With a little help from my ‘friends’

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The mood- and cognition-enhancing effects of illicit substances

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Chapter 1

General Introduction

High levels of competition, workload, and pressure regarding study and work-related performance are very common in students' and young professionals' lives.[1-3] To achieve and maintain high cognitive performance and counteract possible negative mood changes due to stress or pressure, the use of pharmacological interventions to enhance cognitive performance and mood is not uncommon.[4, 5] Cognition enhancement can be seen as overcoming the natural limitations of cognitive capacity,[6] and can include a wide variety of cognitive processes such as creativity, attention or memory. Mood enhancement is commonly associated with "feeling good" or "being happy" but also affects a broader variety of emotional states,[7] such as increasing elation, arousal, or decreasing negative mood states such as anxiety and depression.

While the recreational use of prescription drugs, such as methylphenidate and amphetamines, as cognition and mood enhancers, is known to be fairly prevalent among high school and university students, the prevalence of using illicit substances for the same purpose is even higher.[8] Illicit drug use is defined as the non-medical use of various substances prohibited by international law.[9] These substances include, for instance, cannabis, cocaine, and classical psychedelics, such as Lysergic Acid Diethylamide (LSD). The European drug report of 2020, reported that the use of illicit drug was mostly prevalent among young adults (15-34 years old).[10] The two most prevalent substances used were cannabis and cocaine, with respectively 15.4% (15.8 million) and 2.1% (2.2 million) of young adults having used it in the previous year.[10] The prevalence of LSD use among young adults was 1% (1 million) in 2018.[10]

Cannabis, derived from the *Cannabis Sativa L.* plant, contains several different cannabinoids, of which two main cannabinoids cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) are most widely researched.[11, 12] THC-dominant cannabis is used recreationally to improve mood, i.e., to relieve stress and feel better,[13, 14] but it can induce undesired feelings of anxiety.[11] CBD-dominant cannabis, on the other hand, is used for its anxiolytic properties,[15] and thus decreases negative mood states. It has also been suggested that CBD can counteract THC-induced anxiety.[16] Therefore, it could be suggested that combined use of THC/CBD might be more favourable among users, although the research findings to date is inconclusive.[11]

Cocaine is the active compound present in the leaves of the *Erythroxylum coca* plant.[17] Cocaine in powder form is the purified chemical cocaine hydrochloride[17], which is usually snorted for its stimulating effects.[18] Users claim that cocaine positively affects cognition, creativity, and mood. Cocaine is mostly associated with parties and dance events, but is also used in educational and work-related settings.[19-21] Experimental studies in healthy volunteers indeed showed that cocaine increases positive mood and arousal.[22, 23] Evidence of the effects of cocaine on cognition is mixed[21] and is lacking for creativity.

LSD is a derivative of *ergot*, a parasitic fungus in rye grains, and was synthesised in the lab by the Swiss chemist Albert Hofmann in 1938.[24] LSD in moderate to high doses (75 - 200 mcg) is known for inducing visual hallucinations and a blissful state.[25, 26] Over the past decade, the repeated use of low doses of LSD, called microdosing,[27] increased in popularity allegedly to enhance mood and productivity.[28, 29] Users consume around one-tenth of their blotter to microdose,[27] which means that they consume on average between 6 and 10 mcg of LSD. However, the effectiveness of microdosing with LSD is mainly unknown. In addition, survey studies in users suggest a broad dosing window, including doses between 1.4–50 mcg LSD.[30] This wide range potentially indicates that the optimal dose to achieve the desired effect differs between individuals.[29]

Different mechanisms might underlie some of the claimed beneficial effects of these illicit substances on performance and mood. While THC modulates anxiety by binding to the orthosteric sites as a partial agonist on the cannabinoid type 1 (CB1) receptors located on the amygdala,[31-33] CBD is suggested to decrease THC-induced anxiety by acting as a negative allosteric modulator on these receptors.[31, 32] The psychostimulant effects of cocaine are mainly due to elevated dopamine levels,[34] and enhanced dopamine is associated with enhanced creativity, mood and arousal.[35-37] The underlying mechanism of a microdose of LSD, such as enhanced mood and cognition, might be related to enhanced Brain-derived Neurotrophic Factor (BDNF), a protein involved in neuroplasticity.[38] It is known that a hallucinogenic dose of a serotonergic psychedelic, such as LSD, stimulates serotonin (5-HT2A) receptors located on cortical pyramidal neurons, thereby increasing BDNF,[38] however it is unknown whether a microdose of LSD increases BDNF.

While most of the perceived effects of CBD, cocaine and microdoses of LSD on mood and cognition seem positive, the evidence is mixed[11, 39] or based on user claims.[19, 21, 28, 29] Drug effects experienced by users might be due to adulterants,[40] such as caffeine[41, 42] or NBOMes, novel psychedelic substances.[43] Of note, the subjective effects of NBOMes seem to be similar to LSD, yet also based on user claims. Also, the purity of CBD, cocaine and LSD used can vary greatly.[44-47] Furthermore, previous studies with stimulant drugs show discrepancies between the self-rated performance (subjective) and task-based performance (objective).[48, 49] So one might feel more creative after cocaine use or feel more productive when taking an LSD microdose while actual performance might not be affected when assessed with a cognitive task. Hence, assessment of task-based performance is of added value to the existing self-rated subjective experience.

Additional factors such as an individual's trait and state at the time of substance use might influence the effects of CBD, cocaine and LSD on cognition and mood. A *trait* is a typical tendency to behave, feel, and think across various situations and is considered to be stable over time.[50] A *state* is how one acts, feels, and thinks during a particular situation, at a specific moment in time, and can be affected by manipulating the situation or by the administration of a drug.[50, 51] Both states and traits can have an influence on the positive and negative effects of a substance.[52] For example, an enhanced state of arousal induced by cocaine use might moderate cognitive performance,[53] whereas trait empathy and a positive mood state are positively associated with creative performance.[54, 55] Furthermore, trait and baseline state anxiety might moderate cannabis-induced anxiety.[56, 57] It would be informative for individuals if one could predict drug responses based on trait or state variables, allowing them to estimate whether they are likely to experience positive or negative effects of a drug on cognition and mood.

Aim of this dissertation

The aim of this dissertation was (1) to objectively examine the proclaimed cognition and mood enhancing effects of cocaine, LSD, and CBD in placebo-controlled experimental studies, and (2) to assess whether drug-induced enhancement on cognition and mood depend on

personality traits such as empathy and anxiety; drug-induced states of arousal and positive mood; and baseline state of anxiety.

Methods and outline

All studies described in this dissertation were conducted according to a double-blind, placebo-controlled within-subject design. **Chapter 2** describes a study examining the acute effects of cocaine (300 mg, p.o.) on objective and self-rated creative performance using a variety of creativity tasks. In addition, it was examined whether cocaine's potential effects on creativity was associated with personal factors like trait empathy and cocaine-induced positive mood state. **Chapter 3** describes the acute effects of cocaine (300 mg, p.o.) on prospective memory performance and its potential association with arousal state. **Chapters 4 and 5** describe a dose-finding study with low doses of LSD (0, 5, 10, and 20 µg, p.o.) focusing on objectively measured cognition, self-rated mood states, subjective experiences and inter-individual variation in these subjective and cognitive responses **(Chapter 4)**, and neuroplasticity **(Chapter 5)**. **Chapter 6** describes the acute effects of CBD (13.75 mg, vaporised), THC (13.75 mg) and a combination of THC/CBD (13.75 mg THC + 13.75 mg CBD) on objectively assessed and subjectively rated anxiety states. In addition, it was examined whether these effects are moderated by baseline anxiety state and trait. Finally, **chapter 7** discusses and integrates the findings of the studies described in this dissertation. Clinical implications and future recommendations are given.

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Chapter 2

Cocaine enhances figural, but impairs verbal 'flexible' divergent thinking

Hutten, N.R.P.W., Steenbergen, L., Colzato, L.S., Hommel, B., Theunissen, E.L., Ramaekers, J.G., Kuypers, K.P.C. *European Neuropsychopharmacology*, 2019; *29*(7), 813-824

Abstract

Anecdotal evidence suggests that cocaine use will help overcome creative 'blocks' by enhancing flexible thinking. Given that cocaine is likely to enhance dopamine (DA) levels, which in turn are positively associated with divergent thinking (DT); is a possibility that is tested in the present study. Furthermore, the impact of cocaine is tested on convergent thinking (CT), another aspect of creative thinking, which has been reported to be impaired with high DA levels. It was hypothesized that cocaine would enhance DT and impair CT. A placebo-controlled within-subjects study including 24 healthy poly-drug users was set up to test the influence of oral cocaine (300 mg) on creativity. Verbal CT was assessed with the Remote Associates Task (RAT); figural CT was assessed with the Picture Concepts Task (PCT) and the Tower of London (TOL). Verbal DT was assessed with the Alternative Uses Task (AUT); figural DT was assessed with the Pattern/Line Meanings Task (PLMT). Findings showed that, compared to placebo, cocaine impaired figural CT (TOL) and flexible DT of verbal stimuli (AUT), while it enhanced figural DT (PLMT). No significant effects of cocaine were observed regarding the PCT and RAT. It was demonstrated that cocaine-induced effects on creativity in poly-drug users are stimulus-dependent. Cocaine enhanced performance on figural DT but impaired performance on verbal (flexible) DT. Cocaine impaired CT on only one figural task and but not on the other tasks. As creativity is an important aspect in cognitive therapies, it is important to further understand these discrepancies in creativity task performance.

Introduction

Cocaine is a stimulant drug with the highest lifetime prevalence in Europe as compared to other drugs of abuse.[1] While cocaine is known for its enhancing effects on mood and alertness,[2-4] a large body of anecdotal evidence suggests that people sometimes use it to induce creative flexible thinking, helping to overcome creative 'blocks'.[5-8] These reports have however never been substantiated by means of objective performance measures. Of note, studies have demonstrated a discrepancy between self-rated and computer-assessed cognitive performance during intoxication with similar stimulant substances. For instance, while a mixture of amphetamines did not enhance cognition objectively, participants reported performance enhancement.[9] Similarly, studies often fail to distinguish between enhanced performance versus increased interest in creative tasks and/or experienced creativity.[10]

Similar to the effect of amphetamines, the acute administration of cocaine increases synaptic dopamine (DA) levels,[11] which underlies cocaine's main effects on behaviour and mood,[12, 13] and suggested to play an important role in creativity. Although cocaine acts as a triple reuptake inhibitor, inhibiting serotonin, norepinephrine, and DA reuptake, the boost in creative performance of Parkinson patients after treatment with dopamine replacement therapy[14-18] and the impairment in schizophrenic patients' creativity after DA inhibition[19] supports the suggestion that the dopaminergic system is a key factor in driving creativity. Interestingly, the relation between DA levels and creative performance has been suggested to be dependent on the type of creative process, potentially due to a difference in underlying neuronal mechanisms.[20, 21] The first stage in a typical creative act is 'flexible' *divergent thinking*, also known as brainstorming. It is the ability to come up with multiple solutions or ideas in response to a vaguely defined problem and it is usually quantified with 4 descriptors, i.e., fluency, the amount of ideas generated; originality, the novelty of the generated ideas; flexible thinking, the ability to come up with ideas from different angles (categories); and elaboration, the amount of details the idea contains.[22] The second stage consists in *convergent thinking*; defined as the ability to find the correct solution to a better defined problem.[22, 23] As compared to divergent thinking, convergent thinking emphasizes speed, relies on high accuracy and logic, and performance is independent of the former type.[22]

It has been suggested that the relationship between divergent thinking and DA levels follows an inverted u-shape, with optimal creative performance with medium DA levels.[24] Convergent thinking, in contrast, is assumed to be negatively associated with DA levels, with best performance with low and worst performance with high DA levels.[24] Importantly, however, these relations between DA levels have been reported from verbal creativity tasks, and it remains to be seen whether the observations generalize to nonverbal material. Unfortunately, the distinction between verbal and figural tasks is often not considered in creativity studies, even though different brain networks in different cortical hemispheres are involved in the processing of these stimuli,[25-28] which could imply different DA-creativity relations.

Personal factors like mood *state* and *trait* empathy have been shown to contribute to creative performance,[29-32] and cocaine is known to interact with mood *state*, while experienced effects of cocaine can be associated with a certain personality *trait*.[4] According to the dopaminergic theory of positive affect, there is a positive relationship between levels of affect, DA, and creative (divergent) problem solving; with positive mood being associated with high levels of DA and enhanced verbal and figural divergent thinking, while verbal convergent thinking seems to be impaired by positive mood states.[20, 25, 33-40] The effect of positive mood on figural convergent thinking is unknown. In line with mood *state*, research on *trait* empathy found empathy levels to be positively associated with both, verbal and figural, divergent thinking;[29-32] higher scores on divergent thinking were associated with higher *trait* empathy, while *state* empathy does not correlate with creativity.[41]

The present study was set up to test the acute effects of cocaine on objective and self-rated creative performance and to test whether potential behavioural drug effects are associated with personal factors like mood *state* and *trait* empathy. Based on cocaine's biological and psychological mechanism of action, i.e. elevating dopamine levels and enhancing positive mood, it was hypothesized that cocaine would impair convergent thinking and enhance divergent thinking, as assessed by objective performance measures, and increase subjectively experienced creativity. Secondly, it was hypothesized that drug-induced divergent thinking performance would be associated with drug-induced positive mood and that higher levels of empathy would be associated with enhanced divergent thinking.

Methods

Design

The study design was double-blind, placebo-controlled, within-subject with two treatment conditions, placebo and cocaine HCl (300 mg). Participants were randomly assigned to one of the two treatment condition orders according to a balanced block design. The dosage of cocaine was based on previous experimental studies demonstrating significant cognitive effects.[42-44] Cocaine and placebo were encapsulated and randomized by the GMP licensed company Basic Pharma (Geleen, The Netherlands). Even though the majority of cocaine users prefer nasal as route of administration,[45] cocaine was administered orally because it allows a double-blind administration and it results in a longer intoxication hence a wider test frame. In addition, potential side-effects from nasal administration are prevented in that way.[42] All tests were performed between 1 and 2 hours post-treatment around expected peak concentrations of cocaine.[44] Test days were separated by a minimum wash-out period of 7 days to avoid carry-over effects.

Participants

Participants were 24 healthy recreational poly-drug users (19 males; 5 females), aged 22.2 years on average (*SD*= 2.3). All of them were native speakers of Dutch. Participants indicated their highest level of education to be academic university (*N*= 12; 50%) and university of applied sciences (*N*= 8; 33%), two participants (8%) indicated to have finished secondary education (gymnasium etc.) but did not indicate their current level of education, and one participant indicated to be a student without reporting the level of education. Life time cocaine use was 34.7 times on average (*SD*= 35.1). Experience with the use of other substances such as amphetamines, cannabis, MDMA, LSD, mushrooms, and other psychoactive substances were also reported. Details of drug use history are presented in Table 1.

Table 1. Mean (±SD) drug use of participant in number of uses in life-time and last month.

	Life-time use			**Last month use**		
	Mean (SD)	Range (min-max)	N	Mean (SD)	Range (min-max)	N
Cocaine[a]	34.68 (35.1)	6-150	24	2.88 (2.6)	1-10	12
Amphetamine[a]	12.93 (16.6)	1-50	17	1.75 (1.0)	1-3	4
Cannabis[a]	163.71 (256.9)	1-1000	24	4.9 (4.3)	1-10	7
Ecstasy/MDMA[a]	23.50 (17.6)	2-70	23	1.17 (0.4)	1-2	6
LSD[a]	2.00 (2.0)	1-5	4	-	-	0
Mushrooms[a]	2.13 (1.6)	1-5	16	1 (0.0)	-	2
Other psychoactive substances[b]	2CB (3), Ketamine (30), Methoxetamine(1), 6-APB (1), GHB (30), Truffles (4), Crack cocaine (1)			Ketamine (2), GHB (4), truffles (1)		

[a]Some participants did not quantify their use in words or numbers, some only quantified it in words: Amphetamine: 'sometimes' (1x), 'regularly' (1x); Cannabis: 'yes' (2x), 'often' (1x), 'very often' (1x), 'not a lot' (1x), 'regularly (during a specific period)' (2x); Cocaine: 'yes' (1x), 'very often' (1x), 'more often' (1x), 'regularly' (1x); Ecstasy: 'yes' (1x), 'regularly' (1x), 'more often' (1x); Mushrooms: 'more often' (1x); [b]Other psychoactive substances: the numbers between brackets indicate the number of time the participant used the substance.

Procedures

Participants were recruited through advertisements in university buildings in Maastricht, via a website (digi-prik.nl), local newspaper advertisements, and by word of mouth. Before inclusion, participants underwent a medical screening by a medical supervisor. General health was checked and blood and urine samples were taken for standard blood chemistry, haematology and urinalysis. Inclusion criteria were written informed consent; age 18-40 years; good physical and mental health as determined by medical history and medical examination; BMI between 19-29 kg/m^2. Exclusion criteria were being cocaine naïve; history of drug abuse or addiction as assessed via an extensive interview by an experienced medical supervisor using the DSM-IV criteria; history of psychiatric and neurological disorders as assessed via the medical interview; cardiovascular abnormalities; hypertension; excessive alcohol use defined as drinking more than 21 alcohol consumptions per week; pregnancy or lactation.

Participants were familiarized with tests and test procedures and completed the Empathizing-Systemizing Quotient questionnaire during a training session proceeding the test days. They had to refrain from any drugs at least one week before start of the study until completion of

their testing days. Participants were requested to not consume caffeinated or alcoholic beverages 24 hours prior to testing and to arrive well-rested at the test facilities. Upon arrival they were screened for presence of drugs of abuse in urine (THC/opiates/cocaine/amphetamines/methamphetamines), and alcohol in breath. Women were submitted to a urine pregnancy test. When tests were negative, participants filled out a questionnaire to assess sleep quality and quantity and they had a light standardized breakfast. After breakfast participants were administered a capsule p. o. containing either 300 mg cocaine HCl or placebo. Sixty minutes post-treatment a mood questionnaire and a visual analogue scale (VAS) scale measuring subjective creativity was filled out, a blood sample was taken to determine cocaine concentration afterwards and the test battery consisting of tests of creativity and emotion recognition (results published in Kuypers et al[3]) was presented in the following order: AUT, PLMT, PCT, RAT, TOL and emotion recognition. Between treatment administration and the tests battery participants were seated in a quiet waiting room where they could read a book or watch television.

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and subsequent amendments and it was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University. It was registered in the Dutch Clinical Trial register (number: NTR3998 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=3998). A permit for obtaining, storing and administering cocaine was obtained from the Dutch Drug Enforcement Administration. The present study was carried out in the context of a larger trial on emotion recognition, which for the most part (except for the present data) has been reported elsewhere.[3]

Convergent thinking

Convergent thinking was assessed by means of three tasks, the Remote Associates Task (RAT), the Picture Concept Task (PCT), and the Tower of London (TOL). All tests had parallel versions to avoid learning effects.

The RAT is a *verbal* convergent thinking task based on Mednick[23]. The Dutch version comprised 30 validated items[46] divided into two versions of 15 items, of which 8 were 'easy'

items and 7 'difficult'. Item difficulty categorization was based on a study by Akbari Chermahini et al.[46] Each item consisted of three unrelated words, such as "time", "hair", and "stretch", and participants' task was to identify the common associate ("long") which would result in existing Dutch composite words. There was a time frame of 10 minutes for 15 items. The percentages of correct answers for 'easy' and 'difficult' items were the main dependent variables.

The PCT is a *figural* convergent thinking task consisting of 28 items per parallel version. Each stimulus contained a matrix with 4 to 12 colour pictures shown in two or three rows. Participants' task was to find an association between pictures of the different rows (one picture of each row). They were instructed to provide the correct solution, within a timeframe of 30 seconds per stimulus. Items consisting of 4 - 6 pictures were categorized as 'easy' and items with 9 - 12 pictures as 'difficult'. Percentages correct for 'easy' items and 'difficult' items were taken as dependent measures.

The TOL assesses executive function and planning[47] and is not a 'typical' creativity task. However, it is used as a *figural* convergent thinking task in the present study, because the task contains the rationale of convergent thinking; finding the correct solution to one particular problem. The TOL consists of 44 computer-generated images of beginning- and end-arrangements of three coloured balls on three sticks. Participants' task was to determine as quickly as possible how many ball-movements were needed to get to the end arrangements. Items composed of 2 and 3 steps were categorized as 'easy' and items composed of 4 and 5 steps as 'difficult', two items composed of 6 steps were control items and therefore not included in the analyses. Percentages of correct answers for 'easy' items and 'difficult' items were the main performance measures. Reaction time in seconds was taken as secondary performance measure, for 'easy' and 'difficult' items separately.

Divergent thinking

Divergent thinking was assessed by means of two tasks; the Alternative Uses Task (AUT) and the Pattern/Line Meanings Task (PLMT). Both tasks had parallel versions to avoid familiarization of the stimuli presented.

The AUT is a *verbal* divergent thinking task based on Guilford.[22] Participants were presented with the names of two common household items (e.g., towel and newspaper) and were to generate as many possible alternative uses of these objects as possible within 6 minutes.

The PLMT[48] is a *figural* divergent thinking task consisting of eight black and white drawings, i.e. two parallel versions were created, comprising the uneven line figures and even pattern figures from the original task, or reversed. Participants had to give meaning to a configuration of patterns (4 drawings) or lines (4 drawings) and generate as many explanations for it as possible, trying to be as creative as possible and were allowed 2 minutes response time per item.[49] Patterns and lines were analysed as two different outcome measures.

Dependent outcome variables for both tasks were fluency, originality, ratio, flexibility and elaboration. Fluency was the total number of valid responses. Originality, the uniqueness of responses which was scored with 0, 1, and 2; responses that were given by only 1% of the group counted as unique (2 points), responses given by 5% counted as unusual (1point) and answers given by more than 5% received a score of zero. Summed originality scores served as dependent variable for AUT and PLMT. Ratio (originality/fluency) was also calculated to correct the originality scores for the number of responses that were generated; somebody who gives two 'unusual' original answers and somebody who gives only one 'unique' original answer received a score of '2' on originality in total, while the number of answers differ; the ratio will reflect this difference in quality with the first person getting a lower ratio (1) than the second one (2). Flexibility is the ability to generate a diversity of responses and was measured by combining responses into different numbers of categories. Elaboration was the amount of detail in the answers. Flexibility and elaboration are normally not used as an outcome variable for the PLMT; the present study added these variables to compare verbal and figural divergent thinking.

Subjective Creativity

Participants had to assess their subjective levels of creativity via a Visual Analogue Scale (10 cm); with 0 indicating 'not creative at all' and 10 indicating 'very creative'.

Positive Mood state

Positive Mood was measured by the Profile of Mood States (POMS) questionnaire. Participants were shown 72 adjectives describing a specific mood and they had to rate their current state using 5-point Likert scales, with 0 being 'not at all' to 4 'extremely'. The POMS is a validated scale, comprised of 5 positive and 5 negative affect scales. One of the positive affect scales, positive mood was determined by using composite score of 2 levels of mood (Elation – Depression).[50]

Trait empathy

Trait empathy, the drive to identify mental states and respond to those with an appropriate emotion was assessed using the Empathizing (EQ) and Systemizing quotient (SQ) questionnaire. The EQ scale comprised of 60 statements, of which 20 filler items, in a forced-choice format (i.e., strongly agree; slightly agree; slightly disagree; strongly disagree). The maximum score, indicating very high empathy, is 80.[49, 51, 52]

Pharmacokinetic Assessments

Blood samples were centrifuged at 3500 rpm and resulting plasma was frozen at −20°C until analysis for pharmacokinetic assessments. The determination of cocaine (COC), and its metabolites benzoylecgonine (BZE) and ecgonine methyl ester (EME) were determined in a specialized forensic-toxicological laboratory using validated procedures.[53, 54]

Statistical Analysis

Statistical analyses were performed by means of the statistical package IBM SPSS Statistics (version 24). Data of the convergent thinking tasks were analysed by means of a General Linear Model (GLM) repeated measures (RM) Multivariate Analysis of Variance (MANOVA) with Treatment (2 levels) and Item Difficulty (2 levels) as within-subjects (WS) factors, and Test Day Order (2 levels) as between-subjects (BS) factor. In case of no main effect of Test Day Order, a GLM RM Analysis of Variance (ANOVA) with Treatment (2 levels) and Item Difficulty (2 levels) as within-subjects (WS) factors was conducted. In case of interaction effects paired samples *t*-tests were conducted between treatment conditions, per item difficulty.

Since *trait* empathy is associated with divergent thinking,[32] separate GLM RM Multivariate Analysis of Covariances (MANCOVA) with empathy total score as a covariate to control for empathy levels, Treatment (2 levels) as WS factor and Test Day Order (2 levels) as BS factor were conducted on the dependent variables of the divergent thinking tasks. In case of no main effect of Test Day Order, a GLM RM Analysis of Covariance (ANCOVA) with empathy as covariate and Treatment (2 levels) WS factor was conducted. In case of interaction effects, Pearson correlation analyses were conducted to further explore the relationship between task performance and empathy levels using placebo-change performance scores (cocaine minus placebo).

The effect of Treatment on Positive Mood and subjective creativity was analysed by means of a paired samples t-test. In addition, a series of Pearson correlation analyses including placebo-change scores were conducted to assess the relationship between task performance and positive mood ratings on the one hand, and subjective creativity on the other hand.

Assuming an omnibus $p < 0.05$ and power = 0.8, we estimated including 24 subjects would enable detection of performance differences between cocaine and placebo with an effect size of 0.3 (i.e., a signal change of 0.3 times the standard deviation) in within subject comparisons. The alpha criterion level of statistical significance for all analyses was set at $p= 0.05$. To correct for multiple testing, the alpha criterion was divided by the number of tests per construct; for convergent thinking, the alpha criterion was set at $p= 0.01$ and for divergent thinking the alpha criterions was set at $p= 0.02$. Partial eta squared (partial η^2) is reported in case of significant effects to demonstrate the effect's magnitude, where 0.01 is defined as small, 0.06 as moderate and 0.14 as large. Partial eta squared is based on Cohen's *f* which defines small, medium and large as respectively 0.10, 0.25, and 0.50 which corresponds to η^2 of 0.0099, 0.0588, and 0.1379.[55]

Results

Blood plasma concentrations (mean (SD)) 1h after cocaine administration, at the start of cognitive testing were 0.57 mg/L (0.37) for cocaine, 0.69 mg/L (0.20) for BZE, and 0.22 mg/L (0.11) for EME.

Missing data

Due to noncompliance with task instructions, AUT data were missing for one participant in both treatment conditions. Due to technical issues, computer responses for the TOL were not registered for one participant in the placebo condition. Therefore 23 participants with complete AUT and TOL data-sets entered the analyses. For three participants, a different item of the *trait* empathy EQ scale was missing; these missing values were replaced with the mean value of the other answers on that scale for that participant.

Trait empathy and Positive Mood State

Participants had a mean score of 37.50 (*SD*= 8.23) on *trait* Empathy. Positive mood was significantly elevated (t_{23}= 2.15, p= 0.04) by cocaine (*M*= 14.00, *SE*= 1.52) compared to placebo (*M*= 10.71, *SE*= 0.86).

Subjective Creativity

Paired samples *t*-test revealed that subjective creativity was significantly increased by cocaine compared to placebo (t_{23}= 3.56, p< 0.01); participants felt more creative after cocaine treatment (*M*= 5.84, *SE*= 0.43) compared to placebo (*M*= 4.01, *SE*= 0.36).

Convergent thinking

GLM repeated measures MANOVA showed no main effect of Test Day Order on all the outcome variables of the convergent thinking tasks.

Verbal convergent thinking

GLM repeated measures ANOVA revealed a main effect of Item Difficulty on verbal convergent thinking, measured by the RAT ($F_{1,23}$= 40.39, p< 0.01, η_p^2 = 0.64); indicating that participants had a higher percentage correct for the easy items compared to difficult items. There was no main effect of Treatment ($F_{1,23}$= 0.28, p= 0.60, η_p^2 = 0.01) or Treatment by Item Difficulty interaction ($F_{1,23}$= 0.09, p= 0.76, η_p^2 < 0.01) on percentage correct of the RAT.

Figural convergent thinking

GLM repeated measures ANOVA revealed a main effect of Item Difficulty on all figural convergent thinking tasks; PCT ($F_{1,23}$= 239.41, p< 0.01, η_p^2 = 0.91) and TOL ($F_{1,22}$= 63.13, p< 0.01, η_p^2 = 0.74), indicating that the easy items were answered correctly more often compared to the difficult items. In case of the TOL, a Treatment by Item Difficulty Interaction showed that this effect was dependent on the treatment condition ($F_{1,22}$= 15.43, p< 0.01, η_p^2 = 0.41). Performance on difficult items was impaired (t_{22}= -3.15, p< 0.01) by cocaine (M= 76.87, *SE*= 2.67) compared to placebo (M= 85.43, *SE*= 1.90), leaving performance on easy items unaffected (t_{22}= 1.20, p= 0.24). In addition, there was a main effect of Item Difficulty on reaction time of the TOL ($F_{1,22}$= 147.67, p< 0.01, η_p^2 = 0.87), indicating faster responses to easy items compared to difficult items.

There were no main effects of Treatment on percentage correct of the PCT ($F_{1,23}$= 0.13, p= 0.72, η_p^2 < 0.01) and TOL ($F_{1,22}$= 2.77, p= 0.11, η_p^2 = 0.11) or reaction time of the TOL ($F_{1,22}$= 2.09, p= 0.16, η_p^2 = 0.09); and no Treatment by Item Difficulty interaction effects on percentage correct of the PCT ($F_{1,23}$= 0.55, p= 0.46, η_p^2 = 0.02) and reaction time of the TOL ($F_{1,22}$= 2.39, p= 0.14, η_p^2 = 0.09).

Mean (± SE) scores of the convergent thinking tasks are depicted in Table 2.

Table 2. Mean (±SE) percentage correct on the Remote Association Task (RAT), Picture Concept Task (PCT) and Tower of London (TOL); and TOL reaction time in seconds.

Creativity tests and outcome measures		**Treatment (Mean ±SE)**				N
		Cocaine		**Placebo**		
		Easy	Difficult	Easy	Difficult	
Verbal	RAT (% correct)	58.86 (5.99)	44.64 (4.73)	56.77 (4.69)	39.88 (3.43)	24
Figural	PCT (% correct)	85.69 (2.62)	45.63 (4.51)	86.11 (1.91)	43.33 (3.24)	24
	TOL (% correct)	96.09 (0.91)	76.87 (2.67)	94.13 (1.62)	85.43 (1.90)	23
	TOL (Reaction Time)	5.43 (0.36)	13.11 (1.04)	5.75 (0.38)	14.59 (1.02)	23

Divergent thinking

GLM repeated measures MANOVA showed no main effect of Test Day Order on all the outcome variables of the divergent thinking tasks.

Verbal divergent thinking

GLM repeated measures ANCOVA revealed a main effect of Treatment on flexibility measured by the AUT ($F_{1,21}$= 6.20, p= 0.02, η_p^2 = 0.23), indicating that cocaine reduced flexible thinking when controlled for *trait* empathy. There was an interaction effect of Treatment and the covariate *trait* empathy on flexibility ($F_{1,21}$= 6.15, p= 0.02, η_p^2 = 0.23). A Pearson correlation revealed an association between *trait* empathy and the placebo-change scores of flexibility performance (r_{23}= 0.48; p= 0.02), indicating that participants with higher *trait* empathy were able to generate a higher diversity of responses while under the influence of cocaine compared with placebo (Figure 1, panel A).

There were no Treatment effects on other outcome variables of the AUT; fluency ($F_{1,21}$= 3.32, p= 0.08, η_p^2 = 0.14), originality ($F_{1,21}$= 0.41, p= 0.53, η_p^2 = 0.02), ratio ($F_{1,21}$= 1.17, p= 0.29, η_p^2 = 0.05) and elaboration ($F_{1,21}$< 0.01, p= 0.99, η_p^2 < 0.01). There were no interaction effects of Treatment and *trait* empathy on the other outcome measures of the AUT; fluency ($F_{1,21}$= 3.48, p= 0.08, η_p^2 = 0.14), originality ($F_{1,21}$= 1.06, p= 0.31, η_p^2 = 0.05), ratio ($F_{1,21}$= 0.44, p= 0.52, η_p^2 = 0.02) and elaboration ($F_{1,21}$= 0.03, p= 0.87, η_p^2 < 0.01). Mean (± SE) of the AUT are depicted in figure 2.

Figural divergent thinking

PLMT-'Line' stimuli

GLM repeated measures ANCOVA revealed a Treatment effect on fluency ($F_{1,22}$= 6.70, p= 0.02, η_p^2= 0.23), originality ($F_{1,22}$= 6.09, p= 0.02, η_p^2= 0.22) and flexibility ($F_{1,22}$= 8.29, p< 0.01, η_p^2= 0.27) in the line category of the PLMT. When under the influence of cocaine participants were able to generate more responses, were more original and more divers compared to placebo. Furthermore, analyses revealed an interaction effect of Treatment and *trait* empathy on fluency ($F_{1,22}$= 8.15, p< 0.01, η_p^2= 0.27), originality ($F_{1,22}$= 8.29, p< 0.01, η_p^2= 0.27) and

flexibility ($F_{1,22}$= 10.62, p< 0.01, η_p^2= 0.33) of the line category of the PLMT. Additional Pearson correlations revealed significant associations between the covariate *trait* empathy and placebo-change scores on fluency (r_{24}= 0.52; p< 0.01), originality (r_{24}= 0.52; p< 0.01) and flexibility (r_{24}= 0.57; p< 0.01), indicating that participants with higher *trait* empathy gave more responses and these were also more original and they were able to generate a higher diversity of responses under the influence of cocaine compared to placebo (Figure 1, Panels B-D).

PLMT- 'Pattern' stimuli

GLM repeated measure ANCOVA revealed an interaction effect of Treatment and *trait* empathy on flexibility ($F_{1,22}$= 6.36, p= 0.02, η_p^2= 0.22) of the pattern category. Additional Pearson correlations revealed significant associations between *trait* empathy levels and placebo-change scores on flexibility (r_{24}= 0.47; p= 0.02) in the pattern category, indicating that participants with higher *trait* empathy were able to generate a higher diversity of answers under the influence of cocaine compared to placebo (Figure 1, Panel E).

There were no Treatment effects on fluency ($F_{1,22}$= 2.71, p= 0.11, η_p^2 = 0.11), originality ($F_{1,22}$= 2.19, p= 0.15, η_p^2 = 0.09) and flexibility ($F_{1,22}$= 4.89, p= 0.04, η_p^2= 0.18) for the Patterns category, and no Treatment effects on ratio ($F_{1,22}$< 0.01, p= 0.98, η_p^2 < 0.01; $F_{1,22}$= 0.77, p= 0.39, η_p^2= 0.03) and elaboration ($F_{1,22}$= 0.32, p= 0.56, η_p^2= 0.01; $F_{1,22}$= 1.38, p= 0.25, η_p^2= 0.06) for both the Pattern and Line category respectively. There were no interaction effects of Treatment and *trait* empathy on fluency ($F_{1,22}$= 3.87, p= 0.06, η_p^2= 0.15) and originality ($F_{1,22}$= 3.42, p= 0.08, η_p^2= 0.14) for the pattern category, and no interaction effects on ratio ($F_{1,22}$= 0.01, p= 0.92, η_p^2 < 0.01; $F_{1,22}$= 1.66, p= 0.21, η_p^2= 0.07) and elaboration ($F_{1,22}$= 0.61, p= 0.44, η_p^2= 0.03; $F_{1,22}$= 0.38, p= 0.54, η_p^2= 0.02) for patterns and lines respectively.

Mean (± SE) scores on the PLMT are shown in Figure 2.

Correlational analyses of mood state and self-rated creativity with performance

Pearson correlation analyses revealed an association between the placebo-change scores of Positive Mood and performance measures: TOL Reaction Time for both difficulty levels (easy: r_{23}= -0.63, p< 0.01; difficult: r_{23}=-0.46, p= 0.03), and for fluency (r_{23}= 0.44, p= 0.04) and

originality (r_{23}= 0.48, p= 0.02) on the AUT indicating that participants whose positive mood increased more under the influence of cocaine also became faster in responding in the TOL and were more original and gave more responses on the AUT relative to participants with smaller positive mood increments.

There was a significant correlation between the placebo-change scores (cocaine minus placebo) of self-rated creativity and correct responses on the TOL for the difficult items (r_{23}= -0.50, p= 0.02), indicating that participants who had larger increases in self-rated creativity when under the influence of cocaine showed the largest decrease in performance for the difficult items of the TOL compared to participants who had smaller increases in figural convergent thinking when under the influence of cocaine.

There were no statistically significant correlations between other outcome measures of the creativity tasks and positive mood or subjective creativity (see Table 3 and 4 respectively).

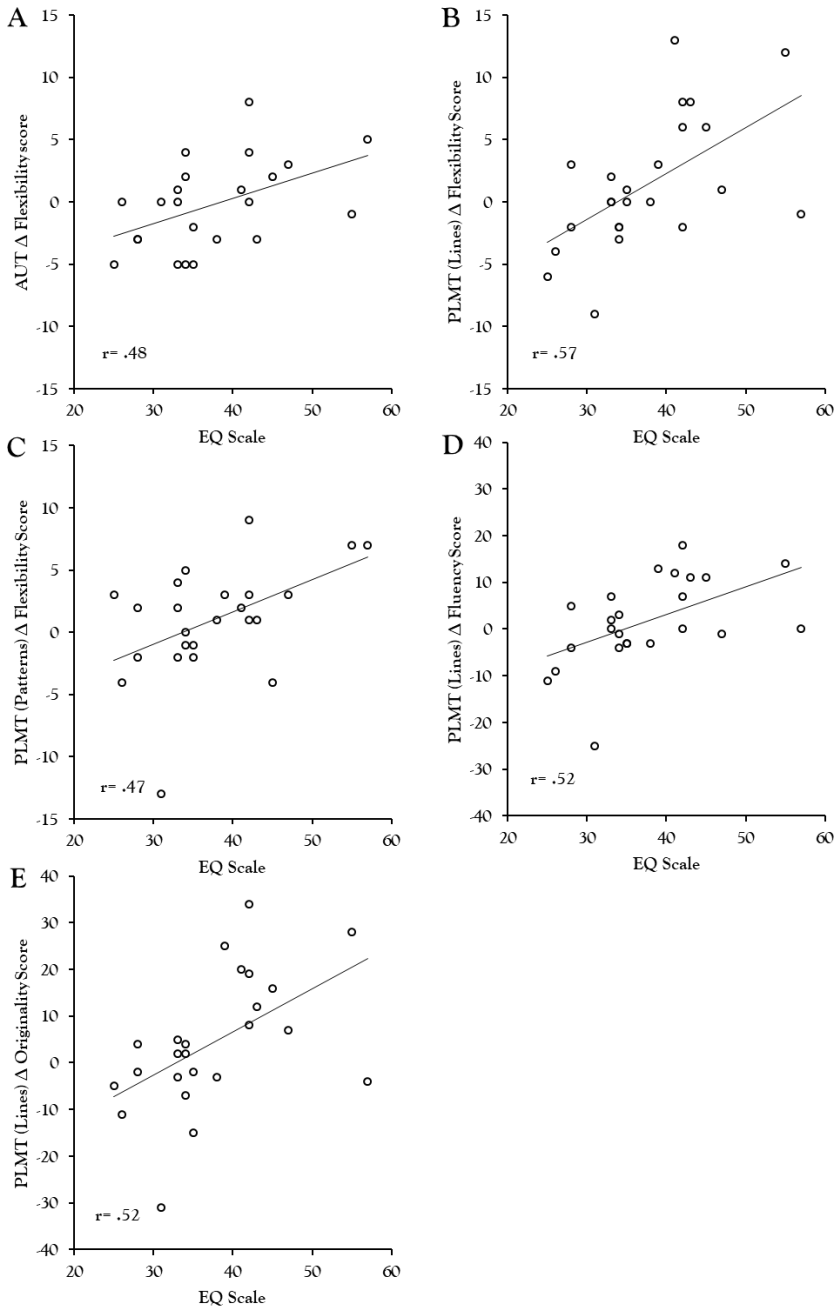

Figure 1. Scatterplots of different scores on divergent thinking tasks (cocaine minus placebo) as a function of Empathy Quotient. Pearson correlations (r) statistically significant at $p<0.05$. EQ: empathy quotient; AUT: alternative uses task; PLMT: pattern/line meanings task.

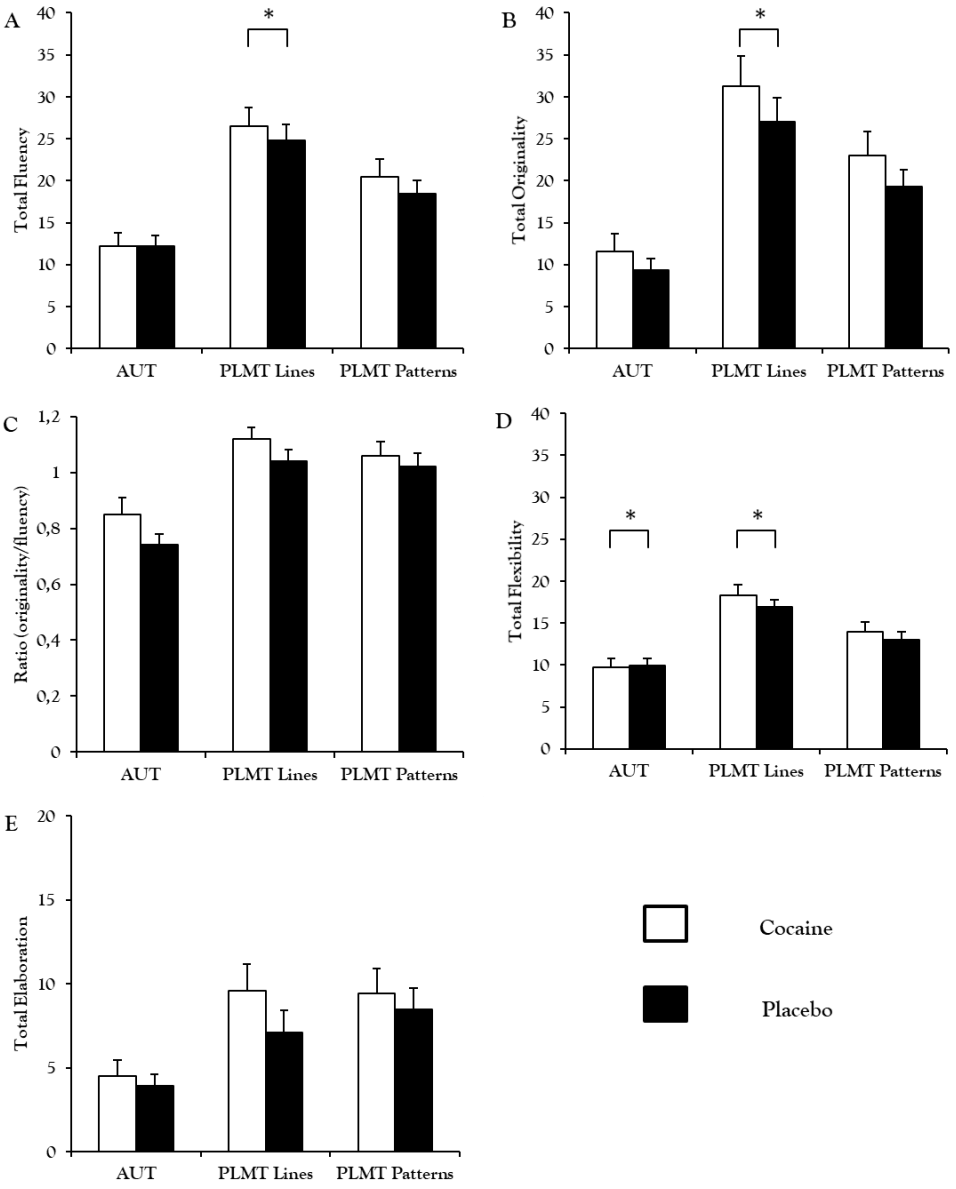

Figure 2. Mean (±SE) scores of fluency (A), originality (B), ratio (C), flexibility (D) and elaboration (E) after cocaine and placebo administration of the AUT and over all items (patterns and lines) of the PLMT. * signifies statistically significant main Treatment at $p < 0.02$. AUT: alternative uses task; PLMT: pattern/line meanings task.

Table 3. Pearson correlations (*r*) between cocaine-induced positive mood ratings and the outcome measures of creativity tasks and corresponding significance levels (*p*); *statistically significant at $p<0.05$

Creativity tasks		**Positive mood**			
Convergent		Easy Items		Difficult Items	
Verbal	RAT (% correct)	$r_{24}= 0.21$	$p= 0.34$	$r_{24}= 0.23$	$p= 0.29$
Figural	PCT (% correct)	$r_{24}= 0.07$	$p= 0.75$	$r_{24}= -0.06$	$p= 0.76$
	TOL (% correct)	$r_{23}= -0.09$	$p= 0.70$	$r_{23}= -0.15$	$p= 0.51$
	TOL (Reaction Time)	$r_{23}= -0.63$	$p< 0.01$	$r_{23}= -0.46$	$p= 0.03$*
Divergent					
Verbal	AUT				
	Fluency	$r_{23}= 0.44$		$p= 0.04$	
	Originality	$r_{23}= 0.48$		$p= 0.02$	
	Ratio	$r_{23}= 0.11$		$p= 0.63$	
	Flexibility	$r_{23}= 0.10$		$p= 0.65$	
	Elaboration	$r_{23}= 0.10$		$p= 0.66$	
Figural	PLMT	Patterns		Lines	
	Fluency	$r_{24}= 0.13$	$p= 0.55$	$r_{24}= 0.25$	$p= 0.24$
	Originality	$r_{24}= 0.23$	$p= 0.29$	$r_{24}= 0.31$	$p= 0.15$
	Ratio	$r_{24}= 0.19$	$p= 0.39$	$r_{24}= 0.30$	$p= 0.15$
	Flexibility	$r_{24}= 0.23$	$p= 0.27$	$r_{24}= 0.19$	$p= 0.38$
	Elaboration	$r_{24}< 0.01$	$p= 0.97$	$r_{24}= 0.07$	$p= 0.76$

Table 4. Pearson correlations between cocaine-induced subjective creativity ratings and the outcome measures of creativity tasks and corresponding significance levels; *statistically significant at $p<0.05$

Creativity tasks		**Subjective creativity**			
Convergent		Easy Items		Difficult Items	
Verbal	RAT (% correct)	r_{24}= 0.19	p= 0.35	r_{24}= -0.09	p= 0.67
Figural	PCT (% correct)	r_{24}= -0.06	p= 0.79	r_{24}= -0.29	p= 0.17
	TOL (% correct)	r_{23}= -0.11	p= 0.60	r_{23}= -0.50	p= 0.02*
	TOL (Reaction Time)	r_{23}= -0.29	p= 0.19	r_{23}= -0.20	p= 0.36
Divergent					
Verbal	AUT				
	Fluency	r_{23}= 0.04		p= 0.87	
	Originality	r_{23}= 0.11		p= 0.61	
	Ratio	r_{23}= 0.07		p= 0.76	
	Flexibility	r_{23}= 0.10		p= 0.65	
	Elaboration	r_{23}= 0.03		p= 0.90	
Figural	PLMT	Patterns		Lines	
	Fluency	r_{24}= -0.08	p= 0.70	r_{24}= 0.08	p= 0.71
	Originality	r_{24}= -0.16	p= 0.46	r_{24}= 0.03	p= 0.88
	Ratio	r_{24}= -0.22	p= 0.30	r_{24}= 0.04	p= 0.86
	Flexibility	r_{24}= -0.23	p= 0.29	r_{24}= 0.03	p= 0.88
	Elaboration	r_{24}= -0.06	p= 0.77	r_{24}= 0.01	p= 0.95

Discussion

The present study aimed to assess the acute effects of cocaine on self-rated creativity and creative task performance and the association between potential behavioural drug effects and personal factors like mood *state* and *trait* empathy in poly-drug users. Based on cocaine's mechanism of action it was expected that creative performance would be impaired during cocaine intoxication. Findings showed a dissociation of cocaine effects on DT with impairment of verbal flexible DT and enhancement of figural DT. CT was in general unaffected by cocaine, only one task (TOL) showed drug-induced impairment for difficult figural stimuli compared to easy stimuli. Cocaine increased self-rated creativity and these ratings were negatively associated with CT, only on difficult figural items. With regard to personal factors it was found that cocaine significantly increased positive mood compared to placebo and this was positively associated with figural CT (TOL) response time and verbal DT (AUT). Higher levels of *trait* empathy were associated with enhanced verbal and figural DT when under the influence of cocaine. Interestingly, the anecdotal relationship between creative performance and cocaine intake reported by users was confirmed in the present study: when under the influence of cocaine, self-rated creativity levels were higher. However, this pattern was not reflected in the objective behavioural data, since cocaine enhanced figural DT only but it impaired verbal flexible DT and figural CT for difficult items, while performance on two other CT tasks was unaffected. These findings highlight the mismatch between subjective experiences and objective performance also demonstrated by other research with psychoactive substances.[9, 56]

The findings on objective measures were not completely in line with the hypotheses, which were based on the biological effects of cocaine and previous creativity research suggesting that high levels of dopamine are in general negatively related to creativity.[20, 24] Important here might be the role of personal factors which also played a role in the present study. Participants whose mood increased the most compared to placebo showed more beneficial effects of cocaine on figural CT and verbal DT performance. Participants who scored higher on *trait* empathy also showed greater positive effects of cocaine on verbal and figural DT. Although this is a highly interesting finding, some caution is needed here. Research has shown that females, score in general higher on *trait* empathy than males,[51] which might indicate that cocaine-induced effects

on DT are stronger in females than in to males. However, the current sample was too small to further explore the association between gender differences and *trait* empathy on creativity. In addition, while we only studied empathy as a personality trait, future studies might also include creativity as a personality trait since it has previously been shown that participants low in trait creativity benefited the most from stimulant-induced creativity enhancement.[57]

A strength of the present study is that creativity was tested with a broad range of tasks addressing verbal and figural, convergent and divergent thinking. In this light, the fact that cocaine only impaired performance on one CT task is interesting and is probably be due to task differences between the PCT and TOL and can perhaps be attributed to the basic cognitive processes that differ between tasks; the PCT asks for semantic associations while the TOL involves a strong spatial component. In addition, the direction of the cocaine effect on divergent thinking was dependent on the presented stimuli; i.e., whereas flexible thinking for figural stimuli was increased, flexibility was decreased when verbal stimuli were presented. A possible explanation for these findings might be sought in the underlying brain networks that are different for verbal and figural stimuli.[25-28] Furthermore, cocaine primarily affected flexibility in DT and while it was previously shown that a DA marker (eye-blink rate) selectively predicted flexible thinking,[24] it might be speculated that only this particular process is affected by DA changes while other outcome measures of DT are not. These findings underline the importance of using a large variety of tasks and stimuli in order to get a complete picture of the effects of a substance on a complex concept such as creativity.

Besides DA levels and personal factors, cocaine affects other neurotransmitters[12, 13] which might have played a role as well.[58] For instance, creativity was reportedly influenced by dexamphetamine,[59] a stimulant drug targeting not only dopamine, but norepinephrine as well.[60] Furthermore, in the study by Farah and colleagues,[59] effects of dexamphetamine on creativity performance were different when taking baseline performance into account; dexamphetamine was beneficial when participants scored low on baseline creativity, while the drug had detrimental effects when baseline creativity was high. Such baseline differences may be explained by baseline DA differences, as reflected in genetic polymorphisms. For example, an interaction between the catechol-O-methyltransferase (COMT) val/met and DA transporter

(DAT) polymorphisms was found to predict individual differences in creativity performance.[61] Regarding effects of cocaine, it is previously shown that cocaine effects on impulsivity are different depending on DBH genotype.[62] Hence, future studies might also include genetics to assess baseline differences in DA levels.

Although this study addressed an important question, only people with prior cocaine experience were included. This could limit the generalizability of the findings towards a drug-naïve population due to premorbid differences between these populations or changes caused in neural networks because of the recreational use of illicit substances.[63, 64] On the other hand, the level of education of the current sample was high which suggests the integrity of cognitive functions. Of interest here would be to include participants who differ in baseline cognitive functioning in future research, since it was previously shown that the cognition-enhancing effects of stimulant drugs is dependent on baseline performance with the highest gain for the low performers.[59] Another interesting point for further research relates to the question which role personal factors that are directly affected by cocaine play in the cocaine-induced effect on creativity. Since previous studies have shown that mood influences creative thinking[20, 25, 33-40] and the present study demonstrates that cocaine enhances positive mood; the possibility of positive mood being e.g. a mediator or moderator in the demonstrated effects on creativity cannot be substantiated. Future studies could include an extra condition in which mood is manipulated by a mood inducing technique and compare with positive mood induced by cocaine on behaviour. Furthermore, in order to understand the neurobiological underpinnings of the cocaine-induced creativity effects on neurotransmitter level, mechanistic studies can combine additional conditions in which cocaine is for instance combined with a dopamine and a serotonin receptor blocker. Previous studies have shown that besides dopamine, serotonin is also involved in creative thinking, with e.g. action of the 5-HT2A receptor leading to enhanced creativity.[41, 65]

To conclude, the present study demonstrated that a single dose of cocaine has different effects on creativity in poly-drug users, depending on a distinction between CT and DT as well as a difference in verbal and figural creativity tasks. Personal factors like positive mood *state* and *trait* empathy play an important role in the effects of cocaine on creativity, both showing to be

positively associated with enhanced divergent thinking. As creativity is shown as an important aspect in cognitive therapies,[66] it is important to further understand the influence of personal factors on creativity and understand the underlying role of neurotransmitters.

Conflict of interest

The authors declare no conflict of interest.

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CRediT authorship contribution statement

Nadia R.P.W. Hutten: Formal analysis, Writing - original draft, Writing - review & editing.

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Chapter 3

A single dose of cocaine enhances prospective memory performance

Hutten, N.R.P.W., Kuypers, K.P.C., van Wel, J.H.P., Theunissen, E.L., Toennes, S.W., Verkes, R.J., Ramaekers, J.G. *Journal of Psychopharmacology*, 2018; *32*(8), 883-892.

Abstract

Prospective memory is the ability to recall intended actions or events at the right time or in the right context. While cannabis is known to impair prospective memory, the acute effect of cocaine is unknown. In addition, it is not clear whether changes in prospective memory represent specific alterations in memory processing or result from more general effects on cognition that spread across multiple domains like arousal and attention. The main objective was therefore to determine whether drug-induced changes in prospective memory are memory specific or associated to more general drug induced changes in attention and arousal. A placebo-controlled, three-way, cross-over study including 15 regular poly-drug users was set up to test the influence of oral cocaine (300 mg) and vaporized cannabis (300 + 150 'booster' microgram/kg bodyweight) on an event-based prospective memory task. Attentional performance was assessed using a divided attention task and subjective arousal was assessed with the Profile of Mood States (POMS) questionnaire. Results showed that cocaine enhanced prospective memory, attention and arousal. Mean performance of prospective memory and attention, as well as levels of arousal were lowest during cannabis as compared with placebo and cocaine as evinced by a significantly increased trend across treatment conditions. Prospective memory performance was only weakly positively associated to measures of attention and arousal. Together, these results indicate that cocaine enhancement of prospective memory performance cannot be fully explained by parallel changes in arousal and attention levels and is likely to represent a direct change in the neural network underlying prospective memory.

Introduction

Acute effects of cannabis and cocaine on cognitive functions of recreational drug users have been repeatedly assessed in placebo controlled experimental studies. These studies have shown that a single dose of delta-9-tetrahydrocannabinol (THC) the principal psychoactive constituent of cannabis, impairs performance in laboratory tasks measuring executive function, impulse control, psychomotor performance,1, 2 attention,1, 3 and memory.3-5 Single doses of cocaine have been shown to impair impulse control, while improving psychomotor function1, 2 and attention.1 Acute effects of cocaine on memory however have not been studied extensively.6 Studies in dependent cocaine users7, 8 and recreational users9 revealed no influence of single doses of cocaine on memory.

Psychostimulants such as cocaine have been used as performance enhancers throughout history.[10] At present, increasing numbers of adults, particularly college students, are misusing psychostimulants primarily for cognitive enhancement.[11] Animal studies have demonstrated that single doses of psychostimulants such as methylphenidate[12, 13] and cocaine[14] enhance learning and memory. Human drug studies on memory enhancement following psychostimulant administration have mostly focused on tasks measuring retrospective memory, i.e. recalling past events or knowledge.[15, 16] Stimulant effects on prospective memory however have hardly been studied so far. Prospective memory involves the capacity and integrity of memory to encode, retrain, and recollect future intentions and actions such as remembering to call a friend, take medication, or go to a meeting, and differs from retrospective memory in that it involves self-initiated retrieval, sometimes cued by an event or time.[17-20] Chronic use of methamphetamine,[21] cocaine and MDMA[22-24] as well as single dose administrations of MDMA[25] have been associated with impairments of prospective memory. Likewise, sedative drugs such as alcohol and cannabis have also been associated with prospective memory deficits both after acute dose administrations[3, 26, 27] as well as after chronic use.[28-31]

Drug effects on prospective memory may represent specific alterations in memory processing or may result from more general effects on cognition that spread across multiple domains.[21] For example, drug induced reductions or increments in arousal and attention may indirectly lead to a decline or boost of memory performance, as high levels of arousal and attention have

been associated with enhanced prospective memory performance.[32, 33] Impairments in prospective memory as observed after acute doses of cannabis[3] and alcohol[26, 27] may thus result from decrements in attention and arousal, rather than from a decrement of memory processing per se. Likewise, memory enhancement as observed for stimulants[15, 16] may reflect an increase in concentration and arousal rather than an improvement in memory processes per se.

The current study was designed to assess the acute influence of single dose of cocaine and of cannabis on prospective memory and to assess whether drug induced changes in prospective memory is associated with drug induced changes in attention and arousal. In order to test these aims a double blind, placebo-controlled, cross-over study was designed. Drug effects on attention and arousal were assessed using a divided attention task and subjective measure of arousal. It was expected that after cannabis administration prospective memory would be impaired, while cocaine administration was expected to improve prospective memory performance.

Methods

Design

The study design was double-blind, placebo-controlled, within-subject with 3 treatment conditions consisting of placebo, 450 µg/kg THC and 300 mg cocaine HCl. The cannabis dose was divided over two doses of 300 and 150 µg/kg THC bodyweight (booster dose) with an interval of approximately 1.5 hours, in order to maintain THC concentrations throughout a 3 hour time window. For a timeline of the study design, see Figure 1. Cannabis was administered through a vaporizer (Volcano) obtained from Storz & Bickel GmbH & Co (Tuttlingen, Germany) and was used according to the manual provided by the producer. Cannabis vaporization is a technique by which cannabis plant material is heated to a temperature where active cannabinoid vapours form. This is considered a safe and effective way of administering cannabis.[34] The vapours are then collected in a detachable plastic balloon of 55cm length. The balloon can be removed from the device and fitted with a mouthpiece for inhalation. Participants were instructed to empty the balloon in 4-5 breaths. After each inhalation, participants had to hold their breath for 10 seconds before exhaling. The vapour was prepared from batches containing 11% THC, a standard potency for cannabis sold at Dutch pharmacies

for medicinal use. As placebo, a herbal plant mixture ('Knaster') was used. The density of the vapour captured in the balloon did not noticeably differ between THC and placebo. Cocaine HCl or placebo was administered in an opaque white capsule. Treatments were administered using a double dummy technique. Conditions were separated by a minimum wash-out period of 7 days to avoid cross-condition contamination. Order of conditions was balanced over participants and sessions.

Participants

The present study was part of a larger trial on the association between drug use and impulse control, of which a large part of the data has been published elsewhere.[2, 35, 36] Initially, 16 healthy poly-drug users from the large trial were included in this part of the study. However, due to non-compliance with the task instructions, one participant was removed from the final sample that entered the data analysis. Participants (14 males; 1 female) were aged 22.8 years (SD= 2.6) on average and with a mean weight of 67.7 (SD= 10.8). The details on their cannabis and cocaine use are depicted in Table 1. Cannabis use history across participants was somewhat equally divided, ranging from use on 1-24 (N= 4); 25-49 (N= 3); 50-74 (N= 3) and 75-100 (N= 5) occasions during the past three months based on cannabis use history groups from [1] Participants also reported a drug use history of ecstasy (60%), amphetamines (33%), mushrooms (47%), LSD (20%) and other drugs (20%).

Table 1. Mean (±SD) drug use in the past 3 months and total years of use.

	Use Previous 3 months				**Years of use**			
	Min	*Max*	*Mean (SD)*	*N*	*Min*	*Max*	*Mean (SD)*	*N*
Cannabis	10	100	50.13 (31.49)	15	3	14	6.5 (2.98)	14*
Cocaine	0	10	3.80 (0.83)	15	1	6	3.62 (1.94)	13*

**Missing data*

Participants were recruited through advertisements in local newspapers and by word of mouth. Before inclusion, participants were examined by a medical supervisor, who checked for general health and took blood samples and a urine sample for standard chemistry and haematology. Inclusion criteria were: written informed consent; age 18-40 years; regular cannabis and cocaine use defined as 2 times per week or more for cannabis and at least 5 times in the past year for cocaine; free from psychotropic medication; good physical health as assessed by a medical

doctor; normal weight as determined by BMI 18-28. Exclusion criteria were: addiction to cocaine according to DSM-IV criteria; presence or history of psychiatric or neurological disorder as assessed during a clinical interview; pregnancy or lactating; cardiovascular abnormalities; excessive drinking or smoking, and hypertension.

Procedures

Participants were familiarized with all tests and procedures during a training session, before the start of the actual test days. They were asked to refrain from all drugs of abuse (except cannabis) at least one week before study start until the last test day. They were asked not to use any caffeinated or alcoholic beverages 24h before testing and to get a normal night of sleep. All participants indicated that they had not smoked cannabis in the morning prior to testing. At 9 AM, prior to the experimental sessions they were screened for presence of drugs of abuse in urine (THC/opiates/cocaine/amphetamines/methamphetamines) and they had to pass a breathalyser ethanol test. Women were given a pregnancy test. When tests were negative (except for cannabis), participants filled out a questionnaire to assess sleep complaints, and had a light standard breakfast. After breakfast at 10 AM participants were administered a capsule containing either 300 mg cocaine HCl or placebo orally. Forty-five minutes after capsule administration, participants inhaled 300 µg/kg bodyweight cannabis or placebo. In between treatments, participants were allowed to read a book or watch television. After conducting laboratory tests, assessing impulsivity, psychomotor performance results published elsewhere[2] and divided attention (DAT at 11.45 AM) participants received a second 'booster dose' of cannabis or placebo (150 µg/kg bodyweight at 12.30 PM) and they proceeded with the second part of the study. After the 'booster dose', a visual analogue scale (VAS) assessing subjective high and the Profile of Mood States (POMS) was administered, followed by the prospective memory task. Blood samples were taken 3 times a day, at baseline, just before the prospective memory task (12:30 PM) and at the end of the test day (13:30 PM). Relative to cocaine and cannabis administration, the second blood sample was collected 2.5 hours post-cocaine administration and immediately after the 2nd cannabis vapour inhalation; the third blood sample was collected 3.5 hours post-cocaine administration and 1 hour after the second

cannabis administration. A schematic representation of the time course of a testing day is represented in Figure1.

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and subsequent amendments and it was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing and administering cocaine and cannabis was obtained from the Dutch drug enforcement administration. Participants signed an informed consent and were paid upon completion of the testing periods for their participation.

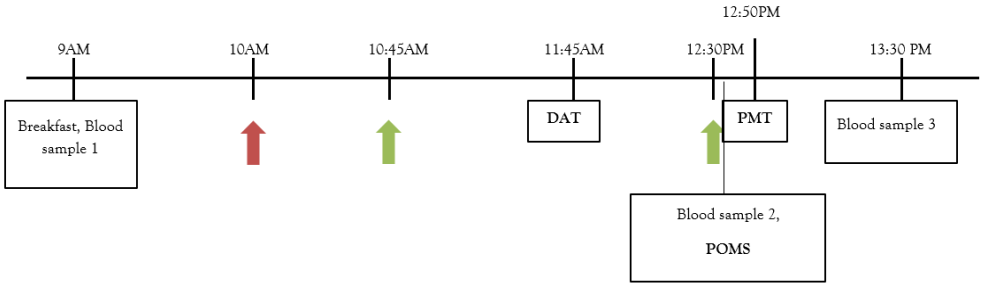

Figure 1. Timeline of the course of a testing day. The red arrow indicates the moment of cocaine (or placebo) capsule administration and the green arrows represent the THC (or Placebo) vapour administration.

Cognitive tasks

Prospective memory task

The event-based prospective memory task (PMT) assessed participants' ability to remember and execute upon the occurrence of a specific future event. Participants were engaged in a foreground task that consisted of pushing as quickly as possible one of two buttons in response to stimuli (letter A or B) presented on a screen. In total, 100 letters were presented with both letters presented equally often. Participants were also given a second prospective task, i.e., to withhold their response during trials that were part of a dynamic memory set. A trial counter that was always present in the left top corner of the screen informed the participants about the number of the trial. In addition, participants were presented at irregular times with a future trial number in the right top corner of the display. Participants were 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 subjects was dynamic and contained

up to three future trial numbers. A trial number in the memory set was replaced by a novel future trial number whenever the actual trial number matched a future trial number in the set. Trials during which participants were expected to respond were classified as Go trials. Trials during which subjects were instructed to withhold a response were classified as No-Go trials (prospective memory trials). The time between the presentation of a future trial number and the actual occurrence of the trial called 'the memory delay'; varied between 1, 2, and 3 minutes, equally divided over all No-Go trials. Each trial lasted for 12 seconds. However, the central letters disappeared upon a button press. Presentations of future trial numbers lasted 4 seconds. In total, the prospective memory task consisted of 68 Go trials and 24 No-Go trials. At the beginning and the end of the task, a total of eight trials were presented during which the memory set was empty. Percentage of correct inhibitions in No-Go trials was the primary performance parameter. Number of correct responses and corresponding reaction time during Go trials were the secondary performance parameters. The PMT lasted for 20 min. Three parallel versions of the PMT were developed for administration during the test sessions to avoid learning effects. The PMT was proven to be sensitive to the acute effects of MDMA.[25, 37]

Divided Attention Task.

The Divided Attention Task (DAT) assessed participants' ability to divide attention between two tasks performed simultaneously.[38] Participants were engaged in a tracking task that measured the ability to control a displayed error signal,[39] which was displayed as a horizontal deviation of a cursor from the midpoint on a horizontal, linear scale. Simultaneously, participants had to monitor 24 single digits which were presented in the corners of the computer screen. Participants were instructed to react to the target number '2' by removing their foot as fast as possible form a pedal switch. Mean absolute tracking error (in mm) and percentage of correct detections (hits %) of the target number were the performance measures.

Subjective measures

Arousal.

Arousal level was measured by the Profile of Mood States (POMS) questionnaire. Participants had to express their level of mood on a 5-point Likert scale, with 0 being 'not at all' to 4 'extremely'. The level of arousal was determined by using a composite score of four levels of

mood; anxiety (9 items), confusion (7 items), fatigue (7 items) and vigor (8 items); accordingly arousal level was calculated through combining the four levels of mood ((anxiety + vigor)−(fatigue + confusion)).[40]

Visual analogue scale (VAS).

Participants had to assess their levels of subjective high via a VAS (10 cm); with 0 indicating 'not high at all' and 10 indicating 'extremely high'.

Pharmacokinetic Assessments

Blood samples to determine cannabis and cocaine concentrations were taken at baseline, 2.5 hours and 3.5 hours after cocaine or placebo capsule administration. Blood samples were centrifuged at 3500 rpm and resulting serum and plasma were frozen at −20°C until analysis for pharmacokinetic assessments. The determination of Δ9-Tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-THC (THC-OH) and, 11-nor-9-carboxy-THC (THC-COOH) was performed in serum; determination of cocaine (COC), and its metabolites benzoylecgonine (BZE) and ecgonine methyl ester (EME) was performed in plasma. Determination took place in a specialized forensic-toxicological laboratory using validated procedures.[41, 42]

Statistical Analysis

Prospective Memory Task and Divided Attention Task data, subjective measures of Arousal and Subjective High, and blood concentrations were checked for normality, using Kolmogorov-Smirnov Tests of Normality. Normality tests per treatment condition showed that reaction time on Go-trials, percentage correct detections (hits %), VAS subjective high and baseline blood levels of THC and its metabolites were not normally distributed. In addition, normality test across all conditions showed that percentage correct in Go-trials, percentage correct in No-Go trials and percentage correct detections (hits %) were not normally distributed.

Normally distributed data were analysed by means of a General Linear Model (GLM) repeated measures Analysis of Variance (ANOVA) version SPSS 24.0 with Treatment (three levels, i.e., placebo, cocaine and cannabis) as the Within-Subjects (WS) factor. In case of main effects, subsequent treatment contrasts were performed. In case of a main Treatment effect, subsequent polynomial contrasts were added as a linear trend analysis across all treatments.

Non-normal distributed data was analysed with a non-parametric Friedman test to test the main effects of treatment, with subsequent Wilcoxon signed-rank test for treatment contrasts, in case of main effects.

A series of correlation analyses were conducted to assess relationships between prospective memory and arousal levels, prospective memory and attention performance (hits % and tracking error); and between arousal levels and attention performance (hits % and tracking error) over all conditions. In addition, correlation analyses were conducted to assess relationship between respectively cannabis and cocaine blood concentrations, and drug-placebo differences of prospective memory failures. The first correlation analysis provides information about the association between prospective memory and arousal and attention, and the association between arousal and attention. The latter provides information on the drug concentration in blood and the acute effects of both drugs on prospective memory performance. Correlation analyses of normally distributed data was analysed by Pearson correlation, while non-normal data distribution was analysed using Kendall's Tau-b correlation.

The alpha criterion level of statistical significance for all analyses was set at $p = 0.05$. Partial eta squared (partial η^2) is reported in case of significant effects to demonstrate the effect's magnitude, where 0.01 is defined as small, 0.06 as moderate and 0.14 as large. Partial eta squared is based on Cohen's *f* which defines small, medium and large as respectively 0.10, 0.25, and 0.50 which corresponds to η^2 of 0.0099, 0.0588, and 0.1379.[43]

Results

Missing data

Due to technical issues, computer responses were not registered resulting in missing data for the DAT in the placebo condition for one person and cocaine condition for another person. Some of the blood samples were missing due to inability to draw blood (see Table 2).

Prospective memory task

GLM repeated measures ANOVA revealed a main effect of Treatment on percentage correct inhibitions of No-Go's ($F_{2,28} = 9.47$, $p < 0.01$, $\eta_p^2 = 0.40$). Subsequent treatment contrasts revealed that percentage of correct inhibitions was higher following cocaine as compared with placebo

(p= 0.03) and as compared with cannabis (p< 0.01). Treatment contrast between cannabis and placebo was not significant. However polynomial contrasts revealed a linear trend across all treatments ($F_{1,14}$= 31.83, p< 0.01), indicating that correct inhibitions during No-Go trials were highest after cocaine, intermediate after placebo and lowest following cannabis treatment. Mean (± SE) performance scores of the percentage correct inhibitions are shown in Figure 2a.

Friedman test revealed Treatment effects on reaction time ($x^2(2)$= 9.73, p< 0.01) and on percentage correct in the Go trials ($x^2(2)$= 10.32, p= 0.01). Subsequent treatment contrasts revealed an impairing effect of cannabis on reaction time (Z= -2.39, p= 0.02) and percentage correct in the Go trials (Z= -2.29, p= 0.02) compared with placebo; and a significant impairing effect of cannabis as compared with cocaine on reaction time (Z= -2.33, p= 0.02) and percentage correct Go trials (Z= -3.02, p< 0.01). There was no significant difference comparing cocaine and placebo treatment on reaction time and percentage correct in Go trials. During cocaine treatment reaction time was fastest (M= 1065.8, SE= 106.02), placebo in between (M= 1179.3, SE= 72.14) and during cannabis responses were the slowest (M=1297.1, SE= 86.61). Percentage correct in the Go trials was highest after cocaine (M= 94.8, SE= 1.41), intermediate after placebo (M= 90.3, SE= 2.87) and lowest after cannabis administration (M=84.2, SE= 3.84).

Divided Attention Task

Friedman test revealed Treatment effects of percentage correct detections (hits %) ($x^2(2)$= 6.68, p= 0.04), subsequent treatment contrasts revealed that percentage of hits was higher during cocaine as compared with cannabis (Z= -2.04, p= 0.04). Treatment contrasts comparing placebo with cocaine and placebo with cannabis were not significant. GLM repeated measures ANOVA revealed a main effect of Treatment on tracking error ($F_{2,24}$= 4.08, p= 0.03, η_p^2=0.25), with subsequent treatment contrasts revealing a reduction in tracking error during cocaine as compared with cannabis (p= 0.01). Again, treatment contrasts comparing placebo with cocaine and placebo with cannabis were not significant. Polynomial contrasts revealed a linear trend across all treatments ($F_{1,12}$= 10.29, p= 0.01), indicating that tracking error was lowest after cocaine, intermediate after placebo and highest following cannabis treatment. Mean (± SE) performance scores for the divided attention task is shown in Figure 2b and 2c.

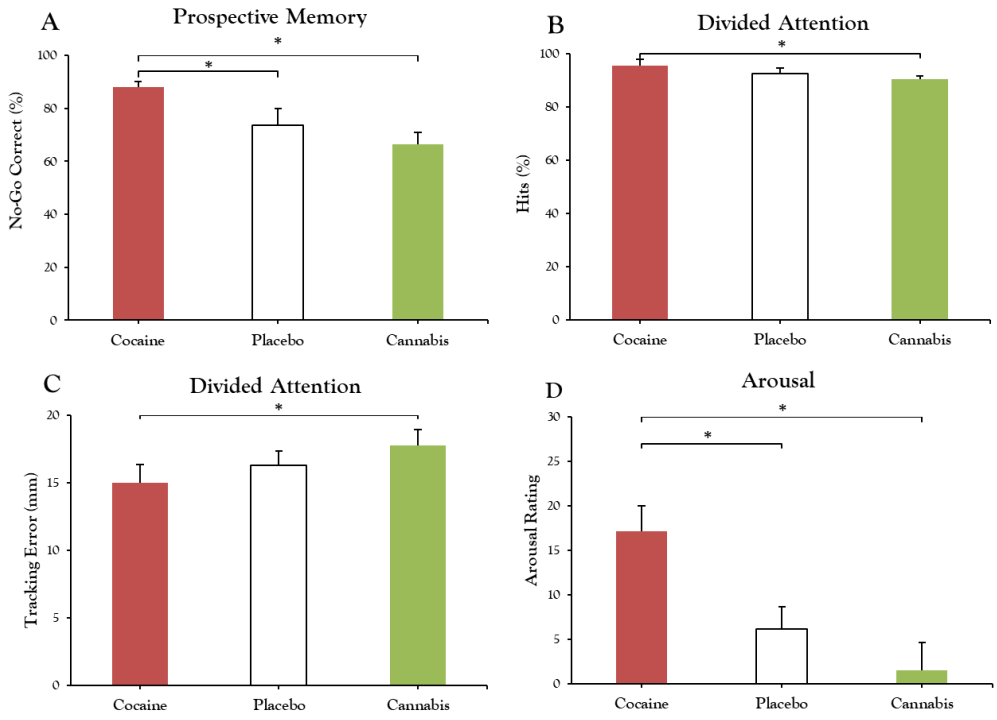

Figure 2. Mean (±SE) percentage correct inhibitions No-Go trials (A), percentage correct detections (B), tracking error (C), and arousal levels (D) after cocaine and cannabis administration and placebo. *significant differences between conditions with $P < 0.05$.

Arousal

Mean (± SE) Arousal levels in every treatment condition are shown in Figure 2d. GLM repeated measures ANOVA revealed a main effect of treatment on arousal levels ($F_{2,28}$= 9.71, p< 0.00, η_p^2= 0.41), subsequent treatment contrasts revealed higher arousal levels following cocaine administration as compared with placebo (p= 0.01) and as compared with cannabis (p< 0.01). Treatment contrast between cannabis and placebo was not significant. Polynomial contrasts revealed a linear trend across all treatments ($F_{1,14}$= 13.84, p< 0.01), indicating that arousal levels were highest after cocaine, intermediate after placebo and lowest following cannabis treatment.

Subjective high

Friedman test revealed Treatment effects of subjective high ($x^2(2)$= 15.17, p< 0.01), subsequent treatment contrasts revealed that both cocaine and cannabis increased subjective high relative

to placebo (Z= -2.20, *p*= 0.03; Z= -3.19, *p*< 0.01). Mean subjective high was higher during cannabis treatment as compared with cocaine (Z= -2.35, *p*= 0.02). Mean levels of subjective high was highest after cannabis administration (M= 5.7, SE= 0.70), intermediate following cocaine (M= 3.3, SE= 0.77) and lowest after placebo (M=1.3, SE= 0.26). Results show that both treatments were significantly intoxicated compared with placebo.

Blood concentrations

Blood concentrations during cocaine, cannabis and placebo treatments are shown in Table 2. The cannabinoid analyses revealed the presence of THC and its metabolites in all conditions at baseline which are a consequence of repeated cannabis use (see Table 1, [41]). Friedman test revealed no significant difference in levels of THC ($x^2(2)$= 2.25, p= 0.33), THC-OH ($x^2(2)$= 0.48, p= 0.79) and THC-COOH ($x^2(2)$= 1.11, p= 0.58) across all three conditions, indicating level of THC and its metabolites are similar for all three conditions at baseline.

Table 2. Mean (±SD) concentrations of THC (ng/mL), cocaine (mg/L) and their main metabolites; BZE= Benzoylecgonine; EME= Ecgoninemethylester.

Condition	Concentration	N	Baseline	N	Before PMT	N	End test day
Placebo	THC	13*	3.4 (7.6)	13*	2.3 (2.3)	13*	2.1 (3.0)
	THC-OH	13*	1.4 (2.7)	13*	0.9 (1.0)	13*	0.9 (1.2)
	THC-COOH	13*	42.7 (58.4)	13*	33.8 (43.7)	13*	36.3 (52.3)
Cannabis	THC	11*	3.3 (5.9)	11*	35.2 (21.3)	11*	8.3 (4.5)
	THC-OH	11*	1.1 (1.6)	11*	5.8 (2.7)	11*	3.3 (1.4)
	THC-COOH	11*	40.6 (51.6)	11*	50.7 (38.9)	11*	41.6 (33.4)
Cocaine	THC	13*	2.3 (3.3)	12*	3.1 (4.3)	12*	2.2 (3.1)
	THC-OH	13*	1.1 (1.6)	12*	1.2 (1.9)	12*	1.0 (1.5)
	THC-COOH	13*	31.2 (39.4)	12*	34.7 (49.6)	12*	30.53 (45.8)
	Cocaine	13*	0.0 (0.0)	13*	0.4 (0.2)	13*	0.1 (0.1)
	BZE	13*	0.0 (0.0)	13*	1.0 (0.3)	13*	1.2 (0.4)
	EME	13*	0.0 (0.0)	13*	0.3 (0.1)	13*	0.2 (0.1)

*Missing samples

Correlations

Kendall's Tau-b correlation analyses revealed significant but low associations between prospective memory and arousal (τ_b= 0.33; p= 0.01), tracking error (τ_b= -0.32; p= 0.04), and correct detections (τ_b= 0.26; p= 0.02); indicating that better prospective memory performance was somewhat associated with higher arousal levels and enhanced attention as shown in Figure 3. In the latter case, the correlation seemed to be driven by 3 outlying data points (see Figure 3c), which seemed like the results of one participant, however these data points represent 3 different participants. Pearson correlations between attention performance and arousal only revealed a low association between tracking error and arousal (r_{43}= -0.34; p= 0.03), indicating that higher levels of arousal are only slightly associated with the primary task variable (tracking) and not associated with the secondary task variable (correct detections) of attention.

Cocaine and THC concentrations in serum were not correlated to performance in the No-Go trials of the prospective memory task, indicating a homogenous participant sample.

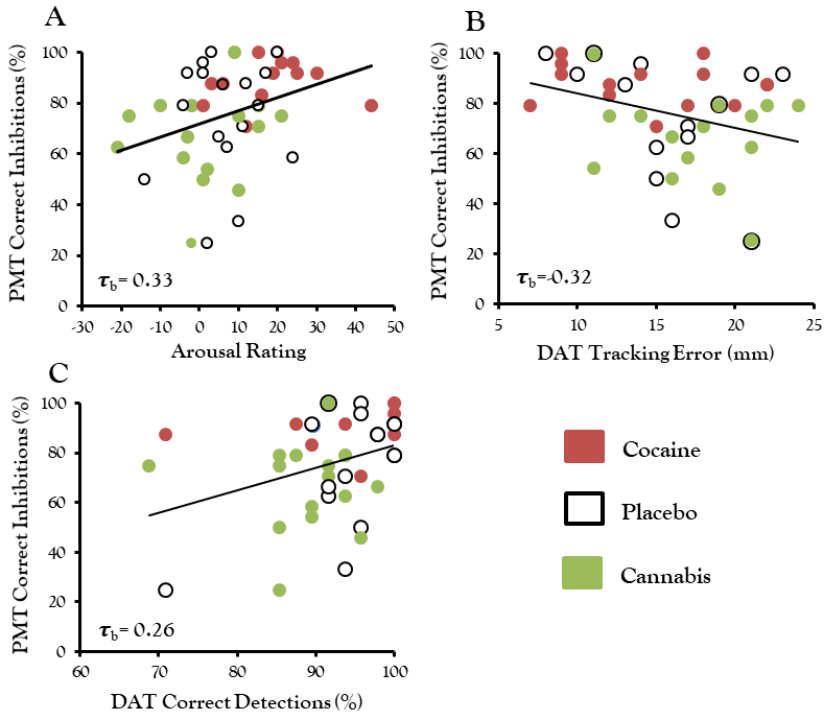

Figure 3. Scatterplots of percentage correct inhibitions No-Go trials as a function of arousal (A), tracking error (B), and correct detections (C). Correlations (τ_b) significant with $P < 0.05$.

Discussion

The current study aimed to assess the influence of single dose administrations of cocaine and cannabis on prospective memory and to determine whether cocaine and cannabis induced changes in prospective memory depend on changes in attention and arousal. Cocaine administration enhanced prospective memory performance relative to placebo and cannabis. Prospective memory performance was lowest after cannabis, in between after placebo and highest after cocaine administration, evinced by a linear trend across treatment conditions. Cocaine also improved performance on the primary (tracking) and secondary (correct detections) task of the divided attention test, relative to cannabis. Tracking performance was lowest in the cannabis condition but increased following placebo and cocaine. After cocaine administration, -subjective arousal levels were increased as compared with placebo and cannabis. Once again arousal levels were lowest in the cannabis condition and increased after placebo and cocaine. Only a small part of the enhancing effects of cocaine on prospective memory can be explained by underlying changes in arousal and attention.

The present study was the first to demonstrate that acute administrations of cocaine can enhance prospective memory. The present study however showed that prospective memory performance was only poorly associated with divided attention performance and arousal. Correlations between prospective memory performance and measures of attention and arousal ranged between τ_b -0.26-0.33. This indicates that these constructs only explained a very small portion of the variance observed in prospective memory performance. Memory enhancement as observed after cocaine in the present study therefore is more likely to have resulted from direct improvement of the prospective memory circuits rather than from a general increase in attention. This finding seems in line with animal studies showing that exposure to cocaine may directly enhance hippocampal function and memory. For example, it was demonstrated that cocaine induces a rapid increase in the formation and accumulation of new dendritic spines in the frontal cortex and that such changes in structural plasticity correlate with an increased ability to learn new stimulus related information.[44] Likewise, Muriach et al[45] demonstrated that cocaine facilitates the learning of new tasks in rats even tough retrieval of information learned prior to cocaine administration was impaired. Such improvement in memory and learning however does not automatically imply that cocaine should be used as a preferred cognition

enhancer. Indeed, many animal studies pointed out that precognitive effects of cocaine may play a role in drug seeking behaviour by strengthening the formation of maladaptive associations between drug use, context and cues.[46] Yet, the present data does suggest that cocaine induced cognition, and therefore enhancement or changes in synaptic plasticity may very well exceed the context of drug reinforcement learning.

Cocaine-induced enhancement of prospective memory might generalize to other domains of memory as well. For example, retrospective and prospective memory processes do not operate completely independent from each other which would allow transfer of procedural deficits in retrospective to prospective memory.[47, 48] Alternatively, individuals with a deficit in prospective memory do often display normal operations of retrospective memory.[49] This suggest that while both memory processes overlap, they are not necessarily interchangeable.[47, 50] Transferability of drug effects between different memory processes therefore may depend on whether a drug acts on a memory mode that is being shared by memory circuits. For example, it has been pointed out that performance on prospective and retrospective memory tasks in part relies on a similar retrieval mode that is located in BA10.[51] We do not know whether cocaine enhanced encoding, retrieval, or storage of information in our participants. Yet, in the case a common retrieval process was positively affected by cocaine, we would expect memory enhancement to appear both in retrospective as well as prospective memory tasks. What is evident is that prospective memory performance heavily relies on working memory processes as the task is very dynamic and requires continuous updating and retrieval of novel information. Working memory and prospective memory processes are not based on the same memory system, but prospective memory requires working memory resources at high demand.[52] Several studies have shown that prospective memory is related to individual working memory capacity.[53-55] As cocaine enhanced prospective memory, it can be expected that cocaine enhances working memory as well.

Memory enhancement has also been demonstrated for stimulant drugs with similar mechanisms of action as cocaine. Adults with ADHD treated with methylphenidate showed improved memory functions when compared with non-medicated patients across a range of memory domains, including prospective memory.[56] Similar effects have been reported in healthy volunteers. A review of methylphenidate studies in healthy volunteers[16] showed that single doses of methylphenidate enhance working memory in 65% of the included studies and

to a lesser extent verbal learning and memory in 31 % of the studies. Cocaine and methylphenidate are also being misused by healthy college students to enhance their study performances although the effectiveness of this approach is largely unknown.[11, 57] Cocaine and methylphenidate share the same dopamine as well as noradrenaline enhancing effects, by blocking dopamine and noradrenaline transporters.[58, 59] Additionally, cocaine also inhibits serotonin transporter. Similarities in their pharmacological profiles seem to indicate that the enhancement of dopamine and noradrenaline levels during cocaine treatment seem to underlie the neurobiological changes in memory.

The influence of cannabis on prospective memory and attentional performance were pretty much as expected. Previous studies already demonstrated that single doses of THC can significantly impair prospective memory[3] and attention.[1] In the present study, performance during cannabis and placebo did not significantly differ from each other when statistically compared with cannabis-placebo contrasts. However, mean performance during cannabis was always worse as compared with placebo, and the trend showing an increase in performance from cannabis, placebo to cocaine administration was highly significant. In addition, THC blood levels after cannabis administration in the present study (mean= 35.2, SD= 21.3) are about equal as blood levels of Theunissen et al[3] (mean= 30.7, SD= 27.4), indicating adequate blood levels were reached after cannabis administration. In the present study, the cannabis condition primarily served as an active control for the placebo condition to widen the coverage of the memory, arousal and attention performance ranging from poor to normal enhancement. As such, the inclusion of cannabis treatment increased the overall reliability of correlational analyses between measures of prospective memory and measures of attention and arousal.

Repeated cannabis use leads to residual concentrations of THC and its metabolites[41] as observed for baseline analyses in the present study. However, we do not expect that these baseline levels would interfere with test performances as these concentrations did not differ across treatment conditions. In addition, concentrations as determined here were found not to be relevant for the observed effects.[1, 60, 61] However, chronic use of cannabis can lead to memory impairments which can last for weeks, months or even years,[62] so stimulants like cocaine might reverse these deficits if present.

The present data revealed that prospective memory enhancing effects of cocaine can only be explained for a small part by enhanced attention and arousal levels. It might be argued that a low association between prospective memory and attention might have resulted from a lack of a very strong drug effects on attentional performance. Indeed, performance on the divided attention task primarily differed between drug conditions and not between drug and placebo treatment. Perhaps drug effects on attention would have appeared more prominent if the sample size had been bigger. However, the cocaine effect on prospective memory and arousal were very robust and significantly different from placebo. But also levels of arousal where only weakly associated to prospective memory performance despite the presence of a strong drug effect on both parameters. The latter therefore seems to indicate the validity of current observations. It would be advisable to replicate the current findings in a larger sample and perhaps with the inclusion of a broader range of memory and attention tests,[33] to further elucidate and differentiate the effects of cocaine.

In summary, current findings suggest that cocaine administration enhances prospective memory performance, while cannabis tends to impair prospective memory. Prospective memory performance was only weakly associated to measures of attention and arousal, suggesting that cocaine exerts a direct influence on memory processing. Replication studies are needed to examine whether the enhancing effects of cocaine are generalizable to other memory domains, and whether other aspects of attention play a role in these effects.

Conflict of interest

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

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Chapter 4

Mood and cognition after administration of low LSD doses in healthy volunteers: a placebo controlled dose-effect finding study

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Abstract

There is a popular interest in microdosing with psychedelics such as LSD. This practice of using one-tenth of a full psychedelic dose according to a specific dosing schedule, anecdotally enhances mood and performance. Nonetheless, controlled research on the efficacy of microdosing is scarce. The main objective of the present dose-finding study was to determine the minimal dose of LSD needed to affect mood and cognition. A placebo-controlled within-subject study including 24 healthy participants, was conducted to assess the acute effects of three LSD doses (5, 10, and 20 mcg) on measures of cognition, mood, and subjective experience, up until 6 hours after administration. Cognition and subjective experience were assessed using the Psychomotor Vigilance Task, Digit Symbol Substitution Test, Cognitive Control Task, Profile of Mood States, and 5-Dimensional Altered States of Consciousness rating scale. LSD showed positive effects in the majority of observations by increasing positive mood (20 mcg), friendliness (5, 20 mcg), arousal (5 mcg), and decreasing attentional lapses (5, 20 mcg). Negative effects manifested as an increase in confusion (20 mcg) and anxiety (5, 20 mcg). Psychedelic-induced changes in waking consciousness were also present (10, 20 mcg). Overall, the present study demonstrated selective, beneficial effects of low doses of LSD on mood and cognition in the majority of observations. The minimal LSD dose at which subjective and performance effects are notable is 5 mcg and the most apparent effects were visible after 20 mcg.

Introduction

Lysergic acid diethylamide (LSD) is a classical psychedelic substance used for recreational purposes[1, 2] and may have therapeutic potential in the treatment of psychiatric conditions like depression and anxiety.[3-6] Clinical studies have demonstrated acute changes in waking consciousness; such as perceptual distortions, increased blissful state and insightfulness, and enhanced mental wellbeing after a single full psychedelic dose of LSD (100-200 mcg) in healthy volunteers.[7-10]

Next to increased attention for the therapeutic potential of full psychedelic doses, the repeated use of low doses (microdosing) has gained general and scientific interest.[11, 12] A microdose is suggested to be one-tenth of a regular consumed dose;[13, 14] small enough not to disturb daily life activities, but large enough to enhance cognitive or emotional processes and wellbeing.[15] Analyses of blotters containing LSD in Brazil (22 samples) and the Netherlands (250 samples) revealed that the average dose of LSD in one 'hit' was respectively 67 mcg LSD tartrate and 85 mcg LSD base, with the latter corresponding with 106 mcg tartrate.[16, 17] The findings suggests that, when users consume 1/10th of their blotter to microdose, they are taking on average between 6-10 mcg of LSD tartrate. However, a recent survey in users suggests that the dosing window is broader, including doses between 1.4–50 mcg LSD.[18] This broad range potentially indicates either that users have little control and knowledge over the doses they take,[19] or the optimal dose to achieve the desired effect differs between individuals.[20] This inter-individual dose-response variability has previously been shown with other psychopharmacological agents like caffeine, alcohol, methylphenidate, and amphetamines.[21-25]

Evidence about mood- and cognition-enhancing effects of microdosing with LSD is mainly limited to anecdotal reports,[19, 26-28] and some older experimental studies that show for example a dose-response increase in mood state and psychomotor tension following a dose of 4 to 40 mcg LSD.[29] Recently, three clinical placebo-controlled studies in healthy volunteers were published showing increased connectivity between the amygdala and middle frontal gyrus (13 mcg LSD tartrate),[30] increased ratings of vigor, the experience of unity and blissful state (26 mcg LSD),[31] and temporal dilation of supra second intervals (10 mcg LSD tartrate).[32] Furthermore, a dose-related reduction in subjectively rated vigilance was shown after 5 to 20

mcg LSD tartrate.[33] None of these studies showed changes in higher-order cognitive performance after a single LSD dose (6.5-26 mcg tartrate).[31-33] However, preliminary findings in a relatively small sample of patients with obsessive-compulsive disorder showed reduced symptoms after a very low dose of psilocybin (25 mcg/kg),[34] suggesting that low doses of a classical psychedelic might improve cognitive control.

Nevertheless, more placebo-controlled studies are needed to examine the effects of low doses of LSD on mood and cognition. While the present study was designed before the two other research groups published their findings on low LSD doses, performance, and mood,[30-33] the primary aim of this study is therefore partly similar. Using a crossover placebo-controlled design including three different low oral doses of LSD (5, 10, and 20 mcg base), we aimed to determine whether, and at which dose, changes in mood, subjective experience, and cognitive processes occur after LSD administration. In addition, we explored the inter-individual variation in subjective and cognitive responses to low doses of LSD. Based on anecdotal reports and previous studies, it was expected that low doses of LSD would increase attention,[19] cognitive control,[34] and positive mood.[30]

Experimental procedures

Design

This study was conducted according to a double-blind, placebo-controlled, within-subject design with four oral doses of 0, 5, 10, and 20 mcg of LSD hydrate on separate test days. Treatment order was randomized across 24 participants. Treatment days were separated by a minimum washout period of 5 days to avoid potential carry-over effects. LSD doses were dissolved in ethanol (96% Vol), resulting in a 1 mL volume on every test day to ensure treatment blinding. The placebo solution consisted of 1 mL of ethanol only.[35]

Participants

Participants were 24 healthy recreational psychedelic drug users (12 males; 12 females), aged 22.8 years on average (*SD*= 3.0). Participants were following higher education, of which one participant completed this level of education. The majority was Caucasian (*N*=21); one participant was half Caucasian, half African American; one participant was Hispanic, and one

participant was Indian. All participants had experience with at least one psychedelic substance: i.e., 2Cs (*N*= 3), DMT (*N*= 1), ketamine (*N*= 1), LSD (*N*= 12), MDMA/ecstasy (*N*= 15), psilocybin (*N*= 20), and salvia (*N*= 1). The mean frequency of psychedelic use in the year before the study was 2.75 times (*SD*= 4.15). Participants (%) also reported previous use of cannabis (96%), amphetamines (29%), cocaine (42%), nicotine (21%), and alcohol (100%).

Procedures

Participants were recruited through advertisements in university buildings in Maastricht, via social media and by word of mouth. Before inclusion, participants were asked to read the information brochure where the study aims, procedures, and design were explained,[36] and they underwent a medical screening by a physician. General health was checked, and blood and urine samples were taken for standard blood chemistry, hematology, and urinalysis.

Inclusion criteria were written informed consent; age 18-40 years; previous experience with a psychedelic drug, but not within the past three months; proficient knowledge of the English language; good physical and mental health as determined by medical history and medical examination; free from psychotropic medication; BMI between 18-28 kg/m^2.

Exclusion criteria were history of drug abuse or addiction as assessed using the DSM-IV criteria; a history of psychiatric and neurological disorders; previous experience with serious side effects to psychedelic drugs (e.g., anxiety or panic attacks); cardiovascular abnormalities; hypertension; psychotic disorder in first-degree relatives; tobacco smoking (> 20 cigarettes/day; excessive alcohol (> 20 alcoholic consumptions/week); pregnancy or lactation.

A training session took place before the test days in order to familiarize participants with the tests and test procedures and to train them to perform with 100% accuracy on a rule-based cognitive control task (CCT).

Participants had to refrain from psychedelic substance use three months before study start, and from other drug use at least one week before study start, until completion of all four testing days. Participants were requested to not consume caffeinated or alcoholic beverages after midnight of the evening before the test days, as well as during the test days. On the test days,

smokers were asked to refrain from nicotine use 2 hours before the start of the test days as well as during the test days. All participants were expected to arrive well rested at the test facilities at 9 AM.

Participants spent the whole day in a quiet room with a window, equipped with a bed, a table, and chairs. All the assessments were conducted in this room. At arrival, participants were screened for the presence of alcohol in breath, drugs of abuse in urine (THC/ opiates/ cocaine/ amphetamines/ methamphetamines), and women were tested for pregnancy. When tests were negative, participants filled out the Groninger Sleep Scale (GSS) to assess sleep quality and quantity of the previous night, baseline subjective measures were taken (Visual Analogue Scales and Profile of Mood States), and it was checked whether participants still performed with 100% accuracy on the CCT. At 10 AM, drug administration took place. Thirty minutes to 6 hours post-administration a test battery consisting of questionnaires and computer tasks was conducted, and blood samples were taken (Table 1). The test battery included additional measurements of creativity and empathy,[37] pain perception,[38] pharmacokinetics and pharmacodynamics,[39] and neuroplasticity.[40]

Table 1. Schematic representation of the measurements on the test days.

	Time relative to drug administration (hours)										
Measurements	-1	0	0.5	1	1.5	2	2.5	3	4	5	6
Drug administration		X									
PVT, DSST						X			X		
CCT 'refresher' and change of rules	X										
CCT							X				
POMS	X			X		X		X		X	
VAS	X		X	X	X	X	X	X	X	X	X
5D-ASC, EDI											X
Blood samples	X		X	X	X	X		X	X		X

Note: PVT: Psychomotor Vigilance Task; DSST: Digit Symbol Substitution Test; CCT: Cognitive Control Task; POMS: Profile of Mood States; VAS: Visual Analogue Scale; 5D-ASC: 5-Dimensional Altered States of Consciousness Rating Scale; EDI: Ego Dissolution Inventory.

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki and subsequent amendments.[36] It was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht

University and registered in the Netherlands Trial Register (number: NTR7102 https://www.trialregister.nl/). A permit for obtaining, storing, and administering LSD was obtained from the Dutch Drug Enforcement Administration. Participants received monetary compensation per hour invested.

Cognitive tasks

Psychomotor Vigilance Task

The Psychomotor Vigilance Task (PVT) is a sustained attention task.[41] Participants had to press a response button as quickly as possible at the onset of a visual stimulus that was presented 100 times at random time intervals for 10 minutes. Outcome measures were mean reaction time (milliseconds) and the number of attention lapses. An attention lapse is the failure to react, or a reaction time exceeding 500 msec.

Digit Symbol Substitution Test

The Digit Symbol Substitution Test (DSST) is a computerized version of the original paper and pencil test taken from the Wechsler Adult Intelligence Scale[42] and was used to assess information processing. This task measures a variety of processes like motor speed, attention, working memory, and visual processing.[43] The participant had to match each digit with a symbol from the encoding list as rapidly as possible for three minutes. Outcomes measures were the number of correctly encoded digits, and the quotient of the number of correct encoded digits and the total encoded digits (ratio).

Cognitive control task

The Cognitive Control Task (CCT) was used to assess participants' level of cognitive control, that is, the ease of shift between habitual and goal-directed behavior.[44] During a training session, before the test days, participants acquired habit-like responding to visual stimuli. Visual stimuli consisted of six different squared patterns on a wooden box. Each pattern corresponded to a key-press (left or right), and a food item (sweet or salty) inside the box. Participants learned, by trial and error, to respond to squared patterns on a computer screen by either pressing left or right key to open the box, and to reveal a food item inside, which was

the reward. If the response was inaccurate, an empty box was shown. The training consisted of 16 blocks of 24 trials. Each block was followed by a questionnaire to assess their insight into the correct associations, i.e., the rules they had to follow to get a reward. When the questionnaire indicated their insight was not 100% accurate by the end of the training session, the training continued until the 100% accuracy level was reached.

A 'refresher' of the rules was given each test day before dose administration, to ascertain accurate responding. The accuracy level was tested with the questionnaire (100% correct). Two and a half hours after drug administration, the participants were told that the rules had changed for half of the stimuli ('devalued' stimuli) and that they had to press the opposite key when presented devalued stimuli. For the other half of the stimuli ('valued' stimuli) the response key did not change. This was a test for goal-directed responding (cognitive control) and consisted of 6 blocks of 24 trials.

The number of correct responses and the reaction time to the stimuli presented as a refresher were used to assess possible baseline differences between dosing days. The outcome measure of cognitive control was the devaluation ratio, the quotient of the percentage of correct responses to valued and devalued items. A score of 100 indicates high cognitive control, whereas a score of zero indicates habitual responding.[44]

Subjective measures

Profile of Mood States

The Profile of Mood States (POMS) is a self-assessment mood questionnaire with 72 adjectives describing specific mood states. Participants had to indicate to what extent the items represented their current mood, on a five-point Likert scale, with zero being "not at all", to four "extremely". Eight mood states (anxiety, depression, anger, vigor, fatigue, confusion, friendliness, and elation) were deducted by calculating the sum score of associated items. Two composite scales were derived, arousal ((anxiety-vigor)-(fatigue+confusion)) and positive mood (elation – depression).[45]

Visual Analogue Scales

Participants had to indicate on nine Visual Analogue Scales (VAS) how they felt by putting a mark on each 10-cm long horizontal scale. There were five statements and four adjectives presented. The statements were: I feel under the influence; Good drug effect; Bad drug effect; I like the drug; and I am feeling high. Each statement had two anchors, which were zero indicating "not at all" and ten "extremely". The adjectives were: Happy, Concentration, Productive, and Time awareness, and had three anchors: -5, 0, and 5 indicating "not at all", "normal", and "extremely". In case of 'Time awareness' "-5" indicated slowed down time perception, "+5" accelerated time perception.

5-Dimensional Altered States of Consciousness Rating Scale

The 5-Dimensional Altered States of Consciousness Rating Scale (5D-ASC) is a 94-item self-report scale that assesses participants' alterations from normal waking consciousness.[46] The participants had to indicate to what extent the statements apply to their own experience compared to normal, by putting a mark on a vertical 10-cm VAS with anchor ends representing the two extremes of the experience; 0 indicating "No, not more than usually" and 10 "Yes, much more than usually".

5D-ASC items are clustered into five key dimensions and eleven associated subscales. Oceanic boundlessness (OB) and related scales: the experience of unity, spiritual experience, blissful state, and insightfulness. Anxious ego dissolution (AED) and related scales: disembodiment, impaired control and cognition, and anxiety. Visionary restructuralization (VR) and related scales: complex imagery, elemental imagery, audio-visual synesthesia, and changed meaning of percepts. The Auditory alterations (AA) and Reduction of vigilance (RV) do not have any subscales. The scores on the five key dimensions, 11 subscales and total score (summed score of the five key dimensions) were taken as outcome measures.

Ego Dissolution Inventory

The Ego Dissolution Inventory (EDI) is an eight-item self-report scale.[47] The participants were asked to mark on a vertical 10-cm line to what extent they had experienced these statements ranging from zero "No, not more than usually" to 10 "Yes I experience this

completely/entirely". For example, one statement being: "I experienced a dissolution of my "self" or ego". The outcome measure (EDI score) was the mean of the scores on the eight items.

Control measures

The Groninger Sleep Scale

The Groninger Sleep Scale (GSS)[48] consists of fifteen dichotomous questions about sleep complaints and one open-ended question about the duration of sleep ('sleep quantity'). The scores on the 15 questions are summed; the resulting score is a measure of sleep quality with zero indicating excellent sleep and 14 poor sleep.

LSD plasma concentrations

Blood samples were centrifuged, and pipetted plasma was frozen at −20°C until analysis for pharmacokinetic analyses. LSD concentrations were determined using ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) as previously described.[49] A different extraction procedure reanalyzed PK samples with an LSD concentration below 5 pg/mL. In brief, aliquots of 150 µL of plasma were extracted with 450 µL methanol. The samples were rigorously mixed and subsequently centrifuged. The supernatant was evaporated under a constant stream of nitrogen and resuspended in 200 µL of mobile phase A and B (10:90 v/v). This extraction reached an LLOQ of 2.5 pg/mL.

Data and Statistical Analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology. Data were analyzed with the statistical program SPSS (version 24.0). Data of the POMS and VAS were baseline-corrected before entering the statistical analyses.

In order to test for main Dose effects, and Dose interaction effects with Participant and Time, data of the PVT, DSST, POMS and VAS was analyzed using a General Linear Model (GLM) Univariate Analysis of Variance (ANOVA) including Dose (4 levels) and Time as fixed factors, and Participant (24 levels) as a random factor. The factor Time had two levels for PVT and DSST, four levels for POMS, and nine levels for VAS. Univariate ANOVA takes into account

all available data, allows for missing values, and estimates fixed effects while adjusting for correlation due to repeated measurements within participants.[50]

In case of a main Dose effect, Bonferroni-corrected Dose contrasts (LSD vs placebo) were conducted to determine the effect of each dose on the respective outcome measure. In case of a Dose by Participant interaction, placebo-LSD difference scores entered binomial tests to investigate the proportion of observations (%) that showed improvement (1) versus impairment (0) on the dependent variable. Observations containing no change were not included in the binomial analysis. The test proportion was set at 50%; a statistically significant test result would indicate that the proportion of observations showing improvement after administration of a specific dose of LSD was higher than 50% ('chance level').

The GSS, 5D-ASC, EDI, and CCT were assessed once each test day and analysed using GLM repeated measures (RM) ANOVA, with Dose as within-subjects factor. Missing values were replaced within a Dose condition. In the 5D-ASC, the participant's average of the remaining items in that sub-scale was used as item imputation; in the CCT the average item score of the total sample was used for unit imputation.

If sphericity was violated, the Greenhouse–Geisser (G-G) correction was used. The alpha criterion level of statistical significance for all analyses was set at $p = 0.05$. Partial eta squared (partial η^2) is based on Cohen's f which defines small, medium, and large as respectively 0.10, 0.25, and 0.0, which corresponds to η^2 of 0.01, 0.06, and 0.14.[51] It is reported in case of statistically significant effects to demonstrate the effect's magnitude.

Results

For details of missing values, see Supplementary material.

Cognitive tasks

Psychomotor Vigilance Test

GLM univariate ANOVA revealed no main Dose effect but an interaction effect between Dose by Participant on number of attentional lapses ($F_{69, 92}$= 5.19, p< 0.01, ηp^2 = 0.80) and mean reaction time ($F_{69, 92}$= 6.09, p< 0.01, ηp^2 = 0.82). The majority of observations on attentional

lapses after 5 mcg (77%), 10 mcg (77%) and 20 mcg (81%) did show a change from placebo and entered the binomial tests. Binomial tests indicated that the proportion of observations containing fewer attentional lapses after administration of 5 and 20 mcg LSD was respectively 76% ($p< 0.01$) and 74% ($p<0.01$), while this was 38% ($p= 0.19$) after 10 mcg LSD. All observations on reaction time after LSD administration differed from placebo and entered the binomial tests. The binomial tests showed a non-significant proportional reduction in reaction time after administration of 5 (65%; $p=0.06$), 10 (60%; $p=0.19$) and 20 mcg (54%; $p=0.66$) of LSD. Mean (SE) number of lapses and reaction time per dose condition is shown in Figure 1A-B.

There was no main Dose effect or Dose by Time interaction effect on the number of attentional lapses or on reaction time (Supplementary Table 1).

Digit Symbol Substitution Test

GLM univariate ANOVA revealed a main Dose effect and Dose by Participant interaction on total number of correctly encoded digits ($F_{3, 69}= 4.38$, $p< 0.01$, $\eta_p^2 = 0.16$; $F_{69, 91}= 2.69$, $p< 0.01$, $\eta_p^2 = 0.67$). Dose contrasts revealed that participants encoded fewer digits correctly after the 20 mcg dose compared to placebo ($p< 0.01$). The majority of observations after 5 mcg (94%), 10 mcg (100%) and 20 mcg (98%) did show a change from placebo and entered binomial tests. Binomial test indicated that the proportion of observations showing a reduction in number of correctly encoded digits after 20 mcg LSD was 79% ($p< 0.01$), while this was 49% ($p= 1.00$) and 52% ($p= 0.89$) after the 5 and 10 mcg respectively.

When the number of correctly encoded digits was corrected for the number of total responses (ratio), there was no Dose effect, indicating that even though participants encoded fewer digits after administration of 20 mcg LSD, they were not less accurate (Supplementary Table 1). Mean (SE) number of correctly encoded digits and ratio per dose condition is shown in Figure 1C-D.

There was no Dose by Time interaction on either outcome variable, and no Dose by Participant interaction on ratio (Supplementary Table 1).

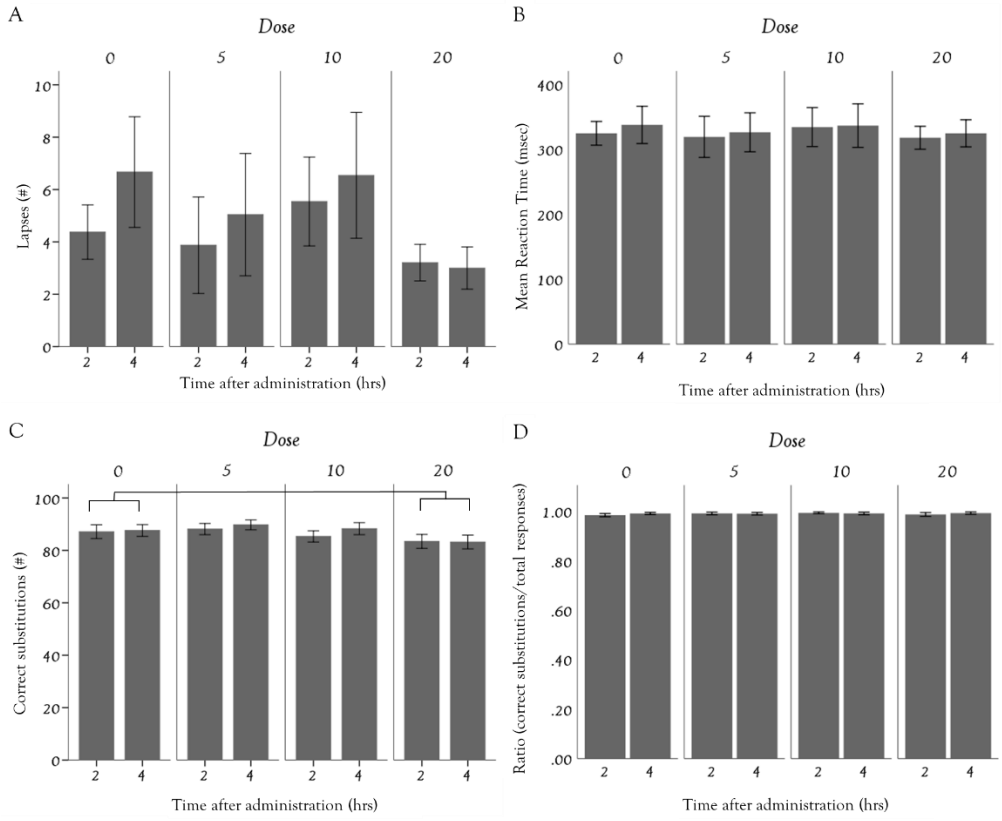

Figure 1. The mean (SE) number of lapses (A) and reaction time (B) of the PVT, and number of correct substitutions (C) and ratio (D) of the DSST per dose condition and time of testing. *signifies statistically significant placebo-LSD contrast ($p \leq 0.05$).

Cognitive Control Task

GLM RM ANOVA did not show a significant baseline difference in performance on the cognitive control variables of the CCT, indicating that all participants remembered the correct responses and had comparable speed of responding in the morning of the different test days (Supplementary Table 1).

G-G corrected GLM RM ANOVA revealed no main Dose effect on the devaluation ratio (Supplementary Table 1), mean (SE) scores of the CCT are shown in Table 2.

Table 2. Mean (SE) of outcome measures of the Cognitive Control Task (CCT) per dose condition

	Placebo	5 mcg	10 mcg	20 mcg
Refresher				
Number correct	33.79 (0.36)	34.63 (0.35)	34.36 (0.25)	34.42 (0.28)
Mean Reaction Time (sec)	2.53 (1.21)	1.62 (0.87)	3.71 (2.60)	3.40 (1.93)
Cognitive Control				
Devaluation Ratio	91.49 (1.62)	87.09 (3.25)	86.75 (2.45)	81.16 (4.71)

Subjective measures

Profile of Mood States

GLM univariate ANOVA revealed significant Dose by Participant interaction effects on all mood states (Supplementary Table 2). Mean (SE) scores are presented in Table 3. Binomial tests indicated that the proportion of observations showing a decrease in anger (10 mcg) and depression (20 mcg) was significantly higher than the proportion of observations that showed an increase in anger and depression, however only half of the total observations showed a change from placebo and have entered these binomial tests (Supplementary Table 3). Furthermore, binomial tests indicated that the proportion of observations showing an increase on the subscales anxiety (5 mcg and 20 mcg), arousal (5 mcg), confusion (10 mcg), friendliness (5 mcg and 20 mcg) and positive mood (20 mcg) was significantly higher than the proportion that showed a decrement on these mood scales. These binomial tests included the majority of observations showing a change from placebo (Supplementary Table 3). There was no significant difference in the proportion of observations that showed an increased or decreased score from placebo on the other mood scales (Supplementary Table 3)

There was no main Dose effect, nor significant Dose by Time interaction on any of the mood states (Supplementary Table 2).

Visual Analogue Scales

GLM univariate ANOVA revealed significant Dose by Participant interaction effects on all nine VASs, and main Dose effects on six VAS-items: I feel Under the influence, High, Good drug effect, Bad drug effect, Liking, and Concentration (Supplementary Table 2). There were

Dose by Time interaction effects on feeling Under the influence, High, Good drug effect, Bad drug effect, Liking, Happy and Productive (Supplementary Table 2).

Dose contrasts revealed that after LSD (10 and 20 mcg) participants felt more Under the influence, more High, scored higher on Good drug effect and Bad drug effect, and Liking (all *p*-values <0.01), while Concentration was rated less after LSD (20 mcg) compared to placebo ($p < 0.01$) (Figure 2A-F). Mean (SE) scores are presented in Table 3.

After both 10 and 20 mcg doses, the majority of observations on the VAS statements showed a change from placebo and entered the binomial tests, except for Bad drug effect where only 34% (10 mcg) and 41% (20 mcg) showed a change from placebo. Binomial tests indicated that the majority of the observations showed increased ratings on Under the influence, High, Good drug effect, Bad drug effect and Liking (Supplementary Table 3). Furthermore, a majority of observations showed an increase in concentration after the 10 mcg LSD dose (59%, $p=0.04$), while the majority showed a decrease in concentration (63%, $p<0.01$) and productivity (61%, $p<0.01$) after the 20 mcg dose. In addition, the majority of observations showed an increase in happiness after 20 mcg (63%, $p<0.01$). There was no significant difference in the proportion of observations that showed an increased or decreased change from placebo on the other VAS scales and on any VAS scales after the 5 mcg dose (Supplementary Table 3).

No main Dose effects or significant Dose contrasts were found for Happiness, Productivity, and Time awareness (Supplementary Table 2). There were no Dose by Time interaction effects for Concentration and Time awareness (Supplementary Table 2).

5-Dimensional Altered States of Consciousness Rating Scale

G-G corrected GLM RM ANOVA revealed a statistically significant main effect of Dose on Total 5D-ASC score, and on four main dimensions: OB, AED, VR, and RV (Supplementary Table 2). Dose contrasts revealed that scores on these dimensions were higher after the 20 mcg dose compared to placebo (all *p*-values < 0.01). The 10 mcg dose only enhanced scores on the AED dimension compared to placebo ($p= 0.04$). There was no main Dose effect on the AA dimension. Mean (SE) scores of the Total 5D-ASC score and the five main dimensions are shown in Figure 2 (Panel G).

There was a significant main Dose effect on five sub-scales: insightfulness, impaired control and cognition, anxiety, complex imagery, and changed meaning of percepts (Supplementary Table 2). Dose contrasts revealed that scores on insightfulness (p= 0.02), impaired control and cognition (p< 0.01), and changed meaning of percepts (p< 0.01) were higher after LSD (20 mcg) compared to placebo; Dose contrasts for the subscales anxiety and complex imagery were not significant after Bonferroni-correction. Mean (SE) scores of the subscales are shown in Table 3.

There was no main Dose effect on the subscales: experience of unity, spiritual experience, blissful state, disembodiment, elemental imagery, and audio-visual synesthesia (Supplementary Table 2).

Ego Dissolution Inventory

G-G corrected GLM RM ANOVA revealed no main Dose effect on the mean score of the EDI ($F_{1.93,44.48}$= 0.73, p= 0.48, ηp^2 = 0.03) (Table 3).

Control measures

The Groninger Sleep Scale

GLM RM ANOVA revealed no significant difference in sleep quality ($F_{3,69}$= 2.19, p= 0.10, ηp^2 = 0.09) and sleep quantity ($F_{3,66}$= 1.65, p= 0.19, ηp^2 = 0.07) the nights before the four test days. Participants slept on average 7.2 hours (SD = 1.5), and had a sleep quality score of 1.4 (SD= 1.5).

LSD plasma concentrations

Due to difficulties with the peripheral venous catheter, blood sample collection resulted in incomplete data sets. Mean (SD) LSD plasma concentrations were 166 pg/mL (51.98), 281 pg/mL (85.44), and 516 pg/mL (196.52) at 1 hour after LSD administration respectively for 5 mcg (N= 14), 10 mcg (N= 18) and 20 mcg(N= 16). The LSD plasma concentrations decreased over time to 53 pg/mL (18.42), 107.99 pg/mL (45.38), and 223 pg/mL (101.81) at 6 hours after LSD administration respectively for 5 mcg (N= 14), 10 mcg (N= 15) and 20 mcg (N= 14).

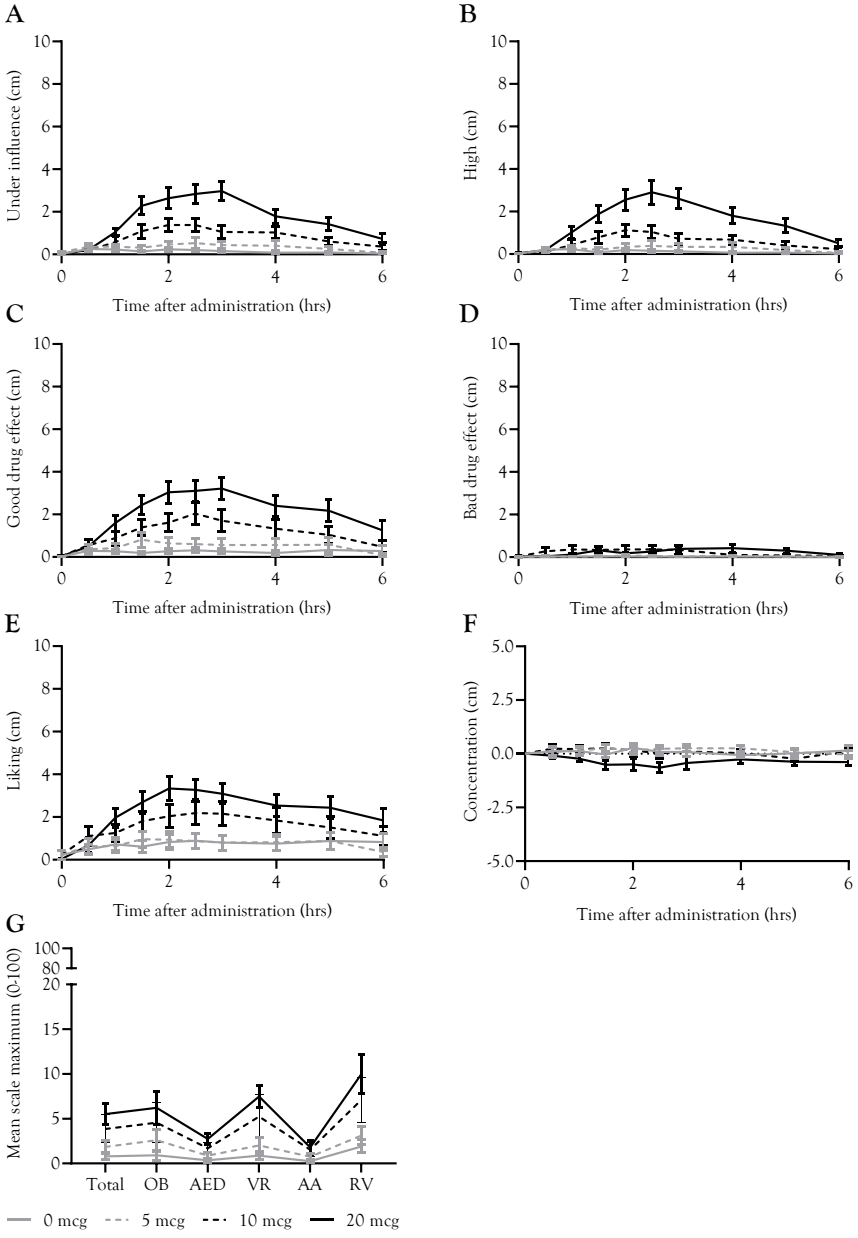

Figure 2. Panels A-F show mean (SE) scores of the VAS: feeling under the influence (A), high (B), good drug effect (C), bad drug effect (D), liking (E), and concentration (F), as a function time after dose administration for the four Drug conditions. Panel G shows means (SE) scores of the total 5D-ASC score, and the five main dimensions: Oceanic Boundlessness (OB), anxious ego dissolution (AED), visionary restructuralization (VR), auditory alterations (AA), and reduction of vigilance (RV) per dose.

Table 3. Mean (SE) scores of subjective states; the baseline-corrected scores of the POMS and VAS, mean scores of5D-ASC (%) and EDI (cm), per dose condition

POMS	**Placebo**	**5 mcg**	**10 mcg**	**20 mcg**
Anger	-0.11 (0.15)	-0.16 (0.12)	-0.79 (0.31)	-0.76 (0.40)
Anxiety	-0.90 (0.34)	0.18 (0.24)	-1.02 (0.55)	0.30 (0.60)
Arousal	-2.16 (0.93)	0.25 (1.44)	-2.38 (1.15)	-1.02 (2.11)
Confusion	0.22 (0.30)	-0.03 (0.40)	0.72 (0.45)	1.49 (1.49)
Depression	-0.18 (0.12)	-0.15 (0.11)	-0.93 (0.65)	-1.00 (0.49)
Elation	0.00 (0.45)	0.23 (0.36)	-0.74 (0.38)	0.48 (0.85)
Fatigue	0.25 (0.30)	-0.55 (0.48)	-0.35 (0.56)	0.67 (0.71)
Friendliness	-0.54 (0.57)	-0.19 (0.40)	-0.59 (0.58)	0.35 (0.87)
Positive Mood	1.77 (0.47)	0.36 (0.39)	0.20 (0.81)	1.48 (1.13)
Vigor	-0.79 (0.64)	-0.51 (0.72)	-0.99 (0.82)	0.83 (1.21)
VAS	**Placebo**	**5 mcg**	**10 mcg**	**20 mcg**
Happy	0.19 (0.03)	0.19 (0.05)	0.20 (0.08)	0.44 (0.06)
Productive	0.06 (0.04)	0.13 (0.06)	0.20 (0.08)	-0.29 (0.09)
Time awareness	0.06 (0.03)	0.06 (0.03)	-0.05 (0.03)	0.04 (0.06)
5D-ASC (%)	**Placebo**	**5 mcg**	**10 mcg**	**20 mcg**
Experience unity	0.77 (0.58)	2.89 (1.61)	3.54 (2.91)	3.79 (1.81)
Spiritual experience	0.54 (0.42)	0.91 (0.65)	2.15 (1.41)	2.62 (1.17)
Blissful state	1.08 (0.75)	4.51 (2.20)	5.02 (2.86)	6.86 (2.66)
Insightfulness	1.42 (0.70)	2.93 (1.42)	3.67 (2.53)	8.38 (2.63)*
Disembodiment	0.29 (0.24)	1.01 (0.67)	3.06 (1.57)	3.69 (1.87)
Impaired control and cognition	0.48 (0.24)	0.90 (0.38)	2.56 (1.23)	4.25 (0.99)*
Anxiety	0.25 (0.15)	0.51 (0.26)	1.44 (0.60)	1.95 (0.72)
Complex imagery	1.05 (0.80)	2.00 (1.23)	5.27 (2.51)	7.51 (2.60)
Elemental imagery	0.99 (0.51)	1.98 (1.00)	4.88 (2.47)	7.19 (3.04)
Audio-visual synesthesia	0.23 (0.16)	1.24 (0.74)	3.49 (1.88)	4.92 (3.03)
Changed meaning of percepts	0.67 (0.35)	1.67 (0.88)	2.92 (1.33)	6.50 (1.81)*
EDI	**Placebo**	**5 mcg**	**10 mcg**	**20 mcg**
	0.12 (0.06)	0.27 (0.17)	0.29 (0.15)	0.31 (0.14)

Note: POMS: Profile of Mood States; VAS: Visual Analogue Scales; 5D-ASC: 5-Dimensional Altered States of Consciousness Rating Scale; EDI: Ego Dissolution Inventory. *indicates significant difference ($p \leq 0.05$) compared to placebo.

Discussion

The aim of this study was to determine whether a low dose of LSD positively affects cognition and mood and if so, at which dose these effects occur. Furthermore, it was aimed to explore whether LSD effects varied inter-individually. Findings showed that speed of information processing was reduced (20 mcg) without affecting the accuracy. LSD increased self-rated drug-related states, i.e., feeling under the influence (10, 20 mcg), good drug effect (10, 20 mcg), bad drug effect (10, 20 mcg), feeling high (10, 20 mcg), and drug liking (10, 20 mcg). Furthermore, LSD (20 mcg) decreased self-rated concentration and induced scores on the majority of the scales measuring changes in psychedelic-induced waking consciousness (5D-ASC). Attention, cognitive control, mood and self-rated levels of happiness, productivity and time awareness were not affected. However, analyses showed inter-individual variability in LSD effects on mood, cognition and subjective drug states. The majority of observations showed enhanced attention (5, 20 mcg), positive mood (20 mcg), friendliness (5, 20 mcg), arousal (5 mcg), happiness (20 mcg), and a decrease in depression (20 mcg) and anger (10 mcg). Of note, this positive effect on the depression (20 mcg) and anger (10 mcg) is based on half of the total observations, as only 48% of the observations showed a change from placebo after LSD administration. Furthermore, an increase in confusion (10 mcg) and anxiety (5, 20 mcg), and reduced feelings of concentration (20 mcg) and productivity (20 mcg) was found in the majority of the observations that were affected by LSD.

The present study is the first to show individual variation to the effects of low doses of LSD on cognition. Low doses of LSD can enhance attention in the majority of observations, which is in line with anecdotal reports. However, LSD reduced the speed of information processing of the DSST, a task requiring higher level of cognitive processes compared to for instance the PVT.[52] In addition, cognitive control was not affected by LSD. Therefore, it is suggested that a low dose of LSD might enhance cognitive performance that requires relatively limited cognitive processing such as sustained attention.[52] Nevertheless, considering the relatively small number of modalities tested, more studies are needed, including a broader range of cognitive functions, to confirm these findings. Yet, our findings stimulate research in patient populations suffering from attentional problems, for instance patients suffering from ADHD.[53]

The present study also showed individual variation to the effects of the different LSD doses on mood. For instance, LSD increased positive mood (20mcg) but also induced unwanted effects such as increased anxiety (5 and 20 mcg), or confusion (10 mcg) in the majority of observations. Both, positive and negative effects are in line with anecdotal reports and related to respectively the motivation to start and stop microdosing.[19, 27, 28] LSD-induced increase in anxiety has previously been demonstrated after full psychedelic doses (100-200 mcg).[5, 6, 54] However, this increase was up to 20 % of the scale maximum,[54] whereas the increase after 20 mcg was only 1% of the scale maximum. Of note, direct comparisons between findings in different studies is difficult due to methodological differences between studies, in this case the use of another mood questionnaire. A previous study showed only an increase in vigor after a slightly higher LSD dose (i.e. 26 mcg of tartrate LSD) but no effect on the other mood scales,[31] however individual responses were not examined. Nevertheless, the present study showed that a low dose of LSD can have positive effects on mood, suggesting that anxiety induced by a low LSD dose does not notably interfere with other activities.

In contrast to anecdotal reports,[11, 27] the present study shows a decrease in self-rated concentration (20 mcg). Although this finding seems to contrast the absence of LSD effects on self-reported concentration reported by Yanakieva et al,[32] they administered lower LSD doses, i.e., a maximum of the 20 mcg tartrate which corresponds to 16 mcg LSD base. It seems to indicate that the tipping point between absence and presence in experienced negative effects lies in general between these two doses. These findings demonstrate the interesting dissociation between objective task performance, which was enhanced on the PVT in 74% of the observations, while the subjective experience showed that 63% of the observations rated performance deterioration. This difference between objective and subjective performance has previously been shown with other substances[55] and it goes against the idea that the effects of low doses of psychedelics would be driven by a placebo effect.[56]

Participants felt under the influence, and they had noticeable alterations from normal waking consciousness as measured by the 5D-ASC after 10 and 20 mcg of LSD. However, when comparing the increased scores on the 5D-ASC of the low LSD doses with full psychedelic doses of LSD (100-200 mcg),[7] it can be noted that the effect-profile on the scales is similar, though the magnitude of the experience is much smaller. For instance, total 5D-ASC-score was

one tenth (4 and 5.5%) of that after 100-200 mcg doses (35-50%). Overall, it can be stated that while participants notice effects, these effects are small and potentially negligible especially compared to high doses.

While no main dose effects were demonstrated on sustained attention and mood, findings showed there was an individual component in the LSD effect, meaning that not all participants demonstrated the cognitive and subjective effects to the same extent. Differences in LSD blood concentrations could be responsible for this individual variation in behaviour. Large variability in LSD blood concentrations was shown after low doses of LSD in the present study, and by Family et al,[33] which is supportive for this line of reasoning. However, the current study had too few samples in order to understand further whether LSD blood concentrations are associated with cognitive performance. Future studies including more samples could address this question. Genetic factors linked to drug metabolism and receptor binding might be another target too. It has for example been shown that responses to stimulant substances such as methylphenidate and amphetamines were associated with variations in the dopamine transporter gene.[24, 25] Future studies can be designed to examine biological underpinnings of inter-individual variation in behavioural responses to low doses of LSD and the potential biological markers associated with these individual differences.

Overall, the present study demonstrated selective, beneficial effects of low doses of LSD on mood and cognition in the majority of observations. Next to that, negative effects like increased anxiety were shown too. The minimal LSD dose at which subjective and performance effects were notable is 5 mcg and the most apparent effects were apparent after 20 mcg. Future studies in patient populations suffering from impaired attention are suggested, including biological parameters involved in LSD receptor binding and metabolism in order to understand the inter-individual variation in response to LSD on cognitive and emotional processes.

Conflict of interest

Authors declare no conflict of interest.

Author disclosures

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Contributors

PD, AF, JR and KK designed the study and wrote the protocol. NH and NM contributed to the acquisition of the data. NH, ET, JR and KK undertook the statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Supplementary Material - *Mood and cognition after administration of low LSD doses*

Missing values.

DSST: Due to technical issues, one measurement was missing in the 5 mcg LSD condition at +4h.

CCT: Due to technical issues, one measurement was missing in the 20 mcg LSD condition.

POMS: Due to technical issues, there were four missing data points; one baseline measurement of one participant in the placebo condition, one measurement at +2h for the 10 and 20 mcg LSD condition of one participant, and one measurement at +3h for the 20 mcg LSD condition for another participant.

VAS: Three participants had in total ten missing data points. For one participant, the rating of Happy was missing at +1h (5 mcg LSD); for a second participant, the ratings of Happy, Concentration, Creative, Productive, and Time Awareness were missing at +4h (20 mcg LSD); and for a third participant, ratings of Under the influence, Good Drug effect, Bad Drug effect, and Liking were missing at +6h (20 mcg LSD).

5D-ASC: Two participants had in total 15 missing data points. One participant failed to fill in one item in the placebo condition, while another participant failed to fill in 14 items (15% of the total questionnaire) during the 20 mcg condition.

GSS: because one participant failed to report the number of hours of sleep on three of the four test days.

Supplementary Table 1. GLM univariate ANOVA tests on the PVT and DSST, and GLM RM ANOVA test on the CCT.

	Main Dose effect			**Dose by Participant interaction effect**			**Dose by Time interaction effect**		
	F (df)	*p*	ηp^2	*F (df)*	*p*	ηp^2	*F (df)*	*p*	ηp^2
PVT									
Attentional lapses	1.41 (3, 69)	0.25	0.06	5.19 (69, 92)	**<0.01**	0.80	1.14 (3, 92)	0.34	0.04
Mean Reaction Time	0.80 (3, 69)	0.50	0.03	6.09 (69, 92)	**<0.01**	0.82	0.51 (3, 92)	0.68	0.02
DSST									
Number correct	4.38 (3, 69.22)	**<0.01**	0.16	2.69 (69, 91)	**<0.01**	0.67	1.03 (3, 91)	0.39	0.03
Ratio	1.08 (3, 69.44)	0.36	0.05	1.35 (69, 91)	0.09	0.51	2.15 (3, 91)	0.10	0.07

	F (df)	*p*	ηp^2
CCT			
Refresher			
Number correct	1.74 (3, 69)	0.17	0.07
Mean Reaction Time	0.29 (3, 69)	0.83	0.01
Cognitive control			
Devaluation Ratio (G-G corrected)	3.03 (2.14, 49.13)	0.06	0.12

Note: PVT: Psychomotor Vigilance Task; DSST: Digit Symbol Substitution Test; CCT: Cognitive Control Task

Supplementary Table 2. GLM univariate ANOVA tests on the POMS and VAS, and GLM RM ANOVA test on the 5D-ASC.

	Main Dose effect			Dose by Participant interaction effect			Dose by Time interaction effect		
POMS	*F (df)*	*p*	ηp^2	*F (df)*	*p*	ηp^2	*F (df)*	*p*	ηp^2
Anger	2.02 (3, 68.02)	0.12	0.08	16.89 (68, 270)	<0.01	0.81	0.63 (9, 270)	0.77	0.02
Anxiety	2.48 (3, 68.04)	0.07	0.10	8.20 (68, 270)	<0.01	0.67	0.51 (9, 270)	0.87	0.02
Arousal	0.74 (3, 68.03)	0.53	0.03	9.12 (68, 270)	<0.01	0.70	0.64 (9, 270)	0.76	0.02
Confusion	2.25 (3, 68.04)	0.09	0.09	6.92 (68, 270)	<0.01	0.64	0.86 (9, 270)	0.56	0.03
Depression	1.43 (3, 68.02)	0.24	0.06	14.46 (68, 270)	<0.01	0.79	0.56 (9, 270)	0.83	0.02
Elation	1.06 (3, 68.04)	0.37	0.05	7.85 (68, 270)	<0.01	0.66	1.81(9, 270)	0.07	0.06
Fatigue	1.87 (3, 68.05)	0.14	0.08	5.74 (67, 270)	<0.01	0.59	0.50 (9, 270)	0.88	0.02
Friendliness	0.73 (3, 68.05)	0.54	0.03	6.60 (68, 270)	<0.01	0.62	1.69 (9, 270)	0.09	0.05
Positive Mood	0.95 (3, 68.04)	0.42	0.04	7.66 (68, 270)	<0.01	0.66	1.28 (9, 270)	0.25	0.04
Vigor	1.16 (3, 68.02)	0.33	0.05	12.50 (68, 270)	<0.01	0.76	2.43 (9, 270)	0.10	0.08
VAS	*F (df)*	*p*	ηp^2	*F (df)*	*p*	ηp^2	*F (df)*	*p*	ηp^2
Under the influence	20.85 (3, 69)	<0.01	0.48	7.56 (69, 736)	<0.01	0.42	6.99 (24, 736)	<0.01	0.19
High	16.94 (3, 69)	<0.01	0.42	9.31 (69, 736)	<0.01	0.47	7.07 (24, 736)	<0.01	0.19
Good drug	12.68 (3, 69)	<0.01	0.36	9.25 (69, 736)	<0.01	0.46	3.24 (69, 736)	<0.01	0.10
Bad drug	2.69 (3, 69)	0.05	0.11	11.67 (69, 735)	<0.01	0.52	2.05 (24, 735)	<0.01	0.06
Liking	7.40 (3, 69)	<0.01	0.24	11.39 (69, 736)	<0.01	0.52	1.98 (24, 736)	<0.01	0.06
Happy	0.70 (3, 69)	0.56	0.03	28.42 (69, 735)	<0.01	0.73	2.62 (24, 735)	<0.01	0.08
Concentration	2.96 (3, 69)	0.04	0.11	10.93 (69, 735)	<0.01	0.51	1.11 (24, 735)	0.33	0.04
Productive	1.62 (3, 69)	0.19	0.07	16.85 (69, 735)	<0.01	0.61	1.77 (24, 735)	0.01	0.05
Time awareness	0.52 (3, 69)	0.67	0.02	4.85 (69, 735)	<0.01	0.31	0.68 (24, 735)	0.87	0.02

5D-ASC (G-G corrected)	*F (df)*	*p*	ηp^2
Total score	9.39 (1.71, 39.38)	**<0.01**	0.29
OB	5.46 (1.74, 40.03)	**0.01**	0.19
Experience unity	1.39 (1.68, 38.64)	0.26	0.06
Spiritual experience	3.20 (1.70, 39.01)	0.06	0.12
Blissful state	1.95 (1.87, 43.04)	0.16	0.08
Insightfulness	4.10 (2.30, 52.94)	**0.02**	0.15
AED	9.78 (2.19, 50.35)	**<0.01**	0.30
Disembodiment	2.55 (1.81, 41.63)	0.10	0.10
Impaired control and cognition	7.38 (2.03, 46.70)	**<0.01**	0.24
Anxiety	4.96 (1.37, 31.39)	**0.02**	0.18
VR	7.24 (1.60, 36.88)	**<0.01**	0.24
Complex imagery	3.66 (1.90, 43.58)	**0.04**	0.14
Elemental imagery	3.15 (1.27, 29.15)	0.08	0.12
Audio-visual synesthesia	1.90 (1.20, 27.56)	0.18	0.08
Changed meaning of percepts	6.54 (1.99, 45.68)	**<0.01**	0.22
AA	2.33 (2.01, 46.21)	0.12	0.09
RV	7.85 (1.63, 37.56)	**<0.01**	0.26

Note: POMS: Profile of Mood States; VAS: Visual Analogue Scales; 5D-ASC: 5-Dimensional Altered States of Consciousness Rating Scale; OB: Oceanic Boundlessness; AED: Anxious ego dissolution; VR: Visionary restructuralization; AA: Auditory Alterations; RV: Reduction of vigilance.

Supplementary Table 3. The percentage of observations included in the binomial tests which showed a change from placebo on the POMS and VAS, and the proportion of observations > placebo after each LSD dose.

	5 mcg LSD			10 mcg LSD			20 mcg LSD		
POMS	Percentage (%) included	Proportion increased (%)	*p*	Percentage (%) included	Proportion increased (%)	*p*	Percentage (%) included	Proportion increased (%)	*p*
Anger	53	49	1.00	48	24	**<0.01**	42	38	0.15
Anxiety	68	77	**<0.01**	85	55	0.44	70	63	**0.05**
Arousal	96	63	**0.02**	91	48	0.83	84	53	0.66
Confusion	78	48	0.82	80	62	**0.04**	80	60	0.11
Depression	32	52	1.00	43	37	0.12	48	30	**0.01**
Elation	90	52	0.75	89	40	0.08	86	57	0.27
Fatigue	74	41	0.15	74	42	0.24	73	54	0.55
Friendliness	90	63	**0.02**	84	47	0.66	86	65	**<0.01**
Positive Mood	90	52	0.75	85	46	0.58	88	63	**0.02**
Vigor	88	57	0.23	88	48	0.74	89	59	0.13
	5 mcg LSD			**10 mcg LSD**			**20 mcg LSD**		
VAS	Percentage (%) included	Proportion increased (%)	*p*	Percentage (%) included	Proportion increased (%)	*p*	Percentage (%) included	Proportion increased (%)	*p*
Under the influence	47	56	0.28	68	84	**<0.01**	87	94	**<0.01**
High	36	58	0.21	58	80	**<0.01**	76	93	**<0.01**
Good drug	45	46	0.54	66	78	**<0.01**	82	91	**<0.01**
Bad drug	17	54	0.74	34	81	**<0.01**	41	91	**<0.01**
Liking	49	50	1.00	71	77	**<0.01**	81	86	**<0.01**
Happy	53	41	0.08	67	53	0.56	79	63	**<0.01**
Concentration	60	56	0.19	71	59	**0.04**	87	37	**<0.01**
Productive	70	49	0.94	69	58	0.07	83	39	**<0.01**
Time awareness	31	48	0.81	38	44	0.37	57	47	0.59

Note: POMS: Profile of Mood States; VAS: Visual Analogue Scales.

Chapter 5

Low doses of LSD acutely increase BDNF blood plasma levels in healthy volunteers

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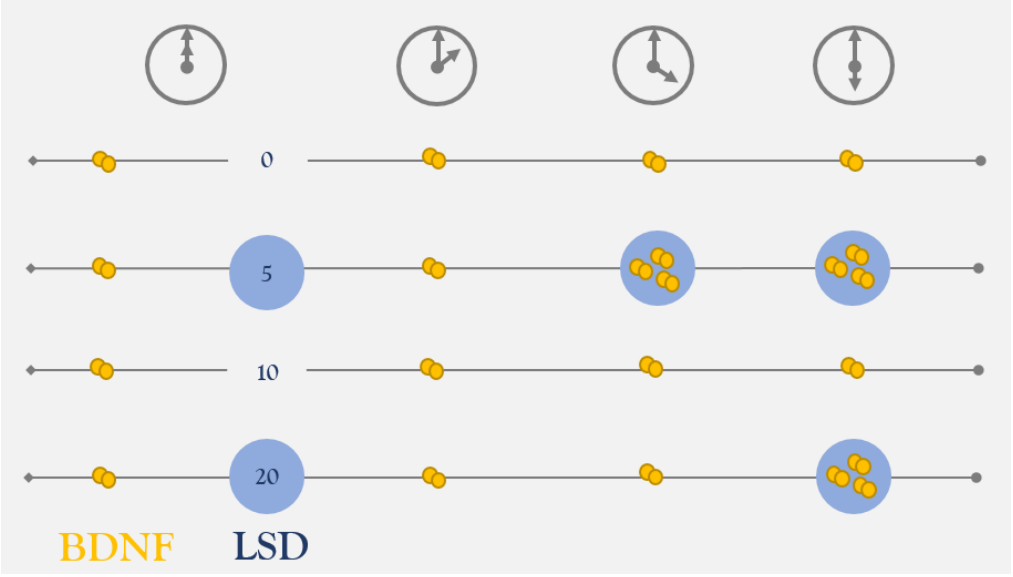
Abstract

Despite preclinical evidence for psychedelic-induced neuroplasticity, confirmation in humans is grossly lacking. Given the increased interest in using low doses of psychedelics for psychiatric indications, and the importance of neuroplasticity in the therapeutic response, this placebo-controlled within-subject study investigated the effect of single low LSD doses (5, 10, and 20 mcg) on circulating BDNF levels, in healthy volunteers. Blood samples were collected every two hours over six hours, and BDNF levels were determined afterward in blood plasma using ELISA. The findings demonstrated an increase in BDNF blood plasma levels at 4 hours (5 mcg) and 6 hours (5 and 20 mcg) compared to the placebo. The finding that LSD acutely increases BDNF levels warrants studies in patient populations.



Introduction

Preclinical research has demonstrated that psychedelic substances, including 2,5-dimethoxy-4-iodoamphetamine (DOI), lysergic acid diethylamide (LSD), N, N-dimethyltryptamine (DMT), and psilocybin, and alkaloids present in ayahuasca (harmine, tetrahydroharmine, and harmaline), affect neuroplasticity after acute, and chronic administration.[1, 2, 3-5] Catlow and colleagues (2013), for example, demonstrated the increased formation of neurons (neurogenesis) in mice' dentate gyrus after an average psilocybin dose of 3.5 mcg/35 g bodyweight (intraperitoneal (i.p.)), while this was slightly decreased after 35 mcg/35 g (psilocybin/bodyweight).[6] Interestingly, when repeatedly given i.p., for four times, interspersed with one week, a higher dose of 52 mcg/35 g (psilocybin/bodyweight) increased neuroplasticity.[2] Chronic administration of twice the ritualistic dose of ayahuasca (150 mL/70 kg bodyweight containing 0.26 mg/kg DMT) in rats, for 28 days, resulted in increased in brain-derived neurotrophic factor (BDNF) levels in the hippocampus of the female rats, compared to control animals.[7]

A recent in vitro study in cultured cortical neurons of animals showed increased forming of new neurites, expressed in a higher number of dendritic branches, and the total length of the arbors, and formation of synapses, after extended (24 h) treatment with a range of psychedelics like DOI, LSD, and DMT.[1] While these effects were similar across psychedelic classes and the dissociative ketamine, LSD was most potent, as shown on a neuritogenesis assay.[1] Also in cultured human cortical neurons, neuro-regenerative effects of DMT,[8] and modulation of proteins involved in dendritic spine formation by 5-MeO-DMT has been shown.[9]

Critical in light of the increased scientific interest in using low psychedelic doses,[10] also known as 'microdosing',[11] preclinical work with DMT has also shown that neuroplastic changes even take place after administration of low DMT doses, that is considered to be sub-hallucinogenic.[1] Examples are morphological changes in the prefrontal cortex of adult rats, and functional changes *ex vivo*.[1] The practice of microdosing entails repeatedly taking low doses, which are usually one-tenth of a recreational dose that causes a psychedelic experience. For LSD, that would, for example, be between 10 and 20 mcg.[12]

User claims suggest the effectivity of self-medication with low doses of psychedelics in the treatment of disorders related to neuroplasticity, including depression.[10] Interestingly, depression has been linked with impairments in neuroplasticity, and pharmacologically-induced symptom improvement is linked with increases in brain-derived neurotrophic factor (BDNF) levels.[13, 14] BDNF is highly expressed in limbic brain regions, which are involved in emotional processes and mood. Of note, Bershad et al[15] recently demonstrated connectivity changes in limbic areas after a low dose of LSD (13 mcg, tartrate). These biological changes correlated positively with the enhanced mood in healthy volunteers.[15]

Together, these findings add scientific evidence to the idea that LSD in low doses could have therapeutic potential in mood-related disorders.[10] Given the interest in BDNF as a key player in several neurodegenerative and neuropsychiatric disorders,[13, 16, 17] and preclinical data showing psychedelics-induced neuroplasticity, even in low doses of psychedelics,[1] the present, double-blind placebo-controlled, within-subjects study aimed to investigate whether LSD base in low doses (0, 5, 10, and 20 mcg) affects BDNF plasma levels in healthy volunteers. Blood samples were collected every two hours over six hours, and BDNF levels were determined afterward in blood plasma using ELISA.[18] Previously it has been demonstrated that blood plasma BDNF concentrations reflect mammalian brain-tissue BDNF levels.[19]

Materials and Methods

Participants were twenty-four recreational psychedelic users who provided informed consent, fell within the inclusion criteria[20] and passed medical screening including standard blood chemistry, hematology and urinalysis, before inclusion.

Test days were scheduled with minimally five days in between. A test day started at 9:00 AM with a screen for the presence of drugs of abuse in urine, and alcohol in the breath; and a urine pregnancy test in women. When tests were negative, a venous catheter was placed to draw blood. LSD (5, 10, and 20 mcg LSD base) was dissolved in 96% ethanol; the placebo consisted of 1 mL of ethanol (96%) without LSD. LSD and placebo were administered orally at 10:00 AM. A dose of 5, 10, and 20 mcg LSD base would be equivalent to respectively 6.2, 12.3, and 24.6 mcg pure LSD tartrate (1:0.5 without any crystal water). Participants were allocated to

unique treatment orders. Blood samples were taken at -0.5h, +2h, +4h and +6h relative to drug administration using BD vacutainer® heparin tubes spray-coated with lithium heparin. Samples were centrifuged, and plasma was transferred into a clean tube and frozen subsequently at −20°C until analysis. BDNF determination was assessed using an ELISA kit (Biosensis Mature BDNF Rapid ELISA kit: human, mouse, rat; Thebarton, Australia).[18] Plasma samples were appropriately diluted (1:20) and detection of BDNF was carried out on a pre-coated mouse monoclonal anti-mature BDNF 96-well plate as described in the manufacturer's protocol. The intra-assay and inter-assay coefficients of variation of this assay are below 10% (intra-assay CV 4.29%, inter-assay CV 7.14%). Samples were analysed in duplicate, and mean values of respective measurements were calculated and used in statistical analyses. All measures were done in blinded fashion. LSD concentrations were determined using ultra-high performance liquid chromatography/ tandem mass spectrometry (UHPLC–MS/MS) as previously described.[21] A different extraction procedure reanalysed samples with an LSD concentration below 5 pg/mL. In brief, aliquots of 150 µL of plasma were extracted with 450 µL methanol. The samples were rigorously mixed and subsequently centrifuged. The supernatant was evaporated under a constant stream of nitrogen and re-suspended in 200 µL of mobile phase A and B (10:90 v/v). An LLOQ of 2.5 pg/mL was reached by this extraction.

This study is part of a more extensive study, including cognitive, psychological, and physiological parameters which are reported elsewhere.[20] The study adhered to the code of ethics on human experimentation,[22] it was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University, and registered in the Dutch Clinical Trial register (number: NTR7102 https://www.trialregister.nl/). A permit for obtaining, storing, and administering LSD was obtained from the Dutch Drug Enforcement Administration.

Statistical analysis

Complete within-subjects (WS) cases entered statistical analyses performed by the statistical program SPSS (version 25.0). Non-parametric Wilcoxon signed-rank (S-R) tests for related samples (placebo versus LSD dose) were conducted on BDNF AUC's and BDNF plasma levels at -0.5h, +2h, +4h and +6h after dose administration of 5, 10 and 20 mcg LSD. In order to

understand at which time points BDNF levels were statistically different, separate Friedman tests per treatment condition were performed, and in case of a main effect, followed by Dunn's tests for pairwise comparisons including baseline versus 2h, 4h, and 6h, 2h-4h, and 4h-6h.

In case of statistically significant effects at alpha= 0.05, effect sizes and their 95% confidence intervals (95% CI) are given; to that end (point-biserial) correlations are calculated for Wilcoxon tests were 0.10, 0.24, and 0.37 signify small, moderate, and large effect sizes;[23] [24] in case of Friedman tests, Cramer's V was calculated where 0.06, 0.17, and 0.29 signify small, moderate, and large effect sizes.[25] The alpha level was corrected for multiple comparisons with sequential Bonferroni in case of Dunn's tests.

Results

Difficulties with the peripheral venous catheter during blood sample collection resulted in missing data. For one participant, no blood samples were collected; for the remaining 23 participants, the percentage of samples over all time points ranged from six to 100%. Only five (21.7%) of the participants had a complete dataset, and therefore, we opted to run the analyses per dose for complete cases (placebo-LSD dose) to be able to perform statistical analyses. In Table 1, the demographic details of participants included in the statistical analyses are presented. Four trapezoidal areas under the curves (AUC) for BDNF were calculated for the three LSD doses and placebo; the same procedure was used for LSD concentrations.

Table 1. Participants' age and gender per complete within-placebo-LSD dose case

LSD dose (mcg)	Number of participants	Mean age (*SD*)	Sex (Male:Female)
5	10	21.5 (3.06)	4:6
10	9	22.89 (2.80)	5:4
20	8	23.75 (2.66)	6:2

Wilcoxon Signed-Rank (S-R) tests revealed a statistically significant difference between AUC BDNF levels following 5 (Z= -2.60, *p*= 0.009, *r*= 0.58, 95% CI [0.11;1.06]) and 20 mcg LSD (Z= -2.52, *p*= 0.01, r= 0.63, 95% CI [0.09;1.17]) compared to placebo; the difference between AUC BDNF levels after 10 mcg LSD and placebo was not significant (Z= -1.01, p=0.31) (Figure 1A). AUC LSD plasma levels for the selection of complete WS cases per dose are shown in Figure

1B for illustrative purposes to show that LSD plasma levels increased with increasing LSD doses.

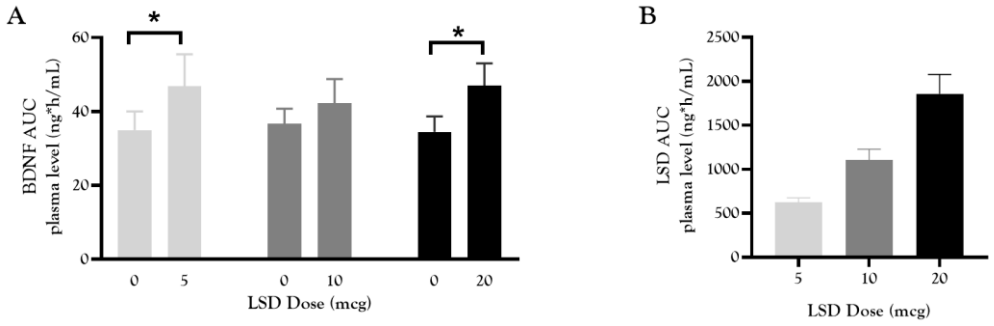


Figure 1. Total mean AUC (SEM) of BDNF (A) and of LSD (B) plasma levels for complete[#] within-subject LSD dose-placebo cases [#]*N(5 mcg LSD)= 10; N(10 mcg LSD)= 9); N(20 mcg LSD)= 8; *indicates statistical significance at p≤0.05*

Wilcoxon S-R tests revealed higher BDNF levels at +4h after administration of 5 mcg (Z= -2.80, p< 0.01, r= 0.63, 95% CI [0.15;1.11]) and 10 mcg LSD (Z= -1.95, p= 0.05, r= 0.46, 95% CI [-0.04;0.97]) compared to placebo. Although analysis including the 10 mcg dose revealed a statistically significant p-value (0.05), the CI included zero, indicating non-significance. Tests at +6h after LSD administration revealed significant effects of 5 mcg (Z= -2.29, p= 0.02, r= 0.51, 95% CI [0.03;0.99]) and 20 mcg (Z= -2.52, p= 0.01, r= 0.63, 95% CI [0.09;1.17]) LSD on BDNF levels compared to placebo (Figure 2 A-C). Corresponding LSD plasma levels are presented in Figure 2 (D-E).

Friedman tests investigating BDNF changes in the function of time demonstrated that BDNF plasma levels remained stable in the placebo conditions throughout the test frame. BDNF plasma levels in the LSD conditions showed their highest levels at four hours after administration of 5 mcg LSD (8.95 ng/mL), and at 6 hours after administration of 10 mcg (8.28 ng/mL) and 20 mcg of LSD (11 49 ng/mL) (Table 2).

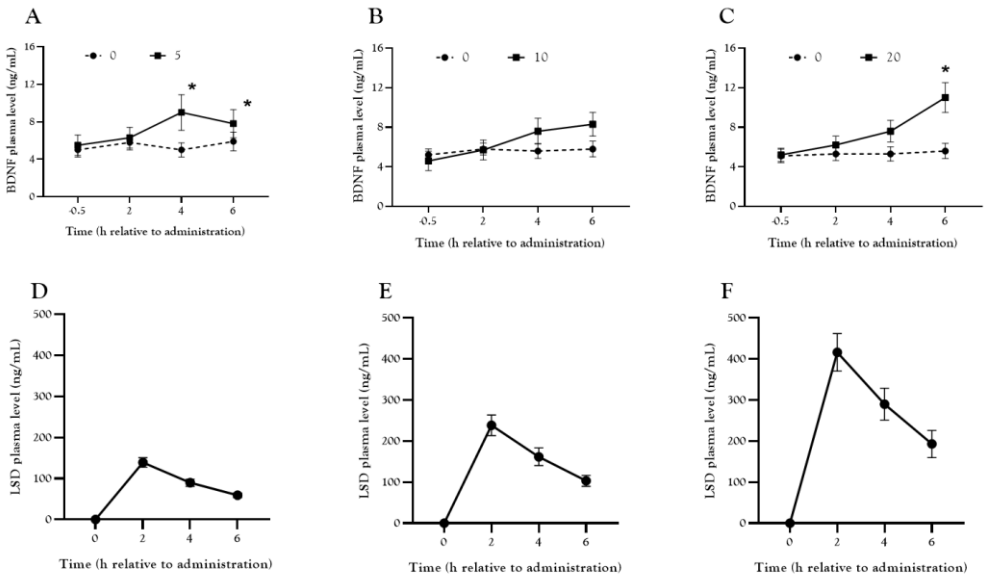


Figure 2. Mean (SEM) BDNF plasma levels for each LSD dose with the corresponding within-subject placebo condition per time of testing (A-C) and corresponding mean (SEM) LSD plasma levels (D-F); **indicates statistical significance at p≤0.05 in A-C; no statistical tests were performed over D-F*

Table 2. Friedman test of Time on BDNF plasma levels in the LSD conditions, and Dunn's pairwise comparisons

		Friedman test Main Time effect		**Dunn's pairwise comparisons (Z(1))**					
LSD dose (mcg)	**Number of participants**	($x^2(3)$)	*p*	Effect size Cramer's V	0-2h	0-4h	0-6h	2-4h	4-6h
5	10	15.72	<0.01	0.51	1.21	3.64*	2.77*	2.42*	0.87
10	9	13.00	<0.01	0.49	1.09	2.56*	3.29*	1.46	0.73
20	8	19.05	<0.01	0.63	0.77	2.13	4.07*	1.36	1.94

'*' signifies statistical significance at a sequential Bonferroni corrected *p*-value of <0.05

Discussion

This study provides preliminary evidence that low doses of LSD increase BDNF plasma levels in healthy volunteers up to 6 hours after administration, suggesting a window of opportunity for a therapeutic response,[26, 27] and cognitive enhancement[28, 29] that might be of use in patient populations. This line of thinking is supported by recent findings with ketamine and ayahuasca (containing the psychedelic DMT) demonstrating increased serum BDNF levels, respectively 24 and 48 hours after a single (high) dose, compared to placebo, which was related to fast antidepressant actions.[27, 30, 31]

Of interest is the different time-course of BDNF levels for the 5 mcg and 20 mcg dose. While BDNF levels peaked at 4 hours for the 5 mcg dose, these levels significantly increased 2 hours later for the 20 mcg dose. Recently Zhang et al[32] showed that a sub-anesthetic dose of IV ketamine (10 mg/kg/2h) increased BDNF in the amygdala, 2 hours after administration, while 40 mg/kg/2h did not affect BDNF levels, while instead elevating levels of other proteins involved in plasticity (cFos, pERK) in the mPFC and hippocampus. Earlier, they showed that IV ketamine (20 mg/kg/2h) induced a *decrease* in BDNF *plasma* in rats, 2 hours after ketamine administration, while 5mg/kg/2h did not affect the levels.[33] Their findings emphasize that BDNF levels undergo time-dependent changes following ketamine administration that can be influenced by the dose and timing of assay,[32] something that might also explain the absence of effects in our study after 10 mcg of LSD. Looking at the BDNF levels in the 20 mcg dose condition suggests that the peak had yet to come. While the multiple assessment points in the present study were are strength, future studies might want to include extra assessment points beyond the 6-hour post-drug period, even assessing the next day to understand the time-course of the effect.

While microdosing implies taking repeated doses of a psychedelic for a prolonged time, the present study only assessed the acute effects of a single administration on BDNF levels. Future studies will have to assess the effects of repeating dosing on neuroplasticity to understand whether this practice is beneficial to neuroplasticity or not. Previous studies investigating the repeated administration of ketamine and classical psychedelic have provided mixed results. Preclinical studies, and studies in ketamine abusers, for example, have shown that long-term

administration decreases the BDNF production in animals and humans.[34, 35] On the other hand, preclinical studies with repeated administration of high doses of serotonergic psychedelics demonstrated increased neuroplasticity.[27]

Concerning the underlying pathway, previously, it was shown that the structural changes induced by psychedelics appear to result from stimulation of the TrkB, mTOR, and 5-HT2A signaling pathways.[36] Ketamine is known to set off a signaling cascade by antagonizing NDMA receptors on presynaptic GABA neurons, resulting in an increased postsynaptic production of BDNF. The intermediate steps are increased presynaptic glutamate release and activation of mTOR pathways.[37-39] Ketamine and LSD might share a final common pathway when it comes to stimulation of BDNF. Future studies might include other proteins as well, to understand the neurobiological pathways underlying neuroplasticity, and the potential (therapeutic) implications of these induced changes. Potential foci might be proteins such as cFos and Perk, implicated in synaptic plasticity and memory formation,[32] as ketamine is known to impact these.

While the present study had a small final sample size, due to the difficulty in collecting blood over the six-hour course the participants were in the lab, the strength was the within-subject set up which neutralized the variation in the placebo-LSD comparisons and the multiple measurements after administration. Besides emphasizing the need to sample BDNF beyond the LSD elimination stage, in addition to including behavioural and imaging measures, future studies could focus on similarities between underlying biological pathways of the well-studied ketamine, and LSD, as it will contribute to understanding the scope of effects LSD, might have, based on ketamine findings. This first evidence of neuroplasticity in humans after low doses of a psychedelic provides a foundation to explore and replicate this finding in patient-populations, to understand the therapeutic value of it, if any.

Competing Interest Statement

The authors declare no conflict of interest

Author Contributions

NRPWH, NLM, PCD, AF, JGR, KPCK conceptualized the study and wrote the protocol, NRPWH, FH, MEL, NV, AE, KPCK collected and/or analyzed the data, NRPWH and KPCK wrote the manuscript, all authors contributed to versions leading up to the final manuscript

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Chapter 6

Cannabis containing equivalent concentrations of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) induces less state anxiety than THC-dominant cannabis

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Abstract

Delta-9-tetrahydrocannabinol (THC), an active component of cannabis, can cause anxiety in some users during intoxication. Cannabidiol (CBD), another constituent of cannabis, has anxiolytic properties suggesting that cannabis products containing CBD in addition to THC may produce less anxiety than THC-only products. Findings to date around this issue have been inconclusive and could conceivably depend on moderating factors such as baseline anxiety levels in users. The present study examined whether anxiety following single doses of vaporized CBD, THC and THC/CBD might be explained by state and trait anxiety levels at baseline. A placebo-controlled, randomised, within-subjects study including 26 healthy recreational cannabis users tested the effects of vaporised cannabis containing THC (13.75 mg THC), CBD (13.75 mg CBD), THC/CBD (13.75 mg THC/13.75 mg CBD), and placebo on anxiety. Self-rated trait anxiety was assessed with the State-Trait Anxiety Inventory (STAI). State levels of anxiety were objectively assessed with a computer-based emotional Stroop task (EST) and subjectively rated with the STAI-state questionnaire and a visual analogue scale. Both THC and THC/CBD significantly increased self-rated state anxiety compared to placebo. State anxiety after THC/CBD was significantly lower than after THC alone. THC-induced anxiety was independent of anxiety at baseline. When baseline anxiety was low, CBD completely counteracted THC-induced anxiety; however, when baseline anxiety was high, CBD did not counteract THC induced anxiety. There were no effects of any treatment condition on the EST. Overall, the study demonstrated that the THC/CBD equivalent cannabis induces less state anxiety than THC-dominant cannabis.

Introduction

With the growing trend to legalise or decriminalise recreational and medical use of cannabis, the prevalence of cannabis consumption is expected to increase.[1, 2] The potency of recreational cannabis products has risen substantially in Europe and the US over the past decade, as shown by higher levels of their psychoactive substance delta-9-tetrahydrocannabinol (THC).[3] THC is mainly used recreationally to induce a subjective feeling of high,[4] but it may also produce undesired feelings such as anxiety.[5-10] Also, medical formulations of THC have been associated with anxiety in patients suffering from HIV wasting disease.[11]

The anxiolytic properties of the non-intoxicating cannabis compound, cannabidiol (CBD), have been well characterized.[12-15] Preclinical studies suggest that CBD has anxiolytic effects under high-stress conditions (i,e foot shock) but not under low-stress conditions.[16, 17] Human studies have also indicated anxiolytic properties of CBD and reported reductions in stress-induced anxiety in healthy volunteers,[7, 18-20] and patients with Parkinson's disease.[21]

Nabiximols, a plant-based medication containing THC and CBD in a ratio of 1:1, is currently prescribed to relieve symptoms of Multiple Sclerosis, chronic pain, and nausea/vomiting. A THC/CBD ratio of 1:1 is believed to provide the best balance between therapeutic effects and adverse effects, such as anxiety.[22] However, some patients using Nabiximols still experienced THC induced anxiety despite the presence of CBD in their formulation.[23, 24] Notably, the anxiolytic effects of CBD tend to occur at much higher dose range (300-800 mg) than would be delivered with therapeutic doses of Nabiximols (e.g. 10-25 mg). Also, clinical studies in healthy volunteers have reported only a minimal impact of CBD on THC-induced anxiety levels with THC to CBD ratios of 1:1 and 1:2.[5, 9, 10] These findings might indicate that other factors play a role in the anxiogenic or anxiolytic effects of THC/CBD.

Potential factors moderating CBD and THC effects on anxiety include both state and trait anxiety. In preclinical studies it appears that high levels of state anxiety may determine the anxiolytic effects of CBD.[16] However, it is unknown whether baseline state anxiety also affects THC and THC/CBD-induced anxiety in humans. In addition, high trait anxiety is positively associated with increased selective attention towards anxiety-related stimuli.[25] This increased bias towards anxiety-related stimuli might also play a role in the increased state anxiety after

THC inhalation. In line with this, those who score high on trait anxiety might experience greater relief of anxiety symptoms when treated with CBD. Furthermore, while CBD appears to produce anxiolytic effects in individual with high states of anxiety,[26, 27] it might be that CBD can only counteract THC-induced anxiety when THC induces high levels of anxiety to begin with. Therefore, it is interesting to explore whether those who experience heightened anxiety after THC inhalation display lower anxiety levels after combined inhalation of THC and CBD.

The present study aimed to compare the effects of inhaled CBD, THC, and THC/CBD on anxiety and whether these effects depend upon moderating factors such as baseline state and trait anxiety levels. Furthermore, we aimed to explore whether individuals who experience high THC-induced anxiety levels display a more substantial reduction in anxiety symptoms when CBD is co-administered.

Methods

Design

This study was part of a more extensive randomised controlled trial investigating THC and CBD effects on cognition and driving performance.[28] This involved a double-blind, placebo-controlled, within-subjects design with four treatment conditions separated by a minimum washout period of 7 days to avoid potential carry-over effects. Treatment conditions were placebo cannabis, THC/CBD containing cannabis (13.75 mg THC and 13.75 mg CBD), THC-only cannabis (13.75 mg THC), and CBD-only cannabis (13.75 mg CBD). The order of treatment conditions was randomised across participants. Cannabis and placebo cannabis were self-administered by vaporisation at 200 °C (Mighty Medic, Storz & Bickel, Tuttlingen, Germany). Participants were instructed to inhale for 5 seconds, hold their breath for 3 seconds, exhale, and repeat this until the vapour was no longer visible, leaving thirty seconds in between.

Participants

Participants were 26 healthy occasional cannabis users (10 males; 16 females), aged 23.1 years on average (*SD*= 2.60). The frequency of cannabis use in the three months prior to study entrance was 10.50 times (*SD*= 13.57). Participants reported to have experience with other substances, including, ecstasy (*N*=7), amphetamines (*N*=1), cocaine (*N*=4), psilocybin (*N*=7),

LSD (*N*=3), and ephedra (*N*=1). The nationality of the participants was German (*N*=13), Dutch (*N*=3), Italian (*N*= 2), Slovenian (*N*=2), British (*N*=1), Finish (*N*=1), Malaysian (*N*=1), Portuguese (*N*=1), Serbian (*N*=1), and Sri Lankan (*N*=1).

Procedures

Participants were recruited through advertisements in university buildings in Maastricht, via social media, and by word of mouth. Before inclusion, participants underwent a medical screening by a physician. Their general health was checked, and blood and urine samples were taken for standard blood chemistry, haematology and urinalysis.

Inclusion criteria were written informed consent; age 20-50 years; occasional cannabis use (defined as; >10 lifetime exposures and <2 per week in the last 12 months); good physical health as determined by medical history and medical examination; absence of any major medical, endocrine, and neurological conditions; BMI between 20-28 kg/m^2. Exclusion criteria were history of drug abuse or addiction; current or history of psychiatric disorder; cardiovascular abnormalities; hypertension; liver dysfunction; any serious prior adverse response to cannabis; pregnancy or lactation.

Prior to the test days, participants visited the test facilities for a training session to familiarise them with the tests and test procedures. They filled out the trait section of the State-Trait Anxiety Inventory questionnaire (STAI-trait). Participants were instructed to abstain from illicit substance use and alcoholic beverages, respectively 7 days and one day before each test day. Instructions for the test days were to have a light breakfast at home, to consume their regular amount of caffeine, and to arrive well rested at the experimental facilities at 9 AM. They were screened for alcohol in breath and drugs of abuse in saliva (cocaine, opiates, benzodiazepine, methamphetamine, amphetamine, MDMA, and THC), and women were tested for pregnancy in urine.

When tests were negative, baseline state anxiety was measured by a Visual Analogue Scale (VAS), a peripheral venous catheter was inserted, and a blood sample was taken. The treatment was vaporised at 9.45 AM. After inhalation, an emotional Stroop task (EST) was conducted,

and participants rated their state anxiety on the state section of the STAI (STAI-state) and VAS. The VAS was assessed repeatedly up to 5.5 hours after inhalation, and blood samples were taken at similar intervals (Table 1).

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki amended in Fortaleza[29] and it was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing, and administering cannabis was obtained from the Dutch Drug Enforcement Administration. Participants were reimbursed for their invested time.

Table 1. Overview of measurements taken during the training session and test days.

		Test days						
		Time relative to administration (minutes)						
Measurements	Training session	Baseline	0	25	130	200	240	320
STAI-trait	X							
STAI-state			X					
VAS		X	X	X	X	X	X	
Emotional Stroop			X					
Blood samples		X	X	X	X	X		X

Note. STAI: State-Trait Anxiety Inventory, VAS: Visual Analogue Scale.

State-Trait Anxiety Inventory (STAI)

The State-Trait Anxiety Inventory (STAI) was used to measure self-rated anxiety. It consists of a trait and a state anxiety section,[30] assessing respectively relatively stable aspects of anxiety proneness and the current anxiety level. Both sections consist of 20 four-point Likert statements with answer options ranging from "almost never" to "almost always" for the trait section and "not at all" to "very much so" for the state section. The scores of both scales are the dependent variables; the min-max scores are 20-80, with a higher score indicating higher anxiety. State anxiety scores higher than 39–40 have been suggested to indicate clinically significant symptoms.[31] Normative state and trait anxiety scores for healthy adults and college students range respectively between 36-38 and 38-40.[32]

Visual Analogue Scale (VAS)

Participants were asked to indicate their level of anxiety throughout the day on a 10-cm long horizontal VAS scale with zero meaning "Not Anxious" and ten "Very Anxious".

Emotional Stroop task (EST)

A computerised emotional Stroop task (EST) was used to assess implicit anxiety. Twenty anxiety-related and twenty anxiety-neutral words[33] were presented in a coloured font (blue, red, green, or yellow) on a grey background. Each word was presented twice in random order resulting in a total of 80 trials. A trial started with a fixation cross, presented for 100 ms in the centre of the screen, followed by the word, which remained visible until a response was made. Participants were instructed to identify the word colour by pressing the correct button on the response box as fast as possible and to ignore the word content.

Prior to these sessions, participants were familiarised with the task procedure during the training session by presenting 40 neutral words twice (80 trials). All word lists used are shown in the Supplement (Table S1).

The primary outcome measure was calculated by subtracting the reaction time for the neutral words from the reaction time of the anxiety-related words, with a positive score indicating increased attentional bias to anxiety-related words. In addition, difference scores were calculated for the number of correct responses between anxiety-related and neutral words as a control measure, with a negative score meaning more correct responses to neutral words and thereby avoiding anxiety-related stimuli, hence indicating anxious behaviour.

Pharmacokinetics

Blood samples were taken at baseline and at 0 (directly), 25, 130, 200, and 320 minutes after treatment inhalation to determine THC, 11-OH-THC, 11-THC-COOH, CBD, and 7-OH-CBD and 7-COOH-CBD concentrations. Blood samples were centrifuged, and plasma was extracted and stored at -20°C until analysis. Plasma was analysed via liquid chromatography-tandem mass spectrometry (LC-MS/MS).[34, 35]

Statistical Analyses

The data and statistical analyses comply with the recommendations on experimental design and analyses in pharmacology. Data were analysed by means of the statistical package IBM SPSS Statistics (version 25).

All data were analysed with Linear Mixed Models (LMMs) with restricted maximum likelihood method (REML). In all analyses, compound symmetry was specified as covariance structure for the repeated factor Treatment (4 levels). In the analysis of VAS, an unstructured covariance structure was further specified for the repeated factor Time (5 levels).

Trait anxiety (STAI-trait) was added as a covariate and a Covariate by Treatment interaction in all models (STAI-state, VAS, and EST) to examine whether trait anxiety moderated Treatment effects. Baseline state anxiety was added as a covariate and as a Covariate by Treatment interaction to the statistical model of the VAS to examine whether baseline state anxiety moderated Treatment effects. Note: no baseline anxiety states were recorded with the STAI-state and EST. When a Covariate by Treatment interaction was statistically non-significant it was removed from the LMM, when subsequently a covariate's main effect was statistically non-significant it was also removed from the model. In case of a significant main Treatment effect, planned pairwise comparisons were performed. In case of a significant Treatment by Time interaction, pairwise comparisons were performed between Treatment conditions at each level of Time. In the case of a treatment by covariate interaction, separate planned pairwise comparisons were performed to examine the effects of Treatment on low (i.e., mean – 1SD), medium (i.e., mean) and high (i.e., mean + 1SD) covariate values respectively.

Because no parallel versions of the emotional Stroop task were used, the data were analysed to examine potential habituation effects using LMMs, including the fixed factors Treatment (4 levels), Test day (4 levels), and Treatment by Test day interaction.

To explore whether individuals who experience high THC-induced anxiety levels display a more substantial reduction in anxiety symptoms when CBD is co-administered, STAI-state and the VAS (peak) difference scores between THC condition and placebo (THC – placebo) were correlated with the difference scores between THC/CBD and THC (THC/CBD – THC).

Since baseline state anxiety played a role in the analyses of state anxiety measured by the VAS, data of the VAS was baseline corrected in this correlation analysis.

To examine the association of blood plasma concentrations with treatment-induced anxiety, difference scores of the STAI-state, VAS (peak) scores, and EST data from placebo (drug – placebo) were correlated with maximum plasma cannabinoid concentrations (Cmax). Since baseline state anxiety played a role in the analyses of state anxiety measured by the VAS, data of the VAS was baseline corrected in this correlation analysis.

The alpha criterion level of statistical significance for all analyses was set at $p = 0.05$. Pairwise comparisons were Bonferroni-corrected by multiplying the *p*-values by 4, the total number of predefined comparisons; THC vs placebo, THC/CBD vs placebo, CBD vs placebo and THC vs THC/CBD. Correlation analyses including blood plasma and induced anxiety were Bonferroni-corrected by multiplying the p-value by 3, total number of active treatment groups (CBD, THC, and THC/CBD). Pearson correlations were used in normally distributed data and Kendall's Tau-b in non-normally distributed data.

Results

STAI-trait anxiety

Overall, participants had a mean score of 33.13 (SD= 7.85) on *trait* anxiety.

STAI-state anxiety

LMM did not reveal significant covariate effects of trait anxiety and trait anxiety by Treatment interaction and therefore were not included in the model. There was a significant main Treatment effect ($F_{3,67.51}$= 17.11, $p< 0.01$); subsequent contrasts revealed that participants felt more anxious in the THC ($p< 0.01$) and THC/CBD ($p< 0.01$) conditions compared to placebo; the THC/CBD-induced anxiety was significantly less compared to THC-induced anxiety ($p= 0.01$) (Figure 1A and Table S3). There was no significant difference in anxiety between CBD and placebo (Table S3).

Pearson correlation analysis revealed that those who experienced greater anxiety in the THC condition showed a larger decrease in anxiety when CBD was co-administered ($r(21)= -0.92$, $p<$

0.01). Figure 1C shows violin plots of THC and THC/CBD induced state anxiety with connected individual data points showing less or more state anxiety after THC/CBD compared to THC.

VAS state anxiety

LMMs revealed significant effects of Treatment, Time, Treatment by Time interaction, and the covariates baseline state anxiety, baseline state anxiety by Treatment and Trait anxiety by Treatment interactions (all: $p < 0.05$, Table S2). There was no main effect of the covariate Trait anxiety. Pairwise comparisons between Treatment conditions (averaged across time points) and at each level of Time are presented in Figure 1C and Table S3. Compared to placebo, THC significantly induced anxiety immediately (Time 0), 25 and 200 minutes after inhalation, while THC/CBD only significantly increased anxiety at 25 minutes after inhalation. The THC/CBD-induced anxiety was significantly less compared to THC-induced anxiety directly after inhalation.

Separate Treatment contrasts for low, medium and high covariate values are shown in Figure 2 and Table 2. The mean differences of THC and placebo on the VAS and the corresponding 95%CI are approximately equal across the different values of the covariates, indicating that THC-enhanced anxiety compared to placebo is independent of baseline and trait anxiety. There was no evidence of an increase in anxiety following THC/CBD inhalation when baseline or trait anxiety was low, but increased anxiety was experienced when baseline or trait anxiety was medium to high. CBD did not counteract THC induced anxiety when baseline anxiety was high, partly counteracted THC-induced anxiety when baseline anxiety was medium, and counteracted THC-induced anxiety completely when baseline anxiety was low. CBD only counteracted THC-induced anxiety when trait anxiety was low. There was no evidence that CBD affects anxiety compared to placebo at all three values of baseline and trait anxiety.

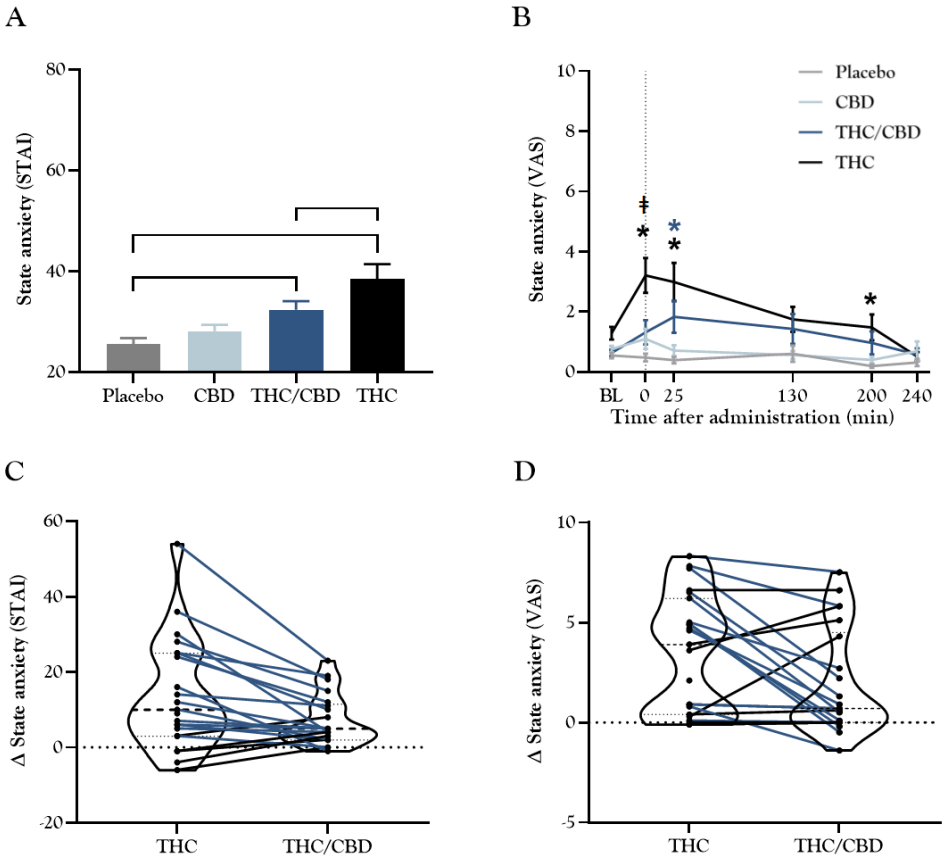

Figure 1. Mean (SE) state anxiety (STAI) per treatment [A]; mean (SE) state anxiety (VAS) per treatment over time [B]; violin plots of drug-placebo differences scores on state anxiety (STAI) [C] and drug-placebo difference peak scores on state anxiety (VAS) [D] with connected individual data points showing less (blue lines) or more (black lines) anxiety after THC/CBD compared to THC. ⊓ significant treatment contrasts, * significant difference between the treatment condition and placebo and ǂ significant difference between THC and THC/CBD condition (p= 0.02). STAI: State-Trait Anxiety Inventory; VAS: Visual Analogue Scale; BL: Baseline.

Kendall Tau-b correlation analyses revealed that those who experienced greater THC-induced state anxiety showed a greater decrease in state anxiety when CBD was co-administered ($\tau(22)$= -0.81, p< 0.01). Figure 1D shows violin plots of THC and THC/CBD induced state anxiety with connected individual data points showing less or more state anxiety after THC/CBD compared to THC.

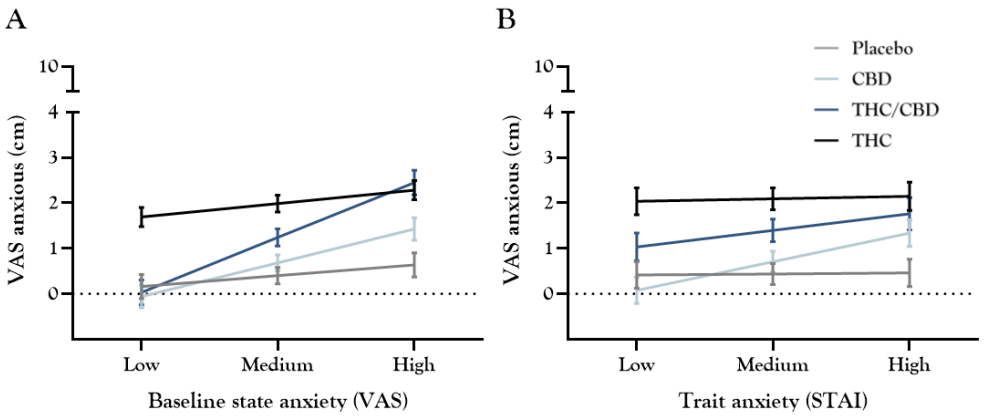

Figure 2. Mean (SE) state anxiety (VAS) per treatment at each level (low, medium, and high) of baseline state [A] and trait [B] anxiety. VAS: Visual Analogue Scale; STAI: State-Trait Anxiety Inventory.

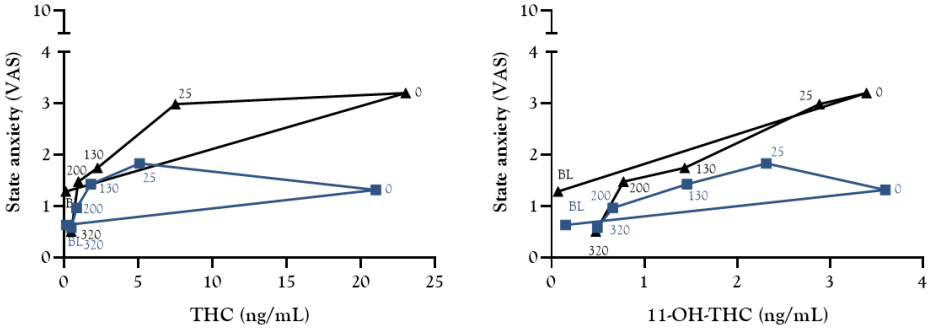

Figure 3. Mean state anxiety scores on the VAS plotted against the average of THC and 11-OH-THC plasma concentrations over time, for the THC and THC/CBD condition. VAS: Visual Analogue Scale; BL: baseline.

Table 2. Bonferroni-corrected pairwise comparisons between Treatment conditions on mean state anxiety (VAS) at each level of baseline state and trait anxiety (low, medium, and high).

	Baseline state anxiety								
	Low			**Medium**			**High**		
Mean VAS	Mean difference	*p*	95% CI	Mean difference	*p*	95% CI	Mean difference	*p*	95% CI
CBD-PLA	-0.22	> 0.99	-0.92, 0.48	0.28	> 0.99	-0.20, 0.77	0.79	0.11	0.09, 1.49
THC/CBD-PLA	-0.13	> 0.99	-0.86, 0.60	0.84	**< 0.01**	0.34, 1.34	1.81	**< 0.01**	1.08, 2.55
THC-PLA	1.53	**< 0.01**	0.88, 2.18	1.59	**< 0.01**	1.09, 2.09	1.65	**< 0.01**	0.99, 2.31
THC-THC/CBD	1.66	**< 0.01**	0.99, 2.33	0.75	**0.02**	0.24, 1.26	-0.17	> 0.99	-0.83, 0.50
	Trait anxiety								
	Low			**Medium**			**High**		
Mean VAS	Mean difference	*p*	95% CI	Mean difference	*p*	95% CI	Mean difference	*p*	95% CI
CBD-PLA	-0.34	> 0.99	-1.03, 0.36	0.27	> 0.99	-0.30, 0.84	0.88	0.06	0.17, 1.58
THC/CBD-PLA	0.62	0.35	-0.09, 1.33	0.96	**< 0.01**	0.36, 1.56	1.30	**< 0.01**	0.50, 2.10
THC-PLA	1.63	**< 0.01**	0.93, 2.32	1.66	**< 0.01**	1.07, 2.24	1.69	**< 0.01**	0.96, 2.42
THC-THC/CBD	1.01	**0.02**	0.29, 1.72	0.70	0.10	0.89, 1.31	0.39	> 0.99	-0.43, 1.20

Note: VAS: Visual Analogue Scale.

Emotional Stroop

There was no habituation effect across test days, and there was no significant main Treatment effect (Table S4), or Trait and Treatment by Trait interaction.

Blood plasma and anxiety measures

Statistical analyses and details of blood plasma concentrations for all time points can be found in Arkell et al.[28] The maximum blood plasma concentrations (SD) of THC, 11-OH-THC, CBD and 7-OH-CBD for all treatment conditions are presented in Table S5.

Correlation analyses revealed that increased state anxiety measured with the STAI correlated positively with maximum plasma concentrations of THC (p= 0.03) and 11-OH-THC (p< 0.01) after the THC, but not after THC/CBD inhalation. All other correlation analyses were non-significant (Table S6). Figure 3 presents counterclockwise hysteresis relationships between state anxiety measured by the VAS and blood plasma concentrations of THC and 11-OH-THC for the THC and THC/CBD conditions.

Discussion

The present study aimed to examine CBD, THC and THC/CBD effects on state anxiety and whether baseline state and trait anxiety levels moderate these effects. A secondary aim was to explore whether participants who experience heightened anxiety following THC inhalation experience a stronger relief of anxiety symptoms when THC is given in combination with CBD. THC and THC/CBD both increased self-rated state anxiety compared to placebo. Directly after inhalation, self-rated state anxiety after THC/CBD was significantly lower compared to THC. CBD, by itself, did not significantly change anxiety ratings on any of the anxiety measures. THC induced anxiety was independent from baseline state and trait anxiety. CBD counteracted THC-induced anxiety completely when baseline anxiety was low, partly counteracted THC-induced anxiety when baseline anxiety was medium, and did not counteract THC induced anxiety when baseline anxiety was high. CBD only counteracted THC-induced anxiety when trait anxiety was low. No treatment effects were found on state anxiety when measured with the EST.

The present study showed that CBD partially blocked THC-induced anxiety when the two drugs were delivered in equivalent concentrations, which is in line with previous studies.[5, 36] This might suggest that increasing the ratio CBD to THC would be even more effective in counteracting the THC-induced effects. However, preclinical studies showed that a higher CBD to THC ratio (5:1) did not augment the anxiogenic-like behaviour of THC, nor did it accentuate the THC effects compared to a ratio of 1:1.[16, 17] Due to the heterogeneity across studies, the literature regarding the CBD effects on THC-induced anxiety are mixed and no clear THC/CBD dose-response relationship is established yet.[37] Next to that THC/CBD interaction effects are also dependent on the order of administration, route of administration, and dose.[38]

Combined treatment of THC and CBD delayed the onset of state anxiety, reduced its magnitude and shortened its duration compared to inhalation of THC alone. A similar pattern with CBD on THC-induced hypothermia was shown in rodents.[17] Possible underlying mechanisms of THC/CBD interactions are complex. THC can modulate anxiety by binding to the orthosteric sites as a partial agonist on the cannabinoid type 1 (CB1) receptors.[39-41] CBD, on the other hand, is suggested to decrease THC-induced anxiety either by acting as a negative allosteric modulator of CB1 receptors,[39, 40] or via inhibiting fatty acid amide hydrolase, thereby increasing anandamide concentrations which then compete with THC for CB1 receptor binding,[42] or by activating Transient Receptor Potential Vanilloid 1 which oppose the effects of CB1 receptors,[43, 44] or via modulation of 5-HT1a receptors.[45] Overall, these possible mechanisms might explain the reduction of THC-induced anxiety, but it does not explain how CBD is able to delay and shorten THC-induced anxiety.

Baseline state and trait anxiety moderated the effects of cannabis on anxiety ratings after cannabis inhalation. This moderation of baseline state and trait anxiety on anxiety experienced after cannabis inhalation may interest patients who use medicinal cannabis or individuals who use cannabis recreationally. In general, the more anxious a person is before inhalation, the less CBD attenuates THC-induced anxiety. Baseline state and trait anxiety plays no role in the effects of THC-only cannabis on anxiety. Thus, if one experiences anxiety after using THC-only cannabis, it may be wise to use a combination THC/CBD cannabis while minimising anxiety before inhalation.

Visual inspection of the individual data points showing the THC-induced and THC/CBD-induced anxiety in the present study demonstrates that most participants experienced less state anxiety following THC/CBD inhalation compared to THC. Furthermore, the present study found that for those who experience heightened anxiety after THC inhalation, CBD seemed to reduce the THC-induced anxiety more efficiently than those who only experienced a small increase in anxiety. Indeed, previous preclinical and clinical studies suggested that CBD might only reduce anxiety or have therapeutic properties when high stress levels are experienced[16] or when severe THC-induced effects are present.[26, 27] However, the strong correlation on the STAI-state questionnaire needs to be interpreted with caution. The STAI-state was only assessed directly after inhalation when THC/CBD induced anxiety had not reached its maximum effect. The VAS, on the other hand, was repeatedly administered over time and thereby able to catch the delayed onset of THC/CBD-induced anxiety. Nevertheless, the inclusion of the peak anxiety states of both THC and THC/CBD in the correlation analysis of the VAS was strong.

THC-induced state anxiety (STAI scores) moderately correlated with THC and 11-OH-THC blood plasma concentrations, but not after THC/CBD inhalation. Therefore, THC and 11-OH-THC in blood plasma was not a good predictor for state anxiety when CBD is co-administered. In line with this, counterclockwise hysteresis loops show that the blood plasma concentration of both THC and 11-OH-THC were equal in the THC and THC/CBD conditions, while state anxiety (VAS scores) differed. These findings indicate that CBD does not alter THC concentrations in blood plasma.

Cannabis did not affect attentional bias towards anxiety-related stimuli, not even in the THC condition. A previous study by Richard et al[33] only found an attentional bias towards anxiety-related stimuli on the emotional Stroop task in a group scoring high on trait and state anxiety but not in a group scoring low on this trait and state. While state anxiety after THC inhalation in the present study was close to being clinically significant,[31] it was lower than state anxiety in the high trait anxiety groups that showed an attentional bias towards anxiety-related stimuli.[33, 46] The lack of THC-induced attentional bias towards anxiety-related stimuli on the EST might be due to the wide range of nationalities included, with the majority not having English as a mother tongue. Or because the present EST was set up using a mixed trial design, whereas

using a block design, the effects on anxiety-related words are more pronounced.[47] Future study needs to examine the effects of THC, CBD and THC/CBD by using a block design and a baseline measure of the emotional Stroop task.

There is a positive effect of CBD on THC-induced anxiety, however there can be negative consequences in daily life. For instance, participants felt more confident to drive following THC/CBD cannabis than when THC-only cannabis was inhaled.[28] However, CBD did not counteract THC-induced impairment on cognition and driving performance.[28] So, one can underestimate the impairing effects of THC/CBD combination on driving performance. So to consume THC/CBD combination one can feel better compared to THC-only cannabis, but the risks remain the same.

This study was not without its limitations. This study was part of a larger trial, and therefore only baseline state anxiety was measured using the VAS scale as it was easier to implement time-wise. Unfortunately, baseline state anxiety was not assessed with the STAI-state and EST. Future studies need to assess baseline state anxiety prior to treatment inhalation for every anxiety measurement used separately. In addition, when cannabis is used medically, one can be under the influence when doing groceries or when giving a speech at work, which can add stress to a situation. As seen in previous studies, CBD only reduces states of anxiety when an explicit stressor is present.[16] The current study had such a real-life stress situation, participants needed to drive on the road after cannabis inhalation which could provoke anxiety in some.[28] However, it was not completely like a real-life situation as an experienced driving instructor was sitting next to them and could take over control at any time. Nevertheless, future studies need to examine whether CBD is able to counteract THC-induced anxiety completely when an explicit stressor is present.

In conclusion, the present study showed that cannabis containing equivalent concentrations of THC and CBD induces less self-rated state anxiety compared to THC-only cannabis in healthy volunteers. Baseline state and trait anxiety moderated THC/CBD-induced anxiety but not THC-induced anxiety. The THC/CBD combination might be more favourable in clinical settings, and it may be a reasonable public health strategy to encourage cannabis breeds containing THC/CBD mixtures where recreational use of cannabis is now legal.

Conflict of interest

Authors declare no conflict of interest.

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Contributors

TA, NH, ET, IM, and JR designed the study and wrote the protocol. TA, RK, and FV contributed to the acquisition of the data. NH, JS, ET, KK and JR undertook the statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Supplementary Material - *Cannabis containing equivalent concentrations of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) induces less state anxiety than THC-dominant cannabis*

Methods

Emotional Stroop task

Table S1. Neutral words used for training session and anxiety-related words with anxiety matched neutral words used for test days.

Neutral Words (Training session)	
Hawk	Ketchup
Cane	Coarse
Muddy	Custom
Truck	Trumpet
Lump	Radiator
Swamp	Highway
Boxer	Whistle
Trunk	Repentant
Alien	Privacy
Rattle	Scissors
Limber	Nursery
Mystic	Pamphlet
Salute	Nonsense
Clumsy	Appliance
Vanity	Sheltered
Spray	Skeptical
Invest	Sentiment
Icebox	Nonchalant
Insect	Thermometer
Hammer	Lighthouse
Anxiety-related	**Anxiety-Matched Neutral**
Weak	Take
Worried	Bramble
Agony	Verse
Panicky	Sections
Failure	Clothes
Nervous	Picture
Helpless	Interest
Terrified	Margarine
Painful	Around
Die	Cup
Sickness	Material
Disease	Library

Tragedy	Whistle
Accident	Instead
Suffering	Something
Cancer	Taller
Paralyzed	Expensive
Despair	Service
Distressed	Understand
Coffin	Lesser

Results

Table S2. Linear Mixed Model of the VAS.

	F (df)	*p*
Treatment	5,15 (3, 60.77)	**<0.01**
Time	7.61 (4, 87)	**<0.01**
Treatment x Time	3.24 (12, 87)	**<0.01**
Covariates		
VAS baseline	36.70 (1, 51.36)	**<0.01**
VAS baseline x Treatment	6.22 (3, 69.03)	**<0.01**
STAI-trait	0.74 (1, 20.95)	0.40
STAI-trait x Treatment	2.88 (3, 58.01)	**0.04**

Note: VAS: Visual Analogue Scale; STAI: State-Trait Anxiety Inventory.

Table S3. Bonferroni-corrected pairwise comparisons between Treatment conditions (STAI-state and VAS) and at each level of Time (VAS).

	CBD-PLA		THC/CBD-PLA		THC-PLA		THC-THC/CBD	
	p	95% CI	*p*	95% CI	*p*	95% CI	*p*	95% CI
STAI-state	0.88	1.46, 6.18	**<0.01**	2.76, 10.89	**<0.01**	9.30, 17.20	**0.01**	-10.54, -2.3
VAS	>0.99	-0.28, 0.75	**<0.01**	0.42, 1.49	**<0.01**	0.98, 2.04	0.18	0.01, 1.10
Time (min)								
0	0.98	-0.40, 1.55	0.24	-0.04, 1.98	**<0.01**	1.69, 3.69	**<0.01**	0.70, 2.76
25	>0.99	-0.81, 1.27	**0.02**	0.51, 2.61	**<0.01**	1.49, 3.61	0.33	-0.13, 2.06
130	>0.99	-0.99, 0.78	0.16	0.04, 1.87	0.10	0.14, 1.96	>0.99	-0.84, 1.03
200	>0.99	-0.60, 0.88	0.09	0.13, 1.66	**0.01**	0.44, 1.95	>0.99	-0.48, 1.07
240	0.85	-0.83, 0.19	0.70	-0.17, 0.89	>0.99	-0.46, 0.58	>0.99	-0.84, 0.23

Note: STAI: State-Trait Anxiety Inventory; VAS: Visual Analogue Scale; CI: confidence interval.

Table S4. Final Linear Mixed Models of the Emotional Stroop without non-significant Trait covariate effects.

Habituation effect	**Test day**		**Test day x Treatment**	
	F (df)	*p*	*F (df)*	*p*
Number correct	0.33 (3, 56.41)	0.80	1.10 (9, 48.59)	0.38
Reaction time	0.38 (3, 49.96)	0.77	0.20 (9, 43.88)	0.99
Treatment effect	**Treatment**			
	F (df)	*p*		
Number correct	0.58 (3, 68.17)	0.63		
Reaction time	0.32 (3, 59.87)	0.81		

Table S5. Maximum blood plasma concentration (SD) of THC, 11-OH-THC, CBD and 7-OH-CBD (ng/mL) for all treatment conditions.

	THC (ng/mL)	11-OH-THC (ng/mL)	CBD (ng/mL)	7-OH-CBD (ng/mL)
Placebo	0.27 (0.55)	0.18 (0.55)	0.07 (0.34)	0.08 (0.40)
THC	22.91 (12.58)	3.35 (2.17)	0.02 (0.07)	<0.01 (<0.01)
THC/CBD	19.98 (3.36)	3.51 (3.46)	13.92 (8.47)	1.18 (1.28)
CBD	1.75 (2.13)	0.85 (1.06)	15.82 (6.92)	0.93 (1.11)

Table S6. Bonferroni-corrected correlation analyses of difference scores (Drug minus placebo) of the STAI-state, baseline-corrected peak scores on the VAS, and emotional Stroop outcome variables with difference values of THC, 11-OH-THC, CBD, and 7-OH-CBD blood plasma concentrations (Drug minus placebo).

	THC	11-OH-THC	CBD	7-OH-CBD
STAI-state				
THC	**$\tau_b(22) = 0.39$, $p = 0.03$**	**$r(21) = 0.66$, $p < 0.01$**	$\tau_b(22) = -0.03$, $p > 0.99$	$\tau_b(22) = -0.13$, $p > 0.99$
THC/CBD	$\tau_b(18) = 0.25$, $p = 0.47$	$\tau_b(18) = 0.16$, $p > 0.99$	$\tau_b(18) = 0.25$, $p = 0.47$	$\tau_b(18) = -0.13$, $p > 0.99$
CBD	$\tau_b(22) = 0.34$, $p = 0.10$	$\tau_b(22) = 0.26$, $p = 0.36$	$\tau_b(22) = 0.30$, $p = 0.18$	$\tau_b(22) = 0.03$, $p > 0.99$
VAS peak levels				
THC	$\tau_b(23) = 0.09$, $p > 0.99$	$\tau_b(22) = 0.04$, $p > 0.99$	$\tau_b(23) = 0.16$, $p > 0.99$	N/A
THC/CBD	$\tau_b(19) = 0.01$, $p > 0.99$	$\tau_b(19) = -0.04$, $p > 0.99$	$\tau_b(19) = -0.06$, $p > 0.99$	$\tau_b(19) = -0.34$, $p = 0.26$
CBD	$\tau_b(22) = -0.15$, $p > 0.99$	$\tau_b(22) = -0.15$, $p > 0.99$	$\tau_b(22) = 0.17$, $p = 0.81$	$\tau_b(22) = -0.19$, $p = 0.82$
Emotional Stroop				
Number correct				
THC	$\tau_b(21) = -0.18$, $p = 0.80$	$r(20) = -0.17$, $p > 0.99$	$\tau_b(21) = 0.17$, $p > 0.99$	$\tau_b(21) < 0.01$, $p > 0.99$
THC/CBD	$r(18) = 0.34$, $p = 0.50$	$\tau_b(18) = 0.01$, $p > 0.99$	$r(18) = 0.27$, $p = 0.83$	$\tau_b(18) = -0.02$, $p > 0.99$
CBD	$\tau_b(21) = -0.05$, $p > 0.99$	$\tau_b(21) = 0.26$, $p = 0.41$	$\tau_b(21) = 0.20$, $p = 0.69$	$\tau_b(21) = 0.01$, $p > 0.99$
Reaction time				
THC	$\tau_b(21) = 0.10$, $p > 0.99$	$\tau_b(20) = 0.08$, $p > 0.99$	$\tau_b(21) = -0.11$, $p > 0.99$	$\tau_b(21) = 0.31$, $p = 0.30$
THC/CBD	$\tau_b(21) = 0.10$, $p > 0.99$	$\tau_b(18) = -0.09$, $p > 0.9$	$r(18) = 0.07$, $p > 0.99$	$\tau_b(18) < 0.01$, $p > 0.99$
CBD	$\tau_b(21) = -0.18$, $p = 0.79$	$\tau_b(21) = -0.09$, $p > 0.99$	$\tau_b(21) = -0.26$, $p = 0.31$	$\tau_b(21) = -0.25$, $p = 0.44$

Note: STAI: State-Trait Anxiety Inventory; VAS: Visual Analogue Scale; τ_b: Kendall Tau correlation coefficient, r: Pearson correlation coefficient.

Chapter 7.

General Discussion

This dissertation aimed to examine the cognition and mood-enhancing effects of cocaine, LSD, and CBD, in healthy volunteers using placebo-controlled experimental studies. In addition, we wanted to assess whether these drug-induced enhancements of cognition and mood depend on personality traits such as empathy and anxiety, drug-induced states of positive mood and arousal, and baseline states of anxiety.

We demonstrated that a single dose of cocaine (300 mg, p.o.) increased positive mood and made participants feel more creative compared to placebo (**chapter 2**). However, the task-based changes in creativity following cocaine administration were process- (convergent and divergent thinking) and stimulus- (verbal and figural) dependent. Regarding personal factors, cocaine-induced positive mood was positively associated with enhanced figural convergent and verbal divergent thinking performance. In addition, higher levels of trait empathy were associated with enhanced verbal and figural divergent thinking when under the influence of cocaine. Cocaine also enhanced prospective memory and divided attention performance and self-rated feelings of arousal (**chapter 3**), with the memory effect being weakly associated with the latter two effects. A microdose of LSD (5 and 20 mcg, p.o.) enhanced sustained attention in the majority of responders to LSD, slowed down information processing (20 mcg, p.o.), but did not affect cognitive control (**chapter 4**). LSD changed self-rated mood states in both positive and negative directions. Increased BDNF blood plasma concentrations were detected after LSD (5 and 20 mcg, p.o.) compared to placebo (**chapter 5**). THC-dominant cannabis (13.75 mg, vaporised) increased state anxiety (**chapter 6**). The addition of CBD (13.75 mg, vaporised) reduced THC-induced anxiety compared to THC-dominant cannabis, yet self-rated anxiety was still higher than after placebo. However, CBD primarily reduced THC-induced anxiety when participants' baseline state and trait anxiety were low, it partly counteracted THC-induced anxiety when baseline state anxiety was medium, and did not counteract THC-induced anxiety when baseline state anxiety was high.

Tolerance and abuse potential

While this dissertation examined the acute effects of a single dose of cocaine, LSD and CBD on mood and cognition; in real life, repeated use of these substances to enhance mood and cognition is common,[1-3] which may result in decreased drug sensitivity through the

development of tolerance, which in turn can lead to addiction. It is widely known that cocaine has a high abuse potential and the use of cocaine has a considerable risk to induce addiction.[4, 5] (However, there is little research examining the abuse potential of microdoses LSD and CBD, and there are no indications so far that the latter two substances have high abuse liability in humans.[6-10]

Repeated use of cocaine (10 or 20 mg/kg every 8h for 4 to 6 days) in rodents[11] and repeated use of low doses of LSD (10-30 mcg twice daily for three days) in humans[12] induced tolerance to the acute effects of these substances, while no tolerance was induced to the acute anxiolytic effects of repeated doses of CBD (5 mg/kg/day) for 6 days[13] and CBD (30 mg/kg/day) for 14 days[14] in rodents. When tolerance to the acute effects of cocaine is experienced, cocaine users tend to increase the dose to get the previously experienced effects such as mood enhancement, which can lead to addictive behaviour.[15] Unlike cocaine, research done in the 50's showed that increasing the dose of LSD after experienced tolerance to microdoses (10-30 mcg, twice daily for three days) produced transient tolerance to the mental effects of a higher dose of LSD (75 mcg) in humans,[12] suggesting little abuse potential. However, survey studies showed that some users microdose daily, sometimes even twice daily.[3, 16] It is not known whether these users experienced tolerance to the acute effects of a microdose of LSD. Experimental and observational studies in humans measuring the effects of microdosing with LSD up to 6 weeks did not report signs of tolerance when microdosing every third day.[17-20] Overall, research on the effects of tolerance, especially in humans is scarce. Therefore it is unknown what the ideal time window should be between doses, and what the exact dose of cocaine, microdose of LSD, and CBD should be to prevent development of tolerance. More research is needed to examine the best dosing practice to avoid the development of tolerance to the acute effects of LSD and CBD in humans for potential therapeutic applications of these substances as described later in this chapter. Of note, it is not recommended to set up experimental research on the effects of tolerance after repeated cocaine use in humans due to ethical reasons.[15]

Besides the development of tolerance to the acute effects of cocaine, chronic use of cocaine can also have long-lasting negative effects on cognition. Having these cognitive impairments while abstaining from cocaine can increase the likelihood of relapse, as users may seek

temporary use of cocaine to reverse these cognitive impairments.[21] Abstinent cocaine-dependent patients showed moderate cognitive impairments, in e.g., attention and memory for up to 12 weeks after discontinuation of cocaine.[22] Chronic use of low doses of LSD (every other day for three months) can also have long-lasting effects as induced hyperactivity, hyperirritability, locomotor activity, anhedonia, and impairment in social interaction persisted three months after cessation of LSD in rodents.[23] However, these unwanted LSD-induced effects may be unlikely to occur in humans, as survey research has shown that users stop microdosing when unwanted effects are experienced.[3] Research suggests that CBD does not impair cognition after chronic use in adult mice,[24] however, no study to date has examined long-term effects of repeated use of CBD in humans. Overall, the effects of chronic use of microdoses LSD and CBD on cognition is not extensively researched, but so far research indicates that no long-term negative effects on cognition occurs, therefore the likelihood of relapse is nihil, if dependence was even present.

Therapeutic indications

Cocaine hydrochloride

Based on its cognition enhancing effect on attention and memory (**chapter 3**), orally administered cocaine hydrochloride could have therapeutic potential for treating symptoms of attention deficit hyperactivity disorders (ADHD) and cognitive decline seen in ageing populations. However, given the abuse potential[4] and the physiological side effects of cocaine, such as increased heart rate and blood pressure,[25, 26] it may not be a 'safe' substance to use in psychiatric therapy. Nevertheless, cocaine hydrochloride up to a dose of 160 mg is approved as a local anaesthetic for diagnostic and surgical procedures of the nasal cavities of adults.[27] However, this approved therapeutic application is relatively harmless, as a soaked pledget with cocaine hydrochloride solution is placed in the nasal cavity for one time use only.[28]

LSD microdoses

Given the effect profile of a microdose of LSD demonstrated in **chapter 4** and **5**, such as enhanced attention performance, positive mood, insightfulness, and changed meaning of percepts (**chapter 4**), increased BDNF concentration in blood (**chapter 5**), a microdose of LSD

could have therapeutic potential to treat mood and attention deficit disorders and might be able to reduce cognitive decline seen in ageing. In addition, emerging evidence of other studies also showed enhanced positive mood,[29, 30] altered brain connectivity in limbic circuits[29] in healthy volunteers and reduced depressive symptoms after LSD microdosing for six weeks.[20] Individuals who microdose psychedelics report enhancement of performance and mood, and relief of symptoms of ADHD and mood disorders as their main motivation for use.[3, 31] Whether microdosing with LSD could be employed as an effective treatment for ADHD, mood disorders, and age-related cognitive decline, however, remains to be examined.

While self-medicating with microdosing of psychedelics was rated to be more effective for depression and anxiety compared to conventional treatment, microdosing was rated less effective than regular doses of a psychedelic substance.[31] This would be in line with studies suggesting that the therapeutic outcome in depression depends on the intensity of the psychedelic experience, which is very low in microdosing.[32] Indeed, administration of a high dose of a psychedelic in patients with treatment-resistant depression and end-of-life anxiety, revealed promising therapeutic effects[33-35] when compared to a very low dose of a psychedelic substance.[35] However, at a 3-month follow-up, the majority of treatment-resistant patients started using antidepressant medication, psychotherapy or recreational psilocybin.[33] This pursuit of follow-up treatment indicates a need for maintenance to sustain a therapeutic response. So one of these potential follow-up treatments might be a psychedelic microdose to sustain the anti-depressant effects after a full dose psychedelic therapy session. The increase in BDNF concentration in blood following a microdose of LSD **(chapter 5)** gives an indication that it could provide an effective follow-up treatment, as enhanced BDNF concentrations in blood resulting from conventional antidepressant medications,[36] the rapid-acting antidepressant ketamine,[37, 38] and the classical psychedelic ayahuasca[39] were linked to relief of depressive symptoms.[40] Future studies need to examine whether a microdose would indeed be effective as a follow-up treatment after a full dose psychedelic therapy session for depression and anxiety.

While the efficacy of microdosing psychedelics for psychiatric conditions has to be investigated in clinical trials, the side effect profile and the treatment schedule seem favourable compared

to conventional medication. Users reported that their traditional stimulants for ADHD caused a crash or rebound after use, including symptoms such as irritability, anxiety, and hyperactivity.[41] Microdosing with psychedelics did not cause such a crash after use.[42] Most common side effects of ADHD stimulants are loss of appetite, difficulties falling asleep, and mood swings, and almost one quarter of patients reported that the side effects were very to extremely bothersome.[43] Common side effects of antidepressants are sexual dysfunction, nausea and diarrhea, increased anxiety and sleep disturbances especially in the first few weeks of treatment, and could lead to cessation of use.[44] Reported side effects of microdosing with LSD are increased anxiety, and feeling overstimulated or difficulties in concentrating at the end of a microdosing day, but did not result in cessation of use.[45] Also, conventional medications for depression take several weeks before effectively reducing symptoms.[46] In addition, cessation of antidepressants can cause withdrawal symptoms which can last for weeks to months.[47] Regarding the frequency of use, it is suggested that microdoses of psychedelics are taken in regular intervals, such as every two or three days,[17] in contrast to traditional medications that usually are taken daily or even multiple times a day.[48, 49] Although microdosing with LSD remains a repeated dosing schedule, it may require fewer administrations than conventional medications. However, the side effect profile and treatment schedule of microdosing with LSD is based on anecdotal reports and clinical evidence is still lacking to date.

We suggest that some guidance of patients is needed when starting a treatment procedure of microdoses of LSD in therapeutic settings for reducing symptoms of mood, attention deficit disorders, or age-related cognitive decline. In **chapter 4**, we showed that mild feelings of anxiety and bad drug effects can occur. In addition, survey studies reported that when an LSD microdose was too high, it could induce mild perceptual alterations, anxiety, and nervousness.[16] These effects may be considered as unwanted effects and could lead to treatment cessation. It can be suggested to follow the three-phase model used in psychedelic-assisted therapy, where a preparation session precedes the session with the administration, which is then followed by an integration session, and a follow-up.[50] However, several clinic visits when microdosing might be unrealistic and pose a significant barrier for many people.[51, 52] Therefore, a first guided session is advisable, so only the first microdose of LSD will be taken under the

supervision of a therapist that can guide the patient when certain feelings may come to the surface, followed by a brief (possible digital) follow-up session to discuss potential unpleasant after-effects that could have occurred the day following a dosing day.

Cannabidiol

Besides the therapeutic efficacy of CBD on anxiety, the therapeutic efficacy is also examined for a wide range of other disorders such as, epilepsy, pain/inflammation, schizophrenia, various substance use disorders, and post-traumatic stress disorder.[53] To date, CBD is approved by the U.S. Food and Drug Administration (FDA) to treat epileptic seizures.[53] Evidence of the efficacy of CBD to treat the other aforementioned disorders is still lacking to date. There are still some concerns regarding potential interactions of CBD with prescribed medications. For instance, abnormal liver functions, somnolence, sedation and pneumonia were reported in epilepsy studies with children, which was suggested to be due to the interaction with other medications used simultaneously.[9] When controlled for the use of other medication, only mild complaints of diarrhoea were mentioned as an adverse event following CBD.[9] The wide range of therapeutic effects examined to date is promising, but more studies are needed to examine the efficacy of the mentioned potential therapeutic indications in patient populations.

Medical cannabis containing THC and CBD in a ratio of 1 to 1, is currently approved and prescribed to relieve symptoms of Multiple Sclerosis, chronic pain, and nausea/vomiting.[54] This THC/CBD ratio of 1 to 1 is believed to provide the best balance between therapeutic and adverse effects, such as anxiety.[54] Nevertheless, anxiety can be experienced using a 1 to 1 THC/CBD ratio.[55, 56] Based on the anxiolytic effects of CBD, one might think that enhancing the CBD concentration compared to THC would reduce anxiety. However, preclinical studies showed that a higher CBD to THC ratio (5 to 1) did not modify the anxiogenic effects of THC compared to a 1 to 1 ratio.[57, 58] Due to the heterogeneity across studies, the literature results regarding the CBD effects on THC-induced anxiety are mixed.[59] Future studies could examine the CBD effects on THC-induced anxiety including multiple THC/CBD ratios in order to establish a dose-response relationship.

Precision medicine

To provide the best therapeutic fit for a patient, more attention needs to be paid to individual state and trait factors that play a role in the effect of substances on mood and cognition in research, as the current chapters **(3 and 6)** demonstrated that individual state anxiety and trait factors, like anxiety and empathy play a role in the effects of THC/CBD on mood and cocaine on creativity. Paying more attention to these individual differences can help develop the best treatment for a patient. For instance, if a patient is anxious, a therapist can offer the patient certain exercises focused on calming down before taking THC/CBD medication. Several other state and trait factors could play a role as well. For example, traits of absorption, openness, and acceptance are associated with positive effects following a high dose of a psychedelic substance, while low levels of the trait openness and surrendering are associated with the likelihood of experiencing negative effects.[60] Different states and traits are researched regarding positive or negative psychedelic experience of high doses of psychedelics,[60] but there is a lack of attention to state and trait factors associated with the experience of other substances such as cocaine, CBD, or microdoses of psychedelics.

Next to state and trait factors, the context in which a moderate to high dose of a psychedelic substance is used plays an important role as well, such as low lighting, music playlists, aesthetically pleasing décor.[61] However, to which extent context plays a role in the use of other substances, such as cocaine, CBD, and a microdose of LSD, is not known. In addition, sex differences are noted in certain disorders, and even some symptoms are tied to the hormonal changes linked with the menstrual cycle,[62] therefore, it can be expected that sex differences also play a role in the treatment effects. It can be a complex interplay between factors and more research is needed to capture the most relevant individual factors in moderating treatment response. Future research needs to compare patients who do and do not respond to a certain pharmacological treatment, find differences in individual factors, and further elucidate which of these differences in individual factors could play a role in the treatment response.

Inter-individual variation in drug responses is common and can also be observed after a microdose of LSD **(chapter 4)**, combinations of THC and CBD **(chapter 6)**, and cocaine **(chapters 2 and 3)**. This indicates that not everyone will benefit from the therapeutic use of

these substances or that some individuals might need a higher dose. A previous survey study reported that microdosers used LSD doses up to 50 mcg,[20] which is no longer considered a microdose.[63] Microdosing protocols for LSD and medical leaflets of pharmaceutical cannabis suggest to "start low and go slow",[17, 64] which implies starting with a low dose and being observative to symptoms that are reduced and additional (unwanted) effects that might arise. The conclusion of this first step is either stay with the dose when it is effective or increase the dose when symptoms are still present, until the symptoms are satisfactorily reduced. However, when increasing the dose, unwanted effects might simultaneously occur. The patient, in dialogue with the therapist, should decide on what the minimum effective dose is, with the best trade-off between positive and negative effects.

Limitations and future directions

Generalisation of findings

The study population in this dissertation consisted of healthy, young (age range 18-32), high-educated individuals who were poly-drug users; which reduces the generalisation of findings to for instance, elderly, patients with mood disorders, and drug-naïve individuals. Those who show cognitive decline may benefit more from the cognitive enhancing effects of a stimulant substance than high-educated individuals. This was e.g., previously shown for the creativity-enhancing effects of dexamphetamine, as the highest gain was recorded for the low performers at baseline.[65] To illustrate, **chapter 3** showed that some healthy participants made no mistakes on the prospective memory and divided attention tasks when receiving a placebo; due to this ceiling effect, no potential cocaine-induced performance improvement could be assessed on these measures. In addition, a review study examining factors that could predict individual reactions to psychedelic substances, suggested that elderly might be less sensitive to psychedelics,[60] however the reason for this lower sensitivity was not explained. Nevertheless, this could indicate that elderly might need a higher dose of a psychedelic substance to have the same cognitive and mood enhancing effects as younger individuals. Furthermore, participants in the current dissertation were not diagnosed with a mood disorder, therefore clinical significant effects of a microdose of LSD on depressed mood could not be revealed. Lastly, all participants in the current dissertation were not drug-naïve. This could have resulted in less

beneficial effects of the substances. For instance, increased experience with a psychedelic substance was associated with less intense effects to the substance, as previous experience with psychedelics may induce compensatory or neuromodulatory effects that buffer against acute cognitive impairments.[60] Therefore, future studies should include people suffering from mental pathologies, and/or elderly who show cognitive decline to get a comprehensive overview of the therapeutic potential of the substances mentioned in this dissertation.

Construct validity

The self-ratings in the current studies of this dissertation are not entirely in line with the findings from task-based measures we used in our experimental designs, which could indicate a difference in concepts assessed with objective tasks and subjective questionnaires. For instance, cocaine induced feelings of being creative, but performance on task-based creativity showed a less consistent picture **(chapter 2)**. A participant's subjective experience of creativity may not be reflected as an objective change in the single constructs of creativity, like convergent and divergent thinking.[66] Furthermore, participants felt less concentrated after 20 mcg LSD than placebo, while sustained attention performance improved **(chapter 4)**, again suggesting a discrepancy between subjective and objective measures. Also, participants felt anxious after THC/CBD and THC inhalation, while objective assessment of anxiety remained unaffected **(chapter 6)**. So when assessing self-rated states of participants, future studies should include a follow-up question asking the participant to self-rate their own performance on the cognitive tasks, to get a good indication of whether the measurement of a construct was in line with the subjective experience of the participant regarding that specific construct.

Ecological validity

Next to the computer tasks assessing cognitive constructs used in the current dissertation, more complex tasks assessing more functional behaviour closer to reality,[67] are of importance when aiming to examine more real-life functioning.[68] During the past decade, there has been a shift from using these 'traditional' neurocognitive computer tasks towards more ecologically valid settings using virtual reality (VR). Performance on a VR-based task has shown to correlate with subjective complaints. For instance, subjective daily memory complaints in mild cognitive

impaired patients correlated better with impaired performance in a VR test assessing memory while sitting in a virtual car than a standard lab-based memory task.[69] Other VR-tasks that have proven to be sensitive to cognitive decline seen in ageing, are for instance the assessment of memory with a shopping list task[70] and of prospective memory with Virtual Week.[71] The subjective claims on cognition enhancement using cocaine, CBD or a microdose of LSD may also positively correlate more strongly with enhanced performance in a VR test than lab-based computer tasks measuring cognitive constructs. In addition, more research is providing evidence for the efficacy of VR-based exposure therapy for several anxiety disorders, and is suggested to be beneficial for some patients who refuse to follow in vivo exposure therapy.[72] Future psychopharmacological research, therefore, needs to use VR paradigms with high ecological validity and use it in psychopharmacological research to understand, next to construct-based effects, more real-life functioning to get a complete picture of the user claims and patients' responses to treatment.

Expectancy effects

Due to media attention on the effects of cocaine on creativity,[73-75] the effects of microdosing with psychedelics on mood and cognition, [76, 77] and the use of CBD products for anxiety,[78, 79] a certain expectation about these substances has been created. These expectations can influence the actual experienced effects. For instance, positive expectations prior to starting to microdose with a psychedelic can predict the positive effects experienced after consumption.[80] One observational study examining the effects of microdosing with psychedelics on subjective experiences, asked participants to 'self-blind' by filling identical capsules with their microdose or nothing (placebo) and place them in identical envelopes with a QR code. The envelopes needed to be shuffled, and with the use of QR codes, they were instructed which envelope, and thus capsule, they were supposed to take. The authors of this study suggested that the acute positive psychological effects experienced with microdosing with psychedelics, could be explained by a placebo effect, as participants noticed they received an 'active' dose and therefore were breaking-blind.[81] However, another research group found that expectations about microdosing with psychedelics assessed prior to the start of microdosing in an online study were not completely in line with the reported effects,[20] which they took as evidence that

observed effects are not merely due to placebo effects. Nevertheless, this does not indicate that expectancy will not play a role in observed effects.

While it is almost impossible to make sure participants do not break blind in placebo-controlled experimental settings with psychoactive substances, adding a measurement of participants' expectations prior to substance administration and comparing the expectations with the observed effects can help control statistically for potential expectancy effects. Next to that, two experimental conditions can be added, creating two active conditions and two placebo conditions, with one condition clearly stating that participants receive the active substance and not a placebo and vice versa. However, these manipulations will have additional study costs, take longer to complete and have a higher risk of drop-outs. Alternatively, one could inform participants that the study examines the effects of multiple types of drugs and that they will receive either a stimulant, a sedative, or a hallucinogen drug, as has been applied by other groups.[82, 83] Either way, it is recommended that placebo-controlled studies should try to control for the expectancy effect.

The studies in this dissertation did not include one of the above mentioned methods to control for expectancy effects, however we doubt that breaking-blind had proportionate effects on the results of our studies. For instance, participants noticed that they were under the influence of LSD of 10 and 20 mcg LSD dose (feeling *under the influence* and *high*; **chapter 4**), but only the 20 mcg dose increased sustained attention **(chapter 4)** and BDNF blood plasma concentrations **(chapter 5)**. If breaking-blind would play a role, these effects should also have been seen with the 10mcg LSD dose. Interestingly, participants did not feel under the influence of 5 mcg LSD **(chapter 4)**, yet, an increase in BDNF concentration was observed **(chapter 5)**. These effects cannot be explained by participants breaking blind.

Conclusion

This dissertation examined the acute effects of cocaine, CBD and microdose of LSD on mood and cognition. It was demonstrated that these substances can enhance certain aspects of cognition and mood, therefore making them candidate therapeutic applications. While some (LSD, CBD) might be more suited than others (cocaine) due to their lower abuse potential,

future research in psychopathological populations can further examine its therapeutic potential. If proven efficacious and safe to use in patients, could be the implementation in therapeutic settings. Next to that, it was demonstrated that state and trait factors play a role regarding the effects of these aforementioned substances. Therefore, future research needs to focus on individual variation so the therapeutic applications can be used to find the best therapeutic fit for a patient.

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Summary

High levels of competition, workload, and pressure regarding study and work-related performance are very common in students' and young professionals' lives. To achieve and maintain high cognitive performance and counteract possible negative mood changes due to stress or pressure, the use of pharmacological interventions to enhance cognitive performance and mood is not uncommon. While the recreational use of prescription drugs, such as methylphenidate and amphetamines, as cognition and mood enhancers, is known to be fairly prevalent among high school and university students, the prevalence of using illicit substances for the same purpose is even higher. These substances include, for instance, cocaine, cannabidiol (CBD) a constituent of cannabis, psychedelic substances like Lysergic Acid Diethylamide (LSD) in small doses ('microdosing'). While most of the perceived effects of cocaine, CBD, and microdoses of LSD on mood and cognition seem positive, the evidence is mixed or based on user claims. Furthermore, additional factors such as an individual's trait and state at the time of substance use might influence the effects of cocaine, CBD and LSD on cognition and mood.

User claims suggest that cocaine allegedly enhances creativity. **Chapter 2** aimed to examine the acute effects of cocaine on task-based and self-rated creative performance using a variety of creativity tasks. In addition, it was examined whether cocaine's potential effects on creativity were associated with personal factors like trait empathy and cocaine-induced positive mood state. A placebo-controlled within-subjects study including 24 healthy poly-drug users was set up to examine these aforementioned aims. Verbal convergent thinking (CT) was assessed with the Remote Associates Task (RAT); figural CT was assessed with the Picture Concepts Task (PCT) and the Tower of London (TOL). Verbal divergent thinking (DT) was assessed with the Alternative Uses Task (AUT); figural DT was assessed with the Pattern/Line Meanings Task (PLMT). Trait empathy was measured with the Empathizing and Systemizing quotient questionnaire and positive mood state was assessed with the Profile of Mood States questionnaire. Findings showed that, compared to placebo, cocaine impaired figural CT (TOL) and flexible DT of verbal stimuli (AUT), while it enhanced figural DT (PLMT). No significant effects of cocaine were observed regarding the PCT and RAT. It was demonstrated that cocaine-induced effects on creativity in poly-drug users are stimulus-dependent. Cocaine enhanced performance on figural DT but impaired performance on verbal (flexible) DT. Cocaine

impaired CT on only one figural task and but not on the other tasks. Cocaine-induced positive mood was positively associated with enhanced figural convergent and verbal divergent thinking performance. In addition, higher levels of trait empathy were associated with enhanced verbal and figural divergent thinking when under the influence of cocaine. As creativity is an important aspect in cognitive therapies, it is important to further understand these discrepancies in creativity task performance.

Next to creativity, user claims suggest that cocaine positively affects cognitive performance. To examine the acute effects of cocaine on cognitive performance, **chapter 3** included a test battery testing both memory and attention, and this study aimed to assess whether the cognitive alterations of cocaine is domain specific or it has a more general effect on cognition. While cannabis is known to impair prospective memory, cannabis served as an active control group. A placebo-controlled, three-way, cross-over study including 15 regular poly-drug users was set up to test the influence of oral cocaine (300 mg) and vaporised cannabis (300+150 'booster' µg/kg bodyweight) on an event-based prospective memory task. Attentional performance was assessed using a divided attention task and subjective arousal was assessed with the Profile of Mood States questionnaire. Results showed that cocaine enhanced prospective memory, attention and arousal. Mean performance of prospective memory and attention, as well as levels of arousal were lowest during treatment with cannabis as compared with placebo and cocaine as evinced by a significantly increased trend across treatment conditions. Prospective memory performance was only weakly positively associated to measures of attention and arousal. Together, these results indicate that cocaine enhancement of prospective memory performance cannot be fully explained by parallel changes in arousal and attention levels, and is likely to represent a direct change in the neural network underlying prospective memory

The practice of microdosing with LSD is believed to enhance mood and performance. **Chapter 4** aimed to determine, by means of a dose-finding study, the minimal dose of LSD needed to affect mood and cognition. A placebo-controlled within-subject study including 24 healthy participants, was conducted to assess the acute effects of three LSD doses (5, 10, and 20 mcg) on measures of cognition, mood, and subjective experience, up until 6 h after administration. Cognition and subjective experience were assessed using the Psychomotor Vigilance Task, Digit

Symbol Substitution Test, Cognitive Control Task, Profile of Mood States, and 5-Dimensional Altered States of Consciousness rating scale. LSD showed positive effects in the majority of observations by increasing positive mood (20 mcg), friendliness (5, 20 mcg), arousal (5 mcg), and decreasing attentional lapses (5, 20 mcg). Negative effects manifested as an increase in confusion (20 mcg) and anxiety (5, 20 mcg). Psychedelic-induced changes in waking consciousness were also present (10, 20 mcg). Overall, the present study demonstrated selective, beneficial effects of low doses of LSD on mood and cognition in the majority of observations. The minimal LSD dose in this study at which subjective and performance effects are notable is 5 mcg and the most apparent effects were visible after 20 mcg.

Despite the preclinical evidence for psychedelic-induced neuroplasticity, confirmation in humans is grossly lacking. Given the increased interest in using low doses of psychedelics for psychiatric indications and the importance of neuroplasticity in the therapeutic response, **chapter 5** aimed to examine the effects of microdoses of LSD on neuroplasticity. A placebo-controlled within-subject study investigated the effect of single low doses of LSD (5, 10, and 20 μg) on circulating Brain Derived Neurotrophic Factor (BDNF) levels in healthy volunteers. Blood samples were collected every 2 h over 6 h, and BDNF levels were determined afterwards in blood plasma using ELISA. The findings demonstrated an increase in BDNF blood plasma levels at 4 h (5 μg) and 6 h (5 and 20 μg) compared to that for the placebo. The finding that LSD acutely increases BDNF levels warrants studies in patient populations.

Delta-9-tetrahydrocannabinol (THC), an active component of cannabis, can cause anxiety in some users during intoxication, while cannabidiol, another constituent of cannabis, has anxiolytic properties. It is suggested that cannabis products containing CBD in addition to THC may produce less anxiety than THC-only products, and may therefore be more favourable among cannabis users. **Chapter 6** examined whether anxiety following single doses of vaporized CBD, THC and THC/CBD might be explained by state and trait anxiety levels at baseline. A placebo-controlled, randomised, within-subjects study including 26 healthy recreational cannabis users tested the effects of vaporised cannabis containing THC (13.75 mg THC), CBD (13.75 mg CBD), THC/CBD (13.75 mg THC/13.75 mg CBD), and placebo on anxiety. Self-rated trait anxiety was assessed with the State-Trait Anxiety Inventory (STAI). State levels of anxiety were objectively assessed with a computer-based emotional Stroop task (EST) and

subjectively rated with the STAI-state questionnaire and a visual analogue scale. Both THC and THC/CBD significantly increased self-rated state anxiety compared to placebo. State anxiety after THC/CBD was significantly lower than after THC alone. THC-induced anxiety was independent of anxiety at baseline. When baseline anxiety was low, CBD completely counteracted THC-induced anxiety; however, when baseline anxiety was high, CBD did not counteract THC induced anxiety. There were no effects of any treatment condition on the EST. Overall, the study demonstrated that the THC/CBD equivalent cannabis induces less state anxiety than THC-dominant cannabis.

Finally, in **chapter 7** the key findings are discussed in a broader context and implications and recommendations for future research are provided. It was concluded that the substances examined in this dissertation can enhance certain aspects of cognition and mood, therefore making them candidate therapeutic applications. While some (LSD, CBD) might be more suited than others (cocaine) due to their lower abuse potential, future research in psychopathological populations can further examine its therapeutic potential. Next steps, if proven efficacious and safe to use in patients, could be the implementation in therapeutic settings. Next to that, it was concluded that state and trait factors play a role regarding the effects of these aforementioned substances. Therefore, future research needs to focus on individual variation so the therapeutic applications can be used to find the best therapeutic fit for a patient.

Samenvatting

Hoge niveaus van competitie, werkbelasting en druk met betrekking tot studie- en werk gerelateerde prestaties zijn zeer gebruikelijk in het leven van studenten en jonge professionals. Om hoge cognitieve prestaties te bereiken en te behouden en mogelijke negatieve stemmingswisselingen als gevolg van stress of druk tegen te gaan, is het gebruik van farmacologische interventies om cognitieve prestaties en stemming te verbeteren niet ongewoon. Het is bekend dat het recreatief gebruik van voorgeschreven geneesmiddelen, zoals methylfenidaat en amfetaminen, als cognitie- en stemmingsverbeteraars tamelijk gangbaar is onder middelbare scholieren en studenten aan universiteiten, maar het gebruik van illegale middelen voor hetzelfde doel is nog omvangrijker. Tot deze stoffen behoren bijvoorbeeld, cocaïne; cannabidiol (CBD) een bestanddeel van cannabis, psychedelische stoffen zoals Lyserginezuurdiëthylamide (LSD) in kleine doses ('microdoseringen'). Hoewel de meeste effecten van cocaïne, CBD en microdoses LSD op stemming en cognitie positief lijken, zijn de bewijzen wisselend of gebaseerd op beweringen van gebruikers. Bovendien kunnen bijkomende factoren, zoals iemands karakter en gemoedstoestand op het moment van het middelengebruik, de effecten van cocaïne, CBD en LSD op cognitie en gemoedstoestand beïnvloeden.

Beweringen van gebruikers suggereren dat cocaïne de creativiteit zou verhogen. **Hoofdstuk 2** onderzocht de acute effecten van cocaïne op taak gebaseerde en zelf gerapporteerde creatieve prestaties met behulp van een variëteit aan creativiteitstaken. Daarnaast werd onderzocht of de mogelijke effecten van cocaïne op creativiteit samenhingen met persoonlijke factoren zoals het persoonlijkheidskenmerk empathie en een cocaïne-geïnduceerde positieve gemoedstoestand. Een placebogecontroleerde within-subjects studie met 24 gezonde poly-druggebruikers werd opgezet om deze bovengenoemde doelstellingen te onderzoeken. Verbaal convergent denken (CT) werd gemeten met de Remote Associates Task (RAT); figurale CT werd gemeten met de Picture Concepts Task (PCT) en de Tower of London (TOL). Verbaal divergent denken (DT) werd gemeten met de Alternative Uses Task (AUT); figurale DT werd gemeten met de Pattern/Lines Meanings Task (PLMT). Persoonlijkheidskenmerk empathie werd gemeten met de Empathizing and Systemizing quotiënt vragenlijst en positieve gemoedstoestand werd gemeten met de Profile of Mood States vragenlijst. De bevindingen toonden aan dat, vergeleken met placebo, cocaïne de figurale CT (TOL) en de flexibele DT van verbale stimuli

(AUT) verminderde, terwijl het de figurale DT (PLMT) versterkte. Er werden geen significante effecten van cocaïne waargenomen met betrekking tot de PCT en RAT. Er werd aangetoond dat cocaïne-geïnduceerde effecten op creativiteit bij polydruggebruikers stimulus-afhankelijk zijn. Cocaïne verbeterde de prestaties bij figurale DT maar verminderde de prestaties bij verbale (flexibele) DT. Cocaïne verminderde CT op slechts één figurale taak, maar niet op de andere taken. Cocaïne-geïnduceerde positieve stemming werd positief geassocieerd met verbeterd figuraal CT en verbaal DT. Bovendien werden hogere niveaus van empathie geassocieerd met verbeterd verbaal en figuraal DT onder invloed van cocaïne. Aangezien creativiteit een belangrijk aspect is in cognitieve therapieën, is het belangrijk om deze discrepanties in creativiteitstaakprestaties verder te begrijpen.

Naast creativiteit, suggereren beweringen van gebruikers dat cocaïne cognitief functioneren positief beïnvloedt. Om de acute effecten van cocaïne op cognitieve prestaties te onderzoeken, omvatte **hoofdstuk 3** een testbatterij waarin zowel geheugen als aandacht werden getest, en het onderzoek probeerde te nagaan of de cognitieve veranderingen van cocaïne domein specifiek zijn of dat het een meer algemeen effect op cognitie heeft. Terwijl bekend is dat cannabis het prospectieve geheugen aantast, diende cannabis als een actieve controlegroep. Er werd een placebogecontroleerd, drieweg, cross-over onderzoek opgezet met 15 regelmatige polydrugsgebruikers om de invloed te testen van orale cocaïne (300 mg) en gevaporiseerde cannabis (300+150 'booster' µg/kg lichaamsgewicht) op een event-gebaseerde prospectieve geheugentaak. De aandachtsprestatie werd gemeten met een verdeelde aandachtstaak en de subjectieve arousal werd gemeten met de Profile of Mood States-vragenlijst. De resultaten toonden aan dat cocaïne het prospectieve geheugen, de aandacht en arousal verhoogde. De gemiddelde prestaties van prospectief geheugen en aandacht, evenals de niveaus van arousal, waren het laagst tijdens de cannabis conditie in vergelijking met placebo en cocaïne, zoals blijkt uit een significant verhoogde trend in alle condities. Prospectieve geheugenprestaties waren slechts zwak positief geassocieerd met metingen van aandacht en arousal. Samen geven deze resultaten aan dat de verbetering van de prospectieve geheugenprestatie door cocaïne niet volledig kan worden verklaard door parallelle veranderingen in arousal en aandachtsniveaus, en waarschijnlijk een directe verandering vertegenwoordigt in het neurale netwerk dat ten grondslag ligt aan prospectief geheugen.

Aangenomen wordt dat de toepassing van LSD in microdoseringen de stemming en het cognitief functioneren verbetert. **Hoofdstuk 4** had als doel om, door middel van een dosis-onderzoek, de minimale dosis LSD te bepalen die nodig is om de stemming en cognitie te beïnvloeden. In een placebogecontroleerde studie, waaraan 24 gezonde deelnemers deelnamen, werden de acute effecten van drie doses LSD (5, 10, en 20 mcg) op cognitie, stemming en subjectieve ervaring gemeten, tot 6 uur na de toediening. Cognitie en subjectieve ervaring werden beoordeeld met behulp van de Psychomotor Vigilance Task, Digit Symbol Substitution Test, Cognitive Control Task, Profile of Mood States, en 5-Dimensional Altered States of Consciousness ratingschaal. LSD vertoonde positieve effecten in het merendeel van de observaties door het verhogen van de positieve stemming (20 mcg), vriendelijkheid (5, 20 mcg), arousal (5 mcg), en het verminderen van aandachtsverlies (5, 20 mcg). Negatieve effecten manifesteerden zich als een toename van verwardheid (20 mcg) en angst (5, 20 mcg). Door psychedelica veroorzaakte veranderingen in het bewustzijn waren ook aanwezig (10, 20 mcg). In het algemeen toonde het huidige onderzoek selectieve, gunstige effecten van lage doses LSD op stemming en cognitie aan bij de meeste observaties. De minimale dosering LSD in het huidige onderzoek waarbij subjectieve en cognitieve-effecten merkbaar zijn is 5 mcg en de meest duidelijke effecten waren zichtbaar na 20 mcg.

Ondanks het preklinische bewijs voor door psychedelica geïnduceerde neuroplasticiteit, ontbreekt de bevestiging daarvan bij mensen nog grotendeels. Gezien de toegenomen interesse in het gebruik van lage doseringen psychedelica voor psychiatrische indicaties en het belang van neuroplasticiteit in de therapeutische respons, was het doel van **hoofdstuk 5** om de effecten van microdoses LSD op neuroplasticiteit te onderzoeken. Een placebo-gecontroleerde studie onderzocht het effect van een enkele lage dosis LSD (5, 10, en 20 μg) op de circulerende Brain Derived Neurotrophic Factor (BDNF) niveaus bij gezonde vrijwilligers. Bloedmonsters werden om de 2 uur afgenomen gedurende 6 uur, en BDNF niveaus werden naderhand bepaald in bloedplasma met behulp van ELISA. De bevindingen toonden een stijging aan van de BDNF bloedplasmaspiegels na 4 uur (5 μg) en na 6 uur (5 en 20 μg) in vergelijking met die voor de placebo. De bevinding dat LSD acuut de BDNF-spiegels verhoogt, is aanleiding voor onderzoek bij patiënten populaties.

Delta-9-tetrahydrocannabinol (THC), een actief bestanddeel van cannabis, kan bij sommige gebruikers angst teweegbrengen, terwijl cannabidiol, een ander bestanddeel van cannabis, anxiolytische eigenschappen heeft. Er wordt gesuggereerd dat cannabisproducten die naast THC ook CBD bevatten, minder angst kunnen veroorzaken dan producten die alleen THC bevatten, en daarom gunstiger zouden kunnen zijn voor cannabisgebruikers. In **hoofdstuk 6** werd onderzocht of angst na een enkele dosis gevaporiseerde CBD, THC en THC/CBD verklaard zou kunnen worden door angst als gemoedstoestand en persoonlijkheidskenmerk tijdens baseline. In een placebogecontroleerd, gerandomiseerd onderzoek met 26 gezonde recreatieve cannabisgebruikers werd het effect op angst onderzocht van gevaporiseerde cannabis met THC (13,75 mg THC), CBD (13,75 mg CBD), THC/CBD (13,75 mg THC/13,75 mg CBD), en placebo. Zelf beoordeelde angst als persoonlijkheidskenmerk werd gemeten met de State-Trait Anxiety Inventory (STAI). Het angstniveau in de gemoedstoestand werd objectief beoordeeld met een computer gebaseerde emotionele Stroop taak (EST) en subjectief beoordeeld met de STAI-state vragenlijst en een visuele analoge schaal. Zowel THC als THC/CBD verhoogden significant de zelf gerapporteerde angst in vergelijking met placebo. De angst na THC/CBD was significant lager dan na THC alleen. De door THC veroorzaakte angst was onafhankelijk van de angst bij aanvang. Wanneer de baseline angst laag was, ging CBD de door THC veroorzaakte angst volledig tegen; wanneer de baseline angst echter hoog was, ging CBD de door THC veroorzaakte angst niet tegen. Er waren geen effecten van de cannabis condities op de EST. In het algemeen toonde het onderzoek aan dat cannabis met een THC/CBD-equivalent minder angst induceert dan cannabis met een THC-dominantie.

Tenslotte worden in **hoofdstuk 7** de belangrijkste bevindingen in een bredere context besproken en worden implicaties en aanbevelingen voor toekomstig onderzoek gegeven. Geconcludeerd werd dat deze stoffen onderzocht in dit proefschrift bepaalde aspecten van cognitie en stemming kunnen versterken, waardoor ze kandidaat zijn voor therapeutische toepassingen. Hoewel sommige (LSD, CBD) door hun lager misbruikpotentieel wellicht geschikter zijn dan andere (cocaïne), kan toekomstig onderzoek bij psychopathologische populaties het therapeutisch potentieel verder onderzoeken. De volgende stap, mits bewezen doeltreffend en veilig voor gebruik bij patiënten, zou de implementatie in therapeutische setting kunnen zijn. Daarnaast werd geconcludeerd dat toestand- en karakterfactoren een rol

spelen bij de effecten van deze stoffen. Daarom moet toekomstig onderzoek zich richten op individuele variatie, zodat de therapeutische toepassingen kunnen worden gebruikt om de beste therapeutische fit voor een patiënt te vinden.

Impact paragraph

Scientific and societal dissemination

To spread the findings of the experimental studies presented in this dissertation to the international research community, recreational substance users, or people who self-medicate with these substances, we strive to publish these results in journals with open access. Four out of five studies presented in this dissertation are published in several international journals, and **chapter 6** is currently submitted. Two papers in particular caught attention as shown by their Altmetric score of 194 (**chapter 5**) and 133 (**chapter 4**). **Chapter 4** is since a year now, in the top 3 most downloaded articles of the *European Neuropsychopharmacology journal.* In addition, the results of all chapters were presented at national and international conferences that hosted many international researchers.

Next to publishing our results in international journals, we were invited to present our experimental findings at Trimbos-institute (national Dutch expertise centre for mental health care, addiction care and social care) and HIT Hot Topics in Liverpool (an organisation delivering interventions on drugs, community safety and other public health concerns).

In addition, we were asked to contribute to radio interviews (Radio 1, L1 radio), documentaries on national and international television (WDR doku, EenVandaag), articles in newspapers and magazines (WIRED, Dagblad de Limburger, vakblad analisten), and several other blogs and internet posts discussing our findings of **chapters 3, 4, and 5**. This media attention led to patients with mental health problems reaching out to us asking whether it is possible to contribute to our research as they were searching for alternative treatment options for their mental health problems, even though our studies were only conducted in healthy participants. An important note: we as researchers need to be clear that we are dealing with psychoactive substances, and that we are not advocating that at this stage these substances can be taken outside a research context, without supervision, and to make sure that the media carries this message as well. Nevertheless, the almost unavoidable media attention will help to further increase the societal, but also scientific interest in the effects of microdosing with psychedelics as a potential alternative treatment option for several disorders.

Drug development

My ultimate goal is to provide evidence or find alternative treatment options for patients who do not respond to treatments currently available. A way to accomplish this goal is to ask people who self-medicate with the substances mentioned in this dissertation about the motives and experienced effects, similar to our previous studies, where we examined the motives to microdosing with psychedelics and self-rated effectiveness of these substances for several disorders. [1,2] In addition, by collaborating with the research and community platforms that provide information on microdosing with psychedelics, for instance Microdosing Institute and Vista+. Alternatively, collect data from internet fora where substance users discuss their experiences with microdosing with psychedelics, cocaine use or CBD use like Erowid or Reddit. While we are not able to advise patients based on the current studies, we can inform therapists and health professionals about the therapeutic potential of these substances. Therapists and health professionals need to know more about these substances when for instance a patient mentions he/she is currently self-medicating or plans to self-medicate. Less than one in five patients discussed the use of microdosing with psychedelics with their therapists, maybe due to the legal status and stigma of these substances.[3]

A number of drug development companies are currently key players in trying to medicalise cannabis and microdosing with LSD. These companies pick up and build upon some of our findings discussed in this dissertation. For instance, three experimental trials are developed to examine the therapeutic potential of repeated microdoses of LSD, partially based upon our findings presented in **chapters 4** and **5**. Mind Medicine (MindMed) Inc., a leading biotech company developing psychedelic-inspired therapies, will finance a randomized placebo-controlled study evaluating the effects of daytime and evening administration of repeated low doses of LSD, to examine whether the time of intake (morning or evening) influences the impact of the substance on sleep, mood and cognition.[84] Another trial financed by MindMed, a phase 2 proof-of-concept study examining the effects of repeated microdoses of LSD (20 mcg) in adult patients with ADHD.[5] Silo Pharma Inc., a developmental biopharmaceutical company focusing on the use of psilocybin as a therapeutic application, will finance a trial examining the effects of repeated low doses of psilocybin and ketamine on cognitive and emotional

dysfunctions in Parkinson's disease and to understand its mechanism of action.[6] Considering the timeline of clinical trials regarding the efficacy of MDMA-assisted psychotherapy to treat PTSD, starting with phase 2 clinical trials in 2004 and the indication that MDMA will be approved by the FDA in 2023,[7-9] we think that the use of low doses a psychedelic substance for treating ADHD and Parkinson's disease (if efficacy is proven) will also take around 10 years before the FDA will approve this as a treatment.

Our finding that cannabis with a THC/CBD ratio of 1 to 1 causes less anxiety than a THC-only cannabis **(chapter 6)** may be of value to GW pharmaceuticals, a company that develops and commercializes pharmaceutical cannabis products. GW pharmaceuticals currently have several clinical trials underway investigating the efficacy of various cannabis-based medications in relation to epilepsy, autism spectrum disorder, glioma, neonatal hypoxic-ischemic encephalopathy, and schizophrenia.[10] They can implement our findings from **chapter 6** into their research protocol, for example, the finding that CBD can completely reduce the unwanted THC-induced anxiety if the person has a low state of anxiety prior to cannabis consumption. They may then choose to implement some relaxation exercises prior to cannabis consumption. This may be especially desirable when conducting research in vulnerable populations, such as children.

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Curriculum Vitae

Nadia R.P.W. Hutten was born on January 19th, 1992 in Kerkrade, the Netherlands. She graduated from secondary education (VWO) at College Rolduc in Kerkrade in 2010. She took a gap year and worked in a garden centre where she developed some garden skills. She continued her education studying health sciences at the Faculty of Health, Medicine, and Life Sciences (FHML) at Maastricht University in 2011. After obtaining the Bachelor's degree in 2014, she started the Master Neuropsychology at the Faculty of Psychology and Neuroscience (FPN). During her research internship, she studied the acute effects of THC on sustained attention and default mode functional connectivity under the supervision of prof. dr. Jan Ramaekers and dr. Eef Theunissen at the department of Neuropsychology and Psychopharmacology (np&pp). After completing her master's degree in 2015, she continued as a research assistant on several projects at np&pp for two years. In September 2017, she worked as a PhD candidate at the department of np&pp till the beginning of 2022. During this project, she obtained her University Teaching Qualification (BKO) in 2021. She continues to work as a post-doctoral researcher in the same department and will examine the effects of microdosing with LSD on resting-state electroencephalography and event-related potentials in healthy volunteers.

Research output

List of publications

As part of this dissertation:

Hutten, N.R.P.W., Arkell, T.R., Vinckenbosch, F., Schepers, J., Kevin, R.C., Theunissen, E.L., Kuypers, K.P.C., McGregor, I.S., & Ramaekers, J.G. (*submitted*). Cannabis containing equivalent concentrations of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) induces less state anxiety than THC-dominant cannabis.

Hutten, N.R.P.W., Mason, N.L., Dolder, P.C., Theunissen, E.L., Holze, F., Liechti, M.E., Varghese, N., Eckert, A., Feilding, A., Ramaekers, J.G., & Kuypers, K.P.C. (2020). Low doses of LSD acutely increase BDNF blood plasma levels in healthy volunteers. *ACS Pharmacology & Translational Science*, *4*(2), 461-466, DOI: 10.1021/acsptsci.0c00099.

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Not as part of this dissertation:

Ramaekers, J.G., **Hutten, N.R.P.W.**, Mason, N.L., Dolder, P.C., Theunissen, E.L., Holze, F., Liechti, M.E., Feilding, A., & Kuypers, K.P.C. (2021). A low dose of lysergic acid diethylamide decreases pain perception in healthy volunteers. *Journal of Psychopharmacology*, *35*(4), 398-405, DOI: 10.1177/0269881120940937.

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Administration in Humans. *Frontiers in Pharmacology*, 9, 713, DOI: 10.3389/fphar.2018.00713.

Presentations conferences and symposia

Low dose of LSD acutely increase BDNF blood plasma levels in healthy volunteers. (2022) Talk presented as part of a mosaic presentation at uniMIND Symposium, Maastricht.

Cannabis containing a combination of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) induces less state anxiety compared to THC-only cannabis. (2021) Poster presented at Dutch Neuroscience Conference, virtual.

Cannabis-induced anxiety; baseline anxiety and anxious personality. (2021) Talk presented at European College of Neuropsychopharmacology Workshop on Neuropsychopharmacology for Early Career Scientists in Europe, virtual.

Cognitive and subjective effects of different low doses of LSD in a placebo-controlled study. (2020) Talk presented at Interdisciplinary Conference on Psychedelic Research, virtual.

A low dose of LSD enhances sustained attention and positive mood, but impairs information processing. (2020) Poster presented at 33rd European College of Neuropsychopharmacology conference, virtual.

Elevator pitch. (2019) Talk presented at EURON Workshop: Drugs and the Brain from Laboratory to Clinic, Crete, Greece.

Self-rated effectiveness of microdosing with psychedelics for mental and physical health problems among microdosers. (2019) Poster presented at EURON Workshop: Drugs and the Brain from Laboratory to Clinic, Crete, Greece.

Low 'micro' doses of LSD – subjective and cognitive effects. (2019) Talk presented at Dutch Neuroscience Conference, Lunteren, the Netherlands.

A single dose of cocaine enhances prospective memory performance. (2018) Poster presented at Faculty of Psychology and Neuroscience Research Day, Maastricht, the Netherlands.

A single dose of cocaine enhances prospective memory performance. (2018) Poster presented at Dutch Neuroscience Conference, Lunteren, the Netherlands.

Invited presentations

What we know about Microdosing with psychedelics. (2019) Talk presented at Hot Topics, Liverpool, United Kingdom.

Lage 'micro' doseringen met LSD – subjectieve en cognitieve effecten. (2019) Talk presented at Trimbos Instituut DIMS dag, Utrecht, the Netherlands.

Masterclass: Microdoseren met psychedelica. (2018) Talk presented at Safe 'n Sound, Gent, Belgium.

A single dose of cocaine enhances prospective memory performance. (2018) Talk presented at The Innbetween, Maastricht, the Netherlands

A single dose of cocaine enhances prospective memory performance. (2018) Talk presented at Maastricht and Memory (M&M) symposium, Maastricht, the Netherlands.

A single dose of cocaine enhances prospective memory performance. (2018) Talk presented at Faculty of Psychology and Neuroscience, Lunch Talk, Maastricht, the Netherlands.