successive letter was one of those from the memory set. Half of the presented letters are part of the memory set. The number of Correct Responses and the mean Reaction Time for correct responses across all sets (on targets and non-targets) are taken as dependent variables. This task is performed with memory sets consisting of 1, 2 and 4 letters, respectively.

The abstract visual pattern learning task (Avipalet) is a memory task for complex visual patterns. Fourteen visual patterns are presented one by one on a computer screen, each for 2 seconds. This procedure is repeated three times. Subsequently participants are presented pairs of patterns, of which one belongs to the earlier presented list. Participants have to decide which pattern belonged to the list they were first shown, by pressing the corresponding button. This procedure is repeated after 20 minutes. The number of Correct Delayed Recognitions and Reaction Time are the dependent variables.

Psychomotor performance

The critical tracking task (CTT) assesses continuous psychomotor reaction time (Jex et al., 1966). CTT measures the participant's ability to control a displayed error signal in a 1st-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 participant is required to make compensatory movements with a progressively higher frequency. Eventually his response frequency lags the error signal by 180 degrees. 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 commonly called "lambda-c" (the "critical frequency"). The reciprocal of this frequency is theoretically the perceptual/motor delay lag for humans operating in closed-loop system. The participant performs this test in five trials on each occasion and the mean Lambda-c is recorded as the final score and is the dependent variable.

The divided attention task (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 center. 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 seconds. 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 variable of the secondary task.

Subjective assessment

The Groningen sleep questionnaire consists of 15 dichotomous questions about sleep complaints and an open question concerning the duration of sleep in order to assess respectively sleep quality and sleep quantity (hours of sleep) (Mulder-Hajonides van der Meulen et al., 1980). The sleep quality score ranges from 0 (best quality of sleep) to 14 (worst quality of sleep) and is based on participants' experienced sleep complaints during the night preceding the test day. Participants completed the sleep questionnaire at the beginning of the test day (Table 2).

The Profile of Mood States (POMS) is a self-assessment mood questionnaire with 72 five point-Likert scale items, representing eight mood states; i.e., Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness and Elation. Two extra scales are derived, i.e. Arousal ((Anxiety + Vigor) – (Fatigue + Confusion)) and Positive mood (Elation – Depression) (de Wit et al., 2002).

Subjective high is measured using a 100 mm visual analogue scale (VAS) with 'not influenced by MDMA at all' at one end and 'very influenced by MDMA' on the other end

of the line. Participants rated their subjective high before the behavioural tasks (1.15 post treatment) and after the behavioural tasks (2.75 h post treatment), as a percentage of the maximum 'high' ever experienced.

Pharmacokinetics

Two blood samples were collected at baseline and before the start of the behavioural tasks at 1 h post T_2 (see Table 2). Blood samples were centrifuged immediately and the serum was subsequently frozen at -20°C until analyses for pharmacokinetic assessments. MDMA/MDA and memantine concentrations were determined using solid-phase extraction and gas chromatography with mass spectrometric detection.

Analysis of memantine

Plasma samples were extracted according to Zarghi et al. (2010) with some modifications. Briefly, one milliliter of plasma was spiked with 30 μL amantadine (1 $\mu\text{g}/\text{mL}$) and 100 μL of NaOH 2N. After homogenization, 3 mL of hexane were added and the samples were vortex-mixed for 20 minutes. Then, samples were centrifuged (5 minutes, 3500 rpm) and organic phase was transferred to a clean tube and evaporated to dryness under N_2 stream (15 psi, 30°C). The residue was dissolved in 100 μL of mobile phase (HCOOH 0.1%/ MeOH, 80/20) and 10 μL were injected in the HPLC/MS. An HPLC (Agilent 1100 Series) coupled to a MS ion trap detector (Bruker Esquire 3000 plus) was used for the analysis of plasma samples. Separation was performed in a XTerra MS C18 (3.5 μm x 2.1 mm x 50 mm, Waters) using HCOOH 0.1% (A) and methanol (B) as a mobile phases. Mass spectrometer was operated in positive ionization mode and ions $[\text{M}+1]$ 163 from 180 (memantine) and 135 from 152 (amantadine) were chosen for quantification. Recoveries of memantine and amantadine (ISTD) were 73% and 84%. The matrix effect was 8 and 11% for each analyte respectively. The limit of detection and quantification were respectively 0.8 ng/mL and 2.4ng/mL. Interassay accuracy and precision were 10.5% and 13.4% and interassay accuracy and precision were lower than 12.5% and 16.7% respectively.

Analysis of MDMA

MDMA was analysed by gas-chromatography coupled to mass spectrometry (GC/MS) following a method previously published (Pizarro et al., 2002).

Statistics

Sample size calculation. A power calculation for detecting a significant effect on the primary parameter of this study, the total number of correct recalled words in the visual verbal learning task, was conducted by means of G-Power (version 3.0). For the calculation of the sample size, the significance level α was set at 0.05, the effect size at 0.30 (determined from previous studies with the visual verbal learning task; e.g. (Kuypers and Ramaekers, 2005a)) and the power at 80%. Based on these numbers, it was shown that 16 participants were sufficient to detect a significant difference between conditions with the $\alpha = 0.05$ level and a power of 80%.

The hypothesis that pre-treatment with memantine would interact with MDMA treatment was tested by means of a General Linear Model (GLM) repeated measures ANOVA analyses - with pre-treatment (two levels: placebo and memantine) and treatment (two levels: placebo and MDMA) as the main within subject factors. Additional drug-placebo contrasts were conducted in case of a significant interaction between pre-treatment (MEM) and treatment (MDMA) to further unfold the nature of the interaction. The alpha criterion significance level was set at $p = 0.05$. All statistical tests were conducted with SPSS version 19.0.

Results

Statistical analysis of behavioural data is described in detail below. A summary of overall effects on subjective and on cognitive performance measures are given in Table 3.

Table 3 Summary of Main Effects of Memantine and MDMA, Interactions between Memantine and MDMA and Mean (SE) Scores Following a GLM Analyses for all Dependent Variables in the Visual verbal learning task (VULT), the Prospective Memory Task (PMT), the Sternberg Memory Test (SMT), the Critical Tracking Task (CTT), the Divided Attention Task (DAT) and the Abstract Visual Pattern Learning Task (Avipalet). Significance ($P < 0.05$) and non-significance (-) of main effects is shown. MEM = Memantine; PLA = Placebo; MDMA = 3,4-methylenedioxymethamphetamine

Variables	GLM ANOVA			MEAN (\pm SE)			
	MEM	MDMA	MEM + MDMA	PLA	MEM	MDMA	MEM + MDMA
VULT – Total Immediate Recall (#)	-	0.017	-	54.07 (3.81)	49.8 (4.50)	48 (4.85)	45.13 (5.20)
VULT – Delayed Recall (#)	-	0.033	-	20.07 (1.70)	17.93 (1.78)	16.4 (2.20)	16.27 (2.31)
VULT – Recognition Reaction Time (ms)	-	-	-	845.03 (26.6)	825.57 (24.43)	829.30 (33.74)	871.04 (38.51)
VULT – Delayed Recognition (#)	-	-	-	27.21 (.79)	26.71 (.87)	26.43 (.86)	26.43 (.95)
PMT – Reaction Time Go (ms)	-	-	-	804.97 (40.41)	850.53 (40.96)	844.27 (41.09)	815.09 (44.94)
PMT – Accuracy No-Go (#)	-	-	-	25.53 (1.17)	26.40 (0.91)	24.87 (0.83)	25.20 (1.00)
SMT – Reaction Time (ms)	-	-	-	543.34 (14.23)	543.01 (16.12)	532.18 (14.55)	534.10 (16.24)
SMT – Correct Response (#)	-	0.046	-	248.07 (2.35)	250.13 (0.64)	253.07 (1.91)	249.60 (2.18)
Avipalet – Reaction Time (ms)	-	-	0.044	1995.63 (127.33)	1855.82 (82.84)	1747.83 (89.76)	1985.93 (106.37)
Avipalet – Correct Response (#)	-	0.015	-	12.29 (0.34)	12.57 (0.34)	11.71 (0.45)	11.5 (0.34)
CTT – Lambda-c	-	-	-	2.78 (0.14)	2.80 (0.13)	2.91 (0.13)	2.87 (0.12)
DAT – Tracking Error (%)	-	-	-	21.05 (1.22)	19.96 (1.13)	19.81 (0.77)	19.26 (1.00)
DAT – Reaction Time (ms)	-	-	-	2158.20 (64.28)	2189.20 (69.15)	2225.13 (79.93)	2226.40 (84.07)
DAT – Control Losses (#)	-	-	-	15.47 (6.24)	15.47 (4.82)	10.40 (3.26)	8.80 (2.99)
DAT – Hits (#)	-	-	-	43.7 (1.22)	44.20 (1.10)	42.87 (1.23)	43.47 (1.16)

Missing data

In all, 15 complete data sets entered statistical analysis, except for the Visual Verbal Learning Task (Recognition and Recognition Reaction Time) and for the Avipalet (Reaction Time and Accuracy), where one participant was excluded due to technical failures during test administration. Three blood samples could not be analysed due to technical failures.

Memory performance

A summary of mean (SE) performances in all memory tasks in each treatment condition and associated GLM analyses are given in Table 3. GLM analysis revealed a main effect of MDMA on Immediate Recall Scores and Delayed Recall Scores in the VULT.

Total Immediate Recall Scores and Delayed Recall Scores were significantly decreased by MDMA ($F[1,14] = 7.8; p = .017$ and $F[1,14] = 5.6; p = .033$, respectively). Participants under the influence of MDMA recalled 6 words less in the immediate recall condition and 4 words less in the delayed recall compared to placebo condition. Delayed Recognition Score and Reaction Time were not affected by MDMA. There was no effect of memantine nor was there any interaction between MDMA and memantine.

Performance during the PMT was not affected by MDMA, memantine or their interaction.

GLM analyses of SMT data revealed a main effect of MDMA on response accuracy. The number of Correct Responses increased during MDMA treatment ($F[1,14] = 4.8; p = .046$). Mean reaction time for Correct Responses on targets and non-targets was not affected by MDMA. There was no effect of memantine or any interaction between MDMA and memantine. A trend in interaction between MDMA and memantine on response accuracy was observed ($F[1,14] = 13.9; p = .068$).

The number of Correct Responses in the Avipalet was significantly decreased by MDMA ($F[1,13] = 7.8; p = .015$). Reaction Time was not affected by MDMA. Correct response or reaction time scores were not affected by memantine, but there was a significant interaction between MDMA and memantine on Reaction Time ($F[1,13] = 4.9; p = .044$). Reaction Time decreased when participants received placebo-MDMA compared to placebo-placebo and placebo-memantine, but increased when memantine was combined with MDMA.

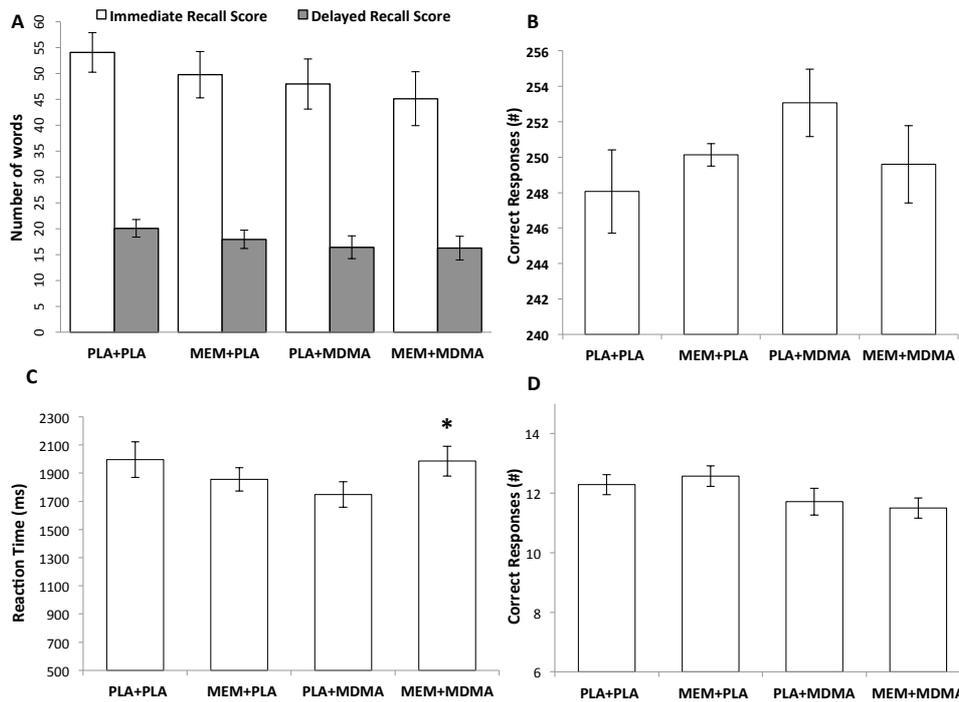


Figure 1 Mean (SE) Immediate and Delayed Recall Scores in the Visual Verbal Learning Task (A), Correct Responses in the Sternberg Memory Task (B) and Reaction Time (C) and Correct Responses (D) in the Abstract Visual Pattern Learning Task are given for each treatment condition. Each treatment condition consisted of a pretreatment (T_1) and a treatment (T_2) (PLA = Placebo; MEM = Memantine; MDMA = 3,4-methylenedioxymethamphetamine; * $p < 0.05$ as indicated by drug-placebo contrasts following a significant main interaction effect of memantine x MDMA in the Abstract Visual Verbal Learning Task).

Psychomotor performance

A summary of mean (SE) performances in all psychomotor tasks in each treatment condition and associated GLM analyses are given in Table 3. MDMA, memantine and their interaction did not affect performance in de CTT and DAT.

Subjective assessment

A summary of mean (SE) subjective rating in all treatment conditions and associated GLM analyses are given in Table 4. There were no significant differences in sleep quality and quantity between any of the treatment conditions as measured by the Groningen sleep scale. Participants' mean (SE) sleep quality and sleep quantity scores were 2.32 (0.39) and 6.82 h (0.28), respectively.

GLM analysis revealed a main effect of MDMA on POMS factor scores. Mean (SE) subjective ratings on the POMS scales in every treatment condition are shown in Table 4. Participants under influence of MDMA experienced more anxiety ($F_{1,13} = 7,1$; $p = 0.019$), vigour ($F_{1,13} = 18,0$; $p = .001$), elation ($F_{1,13} = 9,5$; $p = .009$) and arousal ($F_{1,12} = 14,9$; $p = .002$). POMS ratings were not affected by memantine or the interaction between MDMA and memantine.

There was a significant effect of MDMA on subjective high ratings. Subjective high ($F_{1,13} = 21,7$; $p = .001$) was significantly increased at 1.5 h after MDMA consumption and was also significantly increased ($F_{1,13} = 19,2$; $p = .001$) 2.45 h after treatment with MDMA. Memantine or the interaction between MDMA and memantine did not affect subjective high ratings.

Table 4 Mean (\pm SE) subjective ratings on the Profile of Mood Scales (POMS) and the Visual Analogue Scale (VAS) scales in all 4 treatment conditions. Significance ($P < 0.05$) and non-significance (-) of main effects is shown. MEM = Memantine; MDMA = 3,4-methylenedioxymethamphetamine; PLA = Placebo

POMS Scales	GLM ANOVA			MEAN (\pm SE)			
	MEM	MDMA	MEM + MDMA	PLA	MEM	MDMA	MEM + MDMA
Anxiety	-	0.019	-	4.50 (1.07)	3.93 (1.11)	7.07 (1.50)	7.57 (1.66)
Depression	-	-	-	.57 (.29)	1.86 (1.27)	.86 (0.53)	3.57 (2.00)
Anger	-	-	-	1.36 (.79)	2.14 (.95)	.93 (.24)	2.00 (1.10)
Vigor	-	0.001	-	8.64 (1.60)	8.93 (1.37)	12.93 (1.17)	10.86 (1.50)
Fatigue	-	-	-	3.36 (1.01)	3.21 (1.11)	3.71 (1.77)	3.21 (1.04)
Confusion	-	-	-	4.85 (.94)	5.00 (.85)	6.92 (1.35)	5.85 (1.04)
Positive mood	-	-	-	7.29 (1.21)	6.93 (1.79)	10.07 (1.39)	5.93 (2.87)
Friendliness	-	-	-	14.36 (2.09)	15.57 (1.46)	17.07 (1.62)	16.93 (1.87)
Elation	-	0.009	-	7.86 (1.62)	8.79 (.94)	10.93 (1.18)	9.50 (1.36)
Arousal	-	0.002	-	4.62 (2.41)	4.31 (2.83)	9.39 (3.64)	9.46 (2.39)
Subjective high – VAS 1 (%)	-	0.000	-	6.43 (3.87)	3.00 (1.15)	37.29 (10.77)	45.07 (8.50)
Subjective high – VAS 2 (%)	-	0.001	-	1.15 (0.78)	1.79 (1.21)	12.29 (4.45)	20.93 (5.14)

Pharmacokinetics

Mean drug concentrations in all treatment conditions are shown in Table 5.

Table 5 MDMA and memantine plasma concentrations (mean (SD)) in each of the 4 treatment conditions. PLA = Placebo; MEM = Memantine; MDMA = 3,4-methylenedioxymethamphetamine

Concentration (ng/mL)	PLA	MEM	PLA	MEM
	PLA	PLA	MDMA	MDMA
MEM	N/A	21.6 (7.6)	N/A	21.2 (9.8)
MDMA	N/A	N/A	108 (67)	107 (48)
N	15	15	14	13

Discussion

The goal of this study was to investigate whether MDMA-induced memory impairment can be blocked by pre-treatment with memantine, an alpha 7 nicotinic receptor antagonist. This is the first study that examined the potential of memantine to overcome memory impairment caused by MDMA. Subjects were given single doses of 75 mg MDMA combined with placebo or 20 mg memantine after which several memory tasks were administered.

Single doses of MDMA impaired memory performance in the verbal word learning and abstract visual pattern learning tasks. MDMA decreased immediate and delayed recall scores in the former and decreased the number of Correct Responses in the latter. Delayed Recognition Score was not affected by MDMA. These results confirm results from previous studies that have assessed memory impairment related to acute MDMA exposure using similar memory paradigms (Kuypers et al., 2013a; Kuypers and Ramaekers, 2005a, 2007; van Wel et al., 2011). Performance in the PMT however was not affected by MDMA treatment, which is in contrast with previous studies that reported an increase in prospective memory failures after MDMA treatment (Kuypers et al., 2013;

Ramaekers et al., 2009; van Wel et al., 2011). Although memory accuracy in the PMT task was lowest during MDMA treatment in the present study, the relative difference to placebo was rather small.

MDMA also affected performance in the Sternberg memory task. Response accuracy slightly increased following MDMA while response time remained unaffected. This result contrasts with those of an earlier study that employed the same paradigm but observed no change in accuracy and response time during MDMA treatment (Kuypers et al., 2013a). Performance improvements after MDMA however have been reported repeatedly in other cognitive tasks measuring attention, tracking and motor response time (Kuypers et al., 2007; Lamers et al., 2003; Ramaekers et al., 2006, 2012). In general, performance improvements have been relatively small in magnitude which may contribute the fact the other studies have failed to replicate stimulatory effects of MDMA using similar tests and procedures (Dumont et al., 2008; Dumont and Verkes, 2006; Kuypers et al., 2006a; Ramaekers et al., 2012). Also in the present study, the effects on attention and tracking as measured with CTT and DAT were neutral. Presence or absence of stimulatory effects of MDMA may have depended on factors that have varied between individual studies such time of drug administration, time of task, diurnal rhythms and variations in MDMA blood concentrations. In some studies MDMA was administered in the morning while in other studies administration was done in the afternoon, and sometimes testing proceeded throughout the night (Bosker et al., 2012). Furthermore, MDMA concentrations in blood in other studies ranged between 113.4 ng/mL and 178 ng/mL whereas MDMA concentrations in the present study were somewhat lower.

Single doses of MDMA significantly increased ratings of subjective high and increased positive as well as negative moods as measured by the POMS questionnaire. MDMA raised feelings of vigor, elation and arousal, while also making subjects feel more anxious. These findings are in line with previous studies that also reported a marked effect, both positive and negative, of MDMA administration on mood ratings (Bosker et al., 2010; Kuypers et al., 2013a; van Wel et al., 2012).

Memantine alone did not affect memory, psychomotor function or mood. Overall, memantine did also not alter memory or mood when combined with MDMA. Only one memantine x MDMA interaction reached statistical significance. In the abstract visual pattern learning task, memantine reversed improvements in response time that were evident when MDMA was given alone. The combination of memantine and MDMA however did not affect memory accuracy in this task. If anything, the interaction seems to indicate that memantine may actually reverse some of the stimulating properties of MDMA, rather than the impairing effects of this drug. However, no such interactions were detected in any other tasks that also included response time as a secondary parameter. It is difficult to determine whether the present interaction represents a robust and replicable finding or an isolated chance phenomenon.

Studies showing involvement of the NMDA receptor in acute MDMA intoxication are limited. There have been only two reported accounts for memantine protection against MDMA-induced neurotoxicity (Camarasa et al., 2008; Chipana, Camarasa, et al., 2008). These findings were derived from animal-based research with rodents (Chipana et al., 2006). The 15 mg/kg dose of MDMA that was administered to the rats has been shown to produce similar plasma concentrations of MDMA to those of a human who consumes a 150 mg tablet (Green et al., 2003). The 5mg/kg dose of memantine that was administered to rats would be equivalent to a dose of 63 mg in a 70 kg human, using the interspecies scaling formula (Mordenti and Chappell, 1989), which is a relatively higher dose compared to the memantine dose (20 mg) used in this study. It therefore cannot be excluded that memantine dosing in the present study was too low to replicate the protective effects against MDMA induced memory impairments in rats. Ideally, a follow up study would include more and higher doses of memantine to assess its memory protective potential. In addition, animal studies have attempted to simulate chronic MDMA use and followed an administration schedule that lasted several days (Camarasa et al., 2008), which limits its comparison to acute MDMA studies in humans. It cannot be excluded that protective effects of memantine do not occur immediately after a single dose administration but slowly develop with repeated dosing of memantine and MDMA. As a final limitation it

should be mentioned that our power calculation was based on the primary measure of the VVLT. We therefore cannot exclude the possibility that statistical power for detecting MDMA and memantine effects was lower for secondary measures such as the prospective memory task. More research with larger sample sizes is needed to confirm observations made in this study.

In summary, this study shows that single doses of MDMA impaired memory performance in the visual verbal learning task and abstract visual pattern learning task. These preliminary findings suggest that memantine did not reverse MDMA-induced memory impairment and mood in humans.

Chapter 6

Neurocognitive performance following acute mephedrone administration, with and without alcohol

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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 hypothesized 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 1 hour (T_1) and 4 hours after (T_2) mephedrone administration, by means of the Divided Attention Task, Critical Tracking Task, and the Spatial Memory Test. 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.

Introduction

A large number of new psychoactive substances (NPS) have rapidly spread across Europe and the US over the last decade (Corazza et al., 2013). The United Nations Office on Drugs and Crime (UNODC, 2014) reported that the majority of NPS between 2008 and 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 gained popularity despite legal actions taken to ban their use (EMCDDA, 2015b).

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 as alcohol, tobacco, cannabis, 3,4-methylenedioxyamphetamine (MDMA) and cocaine (Winstock et al., 2011). The data Crime Survey of England and Wales indicates a last year prevalence of 0.6% in individuals aged 16-59 and 1.9% in individuals aged 16-24, which 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, 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.

Similar 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 that of MDMA (López-Arnau et al., 2012). Doses between 25 to 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 and 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., 2011). 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 indicates 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 their 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 eleven non-dependent polydrug users aged between 22 and 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, hematology, 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) BMI within 18.5 –28 kg/m², and (e) presence of CYP2D6 genotypes resulting in the intermediate, extensive or ultrarapid metabolizer 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 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.

Table 1 Participant demographics and history of alcohol and drug use. MDMA = 3,4-methylenedioxymethamphetamine; GHB = Gamma-hydroxybutyrate; BDZ = Benzodiazepine; 2-CB = 4-bromo-2,5-dimethoxyphenethylamine; LSD = Lysergic Acid Diethylamide; METH = Methamphetamine; AMP = Amphetamine

	Mean (SD)	Min.	Max.
Age (years)	28.2 (6.7)	22.0	39.0
BMI (kg/m ²)	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

Design and treatments

The study was conducted according to a double-blind, placebo-controlled, 4-way cross-over design. The four treatment conditions consisted of: mephedrone placebo + alcohol placebo (1), mephedrone + alcohol placebo (2), mephedrone placebo + alcohol (3) and mephedrone + alcohol (4). The order of treatment conditions was balanced over participants and sessions. Conditions were separated by a minimum washout period of 7 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 flavored Fontvella water. Placebo drinks consisted solely of lemon flavored Fontvella water. The total volume to be taken was 350 mL during a period 15 min (117 mL every five 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 (48h) or caffeine-containing beverages (24h) 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, CA-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.

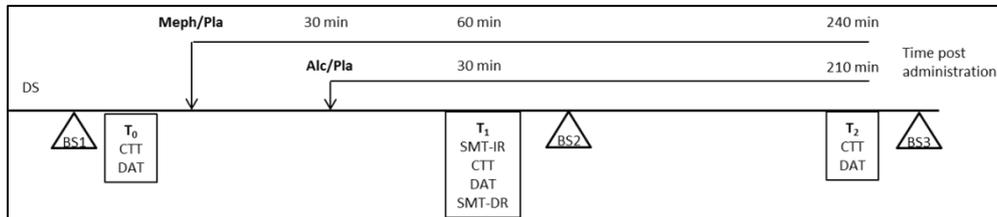


Figure 1 Schematic representation of a test day (DS = Drug Screens; BS = Blood Sample; CTT = Critical Tracking Test; DAT = Divided Attention Test; Meph = Mephedrone; Alc = Alcohol; Pla = Placebo; SMT = Spatial Memory Test; IR = Immediate Relocation; DR = Delayed Relocation).

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.

CTT. 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 seconds. 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

Blood samples (BS) to determine mephedrone and alcohol concentrations in plasma were collected at 3 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 derivatization 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 analyzed by means of a General Linear Model (GLM) repeated measures 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 ($F_{1,10} = 4.99$; $p = 0.050$; $\eta^2_p = 0.333$) 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 ($F_{1,10} = 5.82$; $p = 0.037$; $\eta^2_p = 0.368$) and Delayed Relocation Scores ($F_{1,10} = 15.32$; $p = 0.003$; $\eta^2_p = 0.605$). 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.

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 ($F_{1,10} = 10.78$; $p = 0.008$; $\eta^2_p = 0.519$) on Lambda-c Scores at T_2 . Participants had higher Lambda-c Scores during mephedrone intoxication compared to placebo. Alcohol or AlcoholxMephedrone 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 ($F_{1,10} = 13.69$; $p = 0.004$; $\eta^2 p = 0.578$), Control Loss ($F_{1,10} = 7.49$; $p = 0.021$; $\eta^2 p = 0.428$) and Reaction Time ($F_{1,10} = 13.93$; $p = 0.004$; $\eta^2 p = 0.582$) at T_1 . Alcohol increased Tracking Error and Control Loss in the primary task and Reaction Times 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 ($F_{1,10} = 9.84$; $p = 0.011$; $\eta^2 p = 0.496$) at T_2 , indicating more tracking error during alcohol intoxication. Reaction Time was significantly affected by the interaction between Mephedrone and Alcohol ($F_{1,10} = 12.89$; $p = 0.005$; $\eta^2 p = 0.563$). 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.

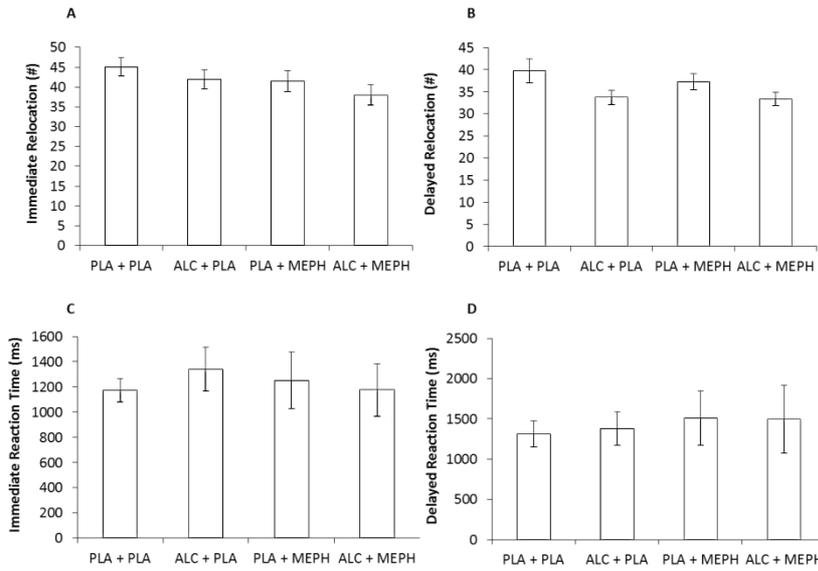


Figure 2 Mean (SEM) Immediate (A) and Delayed Relocation (B) Scores and Immediate (C) and Delayed Reaction Times (D) in the Spatial Memory Test for the four treatment conditions at T₁ (PLA = Placebo; ALC = Alcohol; MEPH = Mephedrone).

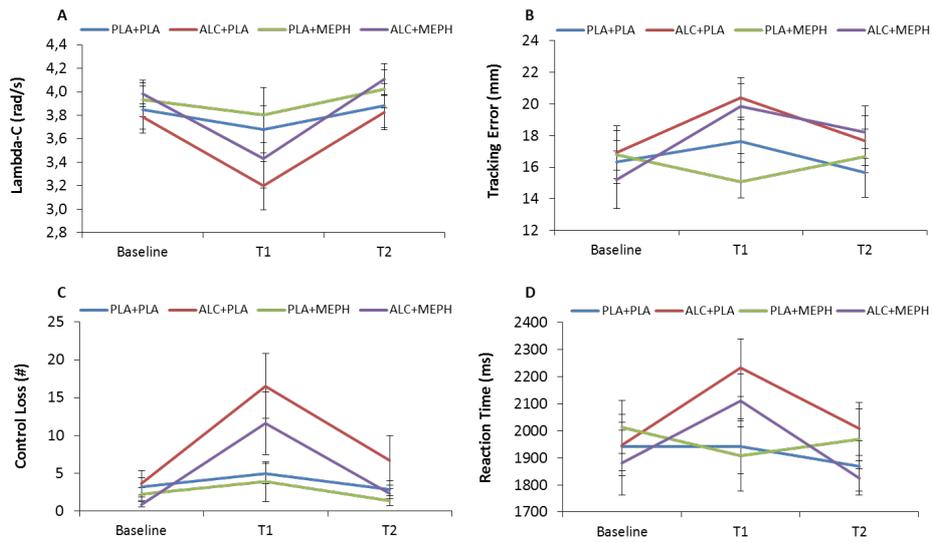


Figure 3 Mean (SEM) Lambda-C Score in the Critical Tracking Test (A), Tracking Error (B), Control Loss (C), and Reaction Time (D) in the Divided Attention Test for each cognitive assessment (PLA = Placebo; ALC = Alcohol; MEPH = Mephedrone).

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.

Table 2 Mean (SE) concentrations of mephedrone and alcohol in plasma at the different time points (N=11). Meph = Mephedrone; Alc = Alcohol; BS = Blood Sample

Concentrations	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)

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 T₁. This finding is in line with data from a naturalistic study in mephedrone users (Freeman et al., 2012) and is also 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 its 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 T₁. 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., 2006a). 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 was 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., 2006a; 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 T₁, whereas tracking error was larger at both T₁ and T₂, 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 T₂. 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 x 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.

Chapter 7

General discussion

The aim of this dissertation was twofold. The first aim was to assess acute drug experiences during exposure to affective cues by means of fMRI and neurocognitive tests. The second aim was to assess acute drug experiences during multiple drug use to examine whether exposure to one drug may block or potentiate the neurocognitive effects of another drug. In this chapter the key findings are presented and discussed in a broader perspective.

Interpretation of affective cues is altered by acute drug exposure

The acute effects of alcohol, cannabis and cocaine administration during exposure to drug marketing cues, aggression cues and emotional face cues are described in chapters **2, 3** and **4**.

Marketing cues

The study described in chapter **2** examined the impact of alcohol and drug marketing cues on the reward circuit in regular alcohol and cannabis users during alcohol and drug intoxication and while sober. Results showed that advertising of alcohol and portrayal of cannabis use and marketing practices elicited striatal activation in the brain's reward circuit in regular alcohol and cannabis users respectively. Acute alcohol and cannabis administration in turn reduced the reward sensitivity of alcohol and cannabis marketing cues. Thus, the influence of drug marketing cues on behavior appears strong when one has not consumed any drugs or alcohol, but much weaker when one is intoxicated. This notion of heightened and blunted reward sensitivity during sobriety and drug intoxication fits well with current notions and models regarding the role of dopamine in motivation and reward processing (Niv, 2007; Schultz, 2007; Volkow and Morales, 2015; Wanat et al., 2009).

The mesolimbic dopamine pathway appears to be crucial for drug-reward processing (Wise, 2009). Mesolimbic dopamine transmission in the VTA and NAcc is regulated by two interacting mechanisms in the brain: synaptic/phasic dopamine and extrasynaptic/tonic dopamine release. Reward cues have been shown to trigger phasic

dopamine release in the striatum (Schultz, 2007). Phasic dopamine release in the NAcc plays a key role in motivational control by providing a ‘teaching’ signal that underlies reinforcement/associative learning or conditioning (Wise, 2009; Zweifel et al., 2009) and an ‘incentive’ signal that promotes immediate reward seeking (Berridge and Robinson, 1998). Specifically, when reward outcome is better than expected, burst (phasic) firing transiently rises above baseline (tonic) firing, but falls below baseline when reward outcome is worse than expected, reflecting a reward error prediction signal (Schultz, 2007). The findings in chapter 2 suggest that drug marketing cues caused an unexpected reward outcome as evinced by an increase in phasic BOLD response to alcohol and drug marketing cues in regular alcohol and cannabis users. This finding stresses the notion that marketing of alcohol and drugs can elicit reward expectations in humans that may lead to repeated use. The current findings imply that activation of the brain reward system through alcohol advertisement and portrayal of drug use might directly increase the motivation to use alcohol or drugs, especially in people who already use.

Measures to eliminate the cues and triggers in advertisements that encourage alcohol consumption would need to be taken on a population level. A reduction or (ideally) complete prohibition on alcohol and drug marketing, as has been the case with tobacco marketing (World Health Organization, 2003), would be effective to reduce brain exposure to reward cues that motivate drug use or induce craving (Lovato et al., 2003a). The reward value of alcohol marketing cues is also associated with branding information (McClure et al., 2004; Schaefer, 2009). Branding influences consumers’ perceptions about a given product and creates an emotional bond to products (Schaefer, 2009), which might further increase reward value and smoking appeal. Plain packaging, i.e., the removal of branding information on packaging as has been done with cigarettes, could also be introduced for alcohol bottles. Plain packing may not only be able to reduce positive perceptions about drinking but also diminish associated reward responses in the brain that motivate alcohol and drug use (Martin, 2014).

Other efforts to further reduce the prevalence of alcohol and drug use could be accomplished through public health campaigns that are focused on raising awareness about the health implications of (regular) alcohol and drug use. The younger population in particular, appears to be more vulnerable to both acute effects of drugs and the effects of drug marketing exposure. Firstly, drug exposure during adolescence can disrupt normal brain development that is needed for proper development of higher-order cognitive functions and can therefore adversely affect mental health and cognitive performance (Niesink and van Laar, 2013; Squeglia et al., 2009). Secondly, alcohol advertising and promotional activities are commonly targeted at adolescents (Cummings et al., 2002; Ling and Glantz, 2002). Adolescents may be more exposed to alcohol and drug marketing through the Internet (Lenhart et al., 2011), especially considering the expansion and interactive nature of social media (Jernigan, 2012), which is also used by the alcohol industry to promote alcohol products (McClure et al., 2016; Moreno and Whitehill, 2014). The impact of alcohol marketing in eliciting positive affective responses in the young population has been linked with a greater likelihood of drinking or intention to drink (Anderson et al., 2009; McClure et al., 2016; Tanski et al., 2015). Taken together, banning of alcohol advertisement specifically targeted at the youth together with health campaigns may be a useful measure to reduce the likelihood of young people starting to drink and encourage a more responsible approach to alcohol consumption.

The phasic BOLD response to marketing cues reduced during alcohol and cannabis intoxication in response to drug-induced increments in tonic dopamine levels. Drugs of abuse trigger an increase in tonic dopamine levels in the striatum (Di Chiara and Imperato, 1988), leading to increased motor responsiveness and action readiness associated with an increased motivational drive (Bromberg-Martin et al., 2010; Spronk et al., 2013). Tonic dopamine also serves as an average reward expectancy signal expressing reward experience history (Niv, 2007). High exposure to rewarding events will lead to an overall increase in tonic dopamine levels and an increase in motivational drive. Conversely, a low exposure to rewarding events would decrease tonic dopamine and motivational drive. Previous studies have shown that motivational drive is both influenced

by reward history (Guitart-Masip et al., 2011) and drug-induced changes in tonic dopamine levels (Beierholm et al., 2013). Alterations in the average reward expectancy signal or tonic dopamine levels are thought to influence reward learning processes governed by phasic dopamine (Cools et al., 2011). Phasic dopamine release is manifested by the discrepancy between expected and received reward, and changes in reward expectancy would affect the magnitude of this discrepancy. Low reward expectancy associated with low tonic dopamine would trigger a large phasic dopamine response to unexpected reward. In contrast, high reward expectancy associated with high levels of tonic dopamine would only trigger a small phasic response to unexpected reward. This notion has been confirmed by a previous pharmacological fMRI study with methylphenidate that assessed the impact of tonic dopamine on reward-related phasic dopamine response in healthy volunteers during a gambling paradigm (Evers et al., submitted). Methylphenidate led to BOLD-related increases in response vigor and reward expectancy in the striatum, and systematically decreased phasic fMRI responses to gain and loss. In light of these previous reports, it can be hypothesized that acute alcohol and cannabis intoxication in regular alcohol and cannabis users respectively led to an increase in tonic dopamine and higher reward expectancy, which attenuated the phasic dopamine to alcohol and cannabis marketing cues. The current findings suggest that medicinal drugs that enhance tonic dopamine levels would be able to reduce the excessive phasic dopamine response to alcohol and drug marketing cues.

Aggression and emotional face cues

Alcohol and cannabis exposure can produce similar effects on the mesolimbic circuitry, but cause different effects outside the reward circuitry. Based on the pharmacological mechanism of the drug (Lüscher and Ungless, 2006; Nestler, 2005), different emotional and behavioral responses to affective cues are expected from stimulants (e.g. cocaine), depressants (e.g. alcohol) and cannabinoids (e.g. cannabis). The study described in chapter 3 examined the impact of aggression cues on self-reported (subjective) aggressive behavior in heavy alcohol and cannabis users during drug intoxication and while sober.

Results showed that exposure to aggression cues during acute alcohol and cannabis intoxication differentially affected subjective aggression; alcohol increased subjective aggression in alcohol users whereas cannabis decreased subjective aggression in cannabis users.

Although it does not directly imply that an increase in subjective aggression or aggressive responding following alcohol intoxication will lead to physical aggression/violence in a real-life setting (i.e. inflict pain on another person), it does suggest that alcohol intoxication is more likely to elicit aggression compared to cannabis intoxication. The expression of intoxicated aggression is influenced through a complex interaction between pharmacological drug characteristics and individual differences (e.g., expectation, genetic make-up, personality) (Beck and Heinz, 2013; Chermack and Blow, 2002; Moss and Tarter, 1993). Acutely, alcohol activates the reward system and increases sensation seeking and/or approach behaviors potentially leading to confrontational and provocative behaviors. Alcohol also decreases threat detection (Hoaken et al., 2003), increases reactivity to pain and the significance of provocation (Pihl et al., 1993) and impairs many aspects of executive cognitive functioning (Peterson et al., 1990). In fact, impairment of executive functioning (e.g., planning, decision-making, problem-solving, behavioral inhibition) mediates the alcohol aggression relationship significantly (Giancola, 2000; Giancola et al., 2012). Taken together, these findings indicate that acute alcohol intoxication can disrupt abilities to regulate emotional and behavioral responses at multiple levels.

The pattern of alcohol consumption plays an important role in the occurrence of aggression. The alcohol group in the current study consisted of heavy drinkers. The frequency of alcohol use impacts the relation between alcohol and aggression. Heavy alcohol intake affects both dopamine and serotonin neurotransmission, which is associated with dysfunctional reward expectation and information processing, and increased impulsivity/poor executive control (Beck and Heinz, 2013; Moeller et al., 2001). Therefore, frequent and heavy alcohol consumption (i.e. binge-drinking) contributes to

the occurrence of intoxicated aggression in chronic alcohol users (Chermack and Blow, 2002; Fals-Stewart, 2003). A reduction of binge-drinking episodes could be an effective countermeasure to prevent aggression from occurring. For example, bars or certain sporting events (or places in which heavy alcohol consumption takes place) should be equipped with breathalyzers and bar tenders/waiters should not sell anymore alcohol when BAC levels are between 0.75 – 1.0 g/L as violent behavior is thought to occur at higher alcohol doses (Astudillo et al., 2010; Duke et al., 2011).

Alternatively, cannabinoids could offer beneficial outcomes for aggressive behavior since they decrease (subjective) aggression implicating the role of the endocannabinoid system in emotional processing. However, it is well known that THC can also impair neurocognitive performance and increase anxiety and paranoia in inexperienced users (Niesink and van Laar, 2013) and would therefore not be a safe choice. Cannabidiol (CBD) in turn is another component of the *Cannabis sativa* plant which is not associated with psychological or adverse cognitive effects and has anxiolytic and possibly anti-psychotic properties (Crippa et al., 2009; Morgan and Curran, 2008). CBD has been shown to decrease anxiety in patients (Bergamaschi et al., 2011; Crippa et al., 2011) and in healthy volunteers (Crippa et al., 2004; Zuardi et al., 1993). Nevertheless, the cannabis breed used in the current study contained around 11% of THC and less than 1% of CBD. It is not clear how the aggression reducing properties are mediated by THC and whether this could also be accomplished with CBD or their combination. There are relatively few human studies that examined THC/CBD ratios on neurocognition and specifically aggressive behavior. Neuroimaging studies that investigated THC/CBD on emotional processing indicate that when administered alone, CBD improved facial affect recognition, while THC impaired this (Hindocha et al., 2015). However, this impairment was attenuated when THC and CBD were combined. CBD might therefore be able to counter the alcohol-induced aggression by reducing reactivity to aggression-provoking cues. Since CBD is associated with few or no adverse effects (Lubman et al., 2015; Niesink and van Laar, 2013), more research is needed to investigate its potential as a therapeutic agent to treat aggression.

Cannabis and cocaine exposure in turn, may also differently affect brain regions involved in emotional reactivity. The study described in chapter 4 examined the impact of emotional facial cues on amygdala reactivity in regular drug users while sober and when intoxicated. The preliminary results suggest that cannabis or cocaine intoxication during exposure to emotional facial cues did not affect amygdala reactivity in regular drug users. Although these findings cannot be generalized to population level based on our small sample size, they do indicate that exposure to emotional facial cues elicit different brain responses compared to non-emotional visual cues and that cannabis and cocaine induce opposing effects on response latencies. Despite this limitation, there is compelling evidence (Ersche et al., 2015; Fernández-Serrano et al., 2010; Kuypers et al., 2015; Miller et al., 2015) that drugs of abuse disrupt the interpretation and recognition of emotions and could therefore lead to maladaptive behaviors in social settings. This can be troublesome in the case of alcohol since it can increase aggressive feelings as implied by the study described in chapter 3. It is also important to assess how emotion processing is affected across different drugs of abuse and whether the findings are consistent across studies. Thus, more research is needed to gain more insight into the acute effects of drugs of abuse on emotional processing.

Multiple drug exposure: one plus one does not always equal two

The acute effect of MDMA and mephedrone when combined with memantine and alcohol respectively are described in chapters 5 and 6.

MDMA and memantine

The study described in chapter 5 examined the potential of the glutamatergic medication, memantine, to overcome memory impairment caused by MDMA as previously suggested by rodent models. The findings indicate that single doses of MDMA decreased verbal and spatial memory performance. MDMA did not affect psychomotor and divided attention performance. Memantine co-administration did not block or reverse memory impairment elicited by MDMA. The current findings imply that the neuropharmacological mechanism involved in MDMA-induced memory impairment in humans is not mediated by glutamate

(NMDA receptors) and/or acetylcholine (alpha-7 nicotinic receptors) as suggested in rodent models. It should be noted however that the pharmacokinetics of MDMA in rats is fundamentally different from the pharmacokinetics of the drug in humans (Green et al, 2012), which could explain the therapeutic effects of memantine found in rats but not in humans.

The effects of MDMA are suggested to be caused by changes in the monoaminergic system, especially serotonin (5-HT) (Han and Gu, 2006). Blockade of 5-HT₂ receptors has been shown to selectively prevent MDMA-induced verbal memory impairment, but not spatial or prospective memory (van Wel et al., 2011). This indicates that MDMA-induced impairment involves several pharmacological mechanisms (Kuypers et al., 2013b; Johannes G Ramaekers et al., 2009; van Wel et al., 2011).

MDMA did not impair psychomotor performance and divided attention in this and other studies (Dumont et al., 2008; Dumont and Verkes, 2006; Kuypers et al., 2006b; Ramaekers et al., 2012). These findings contrasted with other reports that did find performance improvement following MDMA intoxication (Kuypers and Ramaekers, 2007; Lamers et al., 2003; Ramaekers et al., 2006, 2012). The discrepancy in results could be explained by interindividual differences (e.g., genetic profile, gender, drug history) in response to MDMA exposure, which could differently impact MDMA-induced impairment and toxicity (Rietjens et al., 2012). Both pharmacokinetic and pharmacodynamic factors are involved in the individual response to MDMA and should be considered when evaluating both acute and chronic effects (Green and Nutt, 2014). This also suggests that the pharmacodynamic interactions between MDMA and other drugs will differ across individuals. For example co-exposure of MDMA with specific substances (e.g., selective serotonin reuptake inhibitors) in individuals with genetic polymorphisms resulting in poor metabolism status can increase MDMA plasma levels, but can also decrease the formation of toxic metabolites and subsequent cellular damage (Rietjens et al., 2012). A previous study investigating the effects of MDMA on serotonin transporter gene expression and mood in humans indicates that MDMA leads to significant regulatory changes in the expression of serotonergic markers and was more pronounced in females and in carriers

of the 5-HTTLPR I/I genotype (Yubero-Lahoz et al., 2014). Also, both positive and negative correlations were found between MDMA-induced increments in 5-HTT gene expression and subjective ratings of mood (i.e. positive: fatigue, confusion; negative: vigor, arousal). Future research should consider the implications of genetic polymorphisms on MDMA-induced effects (both on a pharmacokinetic and pharmacodynamic level) to classify which polymorphisms play an important role in determining clinical outcome following MDMA exposure. By establishing interindividual vulnerabilities in response to MDMA and other drugs, better individually tailored pharmacological approaches can be developed.

Mephedrone and alcohol

The study described in chapter 6 assessed whether the neurocognitive effects of mephedrone, a so called 'new psychoactive substances' (NPS), after co-administration with alcohol were additive or synergistic. Results indicated that mephedrone improved psychomotor performance, impaired spatial memory and did not affect divided attention performance. Alcohol impaired spatial memory and divided attention. An antagonistic effect of mephedrone on divided attention and an additive effect on spatial memory performance were observed when combined with alcohol. However, stimulatory effects of mephedrone were not sufficient to compensate for the impairing effects of alcohol on most performance parameters.

The acute effects of mephedrone on cognition appear to be similar to those elicited by MDMA. Despite this similarity, it should be noted that mephedrone displays higher brain penetration and a more rapid metabolism and clearance from the brain compared to MDMA (Green and Nutt, 2014). Mephedrone shows greater potency at dopamine transporter sites and causes more dopamine release as compared to MDMA (Simmler et al., 2013). The release of serotonin 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 dopamine and noradrenaline gives it a higher psychostimulant and abuse liability (Green et al., 2014). This may explain why it is frequently taken in a binge-like manner and why users

experience craving after withdrawal (Brunt et al., 2011; Carhart-Harris et al., 2011), especially when it was insufflated. Interviews with current mephedrone users indicate that spontaneous use of mephedrone was frequently associated with larger amounts of alcohol being consumed before mephedrone take (O'Neill and McElrath, 2012). This is very alarming considering that alcohol intoxication impairs cognitive control and decision-making, which might lead to an increase in mephedrone dose or intake of other drugs of abuse. The concomitant use of mephedrone with other substances, whether it is synthetic or classical substances, could be an even greater health risk and can even turn out to be fatal.

The current study was the first placebo-controlled experimental study to assess the acute neurocognitive effects of mephedrone alone and also in combination with alcohol in humans. The rapidly evolving illicit drug market has led to a dramatic increase in the recreational use of NPS or 'designer drugs'. NPS are synthetic analogues of illegal drugs of abuse, which are easy to get, inexpensive and cannot be detected by standard drug or toxicology screens (Baumann et al., 2014). Over 540 different NPS have been reported and this number is thought to increase further (UNODC, 2015). NPS mimic the effects of many classical drugs of abuse, such as opioids ('MT-45') stimulants ('bath salts'), cannabinoids ('spice') and hallucinogens ('N-bombs'). The reality is that we do not know much about the effects of NPS, such as basic toxicology (i.e., the dose-related effects and lethal dose) and human psychopharmacology. Life-threatening cases have been reported for each NPS class (Baumann and Volkow, 2015) and authorities in the United States responded by imposing drug-scheduling legislation (Drug Enforcement Administration, 2014). In Europe, research with NPS in humans is only possible provided they have been prepared according to Good Manufacturing Process (GMP) standards, which is very costly and virtually impossible to be covered by most researchers (Nutt et al., 2013). This hampers all research that is aimed at elucidating the effects of NPS, which could be resolved by creating a special 'research class' schedule I license allowing researchers to investigate any scheduled drug, or by exempting human experimental studies from GMP requirements (Baumann and Volkow, 2015; Green and Nutt, 2014). Current NPS research

needs to be aimed at assessing abuse liability by means of preclinical data from animal models (e.g. drug self-administration), data on pharmacokinetics, bioavailability and metabolism to identify active and toxic metabolites, and the long-term effects after acute and chronic exposure to guide education and interventions (Baumann and Volkow, 2015).

A collective multidisciplinary effort is necessary at an international level considering the rapid increase of recreational use of NPS worldwide. Greater collaboration among preclinical and clinical scientists in both preclinical and clinical studies is needed to determine translational value. Preclinical studies should integrate pharmacodynamics and pharmacokinetic data, by that exposing animals to known or calculable drug concentrations, and thus providing pharmacological data relevant to human drug use (Green and Nutt, 2014). The development of validated and fast screening tools would greatly assist to establish risks of future NPS and speed up health risk assessment. An example of such a coordinated effort is the EU Predicting Risk of Emerging Drugs with In silico and Clinical Toxicology (PREDICT) project (www.predictnps.eu), which is aimed at developing a novel testing system to determine toxicity of NPS based on animal and human data.

Conclusion

This dissertation investigated interactions between acute drug experiences and exposure to affective cues, and assessed drug interactions during multiple drug exposure. The findings discussed in this dissertation indicate that acute exposure to drugs of abuse can alter the interpretation of affective cues. Specifically, alcohol and cannabis marketing cues elicited striatal activation in the mesolimbic reward circuit, whereas striatal activation to these cues was reduced during intoxication. Exposure to aggression cues affected subjective aggression during acute alcohol and cannabis intoxication in opposing ways. Alcohol increased subjective aggression in heavy alcohol users whereas cannabis decreased subjective aggression in regular cannabis users, suggesting that alcohol intoxication is more likely to elicit aggression compared to cannabis intoxication.

The findings discussed in this dissertation also indicate that drug-drug interactions vary between drugs of abuse and across cognitive domains. Memantine co-administration did not block memory impairment elicited by MDMA, implying that the neuropharmacological mechanism involved in MDMA-induced memory impairment in humans is not mediated by glutamate or acetylcholine as suggested by rodent studies. Similar to the neurocognitive effects induced by MDMA, mephedrone improved psychomotor performance, impaired spatial memory and did not affect divided attention. Alcohol co-administration with mephedrone resulted in a reduction of reaction times during divided attention and a greater impairment of spatial memory. The stimulatory effects of mephedrone could not block the impairing effects of alcohol on other performance parameters.

The current findings are important for understanding how neurocognition and the interpretation of affective cues alter during exposure. Regulatory interventions are recommended to reduce the impact of drug use on affective processing during day to day operations.

Summary

Drugs of abuse such as alcohol, cannabis, cocaine, MDMA and mephedrone are often taken for their pleasurable effects. A great number of legal and illegal drugs of abuse exert their reinforcing effects by either directly or indirectly activating the mesolimbic reward circuitry in the brain. Drugs of abuse have been shown to affect brain functioning, impair neurocognitive performance and alter the interpretation and appraisal of affective cues, such as drug marketing cues, emotional face expressions and aggression cues

Exposure to drug marketing cues can lead to positive expectancies towards drug use and increase the intention to consume. The influence of drug marketing cues can change during intoxication and this may change during intoxication. Similarly, affective cues, such as emotional face expressions, and aggression cues may alter the neurocognitive response to acute drug exposure. Acute drug exposure may impair the recognition of fearful or angry face expressions or increase/decrease aggressive behavior following exposure to aggression cues. It is important to investigate whether interactions between drugs of abuse and affective cues change during acute drug intoxication, possibly leading to an increased motivation to use drugs, increased aggression, and impaired interpretation of emotions in face expressions.

Acute drug experience can also be altered during exposure to multiple drugs. The majority of drug users use multiple drugs during the same episode in order to increase positive or decrease negative effects. Alcohol is the most common used drug in conjunction with others, and can considerably increase the harms and even lead to fatal outcomes. Therefore, it is important to examine the underlying neuropharmacological mechanisms during multiple drug exposure to understand their effects on neurocognitive performance.

The main aim of this dissertation was to investigate interactions between acute drug experiences and exposure to affective cues, and to assess drug interactions during multiple drug exposure. Double-blind placebo-controlled experimental studies were conducted involving administration of a broad range of drugs of abuse, such as alcohol, cannabis, cocaine, MDMA and mephedrone. Neurocognitive performance and subjective

effects were measured at peak drug plasma concentrations (T_{max}). The studies described in the first three chapters investigated whether brain activity and neurocognitive performance changes during drug intoxication following exposure to drug marketing cues, emotional face cues and aggression cues. The studies in the following chapters assessed whether exposure to one drug would potentiate or block the effects induced by another drug.

The study in **Chapter 2** examined the impact of alcohol and cannabis marketing on the reward circuit in alcohol and cannabis users while sober and intoxicated. It was predicted that alcohol and cannabis marketing would increase striatal activation when sober and that reward sensitivity would decrease during alcohol and cannabis intoxication. Heavy alcohol and regular cannabis users participated in a mixed factorial study involving administration of alcohol and placebo in the alcohol group, and cannabis and placebo in the cannabis group. Non-drug users served as between group reference. Brain activation after exposure to alcohol and cannabis marketing movies was measured using fMRI and compared between groups while sober and compared to placebo while intoxicated. Implicit alcohol and cannabis cognitions were assessed by means of the Single-Category Implicit Association Test (SC-IAT). Alcohol and cannabis marketing significantly increased striatal BOLD activation across all groups while sober. Striatal activation however decreased during intoxication with alcohol and cannabis. Implicit associations with cannabis marketing cues were significantly more positive in alcohol and cannabis users as compared to non-drug using controls. It was concluded that public advertising of alcohol or cannabis use elicits striatal activation in the brain's reward circuit and suggested that reduction of marketing would reduce brain exposure to reward cues that motivate substance use. Conversely, elevated dopamine levels may protect against the reinforcing potential of marketing.

The study in **Chapter 3** investigated the acute effects of alcohol and cannabis on subjective aggression in alcohol and cannabis users respectively, following aggression exposure. Drug-free controls served as a reference. It was hypothesized that aggression

exposure would increase subjective aggression in alcohol users during alcohol intoxication, whereas it was expected to decrease subjective aggression in cannabis users during cannabis intoxication. Heavy alcohol and regular cannabis users, and controls were included in a mixed factorial study. Alcohol and cannabis users received single doses of alcohol and placebo or cannabis and placebo respectively. Subjective aggression was assessed before and after aggression exposure via the Point-Subtraction Aggression Paradigm (PSAP) and the Single-Category Implicit Association Test (SC-IAT). Testosterone and cortisol levels in response to alcohol/cannabis treatment and aggression exposure were recorded as secondary outcome measures. Subjective aggression significantly increased following aggression exposure in all groups while being sober. Alcohol intoxication increased subjective aggression whereas cannabis decreased the subjective aggression following aggression exposure. Aggressive responses during the PSAP increased following alcohol and decreased following cannabis relative to placebo. Changes in aggressive feeling or response were not correlated to the neuroendocrine response to treatments. It was concluded that alcohol facilitates aggression whereas cannabis diminishes aggression in heavy alcohol and

The study described in **Chapter 4** was aimed to elucidate the acute effect of cannabis and cocaine on amygdala activation following exposure to affective facial stimuli. It was expected that amygdala reactivity to affective stimuli would decrease during cannabis and cocaine intoxication. Regular drug users, participated in a double-blind, placebo controlled, three-way crossover study. Participants received cannabis, cocaine and placebo, after which brain activity was measured by means of an amygdala reactivity fMRI paradigm. Correlations between brain activity and reaction time during task performance were additionally investigated. Results did not reveal any significant amygdala activation during task performance, but increased activity in occipital and temporal brain regions was found following exposure to affective stimuli. Acute cannabis and cocaine intoxication did not affect amygdala activity. Significant positive and negative correlations between BOLD signals and reaction times were revealed in the right inferior occipital, left inferior frontal operculum, right middle temporal, right superior temporal

areas and left amygdala following exposure to threat-related faces during cannabis intoxication. These preliminary findings suggest that amygdala reactivity in regular drug users is not attenuated by cannabis or cocaine intoxication during exposure to affective facial stimuli.

The study in **Chapter 5** investigated whether treatment with memantine can prevent MDMA-induced memory impairment in humans. Recreational MDMA users participated in a double-blind, placebo controlled, four-way crossover study. Participants received both pre-treatment (placebo/memantine 20 mg) (T_1) and treatment (placebo/MDMA 75 mg) (T_2) on separate test days. T_1 preceded T_2 by 120 minutes. Memory function was assessed 90 minutes after T_2 by means of a visual verbal learning task (VVL), a prospective memory task, the Sternberg memory test and the abstract visual pattern learning task (AVIPALET). Profile of Mood State and psychomotor performance were also assessed to control whether MDMA and memantine interactions would selectively pertain to memory or transfer to other domains as well. MDMA significantly impaired performance in the VVL and AVIPALET. Pre-treatment with memantine did not prevent MDMA-induced memory impairment in these two tasks. Both positive (vigor, arousal, elation) and negative effects (anxiety) were increased by MDMA. The responses were not altered by pre-treatment with memantine, which had no effect on memory or mood when given alone. These preliminary results suggest that memantine does not reverse MDMA-induced memory impairment and mood in humans.

The study in **Chapter 6** was designed to assess the effect of mephedrone alone and after co-administration with alcohol on neurocognitive function. It was hypothesized 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. Recreational mephedrone users participated in a double-blind, placebo controlled, four-way crossover study. 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 1

hour (T_1) and 4 hours after (T_2) mephedrone administration, by means of the Divided Attention Task, Critical Tracking Task, and the Spatial Memory Test. 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. The effects of mephedrone on cognition are comparable to those elicited by MDMA. Stimulatory effects of mephedrone were not sufficient to compensate for the impairing effects of alcohol on most performance parameters.

Finally, in **chapter 7** the key findings of the studies are discussed in a broader perspective and implications and recommendations for future research are provided. Firstly, it was concluded that acute exposure to drugs of abuse can impair the interpretation of affective cues. Secondly, it was shown that neurocognitive performance is differently affected across domains during multiple drug exposure and the degree of drug-drug interaction differed across cognitive domains.

Samenvatting

Drugs zoals alcohol, cannabis, cocaïne, MDMA en mephedrone worden vaak gebruikt vanwege hun aangename effecten. Een groot aantal legale en illegale drugs oefenen hun belonende effecten uit door zowel direct als indirect het mesolimbische dopaminerge beloningsstelsel in de hersenen te stimuleren. Het is bekend dat drugs een versturende invloed kunnen hebben op hersenfunctie en neurocognitie. Ook kunnen drugs het vermogen om affectieve stimuli, zoals marketingstimuli (reclame), emoties in gezichtsuitdrukkingen en agressieve beelden, te interpreteren aantasten.

Blootstelling aan drugsgerelateerde marketingstimuli kan leiden tot positieve verwachtingen ten aanzien van drugsgebruik en verhoogt de intentie om te gebruiken. De invloed van drugsmarketingstimuli kan veranderen wanneer men onder invloed is van drugs. Ook kan de neurocognitieve reactie na acute blootstelling aan drugs veranderen door affectieve stimuli zoals emoties in gezichtsuitdrukkingen en agressieve beelden. Acute inname van drugs kan het herkennen van angstige en boze gezichtsuitdrukkingen bemoeilijken en kan leiden tot een toename of afname van agressief gedrag na blootstelling aan agressieve beelden. Het is belangrijk om te onderzoeken of interacties tussen drugs en affectieve stimuli veranderen tijdens acute blootstelling aan drugs. Daarnaast is het belangrijk om te weten of deze interacties leiden tot een verhoogde motivatie om drugs te gebruiken, een toename van agressief gedrag of een verslechterde interpretatie van emoties in gezichtsuitdrukkingen.

De beleving tijdens blootstelling aan drugs kan ook veranderen wanneer er meerdere drugs tegelijkertijd worden ingenomen. De meeste mensen gebruiken meerdere drugs tegelijkertijd om de positieve effecten te versterken of om de negatieve effecten te verlagen. De drug die het meest gecombineerd wordt met andere drugs is alcohol. De combinatie van alcohol en andere drugs kan de schade aanzienlijk vergroten en kan zelfs leiden tot een fatale afloop. Het is daarom belangrijk om de onderliggende neurofarmacologische mechanismen tijdens gelijktijdige blootstelling aan meerdere drugs te onderzoeken om hun effecten op neurocognitieve functies te verduidelijken.

Het doel van dit proefschrift was enerzijds om interacties tussen acute drugservaringen en affectieve cues te onderzoeken, en anderzijds de wisselwerking tussen drugs te evalueren tijdens gelijktijdige blootstelling aan een of meerdere drugs. Dubbel-blind placebo-gecontroleerde studies werden uitgevoerd om de effecten van alcohol, cannabis, cocaïne, MDMA en mephedrone te onderzoeken. Neurocognitieve functies en subjectieve ervaringen werden gemeten wanneer de concentratie van de drug in het bloed het hoogst was. In de eerste drie hoofdstukken werd onderzocht of hersenactiviteit en neurocognitieve functies veranderen na acute drugstoediening en blootstelling aan marketingstimuli, emotionele gezichtsuitdrukkingen en agressieve stimuli. In de volgende twee hoofdstukken werd onderzocht of blootstelling aan een drug de effecten geïnduceerd door een andere drug versterkt of blokkeert.

In de studie beschreven in **hoofdstuk 2** werd de invloed van alcohol- en cannabis-gerelateerde marketingstimuli op het striatale dopaminerge systeem onderzocht bij alcohol- en cannabisgebruikers onder invloed van respectievelijk alcohol en cannabis en tijdens de nuchtere staat. Voorspeld werd dat alcohol- en cannabis-marketingstimuli de striatale activatie in de hersenen zouden verhogen tijdens de nuchtere staat en dat de beloningsgevoeligheid zou verminderen onder de invloed van alcohol en cannabis. Zware alcoholgebruikers en regelmatige cannabisgebruikers deden mee aan een studie en werden opgedeeld in een alcohol-groep en een cannabis-groep. In de alcohol-groep werd alcohol of placebo toegediend, in de cannabis-groep cannabis of placebo. Niet-druggebruikers dienden als referentiegroep. Hersenactiviteit na blootstelling aan alcohol- en cannabis-marketingstimuli werd gemeten met behulp van fMRI. In de nuchtere staat werden de verschillende groepen met elkaar vergeleken, verder werd er een vergelijking met placebo gemaakt wanneer de proefpersonen onder invloed van alcohol en cannabis waren. Impliciete associaties ten aanzien van alcohol- en cannabisstimuli werden gemeten door middel van de Single-Category Implicit Association Test (SC-IAT). Het zien van alcohol- en cannabis-marketingstimuli heeft geleid tot een significante toename in striataal BOLD signaal in alle 3 groepen tijdens de nuchtere staat. Striatale activatie daalde echter onder invloed van alcohol en cannabis. Impliciete associaties met cannabis-

marketing cues waren significant positiever in alcohol- en cannabisgebruikers in vergelijking met de controle groep. In dit hoofdstuk werd geconcludeerd dat reclame voor alcohol- of cannabisgebruik leidt tot activatie van het striatale dopaminerge systeem in de hersenen. Daarnaast is de verwachting dat minder blootstelling aan alcohol- en cannabisreclame ook leidt tot een verminderde prikkeling door stimuli die drugsgebruik motiveren. Marketingstimuli kunnen trek in drugs stimuleren, maar hoge dopamine spiegels kunnen hier mogelijk tegen beschermen.

De studie in **hoofdstuk 3** onderzocht de acute effecten van alcohol en cannabis op subjectieve agressie in alcohol- en cannabisgebruikers na blootstelling aan agressie cues. De hypothese was dat de blootstelling aan agressie cues zou leiden tot een toename in subjectieve agressie bij alcoholgebruikers onder invloed van alcohol, terwijl subjectieve agressie bij cannabisgebruiker zou verlagen onder invloed van cannabis. Zware alcoholgebruikers en regelmatige cannabisgebruikers deden mee aan een studie en werden opgedeeld in een alcohol-groep en een cannabis-groep. In de alcohol-groep werd alcohol of placebo toegediend, in de cannabis-groep cannabis of placebo. Niet-druggebruikers dienden als referentiegroep. Subjectieve agressie werd gemeten voor en na blootstelling aan agressie cues, door middel van Point-Subtraction Aggression Paradigm (PSAP) and the Single-Category Implicit Association Test (SC-IAT). De effecten van alcohol- en cannabistoediening en agressie cues op testosteron- en cortisolspiegels werden geregistreerd als secundaire uitkomstmaten. Subjectieve agressie was significant toegenomen na blootstelling aan agressie cues bij alle groepen tijdens de nuchtere staat. Subjectieve agressie na blootstelling aan agressie cues is toegenomen na alcoholtoediening en afgenomen na cannabistoediening. Agressieve reacties tijdens de PSAP zijn toegenomen na alcohol en afgenomen na cannabis ten opzichte van placebo. Veranderingen in agressieve gevoelens of reacties correleerden niet met de neuro-endocriene reactie na alcohol- en cannabistoediening. Er werd geconcludeerd dat alcohol gevoelens van agressie opwekt terwijl cannabis gevoelens van agressie vermindert in zware alcoholgebruikers en regelmatige cannabisgebruikers.

De in **hoofdstuk 4** beschreven studie was er op gericht om de acute effecten van cannabis en cocaïne op amygdala-activering na blootstelling aan affectieve stimuli met emotionele gezichten te verhelderen. Verwacht werd dat de amygdalareactiviteit na het zien van affectieve stimuli zou afnemen onder invloed van cannabis en cocaïne. Regelmatige drugsgebruikers namen deel aan een dubbelblinde, placebo-gecontroleerde, 3-weg cross-over studie. De deelnemers kregen enkelvoudige doses van cannabis, cocaïne en placebo, waarna hersenactiviteit gemeten werd met behulp van een fMRI taak. Ook werden correlaties tussen hersenactiviteit en reactietijd onderzocht. Resultaten lieten geen significante amygdala-activatie zien na blootstelling aan affectieve stimuli, maar er werd wel verhoogde activiteit in occipitale en temporale hersengebieden gemeten. Cannabis en cocaïne hadden geen invloed op amygdalareactiviteit. Significante positieve en negatieve correlaties tussen BOLD signaal en reactietijden werden gevonden in de rechter inferieure occipitale, linker inferieure frontale operculum, rechter midden temporale en rechter superieure temporale gebieden, en in de linker amygdala na blootstelling aan dreigende gezichten onder invloed van cannabis. Deze voorlopige bevindingen suggereren dat amygdalareactiviteit in reguliere drugsgebruikers niet door cannabis of cocaïne verzwakt wordt na het zien van affectieve stimuli.

De studie in **hoofdstuk 5** onderzocht of behandeling met memantine een door MDMA geïnduceerde verslechtering van het geheugen kan voorkomen. Recreatieve MDMA-gebruikers kregen op verschillende testdagen twee behandelingen toegediend; als eerste placebo of 20 mg memantine (T_1) vervolgens na 120 minuten placebo of 75 mg MDMA (T_2). Geheugenfunctie werd 90 minuten na T_2 onderzocht door middel van een visuele verbale leertaak (VVLT), een prospectieve geheugentaak, de Sternberg memory test en de abstracte visuele patroon leertaak (AVIPALET). Stemming en psychomotorische prestaties werden ook onderzocht om te bepalen of interacties tussen MDMA en memantine selectief betrekking zouden hebben op het geheugen, of dat deze ook naar andere domeinen overgedragen worden. MDMA heeft prestatie tijdens de VVLT en AVIPALET aanzienlijk verminderd. Behandeling met memantine kon de MDMA-geïnduceerde verslechtering van het geheugen in deze twee taken niet voorkomen. Zowel

positieve (kracht, opwinding, opgetogenheid) als negatieve emoties (angst) werden na MDMA versterkt. De reacties waren niet veranderd na voorbehandeling met memantine, wat ook geen invloed op het geheugen of de stemming had wanneer het alleen was toegediend. Deze voorlopige resultaten suggereren dat memantine door MDMA geïnduceerde geheugenverlies en stemmingsveranderingen niet kan omkeren.

De studie in **hoofdstuk 6** is ontworpen om het effect van mephedrone alleen en na gelijktijdige toediening met alcohol op neurocognitieve functies te onderzoeken. De hypothese was dat wanneer mephedrone alleen werd toegediend psychomotorische prestaties zullen verbeteren, maar geheugenprestatie zal verslechteren. Verwacht werd dat de neurocognitieve prestatie verslechtert na gelijktijdige toediening van mephedrone en alcohol. Recreatieve mephedrone-gebruikers kregen een dosis van 200 mg mephedrone of placebo in combinatie met 0.8 g/kg alcohol of placebo. Neurocognitieve prestatie werd gemeten bij aanvang (T_0), 1 uur (T_1) en 4 uur (T_2) na mephedrone toediening, door middel van de Verdeelde Aandacht taak, Critical Tracking taak, en de Spatial Memory taak. Mephedrone verminderde korte termijn ruimtelijk geheugen op T_1 en verbeterde psychomotorische prestaties op T_2 . Mephedrone alleen had geen invloed op verdeelde aandacht, maar toonde een interactie met alcohol op reactietijd in de aandachtstaak bij T_2 . Deze reactietijd nam af wanneer mephedrone tegelijkertijd met alcohol werd toegediend ten opzichte van alcohol alleen. Alcohol verminderde zowel het korte als lange termijn ruimtelijk geheugen op T_1 en verdeelde aandacht op T_1 en T_2 . Psychomotorische prestatie werd niet beïnvloed door alcohol. De huidige bevindingen ondersteunen de hypothese dat mephedrone psychomotorische prestatie verbetert, ruimtelijk geheugen schaadt en geen invloed heeft op verdeelde aandacht. De effecten van mephedrone op cognitie zijn vergelijkbaar met de effecten die door MDMA worden opgewekt. Stimulerende effecten van mephedrone waren niet voldoende om voor de negatieve effecten van alcohol op de meeste prestatieparameters te compenseren.

Tot slot worden in **hoofdstuk 7** de belangrijkste bevindingen van het onderzoek in een breder perspectief besproken, en worden implicaties en aanbevelingen voor

toekomstig onderzoek gegeven. Ten eerste werd er geconcludeerd dat acute blootstelling aan drugs de interpretatie van affectieve signalen kan aantasten. Ten tweede werd er aangetoond dat de neurocognitieve prestatie op verschillende domeinen verschillend wordt beïnvloed tijdens gelijktijdige blootstelling aan meerdere drugs, en dat de mate van drug-drug interactie verschilt per cognitief domein.

Resumen

Hopi biaha ta uza droga, manera alcohol, marihuana (cannabis), cocaina, MDMA (ecstasy, XTC) y mefedrona (mephedrone), pa motibo di e efectonan placentero cu nan tin riba e uzadonan. Un gran cantidad di droga legal y ilegal ta eherce efectonan gratificante door di activa e circuito recompensatorio mesolimbico den e cerebro, sea directamente of indirectamente. Ta conoci cu droga tin efecto negativo riba funcionamiento di cerebro, cu nan ta afecta funcionamiento neurocognitivo y ta altera e capacidad pa interpreta y evalua estimulonan afectivo, manera estimulonan di mercadeo di droga, expresion emocional di cara y estimulonan agresivo.

Exposicion na estimulonan di mercadeo di droga por conduci na expectativanan alentador respecto uzo di droga y intensifica e intencion pa consumie, y esey por cambia na momento cu e uzado ta intoxica. Igualmente, estimulonan afectivo, manera expresion emocional di cara, y estimulonan agresivo, por altera e reaccion neurocognitivo na momento di exposicion agudo na droga. Exposicion agudo na droga por dificulta reconocimiento di expresionnan facial di miedo of di rabia, of fortifica of debilita comportacion agresivo, despues di exposicion na estimulonan agresivo. Ta importante pa investiga si interaccion entre uzo di droga y estimulonan afectivo ta cambia durante intoxicacion agudo, y si esaki ta intensiva e motivacion pa uza droga, ta aumenta comportacion agresivo y ta limita e capacidad pa interpreta emocion den e expresionnan di cara.

E experiencia causa pa uzo agudo di droga tambe por wordo altera den caso di uzo multipel di droga na e mes momento. Mayoria di uzado di droga ta consumi multipel droga pareu den e mesun sesion, pa asina fortifica e efectonan positivo respectivamente debilita e efectonan negativo. E droga di combinacion mas uza ta alcohol. Combinacion di alcohol cu otro droga por aumenta daño considerablemente y te hasta por conduci na un desenlace fatal. P'esey ta importante pa examina e mecanismonan neurofarmacologico subyacente durante uzo multipel di droga, pa compronde nan efecto riba funcionamiento neurocognitivo.

E meta principal di e disertacion aki tabata, di un banda, investiga e interaccionnan entre e experiencianan causa pa uzo agudo di droga y exposicion na estimulonnan afectivo, y, di otro banda, evalua e interaccionnan entre e diferente droganan durante exposicion simultaneo na multipel droga. A haci estudionan experimental tipo dobel ciego controla pa placebo (double-blind placebo-controlled experimental studies), pa investiga e efectonan di un gama amplio di droga, manera alcohol, marihuana, cocaina, MDMA y mefedrona. A investiga funcionamiento neurocognitivo y efectonan subhetivo na momento di concentracion maximo di plasma cu droga den sanger (T_{max}). E estudionan describi den e prome tres capitulonnan a investiga si actividad cerebral y funcionamiento neurocognitivo ta cambia durante intoxicacion despues di exposicion na estimulonnan di mercadeo di droga, estimulonnan di expresion emocional di cara y estimulonnan agresivo. E estudionan den e siguiente capitulonnan a evalua si exposicion na un droga ta fortifica of ta blokea e efectonan causa pa un otro droga.

Den e estudio describi den **Capitulo 2** a investiga e impacto di estimulonnan di mercadeo di alcohol y marihuana riba e circuito recompensatorio di uzadonan di alcohol y marihuana, ora nan ta den estado sobrio y ora nan ta den estado intoxica respectivamente cu alcohol of marihuana. A pronostica cu e estimulonnan di mercadeo di alcohol y marihuana lo aumenta e activacion striatal (striatal activation) den e cerebro den un estado sobrio y cu e sensibilidad recompensatorio lo mengua durante intoxicacion cu alcohol y marihuana. Uzadonan pisa di alcohol y uzadonan di marihuana riba un base regular a participa na un estudio experimental (mixed factorial study). Na e participantenan di e grupo di alcohol a suministra alcohol y placebo, mientras cu na e participantenan di e grupo di marihuana a suministra marihuana y placebo. Hende cu no ta uza droga a fungi como grupo di referencia entre nan. A investiga e activacion cerebral pa medio di fMRI despues cu a somete nan na pelicula cu estimulonnan di mercadeo di alcohol y marihuana. A compara e activacion cerebral entre e gruponan den un estado sobrio y a compara e activacion cerebral cu placebo mientras nan tabata intoxica. Igualmente a midi asociacionnan implicito relaciona cu alcohol y marihuana, pa medio di un Test di Asociacion Implicito di Categoria Simpel (Single-Category Implicit Association

Test (SC-IAT)). Na momento di presencia estimulan di mercadeo di alcohol y marihuana, esaki a conduci na un aumento significativo di e activacion striatal BOLD (striatal BOLD activation) den tur tres grupo den estado sobrio. Sinembargo, e activacion striatal a baha den estado intoxica cu alcohol of marihuana. Asociacionnan implicito cu estimulan di mercadeo di marihuana tabata significativamente mas positivo cerca e uzadonan di alcohol y marihuana, compara cu e grupo di control cu no ta uza droga. A keda conclui cu propaganda masivo pa uzo di alcohol of di marihuana ta conduci na activacion striatal den e circuito recompensatorio di e cerebro. Conforme esey a sugeri cu reduccion di mercadeo al respecto lo encera menos estimulo pa uzo di droga. Si mercadeo por stimula e deseo pa consumi droga, un grado halto di dopamina den cerebro por contraaresta e influencia potencial di mercadeo.

E estudio den **Capitulo 3** a investiga e efectonan agudo di alcohol y marihuana riba agresion subhetivo respectivamente cerca uzadonan di alcohol y uzadonan di marihuana, despues di expone nan na estimulan agresivo. Hende cu no ta uza droga a fungi como grupo di referencia. A plantea e hipotesis cu exposicion na estimulan agresivo lo conduci na un aumento di agresion subhetivo cerca uzadonan di alcohol durante intoxicacion cu alcohol, mientras cu e expectativa tabata cu agresion subhetivo lo disminui cerca uzadonan di marihuana durante intoxicacion cu marihuana. A inclui uzadonan pisa di alcohol y uzadonan di marihuana riba un base regular, como tambe hende cu no ta uza droga, den un estudio experimental (mixed factorial study). Na uzadonan di alcohol a suministra un dosis unico di alcohol y placebo y na uzadonan di marihuana a suministra un dosis unico di marihuana y placebo. A investiga agresion subhetivo tanto prome como despues cu a expone e participantenan na estimulan agresivo, pa medio di un Paradigma di Agresion (Point-Subtraction Aggression Paradigm (PSAP)) y un Test di Asociacion Implicito di Categoria Simpel (Single-Category Implicit Association Test (SC-IAT)). A registra e efectonan di suministro di alcohol y marihuana como tambe di exposicion na estimulan agresivo riba e nivelnan di testosterona y cortisol como midinan di resultado secundario. Agresion subhetivo a aumenta significativa-mente den tur tres grupo despues di exposicion na estimulan agresivo den

estado sobrio. Intoxicacion cu alcohol a aumenta agresion subhetivo mientras cu marihuana a disminui agresion subhetivo despues di exposicion na estimulon agresivo. Reaccion agresivo durante e PSAP-test a aumenta cu alcohol y a disminui cu marihuana, compara cu placebo. Cambio den sentimiento of reaccion agresivo no tabata corela cu reaccion neuroendocrino riba suministro di alcohol y marihuana. A conclui cu alcohol ta aumenta agresion subhetivo cerca uzadonan pisa di alcohol, mientras cu marihuana ta disminui agresion subhetivo cerca uzadonan di marihuana riba un base regular.

E estudio describi den **Capitulo 4** tabatin como obhetivo clarifica e efectonan agudo di marihuana y cocaina riba activacion di amigdala despues di exposicion na estimulon di expresion emocional di cara. E expectativa tabata cu reactividad di amigdala despues di exposicion na estimulon afectivo lo disminui cerca e participantenan durante intoxicacion cu marihuana y cocaina. Uzadonan di droga riba base regular a participa na un estudio experimental (double-blind, placebo controlled, three-way crossover study). A suministra e participantenan un dosis unico di marihuana, di cocaina y di placebo y despues a midi e actividad cerebral pa medio di fMRI. Adicionalmente a investiga corelacion entre actividad cerebral y tempo di reaccion durante eheccion di tarea. E resultadonan di e investigacion no a muestra ningun activacion di amigdala significativo durante eheccion di tarea, pero si a detecta aumento di actividad den e areanan occipital y temporal di e cerebro despues di exposicion na estimulon afectivo. Intoxicacion agudo cu marihuana y cocaina no a conduci na reactividad di amigdala. Si a registra corelacion significativo, tanto positivo como negativo, entre e señalnan BOLD y e temponan di reaccion den e areanan cerebral occipital inferior drechi, operculo frontal inferior robes, temporal intermedio drechi, temporal superior drechi y amigdala robes, despues di exposicion na caranan menasante durante intoxicacion cu marihuana. E resultadonan preliminar aki ta sugeri cu intoxicacion cu marihuana of cocaina no ta debilita reactividad di amigdala cerca uzadonan di droga riba base regular durante exposicion na estimulon afectivo di cara.

E estudio den **Capítulo 5** a investiga si tratamiento cu memantina por preveni deterioro di memoria induci pa MDMA. Uzadonan di MDMA na forma recreativo a participa na un estudio experimental (double-blind, placebo controlled, four-way crossover study). E participantenan a ricibi tanto un tratamiento preliminar (cu placebo/memantina 20 mg) (T_1) como un tratamiento definitivo (cu placebo/MDMA 75 mg) (T_2) riba diferente dia di test. T_1 tabata tuma luga 120 minuut prome cu T_2 . A investiga funcion di memoria 90 minuut despues di T_2 pa medio di un tarea di siñamento visual verbal (visual verbal learning task (VVL)), un tarea di memoria prospectivo, e test di memoria di Sternberg (Sternberg memory test) y e tarea di siñamento segun patronchi visual abstracto (abstract visual pattern learning task (AVIPALET)). Tambe a investiga e perfil di Estado Emocional y rendimiento psicomotorico (Profile of Mood State and psychomotor performance) pa determina si e interaccionnan entre MDMA y memantina selectivamente lo ta liga cu memoria so of si ta transferi nan pa otro area tambe. MDMA significativamente a deteriora prestacion durante e tareanan VVL y AVIPALET. Tratamiento preliminar cu memantina (T_1) no a preveni deterioro di memoria induci pa MDMA den e dos tareanan ey. Tanto emocion positivo (forsa, animo, euforia) como emocion negativo (ansiedad) a incrementa door di MDMA. E reaccionnan no a cambia door di e tratamiento preliminar cu memantina (T_1), y tampoco e no tabatin ningun efecto riba memoria ni emocion ora a suministr'e so. E resultadonan preliminar aki ta sugeri cu memantina no por reverti deterioro di memoria ni cambio di estado emocional induci pa MDMA.

E estudio den **Capítulo 6** a wordo diseña pa investiga e efecto di mefedrona riba funcionamiento neurocognitivo, tanto ora cu suministra mefedrona so como ora cu suministra mefedrona combina cu alcohol. A plantea e hipotesis cu ora cu suministra mefedrona so, e lo mehora prestacion psicomotorico, pero e lo deteriora funcionamiento di memoria. E expectativa tabata cu suministro di mefedrona combina cu alcohol lo afecta funcionamiento neurocognitivo. Uzadonan di mefedrona na forma recreativo a participa na un estudio experimental (double-blind, placebo controlled, four-way crossover study). E participantenan a ricibi un dosis unico di 200 mg di mefedrona of placebo den combinacion cu 0.8g/kg di alcohol of placebo. A investiga funcionamiento neurocognitivo

na inicio di e estudio (T_0), 1 ora despues (T_1) y 4 ora despues (T_2) di suministro di mefedrona, pa medio di e Tarea di Atencion Dividi (Divided Attention Task), e Tarea di Seguimiento Critico (Critical Tracking Task) y e Test di Memoria Espacial (Spatial Memory Test). Intoxicacion cu mefedrona a deteriora memoria espacial di tempo cortico na T_1 y a mehora prestacion psicomotorico na e tarea di seguimento critico na T_2 . Intoxicacion cu mefedrona so no a afecta atencion dividi, pero si a muestra un interaccion cu alcohol den e tempo di reaccion na e tarea di atencion dividi na T_2 . E tempo di reaccion a baha ora cu a suministra mefedrona combina cu alcohol, compara cu ora cu a suministra alcohol so. Intoxicacion cu alcohol a deteriora memoria espacial, tanto di tempo cortico como di tempo largo na T_1 , y a deteriora atencion dividi na T_1 y na T_2 . Intoxicacion cu alcohol no a afecta prestacion psicomotorico (seguimento critico). E resultadonan aki ta sostene e hipotesis cu mefedrona ta mehora prestacion psicomotorico, ta deteriora memoria espacial y no tin efecto riba funcionamiento di atencion dividi. E efectonan di mefedrona riba cognicion ta comparabel cu e efectonan causa pa MDMA. E efectonan estimulante di mefedrona no tabata suficiente pa compensa e efectonan negativo causa pa alcohol na mayoria di e parametronan di prestacion.

Finalmente den **Capitulo 7** ta discuti e resultadonan mas importante di e estudionan den un perspectiva mas amplio. Alabes ta suministra implicacionnan y recomendacionnan pa futuro investigacion. Na prome luga a conclui cu exposicion agudo na droga por afecta e capacidad pa interpreta estimulonnan afectivo. Na di dos luga a keda demostra cu funcionamiento neurocognitivo ta wordo afecta na forma diferente den e diferente areanan durante exposicion simultaneo na multipel droga y cu e grado di interaccion entre droga cu droga tabata diferente den e diferente areanan cognitivo.

Valorisation addendum

This section addresses the impact and relevance of the studies described in this dissertation. Outlined are the way these studies are relevant to society, which target groups can benefit and how research findings have been disseminated.

Societal relevance

The acute and long-term consequences of illicit drug use continue to be a matter of global concern (EMCDDA, 2015b; UNODC, 2015). Drugs of abuse negatively impact cognitive performance and behavior, and increase the risk of developing mental disorders and other drug-related problems (EMCDDA, 2015a; Lammers et al., 2014). Likewise, the use of alcohol has been linked to substance related problems such as aggressive behavior and violence (Beck and Heinz, 2013; Duke et al., 2011). Novel psychoactive substances (NPS) that are flooding current drug markets also elicit a high level of public concern (Baumann and Volkow, 2015; EMCDDA, 2015b; UNODC, 2015). Life-threatening toxidromes have been described for NPS, with symptoms varying from agitation, hallucinations, psychosis, violent behaviors to coma. Drug users intoxicated with NPS represent a significant burden to healthcare professionals as adverse medical consequences are common.

One of the aims of this dissertation was to elucidate the effects of alcohol and cannabis intoxication on neurocognition and the interaction with marketing and aggression exposure. It was shown that exposure to alcohol marketing can activate the brain's reward center and potentially reinforce alcohol use (chapter 2). We have also shown that alcohol intoxication is more likely to elicit feelings of aggression (chapter 3). Furthermore, we have shown that portrayal of cannabis use also activates the reward center and that, other than alcohol, cannabis intoxication decreases feelings of aggression (chapter 3). These findings may imply that alcohol or cannabis users, who wish to stay abstinent, can be negatively influenced by alcohol and drug marketing, by reinforcing alcohol and cannabis use, leading to a vicious cycle of drug-taking behavior.

Another aim of this thesis was to study the influence of a frequently abused NPS (i.e. mephedrone) on neurocognitive function in a placebo-controlled setting. Data from

the current NPS study discard the notion that NPS or “legal highs” are safer and milder than traditional illegal drugs, and show that NPS effects on neurocognition can be as detrimental as those caused by alcohol or traditional drugs of abuse. The distinction between “traditional” and “new drugs” is becoming harder to define as NPS are designed to mimic the effects of traditional, scheduled drugs of abuse.

In fact, this was the very first study design ever that obtained medical ethical approval for acute administration of a NPS in a phase 1 study. This study was conducted in close collaboration with the Institut Hospital del Mar d'Investigacions Mèdiques (IMIM) in Barcelona. As such, this study offers a blueprint for future approaches to determine the mechanism of action of NPS, their toxicity profiles and pharmacological routes following acute administration in a controlled setting. Controlled experimental studies with NPS should generally be promoted because these will offer scientific rationales for future political and societal responses to challenges posed by NPS (Green and Nutt, 2014). Response strategies to the influx and use of NPS often involve drug-scheduling, although this approach is not effective and impedes placebo-controlled research with NPS. Ideally, clinical pharmacokinetic and pharmacodynamics information in animals and humans should be obtained before firm conclusions on the harms of these substances can be drawn. NPS research by means of placebo-controlled experimental studies give relevant and objective information about their acute effects on neurocognition, which can aid in education and prevention messages, as well as therapeutic interventions. This has proven to be a challenge in the past since Ethical Review boards often rule that NPS research should follow the same guidelines that have been established for the development of medicinal products (i.e. Good Manufacturing Process (GMP) requirements). The recent ruling of the European Court of Justice that GMP guidelines should not be extended to clinical research on substances of abuse that have no clinical usage is therefore is very instrumental in promoting further clinical research on acute and long-term effects of NPS in humans.

Target groups

The findings discussed in this dissertation are relevant to multiple target groups. Firstly, researchers in the field of psychopharmacology can benefit from the information that has been added to the existing knowledge on brain mechanisms underlying the reinforcing effects of alcohol and drug marketing, and the impact of alcohol and cannabis intoxication on different measures of aggression. This thesis also demonstrated that glutamate does not play a major role in MDMA-induced memory. This finding supports previous research showing MDMA induced impairment is primarily caused by 5-HT₂ receptor stimulation (van Wel et al., 2011).

Secondly, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the World Health Organization (WHO) can also benefit from the scientific information presented in this dissertation. The data gathered adds to both international and European databases and contributes to objective risk estimates concerning illicit drug use, which applies to both traditional drugs of abuse as well as NPS. These findings will support policymakers in making informed drug policies and strategies, for example regarding marketing of alcohol and other drugs of abuse.

Our findings on the associations between alcohol and aggression also provide scientific support for the introduction of a new bill to allow alcohol and drug testing in perpetrators of violence, which will come into force in the Netherlands in 2017. The bill provides a legal basis for the deployment of alcohol and drug testing in violent offenders. The results of these tests can be taken into account in the conviction and sentencing stage of the criminal justice process. Our findings support the notion that acute effects of alcohol can induce feelings of aggression, provoked as well as unprovoked. Likewise, the use of stimulant drugs such as cocaine and amphetamine, have also been associated with violence (Hoaken and Stewart, 2003; Lammers et al., 2014; Stoddard et al., 2015; Zhao et al., 2015). The study design employed in the current dissertation to measure alcohol-induced aggression could also be helpful in the development of standardized test batteries

to assess drug-induced aggressive behavior in placebo-controlled studies. A standardized battery will promote to reliably identify aggression-inducing properties of drugs of abuse.

Pharmaceutical industries can also benefit from the current findings in the development of dopaminergic drug targets that increase tonic dopamine levels to help minimize the effects of alcohol and drug marketing by reducing motivation to use drugs. Data presented in chapter 2 demonstrates that high tonic levels of dopamine protect against the reinforcing potential of alcohol and cannabis marketing. This suggests that prescription drugs that increase tonic dopamine levels, such as methylphenidate, may be of prophylactic value to alcohol and cannabis abusers to defy alcohol and cannabis marketing exposure in our society. Likewise, data presented in chapter 3 may indicate novel pharmaceutical indicated for cannabis or cannabinoids. Our finding that cannabis reduces feelings of aggression might spark in interest to develop cannabinoid compounds to treat people with aggressive personalities.

Lastly, the current findings are also interesting to the general population and (potential) drug users who would like to be informed about the health risks of drug use. Different consumption patterns are also associated with different levels and types of harm; and more frequent use (i.e., high doses, concurrent use of other substances) are all linked to elevated health risks.

Dissemination and impact

One of the goals of researchers in the field of psychopharmacology and addiction is to assess the acute and long-term effects of psychoactive substances on neurocognition and mood, and identify factors that make people more susceptible to addictions. The ultimate goal being the translation of experimental findings into practical applications aimed at reducing health risks associated with drug exposure by helping people stay in control of their own behavior. We have taken various efforts to ensure that the knowledge gained from our studies is spread across different target groups. Four out of five studies described in this dissertation have been published in several international journals to spread the findings to the international research community. In addition, most results

have been presented at national and international conferences that hosted many researchers from across the globe. The study in chapter 2 was part of the 'Addictions and Lifestyles In Contemporary Europe Reframing Addiction Project (ALICE RAP)' initiated by the European Commission. ALICE RAP was aimed to stimulate a broad and productive debate on science-based policy approaches to addictions. The findings of chapter 2 have been published on the website of ALICE RAP (<http://www.alicerap.eu/>) and were discussed during a scientific debate, 'the A debate'. The A-debate was an interactive discussion of addiction science (on-site and on-line) featuring scientists, policy actors from national and international organizations and clinical professionals, with the objective of presenting and discussing key research findings that came out of the project.

The findings of chapter 3 have also been covered by highly influential newspapers, such as the 'Washington Post', the 'Portland Press Herald' and 'De Morgen'. This ensured dissemination of the research findings to the general public and science communicators. In September 2016, 2 month after publication, the findings in chapter 3 were mentioned by 11 news outlets, 127 tweeters and 19 Facebook pages, which led to a high Altmetric score of 199 (i.e. in top 5% of all research outputs scored by Altmetric).

Finally, the studies were given more international attention by making most of these publications Open Access. Our future goal is to continue disseminating our research findings by means of (inter)national journal publications, (inter)national conference visits and through websites. In addition, the communication of research findings by means of workshops and public debates in order to provide a more interactive atmosphere will keep the scientific community as well as the general public informed.

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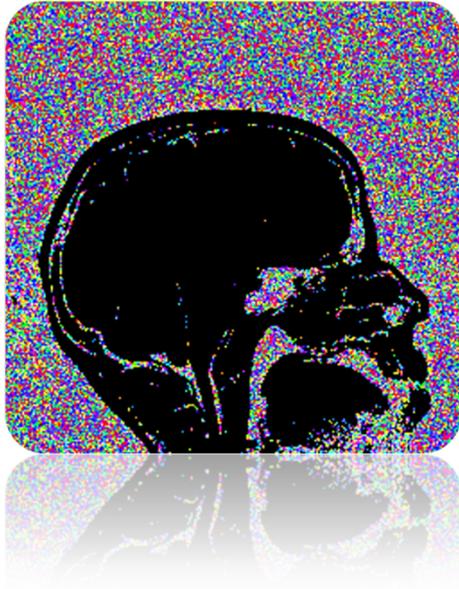
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References

- Adolphs R (2008) Fear, faces, and the human amygdala. *Current Opinion in Neurobiology* 18(2): 166–172.
- Anderson C a and Bushman BJ (2002) Human Aggression. *Annual Review of Psychology* 53(1): 27–51. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11752478>.
- Anderson P, de Bruijn A, Angus K, et al. (2009) Impact of alcohol advertising and media exposure on adolescent alcohol use: a systematic review of longitudinal studies. *Alcohol and alcoholism (Oxford, Oxfordshire)* 44(3): 229–43.
- Angoa-Pérez M, Kane MJ, Briggs DI, et al. (2013) Mephedrone does not damage dopamine nerve endings of the striatum, but enhances the neurotoxicity of methamphetamine, amphetamine, and MDMA. *Journal of Neurochemistry* 125(1): 102–110.
- Anton RF (1999) What is craving? Models and implications for treatment. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism* 23(3): 165–73. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10890811>.
- Aoki Y, Cortese S and Tansella M (2015) Neural bases of atypical emotional face processing in autism: A meta-analysis of fMRI studies. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry* 2975(April): 1–10.
- Aracava Y, Pereira EFR, Maelicke A, et al. (2005) Memantine Blocks $\alpha 7^*$ Nicotinic Acetylcholine Receptors More Potently Than N-Methyl-D-aspartate Receptors in Rat Hippocampal Neurons. *The journal of Pharmacology and Experimental Therapeutics* 312(3): 1195–1205.
- Astudillo M, Kuntsche S, Graham K, et al. (2010) The influence of drinking pattern, at individual and aggregate levels, on alcohol-related negative consequences. *European Addiction Research* 16(3): 115–123.
- Attwood AS, Ohlson C, Benton CP, et al. (2009) Effects of acute alcohol consumption on processing of perceptual cues of emotional expression. *Journal of psychopharmacology (Oxford, England)* 23(1): 23–30.
- Ballard ME, Bedi G and de Wit H (2012) Effects of delta-9-tetrahydrocannabinol on evaluation of emotional images. *Journal of psychopharmacology (Oxford, England)* 26(10): 1289–98.

References

- Ballard ME, Gallo DA and De Wit H (2013) Pre-encoding administration of amphetamine or THC preferentially modulates emotional memory in humans. *Psychopharmacology* 226(3): 515–529.
- Baron RA (1977) *Human aggression*. New York: Plenum.
- Baron-Cohen S, Baron-Cohen S, Ring HA, et al. (1999) Social intelligence in the normal and autistic brain: an fMRI study. *European Journal of Neuroscience* 11(6): 1891–1898.
- Batalla A, Bhattacharyya S, Yücel M, et al. (2013) Structural and Functional Imaging Studies in Chronic Cannabis Users: A Systematic Review of Adolescent and Adult Findings. *PLoS ONE* 8(2).
- Baumann MH and Volkow ND (2015) Abuse of New Psychoactive Substances: Threats and Solutions. *Neuropsychopharmacology*, Nature Publishing Group 41(3): 663–665. Available from: <http://dx.doi.org/10.1038/npp.2015.260>
- 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*, Nature Publishing Group 37(5): 1192–1203. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3306880&tool=pmcentrez&rendertype=abstract>.
- Baumann MH, Solis E, Watterson LR, et al. (2014) Baths Salts, Spice, and Related Designer Drugs: The Science Behind the Headlines. *Journal of Neuroscience* 34(46): 15150–15158. Available from: <http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3223-14.2014>.
- Baylen C a and Rosenberg H (2006) A review of the acute subjective effects of MDMA/ecstasy. *Addiction (Abingdon, England)* 101(7): 933–47. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16771886> (accessed 29 November 2013).
- Beck A and Heinz A (2013) Alkoholbezogene Aggression: Soziale und neurobiologische Faktoren. *Deutsches Arzteblatt International* 110(42): 711–715.
- Beierholm U, Guitart-Masip M, Economides M, et al. (2013) Dopamine modulates reward-related vigor. *Neuropsychopharmacology* 38(8): 1495–503. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3682144&tool=pmcentrez&rendertype=abstract>.
- Belin D, Jonkman S, Dickinson A, et al. (2009) Parallel and interactive learning processes within the basal ganglia: Relevance for the understanding of addiction. *Behavioural Brain Research* 199(1): 89–102.
- Bergamaschi MM, Queiroz RHC, Chagas MHN, et al. (2011) Cannabidiol reduces the

anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* : official publication of the American College of Neuropsychopharmacology 36(6): 1219–26. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3079847&tool=pmcentrez&rendertype=abstract>.

Berger SP, Hall S, Mickalian JD, et al. (1996) Haloperidol antagonism of cue-elicited cocaine craving. *Lancet* 347(9000): 504–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8596268>.

Berridge KC and Robinson TE (1998) What is the role of dopamine in reward: Hedonics, learning, or incentive salience? *Brain Research Reviews* 28: 308–67.

Bhattacharyya S, Morrison PD, Fusar-Poli P, et al. (2010) Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* 35(3): 764–774. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19924114> \n <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3055598/pdf/npp2009184a.pdf>.

Block RI, Farinpour R and Schlechte JA (1991) Effects of chronic marijuana use on testosterone, luteinizing hormone, follicle stimulating hormone, prolactin and cortisol in men and women. *Drug and Alcohol Dependence* 28(2): 121–128. Available from: <http://www.sciencedirect.com/science/article/pii/037687169190068A> (accessed 30 March 2015).

Bolla KI, McCann UD and Ricaurte GA (1998) Memory impairment in abstinent MDMA ('Ecstasy') users. *Neurology* 51(6): 1532–1537. Available from: <http://www.neurology.org/content/51/6/1532.short> (accessed 29 November 2013).

Bond A and Lader M (1974) The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology* 47(3): 211–218.

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(1): 69–76.

Bosker WM, Kuypers KPC, Conen S, et al. (2012) MDMA (ecstasy) effects on actual driving performance before and after sleep deprivation, as function of dose and concentration in blood and oral fluid. *Psychopharmacology* 222(3): 367–76. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3395348&tool=pmcentrez&rendertype=abstract> (accessed 4 June 2013).

Bossong MG, van Berckel BNM, Boellaard R, et al. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology* :

References

- official publication of the American College of Neuropsychopharmacology* 34(3): 759–66. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18754005> (accessed 31 July 2014).
- British Medical Association (2009) *Under the influence: the damaging effect of alcohol marketing on young people*. London: BMA.
- Bromberg-Martin ES, Matsumoto M and Hikosaka O (2010) Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron*, Elsevier Inc. 68(5): 815–834. Available from: <http://dx.doi.org/10.1016/j.neuron.2010.11.022>.
- Brosh T, Sander D, Pourtois G, et al. (2008) Beyond Fear: Rapid Spatial Orienting Toward Positive Emotional Stimuli. *Psychological Science* 19(4): 362–370.
- Brown GL, McGarvey EL, Shirtcliff E a., et al. (2008) Salivary cortisol, dehydroepiandrosterone, and testosterone interrelationships in healthy young males: A pilot study with implications for studies of aggressive behavior. *Psychiatry Research* 159(1–2): 67–76.
- Brown TT and Dobs AS (2002) Endocrine effects of marijuana. *Journal of clinical pharmacology* 42(11 Suppl): 90S–96S.
- Brunt TM, Poortman A, Niesink RJM, et al. (2011) Instability of the ecstasy market and a new kid on the block: mephedrone. *Journal of psychopharmacology (Oxford, England)* 25(11): 1543–7. Available from: <http://jop.sagepub.com/cgi/doi/10.1177/0269881110378370> \n<http://www.ncbi.nlm.nih.gov/pubmed/20826554>.
- Bushman BJ and Cooper HM (1990) Effects of alcohol on human aggression: an integrative research review. *Psychological bulletin* 107(3): 341–354.
- Camarasa J, Marimón JM, Rodrigo T, et al. (2008) Memantine prevents the cognitive impairment induced by 3,4-methylenedioxymethamphetamine in rats. *European journal of pharmacology* 589(1–3): 132–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18582864> (accessed 27 May 2013).
- Carhart-Harris RL, King LA and Nutt DJ (2011) A web-based survey on mephedrone. *Drug and Alcohol Dependence*, Elsevier Ireland Ltd 118(1): 19–22.
- Cassisi JE, Delehant M, Tsoutsouris JS, et al. (1998) Psychophysiological reactivity to alcohol advertising in light and moderate social drinkers. *Addictive Behaviors* 23(2): 267–274.
- Cherek D, Roache J, Egli M, et al. (1993) Acute effects of marijuana smoking on aggressive, escape and point-maintained responding of male drug users. *Psychopharmacology* 111(2): 163–168.

- Cherek DR (1981) Effects of smoking different doses of nicotine on human aggressive behavior. *Psychopharmacology* 75: 339–345.
- Chermack ST and Blow FC (2002) Violence among individuals in substance abuse treatment: The role of alcohol and cocaine consumption. *Drug and Alcohol Dependence* 66(1): 29–37.
- Chipana C, Camarasa J, Pubill D, et al. (2006) Protection against MDMA-induced dopaminergic neurotoxicity in mice by methyllycaconitine: involvement of nicotinic receptors. *Neuropharmacology* 51(4): 885–95.
- Chipana C, Camarasa J, Pubill D, et al. (2008) Memantine prevents MDMA-induced neurotoxicity. *Neurotoxicology* 29(1): 179–83. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17980434> (accessed 24 November 2013).
- Chipana C, Torres I, Camarasa J, et al. (2008) Memantine protects against amphetamine derivatives-induced neurotoxic damage in rodents. *Neuropharmacology* 54(8): 1254–63.
- Cleare AJ and Bond AJ (1995) The Effect of Tryptophan Depletion and Enhancement on Subjective and Behavioral Aggression in Normal-Male Subjects. *Psychopharmacology* 118(1): 72–81.
- Cole JC and Sumnall HR (2003) The pre-clinical behavioural pharmacology of 3,4-methylenedioxyamphetamine (MDMA). *Neuroscience & Biobehavioral Reviews* 27(3): 199–217.
- Cone EJ, Johnson RE, Moore JD, et al. (1986) Acute effects of smoking marijuana on hormones, subjective effects and performance in male human subjects. *Pharmacology Biochemistry and Behavior* 24(6): 1749–1754.
- Cools R and D'Esposito M (2011) Inverted-U shaped dopamine actions on human working memory and cognitive control. *Biological psychiatry* 69(12): 113–125.
- Cools R, Nakamura K and Daw ND (2011) Serotonin and dopamine: unifying affective, motivational, and decision functions. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, Nature Publishing Group 36(1): 98–113.
- 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. *International Journal of Drug Policy*, Elsevier B.V. 24(1): 82–83.
- Cornelius JR, Aizenstein HJ and Hariri AR (2010) Amygdala reactivity is inversely related to level of cannabis use in individuals with comorbid cannabis dependence and major depression. *Addictive Behaviors*, Elsevier Ltd 35(6): 644–646.

References

- Cousijn J, Goudriaan AE, Ridderinkhof KR, et al. (2013) Neural responses associated with cue-reactivity in frequent cannabis users. *Addiction biology* 18(3): 570–80.
- Crane NA, Schuster RM, Fusar-Poli P, et al. (2013) Effects of cannabis on neurocognitive functioning: Recent advances, neurodevelopmental influences, and sex differences. *Neuropsychology Review* 23(2): 117–137.
- Crippa JA, Zuardi AW, Garrido GEJ, et al. (2004) Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 29(2): 417–426.
- Crippa JA, Zuardi AW, Martín-Santos R, et al. (2009) Cannabis and anxiety: a critical review of the evidence. *Hum. Psychopharmacol Clin Exp* 24: 515–523.
- Crippa JA, Derenusson GN, Ferrari TB, et al. (2011) Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *Journal of Psychopharmacology* 25(1): 121–130.
- Crunelle CL, Kaag AM, Van den Munkhof HE, et al. (2015) Dysfunctional amygdala activation and connectivity with the prefrontal cortex in current cocaine users. *Human Brain Mapping* 36(10): 4222–4230.
- Cummings KM, Morley CP, Horan JK, et al. (2002) Marketing to America's youth: evidence from corporate documents. *Tobacco Control* 11(suppl 1): i5--i17.
- Curran HV and Travill RA (1997) Mood and cognitive effects of +/-3,4-methylenedioxyamphetamine (MDMA, 'ecstasy'): week-end 'high' followed by mid-week low. *Addiction* 92(7): 821–831.
- Dal Cin S, Worth KA, Dalton MA, et al. (2008) Youth exposure to alcohol use and brand appearances in popular contemporary movies. *Addiction* 103(12): 1925–1932.
- Danysz W, Parsons CG, Mobius H, et al. (2000) Neuroprotective and Symptomatological Action of Memantine Relevant for Alzheimer ' s Disease - A Unified Glutamatergic Hypothesis on the Mechanism of Action. *Neurotoxicity Research* 2: 85–97.
- de Sousa Fernandes Perna EB, Theunissen EL, Kuypers KPC, et al. (2014) Memory and mood during MDMA intoxication, with and without memantine pretreatment. *Neuropharmacology*, Elsevier Ltd 87: 198–205. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0028390814000938>.
- de Wit H, Enggasser JL and Richards JB (2002) Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 27(5): 813–25. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12431855>.

- Debono M, Ghobadi C, Rostami-Hodjegan A, et al. (2009) Modified-release hydrocortisone to provide circadian cortisol profiles. *Journal of Clinical Endocrinology and Metabolism* 94(5): 1548–1554.
- Deluca P, Davey Z, Corazza O, et al. (2012) Identifying emerging trends in recreational drug use; outcomes from the Psychonaut Web Mapping Project. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, Elsevier Inc. 39(2): 221–226.
- Di Chiara G and Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America* 85(July): 5274–5278.
- 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). *Clinical toxicology (Philadelphia, Pa.)* 53(9): 893–900.
- Doty P and de Wit H (1995) Effect of setting on the reinforcing and subjective effects of ethanol in social drinkers. *Psychopharmacology* 118(1): 19–27.
- Drug Enforcement Administration (2014) *National Forensic Laboratory Information System Special Report: Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2010–2013*. Springfield, VA: U.S. Drug Enforcement Administration.
- Duke AA, Giancola PR, Morris DH, et al. (2011) Alcohol dose and aggression: another reason why drinking more is a bad idea. *Journal of studies on alcohol and drugs* 72(1): 34–43.
- Dumont GJH and Verkes RJ (2006) A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. *Journal of psychopharmacology (Oxford, England)* 20(2): 176–87.
- Dumont GJH, Wezenberg E, Valkenberg MMGJ, et al. (2008) Acute neuropsychological effects of MDMA and ethanol (co-)administration in healthy volunteers. *Psychopharmacology* 197(3): 465–74.
- EMCDDA (2012) *Annual report 2012: the state of the drugs problem in Europe*.
- EMCDDA (2015a) *European Drug Report 2015. European Monitoring of Drugs and Drugs Addiction*.
- EMCDDA (2015b) *New psychoactive substances in Europe: Innovative legal responses. Publications Office of the European Union, Luxembourg*.
- Ersche KD, Barnes A, Simon Jones P, et al. (2011) Abnormal structure of frontostriatal

References

- brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. *Brain* 134(7): 2013–2024.
- Ersche KD, Hagan CC, Smith DG, et al. (2015) In the face of threat: neural and endocrine correlates of impaired facial emotion recognition in cocaine dependence. *Translational psychiatry*, Nature Publishing Group 5(5): e570.
- Everitt BJ and Robbins TW (2013) From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. *Neuroscience and Biobehavioral Reviews*, Elsevier Ltd 37(9): 1946–1954.
- Everitt BJ, Belin D, Economidou D, et al. (2008) Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 363(1507): 3125–35.
- Evers EA, Stiers P and Ramaekers JG (n.d.) High reward expectancy during methylphenidate depresses the dopaminergic response to gain and loss.
- Fals-Stewart W (2003) The occurrence of partner physical aggression on days of alcohol consumption: a longitudinal diary study. *Journal of consulting and clinical psychology* 71(1): 41.
- Fernández-Serrano MJ, Lozano Ó, Pérez-García M, et al. (2010) Impact of severity of drug use on discrete emotions recognition in polysubstance abusers. *Drug and Alcohol Dependence* 109(1–3): 57–64.
- Field M and Cox WM (2008) Attentional bias in addictive behaviors: a review of its development, causes, and consequences. *Drug and alcohol dependence* 97(1–2): 1–20. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18479844> (accessed 11 July 2014).
- Field M, Wiers RW, Christiansen P, et al. (2010) Europe PMC Funders Group Acute Alcohol Effects on Inhibitory Control and Implicit Cognition : Implications for Loss of Control Over Drinking. *Alcohol Clin Exp Res* 34(8): 1346–1352.
- Filbey FM, Schacht JP, Myers US, et al. (2009) Marijuana craving in the brain. *Proceedings of the National Academy of Sciences of the United States of America* 106(31): 13016–21.
- Fitzgerald DA, Angstadt M, Jelsone LM, et al. (2006) Beyond threat: Amygdala reactivity across multiple expressions of facial affect. *NeuroImage* 30(4): 1441–1448.
- Franken IH a, Booij J and van den Brink W (2005) The role of dopamine in human addiction: from reward to motivated attention. *European journal of pharmacology* 526(1–3): 199–206.

- Frankiewicz T and Parsons CG (1999) Memantine restores long term potentiation impaired by tonic N-methyl-d-aspartate (NMDA) receptor activation following reduction of Mg²⁺ In hippocampal slices. *Neuropharmacology* 38(9): 1253–1259.
- 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(4): 792–800. Available from: <http://doi.wiley.com/10.1111/j.1360-0443.2011.03719.x>.
- Frias J, Torres JM, Miranda MT, et al. (2002) Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, beta-endorphin and prolactin in human adults of both sexes. *Alcohol and alcoholism (Oxford, Oxfordshire)* 37(2): 169–173.
- Frith C (2009) Role of facial expressions in social interactions. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364(1535): 3453–3458.
- Fusar-Poli P, Crippa JA, Bhattacharyya S, et al. (2009) Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry* 66(1): 95–105.
- Garcia-Ratés S, Camarasa J, Escubedo E, et al. (2007) Methamphetamine and 3,4-methylenedioxyamphetamine interact with central nicotinic receptors and induce their up-regulation. *Toxicology and applied pharmacology* 223(3): 195–205. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17614110> (accessed 29 November 2013).
- Gauthier I, Tarr MJ, Moylan J, et al. (2000) The Fusiform 'Face Area' is Part of a Network that Processes Faces at the Individual Level. *Journal of Cognitive Neuroscience* 12(3): 495–504.
- Giancola PR (2000) Executive functioning: a conceptual framework for alcohol-related aggression. *Experimental and clinical psychopharmacology* 8(4): 576–597.
- Giancola PR and Chermack ST (1998) Construct validity of laboratory aggression paradigms: A response to Tedeschi and Quigley (1996). *Aggression and Violent Behavior* 3(3): 237–253.
- Giancola PR, Godlaski AJ and Roth RM (2012) Identifying component-processes of executive functioning that serve as risk factors for the alcohol-aggression relation. *Psychology of Addictive Behaviors* 26(2): 201–211.
- Gilman JM, Ramchandani V a, Davis MB, et al. (2008) Why we like to drink: a functional magnetic resonance imaging study of the rewarding and anxiolytic effects of alcohol. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28(18): 4583–91.

References

- Gläscher J (2009) Visualization of group inference data in functional neuroimaging. *Neuroinformatics* 7(1): 73–82.
- Goldstein RZ, Alia-Klein N, Tomasi D, et al. (2009) Anterior cingulate cortex hypoactivations to an emotionally salient task in cocaine addiction. *Proceedings of the National Academy of Sciences of the United States of America* 106(23): 9453–8.
- Gonzalez R (2007) Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. *Neuropsychology Review* 17(3): 347–361.
- Goudriaan AE, de Ruiter MB, van den Brink W, et al. (2010) Brain activation patterns associated with cue reactivity and craving in abstinent problem gamblers, heavy smokers and healthy controls: an fMRI study. *Addiction biology* 15(4): 491–503.
- Gouzoulis-Mayfrank E and Daumann J (2009) Neurotoxicity of drugs of abuse—the case of methylenedioxyamphetamines (MDMA, ecstasy), and amphetamines. *Dialogues in Clinical Neuroscience* 11: 305–317.
- Gowin JL, Swann AC, Moeller FG, et al. (2010) Zolmitriptan and human aggression: Interaction with alcohol. *Psychopharmacology* 210(4): 521–531.
- Green AR and Nutt DJ (2014) Pharmacology should be at the centre of all preclinical and clinical studies on new psychoactive substances (recreational drugs). *Journal of Psychopharmacology* 28(8): 711–718.
- Green AR, Mehan AO, Elliott JM, et al. (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). *Pharmacological reviews* 55(3): 463–508.
- Green AR, King M V, Shortall SE, et al. (2014) The preclinical pharmacology of mephedrone ; not just MDMA by another name. *British Journal of Pharmacology* 171: 2251–2268.
- Greenwald AG, Nosek B a. and Banaji MR (2003) Understanding and using the Implicit Association Test: I. An improved scoring algorithm. *Journal of Personality and Social Psychology* 85(2): 197–216.
- Gruber S a., Rogowska J and Yurgelun-Todd D a. (2009) Altered affective response in marijuana smokers: An FMRI study. *Drug and Alcohol Dependence* 105(1–2): 139–153.
- Guitart-Masip M, Beierholm UR, Dolan R, et al. (2011) Vigor in the face of fluctuating rates of reward: an experimental examination. *Journal of cognitive neuroscience* 23(12): 3933–3938.
- Hamida S Ben, Tracqui A, de Vasconcelos AP, et al. (2009) Ethanol increases the

distribution of MDMA to the rat brain: possible implications in the ethanol-induced potentiation of the psychostimulant effects of MDMA. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)* 12(6): 749–759.

- Han DD and Gu HH (2006) Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC pharmacology* 6: 6.
- Hariri AR, Mattay VS, Tessitore A, et al. (2002) Dextroamphetamine modulates the response of the human amygdala. *Neuropsychopharmacology* 27(0893–133X (Print)): 1036–1040.
- Harms MB, Martin A and Wallace GL (2010) Facial emotion recognition in autism spectrum disorders: A review of behavioral and neuroimaging studies. *Neuropsychology Review* 20(3): 290–322.
- Haxby J V., Hoffman EA and Gobbini MI (2000) The distributed human neural system for face perception. *Trends in Cognitive Sciences* 4(6): 223–233.
- Heinz A, Braus DF, Smolka MN, et al. (2005) Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature neuroscience* 8(1): 20–1. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15592465> (accessed 8 August 2014).
- Heinz a, Mann K, Weinberger DR, et al. (2001) Serotonergic dysfunction, negative mood states, and response to alcohol. *Alcoholism, clinical and experimental research* 25(4): 487–495.
- Heinz AJ, Beck A, Meyer-Lindenberg A, et al. (2011) Cognitive and neurobiological mechanisms of alcohol-related aggression. *Nature reviews. Neuroscience*, Nature Publishing Group 12(7): 400–413.
- Hindocha C, Freeman TP, Schafer G, et al. (2015) Acute effects of delta-9-tetrahydrocannabinol, cannabidiol and their combination on facial emotion recognition: A randomised, double-blind, placebo-controlled study in cannabis users. *European Neuropsychopharmacology*, Elsevier 25(3): 325–334.
- Hoaken PNS and Stewart SH (2003) Drugs of abuse and the elicitation of human aggressive behavior. *Addictive Behaviors* 28(9): 1533–1554.
- Hoaken PNS, Campbell T, Stewart SH, et al. (2003) Effects of Alcohol on Cardiovascular Reactivity and the Mediation of Aggressive Behaviour in Adult Men and Women. *Online* 38(1): 84–92.
- Hogarth L and Duka T (2006) Human nicotine conditioning requires explicit contingency knowledge: Is addictive behaviour cognitively mediated? *Psychopharmacology*

References

184(3–4): 553–566.

Home Office (2014) Drug Misuse: Findings from the 2013/14 Crime Survey for England and Wales. <https://www.gov.uk/government/publications/drug-misuse-findings-from-the-2013-to-2014-csew>.

Home Office Statistical Bulletin (2011) Drug Misuse Declared: Findings from the 2010/11 British Crime Survey. <http://www.homeoffice.gov.uk/publications/scienceresearch-statistics/research-statistics/crime-research/hosb1211/hosb1211?view=Binary>.

Howard RC and Menkes DB (2007) Changes in brain function during acute cannabis intoxication: preliminary findings suggest a mechanism for cannabis-induced violence. *Criminal behaviour and mental health* 17: 113–117.

Ito T, Miller N and Pollock V (1996) Alcohol and aggression: a meta-analysis on the moderating effects of inhibitory cues, triggering events, and self-focused attention. *Psychological bulletin* 120(1): 60–82.

Janes AC, Pizzagalli D a, Richardt S, et al. (2010) Neural substrates of attentional bias for smoking-related cues: an fMRI study. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, Nature Publishing Group 35(12): 2339–45.

Jernigan DH (2012) Who is minding the virtual alcohol store? *Archives of pediatrics & adolescent medicine* 166(9): 866–867.

Jex HR, McDonnell JD and Phatak A V. (1966) A 'critical' tracking task for man-machine research related to the operator's effective delay time. I. Theory and experiments with a first-order divergent controlled element. NASA CR-616. *NASA Contract Rep NASA CR.*: 1–105.

Jongen S, Vuurman E, Ramaekers J, et al. (2014) Alcohol calibration of tests measuring skills related to car driving. *Psychopharmacology* 231(12): 2435–2447. Available from: <http://link.springer.com/10.1007/s00213-013-3408-y>.

Kalechstein AD, De La Garza R, Mahoney JJ, et al. (2007) MDMA use and neurocognition: a meta-analytic review. *Psychopharmacology* 189(4): 531–7.

Karpinski A and Steinman RB (2006) The single category implicit association test as a measure of implicit social cognition. *Journal of personality and social psychology* 91(1): 16–32.

Kelly TH, Foltin RW, Mayr MT, et al. (1994) Effects of Δ^9 -tetrahydrocannabinol and social context on marijuana self-administration by humans. *Pharmacology, Biochemistry and Behavior* 49(3): 763–768.

- Kemmis L, Hall JK, Kingston R, et al. (2007) Impaired fear recognition in regular recreational cocaine users. *Psychopharmacology* 194(2): 151–159.
- 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. *Behavior research methods, instruments, & computers : a journal of the Psychonomic Society, Inc* 31(3): 423–8.
- Kessler RC, Amminger GP, Aguilar-Gaxiola S, et al. (2007) Age of onset of mental disorders: A review of recent literature. *Current Opinion in Psychiatry* 20(4): 359–364.
- King A, Munisamy G, De Wit H, et al. (2006) Attenuated cortisol response to alcohol in heavy social drinkers. *International Journal of Psychophysiology* 59(3): 203–209.
- Kirkpatrick MG and De Wit H (2013) In the company of others: Social factors alter acute alcohol effects. *Psychopharmacology* 230(2): 215–226.
- Klaassen T, Riedel WJ, Deutz NEP, et al. (2002) Mood congruent memory bias induced by tryptophan depletion. *Psychological Medicine* 32(1).
- Kolodny R, Lessin P, Toro G, et al. (1976) Depression of testosterone with acute administration, in: Braude, MC, Szara, S (Eds.). In: Braude MC and Szara S (eds), *Pharmacology of Marijuana*, New York: Raven Press, pp. 217–225.
- Kolodny RC, Masters WH, Robert M. Kolodner RM, et al. (1974) Depression of Plasma Testosterone Levels after Chronic Intensive Marihuana Use. *N Engl J Med* 290: 872–874.
- Koob GF and Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, Nature Publishing Group 35(1): 217–38.
- Kopak a. M, Vartanian L, Hoffmann NG, et al. (2014) The Connections Between Substance Dependence, Offense Type, and Offense Severity. *Journal of Drug Issues* 44(3): 291–307.
- Kouri EM, Pope HG and Lukas SE (1999) Changes in aggressive behavior during withdrawal from long-term marijuana use. *Psychopharmacology* 143(3): 302–308.
- Kufahl PR, Li Z, Risinger RC, et al. (2005) Neural responses to acute cocaine administration in the human brain detected by fMRI. *NeuroImage* 28(4): 904–914.
- Kuypers KPC and Ramaekers JG (2005a) Transient memory impairment after acute dose of 75mg 3,4-Methylene-dioxymethamphetamine. *Journal of psychopharmacology (Oxford, England)* 19(6): 633–9.

References

- Kuypers KPC and Ramaekers JG (2005b) Transient memory impairment after acute dose of 75mg 3,4-Methylene-dioxymethamphetamine. *Journal of psychopharmacology (Oxford, England)* 19(6): 633–9.
- 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(4): 557–63.
- Kuypers KPC, Samyn N and Ramaekers JG (2006a) MDMA and alcohol effects, combined and alone, on objective and subjective measures of actual driving performance and psychomotor function. *Psychopharmacology* 187(4): 467–75.
- Kuypers KPC, Samyn N and Ramaekers JG (2006b) MDMA and alcohol effects, combined and alone, on objective and subjective measures of actual driving performance and psychomotor function. *Psychopharmacology* 187(4): 467–75.
- Kuypers KPC, Wingen M, Samyn N, et al. (2007) Acute effects of nocturnal doses of MDMA on measures of impulsivity and psychomotor performance throughout the night. *Psychopharmacology* 192(1): 111–9.
- Kuypers KPC, Wingen M, Heinecke A, et al. (2011) MDMA intoxication and verbal memory performance: a placebo-controlled pharmaco-MRI study. *Journal of psychopharmacology (Oxford, England)* 25(8): 1053–61.
- Kuypers KPC, de la Torre R, Farre M, et al. (2013a) Inhibition of MDMA-induced increase in cortisol does not prevent acute impairment of verbal memory. *British journal of pharmacology* 168(3): 607–17.
- Kuypers KPC, de la Torre R, Farre M, et al. (2013b) Inhibition of MDMA-induced increase in cortisol does not prevent acute impairment of verbal memory. *British journal of pharmacology* 168(3): 607–17.
- Kuypers KPC, Steenbergen L, Theunissen EL, et al. (2015) Emotion recognition during cocaine intoxication. *European Neuropsychopharmacology, Elsevier* 25(11): 1914–1921.
- Lamers CTJ, Ramaekers JG, Muntjewerff ND, et al. (2003) Dissociable Effects of a Single Dose of Ecstasy (MDMA) on Psychomotor Skills and Attentional Performance. *Journal of Psychopharmacology* 17(4): 379–387.
- Lammers SMM, Soe-Agnie SE, De Haan HA, et al. (2014) Middelengebruik en criminaliteit : een overzicht. *TIJDSCHRIFT VOOR PSYCHIATRIE* 56(1): 32–39.
- Lenhart A, Madden M, Smith A, et al. (2011) Teens, kindness and cruelty on social network sites: How American teens navigate the new world of ‘digital citizenship’. *PewResearchCenter*: 1–86.

- Ling PM and Glantz SA (2002) Why and how the tobacco industry sells cigarettes to young adults: Evidence from industry documents. *American Journal of Public Health* 92(6): 908–916.
- Liu J, Harris A and Kanwisher N (2009) Perception of face parts and face configurations: an fMRI study. *Journal of cognitive neuroscience* 22(1): 203–211.
- López-Arnau R, Martínez-Clemente J, Pubill D, et al. (2012) Neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylene. *British Journal of Medical Psychology* (167): 407–420.
- Lovallo WR, Dickensheets SL, Myers D a, et al. (2000) Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcoholism, clinical and experimental research* 24(5): 651–658.
- Lovato C, Linn G, Stead LF, et al. (2003a) Impact of tobacco advertising and promotion on increasing adolescent smoking behaviours. *Cochrane database of systematic reviews (Online)* (4): CD003439.
- Lovato C, Linn G, Stead LF, et al. (2003b) Impact of tobacco advertising and promotion on increasing adolescent smoking behaviours (Review). *Cochrane Database of Systematic Reviews* (3).
- Lubman DI, Cheetham A and Yücel M (2015) Cannabis and adolescent brain development. *Pharmacology and Therapeutics*, Elsevier Inc. 148: 1–16.
- Lüscher C and Ungless MA (2006) The mechanistic classification of addictive drugs. *PLoS Medicine* 3(11): 2005–2010.
- Maldjian J a, Laurienti PJ and Burdette JH (2004) Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *NeuroImage* 21(1): 450–455.
- Maldjian J a., Laurienti PJ, Kraft R a., et al. (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage* 19(3): 1233–1239.
- Martin L (2014) Effects of plain packaging on decision-making and reward for nicotine. *Neuroscience and Neuroeconomics* 3: 63–73.
- Martin SE, Snyder LB, Hamilton M, et al. (2002) Alcohol advertising and youth. *Alcoholism, clinical and experimental research* 26(6): 900–906.
- Martín-Santos R, Fagundo a B, Crippa J a, et al. (2010) Neuroimaging in cannabis use: a systematic review of the literature. *Psychological medicine* 40(3): 383–98.
- Mayer AR, Franco AR, Ling J, et al. (2007) Assessment and quantification of head motion in

References

- neuropsychiatric functional imaging research as applied to schizophrenia. *Journal of the International Neuropsychological Society* : *JINS* 13(5): 839–45.
- McClernon FJ, Hiott FB, Huettel S a, et al. (2005) Abstinence-induced changes in self-report craving correlate with event-related fMRI responses to smoking cues. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 30(10): 1940–7.
- McClure AC, Tanski SE, Li Zhigang, et al. (2016) Internet Alcohol Marketing and Underage Alcohol Use. *Pediatrics* 137(2): peds.2015-2149.
- McClure SM, Li J, Tomlin D, et al. (2004) Neural correlates of behavioral preference for culturally familiar drinks. *Neuron* 44(2): 379–87.
- Mendelson J, Kuehne J, Ellingboe J, et al. (1974) Plasma testosterone levels before during and after chronic marijuana smoking. *NEngl J Med* 291(20): 1051–1055.
- Mendelson JH, Mello NK and Ellingboe J (1977) Effects of acute alcohol intake on pituitary-gonadal hormones in normal human males. *The Journal of pharmacology and experimental therapeutics* 202(3): 676–682.
- Meneses A (2013) 5-HT systems : Emergent targets for memory formation and memory alterations. *Reviews in the neurosciences* 24(6): 629–664.
- Miczek KA (1978) delta9-tetrahydrocannabinol: antiaggressive effects in mice, rats, and squirrel monkeys. *Science* 199(4336): 1459–1461.
- Miczek KA and Barry H (1977) Comparison of the effects of alcohol, chlordiazepoxide, and delta9-tetrahydrocannabinol on intraspeciesaggression in rats. *Advances in Experimental Medicine and Biology* 85B: 251–264.
- Miller MA, Bershad AK and de Wit H (2015) Drug effects on responses to emotional facial expressions: Recent findings. *Behavioural Pharmacology* 26(6): 571–579.
- Moeller FG, Barratt ES, Ph D, et al. (2001) Reviews and Overviews Psychiatric Aspects of Impulsivity. *Psychiatry Interpersonal and Biological Processes* 158(November): 1783–1793.
- Mohamed WMY, Hamida S Ben, Cassel JC, et al. (2011) MDMA: Interactions with other psychoactive drugs. *Pharmacology Biochemistry and Behavior*, Elsevier B.V. 99(4): 759–774.
- Moore K, Dargan PI, Wood DM, et al. (2013) Do novel psychoactive substances displace established club drugs, supplement them or act as drugs of initiation? The relationship between mephedrone, ecstasy and cocaine. *European Addiction Research* 19(5): 276–282.

- Moore TM and Stuart GL (2005) A review of the literature on marijuana and interpersonal violence. *Aggression and Violent Behavior* 10(2): 171–192.
- Mordenti, J., Chappell W (1989) *Toxicokinetics in New Drug Development*. Pergamon, New York.
- Moreno MA and Whitehill JM (2014) Influence of Social Media on Alcohol Use in Adolescents and Young Adults. *Alcohol Research* 36(1): 91.
- Morgan CJA and Curran HV (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry* 192(4): 306–307.
- Morgan MJ (1999) Memory deficits associated with recreational use of 'ecstasy' (MDMA). *Psychopharmacology* 141(1): 30–6.
- Morgan MJ (2000) Ecstasy (MDMA): a review of its possible persistent psychological effects. *Psychopharmacology* 152(3): 230–248.
- Morgan MJ and Marshall JP (2013) Deficient fear recognition in regular cocaine users is not attributable to elevated impulsivity or conduct disorder prior to cocaine use. *Journal of psychopharmacology (Oxford, England)* 27(6): 526–532.
- Moskowitz H (1973) Laboratory studies of the effects of alcohol on some variables related to driving. *Journal of Safety Research* 5(3): 185–199.
- Moss HB and Tarter RE (1993) Substance Abuse, Aggression, and Violence. *The American Journal on Addictions* 2(2): 149–160.
- Mulder-Hajonides van der Meulen WREH, Weinberg JR, Hollanders JJ, et al. (1980) Measurements of subjective sleep quality. In: Elsevier (ed.), *Fifth European Congress on Sleep Research, Amsterdam*.
- Murdoch D, Pihl RO and Ross D (1990) Alcohol and crimes of violence: Present issues. *International Journal of the Addictions* 25: 1065–1081.
- Myerscough R and Taylor S (1985) The Effects of Marijuana on Human Physical Aggression. *Journal of personality and social psychology* 49(6): 1541–1546.
- Myrick H, Anton RF, Li X, et al. (2004) Differential brain activity in alcoholics and social drinkers to alcohol cues: relationship to craving. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 29(2): 393–402.
- Nestler EJ (2005) Is there a common molecular pathway for addiction? *Nature neuroscience* 8(11): 1445–9.
- Nichols DF, Betts LR and Wilson HR (2010) Decoding of faces and face components in face-

References

- sensitive human visual cortex. *Frontiers in Psychology* 1(JUL): 1–13.
- Niesink RJM and van Laar MW (2013) Does Cannabidiol Protect Against Adverse Psychological Effects of THC? *Frontiers in psychiatry* 4(October): 130.
- Niv Y (2007) Cost, benefit, tonic, phasic: What do response rates tell us about dopamine and motivation? *Annals of the New York Academy of Sciences* 1104: 357–376.
- Nutt DJ, King LA and Nichols DE (2013) Treatment Innovation. 14(August): 577–585.
- 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* (January): 1–10. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27206266>.
- Patel KT, Stevens MC, Meda SA, et al. (2013) Robust changes in reward circuitry during reward loss in current and former cocaine users during performance of a monetary incentive delay task. *Biological Psychiatry*, Elsevier 74(7): 529–537.
- Peterson J, Rothfleisch J, Zelazo P, et al. (1990) Acute alcohol intoxication and cognitive functioning. *Journal of Studies on Alcohol and Drugs* 51(2): 114–22.
- Phan KL, Wager T, Taylor SF, et al. (2002) Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *NeuroImage* 16(2): 331–48.
- Phan KL, Angstadt M, Golden J, et al. (2009) Cannabinoid Modulation of Amygdala Reactivity to Social Signals of Threat in Humans. 28(10): 2313–2319.
- Philip RCM, Whalley HC, Stanfield a C, et al. (2010) Deficits in facial, body movement and vocal emotional processing in autism spectrum disorders. *Psychological medicine* 40(11): 1919–1929.
- Pihl RO, Peterson JB and Lau MA (1993) A biosocial model of the alcohol-aggression relationship. *Journal of Studies on Alcohol* 11: 128–139.
- Pitcher D, Walsh V, Yovel G, et al. (2007) TMS Evidence for the Involvement of the Right Occipital Face Area in Early Face Processing. *Current Biology* 17(18): 1568–1573.
- Pitcher D, Walsh V and Duchaine B (2011) The role of the occipital face area in the cortical face perception network. *Experimental Brain Research* 209(4): 481–493.

- Pizarro N, Ortuno J, Farre M, et al. (2002) Determination of MDMA and its Metabolites in Blood and Urine by Gas Chromatography-Mass Spectrometry and Analysis of Enantiomers by Capillary Electrophoresis. *Journal of Analytical Toxicology* 26(3): 157–165.
- Popma A, Vermeiren R, Geluk C a ML, et al. (2007) Cortisol Moderates the Relationship between Testosterone and Aggression in Delinquent Male Adolescents. *Biological Psychiatry* 61(3): 405–411.
- Power JD, Barnes K a, Snyder AZ, et al. (2012) Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *NeuroImage*, Elsevier Inc. 59(3): 2142–54.
- Preller KH, Herdener M, Schilbach L, et al. (2014) Functional changes of the reward system underlie blunted response to social gaze in cocaine users. *Proceedings of the National Academy of Sciences of the United States of America* 111(7): 2842–7.
- Preller KH, Hulka LM, Vonmoos M, et al. (2014) Impaired emotional empathy and related social network deficits in cocaine users. *Addiction Biology* 19(3): 452–466.
- Psychonaut WebMapping Research Group (2009) *Mephedrone Report*. Institute of Psychiatry, King's College London: London UK.
- Puce A, Allison T, Asgari M, et al. (1996) Differential sensitivity of human visual cortex to faces, letterstrings, and textures: a functional magnetic resonance imaging study. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16(16): 5205–15.
- Quickfall J and Crockford D (2006) Brain neuroimaging in cannabis use: a review. *The Journal of neuropsychiatry and clinical neurosciences* 18(3): 318–332.
- Ramaekers JG, Kuypers KPC and Samyn N (2006) Stimulant effects of 3,4-methylenedioxymethamphetamine (MDMA) 75 mg and methylphenidate 20 mg on actual driving during intoxication and withdrawal. *Addiction (Abingdon, England)* 101(11): 1614–21.
- Ramaekers Johannes G, 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 : official publication of the American College of Neuropsychopharmacology*, Nature Publishing Group 34(7): 1641–8.
- Ramaekers J G, Kauert G, Theunissen EL, et al. (2009) Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of psychopharmacology (Oxford, England)* 23(3): 266–77.

References

- 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.
- Ramaekers JG, Kuypers KPC, Bosker WM, et al. (2012) Effects of stimulant drugs on actual and simulated driving: perspectives from four experimental studies conducted as part of the DRUID research consortium. *Psychopharmacology* 222(3): 413–8.
- Rammes G, Danysz W and Parsons CG (2008) Pharmacodynamics of Memantine: An Update. *Current Neuropharmacology*, Bentham Science Publishers Ltd. 6(1): 55–78.
- Reisberg B, Doody R, Stöffler A, et al. (2003) *Memantine in moderate-to-severe Alzheimer's disease. The New England journal of medicine.*
- Rey A (1964) *L'examen clinique en psychologie*. Paris: Presses Universitaires de France.
- Rietjens SJ, Hondebrink L, Westerink RHS, et al. (2012) Pharmacokinetics and pharmacodynamics of 3,4-methylenedioxyamphetamine (MDMA): interindividual differences due to polymorphisms and drug–drug interactions. *Critical Reviews in Toxicology* 42(10): 854–876.
- Sambeth A, Riedel WJ, Tillie DE, et al. (2009) Memory impairments in humans after acute tryptophan depletion using a novel gelatin-based protein drink. *Journal of psychopharmacology (Oxford, England)* 23(1): 56–64.
- Sarkola T and Eriksson CJP (2003) Testosterone increases in men after a low dose of alcohol. *Alcoholism, clinical and experimental research* 27(4): 682–685.
- Sarkola T, Ahola L, von der Pahlen B, et al. (2001) Lack of effect of alcohol on ethinylestradiol in premenopausal women. *Contraception* 63(1): 19–23.
- Sayette MA, Creswell KG, Dimoff JD, et al. (2012) Alcohol and Group Formation: A Multimodal Investigation of the Effects of Alcohol on Emotion and Social Bonding. *Psychological Science* 23(8): 869–878.
- Schaefer C, Gunn C and Dubowski K (1975) Normal plasma testosterone concentrations after marijuana smoking. *N Engl J Med* 292: 867–868.
- Schaefer M (2009) Neuroeconomics: in search of the neural representation of brands. *Progress in brain research* 178: 241–252.
- Schifano F, Albanese A, Fergus S, et al. (2011) Mephedrone (4-methylmethcathinone; 'Meow meow'): Chemical, pharmacological and clinical issues. *Psychopharmacology* 214(3): 593–602.
- Schmidt CJ and Kehne JH (1990) Neurotoxicity of MDMA: neurochemical effects. *Annals of*

the New York Academy of Sciences 600: 665-80-1.

- Scholey AB, Parrott AC, Buchanan T, et al. (2004) Increased intensity of Ecstasy and polydrug usage in the more experienced recreational Ecstasy/MDMA users: a WWW study. *Addictive behaviors* 29(4): 743-52.
- Schultz W (2007) Multiple dopamine functions at different time courses. *Annual review of neuroscience* 30: 259-88.
- 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.
- Shimono K, Baudry M, Panchenko V, et al. (2002) Chronic multichannel recordings from organotypic hippocampal slice cultures: protection from excitotoxic effects of NMDA by non-competitive NMDA antagonists. *Journal of Neuroscience Methods* 120(2): 193-202.
- Simmler L, Buser T, Donzelli M, et al. (2013) Pharmacological characterization of designer cathinones *in vitro*. *British Journal of Pharmacology* 168(2): 458-470.
- Smith LA, Mäkelä P, Relm J, et al. (2010) Alcohol: equity and social determinants. *Equity, social determinants and public health programmes*: 11-30.
- Smith L a and Foxcroft DR (2009) The effect of alcohol advertising, marketing and portrayal on drinking behaviour in young people: systematic review of prospective cohort studies. *BMC public health* 9: 51.
- Smolka MN, Bühler M, Klein S, et al. (2006) Severity of nicotine dependence modulates cue-induced brain activity in regions involved in motor preparation and imagery. *Psychopharmacology* 184(3-4): 577-88.
- Somainsi L, Manfredini M, Amore M, et al. (2012) Psychobiological responses to unpleasant emotions in cannabis users. *European Archives of Psychiatry and Clinical Neuroscience* 262(1): 47-57.
- Spronk DB, van Wel JHP, Ramaekers JG, et al. (2013) Characterizing the cognitive effects of cocaine: A comprehensive review. *Neuroscience and Biobehavioral Reviews*, Elsevier Ltd 37(8): 1838-1859.
- Squeglia LM, Jacobus J and Tapert SF (2009) The Influence of Substance Use on Adolescent Brain Development. *Clin EEG Neurosci* 40(1): 31-38.
- Sternberg S (1966) High-speed scanning in human memory. *Science (New York, N.Y.)* 153(3736): 652-4.

References

- Stoddard SA, Epstein-Ngo Q, Walton MA, et al. (2015) Substance Use and Violence Among Youth: A Daily Calendar Analysis. *Substance Use & Misuse* 50(3): 328–339.
- Sutherland MT, Carroll AJ, Salmeron BJ, et al. (2013) Individual differences in amygdala reactivity following nicotinic receptor stimulation in abstinent smokers. *NeuroImage*, Elsevier B.V. 66: 585–593.
- Tanski SE, McClure AC, Li Zhigang, et al. (2015) Cued Recall of Alcohol Advertising on Television and Underage Drinking Behavior. *JAMA Pediatrics* 169(3): 264.
- Tapert SF, Cheung EH, Brown GG, et al. (2003) Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Archives of general psychiatry* 60(7): 727–35.
- Taylor SP, Vardaris RM, Rawtich a B, et al. (1976) The effects of alcohol and delta-9 tetrahydrocannabinol on human physical aggression. *Aggressive Behavior* 2: 153–161.
- Terburg D, Morgan B and van Honk J (2009) The testosterone-cortisol ratio: A hormonal marker for proneness to social aggression. *International Journal of Law and Psychiatry*, Elsevier Ltd 32(4): 216–223.
- Thayer JF, Hall M, Sollers JJ, et al. (2006) Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *International Journal of Psychophysiology* 59(3): 244–250.
- Tye JB, Warner KE and Glantz S a (1987) Tobacco advertising and consumption: evidence of a causal relationship. *Journal of public health policy* 8(4): 492–508. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3323236>.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* 15(1): 273–89.
- UNODC (2014) *Global Synthetic Drugs Assessment*. Vienna: UNODC, 2014.
- UNODC (2015) *World Drug Report 2015*. United Nations publication.
- Välimäki M, Tuominen JA, Huhtaniemi I, et al. (1990) The pulsatile secretion of gonadotropins and growth hormone, and the biological activity of luteinizing hormone in men acutely intoxicated with ethanol. *Alcohol Clin Exp Res* 14: 928–931.
- van Wel JHP, Kuypers KPC, Theunissen EL, et al. (2011) Blockade of 5-HT2 receptor selectively prevents MDMA-induced verbal memory impairment. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, Nature Publishing Group 36(9): 1932–9.

- van Wel JHP, Kuypers KPC, Theunissen EL, et al. (2012) Effects of acute MDMA intoxication on mood and impulsivity: role of the 5-HT₂ and 5-HT₁ receptors. *PLoS one* 7(7): e40187.
- Van Wel JHP, Kuypers KPC, Theunissen EL, et al. (2013) Single doses of THC and cocaine decrease proficiency of impulse control in heavy cannabis users. *British Journal of Pharmacology* 170(7): 1410–1420.
- Verdejo-Garcia A, Contreras-Rodríguez O, Fonseca F, et al. (2014) Functional alteration in frontolimbic systems relevant to moral judgment in cocaine-dependent subjects. *Addiction Biology* 19(2): 272–281.
- Volkow ND and Morales M (2015) The Brain on Drugs: From Reward to Addiction. *Cell*, Elsevier Inc. 162(4): 712–725.
- Volkow ND, Wang G-J, Telang F, et al. (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26(24): 6583–8.
- Vollstädt-Klein S, Wichert S, Rabinstein J, et al. (2010) Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. *Addiction (Abingdon, England)* 105(10): 1741–9.
- Wanat MJ, Willuhn I, Clark JJ, et al. (2009) Phasic dopamine release in appetitive behaviors and drug abuse. *Curr Drug Abuse Rev* 2(2): 195.
- Wareing M, Fisk JE and Murphy PN (2000) Working memory deficits in current and previous users of MDMA ('ecstasy'). *British Journal of Psychology* 91(2): 181–188. Available from: <http://doi.wiley.com/10.1348/000712600161772> (accessed 29 November 2013).
- Watson PE, Watson ID and Batt RD (1981) Prediction of Blood Alcohol Concentrations in Human Subjects; Updating the Widmark Equation. *Journal of Studies on Alcohol and Drugs* 42(7): 547–556.
- Whalen PJ (2007) The uncertainty of it all. *Trends in Cognitive Sciences* 11(12): 499–500.
- Winstock, Mitcheson LR, Deluca P, et al. (2011) Mephedrone, new kid for the chop? *Addiction (Abingdon, England)* 106(1): 154–61.
- Winstock, Mitcheson L, Ramsey J, et al. (2011) Mephedrone: use, subjective effects and health risks. *Addiction* 106(11): 1991–1996. Available from: <http://doi.wiley.com/10.1111/j.1360-0443.2011.03502.x>.
- Wise RA (2009) Roles for nigrostriatal-not just mesocorticolimbic-dopamine in reward and addiction. *Trends in Neurosciences* 32(10): 517–524.

References

- Wong DF, Kuwabara H, Schretlen DJ, et al. (2006) Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 31(12): 2716–27.
- World Health Organization (2003) *WHO Framework Convention on Tobacco Control. WHO Framework Convention on Tobacco Control.*
- World Health Organization (2004) *Global Status Report on Alcohol 2004.*
- World Health Organization (2014) *Global status report on alcohol and health 2014.*
- Yacubian J and Büchel C (2009) The genetic basis of individual differences in reward processing and the link to addictive behavior and social cognition. *Neuroscience*, Elsevier Inc. 164(1): 55–71.
- Ylikahri R, Huttunen M, Härkönen M, et al. (1974) Low plasma testosterone values in men during hangover. *Journal of Steroid Biochemistry* 5(7): 655–658.
- Yoo S-S, Choi B-G, Juh R, et al. (2005) Head motion analysis during cognitive fMRI examination: application in patients with schizophrenia. *Neuroscience research* 53(1): 84–90.
- Yubero-Lahoz S, Kuypers KPC, Ramaekers JG, et al. (2014) Changes in serotonin transporter (5-HTT) gene expression in peripheral blood cells after MDMA intake. *Psychopharmacology* 232(11): 1921–1929.
- Zakzanis KK, Campbell Z and Jovanovski D (2007) The neuropsychology of ecstasy (MDMA) use: a quantitative review. *Human psychopharmacology* 22(7): 427–35.
- Zarghi A, Shafaati A, Foroutan SM, et al. (2010) Sensitive and rapid HPLC method for determination of memantine in human plasma using OPA derivatization and fluorescence detection: application to pharmacokinetic studies. *Scientia pharmaceutica* 78(4): 847–56.
- Zhao J, Macdonald S, Borges G, et al. (2015) The rate ratio of injury and aggressive incident for alcohol alone, cocaine alone and simultaneous use before the event: A case-crossover study. *Accident Analysis and Prevention* 75: 137–143.
- Zijlstra F, Veltman DJ, Booij J, et al. (2009) Neurobiological substrates of cue-elicited craving and anhedonia in recently abstinent opioid-dependent males. *Drug and alcohol dependence* 99(1–3): 183–92. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18823721> (accessed 11 August 2014).
- Zuardi AW, Cosme RA, Graeff FG, et al. (1993) Effects of ipsapirone and cannabidiol on human experimental anxiety. *Journal of Psychopharmacology* 7(1): 82–88.

Zweifel LS, Parker JG, Lobb CJ, et al. (2009) Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proceedings of the National Academy of Sciences* 106(18): 7281–7288.

Curriculum Vitae

Elizabeth B. de Sousa Fernandes Perna was born on July 26th, 1988 in Oranjestad, Aruba. She graduated from secondary education (VWO) at Colegio Arubano in Aruba in 2006, and continued her education studying psychology at the Faculty of Psychology and Neuroscience (FPN) at Maastricht University. After obtaining a Bachelor's degree in 2009, she started the Master in Neuropsychology. During her research internship she studied the effects of PDE-5 inhibition in THC-induced cognitive impairment under supervision of Prof. dr. Jan Ramaekers and dr. Eef Theunissen at the department of Neuropsychology and Psychopharmacology. After the completion of her Master thesis, she continued as a research assistant on a similar experiment, however dealing with the effects of MDMA on cognitive performance. These experiences further strengthened her curiosity and interest in the field of psychopharmacology and provided her with knowledge of how research is developed and conducted, with its practicalities, bureaucracies and barriers. She has worked as PhD a candidate at the department of Neuropsychology and Psychopharmacology (FPN) from 2012-2016. Starting from July of 2016, she continues to work as postdoctoral researcher at the same department on the Predicting Risk of Emerging Drugs with In silico and Clinical Toxicology (PREDICT) project (www.predictnps.eu), which is aimed at developing a novel testing system to determine toxicity of New Psychoactive Substances (NPS) based on animal and human data.

List of publications

As part of this dissertation:

De Sousa Fernandes Perna, E.B., Theunissen, E.L., Kuypers, K.P.C., Heckman, P., De La Torre, R., Farre, M. and Ramaekers, J.G., 2014. Memory and mood during MDMA intoxication, with and without memantine pretreatment. *Neuropharmacology*, 87, pp.198-205.

De Sousa Fernandes Perna, E.B., Theunissen, E.L., Kuypers, K.P., Evers, E.A., Stiers, P., Toennes, S.W., Witteman, J., Dalen, W. and Ramaekers, J.G., 2016. Brain reactivity to alcohol and cannabis marketing during sobriety and intoxication. *Addiction biology*.

De Sousa Fernandes Perna, E.B., Theunissen, E.L., Kuypers, K.P.C., Toennes, S.W. and Ramaekers, J.G., 2016. Subjective aggression during alcohol and cannabis intoxication before and after aggression exposure. *Psychopharmacology*, 233(18), pp.3331-3340.

De Sousa Fernandes Perna, E.B., Papaseit, E., Pérez-Mañá, C., Mateus, J., Theunissen, E.L., Kuypers, K.P.C., de la Torre, R., Farré, M. and Ramaekers, J.G., 2016. Neurocognitive performance following acute mephedrone administration, with and without alcohol. *Journal of Psychopharmacology*, p.0269881116662635

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Theunissen, E.L., Heckman, P., **de Sousa Fernandes Perna, E.B.**, Kuypers, K.P.C., Sambeth, A., Blokland, A., Prickaerts, J., Toennes, S.W. and Ramaekers, J.G., 2015. Rivastigmine but not vardenafil reverses cannabis-induced impairment of verbal memory in healthy humans. *Psychopharmacology*, 232(2), pp.343-353.

Witteman, J., Post, H., Tarvainen, M., de Bruijn, A., **de Sousa Fernandes Perna, E.B.**, Ramaekers, J.G. and Wiers, R.W., 2015. Cue reactivity and its relation to craving and

relapse in alcohol dependence: a combined laboratory and field study. *Psychopharmacology*, 232(20), pp.3685-3696.

Kuypers, K.P., Theunissen, E.L., van Wel, J.H., **de Sousa Fernandes Perna, E.B.**, Linssen, A., Sambeth, A., Schultz, B.G. and Ramaekers, J.G., 2016. Verbal Memory Impairment in Polydrug Ecstasy Users: A Clinical Perspective. *PloS one*, 11(2), p.e0149438.

Farré, M., Perez-Mañá, C., **de Sousa, E.**, Mateus, J., Theunisen, E., Kuypers, K., Ramaekers, J., Fonseca, F., Torrens, M., Olesti, E. and de la Torre, R., 2016. Interactions between mephedrone and alcohol in humans: Cardiovascular and subjective effects. *European Psychiatry*, 33, p.S115.

