PHARMACOKINETICS AND PHARMACODYNAMICS OF LYSERGIC ACID DIETHYLAMIDE (LSD) COMPARED WITH STIMULANTS AND ENTACTOGENS IN HEALTHY SUBJECTS

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“There are things known and there are things unknown, and in between are the doors of perception.”

Aldous Huxley

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PREFACE

All research in this thesis is published in peer-reviewed journals and presented in the form of scientific papers. References for each paper are presented within each publication. The general reference list at the end of the thesis is covering the introduction and discussion part. All presented research was performed at the University Hospital Basel and the University of Basel and one paper in collaboration with the University of Maastricht.

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CONTRIBUTIONS

I contributed as lead author or shared lead author to the publications presented in this thesis. For all projects, I took part in the planning, analyzed the data and performed the research together with other research group members, and MD students and master students under my supervision. The microdosing study was conducted in collaboration with the University of Maastricht. I analyzed the PK data and corresponding PD data from a clinical trial that was conducted in the Netherlands at the University of Maastricht. All work was conducted under the supervision of Prof. Dr. Matthias Liechti and with the help of the psychopharmacology research group team.

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SUMMARY

Classic psychedelics such as the semi-synthetic ergoline and serotonin $(5\text{-HT})_{2A}$ receptor agonist lysergic acid diethylamide (LSD) are back in research as potential treatment for various psychiatric and somatic disorders, e.g. depression, anxiety disorders and cluster headache.

This thesis includes three clinical trials to add knowledge to the pharmacology of LSD. The first study investigated the distinction of LSD as prototypical psychedelic from the class of entactogens represented by 3,4-methylenedioxymethamphetamine (MDMA) and the class of stimulants represented by d-amphetamine. Twenty-eight healthy participants were included in a randomized, double-blind, placebo-controlled trial. This study was the first study that used an oral LSD formulation with an exactly known dose and confirmed stability. The second study investigated the dose-effect relationship of LSD and the role of the 5-HT_{2A} receptor by adding the 5-HT_{2A} receptor antagonist ketanserin prior to the administration of a high dose of LSD (200 µg). Sixteen healthy participants were included in a randomized, double-blind, placebo-controlled trial. Treatment conditions were 25 µg, 50 µg, 100 µg, 200 µg LSD, and 200 µg LSD + ketanserin. The third study investigated LSD microdoses by performing a study using 5, 10, and 20 µg LSD in healthy subjects. Twenty-four healthy participants were included in this randomized, placebo-controlled, double-blind trial.

All studies investigated the pharmacokinetics and pharmacokinetic-pharmacodynamic relationship of LSD. Classic pharmacokinetic parameters as well as effect durations, onset, and offset were assessed using modern PK-PD modeling techniques. The first two studies also investigated subjective effects using well established methods and questionnaires such as the 5-dimensions of altered states of consciousness (5D-ASC) scale, the mystical effects questionnaire (MEQ), and visual analogue scales (VASs) to measure subjective effects over time. Furthermore, autonomic effects were assessed including blood pressure, heart rate, body temperature, and pupil size. Additionally, acute levels of brain-derived neurotrophic factor (BDNF) were assessed.

Summarized, results from the present thesis show that LSD can clearly be distinguished from stimulants (e.g. d-amphetamine) and entactogens (e.g. MDMA) in terms of subjective effects. LSD induced significantly higher changes in the acute state of consciousness and promoted higher mystical-type experiences compared to MDMA and d-amphetamine. But, all of the substances produced comparable increases in hemodynamic effects, body temperature, and pupil size, indicating equivalent autonomic responses at the doses used. Furthermore, LSD showed a dose-proportional pharmacokinetic profile for doses from 5-200 µg and effects were closely linked with substance-concentrations in the blood. Subjective effects started at 10 µg LSD suggesting this to be the cut-off for 'microdosing' and the maximum effect was reached at a dose of 100 µg LSD. In addition, 'bad drug effects' seem to be associated with higher doses. Pretreatment with ketanserin effectively prevented the response to 200 µg LSD. The full psychedelic effects of LSD are therefore primarily mediated by 5-HT_{2A} receptor activation.

Findings from this thesis are important for dose finding of LSD and the choice of substance in future research for substance- assisted therapy.

INTRODUCTION

1.1. History & Classification of LSD

Lysergic acid diethylamide (LSD) is one of the most controversial substances in modern history. By 1971, when most research came to halt, over 1000 scientific articles were published (Nichols 2016) and in 2010 an estimated 32 million US residents reported lifetime use of LSD (Krebs and Johansen 2013). Chemically, LSD is classified a semisynthetic ergoline derivative. It is a chiral compound with two stereocenters what theoretically would result in 4 stereoisomeric options, however only one stereoisomer, namely (+)- LSD is psychoactive. LSD is structurally closely related to serotonin and therefore a tryptamine which originate from the amino acid tryptophan.

LSD was first synthesized in 1938 in the laboratories of Sandoz by the Swiss chemist Albert Hofmann during his research on the fungus ergot. The purpose of his research was to find a new cardiovascular stimulant that was more potent than the known coramin (nicotinic acid diethylamide). However, the effects observed in animal experiments lagged behind expectations, so for the time being, the interest in the substance was lost. On April 16th 1943, Albert Hoffmann decided to resynthesize the substance. Shortly after, he experienced the first LSD trip in human history. Three days later, he decided on a test series and ingested a dose of 250 µg LSD tartrate. He later describes the effects as more intense and profound than the first time (Hofmann 1979). This day, the 19th of April, is now famously known as the 'Bicycle Day'. From 1949 to 1966 Sandoz provided LSD under the name Delysid® as a research substance to physicians and psychiatrists all over the world to use as adjunct to psychotherapy or to gain insight into mental processed of psychiatric patients. From there on LSD was used in research to model psychosis and to enhance psychotherapy (Passie et al. 2008; Hofmann 1979). Many of its properties were described in the flood of articles published on LSD during that time period. Due to political reasons LSD was banned by the US government in 1968 (Bonson 2018).

In Switzerland, a small group of therapists had the privilege to conduct LSD and 3,4-methylenedioxymethamphetamine (MDMA) -assisted therapies between 1988-1993 (Gasser 1996). However, it took another 15 years until the first modern study took place in Switzerland. In 2008, the Swiss psychiatrist Peter Gasser administered LSD to 12 patients with anxiety related to a life-threatening disease (Gasser et al. 2014; Gasser et al. 2015). The first modern study administering LSD to healthy volunteers was conducted 5 years later in Basel (Schmid et al. 2015).

1.2. Pharmacology

1.2.1. Mechanism of Action

As main mechanism of action, LSD acts as highly potent partial agonist on the serotonin $(5\text{-HT})_{2A}$ receptor. LSD also binds with high potency to 5-HT_1 receptors whose role in the effects of psychedelics is still unclear (Liechti 2017). Furthermore, LSD binds to adrenergic and dopaminergic receptors at submolecular levels (Rickli et al. 2015) and is the only classic psychedelic that also interacts with the DA system (Rickli et al. 2015; Rickli et al. 2016). The importance of the 5-HT_{2A} receptor in mediating the effects of LSD in humans has previously been demonstrated - in a double blind, placebo-controlled study a moderate dose (100 µg) of LSD was administered after pretreatment with the 5-HT_{2A} receptor antagonist ketanserin. The subjective effects of LSD were fully blocked by ketanserin (Preller et al. 2017). In comparison to the direct agonism of LSD, MDMA as prototypical entactogen, has a more indirect mechanism of action. Its main mechanism of action is mediated via release of 5-HT and inversion of the corresponding uptake transporter. It further releases norepinephrine (NE) and dopamine (DA) via an interaction with the respective monoamine transporter (Rudnick and Wall 1992; Berger et al. 1992). The mechanism of action of LSD is also distinct from a stimulant. Stimulants, for example the prototypical d-amphetamine, mainly enhance DA and further NE neurotransmission by blocking DA and NE transporters and therefore causing an increase of neurotransmitter concentrations in the synaptic cleft (Simmler et al. 2014; Simmler et al. 2013; Kehr et al. 2011).

1.2.2. Pharmacokinetics

Dose	***N***		**K_{01} (1/h)**	**λ_z (1/h)**	**V_d (L)**	**C_{max} (ng/mL)**	**t_{max} (h)**	**$t_{1/2}$ (h)**	**AUC_∞ (ng·h/mL)**	**CL/F (L/h)**
100	24	Geometric mean (95% CI)	1.4 (1.2-4.1)	0.27 (0.24-0.31)	46 (35-76)	1.3 (1.2-1.9)	1.4 (1.3-2.1)	2.6 (2.4-3.0)	8.1 (7.5-11.1)	12.3 (7.8-24)
		Range	0.31-9.9	0.17-0.50	24-270	0.3-3.7	0.4-3.2	1.4-4.2	1-19	5.2-103
200	16	Geometric mean (95% CI)	1.2 (1.68-4.6)	0.27 (0.22-0.35)	37 (32-46)	3.1 (2.6-4.0)	1.5 (1.3-2.4)	2.6 (2.2-3.4)	20.3 (17.3-26.2)	9.9 (8.3-12.8)
		Range	0.27-10	0.12-0.59	18-66	1.9-7.1	0.4-3.8	1.2-5.6	11-39	5.1-18.5

Table 1 Pharmacokinetic parameters for LSD based on compartmental modeling after a single oral dose of 100 and 200 µg LSD in healthy participants. k_{01}, first-order absorption coefficient; λ_z, first order elimination coefficient; V_d volume of distribution; C_{max}, estimated maximum plasma concentration; t_{max}, estimated time to reach C_{max}; $t_{1/2}$, estimated plasma elimination half-life; $AUC\infty$, area under the plasma concentration-time curve from time zero to infinity; CL/F, apparent total clearance. Taken from Dolder et al. 2017 (Dolder et al. 2017a).

LSD is usually taken orally and is rapidly absorbed. Pharmacokinetics show a linear behavior with dose-proportional increases in plasma concentration and first order elimination kinetics (Table 1, Figure 1). C_{max} values are reached approximately after 1.5 hours and the half-life in plasma is approximately 2.5 hours (Dolder et al. 2017a). Bioavailability is estimated to be 71% (Dolder et al. 2017a). An early study investigating LSD concentrations in the cerebrospinal fluid in monkeys found the same concentrations of LSD in the cerebrospinal fluid as in the plasma, suggesting that LSD easily passes the blood brain barrier (Axelrod et al. 1957). LSD is thought to be metabolized to 2-oxo-3-hydroxy-LSD (O-H- LSD) and nor-LSD by cytochrome P450 (CYP) enzymes. However, it is still not entirely clear which enzyme contributes to which metabolite by which mechanism. In vitro studies suggest an involvement of CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 enzymes (Wagmann et al. 2019; Luethi et al. 2019). Unmetabolized LSD and O-H-LSD can be detected in urine (Dolder et al. 2015).

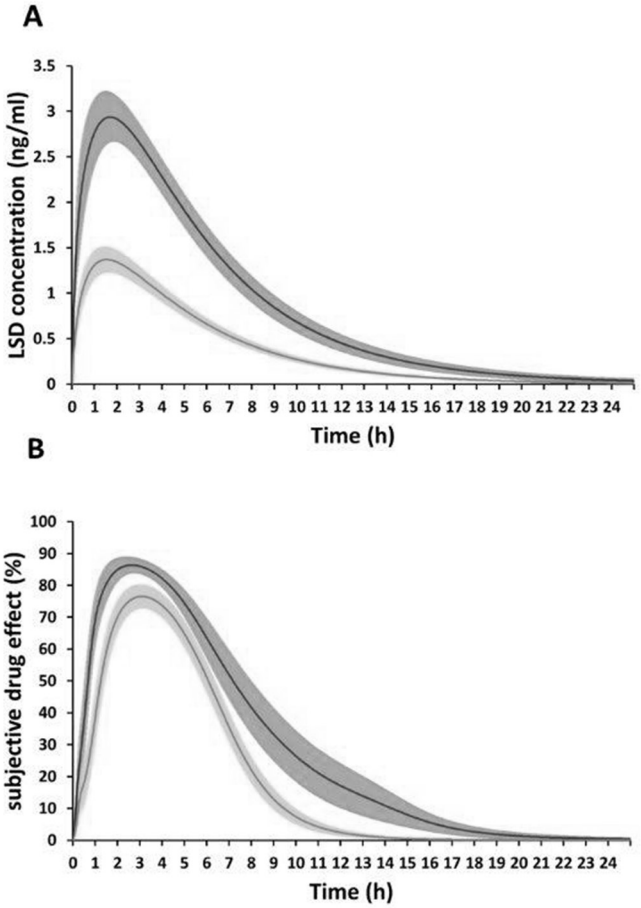

Figure 1 Pharmacokinetics and pharmacodynamics of LSD. LSD concentration-time (A) and subjective effect-time (B) curves. LSD was administered at a dose of 100 (light grey) and 200 µg (dark grey) p.o. to 24 and 16 healthy subjects, respectively, at the time-point t=0. Subjective LSD effects ('any subjective drug effects') were assessed repeatedly using visual analog scales (0-100%) along with blood samples to determine plasma concentrations of LSD (Dolder et al. 2016; Dolder et al. 2017a; Dolder et al. 2015). The LSD concentration curves (A) represent the mean ± SEM of the individual curves fitted to the observed data using a 1-compartment model. The subjective drug effect curves (B) represent the mean ± SEM of the individual curves fitted to the observed data using a sigmoidal E_{max} model linked to the predicted concentrations (Dolder et al. 2017a). Taken from Liechti 2017 (Liechti 2017).

1.2.3. Subjective effects

Studies in healthy participants showed that the onset of acute subjective effects of LSD after oral administration is dose-dependent and started at 0.8 and 0.4 hours for 100 and 200 µg, respectively (Dolder et al. 2017a). The effects peaked at 2.8 and 2.5 hours for 100 and 200 µg, respectively. Effect durations which were also dose-dependent, were 8.2 and 11.2 hours for 100 and 200 µg, respectively (Dolder et al. 2017a). The acute effects of LSD were experienced rather positive, and were expressed by high ratings in 'good drug effect' and 'drug liking' on the corresponding visual analogue scales (VASs) (Schmid et al. 2015; Dolder et al. 2017a). However, with increasing doses also negative effects appeared, which include ratings such as 'bad drug effect' and 'fear' on the corresponding VASs (Liechti 2017; Schmid et al. 2015; Dolder et al. 2017a). In contrast, subjective effects induced by MDMA and d-amphetamine lasted on average 4.2 hours and 4-6 hours, respectively (Vizeli and Liechti 2017; Rush et al. 2001; Brauer et al. 1996). Subjective effects induced by MDMA and d-amphetamine were almost only described as positive, negative subjective effects were mild and transient (Vizeli and Liechti 2017; Dolder et al. 2017b).

LSD induces profound alterations in consciousness that include changes in perception, cognition, thinking, and emotion processing. Perceptual changes include illusions, pseudo-hallucinations, intensified color perception, synesthesia, and alterations in time perception (Passie et al. 2008; Liechti et al. 2017; Schmid et al. 2015). Alterations of thinking may include imaginative thoughts, broader and unusual associations, re-experience of biographic memories, or mystical-type experiences (Passie et al. 2008; Liechti et al. 2017). In modern research these acute subjective effects are mainly assessed using the 5-dimension altered states of consciousness (5D-ASC) scale and the 30 item version of the mystical effects questionnaire (MEQ30). These questionnaires are currently the most important tool to compare altered states of consciousness across laboratories, studies, and substances. LSD induces changes on all scales of the 5D-ASC at a dose of 100 µg however, an oral dose of 200 µg induced significantly greater ratings on scales and dimensions that reflect bliss, changes in the meaning of perceptions and insightfulness compared with 100 µg (Figure 2) (Liechti et al. 2017). A study investigating 75 µg LSD i.v. showed comparable effects as 100 µg LSD in the above-mentioned study (Carhart-Harris et al. 2016b). However, a study comparing 125 mg MDMA with placebo in healthy participants also found marked effects on the 5D-ASC (Hysek et al. 2011). On the MEQ30, an oral dose of 200 µg LSD showed strong effects on all subscales (Liechti et al. 2017).

A single oral dose of LSD was associated with positive long-term effects in healthy participants. Greater alterations in consciousness (reflected by ratings on the 5D-ASC) and mystical-type experiences (reflected by ratings on the MEQ30) were associated with greater ratings of well-being up to 12 months after a high dose of 200 µg LSD (Schmid and Liechti 2018). Furthermore, LSD did not produce relevant changes in personality measures (Schmid and Liechti 2018). These effects are congruent with studies investigating long-term effects of the structurally

similar serotonergic psychedelic psilocybin (Griffiths et al. 2008; Griffiths et al. 2011; Griffiths et al. 2006).

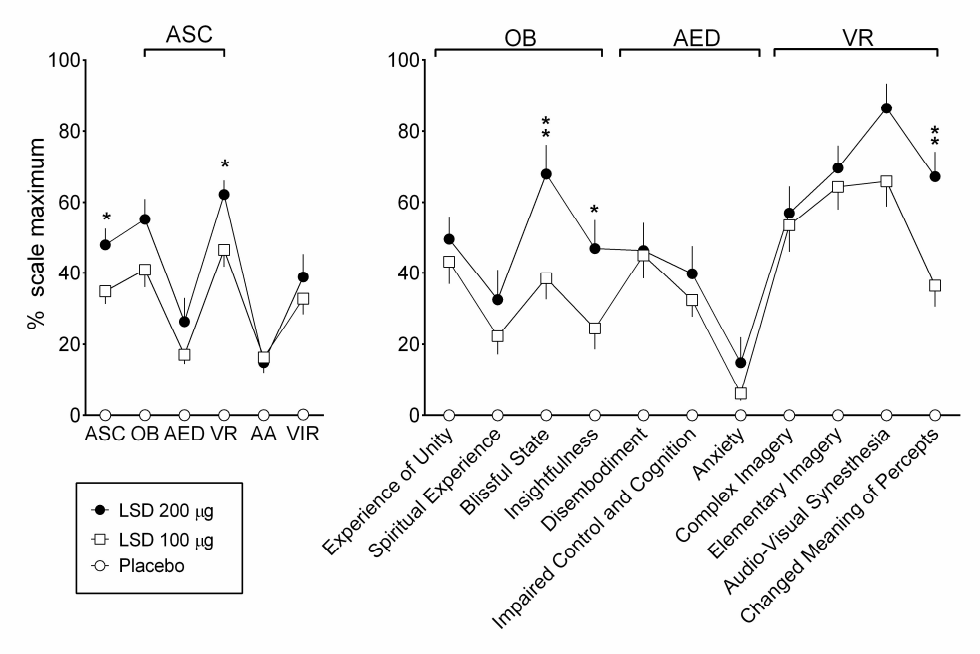

Figure 2 Effects of LSD on the 5 Dimensions of Altered States of Consciousness (5D-ASC) scale. LSD mainly increased ratings of oceanic boundlessness (OB) and visionary restructuralization (VR), with significantly higher ratings for the ASC total score and VR dimension at 200 µg compared with 100 µg. LSD-induced increases in anxious ego-dissolution (AED) and auditory alterations (AA) were relatively small. LSD also produced vigilance reduction (VIR). LSD-induced changes on the 5D-ASC scale were significant compared with placebo for both doses and all of the scales, with the exception of the effects of the 200 µg dose on anxiety. At 200 µg, LSD produced significant and relevantly higher ratings of blissful state, insightfulness, and changed meaning of percepts compared with 100 µg (*$p < 0.05$, **$p < 0.01$, *t*-tests). The data are expressed as the mean ± SEM in 24 subjects and 16 subjects for the 100 and 200 µg doses of LSD, respectively. Taken from Liechti et. al 2017 (Liechti et al. 2017).

1.2.4. Autonomic effects

A double-blind placebo-controlled study in healthy participants comparing the response of 100 µg (N=24) and 200 µg LSD (N=16) with placebo showed that a single dose (100 or 200 µg) moderately increased systolic (SBP) and diastolic (DBP) blood pressure, but also heart rate and body temperature compared to placebo. An average maximum heart rate of 79 and 87 beats/min (bpm) for 100 and 200 µg, respectively; an average maximum SPB of 142 and 148 mmHg for 100 and 200 µg, respectively; an average maximum DBP of 86 and 88 mmHg for 100 and 200 µg, respectively; and an average maximum elevation in body temperature of 0.8 and 0.7°C for 100 and 200 µg, respectively (Dolder et al. 2016) were observed. Another study with patients administering 200 µg LSD observed no effect on blood pressure and heart rate (Gasser et al. 2014). In comparison, a study focusing on the safety of MDMA in healthy subjects revealed for a dose of 125 mg an average maximum SBP of 157 mmHg, a maximum DBP of 93 mmHg and a maximum heart rate of 95 bpm (Vizeli and Liechti 2017). Furthermore, a study investigating acute

autonomic effects of d-amphetamine showed a maximum SBP of 158 mmHg, a maximum DBP of 97 mmHg, and a maximum heartrate of 94 bpm (Dolder et al. 2017b).

A high dose of LSD (200 µg) has been shown to induce an increase in the hormone oxytocin (Schmid et al. 2015). Oxytocin has previously been postulated to contribute to the empathogenic and prosocial effects of MDMA (Ramos et al. 2013). This effect might contribute to some similar empathogenic effects of LSD and MDMA.

1.2.5. Toxicity and adverse effects

LSD shows very low toxicity. There are no documented cases of deaths from an LSD overdose in humans. In documented cases of people overdosing up to 550 times of a usual dose (55 mg), none of the reported cases ended fatal and all patients underwent full recovery (Passie et al. 2008; Haden and Woods 2020; Klock et al. 1975). In animals, the LD_{50} ranges from 0.3mg/kg i.v. in rabbits to 46-60 mg/kg i.v. in mice (Rothlin 1956). LSD possesses little if any abuse liability. Hallucinogens are not self-administered by animals and there is no human LSD dependence syndrome (Passie et al. 2008).

Generally, the primary safety concerns of LSD or generally psychedelics are psychological rather than physiological in nature. Transient anxiety and depressive reactions are thought to be the most common acute adverse events (Passie et al. 2008; Schmid et al. 2015) and are expressed as mild or moderate anticipatory anxiety at the beginning of the onset of the drug effect, however, these reactions usually resolve spontaneously (Schmid et al. 2015). In addition, so called 'bad trips' may occur, which are expressed by a general negative experience. However, these general negative experiences are more likely to occur under uncontrolled conditions (Strassman 1984) and are sometimes called 'challenging experiences' to indicate that they may have partly personal or therapeutic value (Barrett et al. 2016).

Adverse events that may occur after the acute trip include flashbacks which can be defined as episodic and short (seconds or minutes) replications of elements of previous substance-related experiences (Holland and Passie 2011; Passie and Halpern 2014). However, these experiences can be positive or negative. Such phenomena have been reported after the use of many substances and are also prevalent in non-substance using persons and are therefore not psychedelic-specific (Holland and Passie 2011). Clinically significant flashbacks are also defined as hallucinogen persisting perception disorder (HPPD). This disorder is considered very rare and occurs almost exclusively in patients with anxiety disorders and it typically will have a limited course of months to a year (Halpern and Pope 1999; Passie and Halpern 2014; Holland and Passie 2011).

1.2.6. Microdosing

Recently, microdosing psychedelics has gained popularity and refers to the (repeated) consumption of a sub-perceptual dose of a classic psychedelic such as LSD or psilocybin (Kuypers et al. 2019). There are many anecdotal reports and books describing the benefits of this practice to improve mood and cognitive function (Anderson et al. 2019; Passie 2019; Polito and Stevenson 2019; Prochazkova et al. 2018). However, there is only limited data from two placebo-controlled studies assessing the acute effects of single microdose administrations of LSD and on pharmacokinetics of low doses (Family et al. 2020; Yanakieva et al. 2019; Bershad et al. 2019). One randomized, double-blind, placebo-controlled, cross-over clinical study including 20 healthy subjects administering 6.5, 13, and 26 µg LSD tartrate found weak alterations of consciousness at 26 µg LSD tartrate, suggesting a cut off for microdoses at 10 µg LSD base equivalent (Bershad et al. 2019). Another study investigating the pharmacokinetics of LSD only provided limited data due to the low sensitivity of the method used (Family et al. 2020).

1.3. Therapeutic use of LSD

During the 1950s-1970s, LSD had extensively been investigated as treatment for several psychiatric disorders. However, there is currently only one small modern study (n=12) which investigated the therapeutic effects of LSD in anxiety patients associated with a life-threatening disease. This work shows, that LSD-assisted psychotherapy reduces symptoms of anxiety and depression (Gasser et al. 2014; Gasser et al. 2015). Despite the very intensive and broad previous research with LSD, most modern studies investigating the therapeutic effects of classic hallucinogens use psilocybin or ayahuasca. However, studies conducted with psilocybin or ayahuasca show promising results in patients with unipolar depression (Carhart-Harris et al. 2016a; Osorio et al. 2015), anxiety and depression associated with a life-threatening disease (Griffiths et al. 2016; Grob et al. 2011; Ross et al. 2016), obsessive compulsive disorder (Moreno et al. 2006), and substance use disorder (Bogenschutz et al. 2015; Bogenschutz 2013; Johnson et al. 2014). Findings from these studies suggest that the acute psychedelic experience predicts the therapeutic outcome. In detail, one open label study in treatment-resistant depression showed that higher ratings in the 'oceanic boundlessness' dimension of the 5D-ASC are linked with a better therapeutic outcome (Roseman et al. 2017). Another study in depression and anxiety in patients with life-threatening cancer showed that high total mystical experience scores on the MEQ30 are associated with higher reductions in anxiety and depression (Griffiths et al. 2016).

In addition, Switzerland is in a unique legal situation regarding the therapeutic use of psychedelic drugs. Specifically, it is probably the only country in the world where limited administration within the framework of 'compassionate use' of LSD and MDMA is possible (Schmid et al. 2020).

1.4. Significance

LSD is illicitly used for recreational purposes. The lifetime prevalence of LSD consumption for recreational purposes among young adults (15 to 34-year-olds) varies from 0.1% to 5.4% in the EU (EMCDDA 2016) up to 7% in the US population (Krebs and Johansen 2013). But LSD is not only used recreationally, it is currently also being investigated as treatment for several major psychiatric disorders such as depression (NCT03866252) and anxiety (NCT03153579) and moreover somatic diseases such as cluster headaches (NCT0378112). So far, LSD was extensively tested in the 1950s-1970s but modern research is scarce (Liechti 2017). There is only a handful of modern studies investigating the psychological and physiological acute effects of LSD. Especially, the pharmacokinetics and pharmacodynamics of LSD are still poorly characterized and need to be tested within a reasonable sample size. Furthermore, the use of very small 'microdoses' of LSD for cognitive, mood, and creativity enhancement has recently gained popularity and needs investigation.

Besides, the distinction of LSD from other psychoactive substances has not yet been investigated. This is important because other psychoactive substances such as d-amphetamine and MDMA are also used illicitly for recreational purposes and are used or investigated as treatment for psychiatric disorders. For example, MDMA is currently under investigation as medication in patients with PTSD (Mithoefer et al. 2010; Oehen et al. 2013) and d-amphetamine is used in the treatment of ADHD.

This thesis shows data from three recent placebo-controlled cross-over trials investigating the distinction of LSD from MDMA and d-amphetamine, describing the pharmacokinetics and pharmacodynamic-pharmacokinetic relationship of a newly developed oral LSD solution that is currently used in phase II studies (NCT03153579, NCT03866252, NCT0378112), and describing the dose-effect relationship of LSD and of LSD microdoses. The results of these studies will have an impact on many areas of modern hallucinogen and psychiatric research as well as having direct relevance for public health by generating novel data on LSD across a representative dose range.

1.5. Aims & Hypotheses

The main goal of this thesis was to add overall information to the pharmacology of LSD, a substance that is widely used recreationally and likely to return to psychiatry as add-on to psychotherapy for different indications. All studies performed were phase I studies conducted in healthy participants and took place in a highly controlled setting.

A first aim was to distinguish the effects of psychedelics (LSD) from empathogens (MDMA) and stimulants (d-amphetamine). We hypothesized that LSD and MDMA would have some empathogenic subjective effects in common but not d-amphetamine, and that LSD stimulated more diverse psychological effects, compared to MDMA and d-amphetamine, reflected by ratings on the 5D-ASC, VASs, and MEQ30. All substances were hypothesized to show tolerable transient cardiostimulant and thermogenic reactions.

A second aim was to comprehensively describe the pharmacokinetics and corresponding pharmacodynamic effects of LSD for the first time in modern research with exactly known doses. For this purpose, three studies were conducted, the first study described the pharmacokinetics and linked pharmacodynamics of a single dose of LSD. The second study was designed to establish a dose-effect relationship of LSD. We hypothesized increasing subjective effects with increasing doses reflected by ratings on the VAS, the 5D-ASC, and the MEQ30, and that higher doses of LSD were associated with higher plasma concentrations. A further hypothesis was that cardiostimulant and thermogenic reactions were also dose-dependent. The third study was conducted to complete the description of pharmacokinetics and linked pharmacodynamics of very low doses of LSD, so called ‘microdoses’.

A further aim of this thesis was to add knowledge to the role of the 5-HT_{2A} receptor in altered states of consciousness. For this purpose, we added 40 mg of the 5-HT_{2A} receptor antagonist ketanserin as pretreatment to a high dose (200 µg) of LSD, and hypothesized that ketanserin would significantly block LSD-induced alterations in consciousness but not influence plasma concentrations of LSD, thereby suggesting a contributory role of the 5-HT_{2A} receptor to subjective effects.

PUBLICATIONS

2.1. Pharmacokinetics and subjective effects of a novel oral LSD formulation in healthy subjects

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ORIGINAL ARTICLE

Pharmacokinetics and subjective effects of a novel oral LSD formulation in healthy subjects

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Aims: The aim of the present study was to characterize the pharmacokinetics and exposure–subjective response relationship of a novel oral solution of lysergic acid diethylamide (LSD) that was developed for clinical use in research and patients.

Method: LSD (100 µg) was administered in 27 healthy subjects using a placebo-controlled, double-blind, cross-over design. Plasma levels of LSD, nor-LSD, and 2-oxo-3-hydroxy-LSD (O-H-LSD) and subjective drug effects were assessed up to 11.5 hours.

Results: First-order elimination kinetics were observed for LSD. Geometric mean maximum concentration (C_{max}) values (range) of 1.7 (1.0–2.9) ng/mL were reached at a t_{max} (range) of 1.7 (1.0–3.4) hours after drug administration. The plasma half-life ($t_{1/2}$) was 3.6 (2.4–7.3) hours. The AUC_{∞} was 13 (7.1–28) ng·h/mL. No differences in these pharmacokinetic parameters were found between male and female subjects. Plasma O-H-LSD but not nor-LSD (< 0.01 ng/mL) concentrations could be quantified in all subjects. Geometric mean O-H-LSD C_{max} values (range) of 0.11 (0.07–0.19) ng/mL were reached at a t_{max} (range) of 5 (3.2–8) hours. The $t_{1/2}$ and AUC_{∞} values of O-H-LSD were 5.2 (2.6–21) hours and 1.7 (0.85–4.3) ng·h/mL, respectively. The subjective effects of LSD lasted (mean ± SD) for 8.5 ± 2.0 hours (range: 5.3–12.8 h), and peak effects were reached 2.5 ± 0.6 hours (range 1.6–4.3 h) after drug administration. EC_{50} values were 1.0 ± 0.5 ng/mL and 1.9 ± 1.0 ng/mL for "good" and "bad" subjective drug effects, respectively.

Conclusion: The present study characterized the pharmacokinetics of LSD and its main metabolite O-H-LSD. The subjective effects of LSD were closely associated with changes in plasma concentrations over time.

KEYWORDS

concentration–effect relationship, LSD, metabolism, O-H-LSD, pharmacodynamics, pharmacokinetics

1 | INTRODUCTION

Lysergic acid diethylamide (LSD) is a prototypical hallucinogen that has been widely used for recreational and personal purposes.[1] Additionally, LSD is increasingly used in experimental research[2-6] and for the treatment of psychiatric patients.[7,8] However, blood plasma concentrations were not measured in most LSD studies.[3,6] Thus, unknown are the concentrations of LSD at the time points at which

The authors confirm that the PI for this paper is Matthias E. Liechti and that he had direct clinical responsibility for the participants.

pharmacodynamic (PD) outcomes were collected. Only limited data are available on the pharmacokinetics (PK) of LSD. A study in five male subjects reported a mean plasma elimination half-life of LSD of 175 minutes after intravenous administration (2 μg/kg[9]). Another study used non-systematic blood sampling after the administration of 160 μg LSD in 13 subjects up to 2.5–5 hours; however, because of the sparse and short sampling, PK parameters could not be derived.[10] We recently reported the first comprehensive PK data for orally administered LSD. In two studies, the PK of LSD were determined after the administration of 100 and 200 μg LSD in 24 and 16 healthy subjects, respectively.[11-13] However, the formulation that was used in these studies did not have long-term stability. Therefore, we produced a novel oral LSD solution with documented long-term stability (single dose units) and higher content uniformity than is currently being used in experimental studies in healthy subjects (ClinicalTrials.gov no. NCT03604744, NCT03321136, and planned studies), clinical trials in patients (ClinicalTrials.gov no. NCT03153579), and in the context of individually authorized patient treatments (compassionate use in Switzerland). The primary aim of the present study was to describe the PK of this LSD formulation in healthy subjects. A second goal was to describe subjective drug effects of LSD and to link these effects to changes in plasma concentrations over time within-subjects to derive EC_{50} values using PK/PD modelling. We also analysed concentrations of the LSD metabolites 2-oxo-3-hydroxy LSD (O-H-LSD) and *N*-desmethyl-LSD (nor-LSD) in plasma. O-H-LSD and nor-LSD are the main metabolites of LSD that are detected in urine.[12,14-17] However, only one previous study quantified these metabolites in human plasma.[13] Finally, we compared LSD exposure in plasma between the novel solution and previous capsule formulation. The present study is different from our previous PK studies mainly because it used a novel oral formulation with documented content stability.

What is already known about this subject

- There is an increasing number of clinical studies using LSD in humans.
- There is very limited data on the human pharmacokinetics of LSD.
- There are no controlled pharmacokinetic studies with validly defined doses of LSD.

What this study adds

- Subjective responses and pharmacokinetics of LSD and its metabolites are described after controlled administration of a known oral dose of LSD.
- The data serves as a reference to relate plasma concentrations of LSD with its effects in clinical studies and intoxications.

2 | METHODS

2.1 | Study design

We performed a double-blind, placebo-controlled, cross-over study with four experimental 12-hour test sessions (100 μg LSD, 125 mg methylenedioxymethamphetamine [MDMA], 40 mg D-amphetamine and placebo) in a balanced order. The washout periods between sessions were at least 7 days. Only the novel LSD and placebo data are presented here because the PK and PD of the MDMA and D-amphetamine formulations that were used in the study have previously been published[18,19] and because the sampling time was too short to provide full concentration–time curves for these substances with long half-lives.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. The use of LSD in humans was authorized by the Swiss Federal Office for Public Health, Bern, Switzerland. All of the subjects provided written consent before participating in either of the studies, and they were paid for their participation. Participants were informed about acute and potentially lasting effects of LSD.[20,21] The study was registered at ClinicalTrials.gov (NCT03019822).

2.2 | Participants

Twenty-nine healthy participants were recruited from the University of Basel campus via online advertisement. One subject stopped participation after screening and did not complete any of the test sessions, and one subject did not complete the LSD session. The final study sample included 27 subjects (13 males and 14 females) who completed the study. The participants were (mean ± SD) 28 ± 4 years old (range: 25–45 years) with a mean body weight of 71 ± 12 kg (range: 55–97 kg; 80 ± 10 kg in men and 62 ± 6 kg in women). Only healthy subjects who were between 25 and 50 years old were included in the study. The exclusion criteria were the following: pregnancy (urine pregnancy test at screening and before each test session), personal or family (first-degree relative) history of major psychiatric disorders (assessed by the Semi-structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Axis I disorders by the study physician and an additional interview by a trained psychiatrist), the use of medications that may interfere with the study drug, chronic or acute physical illness (based on abnormal physical exam, electrocardiogram or haematological and chemical blood analyses or hypertension >140/90 mmHg), tobacco smoking (>10 cigarettes/day), a lifetime prevalence of illicit drug use >10 times (except for tetrahydrocannabinol [THC]), illicit drug use within the last 2 months, and illicit drug use during the study. We performed urine drug tests at screening and once randomly before one of the test sessions, and no illicit substances were detected during the study. The subjects were asked to abstain from excessive alcohol consumption between test sessions and particularly limit their alcohol use to

one standard drink on the day before the test sessions. Additionally, the participants were not allowed to drink xanthine-containing liquids after midnight before the study day. The participants did not regularly use medications that could potentially interact with the study drug. Five subjects had previously used a hallucinogen, including LSD (three participants), one to four times during their lives, and the other 22 participants were hallucinogen-naïve. A previous study found no difference in the response to LSD between hallucinogen-naïve and moderately experienced subjects (<10 times).[5] THC use prevalence is high in Switzerland (48% in young Swiss men[22]) and previous experience in the absence of dependence was not an exclusion criterion. There is no data on possible interactions of THC and LSD. Subjects were not allowed to use THC during the study. Finally, the study included seven participants who smoked on average 4 ± 3 (range: 1–8) cigarettes/day and 20 non-smokers. Tobacco smoking induces CYP1A2 function, which is involved in the metabolism of LSD in vitro.[23] However, there are no human data.

2.3 | Study procedures

The study included a screening visit, a psychiatric interview, four 12-hour experimental sessions and an end-of-study visit. The experimental sessions were conducted in quiet standard hospital patient rooms. The participants rested in hospital beds except when going to the bathroom. Only one participant and one investigator were present during the experimental sessions per testing room. The participants could interact with the investigator, rest quietly and/or listen to music via headphones, but no other entertainment was provided. LSD or placebo was administered at 10:00 a.m. A small breakfast (two plain croissants), a small lunch (one sandwich), and a dinner (full meal) were served at 8:30 a.m., 1:30 p.m. and 6:00 p.m., respectively. The participants were never alone during the 12-hour session and went home at 10:00 p.m.

2.4 | Study drug

LSD (D-lysergic acid diethylamide base, high-performance liquid chromatography purity >99%, Lipomed AG, Arlesheim, Switzerland) was administered in a single oral dose. Each dose of LSD was formulated as a solution to be administered orally in 1 mL of 96% ethanol according to GMP (batch BZ17-1) and stored free in argon-prefilled vials in the dark at 4°C. The exact analytically confirmed LSD content (mean ± SD) of the formulation was 96.2 ± 0.3 µg (n = 6) after production. Stability of the formulation for longer than the study period was documented in an identically produced previous batch (batch BZ16) by D. Trachsel, Reseachem, Burgdorf, Switzerland. The isomerization of active LSD to inactive iso-LSD occurred to a small degree when the solution was stored at 4°C and resulted in iso-LSD contents (% of initial LSD content) of 0.1%, 0.1%, 1.3%, 3.2% and 3.6% after 4, 6, 12, 18 and 24 months, respectively. No other decomposition products were present. Vials that were stored at room temperature had higher iso-LSD contents of 0%, 3.1%, 3.4%, 6.7% and 9.5% after 2, 4, 6, 12 and 24 months, respectively. The LSD base dose that was used in the present study would correspond to a dose of 118 µg of LSD tartrate (or 125 µg tartrate including crystal water), which is the form of LSD that is more likely to be used when acquired illegally (i.e., in blotter form) for recreational use.

2.5 | Measures

2.5.1 | Blood sampling

Blood was collected into lithium heparin tubes before and 1, 1.5, 2, 3, 3.5, 4.5, 5.5, 6.5, 7.5, 9.5 and 11.5 hours after LSD administration. The blood samples were immediately centrifuged, and the plasma was subsequently stored at −20°C. For long-term storage (1–18 months), the samples were kept at −80°C until analysis. Long-term stability has been shown for LSD when kept under refrigerated or frozen conditions.[14,24]

2.5.2 | Analysis of LSD and metabolite concentrations

LSD, O-H-LSD, nor-LSD and iso-LSD levels were analysed in human plasma by ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS). The UHPLC apparatus was from Shimadzu (Kyoto, Japan) and consisted of four pumps (LC-30AD), a solvent degasser (DGU-20A5R), an autosampler (SIL-30AC), a column oven (CTO-20AC) and a system controller (CBM-20A). An API 5500 QTrap (AB Sciex, Concord, Canada) tandem mass spectrometer equipped with an electrospray interface was used as the detector (see Table S1 for settings).

O-H-LSD, O-H-LSD-d10 and nor-LSD were purchased from TRC (Ontario, Canada). Stock solutions of 1 mg/mL were prepared in dimethylsulfoxide (DMSO). LSD, LSD-d3 and iso-LSD solutions (1 mg/mL in ethanol) were purchased from Lipomed (Basel-Land, Switzerland). Calibration lines of LSD, O-H-LSD, nor-LSD and iso-LSD were prepared in drug-free plasma that contained less than 1% DMSO or ethanol, ranging from 25 (lower limit of quantification [LLOQ]) to 10 000 pg/mL. Quality control samples were prepared at 25, 100, 1000 and 10 000 pg/mL. An accuracy of 85–115% (LLOQ: 80–120%) and precision of less than 15% (LLOQ: 20%) was accepted in this study (Table S2). An aliquot of 50 µL of plasma was extracted with 150 µL acetonitrile that contained 0.1 ng/mL LSD-d3 and 0.25 ng/mL O-H-LSD-d10 of the internal standards (ISs). After rigorous mixing and 30 minutes of centrifugation at 3200g at 10°C, 10 µL of the sample's supernatant was injected into the UHPLC–MS/MS system. The sample was loaded on the analytical column (Kinetex Evo C18, 1.7 µm, 50 × 2.1 mm, Phenomenex, Torrance, CA, USA) using 10% mobile phase B (acetonitrile plus 0.1% formic acid) and 90% mobile phase A (20 mM ammonium bicarbonate adjusted to pH 9 using 25% ammonium hydroxide). The flow rate was increased from 0.1 to 0.6 mL/min within the first 0.5 minutes. In parallel, mobile phase A was delivered using a third pump to dilute the injected sample via a t-union, which was installed

before the analytical column. The flow rate of this pump was thus linearly decreased from 0.5 to 0 mL/min in the first 0.5 minutes of each run. The mobile phase B concentration was increased from 10% to 95% between 0.5 and 2.75 minutes to elute the three analytes. The analytical run was terminated by flushing the column for 0.75 minutes with 95% mobile phase B and reconditioning it for another 0.5 minutes with 10% mobile phase B. This gradient program resulted in a baseline separation of O-H-LSD (1.49 min), nor-LSD (1.71 min) and LSD (1.79 min). Mobile phase B was more slowly increased from 10% to 35% between 0.5 and 4.0 minutes to separate LSD from iso-LSD. Afterwards, the mobile phase B concentration was raised to 95% within 0.5 minutes and kept at this level for an additional minute to flush the analytical column. At the end of the run, the column was reconditioned at 10% mobile phase B for 0.5 minutes. This prolonged gradient program resulted in a baseline separation of LSD (3.8 min) and iso-LSD (4.1 min). Concentrations of iso-LSD were only determined in samples that were collected at 2 hours (i.e., close to the C_{max} of LSD).

All of the analytes were detected by multiple reaction monitoring (MRM) in the positive mode. Analyte-specific settings are given in Table S2. The mass transitions were summed for all of the analytes to increase sensitivity of the method. Gas parameters were set at medium, 20, 30 and 60 for the collision gas, curtain gas, ion source gas 1 and ion source gas 2, respectively. The interface temperature was 500°C, and the ion spray voltage was 5500 V. The system was operated with Analyst 1.6.2 software (AB Sciex, Concord, Canada). Pharmacokinetic data were quantified using MultiQuant 3.0.1 software (AB Sciex, Concord, Canada).

2.5.3 | Subjective mood

Visual Analog Scales (VASs) were repeatedly used to assess subjective effects over time.[4,5,11] The VASs included separate measures for "any drug effect," "good drug effect," "bad drug effect", and "the boundaries between myself and my surroundings seemed to blur" (ego dissolution[21,25]) and were presented as 100 mm horizontal lines (0–100%) marked from "not at all" on the left to "extremely" on the right. The VASs were administered before and 1, 1.5, 2, 3, 3.5, 4.5, 5.5, 6.5, 7.5, 9.5 and 11.5 hours after LSD administration.

2.6 | Pharmacokinetic analyses and pharmacokinetic-pharmacodynamic modelling

All of the analyses were performed using Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA). Pharmacokinetic parameters were estimated using compartmental modelling. A one-compartment model was used with first-order input, first-order elimination, and no lag time. Initial estimates for V_d/F and λ were derived from non-compartmental analyses. The model fit was not relevantly improved by a two-compartment model based on visual inspection of the plots. The one-compartment model also resulted in smaller Akaike information criterion values in all subjects compared with a two-compartment model. A similar one-compartment model but with a lag time was used to determine the parameters for the metabolite O-H-LSD because a better fit could be obtained compared with the no-lag-time model. The PK model was first fitted and evaluated. The predicted concentrations were then used as an input to the PD model by treating the PK parameters as fixed and using the classic PK/PD link model module in WinNonlin. The model used a first-order equilibrium rate constant (k_{eo}) that related the observed PD effects of LSD to the estimated LSD concentrations at the effect site and accounted for the lag between the plasma and effect site concentration curves.[11,26] A sigmoid maximum effect (E_{max}) model (EC_{50}, E_{max}, γ) was selected for all PD effects: $E = (E_{max} \times C_p^h)/(C_p^h + EC_{50}^h)$, in which E is the observed effect, C_p is the plasma LSD concentration, E_{max} is the maximal effect and h is the Hill slope using WinNonlin. EC_{50} and E_{max} estimates were taken from the PK/PD plots.[11] Lower and upper limits for E_{max} were set to 0% and 100%, respectively, for all of the VAS scores. The sigmoidal E_{max} model best described the relationship between estimated effect-site concentrations and LSD effects compared with a simple E_{max} model (plot inspection [Figure S1] and Akaike information criteria). To compare the present PK data for the LSD solution with previous data for LSD capsules,[11] the previously published data were reanalysed similarly to the present data using both compartmental and non-compartmental analyses.

2.7 | Statistical analyses

Comparisons of PK parameters between the solution and capsule were made using t-tests (Statistica 12 software; StatSoft, Tulsa, OK, USA). The onset, t_{max}, offset and effect duration were assessed for the model-predicted "any drug effect" VAS effect–time plots after LSD administration using a threshold of 10% of the maximum individual response using Phoenix WinNonlin 6.4. Associations between peak concentrations and peak effects across subjects were assessed using Pearson correlations.

2.8 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.[27]

3 | RESULTS

3.1 | Pharmacokinetics

Concentrations of LSD and O-H-LSD could be quantified in all of the subjects and at all time points. In contrast, nor-LSD was detected in plasma, but concentrations could not be quantified (<25 pg/mL). Concentrations of iso-LSD were 5% ± 2% of those of LSD, indicating no relevant isomerization/inactivation of LSD. The plasma concentration–time curves for LSD and O-H-LSD are shown

in Figure 1A. The pharmacokinetic parameters are shown in Table 1. Individual PK/PD model-predicted LSD concentration–time curves are shown in Figure 2A. The individual observed LSD concentrations are shown in Figure S2, together with their individual model-predicted curves. Parameters based on non-compartmental analysis are summarized in Table S3. No sex differences in the PK parameters were observed. There were no differences in the PK of LSD between tobacco smokers (<10 cigarettes/day) and non-smokers.

3.2 | Pharmacodynamics and pharmacokinetic-pharmacodynamic modelling

LSD produced robust increases in "any drug effect" (Figure 1B) and "good drug effect" (Figure 1C). Transient "bad drug effect" was reported in some subjects, resulting in a moderate increase in mean group ratings (Figure 1D). LSD also induced "ego dissolution" (Figure S3). The variability in intensity in subjective drug effects is illustrated in the "any drug effect," "good drug effect" and "bad drug effect" curves in Figure 2B–D, respectively. The individual ratings for each subject and time point are shown in Figure S4–S6, respectively, together with the modelled curves. Times of onset and offset of the subjective response, assessed by the "any drug effect" VAS, were (mean ± SD) 0.7 ± 0.2 h (range: 0.3–1.0 h) and 9.1 ± 2.0 h (range: 6.0–13.2 h), respectively. The mean effect duration was 8.5 ± 2.0 h (range: 5.3–12.8 h). The time to peak drug effect was 2.5 ± 0.6 h (range: 1.6–4.3 h).

The predicted concentrations of LSD that produced half-maximal effects (EC_{50} values) and E_{max} values were 1.1 ± 0.4 ng/mL and 91% ± 17% for "any drug effect," 1.0 ± 0.5 ng/mL and 83% ± 20% for "good drug effect," 1.9 ± 1.0 ng/mL and 40% ± 41% for "bad drug effect," and 1.4 ± 0.5 ng/mL and 80% ± 34% for "ego dissolution," respectively (see Table S4 for additional parameter estimates).

The C_{max} of LSD did not correlate with the E_{max} values of the subjective response on any of the VAS when analysed across subjects. Thus, in contrast to the close relationship over time within-subjects, the plasma concentrations of LSD were not associated with the subjective effects of LSD when analysed across subjects after the use of the same dose of LSD in all participants (relatively similar C_{max} and E_{max} values in all subjects).

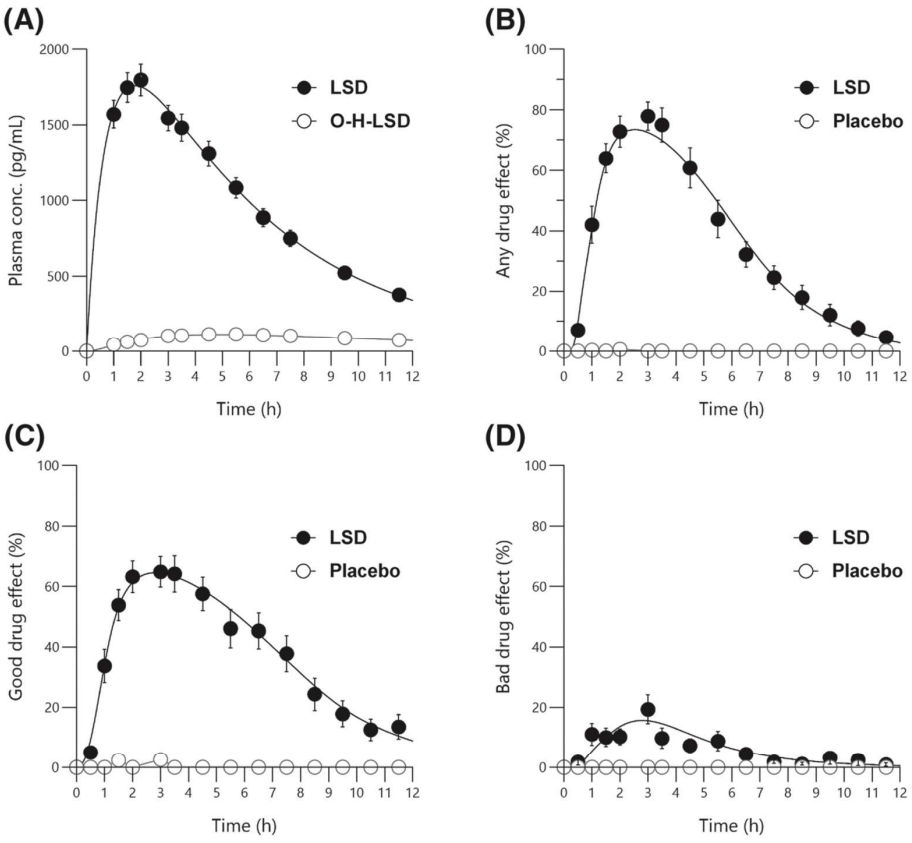

FIGURE 1 Pharmacokinetics and pharmacodynamics of LSD. (A) Plasma LSD and 2-oxo-3-hydroxy LSD (O-H-LSD) concentration–time curves. Nor-LSD levels were below the level of quantification. (B–D) LSD effect–time curves for visual analog scale ratings (0–100%) of (B) "any drug effect," (C) "good drug effect," and (D) "bad drug effect." "Any drug effect" and "good drug effect" were robustly and markedly increased in all subjects and paralleled the changes in LSD concentrations, whereas the mean "bad drug effect" increased only slightly after LSD administration due to moderate anxiety in some but not all subjects. The data are expressed as the mean ± SEM in 27 subjects after administration of 100 µg LSD or placebo at t = 0 h. the lines represent the mean of the individual predictions based on the pharmacokinetic/pharmacodynamic model

TABLE 1 Pharmacokinetic parameters for LSD and O-H-LSD based on compartmental modelling

				K_{01} (1/h)	λ_z (1/h)	V_z/F (L)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_∞ (ng·h/mL)	CL/F (L/h)
LSD	All	27	Geometric mean (95% CI)	1.2 (1.1–1.7)	0.19 (0.18–0.22)	39 (36–47)	1.7 (1.6–2.0)	1.7 (1.6–2.0)	3.6 (3.3–4.3)	13 (12–16)	7.5 (6.9–9.0)
			Range	0.31–3.2	0.10–0.29	23–81	1.0–2.9	1.0–3.4	2.4–7.3	7.1–28	3.6–14
	Male	13	Geometric mean (95% CI)	1.2 (1.0–1.7)	0.18 (0.15–0.23)	42 (34–54)	1.7 (1.4–2.1)	1.8 (1.5–2.2)	3.9 (3.2–5.0)	13 (11–18)	7.4 (6.1–9.9)
			Range	0.31–2.5	0.10–0.28	26–81	1.0–2.9	1.1–3.4	2.4–7.3	7.1–28	3.6–14
	Female	14	Geometric mean (95% CI)	1.3 (1.0–1.9)	0.20 (0.18–0.24)	37 (32–46)	1.8 (1.6–2.1)	1.7 (1.4–2.1)	3.4 (3.0–4.1)	13 (12–16)	7.6 (6.6–9.1)
			Range	0.48–3.2	0.12–0.29	23–61	1.2–2.4	1.0–2.9	2.4–5.7	7.2–22	4.6–14
O-H-LSD	All	27[a]	Geometric mean (95% CI)	0.34 (0.31–0.45)	0.13 (0.12–0.18)	490 (442–646)	0.11 (0.10–0.13)	5.0 (4.7–5.7)	5.2 (4.2–7.8)	1.7 (1.5–2.1)	51 (48–69)
			Range	0.14–0.75	0.03–0.27	197–1294	0.07–0.19	3.2–8.0	2.6–21	0.85–4.3	12–118

AUC_∞, area under the plasma concentration–time curve from time zero to infinity; CL/F, apparent total clearance; C_{max}, estimated maximum plasma concentration; k_{01}, first-order absorption koefficient; $t_{1/2}$, estimated plasma elimination half-life; t_{max}, estimated time to reach C_{max}; V_z/F, volume of distribution; λ_z, first-order elimination coefficient.

[a]Four subjects were not included in the the calculation of λ_z, $t_{1/2}$ and AUC_∞ because the sampling period was too short to correctly determine λz.

3.3 | Pharmacokinetic and pharmacokinetic-pharmacodynamic comparison with LSD capsules

The model-predicted geometric mean (CV%, 95% confidence interval [CI]) C_{max} of the LSD solution was 1.7 (27%, 1.6–2.0) ng/mL and non-significantly ($t_{1,49}$ = 1.6, p = 0.12) higher compared with 1.3 (60%, 1.2–1.9) ng/mL for the LSD capsule that was tested previously in different subjects.[11] The geometric mean (CV%, 95% CI) LSD AUC_∞ values were 13 (34%, 12–16) ng·h/mL and 8.1 (66%, 7.5–11) ng·h/mL for the LSD solution and LSD capsule, respectively, representing a significant difference in overall exposure between the two formulations ($t_{1,49}$ = 3.67, p < 0.001). Additionally, lower inter-individual variability was observed compared with the capsule, indicated by the CV% values and plot of the individual model-predicted LSD concentrations after administration of the two formulations (Figure S7). Additional analyses were performed to explore differences in absorption between the two formulations. Model-estimated t_{max} values (geometric mean [CV%, 95% CI]) were comparable for the solution and the capsule (1.7 [29%, 1.6–2.0] h and 1.4 [82%, 1.3–2.1] h, respectively; $t_{1,49}$ = 0.44, p = 0.66), although greater variability was observed for the capsule. The first-order absorption coefficients were also not significantly different between the solution and the capsule (1.2 [59%, 1.1–1.7] and 1.4 [164%, 1.2–4.1], respectively; $t_{1,49}$ = 1.86, p = 0.07). The onset time that was needed to reach a minimum plasma LSD concentration of 0.3 ng/mL (i.e., the lowest concentration reached in all subjects) was 0.10 (48%, 0.09–0.14) h and 0.12 (164%, 0.11–0.28) h after administration of the solution and capsule, respectively, and significantly shorter ($t_{1,49}$ = 2.08, p = 0.04) and also less variable after administration of the solution compared with the capsule. The $t_{1/2}$ of the LSD solution was 3.6 (33%, 3.3–4.3) h after administration compared with 2.6 (29%, 2.4–3.0) h after capsule administration ($t_{1,49}$ = 3.70, p < 0.001). Non-compartmental analysis confirmed the longer $t_{1/2}$ of LSD in the present study (3.7 [3.4–4.1] h) compared with our previous study that used capsules (2.6 [2.4–3.0] h). PK parameters that were derived from the non-compartmental analysis were comparable to those that were based on the model and are shown for the LSD solution and capsule formulations in Tables S3 and S5, respectively.

The subjective-effects PD parameter estimates for the solution and the capsule did not differ significantly, with the exception that the EC_{50} value for "any drug effect" was lower for the capsule compared with the solution ($t_{1,49}$ = 3.30, p < 0.01; Table S4). Times of onset and offset of the subjective response, mean effect duration, time to peak drug effect and the area under the effect–time curve did not differ significantly between the two formulations (Table S6). However, the time to effect onset presented greater variance for the capsule compared with the solution (Table S6, Figure S8), which is consistent with the PK of the two formulations (Figure S7).

4 | DISCUSSION

The present study mainly characterized the PK and subjective effects of a novel LSD solution that is intended for clinical research and use.

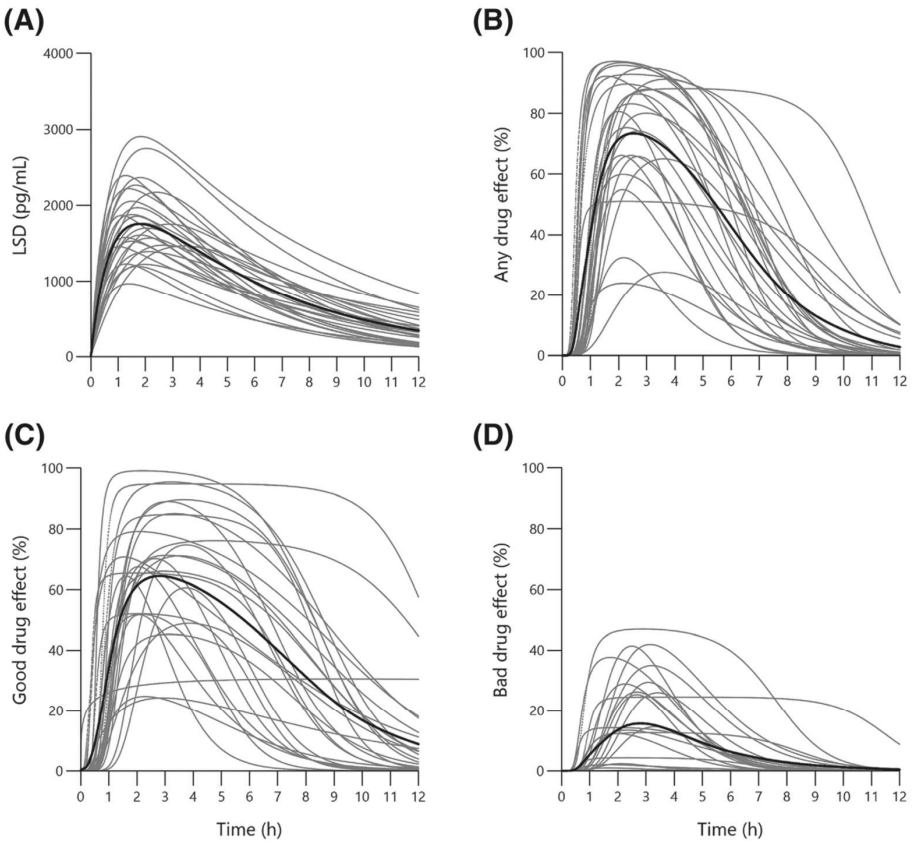

FIGURE 2 Individual pharmacokinetics and pharmacodynamics of LSD. (A) Individual plasma LSD concentration–time curves. (B–D) individual LSD effect–time curves for visual analog scale ratings (0–100%) of (B) "any drug effect," (C) "good drug effect," and (D) "bad drug effect." curves represent the individual pharmacokinetic/pharmacodynamic model predictions in 27 subjects with the mean marked in bold. LSD (100 µg solution) was administered at t = 0 h

The present data on the plasma concentration–time curves of LSD are important because many experimental and therapeutic studies are currently being conducted with this formulation (ClinicalTrials.gov no. NCT03604744, NCT03321136, NCT03019822, NCT03153579, and planned). Many recent LSD studies[3,6,7] have been published without information on the presence of LSD in the human body, and the actual exposure to LSD and exposure–time curves are unknown. The present study also allows an indirect reassessment of the doses that were reported in our previous studies.

The PK and PD parameters that were derived from the present study are generally similar to those in our previous studies that used 100 and 200 µg in capsule form,[11,12] with the exception of higher C_{max} and AUC values. Maximum concentrations of LSD were reached an average of 1.7 hours after administration, and first-order elimination kinetics of LSD were confirmed.[9,11] The subjective drug effects began an average of 0.7 hours after administration, lasted 8.5 hours, and declined in parallel with plasma LSD concentrations.

We expected more rapid absorption and a faster onset of action with the oral solution compared with the capsules. We found that the absorption coefficients and t_{max} values were not significantly different between the two formulations. Thus, the rate-limiting steps to increase LSD concentrations may not be absorption but rather other processes, such as dilution and distribution in the circulation. However, we took only a few blood samples during the expected absorption time period, and we may have missed potential differences between the two formulations. Additionally, a threshold plasma LSD level of 0.3 ng/mL was reached significantly faster after administration of the solution compared with the capsule. This could indicate faster absorption or could be explained by the overall higher LSD concentrations that were reached with the solution. Early studies indicated that the effects of LSD tartrate peak approximately 30 minutes after intravenous administration.[9,28-30] A recent study that evaluated the intravenous administration of LSD base reported that subjective drug effects began within 5–15 minutes and peaked relatively late at 45–90 minutes after infusion of the drug.[3,31] Thus, the time to the maximal response appears to be rather long after both intravenous and oral administration of LSD base. Possible reasons for the long t_{max} may include slow rates of dilution and distribution within the circulation, slow blood–brain barrier passage, slow diffusion to target sites in the brain, and lags in the response mechanism itself.

The terminal $t_{1/2}$ value of LSD should not depend on the type of formulation that is used. The average $t_{1/2}$ of LSD was 3.6 hours in the present study, which is within the range (2.6–3.6 h) that was reported in our previous studies that used capsules.[11] An older small study that evaluated the intravenous administration of LSD (2 µg/kg) calculated a $t_{1/2}$ of 2.9 hours.[9]

The average AUC_∞ value, reflecting total LSD exposure, was 13.3 and 8.1 ng·h/mL in the present study and our previous study,[11] respectively, and thus 1.6-times greater in the present study compared with the previous study that used the same indicated dose of 100 μg of LSD base that was formulated as a capsule.[11] Although the oral drinking solution may have had higher oral bioavailability than the capsule formulation of LSD, the true LSD content of the previously used LSD capsules may have been lower than reported. First, valid longer-term stability data beyond the full study duration were unavailable for the capsules that were used in several previous studies by us and others.[4-6,11,20,21,32-36] Second, after administration of the 200 μg dose in the form of two 100 μg capsules, iso-LSD was detected in plasma,[13] indicating that this inactive decomposition product of LSD was possibly already present in the capsules at the time of their use (although possible formation in the plasma samples cannot be completely excluded). The plasma AUC_{24} values of LSD and iso-LSD of 21 and 9.2 ng·h/mL[13] indicate that an average of 30% of the LSD may have isomerized to inactive iso-LSD in the capsules. Thus, the actual administered doses of LSD may have been 70 and 140 μg LSD base rather than the indicated 100 and 200 μg, respectively. The AUC_∞ values in the previous studies that used 100 and 200 μg doses were 61% and 76%, respectively, of the values that were expected based on the present confirmed 96 μg LSD dose and assuming similar bioavailability. Finally, analytical tests of four unused old LSD capsules that were performed years after study completion suggested a marked reduction of LSD content (remaining amount of LSD = 22 ± 7 μg), indicating a lack of longer-term stability of LSD in this form and that the actual LSD doses that were used were likely already lower than indicated during the studies. Notably, a decrease in content by 15% or even 25% in single capsules would still be compatible with content uniformity, which was documented during production of the capsules.

In summary, based on the results of different quality-control measures, analytical findings (including the present PK data), and the clinical effects of the different formulations,[11] we surmise that the previous studies actually used approximately 60–70 (not 100) μg and 140–150 (not 200) μg of LSD base, corresponding to approximately 80 and 175 μg of LSD tartrate. Additionally, the solution and capsules did not produce significantly different subjective drug effects (i.e., intensity and duration) despite the differences in plasma concentrations. Thus, the actual drug effects in the present study were comparable to previous reports. Although we cannot exclude possible differences in bioavailability of the presently used oral formulation and the previously used capsules, many previously reported clinical and neuroimaging results were likely produced with LSD doses that were lower than reported. Certainly, exposure to LSD in the body, expressed by the AUC, was lower in the previous studies compared with the present study that used the same reported dose. Another consideration is that doses of LSD that were reported in previous studies may not have been very precise or may not have reflected the actual exposure of LSD in the body. This is notably also the case for recent studies that used intravenous dosing with 75 μg hydrophobic LSD base in saline because objective measures of exposure to LSD (i.e., plasma concentrations) were lacking, and the bioavailability of the solution is unknown.[3,25,31,37,38] The clinical response to 75 μg of intravenous LSD was not significantly different from the oral 100 μg dose that was used in our previous studies,[2,21,37] indirectly indicating similar exposure that is comparable to an oral dose of 60–70 μg LSD base.

The present study and discussion illustrate that PK investigations are imperative to confirm the presence and extent of the presence of LSD in the body, particularly when pharmaceutically or pharmacologically poorly characterized formulations of LSD are used in experimental research settings. The present study also described the acute subjective effects of LSD. LSD produced high subjective "good drug effect" in almost all of the subjects. "Bad drug effects" were typically smaller and not present in every subject. Mean EC_{50} values were 1.0 and 1.9 ng/mL for "good drug effects" and "bad drug effects," respectively, indicating that anxiety is associated with higher LSD concentrations. The subjective LSD response was similar in intensity, onset and duration in the present study that used the novel LSD solution compared with a previously used capsule formulation,[11] although a significantly higher plasma LSD concentration was reached in the present study. The only notable difference was lower variance in the time to effect onset for the solution compared with the capsule, which is consistent with the less variable time to reach a minimum plasma concentration.

The present study confirmed the previous finding[11] of a close relationship between LSD concentrations and LSD effects over time within each subject. In contrast, it has previously been shown in detail that concentrations of LSD do not correlate with the response when analysed across subjects in a group of subjects who each received the same dose of LSD.[11] Thus, the plasma concentrations of LSD do not predict the effects of LSD during the time it produces robust and similar effects in all subjects (i.e., little between-subject variability).[11] In contrast, there is a close relationship over time within-subjects, as shown in the PK/PD analysis of the present study.

The present study has limitations. The comparison with the LSD capsules used data from different studies that included different subjects. Small study differences could have contributed to differences in the PK of the two LSD formulations. For example, we used a refined LC–MS/MS assay in the present study, which has higher sensitivity compared with the previous assay.[11,39] Plasma samples were collected at 1.5 hours in the present study but not in the previous study,[11] whereas the previous study had a longer sampling time and included quantified LSD concentrations up to 24 hours in some subjects. Few samples were taken before C_{max}, thus precluding good characterization of the absorption and early distribution phase. However, the half-life of LSD is relatively short and the PK linear, allowing the determination of the PK parameters with high validity within a sampling time of 12 hours. The present study also has notable strengths. The study was relatively large and included both male and female subjects, thus allowing valid comparisons of PK between sexes. A well-characterized formulation of LSD was also used. Quality assurance data were provided, which are typically unavailable or not reported in other studies.

In summary, we present PK data for a novel oral LSD formulation that are useful for interpreting the findings of clinical studies and LSD intoxications.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

F.H. and M.E.L. designed the research. F.H., U.D., P.V., F.M. and S.B. performed the research. F.H., U.D., P.V. and M.E.L. analysed the data. F.H. and M.E.L. wrote the manuscript with input from all of the other authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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2.2. Distinct acute effects of LSD, MDMA, and d-amphetamine in healthy subjects

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ARTICLE OPEN

Distinct acute effects of LSD, MDMA, and D-amphetamine in healthy subjects

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Lysergic acid diethylamide (LSD) is a classic psychedelic, 3,4-methylenedioxymethamphetamine (MDMA) is an empathogen, and D-amphetamine is a classic stimulant. All three substances are used recreationally. LSD and MDMA are being investigated as medications to assist psychotherapy, and D-amphetamine is used for the treatment of attention-deficit/hyperactivity disorder. All three substances induce distinct acute subjective effects. However, differences in acute responses to these prototypical psychoactive substances have not been characterized in a controlled study. We investigated the acute autonomic, subjective, and endocrine effects of single doses of LSD (0.1 mg), MDMA (125 mg), D-amphetamine (40 mg), and placebo in a randomized, double-blind, cross-over study in 28 healthy subjects. All of the substances produced comparable increases in hemodynamic effects, body temperature, and pupil size, indicating equivalent autonomic responses at the doses used. LSD and MDMA increased heart rate more than D-amphetamine, and D-amphetamine increased blood pressure more than LSD and MDMA. LSD induced significantly higher ratings on the 5 Dimensions of Altered States of Consciousness scale and Mystical Experience Questionnaire than MDMA and D-amphetamine. LSD also produced greater subjective drug effects, ego dissolution, introversion, emotional excitation, anxiety, and inactivity than MDMA and D-amphetamine. LSD also induced greater impairments in subjective ratings of concentration, sense of time, and speed of thinking compared with MDMA and D-amphetamine. MDMA produced greater ratings of good drug effects, liking, high, and ego dissolution compared with D-amphetamine. D-Amphetamine increased ratings of activity and concentration compared with LSD. MDMA but not LSD or D-amphetamine increased plasma concentrations of oxytocin. None of the substances altered plasma concentrations of brain-derived neurotrophic factor. These results indicate clearly distinct acute effects of LSD, MDMA, and D-amphetamine and may assist the dose-finding in substance-assisted psychotherapy research.

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INTRODUCTION

Lysergic acid diethylamide (LSD) is a classic serotonergic hallucinogen that has been widely used recreationally [1] and to a limited extent in psychiatric research [2]. LSD acutely induces marked alterations of waking consciousness [3] that have been shown to primarily depend on an interaction with the serotonin 5-hydroxytryptamine-2A (5-HT_{2A}) receptor [4], although LSD also acts on 5-HT_1 and dopamine receptors [5]. Recent clinical trials indicate that the quality of the acute psychedelic experience in response to psilocybin or LSD predicts long-term changes in mental health and well-being in patients and healthy persons [6–11]. For example, greater psilocybin-induced mystical-type experiences and more pronounced and more positive acute alterations of consciousness were associated with lasting antidepressant responses in patients with depression [6, 7]. 3,4-Methylenedioxymethamphetamine (MDMA) is the active compound in the recreational substance ecstasy and is currently investigated as an adjunct to psychotherapy to treat post-traumatic stress disorder (PTSD) [12, 13]. MDMA not only exhibits some amphetamine-like properties but also shows hallucinogenic-like effects and can be considered an intermediate substance between a pure stimulant like D-amphetamine and a pure hallucinogenic drug like LSD. MDMA acutely induces feelings of well-being, love, empathy, and prosociality [14, 15], and produces mild perceptual alterations that are thought to be primarily mediated by the release of serotonin (5-HT) [16, 17] and norepinephrine [18], and the direct activation of 5-HT_{2A} receptors [19]. Additionally, MDMA releases oxytocin [14, 20, 21], which may contribute to the mediation of its prosocial effects [22, 23]. The unique emotional effects of MDMA lead to its classification as an empathogen or entactogen [24], referring to assumingly distinct effects from psychostimulants [25–28]. Psychostimulants such as D-amphetamine and methamphetamine primarily activate dopamine and norepinephrine systems, with only minimal effects on 5-HT [29, 30], and promote stimulation, wakefulness, and concentration without the MDMA-typical emotional effects [25, 27, 28, 31–35]. Although MDMA produces less profound changes in perception compared with classic hallucinogens, it is often also classified as a psychedelic substance. On the other hand, LSD was found to exhibit MDMA-like empathogenic mood effects such as increased closeness, openness, and trust [3], indicating overlapping properties with MDMA [14, 27] potentially

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useful to assist psychotherapy. Whether and how the effects of MDMA are similar or differ from the classic stimulant D-amphetamine and classic hallucinogen LSD have not been studied under double-blind conditions in the same study. Comparative studies, particularly within-subjects comparisons of the acute effects of these prototypical substances, are lacking. Therefore, we compared for the first time the acute subjective, autonomic, and endocrine effects of doses with similar cardiovascular activity ("equivalent" doses) of LSD (0.1 mg), MDMA (125 mg), D-amphetamine (40 mg), and placebo in a cross-over study in healthy subjects. By comparing all three substances using a within-subject design, it is possible to directly assess differences and commonalities of these substances. Moreover, by including different substances with partially overlapping effects, it is also possible to considerably improve blinding. This latter point has been a serious shortcoming of almost all previous studies, which compared effects of MDMA and LSD, respectively, with non-active placebo, which almost inevitably results in unblinding. Dose selection was critical because we could only compare single doses of each substance in this within-subjects study. LSD was used at an intermediate dose of 0.1 mg that is representative of doses that are used recreationally [36] and in research [2]. A higher dose of 0.2 mg LSD has previously been shown to produce greater subjective effects than the 0.1 mg dose [37, 38], but was not used in the present study because it was expected to produce greater alterations of waking consciousness than any of the other substances and would not have allowed brain imaging due to expected anxiety and movement artifacts in the scanner. MDMA was used at a high dose (125 mg) that produces the full range of empathogenic MDMA-typical effects [27] and is considered safe [39], and at the upper range of doses used in research investigating the safety and efficacy of MDMA-assisted psychotherapy in the treatment of PTSD [12] and in experimental studies in healthy participants [27, 39, 40]. Preferred recreational doses are slightly lower and in the range of 80–120 mg [41]. Higher doses are expected to produce largely similar subjective positive responses, but considerably more adverse effects [39, 41]. D-Amphetamine was also used at a rather high dose (40 mg) that is in the upper range of doses that are used in patients and in research [31, 32, 34, 42–44].

The main goal of the present study was to describe and compare the subjective and autonomic effects of all three substances over time and determine plasma concentration–time profiles (pharmacokinetics). We hypothesized that LSD would induce more pronounced and different alterations of waking consciousness, assessed by the 5 Dimensions of Altered States of Consciousness (5D-ASC) scale and Mystical Experience Questionnaire (MEQ) compared with MDMA and D-amphetamine [37]. We predicted that MDMA would produce distinct subjective emotional effects compared with D-amphetamine [25, 27, 28] and induce greater increases in plasma concentrations of oxytocin than LSD and D-amphetamine [3, 14]. Finally, we explored effects on plasma concentrations of brain-derived neurotrophic factor (BDNF), a biomarker that is linked to neurogenesis, because psychedelics have been shown to have neuroregenerative potential and may alter BDNF [45, 46]. Altogether, we tested whether prototypical hallucinogens, empathogens, and psychostimulants are indeed substances with distinct acute-effect profiles in humans for the first time using a head-to-head comparison with the same study and participants.

MATERIALS AND METHODS

Study design

We used a double-blind, placebo-controlled, cross-over design with four experimental test sessions to investigate the responses to 0.1 mg LSD, 125 mg MDMA, 40 mg D-amphetamine, and placebo in 28 healthy participants (14 females, 14 males). The washout period between sessions was at least 10 days. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee northwest Switzerland (EKNZ). The administration of LSD, MDMA, and D-amphetamine in healthy subjects was authorized by the Swiss Federal Office for Public Health, Bern, Switzerland. All of the participants provided written consent before participating in the study, and they were paid for their participation. The study was registered at ClinicalTrials.gov (NCT03019822).

Participants

Twenty-eight healthy subjects (14 men, 14 women; 28 ± 4 years old [mean ± SD]; range, 25–45 years; body weight, 71.5 ± 12.0 kg) were recruited from the University of Basel. Participants who were younger than 25 years old were excluded from participating in the study because of the higher incidence of psychotic disorders and because low age has been associated with more anxious reactions to hallucinogens [47]. Additional exclusion criteria were age >50 years, pregnancy (urine pregnancy test at screening and before each test session), personal or family (first-degree relative) history of major psychiatric disorders (assessed by the Semi-structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Axis I disorders by a trained psychiatrist), the use of medications that may interfere with the study medications (e.g. antidepressants, antipsychotics, sedatives), chronic or acute physical illness (abnormal physical exam, electrocardiogram, or hematological and chemical blood analyses), tobacco smoking (>10 cigarettes/day), lifetime prevalence of illicit drug use >10 times (except for Δ^9-tetrahydrocannabinol), illicit drug use within the last 2 months, and illicit drug use during the study (determined by urine drug tests). A previous study found no difference in the response to LSD between hallucinogen-naive and moderately experienced subjects (<10 times) [3]. However, we wanted to exclude frequent substance users because extensive previous uncontrolled experiences may influence/condition new substance experiences [47]. The participants were asked to abstain from excessive alcohol consumption between test sessions (no more than 10 standard drinks/week) and particularly limit their use to one drink on the day before the test sessions. Additionally, the participants were not allowed to drink xanthine-containing liquids after midnight before the study day.

Five participants had previously used a hallucinogen, including LSD (three participants, 1–4 times), DMT (one participant 4 times), and salvia divinorum (one participant 3 times), eight participants had used MDMA (1–5 times), and 13 participants had previously used a stimulant, including methylphenidate (six participants, 1–3 times), amphetamine (eight participants, 1–2 times), and cocaine (one participant, 4 times). Eight participants had never used any illicit drugs with the exception of cannabis.

We performed urine drug tests at screening and before each test session, and no substances were detected during the study. We did not screen for alcohol use.

Study procedures

The study included a screening visit, a psychiatric interview, four 12-h experimental sessions, and an end-of-study visit. The experimental sessions were conducted in a quiet standard hospital patient room. Only one research subject and one investigator were present during the experimental sessions. The participants could interact with the investigator, rest quietly, or listen to music via headphones, but no other entertainment was provided. LSD, D-amphetamine, or placebo was administered at 9:00 a.m. MDMA or placebo was administered at 9:30 a.m. This was because of the different times to peak effects for each substance so that the functional magnetic resonance imaging (fMRI) scan and other assessments could be performed during the expected time-matched peak drug effects [26, 27, 32, 48, 49]. The fMRI scan

was performed at 11:00 a.m.–12:00 p.m. and the fMRI findings will be published elsewhere. Autonomic and subjective effects were assessed repeatedly throughout the session. Blood was collected to determine endocrine effects and substance concentrations.

Study drugs

LSD (D-lysergic acid diethylamide base, high-performance liquid chromatography purity >99%; Lipomed AG, Arlesheim, Switzerland) was administered in a single intermediate oral dose of 100 µg [50]. D-Amphetamine sulfate (40 mg salt; Hänseler, Herisau, Switzerland) was administered in a relatively high dose in the form of gelatin capsules as a single oral dose that corresponded to 30 mg D-amphetamine base [32]. MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was prepared as gelatin capsules and administered as a single oral dose of 125 mg, which is considered a relatively high dose [28, 40, 51, 52]. Blinding to treatment was guaranteed by using a double-dummy method, with identical capsules and vials that were filled with mannitol and ethanol, respectively, as placebo. At the end of each session and at the end of the study, the participants were asked to retrospectively guess their treatment assignment.

Measures

Subjective effects. Subjective effects were assessed repeatedly using visual analog scales (VASs) 1 and 0.5 h before and 0, 0.5, 1, 1.5, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, and 11 h after drug administration (time specifications correspond to MDMA administration). The VASs included "any drug effect," "good drug effect," "bad drug effect," "drug liking," "drug high," "stimulated," "ego dissolution," "talkative," "open," "concentration," "sense of time," and "speed of thinking" [14]. The VASs were presented as 100-mm horizontal lines (0–100%), marked from "not at all" on the left to "extremely" on the right ("slowed" and "racing" for "speed of thinking"). The VASs for "open," "talkative," "concentration," "sense of time," and "speed of thinking" were bidirectional (±50%), marked from "not at all" on the left (−50) to "normal" in the middle (0) and to "extremely" on the right (+50). The 60-item Adjective Mood Rating Scale (AMRS) [53] was administered 1 h before and 1.5, 4, and 11 h after drug administration. The 5D-ASC scale [54, 55] was administered 11 h after drug administration to retrospectively rate alterations in waking consciousness induced by the drugs. Mystical experiences were assessed using the German version [37] of the 100-item States of Consciousness Questionnaire [56] that includes the 43-item and newer 30-item MEQ (MEQ43 [56] and MEQ30 [57]). The German version of the 49-item Addiction Research Center Inventory (ARCI) [58, 59] was administered 11 h after drug administration. The duration of acute subjective effects was assessed using VAS "any drug effect" effect–time plots and an on/off threshold of 10% of the maximum individual response in Phoenix WinNonlin 6.4. Participants with responses <10% on this scale were not used to determine the effect duration (0, 3, and 4 participants for LSD, MDMA, and D-amphetamine, respectively).

Autonomic effects and adverse effects. Blood pressure, heart rate, and tympanic body temperature were repeatedly measured 1 and 0.5 h before and 0, 0.5, 1, 1.5, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, and 11 h after drug administration (time specifications correspond to MDMA administration) as previously described in detail [60]. Pupil function was measured under standardized dark-light conditions and assessed using a Voltcraft MS-1300 luxmeter (Voltcraft, Hirschau, Germany) after a dark adaption time of 1 min as previously described [61]. Adverse effects were assessed 1 h before and 11 h after drug administration using the 66-item List of Complaints [62]. This scale yields a total adverse effects score and reliably measures physical and general discomfort.

Endocrine effects. Plasma levels of oxytocin were measured at baseline and 1.5, 2.5, 3, and 5 h after MDMA administration. Oxytocin concentrations were measured using the oxytocin enzyme-linked immunosorbent assay (ELISA) kit (ENZO Life Sciences, Ann Arbor, MI) according to the manufacturer's protocol as previously described [63]. The plasma levels of BDNF were measured at baseline and 3 and 5 h after drug administration. Plasma BDNF levels were measured using an ELISA kit (Biosensis Mature BDNF Rapid ELISA kit: human, mouse, rat; Thebarton, Australia) as previously described [64]. Analyses were performed at the end of the study in one batch.

Plasma drug concentrations

The plasma levels of LSD, D-amphetamine, and the LSD metabolite O-H-LSD were measured at baseline and 1, 1.5, 2, 3, 3.5, 4.5, 5.5, 6.5, 7.5, 9.5, and 11.5 h after drug administration. The plasma levels of MDMA and MDMA metabolites 3,4-methylenedioxyamphetamine (MDA) and 4-hydroxy-3-methoxymethamphetamine (HMMA) were measured at baseline and 0, 0.5, 1, 1.5, 2.5, 3, 4, 5, 6, 7, 9, and 11 h after drug administration using liquid chromatography-tandem mass spectrometry as previously described [28, 32, 50]. The data were analyzed using non-compartmental analysis.

Statistical analyses

For measures repeatedly taken over time during each session, we first determined the peak effects (E_{max} and/or E_{min}) or peak changes from baseline (Table 1). The values were then analyzed using repeated-measures analysis of variance, with drug as the sole within-subjects factor, followed by Tukey's post hoc comparisons based on significant main effects. The criterion for significance was $p < 0.05$.

RESULTS

All 28 participants completed the MDMA, D-amphetamine, and placebo session. One participant quit before the final LSD session and only the data from the other sessions was included in the analysis.

Subjective mood effects

Subjective effects were measured over time using VASs (Fig. 1). The corresponding peak responses are presented in Table 1. LSD produced an overall greater response than both MDMA and D-amphetamine, reflected by significantly higher increases in ratings of "any drug effect," "good drug effect," "bad drug effect," and "ego dissolution" compared with MDMA and D-amphetamine. LSD also produced greater "drug liking," "drug high," and "stimulation" than D-amphetamine, whereas the effects of LSD on these scales did not significantly differ from MDMA. MDMA and D-amphetamine but not LSD increased peak ratings of "concentration" compared with placebo and LSD (Table 1). In contrast, LSD induced greater mean reductions over time (Fig. 1) and greater maximal reductions of ratings of talkative, concentration, sense of time, and speed of thinking compared with MDMA and D-amphetamine (Table 1). Only LSD and not MDMA or D-amphetamine induced significant "bad drug effects" compared with placebo. The overall effects ("any drug effect") of LSD, MDMA, and D-amphetamine lasted (mean ± SD) 8.5 ± 2.0 h, 4.4 ± 1.7 h, and 6.2 ± 2.0 h, respectively.

All three drugs similarly increased ratings of feeling "talkative" and "open." MDMA produced higher ratings of "any drug effect," "good drug effect," "drug liking," and "drug high" compared with D-amphetamine.

On the AMRS (Fig. 2, Table 1), LSD produced greater "introversion," "inactivity," "emotional excitation," and "anxiety" compared with MDMA and D-amphetamine. Conversely, MDMA and D-amphetamine increased "extraversion" compared with LSD. D-Amphetamine also increased "activity" and "concentration" compared with LSD.

Table 1. Comparison of the acute effects of LSD, MDMA, D-amphetamine, and placebo

		Placebo (mean ± SEM)	LSD (mean ± SEM)	MDMA (mean ± SEM)	D-Amphetamine (mean ± SEM)	$F_{3,78}$	P
Subjective effects							
VAS (%max)							
Any drug effect	ΔE_{max}	1.6 ± 1.0	87 ± 3.3***	59 ± 5.8***,###,†††	37 ± 4.8***,###,†††	114.94	<0.001
Good drug effect	ΔE_{max}	3.0 ± 2.5	82 ± 3.6***	64 ± 5.9***,##	45 ± 4.8***,###,††	89.09	<0.001
Bad drug effect	ΔE_{max}	0.1 ± 0.1	31 ± 5.3***	8.7 ± 3.1###	4.9 ± 1.9###	18.26	<0.001
Drug liking	ΔE_{max}	2.8 ± 2.4	76 ± 4.4***	64 ± 6.1***	48 ± 5.0***,###,†	63.95	<0.001
Drug high	ΔE_{max}	3.7 ± 2.8	70 ± 5.9***	58 ± 6.5***	41 ± 6.0***,###,†	40.81	<0.001
Stimulated	ΔE_{max}	3.4 ± 2.6	69 ± 6.1***	56 ± 6.6***	46 ± 6.0***,##	40.77	<0.001
Ego dissolution	ΔE_{max}	0.9 ± 0.7	83 ± 10.2***	44 ± 7.9**,###	50 ± 13.0###,†	60.95	<0.001
Open	ΔE_{max}	1.5 ± 1.0	21 ± 3.7***	24 ± 3.4***	22 ± 3.3***	13.02	<0.001
Talkative	ΔE_{max}	1.2 ± 1.0	17 ± 3.2***	20 ± 3.5***	24 ± 3.0***	16.32	<0.001
	ΔE_{min}	−0.5 ± 0.5	−31 ± 3.5***	−12 ± 3.1*,###	−4.7 ± 2.0###	32.05	<0.001
Concentration	ΔE_{max}	0.0 ± 0.0	6.6 ± 2.4	11 ± 3.0**	15 ± 2.8***	7.90	<0.001
	ΔE_{min}	−0.8 ± 0.6	−38 ± 2.6***	−20 ± 3.3***,###	−5.3 ± 1.3###,†††	65.97	<0.001
Sense of time	ΔE_{max}	0.0 ± 0.0	10 ± 3.3**	6.7 ± 2.3	3.3 ± 1.2	4.81	<0.01
	ΔE_{min}	−1.3 ± 1.1	−40 ± 2.5***	−12 ± 3.0**,###	−1.6 ± 0.6###,†	79.92	<0.001
Speed of thinking	ΔE_{max}	0.0 ± 0.0	11 ± 3.4**	9.4 ± 2.9	8.6 ± 2.0	4.16	<0.01
	ΔE_{min}	0.0 ± 0.0	−33 ± 2.9***	−15 ± 3.2***,###	−2.3 ± 0.7###,††	47.91	<0.001
AMRS score							
Activity	ΔE_{max}	0.3 ± 0.3	−0.1 ± 0.3	0.9 ± 0.4	2.2 ± 0.5**,###	6.74	<0.001
Concentration	ΔE_{max}	−0.1 ± 0.3	−0.7 ± 0.7	0.2 ± 0.4	1.5 ± 0.4##	4.05	<0.01
Extroversion	ΔE_{max}	0.2 ± 0.3	−0.1 ± 0.5	2.4 ± 0.4**,###	2.7 ± 0.5***,###	11.70	<0.001
Introversion	ΔE_{max}	0.1 ± 0.1	5.7 ± 0.6***	2.1 ± 0.4**,###	0.8 ± 0.2###	45.37	<0.001
Inactivity	ΔE_{max}	0.4 ± 0.2	4.1 ± 0.6***	1.8 ± 0.4###	0.7 ± 0.2###	19.47	<0.001
Well-being	ΔE_{max}	0.4 ± 0.4	2.9 ± 1.1	4.7 ± 0.7***	4.8 ± 0.8***	7.49	<0.001
Emotional excitation	ΔE_{max}	−0.5 ± 0.3	4.8 ± 1.2***	1.9 ± 0.5##	1.9 ± 0.6*,#	11.66	<0.001
Anxiety	ΔE_{max}	−0.2 ± 0.1	1.3 ± 0.5***	0.3 ± 0.2#	0.1 ± 0.1##	6.76	<0.001
Autonomic effects							
Systolic blood pressure (mmHg)	E_{max}	129 ± 2.1	140 ± 2.6***	149 ± 2.8***,##	161 ± 2.9***,###,†††	70.33	<0.001
Diastolic blood pressure (mmHg)	E_{max}	79 ± 1.3	88 ± 1.4***	89 ± 1.4***	97 ± 1.7***,###,†††	61.42	<0.001
Heart rate (beats/min)	E_{max}	77 ± 2.0	92 ± 3.0***	88 ± 2.3***	87 ± 3.0***	20.20	<0.001
Rate–pressure product (beats·mmHg/min)	E_{max}	9639 ± 329	12725 ± 578***	12707 ± 440***	12042 ± 484***	32.14	<0.001
Body temperature (°C)	E_{max}	37.2 ± 0.1	37.6 ± 0.1***	37.5 ± 0.0***	37.6 ± 0.1***,†	22.76	<0.001
Pupil size (mm)	E_{max}	6.3 ± 0.1	7.0 ± 0.1***	7.1 ± 0.1***	7.1 ± 0.1***	59.23	<0.001
Pupil size after light stimulus (mm)	E_{max}	4.4 ± 0.1	5.4 ± 0.1***	6.3 ± 0.2***,###	5.4 ± 0.1***,†††	110.51	<0.001
Constriction amplitude (mm)	E_{min}	1.7 ± 0.1	1.4 ± 0.1*	0.8 ± 0.1***,###	1.61 ± 0.04†††	49.36	<0.001
LC score							
Acute adverse effects	0–11 h	2.9 ± 1.3	8.15 ± 2.02*	5.43 ± 1.0	5.64 ± 1.37	2.63	NS
Hormones							
BDNF (mU/L)	E_{max}	2974 ± 425	2524 ± 370	3001 ± 423	2153 ± 265	1.27	NS
Oxytocin (pg/mL)	E_{max}	259 ± 62	279 ± 60	809 ± 64***,###	194 ± 35†††	27.36	<0.001

VAS visual analoge scale, *AMRS* Adjective Mood Rating Scale, *LS* List of Complaints, *NS* not significant, E_{max} maximal effect, ΔE_{max} maximal difference from baseline

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with placebo; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared with LSD; †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with MDMA

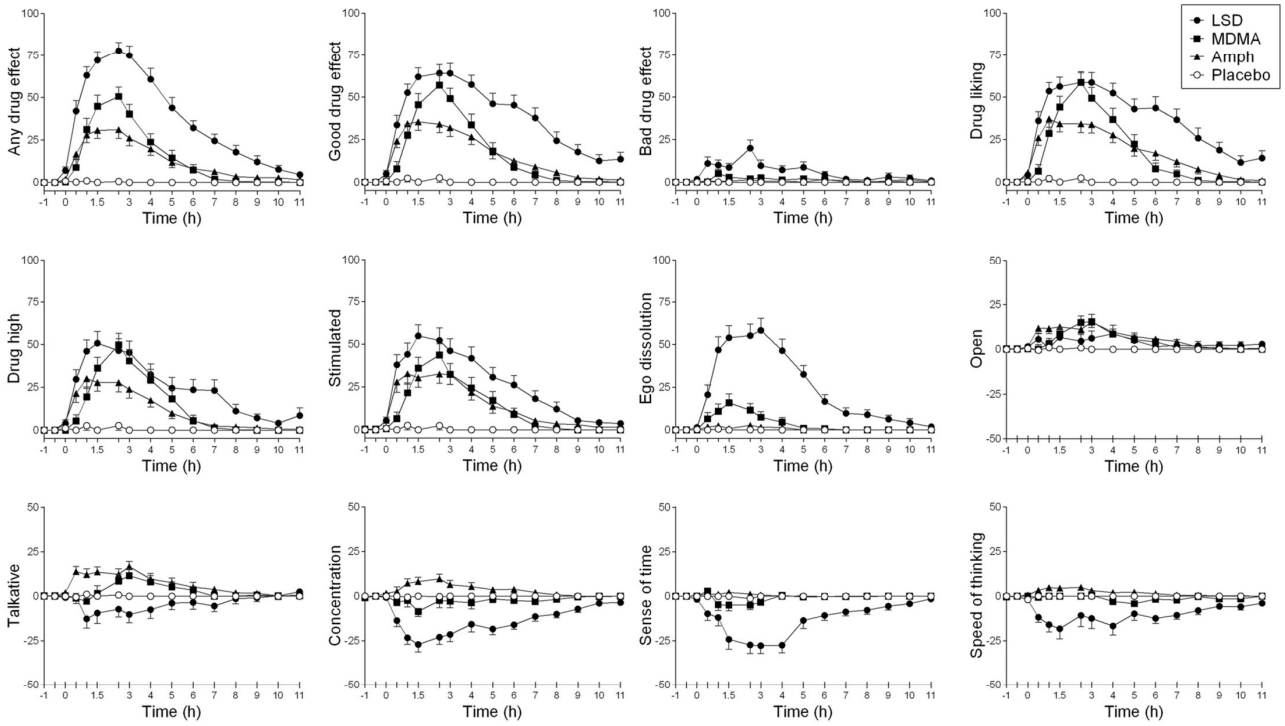

Fig. 1 Subjective effects of LSD, MDMA, and D-amphetamine over time on the VASs. The data are expressed as mean ± SEM. LSD produced significantly greater ratings of "any drug effect," "good drug effect," "bad drug effect," and "ego dissolution" compared with MDMA and D-amphetamine. In contrast, LSD reduced ratings of "talkative," "concentration," "sense of time," and "speed of thinking" compared with MDMA and D-amphetamine. MDMA produced greater ratings of "any drug effect," "good drug effect," "liking," "high," and "ego dissolution" compared with D-amphetamine. The corresponding maximal responses and statistics are shown in Table 1.

LSD was the only drug that induced marked alterations of mind, reflected by large increases on all subscales of the 5D-ASC (Fig. 3a, Supplementary Table S1) compared with placebo, MDMA (Tukey's post hoc tests: $p < 0.001$ for all comparisons), and D-amphetamine ($p < 0.001$ for all comparisons). MDMA only significantly increased ratings of "blissful state" compared with placebo, whereas D-amphetamine had no significant effects on any of the 5D-ASC subscales.

LSD increased ratings on all scales of the MEQ43 and MEQ30 compared with MDMA, D-amphetamine, and placebo ($p < 0.001$ for all comparisons), with the exception of nonsignificant differences in ratings of "deeply felt positive mood" for LSD and MDMA on the MEQ43 (Fig. 3b, Supplementary Table S1). MDMA significantly increased ratings of positive mood and ineffability (difficulty describing the experience in words) on the MEQ43 and MEQ30 compared with placebo ($p < 0.01$). D-Amphetamine moderately increased positive mood ratings on the MEQ43 and MEQ30.

On the ARCI, LSD increased ratings on all subscales that indicated broad (mixed) hallucinogenic, sedative, and euphoriant effects (Supplementary Fig. S1), with the exception of a decrease on the benzedrine group scale, indicating lower stimulation. In contrast, D-amphetamine was the only drug that increased ratings on the benzedrine group scale.

Vital signs and adverse effects

The effects of the drugs on vital signs over time are shown in Fig. 4, and peak effects are shown in Table 1. All active substances significantly increased blood pressure, heart rate, and body temperature compared with placebo. Systolic hypertension > 140 mmHg was seen in 23, 18, 14, and 3 participants after D-amphetamine, MDMA, LSD, and placebo, respectively. Tachykardia >100 beats/min was seen in 5, 5, 7, and 0 participants after D-amphetamine, MDMA, LSD, and placebo, respectively. D-Amphetamine produced a significantly higher increase in blood pressure compared with LSD and MDMA, and LSD and MDMA produced lower heart rate increases than D-amphetamine over the first 4 h, but all three drugs produced overall similar hemodynamic stimulation, considering the similar increases in the rate–pressure product. All three substances increased pupil size (Fig. 4, Table 1). However, only MDMA markedly and significantly impaired normal light-induced pupil constriction compared with placebo (Table 1, Supplementary Fig. S2). Only LSD increased the total acute (0–11 h) adverse effects score on the List of Complaints compared with placebo. Frequently reported adverse effects are presented in Supplementary Table S2. No severe adverse events were observed.

Endocrine effects

MDMA but not LSD or D-amphetamine increased plasma concentrations of oxytocin (Fig. S4, Table 1). None of the substances altered plasma concentrations of BDNF (Fig. S4, Table 1).

Plasma drug concentrations

The concentration–time curves for LSD, O-H-LSD, D-amphetamine, MDMA, MDA, and HMMA are shown in Supplementary Fig. S3. The geometric mean maximum (C_{max}) values (range) for LSD and O-H-LSD were 1.8 (0.99–2.9) and 0.12 (0.07–0.2) ng/ml, respectively. The T_{max} values were 1.6 (1–3.5) and 5.2 (3.1–7.5) h, respectively. The C_{max} values for MDMA, MDA, and HMMA were 236 (158–357), 10.9 (5.3–19), and 160 (43–287) ng/ml, respectively. The corresponding T_{max} values were 3.0 (1.1–5.0), 7.0 (3.0–11), and 2.8 (1.3–6.0) h, respectively. The C_{max} and T_{max} values for D-amphetamine were 100 (68–133) ng/ml and 2.6 (1.0–5.5) h, respectively.

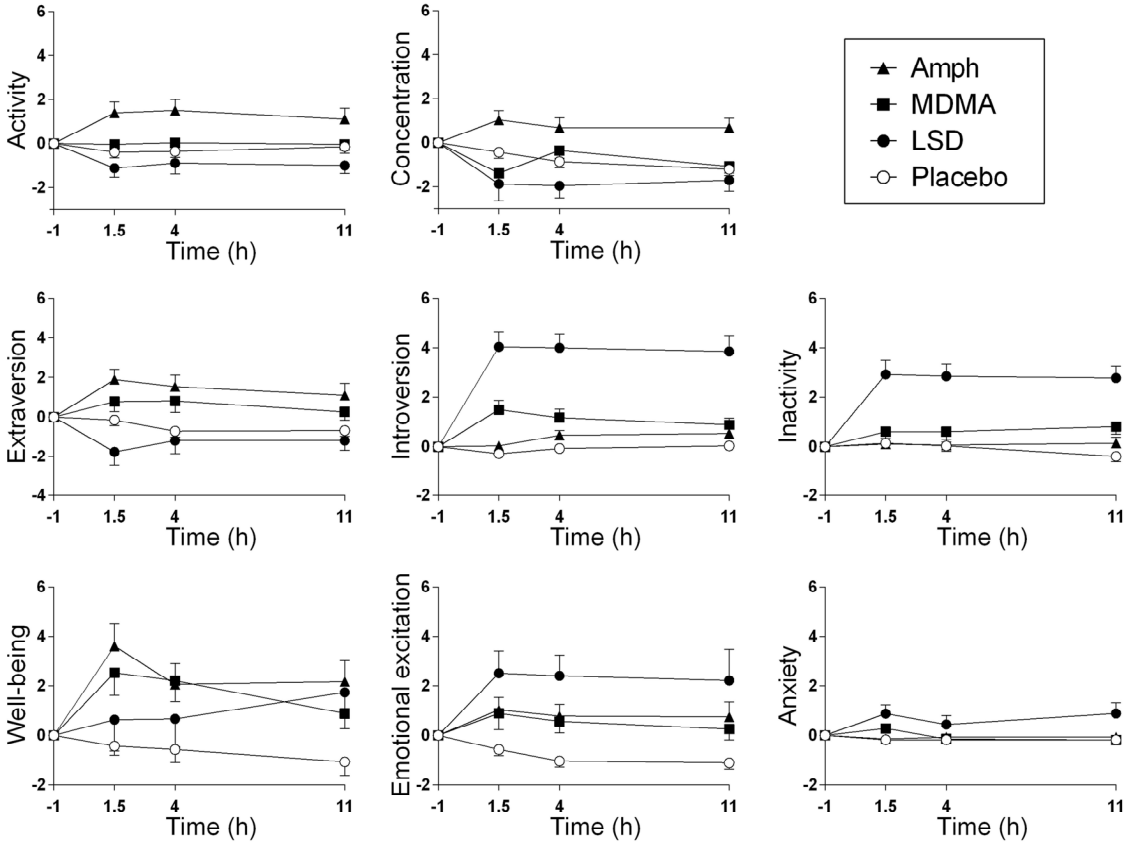

Fig. 2 Subjective effects of LSD, MDMA, and D-amphetamine over time on the AMRS. The data are expressed as mean ± SEM changes from baseline. D-Amphetamine increased ratings of activity and concentration compared with LSD. LSD increased ratings of inactivity compared with MDMA and D-amphetamine. LSD increased introversion and reduced extraversion compared with MDMA and D-amphetamine. MDMA and D-amphetamine increased ratings of well-being compared with placebo, whereas LSD produced no significant effect compared with placebo, and its effects did not differ from MDMA or D-amphetamine. LSD significantly increased emotional excitation and anxiety compared with MDMA and D-amphetamine. The corresponding maximal effects and statistics are shown in Table 1.

Blinding

Data on the participants' retrospective identification of the study substances are shown in Supplementary Table S3. All of the participants correctly identified placebo, 96% correctly identified LSD, 75% correctly identified MDMA, and 75% correctly identified D-amphetamine. MDMA was misclassified as D-amphetamine and vice versa (21%). One participant (4%) misidentified LSD as MDMA and vice versa. One participant (4%) identified D-amphetamine as placebo. Thus, LSD was well distinguished from MDMA and D-amphetamine.

DISCUSSION

As hypothesized, LSD produced stronger and more distinct subjective effects compared with MDMA and D-amphetamine. Specifically, only LSD induced significant and marked alterations of consciousness on all 5D-ASC and MEQ subscales compared with placebo, and responses were also significantly greater compared with MDMA and D-amphetamine. In contrast, MDMA only moderately increased "blissful state" on the 5D-ASC scale and "positive mood" and "ineffability" on the MEQ. D-Amphetamine only weakly increased "positive mood" on the MEQ compared with placebo. Additionally, LSD produced greater overall subjective effects, including both "good drug effects" and "bad drug effects," on the VAS compared with both MDMA and D-amphetamine. Only LSD produced significant "bad drug effects" on the VAS, "anxiety" on the 5D-ASC scale, and "LSD group" effects and "pentobarbital-chlorpromazine-alcohol group" effects on the ARCI compared with placebo. Finally, LSD was correctly identified by 96% and 100% of the participants on the day of administration and at the end of the study, respectively. However, similarities were also observed in the effects of all compounds on scales that measured positive drug effects. All of the drugs produced comparable ratings of "open" and "talkative" on the VAS, and ratings of "drug high," "drug liking," and "stimulated" on the VAS did not differ between LSD and MDMA. The present findings are overall consistent with previous reports on the effects of LSD [3, 4, 38, 50, 65], MDMA [18, 25, 28], and D-amphetamine [32]. In contrast to these previous studies, however, the present study compared the subjective responses to LSD, MDMA, and D-amphetamine using a within-subjects design. Subjective effects of various substances can differ, depending on the comparator that is used. For example, marked effects of MDMA on the 5D-ASC scale compared with inactive placebo have been previously reported [18]. However, when MDMA was compared with LSD in the present study, it induced only minimal and comparatively weak alterations of consciousness.

The present findings have clinical implications. First, acute effects of the LSD-like hallucinogen psilocybin on both the 5D-ASC scale and MEQ also used in the present study have been shown to predict long-term therapeutic outcomes in patients with anxiety and depression in previous studies [6–8]. Similarly, 5D-ASC scale and MEQ ratings correlated with changes in well-being and life satisfaction 1 year after LSD administration in healthy subjects in a previous study [10]. Thus, stronger acute responses to LSD on the 5D-ASC scale and MEQ, as documented in the present study in healthy participants and previously in patients [37], may also predict better therapeutic outcomes in studies that evaluate the

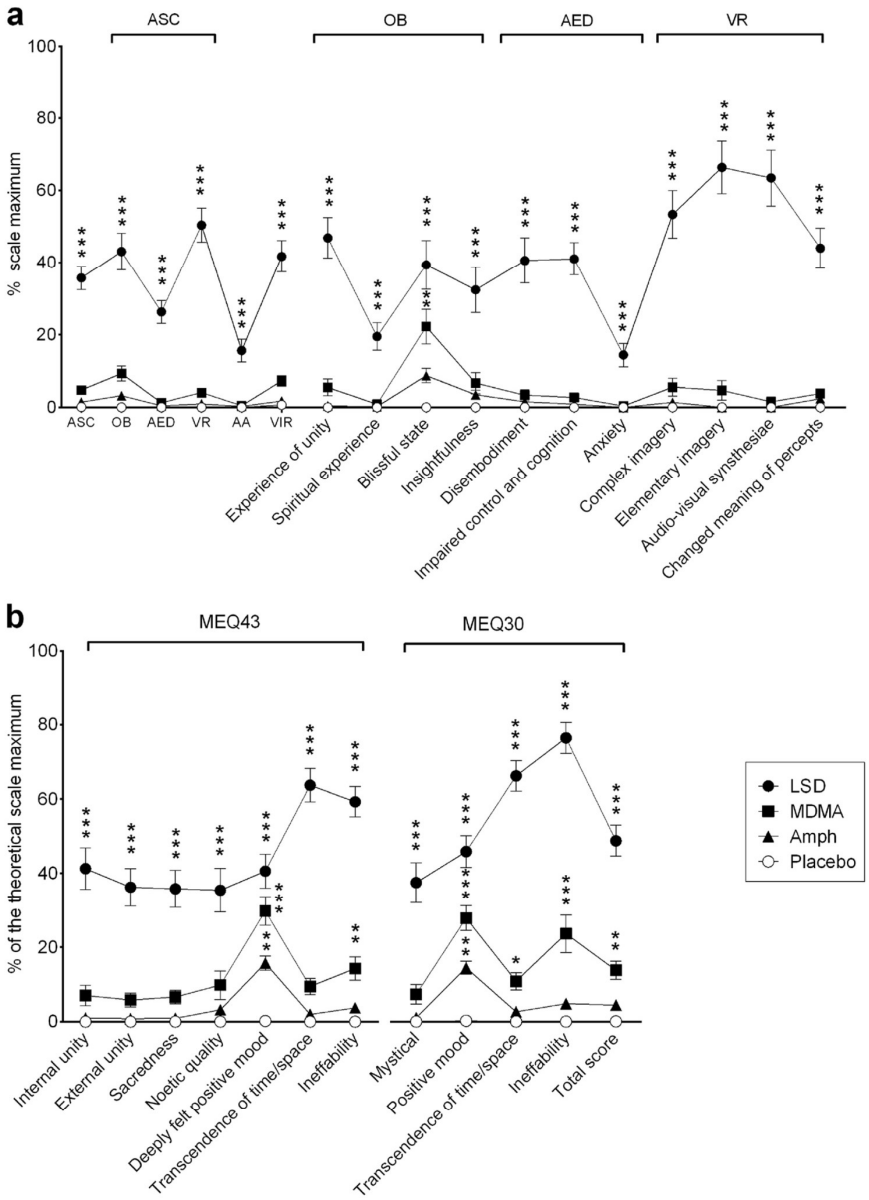

Fig. 3 Subjective effects of LSD, MDMA, and D-amphetamine on the 5D-ASC scale and MEQ. The data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, vs. placebo. **a** LSD produced significantly greater ratings on all dimensions and subscales of the 5D-ASC scale compared with MDMA, D-amphetamine, and placebo. The effects of MDMA tended to be greater than D-amphetamine, but these differences were not statistically significant. MDMA produced significant increases only on the blissful state subscale compared with placebo. The effects of D-amphetamine did not differ significantly from placebo on any of the scales. The corresponding maximal effects and statistics are shown in Table S1. **b** LSD produced significantly higher ratings on all scales of the MEQ43 and MEQ30 compared with MDMA, D-amphetamine, and placebo, with the exception of nonsignificantly different positive mood ratings for LSD and MDMA on the MEQ43. MDMA significantly increased positive mood and ineffability ratings on the MEQ43 and MEQ30 compared with placebo. D-Amphetamine significantly increased positive mood ratings on the MEQ43 and MEQ30, but these effects were significantly lower than MDMA. The corresponding maximal effects and statistics are shown in Table S1.

benefits of LSD-assisted psychotherapy in patients with anxiety and depression [66, 67]. However, this assumption needs to be verified in patients. Second, the present study found that MDMA produced some qualitatively similar (although less pronounced) positive effects compared with LSD, but with lower associated "bad drug effects" and anxiety. Thus, MDMA may produce less untoward effects than LSD, and this may favor its use in patients afraid to take LSD or at risk of adverse reaction (i.e., high neuroticism, high emotional lability, and young age [47]). In fact, MDMA is often used prior to LSD in substance-assisted psychotherapy in Switzerland so that patients can familiarize themselves with substance-induced states [66, 68, 69]. For example, MDMA could be used prior to LSD or psilocybin in substance-assisted psychotherapy so that patients can familiarize themselves with substance-induced states. In fact, MDMA has often been used in the first 1–3 sessions before the use of LSD in substance-assisted psychotherapy in Switzerland.

In the present study, we also directly compared the acute effects of MDMA and D-amphetamine and we hypothesized that MDMA would produce distinct subjective emotional effects compared with D-amphetamine. Previous studies have discussed the extent to which the effects of these amphetamines differ [25, 27, 28, 70]. The present study supports the view that the empathogen MDMA produces at least some clearly distinct effects

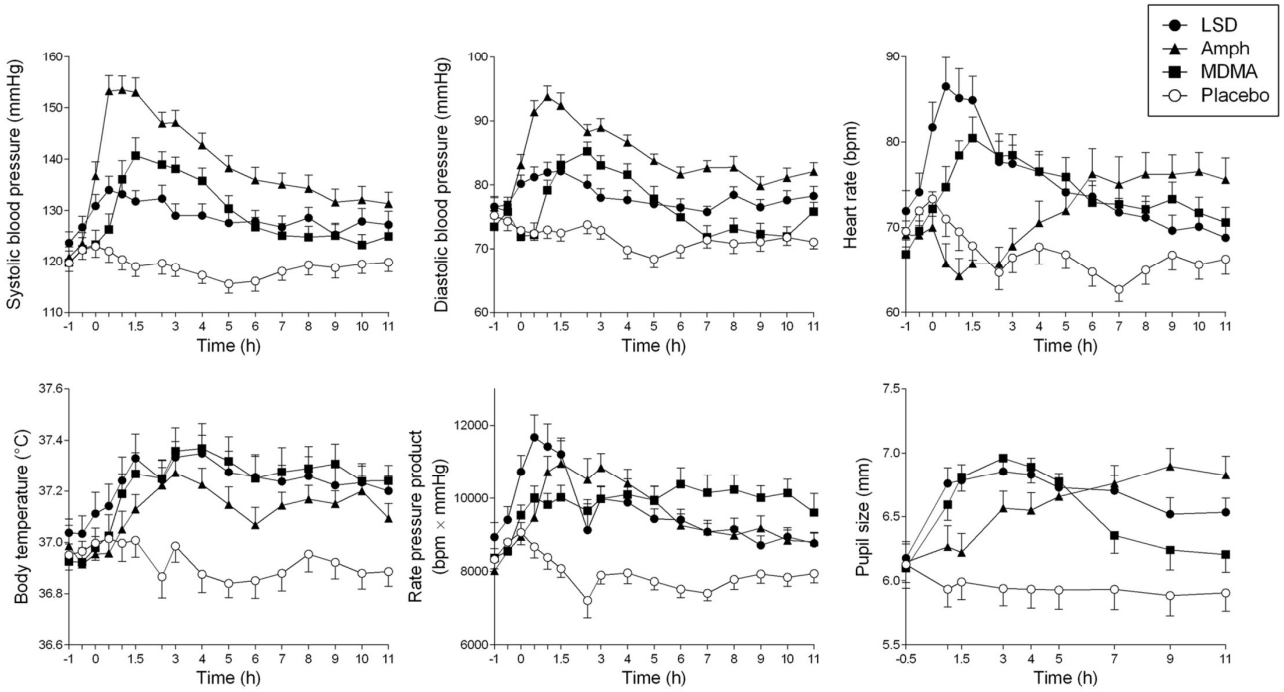

Fig. 4 Autonomic responses to LSD, MDMA, D-amphetamine, and placebo. The data are expressed as mean ± SEM. All of the active substances produced significant sympathomimetic stimulation, reflected by increases in systolic and diastolic blood pressure, heart rate, body temperature, and pupil size. Importantly, the overall hemodynamic response, expressed as the rate–pressure product, was similarly increased by all of the active substances compared with placebo. However, D-amphetamine produced significantly higher increases in blood pressure than LSD and MDMA. Conversely, LSD and MDMA produced greater increases in heart rate than D-amphetamine during the first 4 h. The corresponding maximal effects and statistics are shown in Table 1.

compared with a pure stimulant, such as D-amphetamine. In the present study, MDMA produced greater ratings of "any drug effect," "good drug effect," "drug high," and "drug liking" on the VAS, greater ratings of "positive mood" on the MEQ, and smaller "benzedrine group" effects on the ARCI than D-amphetamine. MDMA also induced greater impairments in "concentration" and "speed of thinking" compared with D-amphetamine.

In contrast and as predicted, MDMA but not D-amphetamine increased plasma oxytocin concentrations, which is thought to be attributable to the MDMA-induced release of 5-HT and 5-HT_{1A} receptor stimulation [23]. Interestingly, the potent 5-HT_{1A} and 5-HT_{2A} receptor agonist LSD [5] did not significantly increase plasma oxytocin levels in the present study, in contrast to a higher dose of LSD and inactive placebo as the comparator in a previous study [3]. Supporting the view of distinct effects of MDMA and D-amphetamine, 75% and 89% of the participants in the present study correctly identified MDMA and D-amphetamine on the day of administration and at the end of the study, respectively. However, MDMA and D-amphetamine also produced overlapping effects, including comparable increases in "open" and "talkative" on the VAS, "well-being" and "extraversion" on the AMRS, and a lack of significant "bad drug effects" or "anxiety" compared with placebo and in contrast to LSD. Similar partly overlapping effects of MDMA and lower doses of D-amphetamine (10–20 mg) have been previously reported [33, 71]. Interestingly, both MDMA and D-amphetamine seemed to produce relatively comparable "empathogenic" effects in the present study, whereas such effects were somewhat more unique to MDMA compared with the stimulant methylphenidate [27, 28]. Thus, MDMA and D-amphetamine are more alike than MDMA and methylphenidate, but this remains to be clarified in future studies. Pharmacologically, D-amphetamine and methylphenidate both activate the dopamine and norepinephrine systems without having relevant effects on 5-HT. However, D-amphetamine also releases monoamines similarly to MDMA, in contrast to the pure uptake inhibitor methylphenidate [29, 72].

In the present study, LSD, MDMA, and D-amphetamine produced comparable sympathomimetic activation, reflected by similar increases in the rate–pressure product, body temperature, and pupil size. Additionally, LSD, MDMA, and D-amphetamine produced comparable amounts of total adverse effects as evidenced by similar scores on the List of Complaints (Table 1), although there were some differences between the substances regarding the specific complaints (Table S2). These findings indicate that the doses of the drugs were similar with regard to sympathomimetic effects, including cardiovascular system stimulation and somatic complaints. The finding that LSD produced relatively pronounced sympathomimetic effects confirmed our previous studies [3, 38] and contradicted the assumption that LSD does not increase blood pressure [67]. On the other hand, the study findings suggest that LSD is capable of inducing greater acute psychological effects (positive and negative) than MDMA and D-amphetamine at doses that are producing comparable somatic adverse responses.

In the present study, we also determined plasma drug concentrations. Peak concentrations of MDMA and D-amphetamine were similar to previous studies that tested identical doses [32, 39, 73]. The full pharmacokinetic data for LSD derived from the present study have been published elsewhere [50]. Importantly, slightly higher plasma concentrations of LSD were documented in the present study compared with a previous study that reportedly used the same dose (0.1 mg) [49]. The higher plasma concentrations in the present study can be explained by the use of a higher dose (0.096 mg) of LSD base (analytically confirmed content and stability) compared with a lower estimated dose of 0.070 mg in previous studies [38, 49], as discussed previously [50].

The main strength and novelty of the present study was that we employed a double-blind, placebo-controlled, within-subjects design that included different active substances and validated pharmacodynamic and substance concentration measurements. The present study also has limitations. We only used one dose

level of each substance. Full dose–response curves would need to be generated for each substance to achieve valid comparisons. However, we used a relatively low dose of LSD compared with the doses of MDMA and D-amphetamine and nevertheless found stronger effects of LSD compared with MDMA and D-amphetamine. Additionally, a previous study that used a higher dose of LSD (0.2 mg) showed significantly greater acute subjective effects of LSD compared with 0.1 mg LSD (the dose used in the present study), but autonomic stimulation was similar between doses [38]. Specifically, the higher dose produced both greater "good drug effect" and "bad drug effect" ratings on the VASs [38] and higher ratings of "blissful state," "insightfulness," and "changed meaning of percepts," but no increase in "anxiety" on the 5D-ASC [37] compared with the lower dose of LSD. Thus, both desired and untoward drug effects were dose-dependent and future multiple dose-level studies will be needed to further define ideal dose ranges. Thus, higher doses of LSD up to 0.2 mg that are already clinically used [2, 67] can be expected to produce even greater subjective effects than the dose (0.1 mg) that was used in the present study. The dose of MDMA that was used in the present study is in the upper range of doses that are used clinically; higher doses would not likely produce stronger positive subjective effects, but would likely result in more adverse somatic responses [39]. Finally, we found that the doses of all of the active substances were equivalent with regard to autonomic stimulation. Nevertheless, there is a need for additional studies including multiple dose levels and additional outcomes such as imaging.

In conclusion, the present study found that LSD induced different and more pronounced alterations of waking consciousness compared with MDMA and D-amphetamine in the same subjects. MDMA also showed partly distinct effects compared with D-amphetamine. The acute-effect profiles of LSD and MDMA will be useful to assist the dose selection for substance-assisted psychotherapy research and to inform patients and researchers on what to expect in terms of positive and negative acute responses to these substances.

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ADDITIONAL INFORMATION

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2.3. Acute dose-dependent effects of lysergic acid diethylamide in a double-blind placebo-controlled study in healthy subjects

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ARTICLE OPEN

Acute dose-dependent effects of lysergic acid diethylamide in a double-blind placebo-controlled study in healthy subjects

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Growing interest has been seen in using lysergic acid diethylamide (LSD) in psychiatric research and therapy. However, no modern studies have evaluated subjective and autonomic effects of different and pharmaceutically well-defined doses of LSD. We used a double-blind, randomized, placebo-controlled, crossover design in 16 healthy subjects (eight women, eight men) who underwent six 25 h sessions and received placebo, LSD (25, 50, 100, and 200 µg), and 200 µg LSD 1 h after administration of the serotonin 5-hydroxytryptamine-2A (5-HT_{2A}) receptor antagonist ketanserin (40 mg). Test days were separated by at least 10 days. Outcome measures included self-rating scales that evaluated subjective effects, autonomic effects, adverse effects, plasma brain-derived neurotrophic factor levels, and pharmacokinetics up to 24 h. The pharmacokinetic-subjective response relationship was evaluated. LSD showed dose-proportional pharmacokinetics and first-order elimination and dose-dependently induced subjective responses starting at the 25 µg dose. A ceiling effect was observed for good drug effects at 100 µg. The 200 µg dose of LSD induced greater ego dissolution than the 100 µg dose and induced significant anxiety. The average duration of subjective effects increased from 6.7 to 11 h with increasing doses of 25–200 µg. LSD moderately increased blood pressure and heart rate. Ketanserin effectively prevented the response to 200 µg LSD. The LSD dose–response curve showed a ceiling effect for subjective good effects, and ego dissolution and anxiety increased further at a dose above 100 µg. These results may assist with dose finding for future LSD research. The full psychedelic effects of LSD are primarily mediated by serotonin 5-HT_{2A} receptor activation.

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INTRODUCTION

Lysergic acid diethylamide (LSD) is a classic serotonergic psychedelic with a broad history of early psychiatric research and recreational use [1, 2]. LSD induces a range of complex alterations of the mind that have been shown to depend on serotonin 5-hydroxytryptamine-2A (5-HT_{2A}) receptor stimulation [3–5]. Renewed interest has been seen in using LSD in psychiatric research and to assist psychotherapy [6–9]. However, all recent placebo-controlled high-dose studies of LSD used only single doses [4, 10–13]. No recent data are available on the acute effects of different well-defined psychoactive doses of LSD in humans and within the same study. Therefore, the present study evaluated acute subjective and autonomic effects of LSD across a range of relevant doses in healthy subjects. In contrast to previous studies [10–12], we used pharmaceutically well-defined doses of LSD. We verified LSD content uniformity of the doses and performed a pharmaceutical stability test. We comprehensively determined plasma LSD concentrations over time to document each individual exposure to LSD and defined the pharmacokinetics of LSD across all doses. Psychedelics can induce neuroregeneration [14]. Therefore, we also measured plasma brain-derived neurotrophic factor (BDNF) levels as a possible biomarker for neurogenesis [15]. Furthermore, we evaluated the role of 5-HT_{2A} receptors in the acute effects of a high dose of LSD by administering the 5-HT_{2A} receptor antagonist ketanserin prior to the administration of 200 µg LSD and compared the acute response to the administration of 200 µg LSD alone. Complex acute subjective effects of LSD were determined using validated psychometric instruments that are used internationally and in trials with patients and have been shown to be useful for predicting therapeutic long-term responses [16–19]. We hypothesized that LSD effects would be dose-dependent and blocked by ketanserin. The present LSD dose–response data may be useful for dose finding in future LSD research in healthy subjects and patients.

METHODS AND MATERIALS

Study design

The study used a double-blind, placebo-controlled, crossover design with six experimental test sessions to investigate the responses to (i) placebo, (ii) 25 µg LSD, (iii) 50 µg LSD, (iv) 100 µg LSD, (v) 200 µg LSD, and (vi) 200 µg LSD 1 h after ketanserin administration (40 mg). Block randomization was used to counterbalance the different dosing conditions. The washout periods between sessions were at least 10 days. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines in Good Clinical Practice and approved by the Ethics Committee of Northwest Switzerland

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(EKNZ) and Swiss Federal Office for Public Health. The study was registered at ClinicalTrials.gov (NCT03321136).

Participants

Sixteen healthy subjects (eight men and eight women; mean age ± SD: 29 ± 6.4 years; range: 25–52 years) were recruited by word of mouth or an advertisement that was posted on the web market platform of the University of Basel. Mean body weight was 69 kg and 78 and 60 kg in male and female participants, respectively. Accordingly, doses/body weight of LSD were 1.3-fold higher in men than women. All of the subjects provided written informed consent and were paid for their participation. Drug administration timing did not consider the menstrual cycle in females for practical reasons. Four women used a hormonal contraceptive and one was menopausal. Exclusion criteria were age <25 years or >65 years, pregnancy (urine pregnancy test at screening and before each test session), personal or family (first-degree relative) history of major psychiatric disorders (assessed by the Semi-structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Axis I disorders by a trained psychiatrist), the use of medications that may interfere with the study medications (e.g., antidepressants, antipsychotics, and sedatives), chronic or acute physical illness (e.g., abnormal physical exam, electrocardiogram, or hematological and chemical blood analyses), tobacco smoking (>10 cigarettes/day), lifetime prevalence of illicit drug use >10 times (except for Δ^9-tetrahydrocannabinol), illicit drug use within the last 2 months, and illicit drug use during the study period (determined by urine drug tests). The participants were asked to consume no more than 10 standard alcoholic drinks/week and have no more than one drink on the day before the test sessions. Six participants had previously used LSD (1–3 times), eight participants had used methylenedioxymethamphetamine (MDMA) (1–5 times), ten participants had previously used a stimulant, including methylphenidate (four participants, 1–2 times), amphetamine (six participants, 1–3 times), and cocaine (two participants, 1–2 times), and one participant had smoked opium (once). Six participants had never used any illicit drugs with the exception of cannabis. Substance use histories are shown in Table S1 in the Supplementary Methods online. Drug of abuse tests performed once during the screening and once during the study in each subject were negative.

Study drugs

LSD base (>99% purity; Lipomed AG, Arlesheim, Switzerland) was administered as an oral solution that was produced according to good manufacturing practice in units that contained 100 or 25 µg LSD in 1 ml of 96% ethanol [20]. The exact analytically confirmed LSD content (mean ± SD) of the 25 and 100 µg formulations was 25.7 ± 0.57 µg ($n = 9$ samples) and 98.7 ± 1.6 µg ($n = 9$ samples), respectively. Stability of the formulation for longer than the study period was documented in an identically produced previous batch [20]. One microgram of LSD base that was used in the present study corresponded to 1.25 µg LSD tartrate that was used recreationally and in other studies [9, 21]. Ketanserin was obtained as the marketed drug Ketensin (20 mg, Janssen-Cilag, Leiden, NL) and encapsulated with opaque capsules to ensure blinding. Placebo consisted of identical opaque capsules that were filled with mannitol. A double-dummy method was used. The subjects received two capsules and two solutions in each session: (i) two placebo capsules and placebo/placebo solutions, (ii) two placebo capsules and 25 µg LSD/placebo solutions, (iii) two placebo capsules and 25 µg LSD/25 µg LSD solutions, (iv) two placebo capsules and 100 µg LSD/placebo solutions, (v) two placebo capsules and 100 µg LSD/100 µg LSD solutions, and (vi) two ketanserin capsules and 100 µg LSD/100 µg LSD solutions. At the end of each session and at the end of the study, the participants were asked to retrospectively guess their treatment assignment.

Study procedures

The study included a screening visit, six 25 h test sessions (each separated by at least 10 days), and an end-of-study visit. The sessions were conducted in a calm hospital room. Only one research subject and one investigator were present during each test session. The test sessions began at 7:45 a.m. A urine sample was taken to verify abstinence from drugs of abuse, and a urine pregnancy test was performed in women. The subjects then underwent baseline measurements. Ketanserin (40 mg) or placebo was administered at 8:00 a.m. LSD or placebo was administered at 9:00 a.m. The outcome measures were repeatedly assessed for 24 h. Standardized lunches and dinners were served at 1:30 p.m. and 6:00 p.m. respectively. The subjects were never alone during the first 16 h after drug administration, and the investigator was in a room next to the subject for up to 24 h. The subjects were sent home the next day at 9:15 a.m.

Subjective drug effects

Subjective effects were assessed repeatedly using visual analog scales (VASs) [12, 13] 1 h before and 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, and 24 h after drug administration. VASs were assessed each time LSD blood concentrations were measured to allow for PK–PD modeling. The Adjective Mood Rating Scale (AMRS) [22] was used 1 h before and 3, 6, 9, 12, and 24 h after drug administration. The 5 Dimensions of Altered States of Consciousness (5D-ASC) scale [23, 24] was used as the primary outcome measure and was administered 24 h after drug administration to retrospectively rate peak drug effects. Mystical experiences were assessed 24 h after drug administration using the States of Consciousness Questionnaire [25, 26] that includes the 43-item Mystical Effects Questionnaire (MEQ43) [25], 30-item Mystical Effects Questionnaire (MEQ30) [27], and subscales for "aesthetic experience" and negative "nadir" effects. Subjective effects measurements are described in detail in Supplementary Methods online.

Autonomic and adverse effects

Blood pressure, heart rate, and tympanic body temperature were repeatedly measured [28]. Adverse effects were assessed 1 h before and 12 and 24 h after drug administration using the list of complaints [29].

Plasma BDNF levels

Plasma BDNF levels were measured at baseline and 6, 12, and 24 h after drug administration using the Biosensis Mature BDNF Rapid ELISA Kit (Thebarton, Australia) [30].

Plasma LSD concentrations

Blood was collected into lithium heparin tubes. The blood samples were immediately centrifuged, and the plasma was subsequently stored at −80 °C until analysis. Plasma concentrations of LSD and O–H–LSD were determined by ultra-high-performance liquid chromatography tandem mass spectrometry with a lower limit of quantification of 5 pg/ml [20].

Pharmacokinetic analyses and pharmacokinetic–pharmacodynamic modeling

Pharmacokinetic (PK) parameters were estimated using a one-compartment model with first-order input, first-order elimination, and no lag time in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA) [20]. The predicted concentrations of LSD were then used as an input to the pharmacodynamic (PD) model by treating the PK parameters as fixed and using a sigmoid maximum effect model in the classic PK/PD link model module in WinNonlin [20]. The time to onset, time to maximal effect, time to offset, and effect duration were assessed for the model-predicted "any drug effect" VAS effect-time plots after LSD administration using a threshold of 10% of the maximum individual response using Phoenix WinNonlin 6.4.

Data analysis

Peak (E_{max} and/or E_{min}) or peak change from baseline (ΔE_{max}) values were determined for repeated measures. The values were then analyzed using repeated-measures analysis of variance, with drug as the within-subjects factor, followed by the Tukey post hoc test. The data were analyzed using Statistica 12 software (StatSoft, Tulsa, OK, USA). The criterion for significance was $p < 0.05$. No correction for multiple testing was applied.

RESULTS

Subjective drug effects

Subjective effects over time on the VAS and AMRS are shown in Fig. 1 and Supplementary Fig. S1, respectively. The corresponding peak responses and statistics are presented in Supplementary Table S2. Alterations of mind and mystical-type effects are shown in Figs. 2, S2, respectively. Statistics are summarized in Supplementary Table S2.

LSD elicited dose-dependent subjective responses starting at the 25 µg dose, which produced significant "any drug effects" compared with placebo ($p < 0.05$). A ceiling effect was reached at the 100 µg dose of LSD on most scales (particularly positive subjective effects), with typically no significant differences between the 100 and 200 µg doses (Figs. 1, 2, S2). However, the 200 µg dose produced significantly greater ego dissolution on the VAS (Fig. 1), greater anxious ego dissolution on the 5D-ASC (Fig. 2), and greater subjectively negative nadir effects (Fig. S2) than the 100 µg dose (all $p < 0.05$). Only the 200 µg dose and not the 100 µg dose induced significant anxiety on the 5D-ASC (Fig. 2) and AMRS (Supplementary Fig. S1; both $p < 0.01$). Thus, only ego dissolution and anxiety increased at an LSD dose above 100 µg. Ketanserin significantly (most $p < 0.001$) reduced the subjective response to high-dose LSD approximately to levels that were observed with the 25 µg dose (Figs. 1, 2, S2). Only a small VAS good drug effect in response to LSD was observed after ketanserin administration, which occurred with a temporal delay compared with the effect of LSD alone (Fig. 1). There was no significant difference in the subjective effects of LSD between LSD-experienced and LSD-naïve participants (Fig. S3).

Autonomic and adverse effects

Autonomic effects over time and respective peak effects are shown in Fig. 3 and Supplementary Table S2, respectively. Frequently reported adverse effects are presented in Supplementary Table S8. LSD moderately but significantly increased blood pressure at doses of 50 µg or higher and heart rate at 100 and 200 µg (Fig. 3). LSD had no effect on body temperature. LSD at doses of 100 and 200 µg increased the total acute (0–12 h) adverse effects score on the List of Complaints compared with placebo and all other conditions. Ketanserin significantly prevented the LSD-induced heart rate response and transiently reduced the LSD-induced blood pressure response up to 6 h. No severe adverse events were observed.

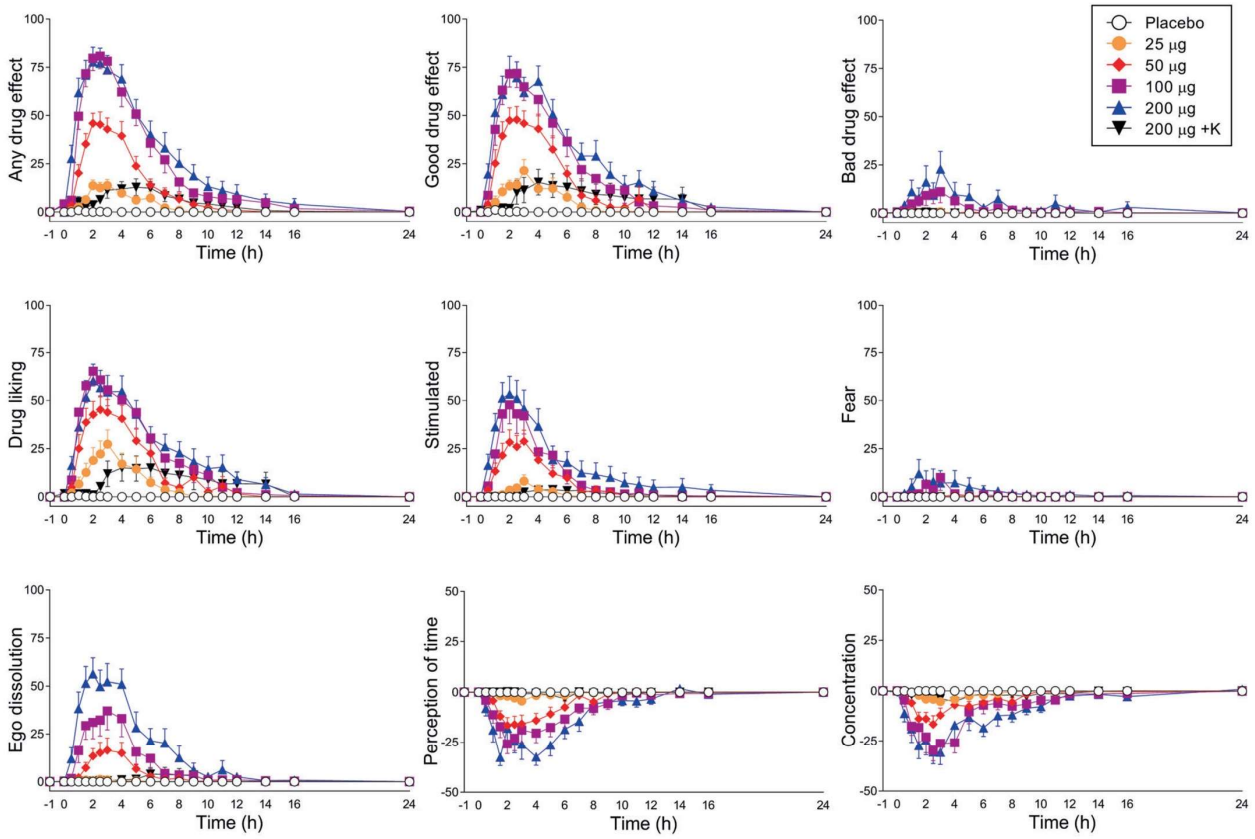

Fig. 1 Acute subjective effects of lysergic acid diethylamide (LSD) over time. LSD (25–200 µg) or placebo was administered at $t = 0$ h. Ketanserin (K) or placebo was administered at $t = -1$ h. LSD dose-dependently induced good drug effects, with a maximum effect reached at the 100 µg dose. The 200 µg dose of LSD did not further increase good drug effects or drug liking compared with the 100 µg dose, but it further increased ego dissolution compared with the 100 µg dose. Ketanserin markedly reduced the response to the high 200 µg dose of LSD approximately to the levels of the 25 µg and delayed the remaining small good drug effect and drug liking response compared with LSD alone. The data are expressed as the mean ± SEM percentage of maximally possible scale scores in 16 subjects. The corresponding maximal responses and statistics are shown in Supplementary Table S2.

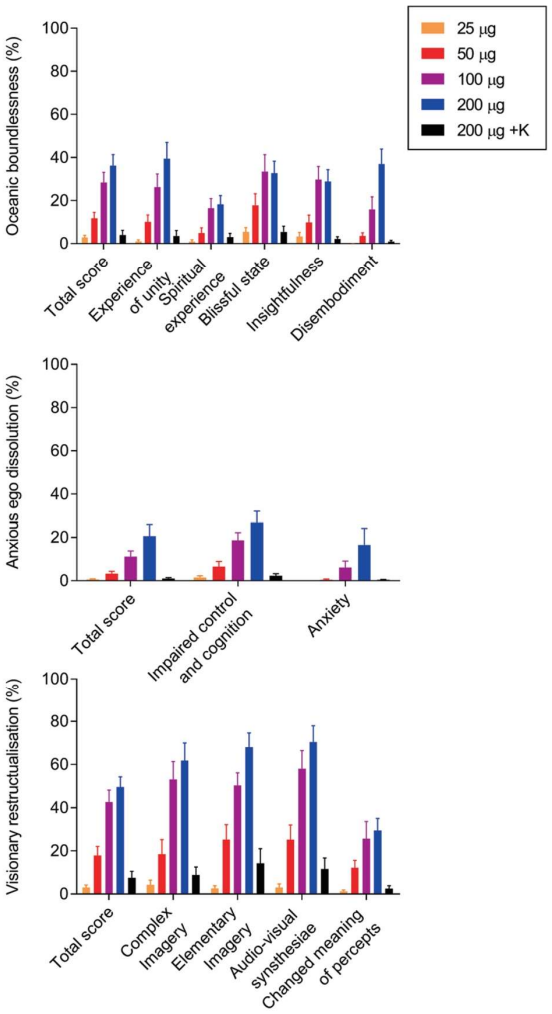

Fig. 2 Acute alterations of mind on the 5 Dimensions of Altered States of Consciousness (5D-ASC) Scale. All lysergic acid diethylamide (LSD) doses (25–200 µg) increased "oceanic boundlessness" and "visionary restructuralization." Only the 50, 100, and 200 µg doses of LSD significantly influenced "anxious ego dissolution." The dose–response curve showed a ceiling effect for oceanic boundlessness and visionary restructuralization ratings at the 100 µg dose. In contrast, ratings of anxious ego dissolution further increased at the 200 µg dose compared with the 100 µg dose. Additionally, only the 200 µg dose and not the 100 µg dose induced significant anxiety. Ketanserin markedly reduced the response to the highest LSD dose (200 µg) approximately to the level of the 25 µg dose. Placebo scores were too low for visualization. The data are expressed as the mean ± SEM percentage of maximally possible scale scores in 16 subjects. Statistics are shown in Supplementary Table S2.

Plasma BDNF levels

LSD increased plasma BDNF at the 200 µg dose compared with placebo (Supplementary Table S2, Fig. S4).

Pharmacokinetics and pharmacokinetic–pharmacodynamic modeling

Concentrations of LSD and its main metabolite 2-oxo-3-hydroxy LSD (O–H–LSD) could be quantified in all of the subjects, at all doses, and at all time-points. Table 1 shows the PK parameters of LSD. Model-predicted LSD concentrations and effects over time are shown in Fig. 4. Plasma LSD concentrations increased proportionally with increasing doses (Fig. 4). The predicted VAS any drug effects and good drug effects of LSD showed a ceiling effect at the 100 µg dose, and higher bad drug effects and greater ego dissolution were reported at the 200 µg dose compared with 100 µg (Fig. 4). The time to onset, time to maximal effect, time to offset, and effect duration are shown in Table S3. Summarized, the time to onset of the LSD response decreased, and the time to offset increased, resulting in longer effect durations with higher doses of LSD (Fig. 4, Table S3). Ketanserin had no significant effect on the PKs of LSD (Table 1). PK parameters based on non-compartmental analyses are shown in Supplementary Tables S4, S5. Parameters for the PK–PD link model are summarized in Supplementary Table S6. Corresponding individual data is presented in Figs. S5–S24. There were no significant difference in the PKs or effects of LSD between male and female participants.

Blinding

Data on the participants' retrospective identification of the LSD dose condition are shown in Supplementary Table S7. Generally, the 100 and 200 µg doses were identified as high doses, but these two doses could not be distinguished. The 25 µg dose of LSD was distinguished from placebo and identified correctly or as the 50 µg dose of LSD by most participants. Ketanserin and LSD together were identified correctly or mistaken as a low dose of LSD but never mistaken for a high dose of LSD.

DISCUSSION

The present study investigated acute effects of LSD using a range of well-defined doses in healthy subjects. Previous recent studies mostly used LSD products that were not developed according to pharmaceutical standards, as discussed elsewhere [20]. Additionally, we determined plasma LSD concentrations as measures of exposure to the substance in the body that are a prerequisite for a valid dose-finding study. We used LSD doses in the psychedelic effect dose range (25–200 µg of LSD base) that were expected to induce full subjective effects of LSD as previously reported by comparable studies that used single-dose levels [4, 10–12]. Plasma LSD concentrations increased proportionally with increasing doses and decreased according to first-order elimination. The PK parameters were consistent with single-dose studies [20, 31]. A preliminary report of a longer terminal elimination half-life of LSD [32] was not confirmed in the present study. We found no sex differences in LSD concentrations or effects consistent with previous studies using no body weight adjustment of LSD doses [20, 31, 32].

LSD dose-dependently increased subjective effects that were largely similar to previous studies that used single-dose levels [10–13, 26]. Importantly, a ceiling effect was reached at higher doses of LSD (>100 µg) with regard to its positive subjective effects, with no difference in good drug effects between the 100 and 200 µg doses. However, the 200 µg dose of LSD produced significantly greater ego dissolution and anxious ego dissolution than the 100 µg dose. Additionally, only the 200 µg dose and not the 100 µg dose of LSD-induced significant anxiety. However, doses above 100 µg may be used if the goal is to induce the experience of ego dissolution or disembodiment. These experiences, however, were produced at doses that also produced more anxiety compared with lower doses. LSD doses of 100 and 200 µg were both subjectively identified as high doses but could not be subjectively distinguished with certainty from each other. Both of these doses can clearly be considered full psychedelic doses and have previously been investigated in healthy subjects [11, 12]. No previous studies directly compared LSD doses of 100 and 200 µg. In contrast to the present findings, we previously reported moderately greater effects of a 200 µg dose of LSD in one study [12] compared with 100 µg in another study [11]. Specifically, 200 µg LSD produced significantly greater total scores on the 5D-ASC scale, including higher ratings of blissful state, insightfulness, and changed meaning of percepts compared with 100 µg [26].

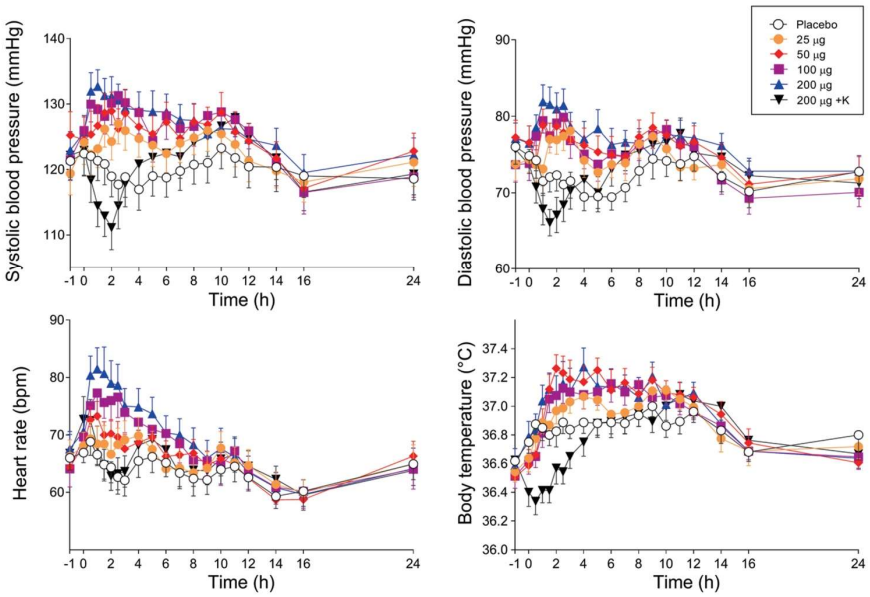

Fig. 3 Acute autonomic effects. Doses of 50, 100, and 200 µg lysergic acid diethylamide (LSD) similarly increased systolic blood pressure compared with placebo. The 100 and 200 µg doses similarly increased diastolic blood pressure and heart rate compared with placebo. Ketanserin (K) transiently decreased blood pressure, heart rate, and body temperature, with a delayed increase to the levels that were reached after the administration of LSD alone. LSD (25–200 µg) or placebo was administered at $t = 0$ h. Ketanserin (K) or placebo was administered at $t = -1$ h. The data are expressed as the mean ± SEM in 16 subjects. Maximal effects and statistics are shown in Supplementary Table S2.

Table 1. Pharmacokinetic parameters for different doses of LSD based on compartmental modeling.

	Dose (µg)		K_{01} (1/h)	λ_z (1/h)	V_z/F (L)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_∞ (ng h/mL)	CL/F (L/h)
LSD	25	Geometric mean (95% CI)	2.0 (1.3–3.2)	0.19 (0.16–0.24)	38 (30–47)	0.49 (0.41–0.58)	1.2 (0.9–1.7)	3.6 (2.9–4.4)	3.5 (2.7–4.5)	7.2 (5.6–9.3)
		Range	0.40–15.1	0.06–0.35	15–102	0.20–0.71	0.29–2.7	2.0–12	1.1–12	2.0–22
	50	Geometric mean (95% CI)	2.1 (1.5–3.0)	0.19 (0.16–0.23)	35 (31–38)	1.1 (0.99–1.2)	1.2 (0.95–1.6)	3.6 (3.0–4.2)	7.4 (6.2–8.9)	6.7 (5.6–8.0)
		Range	0.43–19.8	0.06–0.44	22–46	0.68–1.6	0.25–2.5	1.6–11	4.2–25	2.0–11.8
	100	Geometric mean (95% CI)	1.8 (1.4–2.4)	0.18 (0.15–0.21)	37 (33–42)	2.0 (1.9–2.2)	1.4 (1.2–1.7)	3.9 (3.2–4.7)	15 (12–18)	6.6 (5.4–8.0)
		Range	0.7–4.5	0.06–0.24	22–51	1.7–2.9	0.74–2.5	2.9–12	11–47	2.1–9.4
	200	Geometric mean (95% CI)	1.6 (1.2–2.1)	0.17 (0.14–0.20)	39 (35–43)	3.9 (3.5–4.3)	1.5 (1.3–1.9)	4.1 (3.4–4.9)	31 (25–38)	6.5 (5.3–8.0)
		Range	0.45–5.03	0.06–0.35	25–67	2.5–6.0	0.70–5.0	2.0–11	18–127	1.6–11
	200 + Ketanserin	Geometric mean (95% CI)	2.3 (1.4–3.8)	0.15 (0.13–0.18)	36 (32–40)	4.4 (4.0–4.8)	1.2 (0.85–1.8)	4.5 (3.8–5.3)	36 (30–43)	5.6 (4.6–6.8)
		Range	0.6–20.0	0.06–0.24	23–47	3.1–6.8	0.25–4.1	0.25–4.1	25–110	1.8–7.9

AUC_∞ area under the plasma concentration–time curve from time zero to infinity, CL/F apparent total clearance, C_{max} estimated maximum plasma concentration, $t_{1/2}$ estimated plasma elimination half-life, t_{max} estimated time to reach C_{max}, k_{01} first-order absorption koefficient, λ_z first-order elimination coefficient, V_z/F volume of distribution.

In a previous study, the 200 µg dose of LSD also produced higher ratings of good drug effects, bad drug effects, fear, open, and trust on the VAS compared with 100 µg [11]. There are two explanations for the absence of an LSD dose response for good drug effects in the present study compared with our previous studies. First, the true doses that were used in the previous studies were 60–70 and 150 µg rather than the reported 100 and 200 µg doses because of the use of an unstable formulation with a lower LSD content, as discussed elsewhere [20]. Second, the past comparison was between different subjects and studies [11, 26], whereas the present study used valid within-subject and within-study comparisons. In the present study, we observed a ceiling effect on the dose–response curve. Considering that the previously reported 200 µg dose likely contained only 150 µg of active LSD, additional positive effects may be reached with 150 µg compared with 100 µg. This possibility remains to be tested. One of our recent studies also used an analytically confirmed LSD dose of 100 µg, which produced scores on the VAS and 5D-ASC scale that were nominally higher than those that were reported after 100 µg administration in the present study [13] and more similar to the scores that were reported herein after 200 µg administration. Altogether, the available data support the view that mainly high acute positive effects of LSD can be induced at a 100 µg dose of LSD base. Therefore, we speculate that a dose of 100 µg of LSD may be selected for the treatment of depression or anxiety where higher Oceanic Boundlessness and lower anxiety ratings acutely induced by psychedelics predicted better treatment efficacy [16–19]. The 50 µg dose that was used in the present study also produced substantial positive mood effects and notably only very small and nonsignificant anxious ego dissolution, with no anxiety.

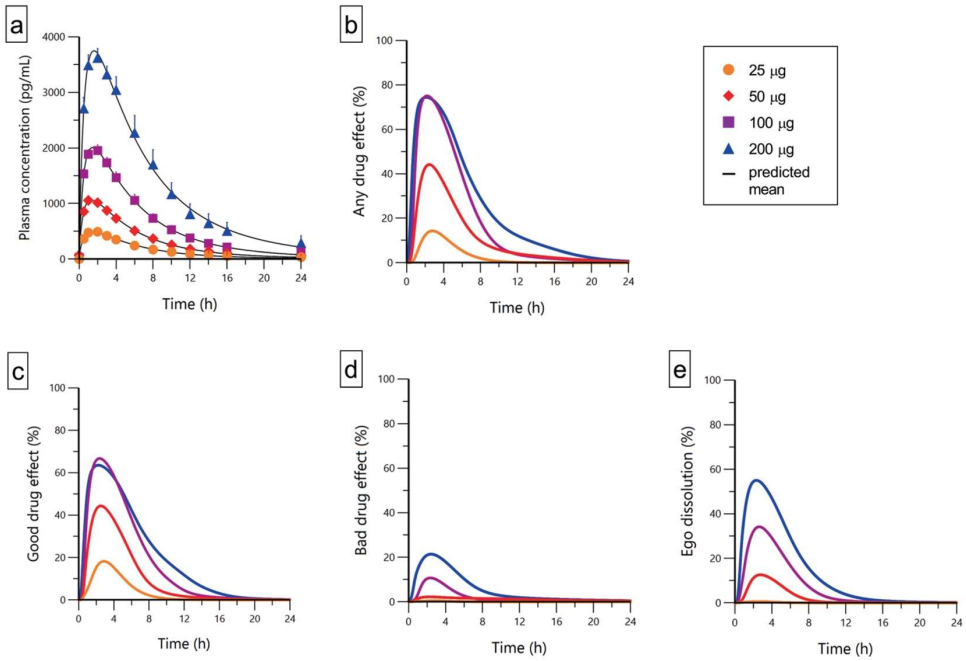

Fig. 4 Pharmacokinetics and subjective effects of lysergic acid diethylamide (LSD). a Plasma LSD concentration-time curves for 25, 50, 100, and 200 µg doses of LSD. **b–e** LSD effect-time curves for Visual Analog Scale ratings (0–100%) of (**b**) "any drug effect," (**c**) "good drug effect," (**d**) "bad drug effect," and (**e**) "ego dissolution." LSD administration resulted in dose-proportional increases in plasma concentrations of LSD, but subjective good drug effects reached a ceiling at the 100 µg dose and did not further increase at the 200 µg dose. In contrast, bad drug effects and ego dissolution increased further at the 200 µg dose compared with 100 µg. Therefore, LSD doses higher than 100 µg produced no further increases in good drug effects but more ego dissolution and anxiety. The data are expressed as the mean ± SEM in 16 subjects. LSD was administered at $t = 0$ h. The lines represent the means of the individual pharmacokinetic–pharmacodynamic (subjective effect) model predictions.

Thus, the 50 µg dose may be useful for inducing a moderately intense and predominantly positive psychedelic experience. This low psychedelic dose would likely be a good starting dose to be used in patients with no previous experience with psychedelics or in subjects who are considered to be more sensitive to the effects of psychedelics [33].

In the present study, LSD produced moderate elevations of arterial blood pressure and heart rate starting at the 50 µg dose that were largely similar to the effects of 100 and 200 µg. Similarly, previous studies that used pharmaceutically not well-characterized doses of 100 and 200 µg LSD found no difference in the acute cardiostimulant effects of these doses [11]. A previous study in patients did not observe any increases in blood pressure using a non-confirmed dose of 200 µg of LSD [34]. Methylenedioxymethamphetamine clearly has more pronounced cardiostimulant effects and a less favorable overall physical safety profile than LSD [13, 35]. In contrast, the psychotropic effects of LSD are significantly greater compared with MDMA [13].

In the present study, administration of the 5-HT_{2A} receptor antagonist ketanserin 1 h before LSD administration markedly reduced the subjective response to the 200 µg LSD dose to levels that were similar to the 25 µg dose. Retrospective reports showed that ketanserin and LSD together were identified correctly by the participants or mistaken as a low dose of LSD but never mistaken for a high dose of LSD. The present findings are consistent with a previous study in which ketanserin administration prior to the administration of 100 µg LSD almost completely prevented the acute effects of LSD [4]. These findings support the view that LSD primarily produces its acute psychedelic effects in humans via 5-HT_{2A} receptor activation [3–5, 36], which was also shown for a high and fully psychedelic dose of LSD. Ketanserin also prevented acute adverse effects of LSD and the LSD-induced heart rate response. However, the weak blood pressure-elevating effects of LSD were only transiently prevented by ketanserin and reappeared later during the LSD response. This observation is consistent with the relatively short half-life of ketanserin (i.e., 2 h) during the first 1–9 h following administration [37, 38].

In the present study, 200 µg LSD significantly increased BDNF plasma concentration compared with placebo with a peak at 6 h. Additionally, there were nonsignificant increases in plasma BDNF after lower doses of LSD or after ketanserin with LSD. In previous studies, 100 µg LSD had no effect on BDNF plasma levels [13] up to 5 h while the psychedelic ayahuasca increased BDNF at 2 days. Further, higher BDNF levels were associated with lower depression ratings after administration of ayahuasca [39]. More research is needed to define the time course of the BDNF response and whether there is a link between psychedelics, BDNF, and the antidepressant response [39].

In addition to providing dose–response data on full psychedelic doses of LSD, the present study further characterized the effects of small doses of LSD [9, 40]. The lowest dose that was used in the present study contained 25 µg of LSD base. This dose produced subjective "any drug effects" that were significantly different from placebo and retrospectively identified as LSD by the majority (>85%) of the participants. Very low doses of LSD have typically been referred to as "microdoses." Psychedelic microdoses have been postulated to have beneficial prolonged effects on mood while producing no or only minimal acute adverse subjective effects [40–43]. Positive long-term effects of psychedelic microdoses remain to be documented [42], and remaining unclear are the LSD doses that actually have no acute subjective effects and thus could be considered microdoses [40]. Very low to low doses of LSD were recently studied in two placebo-controlled trials [9, 21, 44, 45]. One study also provided preliminary PK data [45]. In older healthy volunteers, 5–20 µg of LSD tartrate produced small but significant linear dose-dependent increases in ratings of all of the following: subjective drug effects, vigilance reduction, dizziness, and changes in body feeling [21, 45]. The frequency of

adverse effects of LSD at doses up to 20 µg was not different from placebo. The mean plasma C_{max} values of LSD (non-compartmental analyses) were 0.44 ng/ml ($n = 8$) after the administration of 20 µg of LSD tartrate [45] and 0.51 ng/ml after the administration of 25 µg of LSD base in the present study, indicating comparable dose-proportional peak concentrations. The previous study included younger healthy subjects and found dose-dependent increases in subjective ratings of "feel drug" and "like drug" on VASs and on the 5D-ASC scale after the administration of 6.5, 13, and 26 µg of LSD tartrate [9]. Notably, a 26 µg dose of LSD tartrate would be lower than the 25 µg dose of LSD base (i.e., 31 µg of LSD tartrate equivalent) that was used in the present study. Nevertheless, the 26 µg dose of LSD tartrate produced significant effects on the 5D-ASC scale compared with placebo and nominally greater ratings on the 5D-ASC subscales than the 25 µg dose that was used in the present study. Unfortunately, no plasma LSD concentration data have been published for the 26 µg dose of LSD tartrate [9]. Therefore, a comparison of drug exposures between this previous study and the present study to further validate the dose comparison is not possible. Altogether, the available data from these controlled studies, including the present study that used very small and small doses of LSD, indicate that the 25 µg dose of LSD is clearly acutely psychoactive in the majority of subjects. Doses in the range of 21–30 µg of LSD base may thus be considered "minidoses" rather than "microdoses." Doses of LSD base in the 1–20 µg range may be considered "microdoses" but need further study. However, these doses may already elicit small dose-dependent subjective effects, although they are unlikely to relevantly impair cognition or produce adverse effects [9, 21, 44, 45].

Overall, the present dose–response study characterized a range of LSD doses. Based on the available data, the following dosing terminology may be useful for future LSD research: "microdose" (1–20 µg), "minidose" (21–30 µg), and "psychedelic dose" (>30 µg). Within the psychedelic LSD dose range, good effects likely predominate at doses of 30–100 µg (good-effect dose), whereas ego dissolution and anxiety increase at doses above 100 µg (ego-dissolution dose).

The present study has numerous strengths. Four different doses of LSD were used within subjects and compared with placebo under double-blind conditions in a controlled laboratory setting. A ketanserin-LSD condition was also included to elucidate the mechanism of action of LSD and enhance blinding between the different conditions. We also included equal numbers of male and female participants and used internationally established standardized and validated psychometric outcome measures. The doses of LSD were pharmaceutically well-characterized, and plasma LSD concentrations and PK parameters were determined up to 24 h for all doses. Notwithstanding these strengths, the present study also has limitations. The study used a highly controlled setting and included only healthy subjects. Additionally, participants willing to participate in LSD research are likely to have positive expectations and some participants had past substance experiences. Thus, subjects in different environments and patients with psychiatric disorders may respond differently to LSD.

CONCLUSION

We characterized the effects of LSD at different doses to support the dosing of LSD for research and LSD-assisted therapy. LSD exhibited dose-proportional PKs and first-order elimination. It produced significant dose-dependent subjective responses starting at the 25 µg dose. A ceiling effect was observed for good drug effects at the 100 µg dose. The 200 µg dose induced more ego dissolution but also more anxiety than the 100 µg dose. These results may assist with dose finding for future LSD research. Ketanserin almost completely prevented the response to the high (200 µg) dose of LSD, thus confirming the critical role of 5-HT_{2A} receptors in mediating psychedelic effects of LSD.

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AUTHOR CONTRIBUTIONS

FH, PV, PD, FM, and MEL designed the research. FH, UD, PV, LL, NV, FM, AE, MS, and SB performed the research. FH, UD, PV, and MEL analyzed the data. FH and MEL wrote the paper with input from all of the other authors. All authors gave final approval to the paper.

ADDITIONAL INFORMATION

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2.4. Pharmacokinetics and pharmacodynamics of lysergic acid diethylamide microdoses in healthy participants

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Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide Microdoses in Healthy Participants

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"Microdoses" of lysergic acid diethylamide (LSD) are used recreationally to enhance mood and cognition. Increasing interest has also been seen in developing LSD into a medication. Therefore, we performed a pharmacokinetic-pharmacodynamic study using very low doses of LSD. Single doses of LSD base (5, 10, and 20 µg) and placebo were administered in a double-blind, randomized, placebo-controlled crossover study in 23 healthy participants. Test days were separated by at least 5 days. Plasma levels of LSD and subjective effects were assessed up to 6 hours after administration. Pharmacokinetic parameters were determined using compartmental modeling. Concentration-subjective effect relationships were described using pharmacokinetic-pharmacodynamic modeling. Mean (95% confidence interval) maximal LSD concentrations were 151 pg/mL (127–181), 279 pg/mL (243–320), and 500 pg/mL (413–607) after 5, 10, and 20 µg LSD administration, respectively. Maximal concentrations were reached after 1.1 hours. The mean elimination half-life was 2.7 hours (1.5–6.2). The 5 µg dose of LSD elicited no significant acute subjective effects. The 10 µg dose of LSD significantly increased ratings of "under the influence" and "good drug effect" compared with placebo. These effects began an average of 1.1 hours after 10 µg LSD administration, peaked at 2.5 hours, and ended at 5.1 hours. The 20 µg dose of LSD significantly increased ratings of "under the influence," "good drug effects," and "bad drug effects." LSD concentrations dose-proportionally increased at doses as low as 5–20 µg and decreased with a half-life of 3 hours. The threshold dose of LSD base for psychotropic effects was 10 µg.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Microdosing of lysergic acid diethylamide (LSD) refers to the use of very small doses of LSD to enhance cognition and mood. Pharmacokinetic parameters for very low doses of LSD are lacking.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The pharmacokinetics and pharmacokinetic-pharmacodynamic relationship of LSD doses of 5, 10, and 20 µg were investigated in a double-blind, randomized, placebo-controlled crossover study in healthy participants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Participants began to perceive effects of LSD at a threshold dose of 10 µg. LSD had a half-life of 3 hours. Effects peaked at 1.5–2.5 hours and lasted 5 hours after LSD administration.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The present pharmacokinetic and acute effects data will support the design of further studies that use low-dose LSD in healthy subjects and patients.

Lysergic acid diethylamide (LSD) is a well-known classic serotonergic psychedelic that is widely used for recreational purposes.[1] LSD is well-absorbed,[2,3] and maximal LSD concentrations are reached 1.5–2 hours after oral administration.[4] LSD is mainly metabolized to inactive O-H-LSD, which is renally eliminated.[3] The plasma half-life of LSD is 3–4 hours.[3,4] Increasing interest has been seen in using LSD for the treatment of various disorders, including depression and anxiety,[5] substance use,[6] and cluster headache,[7] among others.[8] LSD "microdosing" has recently become popular.[9,10] The practice of microdosing refers to the use of very low doses of LSD that are taken at 2-to-5-day intervals to improve cognitive function and mood.[11–13] However, little is known about the effects of very low doses of LSD. More data are needed to

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determine specific doses that do not produce subjective effects.[14] A few controlled studies have used defined very low doses of LSD. One recent phase I trial randomly assigned older subjects (mean age: 63 years) 5, 10, and 20 μg LSD tartrate or placebo.[15,16] Only limited pharmacokinetic data, however, were obtained, thus precluding definitions of all pharmacokinetic parameters and pharmacokinetic-dynamic modeling and plasma levels of LSD could not be determined after 5 μg LSD administration because of low sensitivity of the analytical assay. Another controlled study administered LSD tartrate at doses of 6.5, 13, and 26 μg and placebo in a crossover design in four laboratory sessions in 20 healthy young adults.[17] Dose-dependent acute drug effects of LSD were reported. The same authors then showed that the 13 μg LSD tartrate dose increased functional connectivity of the amygdala with frontal brain areas, despite producing only weak effects on mood in 20 young healthy subjects.[18] However, no pharmacokinetic data were obtained in these latter two studies. Therefore, the aim of the present study was to assess the pharmacokinetics and acute effects of LSD and pharmacokinetic-effect relationships at doses of 5, 10, and 20 μg LSD base and placebo in 23 healthy subjects using a sensitive analytical method. Notably, 13 μg LSD tartrate contains 10 μg of LSD base.

METHODS

Study design

The present study used a double-blind, placebo-controlled, crossover design with four experimental 6-hour test sessions to investigate responses to placebo, 5 μg LSD, 10 μg LSD, and 20 μg LSD base. Twenty-four subjects were randomly assigned to 24 possible treatment sequences, counterbalancing all treatments. The washout periods between sessions were at least 5 days. The study was registered at the Dutch Clinical Trial register (no. NTR7102; www.trialregister.nl). Additional data from this study are published elsewhere.[19]

Participants

Participants were recruited from the University of Maastricht campus via advertisement, via social media, and by word of mouth. Only healthy participants who were between 18 and 40 years old, who had a body mass index between 18 and 28 kg/m^2, and who had at least one previous experience with a hallucinogen were included in the study. The exclusion criteria were the following: pregnancy (urine pregnancy test at screening and before each test session) or lactation, a personal history of drug addiction, current or a history of psychiatric disorders or family (first-degree relative) history of major psychiatric disorders, previous experience of serious side effects to psychedelic drugs (anxiety or panic attacks), chronic or acute physical illness (based on abnormal physical exam, electrocardiogram, or hematological and chemical blood analyses or hypertension > 140/90 mmHg), tobacco smoking (> 20 cigarettes/day), excessive drinking (> 20 alcoholic consumptions per week), illicit drug use within the last 3 months, and illicit drug use during the last 7 days prior to the study or during the study. A urine drug test and alcohol breath test were performed at screening and before each test session. No illicit substances were detected during the study. The participants were not allowed to drink alcohol or xanthine-containing liquids after midnight before the study day. Previously used hallucinogens included LSD (n = 12), psilocybin (n = 19), methylenedioxymethamphetamine (MDMA)/ecstasy (n = 15), *N*,*N*-dimethyltryptamine (n = 1), ketamine (n = 1), "2C drugs" (n = 3), and salvia (n = 1).

Study procedures

The study included a screening visit and four experimental sessions (test days). Experimental sessions began at 9:00 AM. An indwelling intravenous catheter was placed in an antecubital vein for blood sampling. A single oral dose of LSD or placebo was administered at 10:00 AM. Autonomic and subjective drug effects were assessed repeatedly throughout the session. Test sessions ended at 4:00 PM. For the analysis of LSD concentrations in plasma, blood samples were collected in lithium heparin tubes before and 0.5, 1, 1.5, 2, 3, 4, and 6 hours after drug administration. Timepoints were selected based on existing pharmacokinetic data on LSD.[4] Blood samples were centrifuged, and plasma was frozen at – 20°C until analysis.

Study drugs

LSD (D-lysergic acid diethylamide base, high-performance liquid chromatography purity > 99%; Lipomed AG, Arlesheim, Switzerland) was manufactured as an oral solution in units that contained 25 μg LSD in 1 mL of 96% ethanol.[4] Stability of the formulation for longer than the study period was documented as described elsewhere.[4] One microgram of LSD base that was used in the present study corresponded to approximately 1.23–1.33 μg of LSD tartrate (depending on the salt form and amount of crystal water), which is the form of LSD that is more likely to be used when acquired illegally (i.e., in blotter form) or was used in two recent studies that used very low doses.[15–17] However, absorption of LSD base likely takes place orally, while LSD base derived from LSD tartrate is likely absorbed when reaching the basic environment of the small intestine. To prepare doses of 5, 10, and 20 μg, 0.2, 0.4, and 0.8 mL of LSD solution, respectively, solution was diluted with ethanol (96% volume) to a final volume of 1 mL. Placebo consisted of 1 mL of ethanol (96% volume) only.

Measures

Analysis of LSD concentrations. Plasma LSD levels were analyzed by ultra-high-performance liquid chromatography tandem mass spectrometry as previously described in detail.[4] Pharmacokinetic samples with an LSD concentration below 5 pg/mL were reanalyzed by a different extraction procedure. Briefly, 150 μL aliquots of plasma were extracted with 450 μL of 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, volume/volume). The lower limit of quantification of 2.5 pg/mL was reached using this extraction method.

Subjective effects. Visual analog scales (VASs) were repeatedly used to assess subjective effects over time.[20–23] The VASs included separate measures for "under the influence" (any drug effect), "good drug effect," and "bad drug effect," and were presented as 10 cm horizontal lines (0–10), marked from "not at all" on the left to "extremely" on the right. These VASs have been shown to be sensitive and reliable measures of the effects of LSD and other psychoactive substances and suitable for pharmacokinetic-pharmacodynamic (PK-PD) analyses.[3,4,22,24–26] The VASs were administered before and 0.5, 1, 1.5, 2, 3, 4, and 6 hours after LSD administration and immediately after blood sampling to provide matched measures of LSD concentrations and effects for the PK-PD modeling.

Pharmacokinetic analyses and PK-PD modeling

A noncompartmental analysis was performed prior to compartmental modeling. Peak plasma concentration (C_{max}) and time to C_{max} were obtained directly from the observed data. The terminal elimination rate constant (λ_z) was estimated by log-linear regression after semilogarithmic transformation of the data using at least three data points of the terminal

linear phase of the concentration-time curve. The area under the concentration-time curve (AUC) from 0–6 hours after dosing (AUC_6) was calculated using the linear up log down method. The AUC to infinity (AUC_∞) was determined by extrapolation of the AUC_6 using λ_z.

Pharmacokinetic parameters were estimated using compartmental modeling in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ). A one-compartment model was applied with first-order input, first-order elimination, and no lag time as previously used to assess the pharmacokinetics of high doses of LSD.[4] Initial estimates for apparent volume of distribution and λ were derived from noncompartmental analyses. The model fit was not improved by a two-compartment model based on visual inspection of the plots and resulted in smaller Akaike information criterion values. The pharmacokinetic model was first fitted and evaluated. The predicted concentrations were then used as an input to the pharmacodynamic model by treating the pharmacokinetic parameters as fixed and using the classic PK/PD link model module in WinNonlin. Thus, the goal was to model the PD parameters using the PK parameters and the observed PD values. The model used a first-order equilibrium rate constant that related the observed pharmacodynamic effects of LSD to the estimated LSD concentrations at the effect site and accounted for the lag between the plasma and effect site concentration curves.[22,27] A sigmoid maximum effect (E_{max}) model (EC_{50}, E_{max}, γ) was selected for all pharmacodynamic effects. Half-maximal effects (EC_{50}) and E_{max} estimates were taken from the PK-PD plots.[4] Lower and upper limits for E_{max} were set to 0 and 10, respectively, for all of the VAS scores. The sigmoidal E_{max} model best described the relationship between estimated effect-site concentrations and LSD effects compared with a simple E_{max} model (plot inspection (**Figure S1**) and Akaike information criteria).

Statistical analyses

The VAS score data were analyzed using repeated-measures analysis of variance, with drug dose as the within-subjects factor (four levels), followed by Tukey *post hoc* comparisons. Scores measured repeatedly over time are expressed as peak (E_{max} and/or minimum effect (E_{min})) values prior to the analysis of variance (Statistica 12 software; StatSoft, Tulsa, OK). The criterion for significance was $P < 0.05$. The time to onset, time to C_{max}, time to offset, and effect duration were assessed for the model-predicted VAS "under the influence" ratings over time plots using a threshold of 25% of the maximum individual response to LSD using Phoenix WinNonlin 6.4.

RESULTS

Study sample

The final study sample included 23 participants (12 males and 11 females) who completed the study. The participants were (mean ± SD) 23 ± 3 years old (range: 19–29 years) with a mean body weight of 70 ± 10 kg (range: 55–87 kg).

Pharmacokinetics

Plasma concentrations of LSD were determined for the 5, 10, and 20 μg LSD doses in 13, 18, and 15 participants, respectively, for whom all blood samples per session could be collected for valid determination of the pharmacokinetic parameters. LSD could be quantified in all samples. The mean predicted and observed LSD concentrations are shown in **Figure 1a**. Individual predicted

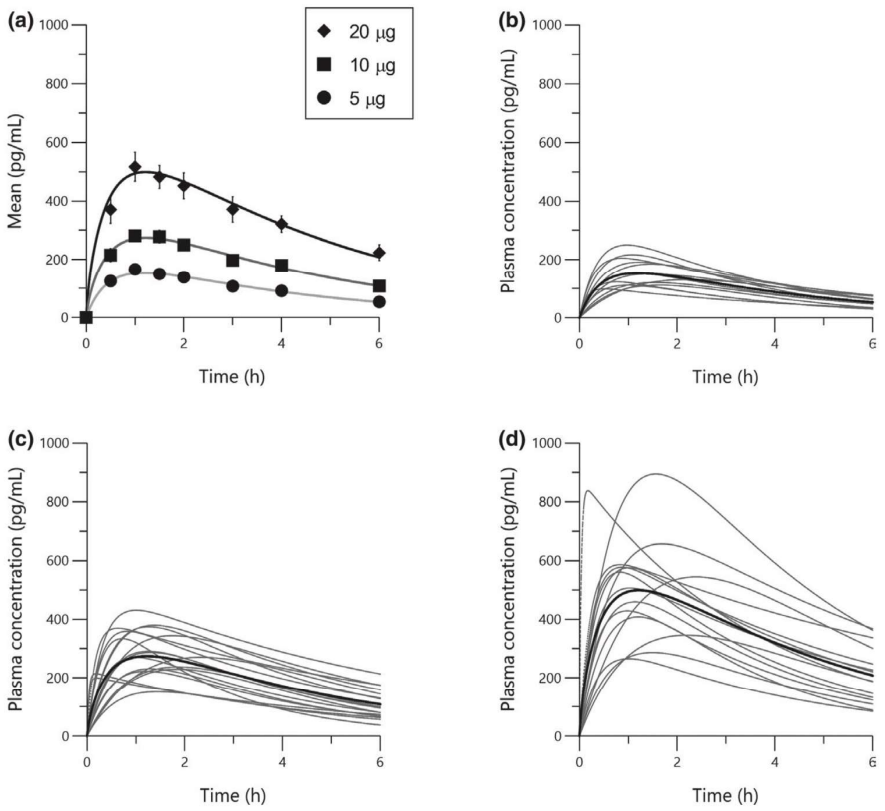

Figure 1 Pharmacokinetics of three very low doses of LSD, 5, 10, and 20 μg, in 13, 18, and 15 subjects, respectively. (**a**) Plasma LSD concentration-time curves representing the mean of the individual pharmacokinetic model predictions. The observed data are expressed as symbols and the mean ± SEM. Dose-linear increases in LSD concentrations were observed. (**b–d**) Predicted individual plasma LSD concentration-time curves shown separately for each subject and the mean marked in bold and illustrating the between-subject variability of LSD concentrations after the administration of (**b**) 5 μg, (**c**) 10 μg, and (**d**) 20 μg LSD. LSD was administered at $t = 0$ hour. h, hours; LSD, lysergic acid diethylamide.

concentrations after the administration of 5, 10, and 20 μg LSD are shown in **Figure 1b–d**, respectively and **Figures S4–S6**. **Figure 1b–d** also illustrate between-subjects variance in the concentrations of LSD for each dose. Coefficients of variation for C_{max} values (**Table 1**) were 30.3%, 28.1%, and 36% for the 5, 10, and 20 μg LSD doses, indicating overall moderate variance and greater variance at the highest dose. The corresponding pharmacokinetic parameters based on compartmental and noncompartmental analyses are shown in **Table 1** and **Table S1**, respectively. LSD concentrations increased proportionally with increasing doses. Elimination occurred according to first-order kinetics. Elimination half-lives were 2.5, 2.7, and 2.9 hours for the 5, 10, and 20 μg doses, respectively, as defined using compartmental analysis (**Table 1**).

Subjective effects

The PK-PD model-predicted subjective effect-time curves for VAS ratings of "under the influence," "good drug effect," and "bad drug effect" are shown in **Figures 2–4**, respectively. Individual predicted effect-time curves for each dose are shown in **Figures 2b,c**, **3b,c** and **4b,c**, respectively and **Figures S5–S13**.

The 5 μg dose of LSD produced no significant acute subjective effects compared with placebo (**Table S2**). The 10 μg dose of LSD significantly increased VAS ratings of "under the influence" and "good drug effect" (both $P < 0.05$, **Table S2**). Time to onset, time to offset, and effect duration of the subjective response were assessed by the VAS "under the influence" as a measure of the overall response to LSD for each dose (**Table 2**). For example, at the 10 μg dose of LSD, subjective effects began an average of 1.1 hours after administration, peaked at 2.5 hours, and ended at 5.1 hours, resulting in an effect duration of 4.0 hours (**Table 2**). The 20 μg dose of LSD significantly increased VAS ratings of "under the influence" ($P < 0.001$), "good drug effect" ($P < 0.001$), and "bad drug effect" ($P < 0.001$) (**Table S2**).

PK-PD modeling parameters are shown in **Table S3**. The predicted concentrations of LSD that produced half-maximal effects (EC_{50} values) were lower for good drug effects (mean ± SD = 0.86 ± 0.7 for 10 μg) compared with bad drug effects (1.6 ± 0.8 for 10 μg; **Table S3**).

Discussion

The present study comprehensively described the pharmacokinetics of low doses of LSD for the first time. Using a sensitive analytical method, full concentration-time curves could be established for the very low single dose of 5 μg LSD base. LSD administration at very low to low doses resulted in dose-proportional changes in plasma LSD concentrations. We also documented first-order elimination kinetics of LSD, confirming past studies that used high doses.[4,22] The average plasma elimination half-lives of LSD were 2.5, 2.7, and 2.9 hours according to the compartmental analysis and 3.0., 3.3, and 3.6 hours according to the noncompartmental analysis for the 5, 10, and 20 μg doses, respectively, and where the noncompartmental analysis may provide a better estimate of the terminal elimination half-life. These half-lives were consistent with the administration of high doses of LSD in four previous pharmacokinetic

Table 1 Pharmacokinetic parameters for different very small doses of LSD based on compartmental modeling

Dose (μg)		*N*	K_{01} (1/hour)	λ_z (1/hour)	V_z/F (L)	C_{max} (pg/mL)	t_{max} (hour)	$t_{1/2}$ (hour)	AUC_∞ (pg·h/mL)	CL/F (L/h)
LSD	5	Geometric mean (95% CI)	1.9 (1.3–2.9)	0.27 (0.23–0.32)	23 (19–28)	151 (127–181)	1.1 (0.91–1.4)	2.5 (2.2–1.4)	800 (658–968)	6.3 (5.2–7.6)
		Range	0.45–7.1	0.18–0.45	14–46	99–250	0.52–2.2	1.5–3.9	513–1,217	4.1–9.7
	10	Geometric mean (95% CI)	2.3 (1.4–4.0)	0.26 (0.21–0.30)	25 (21–30)	279 (243–320)	1.0 (0.71–1.4)	2.7 (2.3–3.2)	1,544 (1,273–1,872)	6.5 (5.3–7.9)
		Range	0.43–27	0.14–0.47	14–48	152–431	0.18–2.3	1.5–4.8	849–3,332	3.0–12
	20	Geometric mean (95% CI)	2.2 (1.3–3.7)	0.24 (0.20–0.29)	29 (23–36)	500 (413–607)	1.1 (0.77–1.5)	2.9 (2.4–3.5)	2,912 (2,271–3,734)	6.9 (5.4–8.8)
		Range	0.41–27	0.11–0.43	13–59	266–894	0.17–2.4	1.6–6.2	1,373–5,742	3.5–15

AUC_∞, area under the plasma concentration-time curve from time zero to infinity; CL/F apparent total clearance; C_{max}, estimated maximum plasma concentration; LSD, lysergic acid diethylamide; $t_{1/2}$, estimated plasma elimination half-life; t_{max}, estimated time to reach C_{max}; V_z/F, volume of distribution during terminal phase; k_{01}, first-order absorption koefficient; λ_z, first order elimination coefficient.

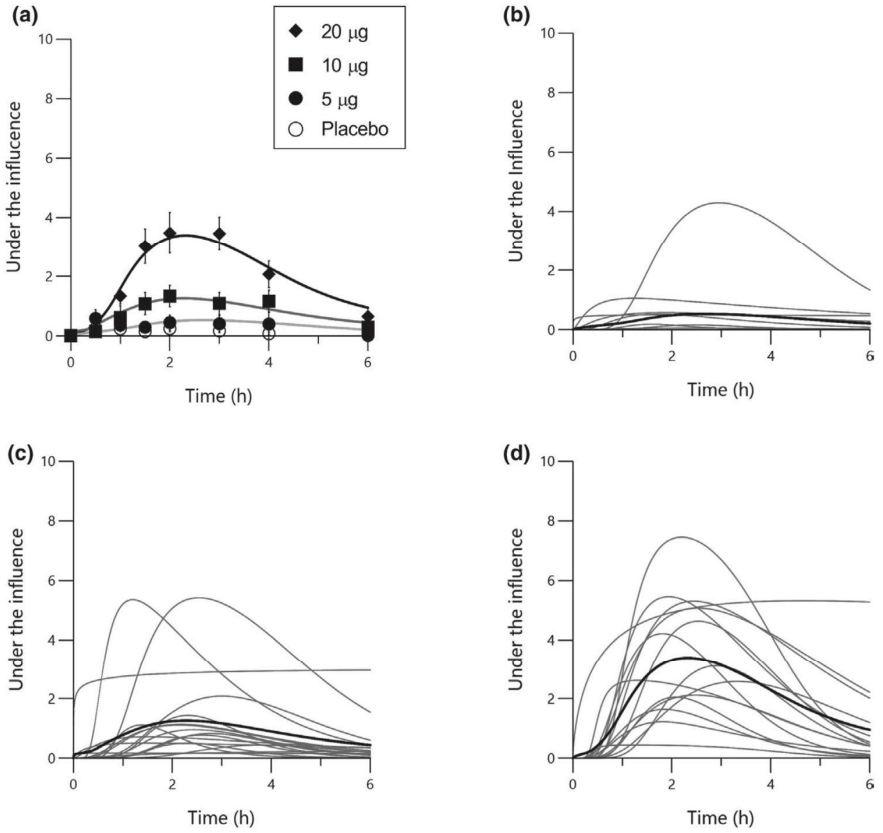

Figure 2 Subjective effects of LSD and placebo over time based on visual analog scale (VAS) ratings from 0–10 for "under the influence" for the 5, 10, and 20 µg LSD doses. (**a**) Effect-time curves represent the mean of the individual pharmacokinetic model predictions. The observed data are expressed as symbols and the mean ± SEM. (**b–d**) Individual LSD effect-time curves for (**b**) 5 µg, (**c**) 10 µg, and (**d**) 20 µg. Predicted individual effect-time curves are shown separately for each subject, and the mean is marked in bold to illustrate the between-subject variability of LSD effects after the administration of (**b**) 5 µg, (**c**) 10 µg, and (**d**) 20 µg LSD. LSD was administered at $t = 0$ hour. The corresponding maximal effect values are shown in **Table S2**. h, hours; LSD, lysergic acid diethylamide.

studies.[4,22,28] Importantly, the longer terminal half-life of 8.9 hours that was described in one preliminary study[3] could not be confirmed by any of the aforementioned studies, including the present study. The relatively short half-life of LSD of approximately 3 hours indicates that LSD does not accumulate in the body with repeated administration (e.g., during "microdosing" when small doses of LSD are used repeatedly), even when used at 24-hour intervals. Additionally, the plasma concentration-time curve of LSD is consistent with its within-subject effect-time curve as documented with the PK-PD modeling in this study for low doses, thus confirming the results with high doses.[4,22] The subjective effects of LSD relatively closely mirror LSD concentrations in healthy subjects. Psychotropic effects of LSD are generally present as long as LSD is present in the body. Accordingly, no acute tolerance occurs as with other psychoactive substances, such as MDMA, in which the drug is present in plasma in high concentrations for several hours beyond its acute psychoactive effects.[29] LSD concentrations and its effects are closely linked within subjects as evidenced by the good PK-PD model fit. Greater variance in the effects of LSD is observed between individuals. However, the variance in plasma concentrations between subjects at a given dose was surprisingly small in the present study, indicated by the coefficients of variation for the C_{max} values of 30–36%. Similar low variability in plasma has previously been reported with the same formulation of LSD base when used at high doses.[4] In contrast, higher variation was seen with older and less stable formulations that were used in older studies[3,22] and would be expected with non-controlled recreational products. This observation indicates that more consistent exposure to the drug is produced with the novel formulation of LSD used in the present and some recent studies,[4] which may then likely result in more consistent and predictable effects compared with past and less well-characterized pharmaceutical preparations. Using pharmaceutical formulations of LSD with confirmed content and stability and documenting consistent pharmacokinetic characteristics will be important for LSD research and the further development of LSD as a pharmaceutical product. We suggest that researchers use LSD formulations with known pharmacokinetic characteristics or obtain such data during their studies when using novel preparations to validate the doses that are used and allow reliable comparisons with other studies as discussed and suggested previously.[4,14,17]

Limited preliminary pharmacokinetic data on low-dose LSD tartrate administration have previously been published in older healthy volunteers (mean age: 63 years).[15] However, the use of an analytical

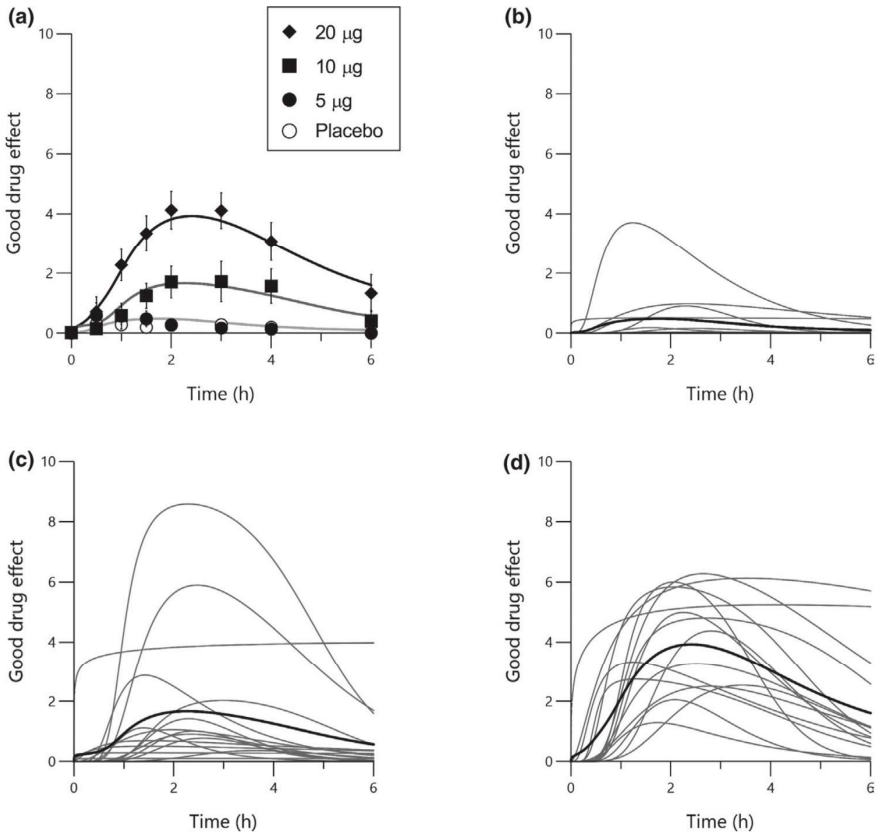

Figure 3 Subjective effects of LSD and placebo over time based on visual analog scale (VAS) ratings from 0–10 for "good drug effect" for the 5, 10, and 20 µg LSD doses. (**a**) Effect-time curves represent the mean of the individual pharmacokinetic model predictions. The observed data are expressed as symbols and the mean ± SEM. (**b–d**) Individual LSD effect-time curves for (**b**) 5 µg, (**c**) 10 µg, and (**d**) 20 µg. Predicted individual effect-time curves are shown separately for each subject, and the mean is marked in bold to illustrate the between-subject variability of LSD effects after the administration of (**b**) 5 µg, (**c**) 10 µg, and (**d**) 20 µg LSD. LSD was administered at $t = 0$ h. The corresponding maximal effect values are shown in **Table S2**. h, hours; LSD, lysergic acid diethylamide.

method with a lower limit of quantification of 200 pg/mL in that previous study compared with 2.5 pg/mL in the present study did not allow for the sensitive and valid quantification of very low LSD concentrations. We also took eight blood samples within the first critical 6 hours after LSD administration compared with five samples in the previous study. Altogether, only the sensitive analytical method and more frequent sampling allowed valid determinations of pharmacokinetic parameters of very low doses of LSD in the present study. Nevertheless, the present data and previously published C_{max} and AUC values for 10 and 20 µg LSD base equivalent doses are comparable.[15] Additionally, pharmacokinetic data for the 5 µg LSD base dose and half-lives are presented here for the first time.

In addition to being the first comprehensive description of the pharmacokinetics of LSD, we confirmed the results of a previous study of the subjective effects of 5–20 µg LSD microdoses. Specifically, the 5 µg dose of LSD base in the present study had no significant acute subjective effects in healthy young subjects, confirming the absence of relevant effects of an equivalent 6.5 µg dose of LSD tartrate.[17] The 10 µg dose of LSD base that was used in the present study induced subjective feelings of "under the influence" and "good drug effects," consistent with the increase in "feel good" at the drug peak effect that was seen with 13 µg LSD tartrate.[17] Interestingly, although having negligible subjective effects, 13 µg LSD tartrate (equivalent to 10 µg LSD base) has been shown to alter brain connectivity in the limbic system.[18] These data indicate that healthy subjects begin to subjectively perceive effects of LSD at a threshold dose of 10 µg LSD base. Thus, doses < 10 µg LSD base could be considered subperceptual and would qualify as "microdoses."[14] Doses of 10 µg LSD base could also likely be used safely in future studies that use multiple dosing and/or administration in patients when aiming at producing no acute perceptual effects.

The higher 20 µg dose of LSD base that was used in the present study and the equivalent 26 µg dose of LSD tartrate that was used previously[17] both induced weak but clearly significant subjective effects compared with placebo under double-blind controlled conditions.[17] These data indicate small but measurable psychedelic-like effects at a 20 µg LSD base equivalent.[17] Thus, 20 µg LSD base could be considered a very low to low psychedelic dose. Doses of 5–10 µg LSD appear to be without relevant subjective effects. However, requiring further investigation is how well the subjective effects of 10–25 µg doses are tolerated by participants in studies with less close monitoring after drug administration compared with the present study. The present study indicates that relevant alterations of the mind can be induced by 20 µg LSD in some study participants. Additionally,

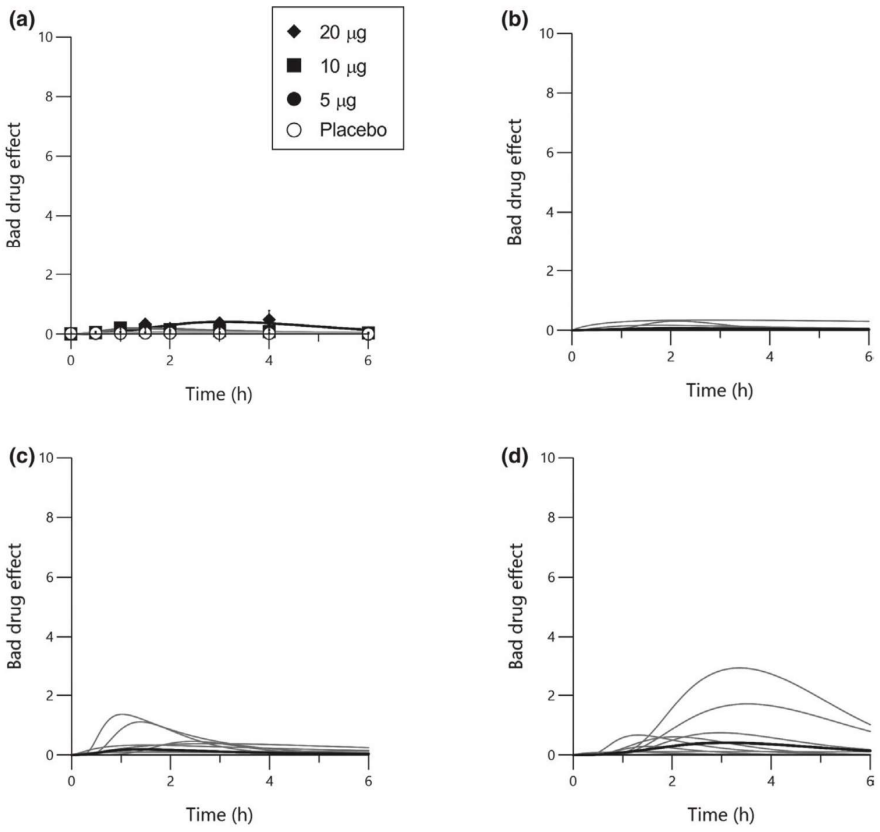

Figure 4 Subjective effects of LSD and placebo over time based on visual analog scale (VAS) ratings from 0–10 for "bad drug effect" for the 5, 10, and 20 µg LSD doses. (**a**) Effect-time curves represent the mean of the individual pharmacokinetic model predictions. The observed data are expressed as symbols and the mean ± SEM. (**b–d**) Individual LSD effect-time curves for (**b**) 5 µg, (**c**) 10 µg, and (**d**) 20 µg. Predicted individual effect-time curves are shown separately for each subject, and the mean is marked in bold to illustrate the between-subject variability of LSD effects after the administration of (**b**) 5 µg, (**c**) 10 µg, and (**d**) 20 µg LSD. LSD was administered at t = 0 hour. The corresponding maximal effects and statistics are shown in **Table S2**. h, hours; LSD, lysergic acid diethylamide.

the participants in the present study were healthy, and such responses may differ in clinical patients.

In the present study, we found that the subjective effects of 5–20 µg LSD base began an average of ~ 1 hour after administration, peak at 1.5–2.5 hours, and lasted 5 hours. Most of the subjects reported no subjective response to the 5 µg LSD base dose. The PK-PD modeling yielded lower EC_{50} values for "under the influence" and "good drug effect" compared with "bad drug effects," thus confirming previous studies that used 100 and 200 µg LSD[4,22] and indicating that bad drug effects are associated with higher LSD concentrations.

The present study has several strengths. Three different doses of LSD were used within subjects and compared with placebo under double-blind conditions in a controlled laboratory setting. Additionally, we used a very sensitive and validated analytical method.[4] The study included several assessments of the acute pharmacodynamics of LSD, which allowed the PK-PD modeling of different aspects of the acute subjective response to LSD. The present study also has important

Table 2 Characteristics of the subjective response ("under the influence") to different small doses of LSD

	5 µg LSD	10 µg LSD	20 µg LSD
Time to onset (hour)	0.71 ± 0.58 (0.25–1.6)[a]	1.1 ± 0.52 (0.35–2.3)[b]	0.85 ± 0.41 (0.10–1.7)
Time to offset (hour)	5.4 ± 0.57 (4.6–5.9)[a]	5.1 ± 0.94 (2.9–6.0)[b]	5.2 ± 0.62 (4.2–6.0)
Effect duration (hour)	4.7 ± 0.96 (3.7–5.6)[a]	4.0 ± 0.97 (2.3–5.6)[b]	4.3 ± 0.57 (3.2–5.0)
Time to maximal effect (hour)	1.5 ± 1.2 (0–3.7)	2.5 ± 1.6 (0–6)	2.3 ± 0.84 (1.2–4.6)
Maximal effect	0.57 ± 1.2 (0–4.3)	1.4 ± 1.6 (0–5.4)	3.6 ± 2.0 (0.44–7.5)
Area under effect-time curve	2.2 ± 4.1 (0–15)	4.9 ± 5.9 (0–19)	12 ± 8 (1.7–29)

Parameters are for the VAS "under the influence" as predicted by the pharmacokinetic-pharmacodynamic link model. The threshold to determine times to onset was set individually at 25% of the individual maximal response. Values are mean ± SD (range).
LSD, lysergic acid diethylamide; VAS, visual analog scale.
[a] For four subjects. Ratings of other subjects were too low to define onset and offset. [b] For 13 subjects. Ratings of other subjects were too low to define onset and offset.

limitations. The primary focus of the clinical trial was on psychological measures. Plasma samples could not be obtained from all 23 participants or for all doses because of technical problems. Therefore, the pharmacokinetic analyses included different total numbers of subjects for each dose and partly different subjects. Therefore, confirmative pharmacokinetic studies are needed with more comprehensive blood sampling. Furthermore, the present study used a standardized formulation with an exactly known content of LSD base, while recreational users use noncontrolled products containing mainly LSD tartrate. Thus, the present data may not reflect the PK of LSD when used recreationally.

In summary, we newly described the pharmacokinetics of three very low doses of LSD base in healthy subjects, provided PK-PD modeling data, and confirmed the previously reported dose-linear subjective effects of LSD.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

M.E.L. is a consultant for Mind Medicine, Inc., which had no role in funding or conduct of the present study. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

F.H. and M.E.L. wrote the manuscript. F.H., M.E.L., N.R.P.W.H., P.C.D., A.F., J.G.R., and K.P.C.K. designed the research. N.R.P.W.H., N.L.M., E.L.T., P.C.D., and U.D. performed the research. F.H. and M.E.L. analyzed the data.

ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University. The use of LSD in humans was authorized by the Dutch Drug Enforcement Administration.

INFORMED CONSENT

All of the subjects provided written consent before participating in the study and were paid for their participation.

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DISCUSSION, CONCLUSION & OUTLOOK

The scope of the present thesis describes comprehensively the acute effects and the pharmacokinetics of different doses of LSD in healthy participants, and shows that LSD exhibits clearly distinct effects compared with entactogens and stimulants. In detail, this thesis covers data from three clinical trials. One comparing a single dose of LSD to d-amphetamine and MDMA, one investigating the dose-effect relationship of LSD, and one investigating the pharmacokinetics and corresponding subjective effects of low to very low doses of LSD. The study findings are detailed in the published papers above. Here is a brief discussion of the whole work, a conclusion and an outlook.

Firstly, all mentioned studies used an oral LSD formulation that was developed according to pharmaceutical standards and in all studies plasma LSD concentrations as measures of exposure were determined. The pharmacokinetic parameters and properties of the 100 µg dose were described in detail in the first publication. The pharmacokinetic parameters that were derived from the study were generally similar to those in previous studies that used 100 and 200 µg in capsule form (Dolder et al. 2017a; Dolder et al. 2015), with the exception of higher C_{max} and AUC values. The average AUC∞ value, reflecting total LSD exposure, was 1.6-times greater with the oral drinking solution compared with a previous study that used the same indicated dose of 100 µg of LSD base formulated as a capsule (Dolder et al. 2017a). Therefore, the true LSD content of the LSD capsules used in previous studies may have been lower than reported. Maximum concentrations of LSD were reached an average of 1.5 h after administration, and first-order elimination kinetics of LSD were confirmed (Aghajanian and Bing 1964; Dolder et al. 2017a). This shows the importance of analytically confirmed and stable formulations. Many recent LSD studies (Gasser et al. 2015; Carhart-Harris et al. 2016c; Preller et al. 2017) have been published without information on the presence of LSD in the human body, and the actual exposure to LSD and exposure-time curves are unknown. In the LSD dose-response study and the microdosing study, plasma LSD concentrations of 5- 200 µg increased proportionally with increasing doses. We therefore provided the first comprehensive description of the pharmacokinetics of very low to low doses of LSD. Using a sensitive analytical method with a lower limit of quantification of 2.5 pg/ml, full concentration-time curves could be established for the very low single dose of 5 µg LSD base. Previous research used a lower limit of quantification of 200 pg/ml and was therefore not able to provide pharmacokinetic data for a dose as low as 5 µg LSD base (Family et al. 2020).

In the present studies, we found the onset of subjective effects of LSD base to be dose-dependent starting the earlier the higher the dose. That is, on average between 25 minutes and 1 hour. Subjective effects peaked around 2.5 hours for all doses and lasted dose-dependently between 4 and 11 hours on average. The pharmacokinetic-pharmacodynamic modeling yielded lower EC_{50} values for overall any subjective and good subjective effects compared with bad drug effects and anxiety, thus indicating that bad drug effects and anxiety are associated with higher LSD concentrations. Additionally, the plasma concentration-time curve of all LSD doses is consistent with its within-subject effect-time curve as documented with the pharmacokinetic-

pharmacodynamic modeling. Psychotropic effects of LSD are generally present as long as LSD is present in the body. Accordingly, no acute tolerance occurs as with other psychoactive substances such as MDMA, in which the drug is present in plasma in high concentrations for several hours beyond its acute psychoactive effects (Hysek et al. 2011).

Regarding microdoses, we provided the first comprehensive description of the pharmacokinetics of a LSD dose as low as of 5 µg LSD, and in addition we confirmed the results of a previous study of the subjective effects of 5-20 µg LSD. Specifically, the 5 µg dose of LSD base in the present study had no significant acute subjective effects in healthy young subjects, confirming the absence of relevant effects of an equivalent of 6.5 µg dose of LSD tartrate (Bershad et al. 2019). The 10 µg dose of LSD base that was used in the present study induced subjective peak drug feelings that was seen with 13 µg LSD tartrate (Bershad et al. 2019). Interestingly, although having negligible subjective effects, 13 µg LSD tartrate (equivalent to 10 µg LSD base) has been shown to alter brain connectivity in the limbic system (Bershad et al. 2020).

In terms of alterations in consciousness, LSD produced stronger and more distinct subjective effects compared with MDMA and d-amphetamine. Specifically, only LSD induced significant and marked alterations of consciousness compared with placebo, and responses were also significantly greater compared with MDMA and d-amphetamine. MDMA only moderately increased 'blissful state' on the 5D-ASC scale and 'positive mood' and 'ineffability' on the MEQ30. D-amphetamine only weakly increased 'positive mood' on the MEQ30 compared with placebo. Additionally, LSD produced greater overall subjective effects compared with both MDMA and d-amphetamine. Only LSD produced significant 'bad drug effects' and 'anxiety'. The present findings are overall consistent with previous reports on the effects of LSD (Schmid et al. 2015; Dolder et al. 2016; Carhart-Harris et al. 2016b; Preller et al. 2017), MDMA (Hysek et al. 2011; Dolder et al. 2018; Bershad et al. 2016), and d-amphetamine (Dolder et al. 2017b). Subjective effects of various substances can differ, depending on the comparator that is used. For example, marked effects of MDMA on the 5D-ASC scale compared with inactive placebo have been previously reported (Hysek et al. 2011). In contrast, LSD induced subjective effects that were largely similar to previous studies that used single dose levels (Dolder et al. 2016; Schmid et al. 2015; Liechti et al. 2017; Carhart-Harris et al. 2016b). Furthermore, a ceiling effect was reached at higher doses of LSD (> 100 µg) with regard to its positive subjective effects and any drug effects, with no difference in any and good drug effects between the 100 and 200 µg doses. However, the 200 µg dose of LSD produced significantly greater ego-dissolution and anxious ego-dissolution than the 100 µg dose. Additionally, only the 200 µg dose and not the 100 µg dose of LSD induced significant anxiety. LSD doses of 100 and 200 µg were both subjectively identified as high doses but could not be subjectively distinguished with certainty from each other. Both of these doses can clearly be considered full psychedelic doses and have previously been investigated in healthy subjects (Dolder et al. 2016; Schmid et al. 2015). No previous studies directly compared LSD doses of 100 and 200 µg. In contrast to the present findings, in one previous study moderately greater

effects of a 200 µg dose of LSD were reported (Schmid et al. 2015) compared with 100 µg in another study (Dolder et al. 2016). However, this was a study comparing different studies and subjects (Dolder et al. 2016; Liechti et al. 2017). Furthermore, the true LSD doses used in these studies were lower than reported, as discussed above. The first study comparing LSD, MDMA and d-amphetamine produced scores on the VAS and 5D-ASC scale that were nominally higher than those that were reported after 100 µg administration in the LSD dose response study and more similar to the scores that were reported herein after 200 µg administration. This available data supports the view that high acute mainly positive effects of LSD can be induced at a 100 µg dose of LSD base.

Overall, the present dose-response studies characterized a full range of LSD doses. Based on the available data, the following dosing terminology may be useful for future LSD research: 'microdose' (1-10 µg), 'minidose' (20-30 µg), and 'psychedelic dose' (> 30 µg). Within the psychedelic LSD dose range, good effects likely predominate at doses of 30-100 µg (good-effect dose), whereas ego-dissolution and anxiety increase at doses above 100 µg (ego-dissolution dose). However, the study using very low doses the data indicates that subjects begin to subjectively perceive effects of LSD at a threshold dose of 10 µg LSD base. Thus, doses of 10 µg LSD base could be considered the cut off for perceptual changes.

With the doses used, LSD, MDMA, and D-amphetamine produced comparable sympathomimetic activation, reflected by similar increases in the rate-pressure product, body temperature, and pupil size. These findings indicate that the doses of the drugs were similar with regard to sympathomimetic effects. The finding that LSD produced sympathomimetic effects confirmed previous studies (Dolder et al. 2016; Schmid et al. 2015) and contradicted the assumption that LSD does not increase blood pressure (Gasser et al. 2014). With respect to dose-response, LSD produced elevations of arterial blood pressure and heart rate starting at the 50 µg dose that were largely similar to the effects of 100 and 200 µg. Similarly, previous studies that used pharmaceutically not well characterized doses of 100 and 200 µg LSD found no difference in the acute cardiostimulant effects of these doses (Dolder et al. 2016).

In addition, administration of the 5-HT_{2A} receptor antagonist ketanserin 1 h before LSD administration markedly reduced the subjective response to the 200 µg LSD dose to levels that were similar to the 25 µg dose. These findings support the view that LSD primarily produces its acute psychedelic effects in humans via 5-HT_{2A} receptor activation (Preller et al. 2018; Barrett et al. 2018; Preller et al. 2017; Kraehenmann et al. 2017), which was also shown for a high and fully psychedelic dose of LSD. Ketanserin also prevented the acute LSD-induced heart rate response. However, the weak blood pressure-elevating effects of LSD were only transiently prevented by ketanserin and reappeared later during the LSD response. This observation is consistent with the relatively short half-life of ketanserin (i.e., 2 h) during the first 1-9 h following administration (Persson et al. 1991; Reimann et al. 1983).

BDNF plasma concentration was significantly increased by 200 µg LSD compared with placebo with a peak at 6 h. Additionally, there were non-significant increases in plasma BDNF after lower doses of LSD or after ketanserin with LSD. In the LSD, MDMA, d-amphetamine comparison study 100 µg LSD had no effect on BDNF plasma levels, nor did MDMA and d-amphetamine. These observations suggest a possible dose-dependent effect regarding the BDNF increase induced by LSD. Previous studies using the psychedelic ayahuasca showed increases in BDNF at two days (de Almeida et al. 2019). Overall, more research is needed to define the time course of the BDNF response and whether there is a link between psychedelics and BDNF, and the potential antidepressant response (de Almeida et al. 2019).

As predicted, MDMA increased plasma oxytocin concentrations, which is thought to be attributable to the MDMA-induced release of serotonin and 5-HT_{1A} receptor stimulation (Thompson et al. 2007). Interestingly, a dose of 100 µg of the potent 5-HT_{1A} and 5-HT_{2A} receptor agonist LSD (Rickli et al. 2016) did not significantly increase plasma oxytocin levels in the present study, in contrast to a higher dose of LSD (200 µg) and inactive placebo as the comparator in a previous study (Schmid et al. 2015). Taken together, this could imply a dose-dependency regarding the oxytocin release stimulation by LSD.

The present findings also have clinical implications. First, acute effects of the serotonergic hallucinogen psilocybin on both the 5D-ASC scale and the MEQ have been shown to predict long-term therapeutic outcomes in patients with anxiety and depression in previous studies (Griffiths et al. 2016; Ross et al. 2016; Roseman et al. 2017). Similarly, 5D-ASC scale and MEQ ratings correlated with changes in well-being and life satisfaction 1 year after LSD administration in healthy subjects in a previous study (Schmid and Liechti 2018). Thus, stronger acute responses to LSD on the 5D-ASC scale and MEQ, as documented in the present studies in healthy participants and previously in patients (Liechti et al. 2017), may also predict better therapeutic outcomes in studies that evaluate the benefits of LSD-assisted psychotherapy. However, this assumption needs to be verified in patients. Therefore, we speculate that a dose of 100 µg of LSD may be selected for the treatment of depression or anxiety where higher 'Oceanic Boundlessness' and lower anxiety ratings are acutely induced which are associated with better therapeutic outcomes in patients with treatment-resistant depression (Roseman et al. 2017).

One of the present studies found that MDMA produced some qualitatively similar (although less pronounced) positive effects compared with LSD but with lower associated 'bad drug effects' and anxiety. Thus, MDMA may produce less untoward effects than LSD, and this may favor its use in patients afraid to take LSD or at risk of adverse psychological reaction (i.e., high neuroticism, high emotional lability, and young age (Studerus et al. 2012)). In fact, MDMA is often used prior to LSD in substance-assisted psychotherapy in Switzerland so that patients can familiarize themselves with substance-induced states (Gasser 1996; Oehen et al. 2013; Gasser et al. 2015; Schmid et al. 2020). The 50 µg dose that was used in one of the present studies also

produced substantial positive mood effects and notably only very small and nonsignificant anxious ego-dissolution, with no anxiety. Thus, the 50 µg dose may be useful for inducing a moderately intense and predominantly positive psychedelic experience. This low psychedelic dose would also likely be a good starting dose to be used in patients with no previous experience with psychedelics.

The present thesis has numerous strengths. Overall, seven different doses of LSD were used and compared with placebo under double-blind conditions in a controlled laboratory setting. A ketanserin-LSD condition was also included to elucidate the mechanism of action of LSD. In all studies, equal numbers of male and female participants were included and internationally established standardized and validated psychometric outcome measures were used. The doses of LSD were pharmaceutically well-characterized, and plasma LSD concentrations and PK parameters were determined for all doses. Additionally, we used a very sensitive and validated analytical method. The studies included several assessments of the acute pharmacodynamics of LSD, which also allowed the pharmacokinetic-pharmacodynamic modeling of different aspects of the acute subjective response to LSD.

Notwithstanding these strengths, the present thesis also has limitations. All studies used a highly controlled setting and included only healthy subjects. Additionally, participants willing to participate in LSD research are likely to have positive expectations and some participants had past, but limited, substance experiences. Furthermore, the studies were conducted in a one-to-one setting, whereas therapeutic interventions may take place in group-settings (Schmid et al. 2020). Thus, subjects in different environments and patients with psychiatric disorders may respond differently to LSD.

Conclusion & Outlook

This thesis focused on the pharmacology of LSD in comparison to entactogens and stimulants. Summarized, we comprehensively described the pharmacokinetics of doses of 5-200 µg LSD, we established pharmacokinetic-pharmacodynamic relationships for all tested doses, and we distinguished subjective effects of LSD from MDMA and d-amphetamine. Furthermore, a comprehensive dose-effect relationship of LSD was described yielding in a ceiling effect at 100 µg LSD for any and good drug effects, suggesting this dose to be optimal for therapeutic use. Regarding the pharmacokinetic properties, we suggest that researchers use LSD formulations with known pharmacokinetic characteristics or obtain such data during their studies when using novel preparations to validate the doses that are used and allow reliable comparisons with other studies (Bershad et al. 2019; Kuypers et al. 2019). Additionally, the possibility of different salt forms of LSD such as base and tartrate should be noted. One µg of LSD base corresponds therefore to approximately 1.46 µg LSD tartrate.

There are several further comparisons that also have to be investigated. For example, LSD and psilocybin are investigated as potential treatment for the same indications, but so far, no modern study directly investigated the differences and similarities of both substances using well validated and established tools. The same accounts for other classic psychedelics such as DMT and mescaline.

In the coming years, three trials using the newly developed oral LSD solution (ClinicalTrials.gov no. NCT03866252 NCT03153579, NCT0378112) will end, and provide the first data on the effectiveness of LSD for the treatment of anxiety, depression, and cluster headache. This will be important milestones for LSD research. Therefore, using pharmaceutical formulations of LSD with confirmed content and stability and documenting consistent pharmacokinetic characteristics will be important for LSD research and the further development of LSD as a pharmaceutical product.

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APPENDIX

3.1. Curriculum Vitae

Personal Information

Name	Holze
First name	Friederike Sophie
Address	Klosiweg 13 3904 Naters
Nationality	Swiss
Date of birth	28th September 1990
Mobile	+41 76 471 84 11
E-Mail	f_holze@msn.com
OrcID iD	orcid.org/0000-0003-3143-1519

Education

09/2016	**Swiss Federal Diploma in Pharmacy** (GLN: 7601003883466)
08/2014 – 08/2016	**Master of Science in Pharmacy**, University of Basel
	Masterthesis, Pharmaceutical science, Clinical pharmacology and toxicology, University of Basel: *„The effects of alcohol (beer) on social cognition"*
08/2011 – 07/2014	**Bachelor of Science in Pharmaceutical Sciences**, University of Basel
08/2008 – 06/2011	**Matura**, Secondary school: Gymnasium Spiritus Sanctus, Brig, VS, Focus: Biology and Chemistry
08/2007 – 06/2008	EF High School Year in Slayton, Minnesota (USA)
08/2005 – 06/2007	Secondary school: Gymnasium Spiritus Sanctus, Brig, VS

Employment history

09/2017 – 05/2021	**PhD student in Medical Sciences**, Clinical pharmacology and toxicology, Psychopharmacology, University of Basel, University Hospital Basel, Basel, Switzerland. The objective of the PhD project is the pharmacokinetics and pharmacodynamics of LSD compared with stimulants in healthy subjects. The supervision have Prof. Matthias Liechti and Prof. Stephan Kraehenbuehl.
10/2016 – 06/2017	**Pharmacist** at production non-sterile, Kantonsapotheke Zürich
08/2015 – 06/2016	**Pharmacist-in-training** at Greifen Apotheke, Basel

Institutional responsibilities

Planning, conducting, organizing, and support of clinical studies.
Training and introduction of new PhD students and interns.

Supervision of students/junior researchers

01/2021 – now	Master's thesis in Pharmacy of Marc Roder (University of Basel)
08/2020 – now	Master's thesis in Medicine of Isidora Avedisian (University of Basel)
01/2020 – 06/2020	Master's thesis in Pharmacy of Shabnam Khan (University of Basel)
09/2019 –12/2020	Master's thesis in Medicine of Sebastian Silva (University of Basel)
08/2019 –02/2020	Internship and Bachelor's thesis of Vanja Toedtli (Zurich University of Applied Science)
01/2019 – 06/2019	Master's thesis in Pharmacy of Melanie Stocker (University of Basel)
10/2018 – 10/2019	MD thesis of Toya Caluori (University of Basel)
01/2018 – 04/2018	Internship of Robert Widmer (University of Basel)
02/2018 – 12/2018	Civilian service including MD thesis of Raoul Dürig (University of Basel)

Awards

09/2019	**Young Researchers Award** of International Conference of the European Foundation for Psychedelic Science (Mind Foundation), Insight Conference 2019, Berlin, Germany Oral presentation about the *Direct within-subject comparison of the acute subjective and autonomic effects of LSD, MDMA, and amphetamine in healthy subjects*
05/2019	**Runner Up – Young Investigator Award** of International Congress of the 39th European Association of Poison Centers and Clinical Toxicologists (EAPCCT) Congress 2019, Napoli, Italy Oral presentation about the *Pharmacokinetics of an oral LSD solution in healthy subjects*

Personal skills

Language skills

German	native
English	fluent skills in writing and conversation
French	basics
Italian	basics

Digital skills

Microsoft Office	Excel, Word, PowerPoint
Statistic-Software	R, GraphPad PRISM, Statistica

Clinical courses

Good Clinical Practice	Basics and Advanced (University Hospital Basel)

3.2. Publication List

Publications in peer-reviewed scientific journals

Müller F, **Holze F**, Dolder P, Ley L, Vizeli P, Soltermann A, Liechti ME, Borgwardt S. *MDMA-induced changes in within-network connectivity contradict the specificity of these alterations for the effects of serotonergic hallucinogens*. Neuropsychopharmacology. **2020** Nov 20. doi: 10.1038/s41386-020-00906-2

Müller F, Mühlhauser M, **Holze F**, Lang UE, Walter M, Liechti ME, Borgwardt S. *Treatment of a complex personality disorder using repeated doses of LSD-A case report on significant improvements in the absence of acute drug effects*. Front Psychiatry. **2020** Oct 22;11:573953. doi: 10.3389/fpsyt.2020.573953

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Ramaekers JG, Hutten N, Mason NL, Dolder P, Theunissen EL, **Holze F**, Liechti ME, Feilding A, Kuypers KP. *A low dose of lysergic acid diethylamide decreases pain perception in healthy volunteers*. J Psychopharmacol. **2020** Aug 25:26988112094093

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Steuer AE, Kaelin D, Boxler MI, Eisenbeiss L, **Holze F**, Vizeli P, Czerwinska J, Dargan PI, Abbate V, Liechti ME, Kraemer T. *Comparative Untargeted Metabolomics Analysis of the Psychostimulants 3,4-Methylenedioxy-Methamphetamine (MDMA), Amphetamine, and the*

Novel Psychoactive Substance Mephedrone after Controlled Drug Administration to Humans. Metabolites **2020** Jul 27;10(8):306

Holze F, Vizeli P, Müller F, Ley L, Dürig R, Varghese N, Eckert A, Borgwardt, Liechti ME. *Distinct acute effects of LSD, MDMA, and D-amphetamine in healthy subjects*. Neuropsychopharmacology **2020** Feb; 45(3): 462–471

Holze F, Duthaler U, Vizeli P, Müller F, Borgwardt, Liechti ME. *Pharmacokinetics and subjective effects of a novel oral LSD formulation in healthy subjects*. Br. J. Clin. Pharmacol **2019**;85(7):1474-83.

Dolder PC, **Holze F**, Liakoni E, Harder S, Schmid Y, Liechti ME. *Alcohol acutely enhances decoding of positive emotions and emotional concern for positive stimuli and facilitates the viewing of sexual images*. Psychopharmacology (Berl). **2017** Jan;234(1):41-51.

Peer-reviewed conference proceedings

Holze F, Duthaler U, Vizeli P, Liechti ME. *Pharmacokinetics of an oral lysergic acid diethylamide (LSD) solution in healthy subjects*. Clin Toxicol, **2019**. 57(6), 531-531.

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