SAFETY PHARMACOLOGY AND PHARMACOGENETICS OF 3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA)

Inauguraldissertation

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

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aus Basel (BS)

2020

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag von

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Basel, den 18.02.2020

Prof. Dr. Martin Spiess
Dekan der Philosophisch-Naturwissenschaftlichen Fakultät

—

“It is astounding how little we know our brains, but we live in them.”

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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.

ACKNOWLEDGEMENTS

Right at the beginning I want to take the opportunity to express my gratitude to all those who have made this thesis possible. During my PhD thesis I had the pleasure to meet and work with many great people. Not only as co-worker or subjects in clinical trials, but as friends. The joy of coming to your workplace lays not in work, but in the team you work with. I am very grateful to all who have accompanied me during this fascinating time as mentors and supervisors, colleagues, friends, and family.

My first thank goes to Prof. Dr. med. Matthias Liechti. As head of the psychopharmacology group he created a highly appreciated stimulating but relaxed environment. As a mentor he taught me a lot, sometimes even about the topic of this thesis. Thank you, Matthias, for letting me climb high and free, but always holding the ropes.

I would also like to thank Prof. Dr. Stephan Krähenbühl for his support and helpful remarks as well as for taking the role of the faculty representative. I am also very grateful to Prof. Dr. Jörg Huwyler who has kindly offered to complete my dissertation committee as co-referee.

A very big thank goes to Patrick Dolder for a very appreciated friendship as well as sharing and optimizing the best “office”. Your support in the beginning of my PhD was superb and helped me a lot to get everything up and high-flying.

My warmest gratitude goes to Friederike Holze. Friede, thank you for the great time as office partner and friend. It was a pleasure to play in the same team and it is the biggest drop of bitterness to leave this office and join another without you.

Many thanks go to Laura Ley, for always give me a break and sharing her different points of view that led to endless discussions and learning sessions. I enjoyed it very much.

Furthermore, it has been my utmost pleasure to get acquainted with the finest ladies and gentlemen who ever walked through the halls of the DBM – Deborah Rudin, Karolina Kolaczynska, Gerda Sanvee, Noemi Roos, Anna Rickli, Bea Vetter, Dr. Dino Lüthi, Urs Duthaler, Fabio Bachmann, Riccardo Mancuso, and Milijenko Panajatovic. We had a lot of fun partying, zebraing, playing cards, skiing, and get entangled in engaging discussions. I will miss the trips to the lab, but also your trips in/to the ASZ.

Special thanks go to Cedric Hysek for getting the swell going with all previous clinical studies with MDMA, on whose data I could learn to surf.

Further, our clinical studies would not have been possible without the wonderful support of Silke Purschke, Vanessa Grassedonio, Claudia Bläsi, Astrid Roesler, Claudia Sünderhauf, Tanja Grandinetti, Florian Pfefferkorn, and Joyce Santos de Jesus. I owe you all a great debt of gratitude.

A thank in a special rhombus form goes to Petra Strajhar. Furthermore, I want to specially thank Felix Hammann for his valuable stock market lessons and five minutes of his precious time.

I also very much appreciated the lead of Felix Müller. As our study psychiatrist he took good care of our subjects but more importantly of us.

I would also like to thank various people who kept the daily live going and interesting: Anne Leuppi-Taegtmeyer, Manuel Haschke, Alexandra Rätz-Bravo, Annette Harings-Kaim, Barbara Zimmermanns, and of course Evelyne Rudin.

I want to also express my gratitude to all with whom I could form collaborations for various projects: Prof. Victoria Risbrough, Dr. Richard (Pete) Sharp, Prof. Henriette Meyer zu Schwabedissen, and Dr. Erich Studerus.

An enormous thank goes to the motivated interns, and pharmacy and medical students, Florian Hirt, Isabelle Straumann, Toya Caluori, Samuel Harder, Robert Widmer, Cedric Stoll, Raoul Düring, and Vanja Toedtli for their invaluable work which I happened to have the pleasure of supervising.

My very personal thanks go to my friends and family. Especially, to my parents Andreas and Marianne, and my sister Fabienne for their endless garing support and encouragement. A special thank goes also to Jonas Grüter and Dario Grenacher for their help in elaborating many practical problem solutions as well as for the stimulating discussions.

My greatest and heartfelt gratitude goes to Yasmin Schmid for her complete confidence in me, her patience, guidance, and dedication. It was much needed and infinitely appreciated.

Thank-you!

CONTRIBUTIONS

I contributed as lead author to the publications presented in this thesis with the exception of the CYP2D6 pharmacogenetics of MDMA. However, I included the CYP2D6 publication for the sake of completeness and because I contributed with genotyping and substantial analysis. For the other projects, I took part in the planning, analyzed the data and performed the genotyping together with master students under my supervision. The genotyping was conducted in collaboration with the biopharmacy group of the University of Basel. I analyzed pooled data from clinical studies with MDMA conducted at the University Hospital Basel by my supervisor Prof. Matthias Liechti with the help of the psychopharmacology team.

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SUMMARY

Psychoactive substances such as the ring substituted phenylethylamine 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”) are widely used in recreational settings. Additionally, recent research highlights substance-assisted psychotherapy as potential new effective treatment for various psychiatric disorders, e.g. post-traumatic stress disorder (PTSD). MDMA releases and inhibits the uptake of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) via an interaction with the respective monoamine transporter. Additionally, MDMA increases blood levels of the hormone oxytocin. Through these mechanisms, MDMA produces autonomic and distinct psychological effects such as increased empathy and sociability - effects that may prove to be helpful in psychotherapy. Despite the widespread recreational use and growing interest in using MDMA for medical purposes, interindividual differences in the response to MDMA are not elucidated. Genetic variants, such as single-nucleotide polymorphisms (SNPs) may influence the individual effects of MDMA. To address this matter, we used a uniquely large population of up to 166 subjects assembled from pooled but highly standardized phase I MDMA studies and conducted in-depth analyses on the clinical safety and on the influence of different genetic variations on the effects of MDMA.

The first part of the present thesis was to evaluate the clinical safety pharmacology of single-dose administrations of 75 or 125 mg of MDMA. In up to a third of the subjects, administration of MDMA showed notable increases in maximum systolic blood pressure (>160 mmHg), heart rate (>100 bpm), and body temperature (>38 °C). Those effects on autonomic measures were significantly greater in subjects receiving 125 mg of MDMA. Acute and subacute adverse reactions such as headache, bruxism or lack of appetite were also dose-dependent and more frequent in women than men. However, no extreme outliers were observed, and the use of MDMA was considered as safe in controlled clinical settings. Nevertheless, we suggest a lower therapeutic dose for women. Due to the sympathomimetic stimulation, risks of MDMA might be higher in patients with cardiovascular diseases and should be further investigated in psychiatric patients with comorbidities.

In the second part of this thesis focus was laid on the identification of pharmacogenetic roles in the effects of MDMA. Specifically, the influence of genetic variants within genes coding for relevant cytochromes P450 (CYPs), and pharmacodynamic targets such as the 5-HT, NE, and DA system, and oxytocin receptors, on the response to MDMA was tested. We found that CYP2D6 poor metabolizers (PMs) exhibited increased plasma levels of MDMA, leading to accelerated cardiovascular and psychostimulant responses to acute MDMA administration. Polymorphisms in CYP2D6, CYP1A2, CYP2C19, and CYP2B6 altered the metabolism of MDMA to 3,4-methylenedioxyamphetamine (MDA), but showed no clinical relevance. In additional analyses, moderating effects for MDMA-induced feelings of trust and desire for company between variations of an oxytocin receptor single nucleotide polymorphism (SNP OXTR rs1042778) were shown.

To our knowledge, investigations assessing the influence of the monoamine system gene variations on the effects of MDMA were mostly the first on this matter. Subsequently, results had to be rigorously corrected for statistical errors and tested for specific hypothesis. Most of the tested genetic polymorphisms in the 5-HT (7 SNPs and 1 repeat polymorphism), NE (5 SNPs), and DA (10 SNPs and 1 repeat polymorphism) systems did not alter the effects of MDMA when adjusting for multiple comparisons. Only SNPs in the NE transporter gene SLC6A2 (rs1861647, rs2242446, and rs36029) significantly altered the acute MDMA-induced cardiovascular response.

In summary, apart from variations within CYPs, genetic polymorphisms seem to play a subordinate role in the acute MDMA effects and are unlikely to sum up all interindividual variations.

Results from the present thesis showed that MDMA was overall safe and well-tolerated with only moderate adverse effects in a clinical setting. Furthermore, pharmacogenetic analysis highlighted possible relevant genetic variations for the pharmacokinetic and pharmacodynamics effects of MDMA and point out targets of interest, which can define the scope of future studies with MDMA.

INTRODUCTION

1.1. History & Classification of MDMA

The synthetic amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) is commonly known as the main compound in the recreational drug “ecstasy”. According to the United Nations drug report, up to 40 million people between 15 – 64 years had used “ecstasy” in the past year worldwide. Prevalence rates are especially high in Australia, North America, and Western and Central Europe with 2.2%, 0.9%, and 0.9%, respectively (United Nations Office On Drugs And Crime, 2019). In 2016, a study in Switzerland showed that Swiss people over 15 years had a life time prevalence of “ecstasy” use of 3.9% with a maximum of 9.7% in the population between 25-34 years (Gmel G., 2017).

MDMA was first synthesized 1912 and patented 1914 by the pharmaceutical company Merck. It was issued as an appetite suppressant but never released commercially (Green et al., 1995; Benzenhofer and Passie, 2006). In the 1950ies it was shortly tested by the US military as “truth serum”, before the substance was introduced in psychotherapy in the late 1970ies (Benzenhofer and Passie, 2006). MDMA effects are known on one side to reduce fear, negative affect, and defensiveness, and on the other side to promote relaxation, emotional sensitivity, and empathy (Greer and Strassman, 1985; Grinspoon and Bakalar, 1986; Shulgin, 1986; Doblin, 2002). Psychotherapists saw a great potential in these facilitating effects for therapeutic communication. The effects of MDMA were clearly distinguished from the stimulant class of the amphetamines and therefore classified as a new psychoactive substance class called the “entactogens” (Nichols, 1986). MDMA was not limited to the therapeutic setting and gained popularity under the name “ecstasy” in the new up-coming “rave” movement (Schwartz and Miller, 1997). However, the US government declared MDMA a drug of abuse and it was thereafter banned from most of the United Nations members in 1985 (Saunders and Walder, 1994). Despite the global ban, researchers and psychiatrist in Switzerland were periodically still permitted to use illegal substances under certain strict circumstances. In recent years, the research with MDMA and its therapeutic potential experienced also a revival in the US (Oehen et al., 2013; Mithoefer et al., 2016; Mithoefer et al., 2018). In 2018, two phase III trials started in the US with the goal of turning MDMA into a medically used substance for the assisted treatment of chronic post-traumatic stress disorder (PTSD; Mithoefer et al., 2019).

1.2. Pharmacology of MDMA

The onset of the acute effects of MDMA after oral administration is at a mean time of 33 minutes after oral intake and reaches peak effects after 1.6 h. The mean effect duration is lasting 4.2 h (Vizeli and Liechti, 2017). As all phenethylamines, MDMA contains a chiral center as it can form two enantiomers. The S-MDMA isoform is described to be more pharmacologically active than the R-MDMA (Pizarro et al., 2004). As main mechanism of action, MDMA interacts with multiple neurotransmitter systems causing an indirect acute efflux of presynaptic monoamine transmitters such as serotonin (5-HT), norepinephrine (NE), and dopamine (DA) into the synaptic cleft. This rapid release is triggered by an interaction on the monoamine transporter side. MDMA implies a reverse of the monoamine reuptake-carrier (Berger et al., 1992; Rudnick and Wall, 1992). The most potent affinity to monoamine transporters is shown for the NE transporter (NET; IC_{50}: 0.447 µM), followed by the affinity to the 5-HT transporter (SERT; IC_{50}: 1.36 µM), and 10 times weaker for the DA transporter (DAT; IC_{50}: 17 µM; Simmler et al., 2013). Less important mechanisms of action might lie in the weak affinity of MDMA to 5-HT_2 -, α_2-adrenergic, M_1-muscarinic, and H_1-histamine receptors and the small inhibition of the monoamine oxidase enzyme (MAO; Battaglia et al., 1988; Green et al., 1995; Liechti and Vollenweider, 2000a; b; 2001; Hysek et al., 2012a). The typical acute psychotropic effects of MDMA including feelings of well-being, trust, and euphoria are a result of the differential release of the aforementioned neurotransmitters (Liechti et al., 2000a; Hysek et al., 2011; Hysek et al., 2012d). Further, MDMA produces a rise in oxytocin plasma levels (Thompson et al., 2007; Dumont et al., 2009; Hysek et al., 2014a; Francis et al., 2016). The peptide hormone oxytocin is known to play a key role in regulating emotion processing and social behavior (Neumann, 2008; Meyer-Lindenberg et al., 2011; Kuypers et al., 2014). Due to enhancement of emotional empathy and prosocial behavior, MDMA is called an “entactogen” or “empathogen” (Nichols, 1986; Hysek et al., 2014a). However, whether oxytocin is a substantial mediator of the effects of MDMA in humans is inconclusive (Kuypers et al., 2014). Studies have assessed the effects of MDMA on emotion recognition relating to the cognitive aspects of empathy (Bedi et al., 2009; Hysek et al., 2012b). MDMA selectively impaired the recognition of negative emotions, while other stimulants such as methylphenidate enhanced the recognition of emotions regardless of the valence (Schmid et al., 2014; Wardle and de Wit, 2014; Dolder et al., 2018; Schmidt et al., 2018). In clinical studies, subjective effects of MDMA are mostly perceived as positive, with minimal bad drug effects (Dolder et al., 2018; **Figure 1**). Women experienced more overall drug effects but seem also more prone to negative drug experiences under MDMA than men (Liechti et al., 2001a).

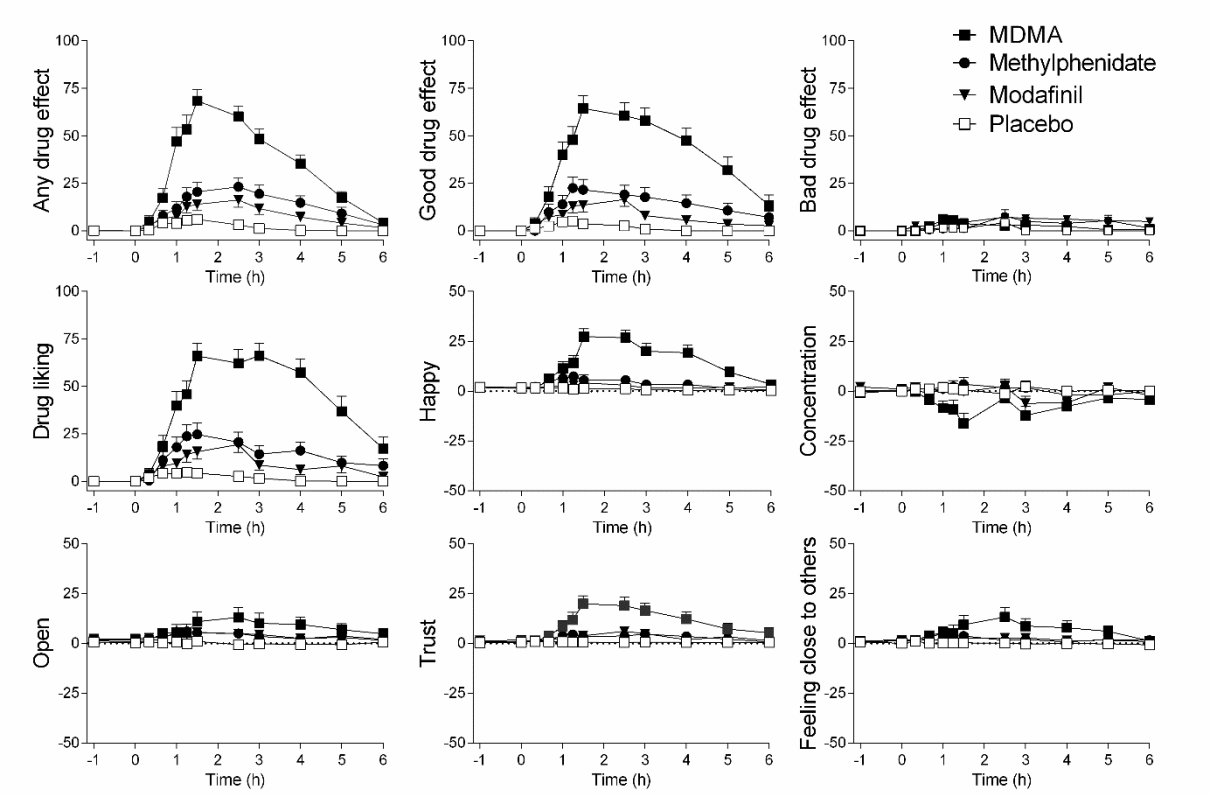

Figure 1 Subjective effects of MDMA (125 mg), methylphenidate (60 mg), modafinil (600mg), and placebo on the visual analog scales (VASs). MDMA produced greater subjective effect ratings for any drug effects, good drug effects, drug liking, happiness, trust, and feeling close to others than methylphenidate, modafinil, and placebo. None of the substances produced significant bad drug effects compared with placebo. The data are expressed as the mean ± SEM in 24 subjects. The substance was administered at t = 0. (Dolder et al., 2018)

A single dose of 125 mg of MDMA increases systolic (SBP) and diastolic (DBP) blood pressure (**Figure 2**; Dolder et al., 2018), but also heart rate and body temperature compared to placebo (Liechti et al., 2001a; Dolder et al., 2018). In a placebo-controlled double-blind study in 8 healthy adults, mean heart rate was elevated by 30 beats/min (bpm) and the cardiac output by 2 l/min after administration of 1.5 mg/kg MDMA (Lester et al., 2000). MDMA induced a significant rise in body temperature of about 0.2-0.8° C (Liechti, 2014). Further acute sympathomimetic effects include pupil dilation, trismus and bruxism, nystagmus, loss of appetite, and in males, possible erectile dysfunction (Downing, 1986; Hysek and Liechti, 2012).

Taken together, responses to MDMA are both stimulating with amphetamine-like sympathomimetic effects, and "entactogen" with oxytocin-like sociable effects (**Figure 1 – 2**).

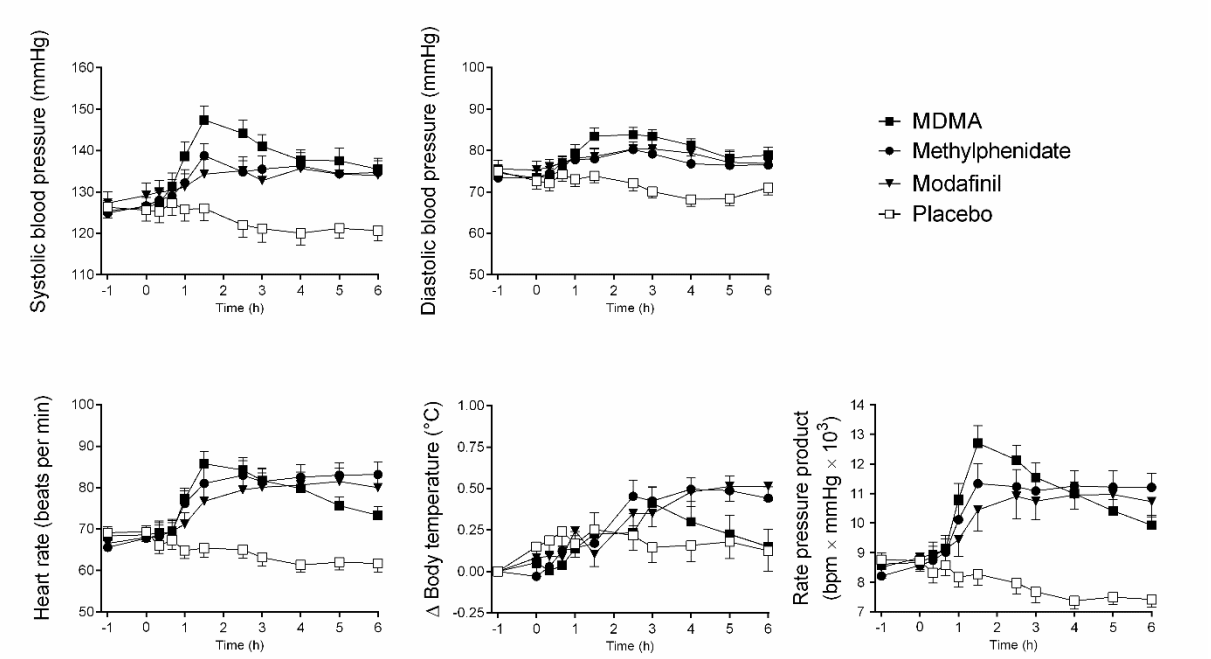

Figure 2 Autonomic responses to MDMA (125 mg), methylphenidate (60 mg), modafinil (600 mg), and placebo. MDMA showed higher increases in blood pressure than methylphenidate, modafinil, and placebo. The overall hemodynamic response, expressed as the rate-pressure product, similarly increased after all active treatments compared with placebo. The data are expressed as the mean ± SEM in 24 subjects. The substance was administered at t = 0. (Dolder et al., 2018)

1.3. Metabolism & Pharmacokinetics of MDMA

PARAMETER	C_{MAX}	T_{MAX}	AUC_{27H}	AUC_{∞}	K_E	$T_{1/2B}$	CL_P	CL_H	CL_R
UNIT	µg/L	h_{median}	µg×h/L	µg×h/L	1/h	h	L/h	L/h	L/h
MEAN VALUE ± SD	233 ± 45	1.5	2542 ± 469	2866 ± 579	0.086 ± 0.018	8.3 ± 1.4	36.3 ± 8.4	26.9 ± 5.6	9.4 ± 3.8

Table 1 Pharmacokinetic parameters after a single oral dose of 100 mg MDMA in seven healthy male subjects (Segura et al., 2005). C_{max}; maximum plasma concentration; T_{max}, time to reach C_{max}; AUC, area under the time-concentration curve; K_e, elimination rate constant; $T_{1/2\beta}$, elimination half-life; CL_p, plasmatic clearance; CL_h, hepatic clearance; CL_r, renal clearance.

MDMA is usually taken orally and rapidly absorbed (de la Torre et al., 2000a). The major metabolic pathway is mainly regulated by cytochrome P450 (CYP) 2D6 and catechol-O-methyltransferase (COMT; **Figure 3**), leading over an O-demethylenation to 3,4-dihydroxymethamphetamine (HHMA). This step is predominantly mediated by CYP2D6 (Farre et al., 2004; Meyer et al., 2008). The O-demethylenation is regulated for about 30% by CYP2D6. MDMA is an uncompetitive mechanism-based inhibitor (MBI) of CYP2D6, resulting in an irreversible inhibition within 2 hours, and reaching phenotypical poor metabolizer activity (Farre et al., 2004; O'Mathuna et al., 2008). CYP2D6 activity recovers only after ten days with a recovery half-life of about 47 hours (O'Mathuna et al., 2008). The non-linearity of MDMA pharmacokinetics implies that relatively small increases in the dose of MDMA ingested are translated to disproportionate rises in MDMA plasma concentrations. Hence subjects with a higher dose might be more prone to develop acute toxicity (de la Torre et al., 2000a; de la Torre et al., 2000b). As shown in **Figure 3,** the minor pathway leads over N-demethylation regulated by CYP2B6, but also partly by CYP1A2 and to some extent by CYP2D6, to 3,4-methylendioxyamphetamine (MDA) and through O-demethylenation to 3,4-dihydroxyamphetamine (HHA; de la Torre et al., 2000b). HHMA and HHA is further O-methylated by COMT to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA), respectively, and conjugated by phase II enzymes in an O-glucuronidation- or O-sulfation form before they are excreted in urine (Ensslin et al., 1996). Furthermore, approximately 15% of the administered MDMA dose is excreted unchanged in urine (Abraham et al., 2009). CYP2C19 was observed having a preference for the S-enantiomer. This enantioselective metabolism may be an explanation for the enantioselective pharmacokinetics of MDMA (Meyer et al., 2008).

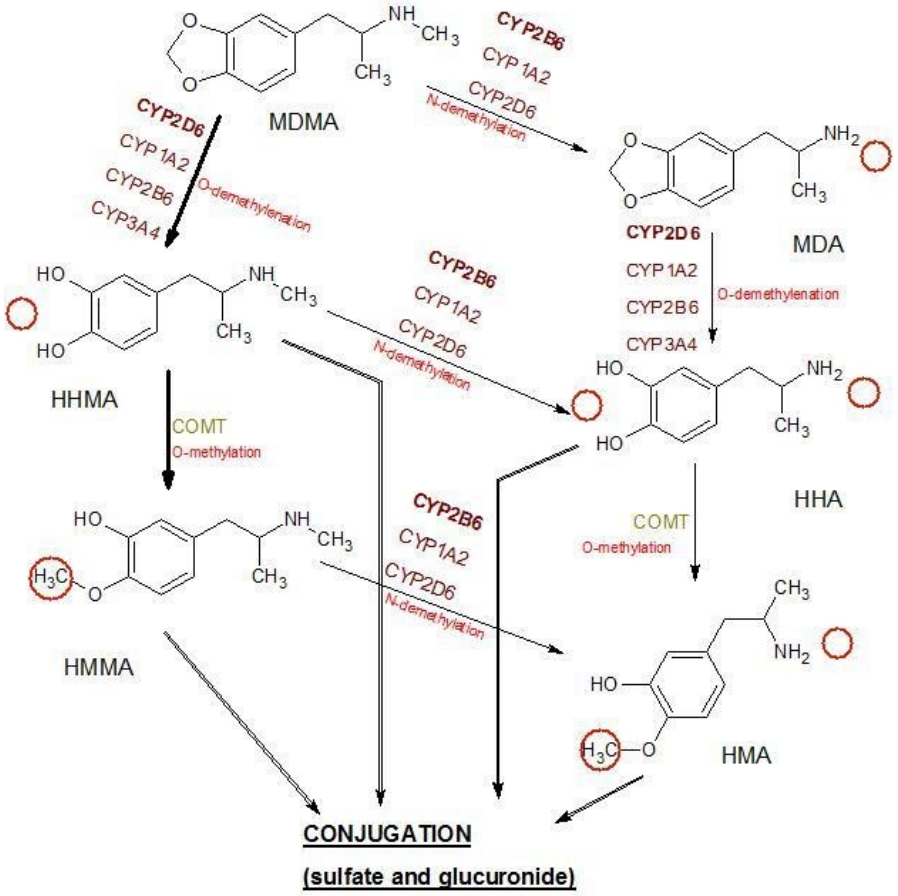

Figure 3. Simplified hepatic metabolism pathways of racemic MDMA in human. Bold arrows mark the major metabolic pathway. Red circles show the detached/added part by metabolic action. CYP2C19 is not shown in this figure, because it is not equally important for both MDMA enantiomer.

1.4. Safety & Toxicology of MDMA

"All things are poison, and nothing is without poison, the dosage alone makes it so a thing is not a poison." This famous quote by Paracelsus seems to be only partially true for MDMA. At least, there is evidence of an idiosyncratic drug reaction in cases of severe liver damage (Henry et al., 1992; Ellis et al., 1996; Antolino-Lobo et al., 2011; Atayan et al., 2015; Maharaj et al., 2015). Cases of excessive regular use or 40-50 tablets of MDMA on one occasion did not end fatally, while on the other hand reported consumption of only one pill caused mortality (Henry et al., 1992; Parrott et al., 2001). It is, however, not reported how much active substance was present in those cases. In general, it is shown that the metabolites HHMA and HHA are more cytotoxic than the parent compound by forming reactive oxygen and nitrogen species (Kreth et al., 2000; Monks et al., 2004; Carmo et al., 2006; Antolino-Lobo et al., 2010). Furthermore, both MDMA and its active metabolite MDA are thought to be serotonergic neurotoxins, but did not manifest any neurotoxicity directly injected into the brain (Esteban et al., 2001). It is also postulated that minor systemic metabolites may be directly responsible for the neurotoxicity. Animal studies showed MDMA-related gradual loss of serotonergic axon terminals as well as cognitive functions (Ricaurte et al., 1985; de la Torre and Farre, 2004; Puerta et al., 2009). In human, neuroimaging studies found serotonergic deficits and memory impairments in heavy MDMA users (Gouzoulis-Mayfrank and Daumann, 2006; Parrott, 2013). However, in some cases partial reconstitution may occur after abstinence or cognitive impairment may even never become an issue (Halpern et al., 2004; Wagner et al., 2013).

In general, the extent of toxicity of MDMA is difficult to determine. In most of the known MDMA-related emergencies, other substances like alcohol, cocaine, nicotine and many further play a more or less relevant role (Liechti et al., 2005; Antolino-Lobo et al., 2011; Roxburgh and Lappin, 2019). In fact, evidence-based analyses placed MDMA in a rather "safe" spot in relation to other illicit drugs (Nutt et al., 2007; Nutt et al., 2010). Nevertheless, among several MDMA adverse effects like tachyarrhythmia, loss of consciousness, depressive mood, dizziness or weakness and anxiety, hyperthermia and hyponatremia are considered to be the most life-threatening acute physiological consequences of MDMA intoxications (Henry, 1992; Henry et al., 1992; Kalant, 2001; Liechti et al., 2001a). While moderate elevated body temperatures (>38 °C) are to be expected in controlled settings, body temperatures over 42 °C have been reported in emergency cases (Green et al., 1995; Ellis et al., 1996; Liechti, 2014). It is suggested that MDMA stimulates the hypothalamic-pituitary-adrenal axis, the 5-HT, and the sympathetic system, and the hereby caused increases in cortisol, 5-HT, and NE levels contribute to the increase in body temperature (Liechti et al., 2000b; Hysek et al., 2011; Seibert et al., 2014). However, the mediation role of 5-HT in the thermogenic response to MDMA is somewhat unclear (Liechti et al., 2000b; Liechti and Vollenweider, 2000b; Farre et al., 2007), while the role of NE and α_1- and β_3-adrenergic receptors seems to be more conclusive (Sprague et al., 2003; Hysek et al., 2011; Hysek et al., 2012c; Hysek et al., 2013). Hyperthermia is followed by vasodilatation inducing

cutaneous blood flow and therefore enhancing the heat dissipation. The vasoconstriction effect of MDMA impairs this counteraction of the body (Pedersen and Blessing, 2001; Mills et al., 2004). In addition, the activation of the mitochondrial uncoupling protein 3 (UCP 3) induced by NE-release could represent a further heat generation mechanism (Mills et al., 2004; Sprague et al., 2004; Parrott, 2012). However, another factor is closely related to life threatening hyperthermia. MDMA is often consumed in crowded, hot environments such as raves (Schwartz and Miller, 1997). High ambient temperatures, prolonged dancing, and dehydration take a significant account to the MDMA-induced hyperthermia (Patel et al., 2005; Capela et al., 2006; Parrott, 2012). To counteract dehydration, women in particular should be careful and replace hypotonic water with an electrolyte-containing drink, as MDMA-induced hyponatremia is another potentially life-threatening condition that is fostered by excessive fluid intake (Rosenson et al., 2007). MDMA-related hyponatremia is possibly caused by the inexpedient secretion of arginine vasopressin (AVP) by HMMA inducing water retention in combination with extreme physical activity and increased body temperature (Holden and Jackson, 1996; Henry et al., 1998; Cherney et al., 2002; Fallon et al., 2002; Hartung et al., 2002). This phenomenon is seen to be more frequently occurring in women (Rosenson et al., 2007; Bora et al., 2016).

Substances that are widely used recreationally are often prone to lead to dependency. MDMA is popular in recreational settings like night clubs and “raves” (Schwartz and Miller, 1997), however, its dependence potential is questionable (Degenhardt et al., 2010). In a review in a cohort of over 6700 ecstasy users a minority was concerned about their use pattern (Degenhardt et al., 2010). MDMA seems to be a less potent reinforcer than other drugs, interestingly also significantly less than other popular stimulants (Degenhardt et al., 2010). The dependency profile of MDMA is arguably more similar to the hallucinogen class, which are hardly addictive, but not undisputed (Liechti and Vollenweider, 2000b; Cottler et al., 2009). An explanation for this matter might be that drug dependency is directly or indirectly tied to a dopaminergic pathway of action (Nestler, 2005). Despite dopaminergic activity, the effects of MDMA rely more on the norepinephrine and serotonergic mechanism of action (Hysek et al., 2012d; Simmler et al., 2013). Even though MDMA demonstrated self-administration behavior in rats, the 5-HT release was also found to attenuate the reinforcing effects compared with other amphetamines (Bankson and Yamamoto, 2004; Schenk et al., 2007).

1.5. Pharmacogenetics of MDMA

As mentioned above, metabolism and transporter/receptor as mechanism of action seem to influence the effects of MDMA. Some of these players can be differently expressed due to different genetic disposition and therefore elicit interindividual differences in the response to MDMA exposure. The various genotypes are mostly caused by so-called single nucleotide polymorphisms (SNPs) but also gene deletions or duplications. The predominantly involved phase I enzyme for the metabolism of MDMA, CYP2D6, exhibits a high polymorphism in phenotypes with several different observed genotypes. Genetic poor metabolizers (PM) are observed in 5-10%, intermediate metabolizers (IM) in 10-17%, extensive metabolizers (EM) in 70-80%, and ultra-rapid metabolizers in 3-5% of the Caucasian population (Sachse et al., 1997; Hicks et al., 2013; Preissner et al., 2013). CYP2D6 PMs were typically not represented in previous studies that evaluated the pharmacokinetics of MDMA. Hence, an MDMA pharmacokinetic study not including PMs found no effects of CYP2D6 genotype on plasma MDMA concentrations or its associated physiological response (Pardo-Lozano et al., 2012). In another study, a subject categorized as PM with a low enzyme activity displayed three times higher MDMA concentrations compared to normal activity EMs (de la Torre et al., 2005). In line with this, higher HMMA plasma levels were observed in subjects with two functional alleles of CYP2D6 (Pardo-Lozano et al., 2012). However, it was postulated that the autoinhibitory behavior of MDMA on CYP2D6 implies that subjects, irrespective of their genotype, are phenocopied into PMs. It is even suggested that the PM genotype of CYP2D6 prevents long-term neurotoxicity, since the toxicity was linked to minor metabolites of MDMA after its methylenedioxyphenyl ring-opening by CYP2D6 (de la Torre and Farre, 2004; Carmo et al., 2006). The phase II enzyme COMT shows a well-studied SNP that results in an amino acid exchange from valine (Val) to methionine (Met) in position 158 of the amino acid sequence (Green et al., 2003). The Val-allele is associated with a high, and the Met-allele with a low activity of the COMT enzyme. An association has been observed between low COMT activity and a higher increase in plasma cortisol concentration and lower plasma sodium compared to high COMT activity after MDMA administration (Aitchison et al., 2012; Wolff et al., 2012). Surprisingly, a study in 27 healthy subjects has shown greater cardiovascular effects and lower negative subjective effects such as dizziness, sedation, and anxiety in carriers with the high functionality COMT genotype after MDMA administration (Pardo-Lozano et al., 2012). The same study found also greater cardiovascular effects in long allele carriers of the 5-HT-transporter-linked polymorphic region (5-HTTLPR) compared to exclusive short allele carriers (Pardo-Lozano et al., 2012). Other pharmacodynamic targets of MDMA were not investigated yet, except an oxytocin receptor gene (OXTR) polymorphism defined by the SNP rs53576. Bershad et al. found that sociability did not increase in individuals carrying the rs53576 AA genotype vs individuals with at least one G allele after a high dose (1.5 mg/kg) of MDMA (Bershad et al., 2016b). In addition, recent research has started to determine potential genetic differences that may underlie the individual response to d-amphetamine (Dlugos et al., 2007; Hamidovic et al.,

2010a; b; Dlugos et al., 2011). For example, it has been shown that polymorphisms in the DA transporter (Solute Carrier 6A3, SLC6A3) were associated with stimulant effects of amphetamine. Specifically, individuals with the C/C genotype at rs460000 reported twofold higher stimulation and euphoria relative to the A/A or A/C genotype group (Hamidovic et al., 2010b). Different studies from the same group reported many positive associations between SNPs and subjective effects of d-amphetamine although these could not all be replicated in a larger study sample (Hart et al., 2013). While many gene polymorphism-drug effect associations were clearly not confirmed, some interesting ones for MDMA were reconfirmed including DAT (3'UTRVNTR, rs460000) and trends for NET rs1861647 (Hart et al., 2013).

1.6. Significance

In Switzerland, 3.9% of the population reported having used Ecstasy at least once in their life (Gmel G., 2017). MDMA is the active substance usually found in Ecstasy pills. But MDMA is not only recreationally used, it is also being evaluated as a treatment for post-traumatic stress disorder in several countries including Switzerland (Oehen et al., 2013; Mithoefer et al., 2016; Mithoefer et al., 2018). MDMA has also been suggested to be useful to treat and study mood disorders due to its potential to increase empathy and rapidly elevate mood (Brensilver et al., 2012). So far, MDMA was tested in several studies with healthy subjects and patients. However, the safety pharmacology and pharmacogenetics of MDMA is poorly characterized and needs to be tested within a reasonable sample size. Linking genetics to MDMA effect variability helps to elucidate individual drug responses and toxicity. Unfortunately, such linking studies are scarce or absent for many polymorphisms on a pharmacokinetic and -dynamic level. Previous pharmacogenetic studies with MDMA did not reach a sufficient sample size to come to a revealing conclusion. More research is necessary to unravel which polymorphisms are of clinical relevance. This thesis shows several new analyses within the largest pooled but consistent study cohort of healthy subjects tested with MDMA.

1.7. Aims & Hypothesis

The main goal of this thesis was to add completive information to the safety pharmacological and pharmacogenetics of MDMA, a substance that is widely used recreationally and likely to be used soon as a medication for the treatment of PTSD.

The first aim was to point out the frequency and magnitude of adverse events of one or two single-dose administrations of MDMA at doses similar or equal to the ones used in MDMA-assisted therapy. We hypothesized that MDMA would produce predominantly acute positive mood effects and tolerable transient cardiostimulant and thermogenic reactions. Changes in liver enzymes or creatine levels as a sign of liver damage or renal failure were not expected in a controlled setting.

Our hypotheses for the different pharmacogenetic studies were diverse and depended on the target and a few preliminary findings from earlier studies of effects of different genetic polymorphisms on the effects of stimulants. In general, we only investigated the influence of genetic polymorphisms that were prominent in the Caucasian population (>1%) and that were thought to be functional.

We hypothesized that the pharmacokinetics would be influenced according to the genetically determined activity levels of the CYPs and the involved MDMA metabolism pathways.

Specifically, we expected that CYP2D6 poor metabolizers would show higher concentrations of MDMA than normal metabolizers, and tobacco smokers with the inducible variant of CYP1A2 would likely show a higher formation of MDA compared to non-smokers and smokers with the non-inducible variant of CYP1A2. If the genetic polymorphisms of the MDMA-metabolizing CYPs would also alter pharmacodynamic parameters was an additional aim of this study.

Many data are available on interactions between monoamine transporter (NET, SERT, DAT) inhibitors and MDMA. These observations together with rare existing pharmacogenetic data on the effects of genetic polymorphism on stimulant drugs and / or MDMA led to our hypotheses for polymorphisms in genes coding for the respective monoamine system. Two smaller previous studies highlighted modification of the cardiovascular effects and the anxiety felt after MDMA administration caused by 5-HTTLPR polymorphisms (see 1.5 Pharmacogenetics). We expected to replicate those previous results. Variations within genes coding for the serotonin system were additionally tested for alterations to the acute responses to MDMA, since the serotonin system is thought to play a major role in the mediation of most MDMA effects. For the impact of genotypes within the NE and DA system on the effects of MDMA, no preliminary results were existing. However, since NE was shown to be critically involved in the mediation of the cardio- and psychostimulant effects of MDMA, we suspected a modification in the MDMA-induced increase of subjective stimulant effects and the rate-pressure product. The interaction of MDMA with the DA system is suspected be vague responsible for the acute effects of MDMA. Because of previous interaction studies and low affinity to the DAT, we hypothesized none to minimal influence of variants within genes coding for the DA system on the acute effects of MDMA.

Because oxytocin is known to influence prosociality, the hypothesis for the OXTR polymorphism was that we would observe changes in the prosocial effects of MDMA and replicate previous findings regarding the role of an OXTR SNP and the prosocial response to MDMA. Therefore, we focused on visual analog scales (VAS) like “trust” and “closeness to others” as well as several tests to assess altruism, and emotional and cognitive empathy.

Due to the explanatory nature of the pharmacogenetic studies in this thesis, we accounted for multiple comparisons in the statistical results.

PUBLICATIONS

2.1. Safety pharmacology of acute MDMA administration in healthy subjects

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Journal of Psychopharmacology, 2017, Vol. 31, 576 - 588

Original Paper

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Journal of Psychopharmacology
2017, Vol. 31(5) 576–588

DOI: 10.1177/0269881117691569
journals.sagepub.com/home/jop

Abstract

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is being investigated in MDMA-assisted psychotherapy. The present study characterized the safety pharmacology of single-dose administrations of MDMA (75 or 125 mg) using data from nine double-blind, placebo-controlled, crossover studies performed in the same laboratory in a total of 166 healthy subjects. The duration of the subjective effects was 4.2 ± 1.3 h (range: 1.4–8.2 h). The 125 mg dose of MDMA produced greater 'good drug effect' ratings than 75 mg. MDMA produced moderate and transient 'bad drug effect' ratings, which were greater in women than in men. MDMA increased systolic blood pressure to >160 mmHg, heart rate >100 beats/min, and body temperature >38°C in 33%, 29% and 19% of the subjects, respectively. These proportions of subjects with hypertension (>160 mmHg), tachycardia, and body temperature >38°C were all significantly greater after 125 mg MDMA compared with the 75 mg dose. Acute and subacute adverse effects of MDMA as assessed by the List of Complaints were dose-dependent and more frequent in females. MDMA did not affect liver or kidney function at EOS 29 ± 22 days after use. No serious adverse events occurred. In conclusion, MDMA produced predominantly acute positive subjective drug effects. Bad subjective drug effects and other adverse effects were significantly more common in women. MDMA administration was overall safe in physically and psychiatrically healthy subjects and in a medical setting. However, the risks of MDMA are likely higher in patients with cardiovascular disease and remain to be investigated in patients with psychiatric disorders.

Keywords

3,4-methylenedioxymethamphetamine, safety, adverse effect, Phase I, human

Introduction

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is used recreationally and investigated clinically as a medication in MDMA-assisted psychotherapy in patients with post-traumatic stress disorder (PTSD) (Amoroso and Workman, 2016; Kupferschmidt, 2014; Mithoefer et al., 2010, 2016; Oehen et al., 2013; Sessa and Nutt, 2015). The future use of MDMA in psychiatric practice will depend on its efficacy in specific disorders and its safety of use. The benefits and harms associated with MDMA have been previously discussed (Doblin et al., 2014; Parrott, 2013a, 2014). Currently, however, sufficient data from clinical Phase I–III studies have not been published in peer-reviewed journals. Phase III studies are currently being planned (MAPS, 2016), and more information on the clinical safety of MDMA is needed (Mithoefer et al., 2016). Therefore, the present study sought to provide data on the safety pharmacology of single-dose administrations of MDMA. We primarily addressed the acute effects during the MDMA response (0–6 h) and the subacute adverse effects up to 24 h after the administration of MDMA. We also included data on any adverse events that occurred during the entire clinical studies including blood laboratory values obtained at the end of study (EOS) visit. These data were collected from a series of Phase I clinical studies that were conducted in our laboratory and used the same standardized data recording methods. These studies used one or two single-dose administrations of MDMA at doses equal or similar to those used in MDMA-assisted psychotherapy (Mithoefer et al., 2010; Oehen et al., 2013) and in subjects with no or minimal prior ecstasy use, which is also likely the case when MDMA is used in patients. The aims of the study were to describe subjective effects (self-rated good and bad drug effects), the duration of the acute MDMA response, cardiovascular and hyperthermic effects, and acute and subacute adverse effects, and lasting effects on laboratory indices of liver and kidney function. We also tested the moderating effects of dose (de la Torre et al., 2000; Kolbrich et al., 2008a; Schmid et al., 2014) and sex (Liechti et al., 2001; Pardo-Lozano et al., 2012; Reneman et al., 2001; Simmler et al., 2011; Verheyden et al., 2002) on these acute responses to MDMA.

The pharmacology of MDMA has been relatively well-studied. MDMA mainly induces the release of presynaptic serotonin (5-hydroxytryptamine (5-HT)) and to a lesser extent norepinephrine and dopamine through interactions with the corresponding monoamine transporters (Hysek et al., 2012d; Rothman et al., 2001; Simmler et al., 2013). MDMA is generally classified as an 'entactogen' or 'empathogen' because its socio-emotional effects differ from those of prototypic stimulants (Bershad et al., 2016;

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Hysek et al., 2014b; Schmid et al., 2014) and it produces fewer perceptual alterations than hallucinogens (Schmid et al., 2015a). Specifically, MDMA increases feelings of closeness to others, trust and openness and enhances emotional empathy for positive situations (Hysek et al., 2014a; Schmid et al., 2014). Such effects are not observed with stimulants that predominantly act on the dopamine system (Schmid et al., 2014). MDMA (Baggott et al., 2016) but not D-amphetamine (Childs et al., 2016) decreased social anxiety. MDMA selectively impaired the recognition of negative emotions (Bedi et al., 2010; Hysek et al., 2014a; Schmid et al., 2014; Wardle et al., 2014), whereas such stimulants as amphetamine and methylphenidate nonselectively enhanced the recognition of emotions (Hysek et al., 2014b; Schmid et al., 2014; Wardle et al., 2012). These findings indicate that MDMA produces unique effects on emotion processing in healthy subjects that are likely linked to its predominant effects on the 5-HT system (Bershad et al., 2016; Hysek et al., 2012b, 2012d) and may be useful in MDMA-assisted psychotherapy (Sessa, 2016).

Earlier studies that described the acute effects of MDMA in healthy volunteers have previously been reviewed (Dumont and Verkes, 2006), but many more have been performed since then (Carhart-Harris et al., 2014; de Sousa Fernandes Perna et al., 2014; Dumont et al., 2008, 2009; Farre et al., 2007, 2015; Hasler et al., 2009; Hysek et al., 2011; 2012a, 2012c, 2012d, 2013, 2014b; Kirkpatrick et al., 2012, 2014a, 2014b; Kolbrich et al., 2008a; Kuypers et al., 2014; Pardo-Lozano et al., 2012; Parrott et al., 2011; Peiro et al., 2013; Schmid et al., 2014, 2015b; van Wel et al., 2012). However, all of these individual studies were relatively small (n = 6–30) in particular to characterize adverse events that are not very common (<10%), and safety aspects were not the primary endpoint or were not described. Additionally, we are unaware of any larger data analyses (n > 100) that focused on the clinical safety profile of acute MDMA administration. The present analyses used a relatively large sample size including data from 166 subjects (equal numbers of males and females) and 166 administrations of MDMA alone and 112 administrations of MDMA with another substance. In contrast to compilations of data from different laboratories, the present data set used the same doses and formulations of MDMA and the same standardized outcome measures across all the individual studies including measures of plasma levels of MDMA. We hypothesized that MDMA would produce predominantly positive mood effects (Baggott et al., 2016; Hysek et al., 2014a; Kolbrich et al., 2008a) and cardiostimulant (Schmid et al., 2016) and thermogenic (Liechti, 2014) responses. Renal failure and hepatotoxicity are potential consequences of uncontrolled ecstasy use (Ben-Abraham et al., 2003; Liechti et al., 2005), but we did not expect to see changes in liver enzymes or creatinine levels after single doses of MDMA in a controlled setting.

Methods

Study design

This was a pooled analysis of nine Phase I double-blind, placebo-controlled, crossover studies in healthy subjects (Hysek and Liechti, 2012; Hysek et al., 2011, 2012a, 2012c, 2012d, 2014b; Schmid et al., 2014, 2015b). These studies were all conducted at the University Hospital Basel and included a total of 166 subjects who were all psychiatrically screened and healthy. The aim of the pooled analysis was to assess the safety pharmacology of one or two single doses of MDMA in healthy subjects with no regular MDMA use and no or minimal previous use. Seven studies each included 16 subjects (total of 112 subjects) who received 125 mg MDMA twice, once alone and once after pretreatment with a medication (Hysek and Liechti, 2012; Hysek et al., 2011, 2012a, 2012c, 2012d; 2014b; Schmid et al., 2015b). An additional unpublished study included 24 subjects who received 125 mg MDMA once without pretreatment (ClinTrials.gov ID: NCT01951508). Lastly, one study included 30 subjects who received a single dose of 75 mg MDMA without pretreatment (Schmid et al., 2014). The focus of this analysis is on the acute effects of MDMA alone. Effects of MDMA with pretreatments are shown in the Supplementary Material online. In all of the studies, the washout periods between the MDMA single-dose administrations were at least seven days to exclude carry-over effects. The studies were all registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01771874, NCT01951508 and NCT01616407). All of the studies were approved by the local ethics committee and Swiss Agency for Therapeutic Products (Swissmedic). The studies were conducted in accordance with the Declaration of Helsinki. MDMA administration in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Informed consent was obtained from all of the participants who were included in the studies. All of the subjects were paid for their participation.

Subjects

A total of 166 healthy European/Caucasian subjects, aged 18–45 years (mean ± SD = 24.5 ± 4 years) were mostly recruited from the University of Basel campus and included in the studies. The mean ± SD body weight was 69 ± 10 kg (range: 46–95 kg). Thirty subjects (15 men, 15 women) received a single 75 mg dose of MDMA. One hundred and thirty-six subjects received a single 125 mg dose of MDMA. In these 136 subjects, MDMA was administered as a single dose in 24 subjects (12 men, 12 women) and as two single doses of 125 mg (once alone and once combined with another drug on two separate occasions at least seven days apart) in 112 subjects (56 men, 56 women), resulting in total exposure of 250 mg MDMA. The time interval between the two MDMA administrations was 26 ± 17 days.

The detailed exclusion criteria were reported elsewhere (Hysek and Liechti, 2012; Hysek et al., 2012a, 2012c, 2012d), including a history of psychiatric disorders, physical illness, a lifetime history of using illicit drugs more than five times (with the exception of past cannabis use), illicit drug use within the last two months, and illicit drug use during the study, determined by urine tests that were conducted before the test sessions. Fifty-nine subjects had prior drug experience (1–5 times), of which 34 subjects had previously used MDMA (1–4 times).

Study drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 75 or 125 mg prepared as gelatin capsules (25 or 100 mg, Bichsel Laboratories, Interlaken, Switzerland). Similar amounts of MDMA are found in ecstasy pills

(Brunt et al., 2012) and have been or are being used in clinical studies in patients (Mithoefer et al., 2010; Oehen et al., 2013). Male and female subjects were treated with the same 125 mg dose irrespective of body weight in the clinical studies in patients (Mithoefer et al., 2010; Oehen et al., 2013). Similarly, in the present study, the doses were not adjusted for body weight or sex. The dose per body weight (mean ± SD) was 1.7 ± 0.4 mg/kg (range: 0.8–2.7 mg/kg). For the 75 mg dose of MDMA, the dose per body weight was 1.0 ± 0.1 mg/kg for men and 1.2 ± 0.1 mg/kg for women. For the 125 mg dose of MDMA, the dose per body weight was 1.7 ± 0.2 mg/kg for men and 2.1 ± 0.3 mg/kg for women.

Pharmacodynamic measures

Visual analogue scales (VASs) were repeatedly used to assess subjective effects over time (Hysek et al., 2014a). The VASs included 'any drug effect', 'good drug effect' and 'bad drug effect'. The VASs 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 applied before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h after MDMA or placebo administration. In the study that evaluated 75 mg MDMA, the 0.33 h time point is missing. In one study that evaluated 125 mg MDMA ($n = 24$), the 2 and 2.5 h time points are missing. The onset, offset and duration of the subjective response were determined using the VAS 'any drug effect'-time curve, with 10% of the individual maximal response as the threshold, in Phoenix WinNonlin (version 6.4, Pharsight, Certara L.P., St. Louis, MO, USA). Because subjective effects were assessed only up to 6 h, the offset in two subjects with an effect >10% at 6 h was determined by log-extrapolation. Four subjects were excluded from this analysis because they reported no subjective effects.

Blood pressure, heart rate and body temperature were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h after MDMA or placebo administration. Systolic and diastolic blood pressure and heart rate were measured using an automatic oscillometric device (OMRON Healthcare Europe NA, Hoofddorp, Netherlands). The measurements were performed in duplicate at an interval of 1 min and after a resting time of at least 10 min. The averages were calculated for analysis. Core (tympanic) temperature was measured using a Genius™ two ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA). In the study that evaluated 75 mg MDMA, the 0.33 h and 2.5 h time points are missing. In one study that evaluated 125 mg MDMA ($n = 24$), the 2 h time point is missing. Criteria for grouping subjects at least >90, 100 and 110 mmHg for diastolic and >140, 160 and 180 mmHg for systolic hypertension grade I–III, respectively. Tachycardia was defined as >100 beats/min. Hyperthermia and hyperpyrexia were defined as tympanic body temperature >38°C and 40°C, respectively.

Acute and subacute adverse effects were assessed using the list of complaints (Hysek et al., 2012a; Zerssen, 1976). The scale consisted of 66 items, yielding a total adverse effects score (non-weighted sum of the item answers) that reliably measures physical and general discomfort. Bruxism (item 66, a common side effect of MDMA) was included in the List of Complaints that was used in 134 subjects. The List of Complaints was administered before and 3–6 (acute adverse effects up to 6 h) and 24 h (subacute adverse effects up to 24 h) after MDMA or placebo administration. Additionally, participants were asked at the beginning of each study session and at the EOS visit to report any adverse events for the periods from 24 h until 14 days after administration.

Plasma concentrations of MDMA

Blood samples were collected 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4 and 6 h after administration of MDMA or placebo. Plasma concentrations of MDMA were determined as previously described (Hysek et al., 2012d). Peak plasma concentrations (C_{max}) were obtained directly from the observed data. The area under the concentration-time curve (AUC) from 0 to 6 h after dosing (AUC_6) was calculated using the linear-log trapezoidal method in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA).

Blood sampling and EOS visit

Blood chemistry and blood cell count tests were performed at the screening visit at the start of the study and at the EOS visit, which were separated by 88 ± 50 days. The EOS visit including the blood sampling took place at variable time intervals (29 ± 22 days) after the last MDMA administration and after one or two administrations of MDMA with or without pretreatments. The analyses were performed using standard assays according to Good Laboratory Practice by the Laboratory Medicine Department of the hospital. The glomerular filtration rate was determined by the Cockcroft–Gault Equation using plasma creatinine concentrations, age and sex of the subject. At the EOS visit, the participants were asked to retrospectively rate the duration of the subjective response to MDMA, whether the experience was positive or negative, whether the controlled clinical setting influenced their experience, and whether they considered taking MDMA again and in what setting. The participants were also asked whether they experienced 'flashbacks'. These questions were asked in 141 subjects.

Statistical analyses

The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). Repeated-measures analyses of variance (ANOVAs) with drug (MDMA alone without pretreatment *vs*. placebo) as the within-subjects factor and dose (75 *vs*. 125 mg) as the between-subjects factor were used to evaluate all the effects of MDMA compared with placebo (main effect of drug) and dose–response effects (drug × dose interactions). Sex differences were explored by adding sex as an additional between-subjects factor to the analyses. Tukey post hoc tests were used based on significant main effects or interactions in the ANOVA. Finally, dose per body weight and peak plasma concentrations of MDMA were used as a covariate to test whether sex differences were confounded by dose per body weight or plasma concentrations of MDMA, respectively. The latter analysis also accounted for potential differences in metabolism (Schmid et al., 2016, Vizeli et al., 2017) and non-linear pharmacokinetics. Fisher's exact tests were used to compare proportions. Differences in kidney and liver function and blood cell counts between the screening and EOS visit measures were analysed using paired *t*-tests. The level of significance was set to $p < 0.05$.

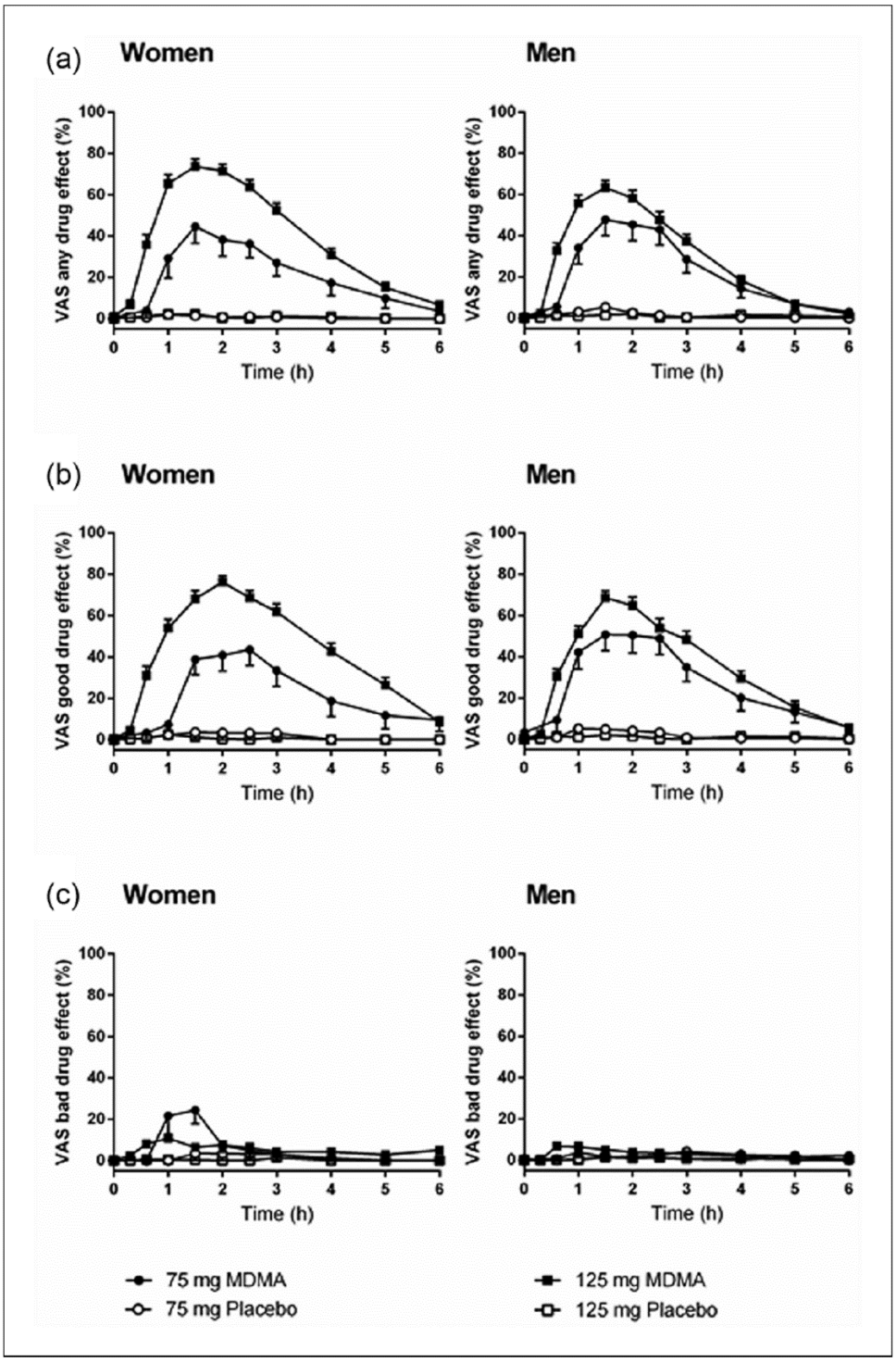

Figure 1. Subjective effects of MDMA. (a) The overall subjective effect ('any drug effect') began after a mean time of 33 min, reached its peak after 1.6 h, and lasted 4.2 h (effect duration from onset to offset with a threshold of 10% of the peak). (a), (b) MDMA produced greater 'any drug effect' and 'good drug effect' ratings at 125 mg compared with 75 mg. (c) MDMA produced moderate and transient 'bad drug effect' ratings that were not dose-dependent but greater in women than in men. The data are expressed as the mean ± SEM in 15 women and 15 men for the 75 mg dose and 68 women and 68 men for the 125 mg dose.

MDMA: 3,4-methylenedioxymethamphetamine; VAS: visual analogue scale.

Results

Acute subjective effects of MDMA

The time course of the subjective response to MDMA alone is shown in Figure. 1. The onset of effects and peak effect were 33 ± 24 min (mean ± SD) and 1.6 ± 0.8 h after MDMA administration, respectively. The duration of the subjective drug effects was 4.2 ± 1.3 h (range: 1.4–8.2 h). No sex or dose differences were found in the duration of the subjective effects. At the EOS visit, the subjects retrospectively indicated that the duration of their subjective response to MDMA was 4.0 ± 2.9 h. MDMA significantly increased ratings of 'any drug effect' and 'good drug effect' compared with placebo (Table 1, Figure 1). The 125 mg dose of MDMA produced higher 'any drug effect' and 'good drug effect' ratings compared with the 75 mg dose of MDMA (Table 1, Figure 1). A nearly significant drug × sex interaction in the ANOVA indicated that women tended to give greater 'any drug effect' ratings than men (Table 1, Figure 1). MDMA increased ratings of 'bad drug effect'. 'Bad drug effect' ratings were not dose-dependent in contrast to 'good drug effect' ratings. 'Bad drug effect' ratings were greater in women than in men. When we accounted for the higher mg/kg dose in women by adding it as a covariate in the analysis, women still gave significantly greater 'bad drug effect' ratings than men after MDMA administration compared with placebo ($F_{1,162} = 20.0$; $p < 0.001$). The sex difference also remained significant after correcting for differences in peak plasma concentrations of MDMA ($F_{1,162} = 21.5$; $p < 0.001$).

Acute effects of MDMA on vital signs

MDMA alone acutely increased blood pressure, heart rate and body temperature (Table 2). A significant dose–response effect of MDMA on blood pressure but not heart rate or body temperature was found (Table 2). The acute effect of MDMA on systolic blood pressure, heart rate and body temperature peaked after 1.7 ± 0.9 h, 2.0 ± 1.4 h and 2.4 ± 1.2 h, respectively. No sex differences in the effects of MDMA on vital signs were observed. However, when we accounted for the lower mg/kg dose in males compared with females by adding it to the analysis (i.e. dose normalization), men presented greater increases in systolic blood pressure compared with women ($F_{1,164} = 6.08$; $p < 0.05$). Blood pressure increases were also greater in men than women when correcting for plasma concentrations of MDMA ($F_{1,164} = 8.28$; $p < 0.01$). After MDMA administration, 54 subjects (33%) and seven subjects (4%) had systolic blood pressure >160 mmHg and 180 mmHg, respectively. The proportion of subjects with systolic blood pressure >160 mmHg was significantly greater after 125 mg MDMA administration compared with 75 mg ($p < 0.01$). Forty-eight subjects (29%) presented tachycardia (heart rate >100 beats/min). The proportion of subjects with heart rate >100 beats/min was significantly greater after 125 mg MDMA administration compared with 75 mg ($p < 0.05$). MDMA increased body temperature to >38°C in 32 subjects (19%), up to a maximum of 39.1°C. The proportion of subjects with body temperature >38°C was significantly greater after 125 mg MDMA administration compared with 75 mg ($p < 0.01$). The mean room temperature was 22.7 ± 0.6°C. The effects of co-administering MDMA with other medications on vital signs are shown in Supplementary Table S1. No hypertensive urgencies or incidents of hyperpyrexia (>40°C) occurred. The maximal diastolic and systolic blood pressure values among the 166 administrations of MDMA alone were 130 and 196 mmHg, respectively. The maximal diastolic and systolic blood pressure values among the 112 administrations of MDMA plus a pretreatment were 119 and 200 mmHg, respectively. The maximal observed body temperature value among all 278 administrations of MDMA alone or with pretreatment was 39.1°C.

Adverse effects of MDMA

MDMA produced significant acute and subacute adverse effects on the List of Complaints compared with placebo (Tables 1 and 2). The 125 mg dose of MDMA increased acute and subacute List of Complaints total scores significantly more than the 75 mg dose (Table 2). MDMA increased acute List of Complaints ratings more in women (8.8 ± 5.9) than in men (6.4 ± 6.8; Table 1). MDMA also increased subacute List of Complaints scores more in women (5.4 ± 5.5) than in men (3.0 ± 5.0; Table 1). The difference in the acute and subacute adverse effects between women and men remained significant after correcting for differences in the mg/kg dose of MDMA ($F_{1,162} = 6.27$; $p < 0.05$ and $F_{1,162} = 6.21$; $p < 0.05$, respectively) or differences in the plasma concentration of MDMA ($F_{1,162} = 6.13$; $p < 0.05$ and $F_{1,162} = 4.63$; $p < 0.05$, respectively).

Frequent and relevant complaints after MDMA and placebo administration are shown in Table 3. The most frequent acute adverse effects after MDMA administration included lack of appetite, dry mouth, difficulty concentrating, cold feet, sweating, bruxism, restless legs, dizziness and hot flushes. Significant subacute (24 h) adverse effects of MDMA included headache, lack of appetite, lack of energy, dry mouth, difficulty concentrating and bruxism. Acute dry mouth, difficulty concentrating, sweating and bruxism were more frequently observed after 125 mg MDMA compared with 75 mg. Acute anxiety was reported by nine subjects (6%) after the 125 mg dose and none of the subjects after the 75 mg dose (Supplementary Table S2). No serious adverse events were reported. Additional adverse events that were reported for the periods from 24 h until 7–14 days after drug administration included the following (0% if not mentioned): headache, 6% after 125 mg MDMA, 7% after 75 mg MDMA, and 5% after placebo; depressed mood, 4% after 125 mg MDMA; common cold: 2% after 125 mg MDMA, 4% after placebo; dysmenorrhoea: 10% after 75 mg MDMA; diarrhoea: 2% after 125 mg MDMA; dizziness: 2% after 125 mg MDMA; gastroenteritis: 3% after 75 mg MDMA, 1% after placebo; emesis: 2% after 125 mg MDMA; toothache: 2% after 125 mg MDMA; jaw muscle soreness: 1% after 125 mg MDMA; migraine: 1% after 125 mg MDMA; back pain: 1% after 125 mg MDMA; sinusitis: 1% after 125 mg MDMA; abdominal pain: 1% after 125 mg MDMA, 1% after placebo; flu: 1% after 125 mg MDMA; bronchitis: 3% after 75 mg MDMA. These adverse events were not reported significantly more frequently after MDMA administration compared with placebo. There were several adverse events that occurred only after placebo: sleep walking, herpes zoster, insomnia, syncope, upper ankle joint distortion, pneumonia, nausea, inflamed mosquito bites, infection of the eye, dry cough, and abscess in the inguinal region combined with tinea of the body (each in 1% of the subjects). In the studies that used 125 mg MDMA, a total of 12 subjects (9%) reported flashbacks 1–3 times and eight times in

Table 1. Subjective effects and adverse effects in men and women.

E_{max}, mean ± SD	Women				Men				Drug		Drug × Dose		Drug × Sex		Drug × Dose × Sex	
	75 mg *n* = 15		125 mg *n* = 68		75 mg *n* = 15		125 mg *n* = 68		$F_{1,162}$	*p* =	$F_{1,162}$	*p* =	$F_{1,162}$	*p* =	$F_{1,162}$	*p* =
	Placebo	MDMA	Placebo	MDMA	Placebo	MDMA	Placebo	MDMA								
Any drug effect	3 ± 6	59 ± 29***	4 ± 8	85 ± 20***###	6 ± 9	56 ± 32***	4 ± 13	73 ± 24***#	652	<0.001	20	<0.001	3.4	0.068	0.2	NS
Good drug effect	6 ± 13	60 ± 28***	3 ± 10	82 ± 25***##	7 ± 12	57 ± 33***	4 ± 15	77 ± 23***##	585	<0.001	20	<0.01	0.9	NS	0.04	NS
Bad drug effect	4 ± 13	35 ± 33***	2 ± 10	24 ± 24***	6 ± 16	7 ± 11+++	2 ± 10	13 ± 22***+	44	<0.001	0.001	NS	16	<0.001	3.6	0.059
List of complaints score, mean ± SD																
Before, *n*	1.5 ± 1.6	2.1 ± 2.5	1.6 ± 2.0	1.6 ± 1.8	0.7 ± 1.4	0.7 ± 1.3	1.4 ± 2.4	1.2 ± 1.8	0.1	NS	0.7	NS	0.5	NS	0.2	NS
Acute, up to 6 h, *n*	2.2 ± 2.2	8.2 ± 4.6**	2.2 ± 2.3	11.6 ± 6.1***	1 ± 1.7	3 ± 2.4+	1.1 ± 2.1	8.5 ± 6.9***##++	99	<0.001	12	<0.001	5.9	<0.05	0.6	NS
Subacute, up to 24 h, *n*	0.9 ± 1.5	4.1 ± 5.2	1.2 ± 2.1	7.1 ± 5.7***	1 ± 1.6	1.6 ± 2.4	0.6 ± 2.3	4.2 ± 5.1***+++	41	<0.001	7.3	<0.01	5.8	<0.05	0.02	NS
MDMA plasma concentration, mean ± SD									Dose		Sex		Dose × Sex			
C_{max}, ng/mL	133 ± 27		252 ± 40###		116 ± 29		195 ± 37###+++		180.97	<0.001	24.99	<0.001	7.5	<0.01		
AUC_6, ng*h/mL	547 ± 127		1058 ± 185###		493 ± 113		838 ± 160###+++		164.85	<0.001	16.79	<0.001	6.14	<0.05		

**$p < 0.01$, ***$p < 0.001$ compared with placebo.
#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared with 75 mg.
+$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$ compared with women.
n: number of subjects; SD: standard deviation; E_{max}: maximal effect; MDMA: 3,4-methylenedioxymethamphetamine; C_{max}: peak plasma concentration; AUC_6: area under concentration-time curve.

Table 2. Maximal effects on vital signs and adverse effects.

MDMA dose	Placebo	MDMA	Placebo	MDMA	Drug		Drug × dose	
	75 mg		125 mg		$F_{1,165}$	p =	$F_{1,164}$	p =
	n = 30		n = 136					
Diastolic blood pressure, mean ± SD, mmHg	77 ± 5	84 ± 8**	79 ± 8	93 ± 10***###	292	<0.001	17.9	<0.001
>90, *n* (%)	0 (0)	7 (23)*	13 (8)	83 (61)***###				
>100, *n* (%)	0 (0)	1 (3)	1 (1)	30 (23)***#				
>110, *n* (%)	0 (0)	0 (0)	0 (0)	4 (3)				
Max, mmHg	90	101	104	130				
Systolic blood pressure, mean ± SD, mmHg	131 ± 11	145 ± 14***	133 ± 15	157 ± 15***###	486	<0.001	15.2	<0.001
>140, *n* (%)	7 (23)	19 (63)**	32 (24)	122 (90)***###				
>160, *n* (%)	0 (0)	3 (10)	8 (6)	51 (38)***##				
>180, *n* (%)	0 (0)	0 (0)	0 (0)	7 (5)*				
Max, mmHg	153	176	175	196				
Heart rate, mean ± SD, beats/min	69 ± 9	83 ± 17***	76 ± 12	95 ± 17***	267	<0.001	2.38	NS
>100, *n* (%)	0 (0)	3 (10)	3 (2)	45 (33)***#				
Max, beats/min	95	134	117	145				
Body temperature, mean ± SD, °C	37.0 ± 0.3	37.2 ± 0.3	37.4 ± 0.5	37.7 ± 0.5***	44.4	<0.001	2.43	NS
>38, *n* (%)	0 (0)	0 (0)	12 (9)	32 (24)**##				
Max, °C	37.7	37.6	38.7	39.1				
List of complaints score								
before, mean ± SD, *n*	1.1 ± 1.5	1.4 ± 2.1	1.5 ± 2.2	1.4 ± 1.8	0.01	NS	0.66	NS
acute, up to 6h, mean ± SD, *n*	1.6 ± 2.1	5.6 ± 4.5**	1.7 ± 2.3	10.0 ± 6.6***###	93.0	<0.001	11.9	<0.001
subacute, up to 24h, mean ± SD, *n*	0.9 ± 1.5	2.9 ± 4.1	0.9 ± 2.2	5.7 ± 5.6***##	35.5	<0.001	6.91	<0.01

n: number of subjects/complaints; SD: standard deviation; MDMA: 3,4-methylenedioxymethamphetamine; NS: not significant.
*p < 0.05, **p < 0.01, ***p < 0.001 compared with placebo.
#p < 0.05, ##p < 0.01, ###p < 0.001 compared with 75 mg.

one subject. Flashbacks reportedly occurred 26 ± 15 h after MDMA administration (range: 8–50 h). No sex differences were found in the reports of adverse events or flashbacks.

Plasma concentrations of MDMA

Plasma concentrations of MDMA for both doses and sexes are shown in Table 1. Peak plasma concentrations of MDMA were significantly and on average 1.8-fold higher after the 125 mg dose (224.1 ng/mL) compared with the 75 mg dose (124.5 ng/mL). Statistical comparison of the dose-normalized C_{max} values revealed a significant difference in the total sample ($F_{1,161}$ = 4.19; p < 0.05), which was significant only in women (p < 0.05), indicating nonlinear pharmacokinetics, but not in men (p = 1.0). Peak concentrations were on average 1.9- and 1.7-fold higher after the 125 mg compared with the 75 mg dose in women and men, respectively. At the same 125 mg dose, women showed on average 1.29- and 1.26-fold higher C_{max} and AUC_6 levels compared with men, respectively.

Effects of MDMA on kidney and liver function and changes in blood cell counts

At EOS and 30 ± 22 days after the last of one or two administrations of MDMA plus pretreatments in some of the studies, plasma creatinine levels and the estimated glomerular filtration rate were unchanged compared with the start of the study and before MDMA administration (Table 4). Similarly, plasma levels of aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transpeptidase were similar at the screening and at the EOS visits. Red blood cell counts and haemoglobin levels decreased, and thrombocyte levels increased during the study. White blood cell counts remained unchanged. Red blood cells and haemoglobin were reduced significantly more in women than in men ($F_{1,159}$ = 8.12; p < 0.01 and $F_{1,159}$ = 12.9; p < 0.001). The decrease did not reach statistical significance in men.

Subjects' interest in using MDMA again

Eighty per cent of the subjects were MDMA-naive, and the rest had very limited experience with MDMA (i.e. ⩽4 exposures at most). One hundred and forty-one subjects were asked whether they would consider taking MDMA again. Twenty-seven subjects (19%) reported that they would probably not take MDMA again under any circumstances. Sixty-six subjects (47%) reported that they would not use MDMA in a recreational setting but might consider participating in another study with MDMA administration under controlled conditions. Forty-eight subjects (34%) indicated that they may consider taking MDMA in a recreational setting but only if the identity, purity and dose were known. Twenty-four of these 48 subjects (50%) had taken illicit drugs previously, including MDMA. Only 13 subjects considered taking MDMA in a club setting. Ninety-five subjects (67%) reported a positive overall MDMA experience, 25 subjects (18%)

Table 3. Acute and subacute adverse effects of MDMA (total *n* = 166).

	Placebo			MDMA		
		Acute	Subacute		Acute	Subacute
	0 h	Up to 6 h	24 h	0 h	Up to 6 h	24 h
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Lack of appetite	2 (1)	3 (2)	1 (1)	4 (2)	98 (59)***	52 (31)***
Dry mouth	1 (1)	3 (2)	3 (2)	1 (1)	91 (55)***	37 (23)***
Difficulty concentrating	6 (4)	10 (6)	4 (2)	5 (3)	76 (46)***	35 (22)***
Cold feet	8 (5)	7 (4)	2 (1)	10 (6)	69 (42)***	10 (6)*
Sweating	2 (1)	0 (0)	0 (0)	0 (0)	68 (41)***	7 (4)*
Bruxism[a]	2 (2)	1 (1)	1 (1)	3 (2)	54 (40)***	19 (14)***
Restless legs	1 (1)	2 (1)	2 (1)	2 (1)	62 (37)***	12 (7)*
Dizziness	2 (1)	2 (1)	3 (2)	5 (3)	57 (34)***	12 (7)*
Hot flushes	1 (1)	0 (0)	0 (0)	1 (1)	52 (31)***	12 (7)***
Headache	9 (5)	27 (16)	25 (15)	8 (5)	42 (25)	55 (33)***
Heart palpitation	1 (1)	2 (1)	1 (1)	1 (1)	40 (24)***	11 (7)**
Lack of energy	9 (5)	23 (14)	5 (3)	8 (5)	38 (23)*	49 (30)***
Nausea	3 (2)	2 (1)	1 (1)	2 (1)	19 (11)***	9 (6)*
Anxiety	0 (0)	0 (0)	0 (0)	2 (1)	9 (6)**	3 (2)

[a]*n* = 134.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with placebo (Fisher's exact test).
MDMA: 3,4-methylenedioxymethamphetamine; *n*: number of subjects.

Table 4. Chronic effects of MDMA on kidney and liver function and blood cell counts.

Kidney and liver function	Screening	EOS	*t*-test	
	n = 164[a]		*t*	*p* =
Creatinine, normal: <97 µm				
Mean ± SD, µm (range)	74 ± 13 (47–115)	73 ± 12 (47–108)	1.89	NS
Glomerular filtration rate C_{CR}, normal: > 90 mL/min				
Mean ± SD, mL/min (range)	122±22 (70–202)	125±24 (64–220)	−1.95	NS
Aspartate aminotransaminase, normal: <34 U/L				
Mean ± SD, U/L (range)	25 ± 7 (14–64)	26 ± 11 (9–131)	−0.93	NS
Alanine aminotransferase, normal: <59 U/L				
Mean ± SD, U/L (range)	19 ± 9 (6–64)	20 ± 10 (5–82)	−0.44	NS
γ-glutamyl transpeptidase, normal: <68 U/L				
Mean ± SD, U/L (range)	19 ± 8 (7–56)	18 ± 8 (7–51)	2.73	< 0.01
Blood cell counts	*n* = 161[b]			
White blood cells, normal: 3.5–10.0 × 10^9/L				
Mean ± SD, ×10^9/L (range)	6.6 ± 1.8 (3.2–14.6)	6.4 ± 1.8 (2.6–16.4)	1.54	NS
Red blood cells, normal: 4.2–6.3 × 10^{12}/L				
Mean ± SD, ×10^{12}/L (range)	4.7 ± 0.4 (3.8–6.1)	4.6 ± 0.4 (3.8–5.8)	4.77	< 0.001
Haemoglobin, normal: 120–180 g/L				
Mean ± SD, g/L (range)	144 ± 12 (121–174)	140 ± 14 (106–181)	6.33	< 0.001
Thrombocytes, normal: 150–450 ×10^9/L				
Mean ± SD, ×10^9/L (range)	263 ± 53 (145–438)	276 ± 56 (164–481)	−4.27	< 0.001

MDMA: 3,4-methylenedioxymethamphetamine; EOS: end of study; *n*: number of subjects; SD: standard deviation; NS: not significant.
[a]Data from two subjects missing.
[b]Data from five subjects missing.

reported a neutral experience and 21 subjects (15%) reported a disappointing or bad experience. No sex differences were observed. Fifty-one subjects (36%) reported that the controlled setting had no impact on their experience, whereas 90 subjects (64%) reported that the controlled setting was important for their type of experience and was reassuring and made them feel safe.

Women rated the setting as significantly more important than men ($F_{1,139} = 12.4$; $p < 0.001$).

Discussion

The present study analysed pooled data from nine placebo-controlled Phase I studies and characterized the acute effects of MDMA in healthy subjects, with a focus on tolerability and clinical safety. The safety considerations included aspects of psychological and physical harm. The present study showed clearly greater positive than negative acute subjective effects. MDMA induced a state of predominantly positive mood across different laboratories (Baggott et al., 2016; Bershad et al., 2016; Dumont and Verkes, 2006; Farre et al., 2007; Hysek et al., 2014a; Kamilar-Britt and Bedi, 2015; Kirkpatrick et al., 2012, 2014a; Kolbrich et al., 2008a; Kuypers et al., 2014; Tancer and Johanson, 2003), with one exception (Parrott et al., 2011). Modest apprehension anxiety was present in some subjects as previously reported (Dumont and Verkes, 2006; Liechti et al., 2001), whereas social anxiety has been shown to decrease (Baggott et al., 2016). In the present study, anxiety was reported in the List of Complaints as an acute adverse effect by 7% of the subjects after 125 mg MDMA administration but not after 75 mg. In our experiments, anxiety could be reduced by verbal support in all of the subjects, and benzodiazepines were not used. There were no cases of severe anxiety or panic attacks. Psychological distress was minimal. Overall positive subjective experiences were retrospectively reported in two-thirds of the participants in the present study, whereas 15% of the subjects were rather disappointed by the effects of MDMA or had bad experiences. In contrast to the findings from the present controlled studies, panic attacks and agitation/aggression are common psychiatric complications in recreational ecstasy users who present to emergency departments (Halpern et al., 2011; Liechti et al., 2005; Wood et al., 2016).

The present study also determined the exact time course of the overall subjective response to MDMA. The average onset time, peak time and effect duration (33 min, 1.6 h and 4.2 h, respectively) were comparable to previous studies (Farre et al., 2004; Hysek and Liechti, 2012; Kolbrich et al., 2008a; Liechti et al., 2001; Mithoefer et al., 2010). The effect duration represented the time an individual experienced a subjective drug effect ⩾10% of his/her own peak response. It has been suggested that the duration of the effect of MDMA (125 mg) could be prolonged by another dose (62.5 mg) 2 h after the initial dose (Mithoefer et al., 2010; Sessa, 2016). However, the short duration of action of MDMA relative to its long plasma half-life (Hysek et al., 2011; Kolbrich et al., 2008b) is attributable to acute pharmacological tolerance. Thus, the subjective effects decline despite high concentrations of MDMA in the body (Hysek et al., 2011, 2012a). Therefore, adding more MDMA to increase the MDMA concentration in the body might not relevantly prolong the limited effect duration and may increase adverse effects (Peiro et al., 2013). In line with this view, the effect duration of the 75 and 125 mg dose of MDMA did not differ in the present study. In contrast to MDMA, acute pharmacological tolerance was not observed with lysergic acid diethylamide (LSD), which is also investigated in substance-assisted psychotherapy (Gasser et al., 2014), and the effect duration of LSD was shown to be dose-dependent (Dolder et al., 2015, 2016).

In terms of potential physical harm, MDMA induced sympathomimetic activation. MDMA produced at least moderate hypertension (systolic blood pressure >160 mmHg) and tachycardia (heart rate >100 beats/min) in one-third of the participants. Thus, although the participants were resting, they presented changes in vital signs that were similar to moderate physical activity. Transient severe hypertension (systolic blood pressure >180 mmHg) was observed in 5% of the participants who received 125 mg MDMA. Severe hypertension may lead to complications including stroke and heart attacks in vulnerable persons. No other signs or symptoms of hypertensive crises (severe headache, shortness of breath, or nosebleeds) were observed in the present study. Similarly, a previous analysis that included 74 subjects from laboratory studies found systolic blood pressure ⩾160 mmHg and ⩾180 mmHg in 32% and 9% of the subjects, respectively (Liechti et al., 2001). Other stimulant drugs, such as methamphetamine, D-amphetamine and methylphenidate, produced comparable cardiovascular stimulation to MDMA when administered as single oral doses (Bershad et al., 2015; Brauer et al., 1996; Hysek et al., 2014b; Kirkpatrick et al., 2012; Martin et al., 1971; Schmid et al., 2014; Wardle and De Wit, 2012).

The present study also found statistically significant MDMA-induced increases in body temperature, but body temperatures did not increase to >39.1°C, consistent with previous studies (Freedman et al., 2005; Kolbrich et al., 2008a; Liechti, 2014). There can be considerable variance in the thermal reactions to acute MDMA (Kolbrich et al., 2008a; Liechti, 2014). Hyperpyrexia (>40°C) is rare but represents the most important life-threatening complication of recreational MDMA use (Grunau et al., 2010; Halpern et al., 2011; Henry et al., 1992; Liechti, 2014; Liechti et al., 2005; Wood et al., 2016). No controlled clinical study of MDMA has reported MDMA-induced hyperpyrexia, possibly because the participants were treated with moderate single doses at rest, were well-hydrated and were not in a hot or crowed environment, unlike in some recreational settings that are known to increase the risk of hyperpyrexia (Dafters, 1995).

In the present study, the participants reported a series of MDMA-induced acute and subacute adverse effects. These effects were consistent with moderate sympathomimetic toxicity, including lack of appetite, dry mouth, cold feet, sweating, restlessness and heart palpitation. The most frequently reported adverse effects 24 h after MDMA administration were headache, lack of energy and lack of appetite. Depressed mood, including emotional irritability, lack of energy, brooding and bad dreams, has previously been reported in up to one-third of subjects, lasting up to three days in some subjects (Liechti et al., 2001). Anxiety, difficulty concentrating, irritability and loss of appetite were also noted in the week following MDMA use in psychotherapy (Mithoefer et al., 2010; Oehen et al., 2013). Similarly, ecstasy users reported mid-week depression following weekend MDMA use (Verheyden et al., 2002).

Hallucinogen persisting perception disorder has been described following recreational ecstasy use (Litjens et al., 2014). One hundred and forty-one participants in the present study were asked at the end of the study whether they experienced flashbacks. Twelve subjects (9%) reported flashbacks, but only within 8–50 h after drug administration. Thus, our study found no evidence of persisting perceptual alterations after MDMA administration in a controlled setting.

In the present study, MDMA did not influence levels of liver enzymes on average one month after administration. Although direct hepatotoxic effects of MDMA are unlikely at the doses used, there is evidence of rare idiosyncratic hepatotoxicity (Atayan et al., 2015; Ellis et al., 1996; Henry et al., 1992; Maharaj et al., 2015). This type of hepatotoxicity is observed with many marketed medications. The decrease in red blood cells and increase in thrombocytes that were observed over the time course of the present study were attributable to the sampling of blood (400–600 mL) for pharmacokinetic analyses and not a consequence of MDMA use.

Although the 125 mg dose of MDMA was 1.67-fold higher than the 75 mg dose, it produced 1.8-fold higher C_{max} and AUC_6 values than the 75 mg dose, indicating nonlinear dose-exposure relationship. This finding is in line with previous studies (de la Torre et al., 2000; Kolbrich et al., 2008a; Schmid et al., 2014) but statistically significant only in women and not in men at the doses tested in the present study. At the same fixed 125 mg dose of MDMA, women showed 1.29-fold higher C_{max} levels than men, which could be explained mainly by their 1.24-fold lower body weight. In the present study, subjective 'bad drug effect' ratings and other adverse acute and after-effects were greater in women than in men. These negative effects of MDMA were significantly greater in women than men even when correcting for the higher dose per body weight or significantly higher plasma levels in women compared with men. Similarly, greater acute and subacute adverse effects were previously reported in women compared with men in an analysis of studies that used body weight-adjusted dosing of MDMA (Liechti et al., 2001). Previous studies also found greater MDMA-induced negative subjective effects, including 'fear of loss of body control' and 'thought disorders' (Liechti et al., 2001), as well as dizziness and sedation (Pardo-Lozano et al., 2012), in women than in men. Higher midweek depression scores in women than in men following weekend MDMA use have also been reported (Verheyden et al., 2002). Women may be more susceptible to the adverse effects of MDMA on the serotonin system (Reneman et al., 2001). Men presented higher acute blood pressure responses than women (Liechti et al., 2001), consistent with the greater cardiovascular stimulant effects of MDMA in men than in women, after dose-normalization in the present study. In contrast, women presented higher heart rates than men in another smaller study (Pardo-Lozano et al., 2012). To our knowledge, no other laboratory studies have specifically reported sex differences in the acute subjective and physiological effects of MDMA. The present findings may be useful for dose selection in MDMA-assisted psychotherapy in patients with PTSD. Women had similar adverse effects scores on the List of Complaints after 75 mg MDMA administration compared with men that received 125 mg, suggesting that the lower dose should be used in female patients. However, significantly higher subjective 'good drug effect' ratings and comparable 'bad drug effect' ratings were reported in women after 125 mg MDMA administration compared with 75 mg. Based on our data, we suggest that a fixed dose of 100 mg may be a good choice in women and comparable to the 125 mg dose in men. This dose adjustment would also account for the approximately 1.3 times higher plasma peak levels of MDMA in women compared with men at the 125 mg dose shown in the present study. Doses up to 187.5 mg have been safely used in mostly women in MDMA-assisted psychotherapy (Mithoefer et al., 2010; Oehen et al., 2013). Whether these high doses are indeed needed in female patients to achieve therapeutic effects requires further study.

The present safety data can in part be applied to the use of MDMA in patients. In patients, MDMA is typically used sporadically 2–3 times and spaced several weeks apart in addition to non-substance assisted psychotherapy (Mithoefer et al., 2010; Oehen et al., 2013; Sessa, 2016). The same 125 mg dose of MDMA was used in the present study and in MDMA-assisted psychotherapy (Mithoefer et al., 2010; Oehen et al., 2013). The study participants typically had no or very little previous MDMA experience, similar to patients. In the research setting that is used in our laboratory, a research assistant is always present with the participants, similar to a therapy session (Mithoefer et al., 2010; Oehen et al., 2013), in contrast to some other experimental settings (Kirkpatrick et al., 2014a). However, the subjective response to MDMA may be different in patients with psychiatric disorders compared with subjects who are screened to be psychiatrically healthy. Consistent with the present data, there have been no reports to date of serious adverse reactions in clinical MDMA studies (Doblin et al., 2014; Mithoefer et al., 2010; Oehen et al., 2013).

The present study has limitations. We included only psychiatrically healthy subjects and the risks of using MDMA in persons with psychiatric disorders may be different (Greer and Tolbert, 1986; Parrott, 2007; Vollenweider et al., 1998) and also need to be investigated. For example, the use of MDMA has been reported to acutely induce negative moods and cognitions, and other undesirable psychological effects in psychotherapy, (Greer and Tolbert, 1986; Parrott, 2007). We included mostly young subjects, but older patients with mainly more cardiovascular risk factors may also be treated in MDMA-assisted psychotherapy. Hyponatraemia is frequently observed in ecstasy intoxications (Halpern et al., 2011; Hartung et al., 2002; Rosenson et al., 2007) but was not assessed in the present study. Additionally, we did not evaluate neurocognitive function or any other correlates of neurotoxicity or long-term problems (Kuypers et al., 2016; Mueller et al., 2016; Parrott, 2013b, 2014; Rogers et al., 2009). Laboratory markers of liver and renal function were only assessed at the end of the study and on average one month after the last MDMA administration not excluding short-lasting transient changes in these parameters. Finally, the sample size of the present pooled analysis of 166 administrations of MDMA alone or 278 administrations of MDMA alone or with a pretreatment was too small to exclude infrequent (0.1–1%) or rare (<0.1%) adverse events. For example, we observed no event of hyperpyrexia among the 278 MDMA administrations and the 95% confidence interval for no event among 278 observations calculated using the binomial distribution is 0–1.3%.

Conclusion

Single-dose administration of MDMA was safe in healthy subjects and in a controlled clinical setting. Acute subjective effects were predominantly positive. Subjective adverse effects were more frequently reported in women than in men. A fixed dose of 100 mg is likely more appropriate for women and comparable to the 125 mg dose in men. These safety data do not raise any concerns related to further studies of MDMA as an adjunct to psychotherapy in controlled medical environments. However,

MDMA dose-dependently induced cardiovascular stimulation, which may be greater in men and should be considered a significant risk for patients with cardiovascular disease. Finally, the risks and benefits of using MDMA in patients with psychiatric disorders need further study.

Acknowledgements

The authors acknowledge the assistance of CM Hysek, A Rickli, Y Schmid and PC Dolder in conducting the clinical studies and M Arends for text editing. PV analysed the data and wrote the manuscript. MEL conceived the study, obtained funding, analysed the data and wrote the manuscript.

Declaration of conflicting interests

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

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Swiss National Science Foundation (grant nos. 323230_126231, 32323B_144996 and 320030_149493).

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2.2. CYP2D6 function moderates the pharmacokinetics and pharmacodynamics of MDMA in a controlled study in healthy subjects

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Pharmacogenetics and Genomics, 2016, Vol. 26, 397-401

CYP2D6 function moderates the pharmacokinetics and pharmacodynamics of 3,4-methylene-dioxymethamphetamine in a controlled study in healthy individuals

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The role of genetic polymorphisms in cytochrome (CYP) 2D6 involved in the metabolism of 3,4-methylene-dioxymethamphetamine (MDMA, ecstasy) is unclear. Effects of genetic variants in CYP2D6 on the pharmacokinetics and pharmacodynamic effects of MDMA were characterized in 139 healthy individuals (70 men, 69 women) in a pooled analysis of eight double-blind, placebo-controlled crossover studies. In CYP2D6 poor metabolizers, the maximum concentrations (C_{max}) of MDMA and its active metabolite 3,4-methylene-dioxyamphetamine were +15 and +50% higher, respectively, compared with extensive metabolizers and the C_{max} of the inactive metabolite 4-hydroxy-3-methoxymethamphetamine was 50–70% lower. Blood pressure and subjective drug effects increased more rapidly after MDMA administration in poor metabolizers than in extensive metabolizers. In conclusion, the disposition of MDMA and its effects in humans are altered by polymorphic CYP2D6 activity, but the effects are small because of the autoinhibition of CYP2D6.
***Pharmacogenetics and Genomics* 26:397–401**

Pharmacogenetics and Genomics 2016, **26**:397–401

Keywords: cytochrome P450, CYP2D6, 4-hydroxy-3-methoxymethamphetamine, 3,4-methylene-dioxyamphetamine, 3,4-methylene-dioxymethamphetamine, pharmacokinetics

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Received 4 February 2016 **Accepted** 5 May 2016

Interindividual differences in the clinical toxicity of 3,4-methylene-dioxymethamphetamine (MDMA) may result from alterations in drug-metabolizing enzymes, such as cytochrome P450 monooxygenases (CYPs), that are involved in the metabolism of MDMA [1,2] shown in Supplementary Fig. S1, Supplemental digital content 1, *http://links.lww.com/FPC/B41*.

Only limited controlled data are available on the pharmacogenetics/toxicogenetics of MDMA [2]. The aim of the present study was to investigate the role of CYP2D6 in the PK of MDMA in a prospectively designed pooled analysis of eight double-blind, placebo-controlled, crossover studies in a total of 139 healthy individuals (methods shown in Supplemental digital content 1, *http://links.lww.com/FPC/B41*). This is the first study with a meaningful sample size and the inclusion of several individuals with relevantly impaired function. We also performed both genotyping and phenotyping and used genetic activity scores [3] for CYP2D6 activity classification.

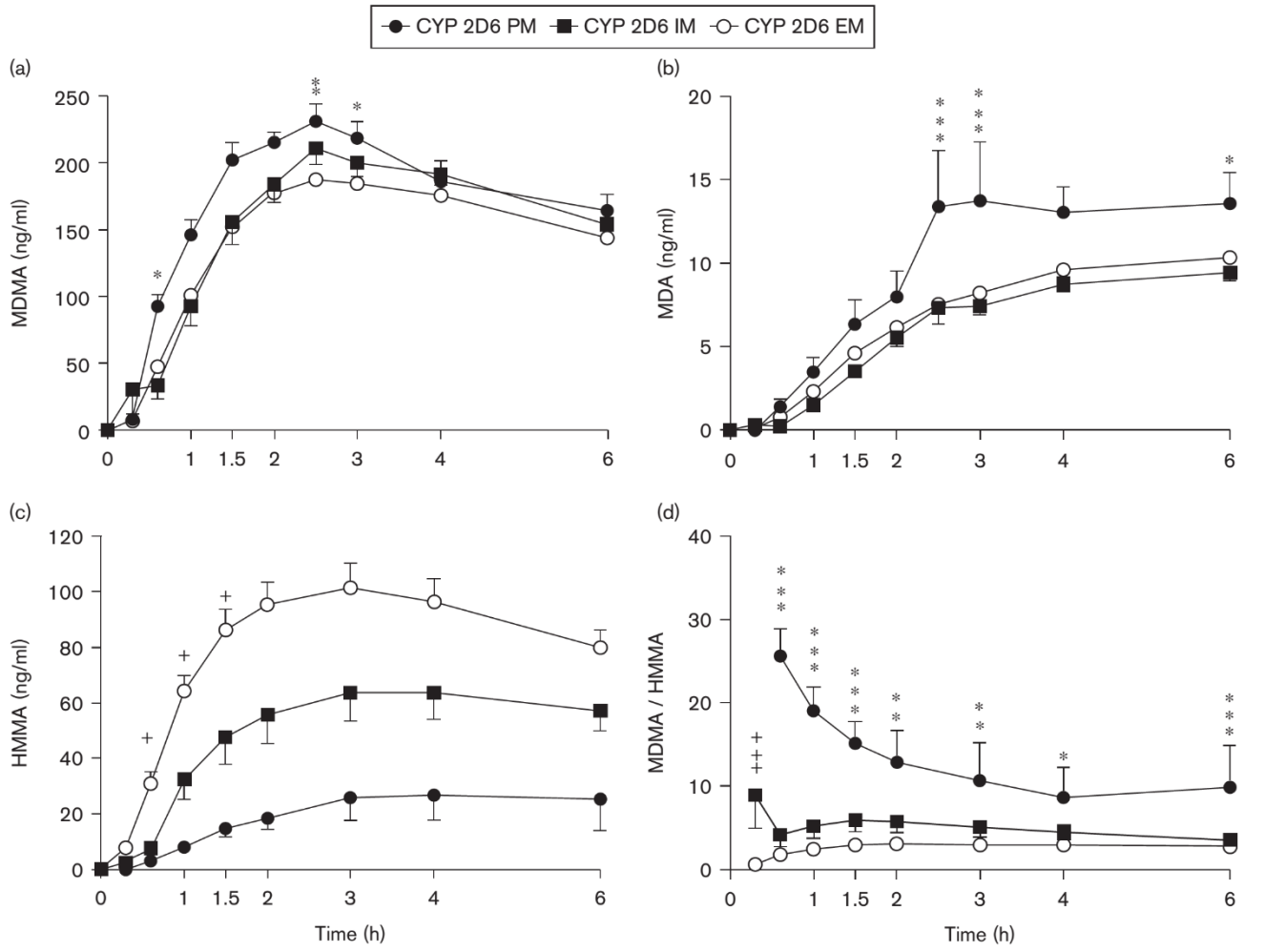
We found that CYP2D6 activity significantly altered plasma MDMA levels up to 3 h after drug administration (i.e. during drug absorption/distribution), but not beyond 3 h (i.e. during drug elimination; Supplementary Table S1, Supplemental digital content 1, *http://links.lww.com/FPC/B41*, Fig. 1a and Supplementary Fig. S2c, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). A significant main effect of the CYP2D6 genotype on the C_{max} of MDMA was found ($F_{2,136}=4.19$, $P=0.02$), with higher C_{max} values in CYP2D6 poor metabolizers (PMs) compared with extensive metabolizers (EMs; $P=0.049$; Supplementary Fig. S1a, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6 activity score similarly altered the C_{max} of MDMA (Supplementary Table S1 and Supplementary Fig. S2b, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). MDMA area under the concentration–time curve up to 6 h (AUC_6) values also varied across CYP2D6 genotype groups ($F_{2,136}=5.25$, $P<0.01$), with PMs showing higher AUC_6 values compared with EMs ($P<0.01$; Supplementary Fig. S2d, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). MDMA AUC_6 values also differed between genotype-based CYP2D6 activity groups (Supplementary Table S1 and Supplementary Fig. S2e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP 2D6 genotype

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (*www.pharmacogeneticsandgenomics.com*).

1744-6872

DOI: 10.1097/FPC.0000000000000231

Fig. 1



CYP2D6 phenotypes predicted by genotyping altered the pharmacokinetics of MDMA (a), MDA (b), HMMA (c), and the MDMA/HMMA ratio (d). Lower CYP2D6 function as in PMs resulted in higher MDMA (a) and MDA (b) plasma levels, lower HMMA plasma levels (c), and higher MDMA/HMMA plasma concentration ratios (d) compared with higher CYP2D6 function as in EMs. The data are expressed as the mean ± SEM in seven PMs, 19 IMs, and 113 EMs for MDMA and MDA. MDMA was administered at $t = 0$ h. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for PMs compared with EMs and $^{+}P < 0.05$ for EMs compared with IMs at the corresponding time. CYP2D6, cytochrome 2D6; EMs, extensive metabolizers; HMMA, 4-hydroxy-3-methoxymethamphetamine; IM, intermediate metabolizers; MDA, 3,4-methylene-dioxyamphetamine; MDMA, 3,4-methylene-dioxymethamphetamine; PMs, poor metabolizers.

altered the concentration–time curve of the minor active metabolite 3,4-methylene-dioxyamphetamine (MDA) (Fig. 1b). The C_{max} and AUC_6 of MDA varied across genotypes ($F_{2,136} = 8.82$, $P < 0.01$, and $F_{2,136} = 9.09$, $P < 0.001$, respectively), which were higher in PMs compared with intermediate metabolizers (IMs; $P < 0.001$) and EMs ($P < 0.001$; Supplementary Fig. S3a and d, Supplemental Digital Content 1, *http://links.lww.com/FPC/B41*). The C_{max} and AUC_6 of MDA were also higher in individuals with a CYP2D6 activity score of 0 compared with all of the other CYP2D6 activity groups (Supplementary Table S1 and Supplementary Fig. S2b, c, e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6 genotype influenced the concentration–time curve of the inactive metabolite 4-hydroxy-3-methoxymethamphetamine (HMMA; Fig. 1c). The CYP2D6 genotype altered the C_{max} and AUC_6 of HMMA ($F_{2,73} = 3.50$, $P = 0.03$ and 5.22, $P < 0.01$; Supplementary Table S1 and Supplementary Fig. S4a and d, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). C_{max} and AUC_6 of HMMA were higher in individuals with high CYP2D6 activity (activity score of 2) compared with individuals with low activity (activity score of 0.5; Supplementary Table S1 and Supplementary Fig. S4b, c, e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6 genotype altered the unconjugated HMMA concentrations similar to those of HMMA (Supplementary Table S2 and Supplementary Fig. S5, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6

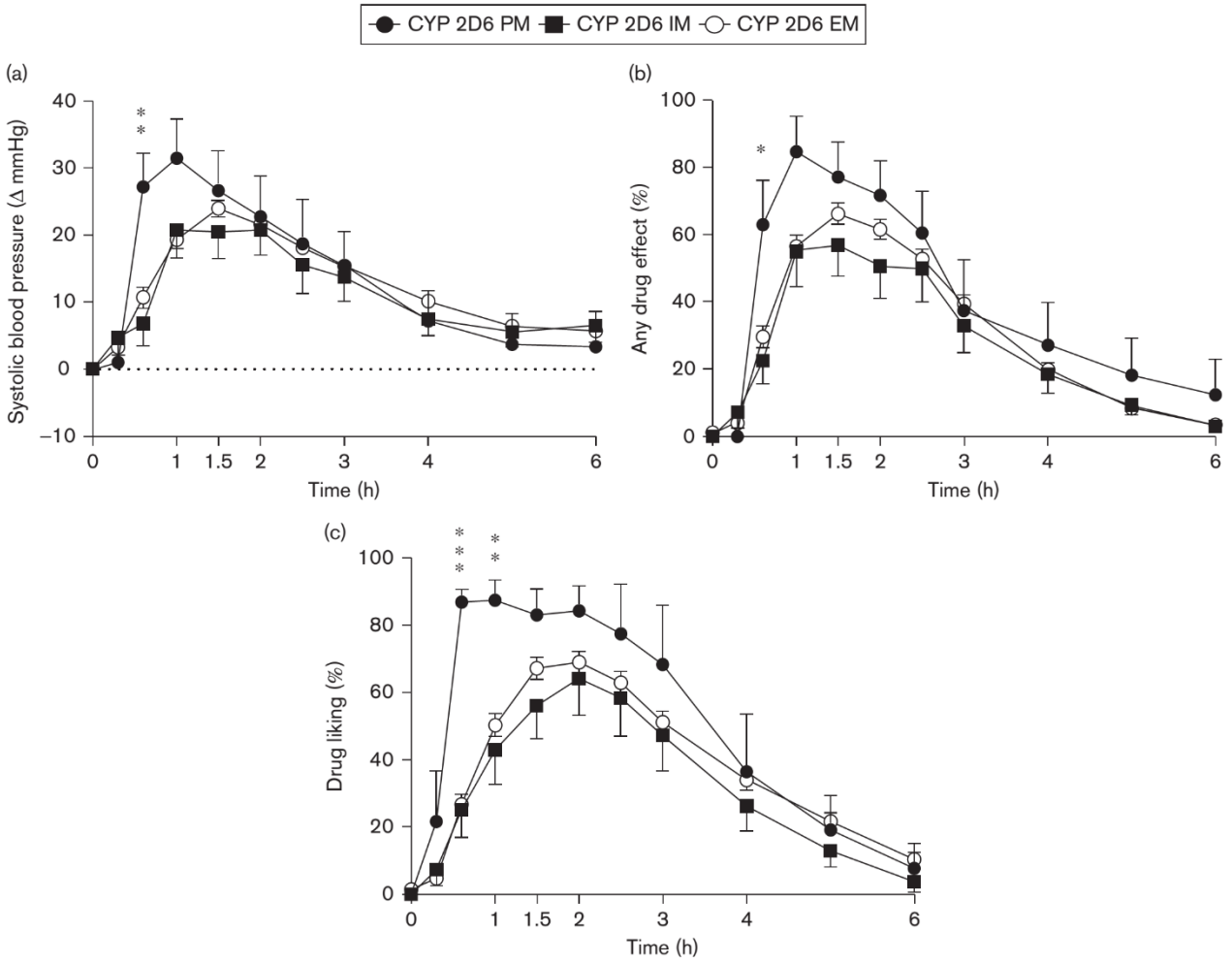
genotype altered the MDMA/HMMA C_{max} and AUC_6 ratios ($F_{2,73} = 10.98$ and 10.08, both $P < 0.001$; Fig. 1d). CYP2D6 PMs had higher MDMA/HMMA C_{max} and AUC_6 ratios compared with IMs ($P < 0.01$ and $P < 0.05$) and EMs (both $P < 0.001$; Fig. 1d, and Supplementary Fig. S6a and d, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). Individuals with a CYP2D6 activity score of 2 had lower MDMA/HMMA C_{max} and AUC_6 ratios compared with individuals with activity scores of 0, 0.5, and 1 (Supplementary Table S1 and Supplementary Fig. S6b, c, e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The effects of CYP2D6 on the biotransformation of MDMA to HMMA over time are also evident in the hysteresis plots in Supplementary Fig. S8, Supplemental digital content 1, *http://links.lww.com/FPC/B41*. A correlation was found between the AUC_6 values of MDMA and MDA ($R_s = 0.18$, $P < 0.05$, $N = 139$), indicating that higher MDMA levels resulted in higher MDA levels. In contrast, higher AUC_6 values of MDMA were negatively correlated with those of HMMA ($R_s = -0.33$, $P < 0.01$, $N = 76$) or unconjugated HMMA ($R_s = -0.39$, $P < 0.001$, $N = 124$), consistent with the impaired conversion of MDMA into HMMA.

CYP2D6 activity influenced both the cardiovascular and the psychotropic responses to MDMA. Both the MDMA-induced blood pressure response and subjective drug effects increased more rapidly in genotype-based CYP2D6 PMs compared with IMs and EMs (Fig. 2), reflected by group differences early in time after MDMA administration, whereas maximal effects did not differ. Elevations in systolic blood pressure were greater in PMs compared with IMs ($P = 0.02$) and EMs ($P = 0.01$) at 0.6 h ($F_{2,135} = 3.50$, $P = 0.03$) and also tended to be greater at 1 h ($F_{2,135} = 2.49$, $P = 0.09$) after drug administration. Subjective 'any drug effect' ratings were higher in PMs compared with both IMs and EMs at 0.6 h ($P < 0.05$). 'Drug liking' ratings were higher in PMs compared with both IMs and EMs at 0.6 h ($P < 0.001$) and 1 h ($P < 0.01$) and tended to be higher at 1.5 h. No effects of the CYP2D6 genotype were found on heart rate or body temperature. The MDMA/HMMA ratio at 0.6 h, which inversely reflects CYP2D6 activity, was associated with the MDMA-induced elevations in systolic blood pressure ($R_s = 0.41$, $P < 0.001$, $N = 76$), any drug effect ($R_s = 0.42$, $P < 0.001$), and drug liking ($R_s = 0.40$, $P < 0.001$) at 0.6 h. The association remained significant for systolic blood pressure up to 1.5 h and for any drug effect and drug liking up to 4 and 6 h, respectively.

As expected, the CYP2D6 phenotype was associated with the CYP2D6 genotype. The DM/DX ratio in the group of PMs with an activity score 0 was significantly greater than in all of the other activity score groups, but these ratios did not differ between IMs and the different EM groups or within the EM groups (Supplementary Table S1 and Supplementary Fig. S9, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). Despite some discordance between individual genotypes and phenotypes, the effects of the CYP2D6 genotype (Fig. 1) and the CYP2D6 phenotype (Supplementary Fig. S10, Supplemental digital content 1, *http://links.lww.com/FPC/B41*) groups on MDMA and metabolite plasma–time curves were very similar. Additional findings are presented in the Supplementary material, Supplemental digital content 1, *http://links.lww.com/FPC/B41*.

Taken together, the present study found an increase in MDMA exposure and more rapid increases in the cardiostimulant and psychotropic effects of MDMA in individuals with poor CYP2D6 function. The mean C_{max} levels of MDMA in the seven CYP2D6 PMs in the present study were only 1.15 times higher than in 111 EMs, and differences in the PK or subjective and blood pressure responses to MDMA were only present in the first hour after MDMA administration. Thus, interindividual differences in CYP2D6 function have a small and transient effect on the plasma concentration of MDMA and its pharmacodynamic effects. These findings are consistent with the mechanism-based inhibition of CYP2D6 by MDMA, turning all individuals into functional CYP2D6 PMs within 1 h after MDMA administration [4,5]. Consistent with our experimental data, a physiologically based PK model estimated that the absence of CYP2D6 function in PMs would increase MDMA C_{max} levels by only 36% [4]. Consistent with the findings in genetically impaired CYP2D6 PMs in the present study, the pharmacological inhibition of CYP2D6 function increased the C_{max} and AUC values of MDMA by 15–20 and 10–30%, respectively [6,7]. The mean HMMA AUC_6 in CYP2D6 PMs was four times lower (24%) compared with EMs. This more pronounced effect of the CYP2D6 genotype on HMMA concentrations compared with MDMA concentrations could be clinically relevant because metabolites that are formed by CYP2D6, including HHMA and HMMA, have been implicated in MDMA-induced neurotoxicity and hepatotoxicity [8], hyponatremia, and hyperthermia [9]. Low CYP2D6 activity was associated with higher MDA levels. Although CYP2D6 has been shown to be involved in the *N*-demethylation of MDMA to MDA in-vitro [10], individuals with low CYP2D6 function appear to produce more MDA because the main metabolic pathway of CYP2D6-mediated MDMA-HMMA conversion is less active, thus resulting in higher MDMA levels and compensatory MDA formation. Altogether, statistically significant but only moderate clinical effects of CYP2D6 genetics or pharmacological CYP2D6 inhibition on MDMA disposition were found, effects that are attributable to the self-inhibition of CYP2D6 and phenoconversion. CYP2D6 PMs were not overrepresented in fatalities associated with ecstasy [11]. However, individuals may be at increased risk when more than one CYP system is genetically impaired or pharmacologically

Fig. 2



CYP2D6 phenotypes predicted by genotyping modulated the blood pressure and subjective responses to MDMA. Systolic blood pressure (a) and subjective effects, including any drug effects (b) and drug liking (c), increased more rapidly in CYP2D6 PMs compared with IMs and EMs. The data are expressed as the mean ± SEM in seven PMs, 19 IMs, and 113 EMs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, PMs compared with IMs or EMs at the corresponding time. CYP2D6, cytochrome 2D6; EMs, extensive metabolizers; IM, intermediate metabolizers; MDMA, 3,4-methylenedioxymethamphetamine; PMs, poor metabolizers.

inhibited. Severe or fatal MDMA toxicity and high plasma exposure to MDMA were noted in cases of co-use with ritonavir, which blocks not only CYP2D6 but also CYP2B6 and CYP3A4. In fact, because CYP2D6 is inhibited by MDMA itself in all individuals, polymorphisms in other CYPs (e.g. CYP1A2, CYP2C19, and CYP2B6) and the pharmacological inhibition of CYP3A4 may actually be more clinically relevant than CYP2D6 genetics. Supporting this view, CYP1A2 function was shown to increase after MDMA administration in another study [12], which possibly compensated for the inhibition of CYP2D6 function by MDMA. We have assessed the effects of CYPA12, CYP2C19, and CYP2B6 polymorphisms on the PK of MDMA in this study and the findings will be published separately. There were no moderating effects of these polymorphisms on the effects of CYP2D6.

The present study has limitations. We analyzed a pooled sample of different studies and different doses and the different CYP2D6 activity groups were not equally represented across studies and dose groups. In addition, there were only seven PMs and all were in the high-dose group. However, an analysis of the high-dose group only produced similar results as the analysis of the total sample.

In conclusion, the present study evaluated the CP2D6 pharmacogenetics of the PK of an important recreational substance in a unique cohort that included a relatively large number of individuals and a wide spectrum of CYP functions, including CYP2D6 PMs. The study found

only moderate increases in the plasma exposure to MDMA and its active metabolite MDA and a more rapid onset of associated blood pressure and psychotropic effects in individuals with poor CYP2D6 function. Although CYP2D6 PMs may be at increased risk for MDMA toxicity, the self-inhibition of CYP2D6 reduces the impact of the CYP2D6 genotype on MDMA PK.

Acknowledgements

This study was supported by the Swiss National Science Foundation (grant no. 320030_1449493).

Conflicts of interest

There are no conflicts of interest.

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2.3. Pharmacogenetics of Ecstasy: CYP1A2, CYP2C19, and CYP2B6 polymorphisms moderate pharmacokinetics of MDMA in healthy subjects

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European Neuropsychopharmacology, 2017, Vol. 27, 232-238

European Neuropsychopharmacology (2017) 27, 232–238

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Pharmacogenetics of ecstasy: CYP1A2, CYP2C19, and CYP2B6 polymorphisms moderate pharmacokinetics of MDMA in healthy subjects ☆

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Received 19 May 2016; received in revised form 1 December 2016; accepted 5 January 2017

KEYWORDS
3,4-Methylenedioxy-methamphetamine;
3,4-Methylenedioxy-yamphetamine;
Pharmacokinetics;
CYP1A2;
CYP2C19;
CYP2B6

Abstract
In vitro studies showed that CYP2C19, CYP2B6, and CYP1A2 contribute to the metabolism of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) to 3,4-methylenedioxyamphetamine (MDA). However, the role of genetic polymorphisms in CYP2C19, CYP2B6, and CYP1A2 in the metabolism of MDMA in humans is unknown. The effects of genetic variants in these CYP enzymes on the pharmacokinetics and pharmacodynamics of MDMA were characterized in 139 healthy subjects (69 male, 70 female) in a pooled analysis of eight double-blind, placebo-controlled studies. MDMA-MDA conversion was positively associated with genotypes known to convey higher CYP2C19 or CYP2B6 activities. Additionally, CYP2C19 poor metabolizers showed greater cardiovascular responses to MDMA compared with other CYP2C19 genotypes. Furthermore, the maximum concentration of MDA was higher in tobacco smokers that harbored the inducible CYP1A2 rs762551 A/A genotype compared with the non-inducible C-allele carriers. The findings indicate that CYP2C19, CYP2B6, and CYP1A2 contribute to the metabolism of MDMA to MDA in humans. Additionally, genetic polymorphisms in CYP2C19 may moderate the cardiovascular toxicity of MDMA.

☆Trial registration: ClinicalTrials.gov: http://www.clinicaltrials.gov, No: NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT013861177, NCT01465685, and NCT01771874.

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http://dx.doi.org/10.1016/j.euroneuro.2017.01.008
0924-977X/

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) produces feelings of well-being, enhanced emotional empathy, and prosociality (Hysek et al., 2014a) and is used recreationally and as an adjunct to psychotherapy (Oehen et al., 2013). The recreational use of ecstasy has been associated with potentially severe toxicity, including agitation, hypertension, and hyperthermia (Halpern et al., 2011; Liechti, 2014; Liechti et al., 2005). Individually increased vulnerability to the clinical toxicity of MDMA may result from alterations in drug-metabolizing enzymes, such as cytochrome P450 monooxygenases (CYPs), that are involved in the metabolism of MDMA (de la Torre et al., 2012; Rietjens et al., 2012). Specifically, MDMA is O-demethylenated primarily by CYP2D6 to 3,4-dihydroxymethamphetamine (HHMA), which is then O-methylated to 4-hydroxy-3-methoxymethamphetamine (HMMA) by catechol-O-methyltransferase (COMT) (de la Torre et al., 2012; Kreth et al., 2000; Meyer et al., 2008; Segura et al., 2005), the main inactive metabolite of MDMA in humans (Kolbrich et al., 2008; Schindler et al., 2014). Additionally, MDMA is N-demethylated by CYP1A2, CYP2B6, CYP3A4, and CYP2C19 (Kreth et al., 2000; Meyer et al., 2008) to the minor active metabolite 3,4-methylenedioxyamphetamine (MDA) (de la Torre et al., 2004; Hysek et al., 2012c; Kolbrich et al., 2008).

Only limited controlled data are available on the pharmacogenetics/toxicogenetics of MDMA (Pardo-Lozano et al., 2012; Rietjens et al., 2012). The pharmacokinetics (PK) of a drug is in part determined by genetic variants in drug-metabolizing enzymes. Genetic polymorphisms in CYP2D6 have been shown to influence MDMA metabolism in humans (de la Torre et al., 2005, 2012; Hysek et al., 2013, 2014b; O'Mathuna et al., 2008; Schmid et al., 2016; Yang et al., 2006) but the role of other CYPs is unknown. In vitro studies indicate that CYP2D6 is responsible for most of the clearance of MDMA, but CYP1A2, CYP2B6, and CYP2C19 also contribute to the N-demethylation of MDMA to MDA, and their role may become more important in cases of overdose (Meyer et al., 2008) or in CYP2D6 poor metabolizers (PMs; de la Torre et al., 2012). However, the effects of polymorphisms in these CYPs on the metabolism of MDMA in humans have not yet been investigated. Therefore, the aim of the present study was to explore whether genetic variants in the CYP2C19, CYP2B6, and CYP1A2 genes alter the conversion of MDMA to MDA in humans. No common CYP1A2 loss-of-function polymorphisms have been identified to date. However, CYP1A2 is inducible by tobacco smoking in subjects with the common single-nucleotide polymorphism (SNP) rs762551 A/A genotype compared with the C/A and C/C genotypes (Sachse et al., 1999). Therefore, we tested whether MDA formation is greater in tobacco smokers who carry the A/A genotype to assess the contribution of CYP1A2 to the metabolism of MDMA for the first time in humans. Finally, we tested whether CYP2C19, CYP2B6 or CYP1A2 genotype influenced the pharmacodynamics of MDMA.

2. Experimental procedures

2.1. Study design

This was a prospectively designed pooled analysis of eight double-blind, placebo-controlled, crossover studies in healthy subjects (Hysek et al., 2012a, 2013, 2012b, 2011, 2012c, 2014b; Schmid et al., 2014, 2015) including a total of 142 subjects. The prespecified primary endpoint of the pooled analysis was to assess the effects of polymorphisms in CYP enzymes on the PK of MDMA in all of the studies. In seven studies each including 16 subjects, a total of 112 subjects received MDMA at a dose of 125 mg, placebo, one of eight pretreatments plus MDMA, or the pretreatment alone (Hysek et al., 2012a, 2013, 2012b, 2011, 2012c, 2014b; Schmid et al., 2015). In one study, 30 subjects received MDMA at a dose of 75 mg, placebo, or methylphenidate (Schmid et al., 2014). Washout periods between treatment periods were at least 7 days. Only data after the administration of MDMA alone without other treatments were included in this analysis and the washout was considered sufficiently long to exclude any effects of the other treatments on the effects of MDMA alone. All of the studies were registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01616407, NCT01465685, and NCT01771874). All of the studies were approved by the local ethics committee and the Swiss Agency for Therapeutic Products (Swissmedic) and conducted in accordance with the Declaration of Helsinki. The administration of MDMA in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Informed consent was obtained from all participants included in the studies.

2.2. Subjects

A total of 142 healthy European/Caucasian subjects, aged 18–45 years, were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, one participant did not give consent for genotyping, and a full concentration-time profile could not be obtained in one participant, resulting in data from 139 participants (69 male, 70 female, mean age ±SD: 24.9±4.1 years; range: 18–44 years) that were included in the analysis. A total of 110 subjects (54 male, 56 female) received 125 mg MDMA (mean±SD: 1.9±0.3 mg/kg), and 29 subjects (15 male, 14 female) received 75 mg MDMA (1.1±0.1 mg/kg) (Hysek et al., 2012a, 2012b, 2012c; Hysek and Liechti, 2012). The exclusion criteria were a history of psychiatric disorders, physical illness, a lifetime history of using illicit drugs more than five times (with the exception of past cannabis use), illicit drug use within the last 2 months, illicit drug use during the study, determined by urine tests that were conducted before the test sessions, and the use of drugs that interact with CYP function. Tobacco smoking (>10 cigarettes/day) was an exclusion criterion, but light tobacco smokers (6–10 cigarettes/day) and very light tobacco smokers (1–5 cigarettes/day) were included in the study. The detailed exclusion criteria were reported elsewhere (Hysek et al., 2012a, 2012b, 2012c; Hysek and Liechti, 2012).

2.3. Study drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 or 75 mg. Similar doses are found in ecstasy pills (Brunt et al., 2012) and have been used in clinical studies (Oehen et al., 2013). The dose range was 0.8–2.7 mg/kg (mean=1.7 mg/kg).

2.4. Blood sampling and drug analysis

Blood samples were collected in lithium heparin tubes 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, and 6 h after administration of MDMA or placebo and immediately centrifuged. Plasma was stored at -20° C until analysis. Plasma concentrations of MDMA, MDA, and HMMA were determined as previously described (Hysek et al., 2013, 2012c). HMMA concentrations were determined after enzymatic deglucuronidation in 76

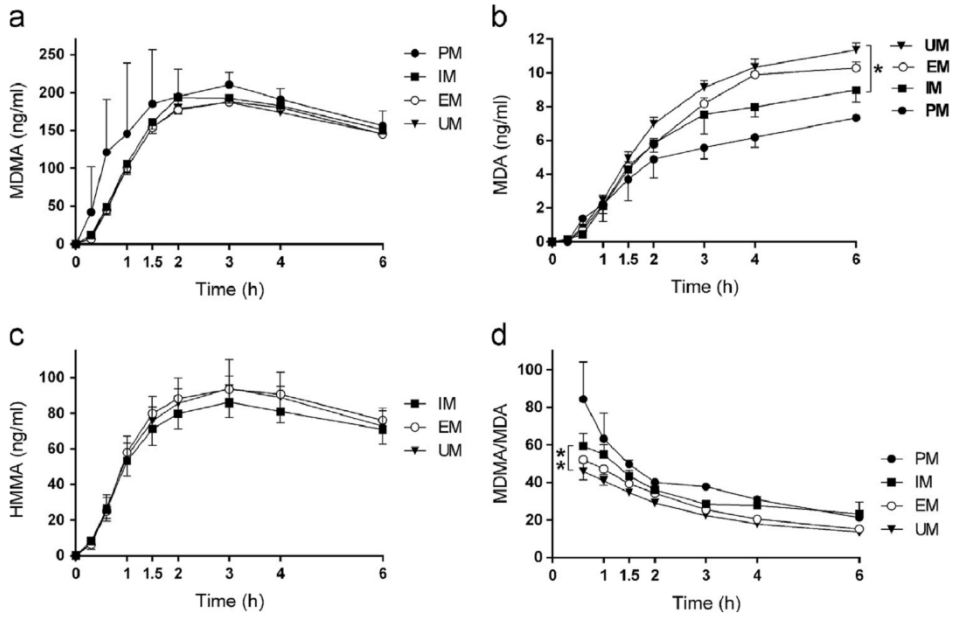

Figure 1 Effect of the CYP2C19 polymorphism on the plasma concentrations of MDMA **(a)**, MDA **(b)**, HMMA **(c)** and the MDMA/MDA ratio **(d)**. The MDMA/MDA ratio **(d)** decreased with increasing CYP2C19 function, while MDA levels **(b)** increased with decreasing CYP2C19 function, indicating influence on the *N*-demthylation of MDMA to MDA. The data are expressed as mean ± SEM in 2 PMs, 24 IMs, 66 EMs and 47 UMs (except for HMMA). *$P<0.05$ for the AUC_6 of MDA in UM vs. IM based on a significant main effect over all genotypes ($F_{3,135}=3.54$, $P<0.05$). **$P<0.01$ for the MDMA/MDA AUC_6 ratio in UM vs. IM based on a significant main effect over all genotypes ($F_{3,135}=5.55$, $P<0.01$).

subjects. The lower limit of quantification concentrations were 1 ng/ml for all analytes (Hysek et al., 2012c).

2.5. Pharmacodynamic measures

Blood pressure, heart rate, and body temperature were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, and 6 h after MDMA or placebo administration as previously described (Hysek and Liechti, 2012; Hysek et al., 2011). The rate pressure product (RPP), a measure of the overall cardiostimulant effects, was calculated as systolic blood pressure × heart rate. Core (tympanic) temperature was assessed using a GENIUS™ 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA). Subjective effects were measured using Visual Analog Scales (VAS) (Hysek et al., 2012a, 2012b, 2012c; Hysek and Liechti, 2012).

2.6. Genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. Genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne, Switzerland). CYP2C19 TaqMan drug metabolism genotyping assays were used to determine the most common loss-of-function SNPs rs4244285 (CYP2C19*2, c.681G>A, assay: C_25986767_70) and rs28399504 (CYP2C19*4, c.1A>G, assay: C_30634136_10) and gain-of-function SNP rs12248560 (CYP2C19*17, c.806C>T, assay: C_469857_10) (Hicks et al., 2013). Predicted CYP2C19 PMs included CYP2C19*2/*2, intermediate metabolizers (IMs) included CYP2C19*1/*2 and CYP2C19*2/*17, extensive metabolizers (EMs) included CYP2C19*1/*1, and ultra-rapid metabolizers (UMs) included both CYP2C19*17/*17 and CYP2C19*1/*17 (Hicks et al., 2013). For CYP1A2, the CYP1A2 TaqMan drug metabolism genotyping assay (C_8881221_40) was used to determine the common SNP rs762551, the sole variant of the CYP1A2*1F haplotype. For CYP2B6, we determined the reduced-activity SNP rs3745274 (516G>T, CYP2B6*6 or CYP2B6*9, assay: C_7817765_60).

2.7. Pharmacokinetic analyses

Peak plasma concentrations (C_{max}) were obtained directly from the observed data. The area under the concentration-time curve (AUC) from 0 to 6 h after dosing (AUC_6) was calculated using the linear trapezoidal method. Plasma concentrations were determined up to 6 h after MDMA administration because the aim of the study was to assess potential changes in MDMA plasma levels while relevant pharmacodynamics effects or MDMA are present (Hysek et al., 2011, 2012c).

2.8. Statistical analyses

The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). Group differences were analyzed using one-way analysis of variance (ANOVA), with genotype as between-subjects group factors, followed by the Tukey *post hoc* test. Smoking status was included as factor with CYP1A2 genotype. To account for differences in body weight and dosing, plasma levels were dose-normalized to the mean dose per body weight (1.7 mg/kg), and the mg/kg dose of MDMA was included as a covariate in the analysis of the pharmacodynamic effects. Sensitivity analyses were also conducted using only the 125 mg MDMA dose to exclude confounding by dose level. Additionally, CYP2D6 activity was determined using the dextromethorphan/dextrorphan ratio (Schmid et al., 2016) and the analyses were replicated in 111 subjects phenotyped as CYP2D6 EMs and after exclusion of 19 IMs and 9 PMs to exclude confounding by CYP2D6 activity.

3. Results

3.1. Effects of CYP2C19

Effects of the CYP2C19 genotype on the pharmacokinetics of MDMA are shown in Figure 1 and Supplementary Table S1. MDMA plasma levels increased more rapidly in the two CYP2C19 PMs (Figure 1a) but this effect was not significant. The CYP2C19 genotype significantly influenced the AUC_6 of MDA ($F_{3,135}=3.54$, $P<0.05$, Figure 1b) and the MDMA/MDA AUC_6 ratio ($F_{3,135}=5.55$, $P<0.01$, Figure 1d), but not HMMA concentrations (Figure 1c). CYP2C19 genotype altered the E_{max} of the RPP ($F_{3,134}=2.92$, $P<0.05$) with higher RPP values in CYP2C190 PMs compared with IMs and UMs (both $P<0.05$, Figure 2). CYP2C19 genotype had no effects on body temperature or any of the subjective effects of MDMA.

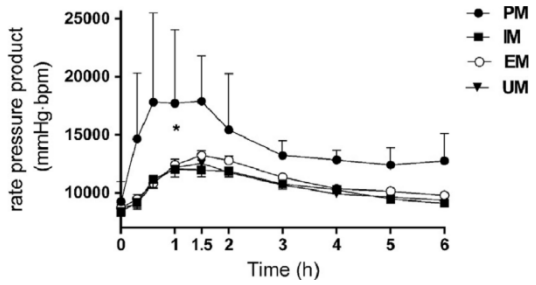

Figure 2 Effect of CYP2C19 polymorphism on cardiovascular stimulation. The heart rate systolic blood pressure product increased more in the two CYP2C19 PMs compared to IMs or UMs (*for both $P<0.05$ based on a significant main effect of genotype on E_{max} values: $F_{3,134}=2.92$, $P<0.05$). The data are expressed as the mean ± SEM in 2 PMs, 24 IMs, 66 EMs and 47 UMs.

3.2. Effects of CYP2B6

Effects of the CYP2B6 rs3745274 SNP (G/G vs. G/T vs. T/T) on the pharmacokinetics of MDMA are shown in Figure 3 and Supplementary Table S2. The CYP2B6 genotype significantly altered the MDMA C_{max} ($F_{2,136}=3.72$, $P<0.05$, Figure 3a), with a higher concentration in subjects within the T/T compared to the G/G genotype ($P<0.05$). The CYP2B6 genotype significantly influenced the MDMA/MDA AUC_6 ratio ($F_{2,136}=3.67$, $P<0.05$, Figure 3d) with higher ratios in the T/T vs. G/T or G/G group (both $P<0.05$), but had no significant effects on plasma levels of MDA (Figure 3b) or HMMA (Figure 3c). CYP2B6 genotype did not alter the autonomic or subjective effects of MDMA.

3.3. Interacting effects of CYP1A2 and smoking

Smoking status interacted with CYP1A2 genotype (inducible rs762551 A/A vs. non-inducible A/C and C/C) to affect MDA C_{max} and AUC_6 values ($F_{5,133}=5.56$, $P<0.001$ and 4.04, $P<0.01$, respectively; Table S3 and Figure 4). Smoking status altered MDA formation only in subjects with the inducible rs762551 A/A genotype, with higher MDA formation in light tobacco smokers (6–10 cigarettes/day) compared with nonsmokers and very light smokers (1–5 cigarettes/day, both $P<0.001$; Figure 4). No effect of

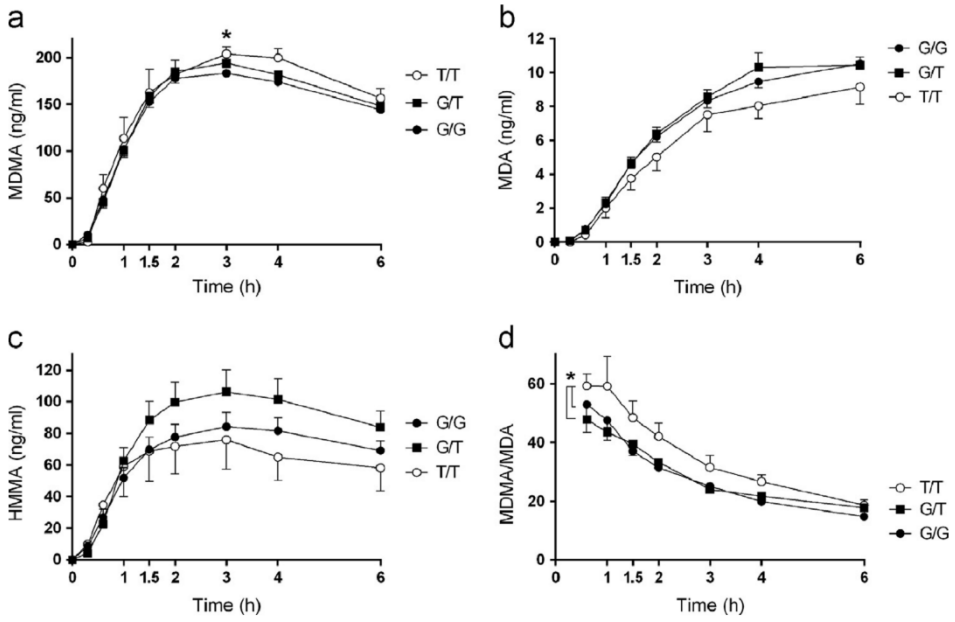

Figure 3 Effect of CYP2B6 genotype on the plasma concentrations of MDMA **(a)**, MDA **(b)**, HMMA **(c)** and the MDMA/MDA ratio **(d)**. Maximal MDMA concentrations were higher in the reduced-function CYP2B6 rs3745274 T/T genotype group compared with the normal functioning G/G and G/T genotype groups **(a)** (*for both $P<0.05$). The CYP2B6 genotype also significantly influenced the MDMA/MDA ratio **(d)** with higher ratios in the T/T vs. G/T groups and T/T vs. G/G group (*for both $P<0.05$), but had no significant effects on plasma levels of MDA **(b)** or HMMA **(c)**. The data are expressed as mean ± SEM in 78G/G, 51 T/T and 10 T/T for **(a)**, **(b)**, and **(d)** and in 42 G/G, 29 G/T and 5 T/T for **(c)**.

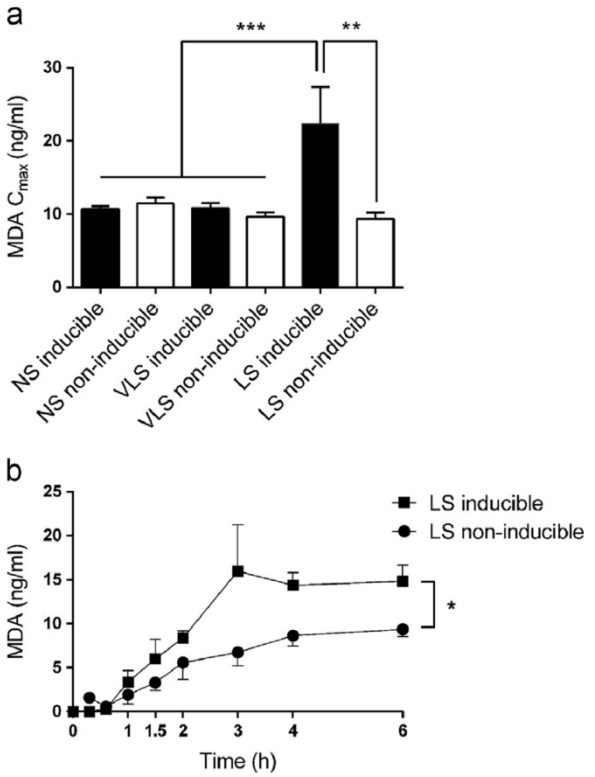

Figure 4 Effects of the CYP1A2 SNP rs762551 and smoking status on MDA plasma levels. Maximal plasma concentrations of MDA were higher in light smokers (LS; 6–10 cigarettes/day) with the inducible CYP1A2 rs762551 A/A genotype compared with nonsmokers (NS), very light smokers (VLS; 1–5 cigarettes/day), and smokers with the non-inducible CYP1A2 rs762551 A/C and C/C genotypes **(a and b)**. The data are expressed as the mean ± SEM in 57 inducible NS, 50 non-inducible NS, 9 inducible VLS, 16 non-inducible VLS, 4 inducible LS, 3 non-inducible LS. $^{**}P<0.01$, $^{***}P<0.001$.

smoking status on MDA levels was found in subjects with the rs762551 A/C and C/C genotypes. There were no effects of CYP1A2 genotype or smoking or interaction on the plasma concentrations of MDMA or HMMA (Table S3) or on the pharmacodynamic autonomic and subjective effects of MDMA.

3.4. Effect of dose and dose normalization

As expected, peak plasma concentrations of MDMA were greater after the 125 mg dose vs. the 75 mg dose (mean ± SD: 230 ± 46 vs. 125 ± 29 ng/ml; $F_{1,137}=140.20$, $P<0.001$). Consistently, the 125 mg dose produced greater subjective peak drug effects (80 ± 23 vs. 57 ± 30%; $F_{1,137}=21.5$, $P<0.001$) and cardiovascular stimulant peak responses (RPP = 14728 ± 3278 vs. 12067 ± 3159 mmHg × bpm; $F_{1,137}=15.3$, $P<0.001$). After dose normalization, the subjective and cardiovascular effects of MDMA did not differ between the dose groups. However, dose-normalized C_{max} values of MDMA were near-significantly greater at the 125 mg compared with the 75 mg dose ($F_{1,137}=4.08$, $P=0.05$) indicating a trend towards nonlinear pharmacokinetics at the doses used in this study.

4. Discussion

The present study described the pharmacogenetics of CYP1A2, CYP2C19 and CYP2B6 in the disposition of MDMA in healthy human subjects. We documented a role for CYP2C19 and CYP2B6 in the conversion of MDMA to MDA in humans, confirming in vitro metabolism studies (Kreth et al., 2000; Meyer et al., 2008). The MDMA/MDA AUC6 ratio was greater in subjects with low CYP2C19 or low CYP2B6 function, consistent with a contributing role for both CYP2C19 and CYP2B6 in the N-demethylation of MDMA to MDA in humans and confirming in vitro studies (Kreth et al., 2000; Meyer et al., 2008). Additionally, subjects with genetically determined low CYP2C19 function showed a more rapid and greater cardiovascular response to MDMA, although only two subjects with CYP2C19 PM genotype were included in the present study. In contrast to the CYP2C19 genotype, the CYP2B6 genotype altered MDMA concentrations later in time 3–4 h after drug administration. This finding may indicate that CYP2B6 becomes more important when CYP2D6 function decreases over time due to auto-inhibition by MDMA (de la Torre et al., 2012; O'Mathuna et al., 2008; Schmid et al., 2016; Yang et al., 2006). MDA is pharmacologically active in vitro (Hysek et al., 2012c; Rickli et al., 2015) and in rats (Schindler et al., 2014). One might therefore predict that alterations in the conversion of MDMA to MDA should not have a relevant effect on the pharmacodynamics of MDMA. However, the present study showed greater cardiostimulant effects of MDMA in subjects with slower MDMA to MDA conversion suggesting that MDMA contributes more to the cardiostimulant effects of MDMA than MDA.

Similar to CYP2C19 and CYP2B6, CYP1A2 contributes to the N-demethylation of MDMA to MDA in vitro (Meyer et al., 2008). CYP1A2 can be induced by tobacco smoking (Sachse et al., 1999). CYP1A2 activity increased with the number of cigarettes smoked per day (Dobrinas et al., 2011) and normalized with a half-life of 39 h when smoking is stopped (Faber and Fuhr, 2004). Additionally, CYP1A2 function is greater in smokers with the inducible SNP rs762551 A/A genotype compared with smokers with the non-inducible A/C and C/C genotypes (Sachse et al., 1999). We found higher MDA levels in tobacco smokers with the inducible vs. non-inducible genotypes and compared with nonsmokers. This finding indicates that CYP1A2 contributes to the N-demethylation of MDMA to MDA in humans. However, CYP1A2 genetics did not alter the response to MDMA.

Overall, polymorphism in CYP1A2, CYP2C19, and CYP2B6 influenced the metabolism of MDMA but none of the polymorphism altered the subjective response to MDMA.

The present study has several limitations. Although it is a relatively large study, it included only two subjects with the 2C19 PM genotype. While consistent with the higher concentrations of MDMA, the greater cardiostimulant response to MDMA in these two subjects may represent a chance finding. Similarly, there were only 4 light smokers with the inducible CYP1A2 genotype and this interaction of CYP1A2 and smoking

in the metabolism of MDMA needs to be confirmed in a larger study. We also included only smokers (<10 cigarettes/day) and it is likely that heavy smokers would show greater CYP1A2 induction (Dobrinas et al., 2011).

Although impairments in CYP2C19 or CYP2B6 alone may have only small effects on MDMA pharmacokinetics and its effects, the presence of multiple enzymes with impaired function such as combinations of 2D6 PM with CYP2C19 PM and CYP2B6 T/T may result in more pronounced consequences. The present study did not include subjects with such rare combinations that may predispose to MDMA toxicity.

Plasma for pharmacokinetic analyses was sampled only up to 6 h. However, this time covers the actual pharmacodynamic effects of MDMA which are shorter than its presence in plasma due to acute tolerance. Finally, we tested only doses of MDMA up to 125 mg which is in the upper range of recreational doses (Brunt et al., 2012) and identical to the dose used in clinical studies (Mithoefer et al., 2010; Oehen et al., 2013) but may not represent all cases of overdosing.

In conclusion, the results indicate that CYP1A2, CYP2C19, and CYP2B6 contribute to the conversion of MDMA to MDA in humans. Additionally, genetic polymorphisms in CYP2C19 may play a role in the clinical toxicity of MDMA.

Role of funding source

This study was supported by the Swiss National Science Foundation (Grant no. 320030_149493).

Contributors

YS, PV, and MEL designed the study. MEL obtained funding. YS, PV, and KP performed the research. YS, PV, KP, HMS, and MEL analyzed the data. PV, YS, and MEL wrote the manuscript. All the authors reviewed and approved the manuscript.

Conflict of interest

The authors do not have any conflicts of interest to declare for this work.

Acknowledgement

The authors acknowledge the assistance of C. M. Hysek, L. D. Simmler and A. Rickli in study management and analysis of plasma drug concentrations.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2017.01.008.

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2.4. Oxytocin receptor gene variations and socio-emotional effects of MDMA: a pooled analysis of controlled studies in healthy subjects

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PLoS One, 2018, Vol. 13, e0199384

PLOS ONE

RESEARCH ARTICLE

Oxytocin receptor gene variations and socio-emotional effects of MDMA: A pooled analysis of controlled studies in healthy subjects

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Citation: Vizeli P, Liechti ME (2018) Oxytocin receptor gene variations and socio-emotional effects of MDMA: A pooled analysis of controlled studies in healthy subjects. PLoS ONE 13(6): e0199384. https://doi.org/10.1371/journal.pone.0199384

Editor: Alexandra Kavushansky, Technion Israel Institute of Technology, ISRAEL

Received: November 23, 2017

Accepted: March 26, 2018

Published: June 18, 2018

Data Availability Statement: All relevant data are within the paper and its supplement.

Funding: This study was supported by the Swiss National Science Foundation (grant no. 320030_149493 and 320030_170249 to MEL). http://www.snf.ch. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Methylenedioxymethamphetamine (MDMA) increases oxytocin, empathy, and prosociality. Oxytocin plays a critical role in emotion processing and social behavior and has been shown to mediate the prosocial effects of MDMA in animals. Genetic variants, such as single-nucleotide polymorphisms (SNPs), of the oxytocin receptor (OXTR) may influence the emotional and social effects of MDMA in humans. The effects of common genetic variants of the OXTR (*rs53576*, *rs1042778*, and *rs2254298* SNPs) on the emotional, empathogenic, and prosocial effects of MDMA were characterized in up to 132 healthy subjects in a pooled analysis of eight double-blind, placebo-controlled studies. In a subset of 53 subjects, MDMA produced significantly greater feelings of trust in *rs1042778* TT genotypes compared with G allele carriers. The *rs53576* and *rs225498* SNPs did not moderate the subjective effects of MDMA in up to 132 subjects. None of the SNPs moderated MDMA-induced impairments in negative facial emotion recognition or enhancements in emotional empathy in the Multifaceted Empathy Test in 69 subjects. MDMA significantly increased plasma oxytocin concentrations. MDMA and oxytocin concentrations did not differ between OXTR gene variants. The present results provide preliminary evidence that OXTR gene variations may modulate aspects of the prosocial subjective effects of MDMA in humans. However, interpretation should be cautious due to the small sample size. Additionally, OXTR SNPs did not moderate the subjective overall effect of MDMA (any drug effect) or feelings of "closeness to others".

Trial registration: ClinicalTrials.gov: http://www.clinicaltrials.gov, No: NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01771874, and NCT01951508.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is recreationally used for its effects on empathic feelings and sociability [1, 2]. MDMA has also been shown to reduce the perception of negative emotions and enhance empathy [1, 3–6], effects that could potentially be useful in MDMA-assisted psychotherapy [7]. MDMA mainly causes the release of serotonin, norepinephrine, and dopamine [8, 9] but also increases oxytocin [1, 10–14]. Many similarities

are seen in the effects of MDMA and oxytocin on emotion processing and social behavior. For example, MDMA improves the identification of positive facial emotions and impairs the recognition of negative facial emotions [1, 4, 12, 15]. Similar effects were observed after intranasal administration of oxytocin [16, 17]. Intranasal administration of oxytocin increased trust and productive communication [18, 19]. MDMA similarly increased feelings of trust, openness, and closeness to others [1, 3]. Oxytocin increased generosity, and MDMA increased prosocial economic behavior [1]. One study showed significant within-subject correlations between MDMA-induced changes in prosocial feelings and changes in plasma oxytocin concentrations [10] implicating oxytocin as a mediator of the prosocial effects of MDMA. However, many other studies failed to find associations between oxytocin concentrations and the subjective, emotional, empathic, or prosocial effects of MDMA across subjects [1, 4, 12, 20, 21]. Animal studies showed that MDMA-induced prosocial effects in rats and mice could be blocked by oxytocin receptor (OXTR) antagonists [13, 22] also supporting a role for oxytocin in the prosocial effects of MDMA in animals. However, whether oxytocin is indeed a mediator of the effects of MDMA in humans is unclear. Oxytocin receptor blockade in the human brain may be challenging because OXTR blockers, such as atosiban, do not cross the blood-brain barrier. Correlational analyses are also problematic because plasma oxytocin concentrations may not necessarily reflect oxytocin levels in the brain [23].

While MDMA induces positive mood effects and prosociality in most subjects, negative mood effects have also been reported [24–27]. Genetic variations could explain some of the interindividual differences in the response to MDMA. Specifically, several genetic variations of the OXTR that are caused by single-nucleotide polymorphisms (SNPs) are associated with human social behavior or traits [28, 29]. One approach to indirectly testing the role of oxytocin in the effects of MDMA is to evaluate the moderating role of different SNPs of the OXTR gene in the prosocial and empathogenic effects of MDMA. In one study on the role of OXTR gene variants in the effects of MDMA, MDMA increased sociability in carriers of the G allele of the OXTR *rs53576* SNP but not in individuals with the AA genotype [30]. Furthermore in studies without MDMA, the common GG variant of *rs53576* has been associated with greater empathy and trust, seeking emotional support in times of distress, and less stress reactivity [31]. In contrast, carriers of the A allele presented lower sociability and lower sensitivity to oxytocin-induced enhancements in emotion recognition [32]. In addition to the *rs53576* SNP, two of the most studied SNPs of the OXTR gene are *rs1042778* and *rs2254298*. These SNPs were more likely to show an association to prosociality or other effects of oxytocin than other SNPs studied. For example, among 15 SNPs, the *rs1042778* showed the most significant associations with prosociality [33]. Carriers of the G allele of the *rs1042778* SNP presented greater prosocial behavior in an economic exchange game also used in the present study [33] and more sensitive parenting [34]. The *rs2254298* SNP has been associated with autism, attachment behavior, and depression [35–37] and *rs2254298* and *rs53576* were the most informative among 27 OXTR SNPs in a small sample of 38 subjects to predict responses to oxytocin in autism [38].

In the present study, we exploratorily investigated whether the OXTR *rs53576*, *rs1042778*, and *rs2254298* SNPs influence the social subjective, emotional, empathic, and prosocial effects of MDMA. Additionally, we sought to replicate previous findings regarding the role of the OXTR *rs53576* SNP in the prosocial response to MDMA [30].

Materials and methods

Study design

This was a pooled analysis of eight Phase I double-blind, placebo-controlled, crossover studies in healthy subjects that used similar methods [8, 39–45]. These studies included a total of 136

healthy subjects and were conducted between April 2009 and December 2014. Seven studies each included 16 subjects (112 total subjects) who received 125 mg MDMA twice, once alone and once after pretreatment with a medication [8, 39–43, 45]. However, in the present analysis, only data from the MDMA-alone and placebo sessions were used. An additional study included 24 subjects who received 125 mg MDMA once without pretreatment [44]. In all of the studies, the washout periods between the single dose administrations were at least 7 days to exclude carry-over effects. The studies were all registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01771874, and NCT01951508). All of the studies were approved by the local ethics committee and Swiss Agency for Therapeutic Products (Swissmedic). The studies were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all of the participants who were included in the studies. All of the subjects were paid for their participation. Pharmacokinetic and safety data from the same studies have been reported elsewhere [26, 46, 47].

Subjects

A total of 136 healthy subjects of European descent, aged 18–44 years (mean ± SD = 24.8 ± 4 years), were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, and three participants did not give consent for genotyping, resulting in the analysis of data from 132 subjects (64 men, 68 women). The mean ± SD body weight was 68 ± 10 kg (range: 46–90 kg).

The detailed exclusion criteria were reported elsewhere [8, 39, 40, 42] and included a history of psychiatric disorders, physical illness, a lifetime history of using illicit drugs more than five times (with the exception of past cannabis use), illicit drug use within the last 2 months, and illicit drug use during the study, determined by urine tests before the test sessions.

Study drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 mg that was prepared as gelatin capsules (25 and 100 mg, Bichsel Laboratories, Interlaken, Switzerland). Similar amounts of MDMA are found in ecstasy pills and have been used in clinical studies in patients [7]. The doses were not adjusted for body weight or sex. The dose per body weight (mean ± SD) was 1.9 ± 0.3 mg/kg (1.7 ± 0.2 mg/kg for men and 2.1 ± 0.3 mg/kg for women; range: 1.4–2.7 mg/kg).

Genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. Genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne, Switzerland). The *OXTR* is located on the short arm of chromosome 3 (3p25) and has three introns and four exons. We genotyped three OXTR SNPs: *rs53576* (c.922+4581T>C, position [GRCh37]: chr3:8804371, assay: C___3290335_10), *rs1042778* (c.*118C>A, position [GRCh37]: chr3:8794545, assay: C___7622140_30), and *rs2254298* (c.922+6724C>T, position [GRCh37]: chr3:8802228, assay: C__15981334_10). Linkage disequilibrium analysis between the SNPs were performed using Haploview (Broad Institute).

Subjective effects

Visual Analog Scales (VASs) were repeatedly applied to assess subjective effects over time [1]. The VAS "any drug effect" was presented as 100 mm horizontal lines (0–100%), marked from

"not at all" on the left to "extremely" on the right. The VASs "closeness to others," "trust," "want to be hugged," "want to hug," "want to be alone," and "want to be with others" were bidirectional (±50%). "Trust," "want to be hugged," "want to hug," "want to be alone," and"want to be with others" were assessed in 53 subjects. The VASs were administered before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration.

Emotion recognition

To measure emotion recognition, we used the Facial Emotion Recognition Task (FERT), which is sensitive to the effects of MDMA [3, 5, 12, 43] and other serotonergic substances [48]. The task included 10 neutral faces and 160 faces that expressed one of four basic emotions (i.e., happiness, sadness, anger, and fear), with pictures morphed between 0% (neutral) and 100% in 10% steps. Two female and two male pictures were used for each of the four emotions. The stimuli were presented in random order for 500 ms and then were replaced by the rating screen where the participants had to indicate the correct emotion. The outcome measure was accuracy (proportion correct) and misclassification (emotions that were indicated incorrectly). The FERT was performed 90 min after drug administration. FERT data were available from 69 subjects. The genotype distribution for this subsample was: *rs1042778* (28 GG, 33 GT, 8 TT), *rs53576* (30 GG, 25 AG, 14 AA), and *rs2254298* (57 GG, 12 AG/AA).

Empathy

The Multifaceted Empathy Test (MET) is a reliable and valid task that assesses the cognitive and emotional aspects of empathy [49]. The MET is sensitive to oxytocin [17], MDMA [1, 3, 20], and other psychoactive substances [48]. The computer-assisted test consisted of 40 photographs that showed people in emotionally charged situations. To assess cognitive empathy, the participants were required to infer the mental state of the subject in each scene and indicate the correct mental state from a list of four responses. Cognitive empathy was defined as the percentage of correct responses relative to total responses. To measure emotional empathy, the subjects were asked to rate how much they were feeling for an individual in each scene (i.e., explicit emotional empathy) and how much they were aroused by each scene (i.e., implicit emotional empathy) on a 1–9 point scale. The latter rating provides an inherent additional assessment of emotional empathy, which is considered to reduce the likelihood of socially desirable answers. The three aspects of empathy were each tested with 20 stimuli with positive valence and 20 stimuli with negative valence, resulting in a total of 120 trials. The MET was performed 90–180 min after drug administration. MET data were available from 69 subjects. The genotype distribution for this subsample was: *rs1042778* (28 GG, 33 GT, 8 TT), *rs53576* (30 GG, 25 AG, 14 AA), and *rs2254298* (57 GG, 12 AG/AA).

Prosociality

We used the paper version of the validated Social Value Orientation (SVO) test to assess social behavior [50]. The SVO test is sensitive to MDMA [1] and other psychoactive substances [48]. In this economic resource allocation task, prosociality is defined as behavior that maximizes the sum of resources for the self and others and minimizes the difference between the two. The test consists of six primary and nine secondary SVO slider items with a resource allocation choice over a defined continuum of joint payoffs [50]. The participants were instructed to choose a resource allocation that defined their most preferred joint distribution between themselves and another person. The allocated funds had real value, and four randomly selected subjects received the funds they earned. Mean allocations for the self and the other were calculated [1, 50], and the inverse tangent of the ratio of these two means produced an angle that

indicated the participants' SVO index. A smaller SVO angle indicates more individualistic or competitive behavior, and a larger SVO angle indicates more prosocial or even altruistic behavior. The nine secondary items were used to differentiate between two different prosocial motivations (inequality aversion and joint gain maximization). The inequality-aversion index was calculated as previously described [50]. An index of 0 indicates perfect inequality aversion, and 1 indicates maximal preference for joint gain maximization. The SVO test was performed 3–4 h after drug administration. SVO primary data and the inequality-aversion index were available from 69 and 33 subjects, respectively. The genotype distribution for this subsample was: *rs1042778* (28 GG, 33 GT, 8 TT), *rs53576* (30 GG, 25 AG, 14 AA), and *rs2254298* (57 GG, 12 AG/AA) and *rs1042778* (15 GG, 15 GT, 3 TT), *rs53576* (12 GG, 15 AG, 6 AA), and *rs2254298* (26 GG, 7 AG/AA), respectively.

Plasma concentrations of oxytocin and MDMA

The plasma concentration of oxytocin has been shown to peak 2 h after MDMA administration [10] and was therefore measured at baseline and 2 h after drug administration and analyzed as described previously [4, 23, 51]. The plasma level of MDMA was determined 1 h before and 0.5, 1, 1.5, 2, 3, 4, and 6 h after drug administration and analyzed as described previously [8].

Statistical analysis

Subjective drug effects on the VASs were determined as the area under the effect-time curve from 0 to 6 h ($AUEC_6$) after drug administration using the trapezoidal method in Phoenix WinNonlin (version 6.4, Pharsight, Certara L.P., St. Louis, MO, USA). The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). The effects of MDMA on subjective effect ratings and plasma oxytocin concentrations were expressed as differences from placebo. Repeated-measured analyses of variance (ANOVAs), with drug (MDMA *vs*. placebo) as the within-subjects factor, were used to evaluate drug effects. One-way ANOVAs, with genotype group as the between-subjects factor, followed by the Tukey post hoc test were used to evaluate the effects of genotype on the effects of MDMA (differences from placebo). Additional ANOVAs including plasma concentrations of MDMA and/or oxytocin as covariates were conducted as well as sex differences were included by adding sex as an additional between-subjects factor to the analyses to exclude confounding by any of these variables. This showed that the results were not confounded by oxytocin plasma levels, or sex, or differences in plasma concentrations of MDMA, that corrects for differences in body weight, dosing, or/and known and unknown activity differences in metabolizing enzymes [46, 47]. The reported results are from analysis with MDMA plasma concentration AUC_6 and oxytocin plasma concentration change at 2 h as covariates with the exception of the VAS "any drug effect" and "closeness to others" for which only MDMA plasma concentration AUC_6 was corrected due to the lack of oxytocin plasma concentration data for subjects from the first two studies [8, 41]. The level of significance was set to $p < 0.05$. The Nyholt correction method was used to account for multiple comparisons and flagged separately [52]. We thereby corrected for the 7 VASs, 3 emotions in the FERT, emotional empathy in the MET, and 2 items in the SVO which have all been shown sensitive to the effects of MDMA and for each of the 3 tested SNPs ([7+3 +1+2] × 3), resulting in 39 variables and an effective number of independent variables (V_{eff}) of 28.96 according to Nyholt and a corrected significance threshold to keep Type I error rate at 5% of $p < 0.0017$. For the analysis of the *rs2254298* SNP, the AA and AG genotypes were pooled as A allele carriers because only one subject had the AA genotype.

Results

Genotyping

The distribution of the alleles and genotypes did not differ from distributions reported elsewhere in Caucasian cohorts (Ensembl database release 86, Oct 2016). The rare allele frequencies for *rs53576*, *rs1042778*, and *rs2254298* were A (93 [35%]), T (101 [38%]), and A (20 [11%]), respectively. No linkage disequilibrium was detected between the tested SNPs (S1 Fig).

Subjective effects

On the VASs, MDMA increased the $AUEC_6$ values for "any drug effect" ($F_{1,131} = 544$, $p < 0.001$), "closeness" ($F_{1,131} = 57$, $p < 0.001$), "trust" ($F_{1,52} = 33$; $p < 0.001$), "want to be hugged" ($F_{1,52} = 6.6$, $p < 0.05$), "want to hug" ($F_{1,52} = 7.6$, $p < 0.01$), and "want to be with others" ($F_{1,52} = 20$, $p < 0.001$) and decreased feelings of "want to be alone" ($F_{1,52} = 21$, $p < 0.001$, Fig 1, Table 1).

The effects of the OXTR *rs1042778* SNP on the subjective effects of MDMA are shown in Table 1, Fig 1, and S1 Table. MDMA produced increases in "trust" ($F_{1,49} = 14$, $p < 0.001$), "want to be hugged" ($F_{1,49} = 5.3$, $p < 0.05$), and "want to be with others" ($F_{1,49} = 6.5$, $p < 0.05$) in the TT genotype group compared with the G allele carriers. MDMA lowered ratings of "wanting to be alone" more in subjects with the TT genotype compared with G allele carriers ($F_{1,49} = 4.5$, $p < 0.05$). Using Nyholt correction for the multiple comparisons, only MDMA effects on "trust" was significantly altered by the *rs1042778* SNP. The OXTR *rs53576* and *rs2254298* SNPs did not alter the subjective effects of MDMA (Table 1).

Emotion recognition

On the FERT, MDMA impaired the recognition of fearful ($F_{1,68} = 47$, $p < 0.001$), sad ($F_{1,68} = 14$, $p < 0.001$), and angry ($F_{1,68} = 16$, $p < 0.001$) faces compared with placebo. None of the OXTR gene variants moderated the effects of MDMA on the FERT.

Empathy

MDMA increased explicit emotional empathy for positive emotions ($F_{1,68} = 7.6$, $p < 0.01$) compared with placebo. None of the OXTR gene variants altered the effects of MDMA on the MET.

Prosociality

MDMA produced a near-significant trend toward an increase in the SVO angle compared with placebo ($F_{1,68} = 3.1$, $p = 0.08$). MDMA reduced the inequality-aversion index ($F_{1,32} = 9.3$, $p < 0.01$) in subjects with a prosocial orientation, indicating a shift from joint gain maximization to inequality aversion. The *rs53576* and *rs1042778* SNPs moderated the MDMA-induced increase in inequality aversion (S2 Fig). MDMA significantly reduced the inequality-aversion index in the *rs53576* AA genotype group compared with G allele carriers ($F_{1,31} = 9.4$, $p < 0.01$). MDMA also reduced inequality-aversion in the *rs1042778* GG genotype group compared with T allele carriers ($F_{1,29} = 5.6$, $p < 0.05$). T allele carriers who received placebo also had a lower inequality-aversion index (corresponding to greater inequality-aversion) compared with subjects with the GG genotype ($p < 0.05$). However, if corrected for multiple comparisons none of the OXTR genetics influence SVO findings significantly.

Plasma concentrations of oxytocin and MDMA

Plasma oxytocin and MDMA concentrations are shown in Table 1. Oxytocin concentrations were significantly elevated 2 h after MDMA administration compared with placebo (placebo:

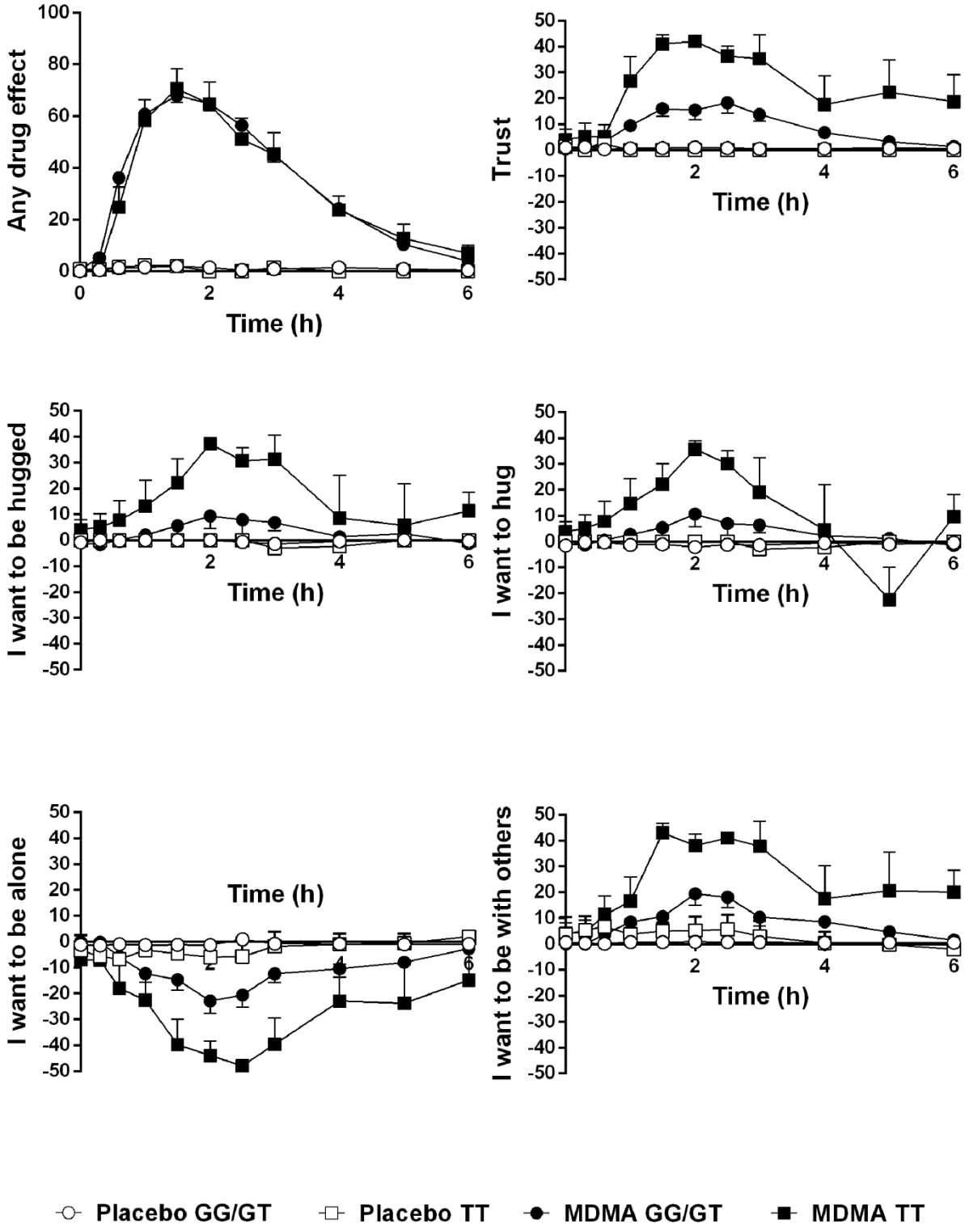

Fig 1. Effect of the OXTR rs1042778 SNP on the subjective effects of MDMA. MDMA produced greater "trust," "want to be hugged," "want to hug," and "want to be with others" and reduced "want to be alone" in the TT group (n = 5) compared with the GG/GT group (n = 48, Table 1 and S1 Table). The data are expressed as mean ± SEM. MDMA or placebo was administered at time = 0.

https://doi.org/10.1371/journal.pone.0199384.g001

Table 1. Effects of oxytocin receptor rs53576, rs1042778, and rs2254298 polymorphism groups on the response to MDMA (mean±SD and statistics).

	SNP rs1042778					SNP rs53576					SNP rs2254298				
	GG/GT	TT	F	p value	p value[a]	GA/GG	AA	F	p value	p value[a]	GG	AA/AG	F	p value	p value[a]
N (%)	113 (86)	19 (14)				113 (86)	19 (14)				105 (80)	27 (20)			
Male, N (%)	57 (50)	7 (37)				52 (46)	12 (63)				51 (49)	13 (48)			
MDMA plasma concentration AUC_6 (ng*h/mL)	954 ±212	924 ±179	0.34	NS	NS	956 ±205	914 ±219	0.68	NS	NS	941 ±208	985 ±200	0.99	NS	NS
MDMA peak concentrations (ng/mL)	225 ±49	223±41	0.02	NS	NS	226 ±47	214 ±53	1	NS	NS	222 ±47	235 ±50	1.8	NS	NS
Oxytocin Δplasma concentration at 2h (pg/mL)[b]	53±63	64±101	0.33	NS	NS	55±71	53±60	0.01	NS	NS	53±68	63±74	0.35	NS	NS
Visual Analog Scale rating $\Delta AUEC_6$															
Any drug effect	195 ±95	196 ±107	0.16	NS	NS	193 ±98	205 ±88	1.14	NS	NS	188 ±95	221 ±97	1.55	NS	NS
Closeness to others	37±57	64±87	3.89	0.051	NS	39±60	54±74	1.59	NS	NS	41±65	41±51	0.07	NS	NS
Trust[c]	46±64	153±65	14.03	<0.001	0.014	58±75	52±59	0.02	NS	NS	50±72	86±60	1.06	NS	NS
Want to be hugged[c]	20±68	105 ±129	5.27	0.026	NS	29±80	23±78	0.03	NS	NS	26±82	37±59	0.02	NS	NS
Want to hug[c]	22±65	63±101	1.53	NS	NS	29±70	18±66	0.01	NS	NS	26±72	26±57	0.15	NS	NS
Want to be alone[c]	-55 ±97	-153 ±108	4.49	0.039	NS	-55 ±96	-91 ±116	2.62	NS	NS	-58 ±105	-93 ±78	0.28	NS	NS
Want to be with others[c]	43±77	140 ±120	7.28	0.010	NS	47±85	67±88	1.56	NS	NS	48±91	70±45	0.09	NS	NS

N, number of subjects; AUEC, area under the effect-time curve; SD, standard deviation; NS, not significant; Δ, values are change scores from placebo.

[a]p value additionally corrected for multiple comparisons according to the Nyholt correction.

[b]N = 101 (rs1042778: 87 GG/GT, 14 TT; rs53576: 83 GG/AG, 18 AA; rs2254298: 81 GG, 20 AG/AA).

[c]N = 53 (rs1042778: 48 GG/GT, 5 TT; rs53576: 40 GG/AG, 13 AA; rs2254298: 44 GG, 9 AG/AA).

https://doi.org/10.1371/journal.pone.0199384.t001

19 ± 39 pg/ml; MDMA: 74 ± 70 pg/ml; $F_{1,99} = 62$, $p < 0.001$). Plasma oxytocin and MDMA concentrations similarly increased across all OXTR gene variants (Table 1). MDMA peak concentrations and AUC_6 values were (mean ± SD) 224 ± 48 ng/mL and 950 ± 207 ng×h/mL in the total of 132 subjects. There was acute tolerance to the subjective response to MDMA.

Discussion

The main finding of this study was that the OXTR *rs1042778* SNP influenced the typical empathogenic and prosocial feelings that are produced by MDMA, including enhancements of "trust". Similar modulation of the prosocial effects of MDMA was previously reported for the OXTR *rs53576* SNP [30] but not *rs1042778* SNP. These findings suggest that humans may respond differently to the typical subjective effects of MDMA, depending on their OXTR genetics. The results indirectly indicate a possible role for oxytocin in the subjective effects of MDMA, similar to its interoceptive effects in rats [53]. Animal studies have shown that oxytocin mediates the prosocial effects of MDMA [13, 14, 54]. We observed lower subjective prosociality after MDMA administration in carriers of the G allele of the *rs1042778* SNP. Greater prosociality [33] and lower antisocial behavior [55] have been associated with the G allele in the absence of treatment. Our placebo condition was unsuitable for assessing differences in prosociality between subjects in the absence of treatment. Altogether, however, the findings are consistent with the notion that subjects with lower sociality may respond more to the prosocial effects of MDMA or oxytocin [56].

In the present study, we failed to replicate a previous finding of moderation of the subjective prosocial effects of MDMA by the *rs53576* SNP [30]. This previous study showed that carriers of the AA genotype at the *rs53576* locus were not susceptible to the prosocial effect of 1.5 mg/kg MDMA [30]. However, this previous study assessed "sociability" as a combined outcome of several VASs, including "friendly," "sociable," "confident," "playful," and "loving" using principal component analysis [30]. Additionally, the effect of *rs53576* was observed only at a dose of 1.5 mg/kg MDMA, whereas opposite effects were observed with 0.75 mg/kg MDMA [30]. The discrepant findings between these two studies may be partially explained by the different scales that were used. The findings may also indicate that the MDMA effect modulation by different OXTR SNPs is not very robust across studies and rather small.

The effects of MDMA on the FERT and MET in the present study were consistent with studies by other researchers who used the same tests [5, 20]. The present study found that the *rs53576*, *rs1042778*, and *rs2254298* SNPs did not influence MDMA-induced impairments in the recognition of fearful, sad, and angry faces or increases in emotional empathy. No other data are available on the effects of other OXTR gene variants on MDMA-induced changes in tests of emotion processing or empathy. In contrast to the present study, the effect of intranasal oxytocin on dynamic face recognition has previously been shown to be modulated by OXTR SNP haplotypes, including the SNPs that were studied herein [32].

On the SVO test, MDMA produced a trend toward an increase in prosocial behavior and increased inequality aversion. We previously reported a significant increase in prosociality and a trend toward an increase in inequality aversion from a subset of the present data [1]. A novel finding of the present study was that MDMA increased inequality-aversion and thus a preference for fairness only in subjects with the *rs53576* AA or *rs1042778* GG genotypes, indicating a role for these OXTR gene variants in MDMA's effect on social behavior. However, the MDMA-induced increase in preference for fairness in *rs1042778* GG subjects appears to conflict with the smaller increase in trust in these subjects (S1 Table). Additionally, *rs1042778* GG individuals presented lower inequality aversion compared with T allele carriers who received placebo. Higher prosociality on the SVO test has previously been reported in G allele carriers [33], but differences in the inequality-aversion index were not studied because this scale was only added later to the SVO test [50]. Furthermore, the OXTR *rs1042778*, *rs53576*, and *rs2254298* SNPs had no effects in two other economic games (i.e., dictator game and trust game; [57]). The findings in the SVO tests are conclusive but need to be interpreted with caution, since they did not survive the correction for multiple comparisons and the total number of subjects in this subset was reduced to 33 due to the limiting calculation of the inequality aversion [50].

The present study has several limitations. First, the study was mostly exploratory and the findings would need to be confirmed in larger studies. Second, not all outcome measures were used in all of the subjects, thus limiting the sample size and also increasing the risk of Type I errors. Third, we tested only three OXTR SNPs. Other SNPs or haplotypes may also play a role [32]. Fourth, MDMA causes the release of oxytocin, monoamines [8, 9], and arginine vasopressin [51]. The latter two are well known to influence social cognition and behavior [58]. MDMA also increases cortisol and other corticosteroids [59, 60] and oxytocin and cortisol may interact to influence the response to MDMA and these interactions need further study [21]. Finally, cultural and early environmental background plays an uncertain role in the results of genetic studies, especially studies of OXTRs. For example, studies of the *rs2254298* SNP reported different results in Caucasian and Asian subjects [35, 61].

The present findings of individual differences in the response to MDMA that depended on OXTR genetics need to be confirmed and might have implications for MDMA-assisted psychotherapy [7] and may contribute to more personalized treatment. Therapeutic studies that

use MDMA in patients should genotype OXTR SNPs and test for polymorphisms of the genes that regulate the metabolism of MDMA [46, 47].

Conclusion

The OXTR *rs1042778* SNP but not the *rs53576* or *rs2254298* SNPs altered the typical MDMA-induced feelings of trust. A previous finding of a moderating influence of the rs53576 on the socio-emotional effect of MDMA could not be replicated indicating a chance finding. Additionally, after correction for multiple comparisons OXTR SNPs did not moderate the subjective overall effect of MDMA (any drug effect) or feelings of "closeness to others" in the total larger study sample of 132 subjects. Thus, the results are preliminary and should be interpreted with caution due to multiple comparisons and small genotype group sample sizes.

Supporting information

S1 Fig. Linkage disequilibrium across the determined SNPs. Estimates of the square of the correlation coefficient (r^2) were calculated for each pairwise comparison of SNPs based on data from our study cohort.
(TIF)

S2 Fig. OXTR rs53576 and rs104278 SNPs moderate the effects of MDMA on the SVO Inequality-aversion index. (a) MDMA reduced the inequality-aversion index in the OXTR rs53576 AA genotype group (n = 6) but not in the GG/GA genotype group (n = 27, $^{**}p < 0.01$). **(b)** MDMA reduced the inequality-aversion index in the OXTR rs1042778 GG genotype group (n = 15) but not in the TT/TG genotype group (n = 18, $^{*}p < 0.05$, $^{**}p < 0.01$). The data are expressed as mean ± SEM. If corrected for multiple comparisons none of the OXTR genetics significantly influence SVO findings. An inequality-aversion index of 0 indicates perfect inequality aversion (maximal fairness), and 1 indicates maximal preference for joint gain maximization in subjects with a prosocial value orientation.
(TIF)

S1 Table. Effects of oxytocin receptor rs53576 and rs1042778 polymorphisms (all allele groups) on the response to MDMA (mean±SD and statistics). N, number of subjects; AUEC, area under the effect-time curve; SD, standard deviation; NS, not significant; D, values are change scores from placebo; **p value < 0.01 compared to rs1042778 TT. ^{a}p value additionally corrected for multiple comparisons according to the Nyholt correction. ^{b}N = 101 (rs1042778: 39 GG, 48 GT, 14 TT; rs53576: 44 GG, 39 AG, 18 AA; rs2254298: 80 GG, 21 AG/AA). ^{c}N = 53 (rs1042778: 21 GG, 27 GT, 5 TT; rs53576: 23 GG, 17 AG, 13 AA; rs2254298: 44 GG, 9 AG/AA).
(XLSX)

Acknowledgments

The authors thank and acknowledge the assistance of C. M. Hysek, A. Rickli, Y. Schmid, and P. C. Dolder in conducting the clinical studies and M. Arends for text editing.

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2.5. No major role of norepinephrine transporter gene variations in the cardiostimulant effects of MDMA

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European Journal of Clinical Pharmacology, 2018, Vol. 74, 275-283

European Journal of Clinical Pharmacology (2018) 74:275–283
https://doi.org/10.1007/s00228-017-2392-2

PHARMACOGENETICS

No major role of norepinephrine transporter gene variations in the cardiostimulant effects of MDMA

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Received: 21 July 2017 / Accepted: 27 November 2017 / Published online: 2 December 2017

Abstract

Purpose Methylenedioxymethamphetamine (MDMA, ecstasy) is used recreationally and frequently leads to sympathomimetic toxicity. MDMA produces cardiovascular and subjective stimulant effects that were shown to partially depend on the norepinephrine transporter (NET)-mediated release of norepinephrine and stimulation of α_1-adrenergic receptors. Genetic variants, such as single-nucleotide polymorphisms (SNPs), of the NET gene (*SLC6A2*) may explain interindividual differences in the acute stimulant-type responses to MDMA in humans.

Methods We characterized the effects of common genetic variants of the *SLC6A2* gene (rs168924, rs47958, rs1861647, rs2242446, and rs36029) on cardiovascular and subjective stimulation after MDMA administration in 124 healthy subjects in a pooled analysis of eight double-blind, placebo-controlled studies.

Results Carriers of the GG genotype of the *SLC6A2* rs1861647 SNP presented higher elevations of heart rate and rate-pressure product after MDMA than subjects with one or no G alleles. Subjects with a C allele in the *SLC6A2* rs2242446 SNP presented higher elevations of the heart rate after MDMA administration compared with the TT genotype. Subjects with the AA genotype of the *SLC6A2* rs36029 SNP presented higher elevations of mean arterial pressure and rate pressure product after MDMA administration than carriers of the G allele. The *SLC6A2* rs168924 and rs47958 SNPs did not alter the response to MDMA.

Conclusions Genetic polymorphisms of the SLC6A2 gene weakly moderated the acute cardiovascular response to MDMA in controlled studies and may play a minor role in adverse cardiovascular events when MDMA is used recreationally.

Keywords MDMA · Norepinephrine transporter · SLC6A2 · Pharmacogenetics · Cardiostimulation

Introduction

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is used recreationally for its ability to enhance empathic feelings and sociability [1, 2]. MDMA has also been investigated as a treatment for posttraumatic stress disorder [3, 4]. However, MDMA produces adverse effects, including cardio- and psychostimulant effects to varying degrees [5–7]. The sympathomimetic effects of MDMA vary across subjects, and high blood pressure responses were observed in a few subjects [6, 8]. The response to MDMA varies between subjects, and genetic variations may explain some of this interindividual variation [9–11]. For example, genetic variations of the enzymes that are involved in MDMA metabolism (mainly CYP2D6) have been shown to affect plasma levels of MDMA and its metabolites [9, 10, 12] and moderate the pharmacokinetics and partially the pharmacodynamics of MDMA. However, genetic variants of the pharmacological targets of MDMA [7, 13] may also moderate its pharmacodynamic effects, but such effects have not yet been studied.

MDMA interacts with presynaptic monoamine transporters and mainly causes the transporter-mediated efflux of serotonin (5-hydroxytryptamine [5-HT]) and norepinephrine (NE; [14–17]). The transporter-mediated efflux

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00228-017-2392-2) contains supplementary material, which is available to authorized users.

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of NE has been suggested to critically contribute to the cardio- and psychostimulant effects of MDMA [7, 13, 18, 19]. The solute carrier family 6 (neurotransmitter transporter, NE transporter [NET]), member 2 (*SLC6A2*) is a crucial player in the noradrenergic system and involved in the mechanism of action of MDMA in humans. Inhibition of the NET significantly attenuated the sympathomimetic stimulant-like effects of MDMA and other stimulant-type substances [7, 20]. In a controlled study in healthy subjects, the NET inhibitor reboxetine reduced the MDMA-induced elevations of blood pressure and heart rate and subjective stimulation and increased the pupil diameter at rest and after light [7, 21], indicating a role for the NET in mediating the MDMA response [22]. Similarly, pretreatment with the NET inhibitor atomoxetine attenuated D-amphetamine-induced elevations of blood pressure and self-reported ratings of feeling "stimulated" [20]. Another study in humans showed a similar reduction of cardiostimulant responses to cocaine after treatment with the NET inhibitor atomoxetine [23].

Several genetic variations of the *SLC6A2* gene that are caused by single-nucleotide polymorphisms (SNPs) are associated with different functional phenotypes. However, the roles of these genotypes in the effects MDMA have not yet been investigated. Therefore, we focused on validated, polymorphic (minor allele frequency in Caucasians > 0.1), and potentially functionally relevant variants of *SLC6A2*. Specifically, the G allele of the *SLC6A2* rs168924 SNP was associated with hypertension in Japanese patients [24] but lower blood pressure in Caucasians [25]. Subjects with the AA genotype of the *SLC6A2* rs1861647 SNP or CC genotype of the *SLC6A2* rs47958 SNP had higher subjective elation scores in response to D-amphetamine compared with carriers of the G allele [26] or A allele [27], respectively. The *SLC6A2* rs2242446 SNP was shown to influence blood pressure during exercise [28]. Additionally, an association was found between the rs2242446 SNP and recurrent depression [29] and antidepressant response to the 5-HT/NE transporter inhibitor milnacipran [30]. Finally, the *SLC6A2* rs36029 SNP was shown to be significantly associated with alcohol dependence [31].

The present study investigated the impact of genotypes within the noradrenergic system on the effects of MDMA. We evaluated whether the *SLC6A2* rs168924, rs47958, rs1861647, rs2242446, and rs36029 SNPs influence the cardiovascular and subjective stimulant effects of MDMA. MDMA-induced peak increases in the rate-pressure product (RPP) and subjective ratings of stimulation were considered the two primary endpoints. Plasma concentrations of MDMA and NE were determined to exclude possible confounding effects on the influence of genotype.

Methods

Study design

This was a pooled analysis of eight Phase I double-blind, placebo-controlled, crossover studies in healthy subjects that used similar methods [7, 13, 21, 32–36]. These studies included a total of 136 healthy subjects. Seven studies included 16 subjects each, for a total of 112 subjects, who received 125 mg MDMA twice, once alone, and once after pretreatment with a medication [7, 13, 21, 32–36]. An additional study included 24 subjects who received 125 mg MDMA once alone, placebo, or other treatments [36]. In the present analysis, only data from the MDMA-alone and placebo sessions were used. In all of the studies, the washout periods between single-dose administrations of MDMA were at least 7 days to exclude carryover effects. The studies were all registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01771874, and NCT01951508). All of the studies were approved by the local ethics committee and Swiss Agency for Therapeutic Products (Swissmedic). The studies were conducted in accordance with the Declaration of Helsinki. MDMA administration in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Informed consent was obtained from all of the participants who were included in the studies. All of the subjects were paid for their participation. Pharmacokinetic and safety data from these studies have been reported elsewhere [6, 9, 10]. In all studies, test sessions took place in a quiet hospital research ward with no more than two research subjects present per session. The participants were comfortably lying in hospital beds and were mostly listening to music and did not engage in physical activities. MDMA was given without food in the fasting state in the morning at 8:00–9:00 a.m.. A small standardized lunch was served at 12:00–1:00 p.m.

Subjects

A total of 136 healthy European/Caucasian subjects, 18–44 years old (mean ± SD = 24.8 ± 4 years), were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, three participants did not give consent for genotyping, and eight subjects participated twice, and only the first participation was included, resulting in data from 124 subjects. The mean ± SD body weight was 68 ± 10 kg (range 46–90 kg).

The exclusion criteria included a history of psychiatric disorders, physical illness, a lifetime history of using illicit drugs more than five times (with the exception of past cannabis use), illicit drug use within the past 2 months, and illicit drug use during the study, determined by urine tests that were

conducted before the test sessions as reported in detail elsewhere [13, 21, 32, 33]. Thirty-eight subjects had prior illicit drug experiences (1–5 times), of which 16 subjects had previously used MDMA (1–2 times), 7 amphetamine or methamphetamine (1 time), 9 cocaine (1–3 times), 6 lysergic acid diethylamide (1 time), and 11 psilocybin (1–4 times).

Study drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 mg, prepared as gelatin capsules (Bichsel Laboratories, Interlaken, Switzerland). Similar amounts of MDMA are found in ecstasy pills [37] and have been used in clinical studies in patients [3, 4]. The doses were not adjusted for body weight or sex. The dose per body weight (mean ± SD) was 1.9 ± 0.3 mg/kg (1.7 ± 0.2 mg/kg for men and 2.1 ± 0.3 mg/kg for women, range 1.4–2.7 mg/kg).

Cardiovascular effects

Blood pressure and heart rate were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Systolic and diastolic blood pressure and heart rate were measured using an automatic oscillometric device (OMRON Healthcare Europe NA, Hoofddorp, Netherlands). The measurements were performed in duplicate at an interval of 1 min and after a resting time of at least 10 min. The averages were calculated for the analysis. Mean arterial pressure (MAP) was calculated as diastolic blood pressure + (systolic blood pressure − diastolic blood pressure) / 3. The RPP was calculated as *systolic blood pressure* × *heart rate* and was considered the primary cardiovascular measure that reflected overall cardiovascular stimulation.

Subjective effects

To assess subjective stimulation, a visual analog scale of "stimulated" was presented as a 100-mm horizontal line (0–100%), marked from "not at all" on the left to "extremely" on the right [1]. The scale was administered before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration.

Plasma concentrations of MDMA and norepinephrine

Plasma levels of MDMA were determined before and 0.5, 1, 1.5, 2, 3, 4, and 6 h after drug administration [34]. Plasma levels of NE were measured before and 2 h after drug administration as described previously [7, 38].

Pupillometry

Pupillometry was performed 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Pupil function was measured under standardized dark-light conditions using a hand-held PRL-200 infrared pupillometer (NeurOptics, Irvine, CA) as reported previously in detail [21]. Dark-adapted pupil diameter and minimal pupil diameter after a light stimulus were assessed.

Genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. Genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne, Switzerland) and the TaqMan Genotyping Master Mix. Fluorescence was detected using the ViiA7 real-time PCR system. We assayed the following *SLC6A2* SNPs: rs168924 (assay: C____581568_10), rs1861647 (assay: C___1232469_30), rs47958 (0.39, assay: C___3020083_10), rs2242446 (assay: C__26354911_10), rs36029 (C___1232432_10). We also assayed the following ADRA1A SNP: rs1048101 (assay: C___2696454_30). However, due to inconsistency with the Hardy-Weinberg equilibrium, we excluded the ADRA1A rs1048101 SNP from further analysis. The rs1861647 genotype could not be determined in one subject.

Statistical analysis

The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). For repeatedly measured data, peak effects (E_{max}) and areas under the effect-time curve (AUEC) from 0 to 6 h values were determined for MDMA and placebo. Differences in E_{max} and AUEC values (MDMA-placebo) were then analyzed using one-way analysis of variance (ANOVA), with genotype as the between-group factor, followed by the Tukey post hoc test. The primary analysis did not control for the multiple comparisons, but a secondary analysis was conducted using Bonferroni correction for the five SNPs. To account for differences in plasma concentrations of MDMA that were caused by differences in body weight, dosing, or metabolizing enzymes [9, 10], the area under the MDMA plasma concentration-time curve from 0 to 6 h (AUC) was included as a covariate in the ANOVAs, and we report the corrected statistics. Additionally, moderating effects of sex were explored by adding sex as a between-subjects factor in the ANOVAs. E_{max} values were obtained directly from the observed data, and AUC and AUEC curves were calculated using the linear-log trapezoidal method in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ).

Results

Effects of the SNPs on the maximum response (E_{max}) to MDMA are shown in Table 1. Supplementary Table S1 shows the data without adjustment for MDMA plasma concentrations. Supplementary Table S2 shows effects of the SNPs on the overall response to MDMA (AUEC values).

Genotyping

The distribution of the alleles and genotypes did not differ from the distributions that were reported elsewhere in Caucasian cohorts (Ensembl database release 88, Mar 2017). The minor allele frequencies for rs168924, rs47958, rs1861647, rs2242446, rs36029, and rs1048101 were G (29 [13%]), A (112 [45%]), A (79 [32%]), C (80 [33%]), G (100 [40%]), and G (107 [43%]), respectively. The tested genetic variants were consistent with the Hardy-Weinberg equilibrium ($p > 0.05$) with the exception of rs1048101 ($p = 0.01$).

Plasma concentrations of MDMA and norepinephrine

Plasma concentrations of MDMA and norepinephrine did not differ between the different genotype groups (Table 1 and Supplementary Table S1).

Subjective effects

None of the examined polymorphisms influenced subjective stimulation that was induced by MDMA (Table 1, Supplementary Tables S1 and S2).

Pupillary effects

None of the examined polymorphisms influenced the MDMA-induced change in pupillary size before and after light stimulus (Table 1 and Supplementary Tables S1 and S2).

Cardiovascular effects

The effects of the polymorphisms on elevations of MAP, heart rate, and RPP in response to MDMA (adjusted for differences in plasma MDMA concentrations) are shown in Table 1 and Fig. 1. The rs1861647 SNP located in *SLC6A2* significantly altered the elevations of heart rate and RPP after MDMA administration. The effect on the heart rate remained significant after Bonferroni correction for multiple testing ($p < 0.05$). Subjects with the GG genotype had significantly higher elevations of heart rate and RPP after MDMA administration than subjects with the AG genotype. When we combined the AA and AG genotype groups, subjects with the GG genotype presented higher elevations of heart rate and RPP than carriers of the minor A allele ($F_{1,120} = 9.79$, $p < 0.01$ and $F_{1,120} = 7.53$, $p < 0.01$, respectively). These effects remained significant after Bonferroni correction for multiple testing ($p < 0.02$ and $p < 0.04$, respectively).

The rs2242446 SNP significantly moderated the elevation of RPP after MDMA administration, which was attributable to significant moderating effects on MDMA-induced changes in MAP and heart rate. The CC genotype presented elevations of heart rate, MAP, and RPP after MDMA administration compared with the TT genotype. When we combined the rs2242446 CT and CC genotype groups, subjects with the TT genotype presented lower elevations of heart rate and RPP than carriers of the C allele ($F_{1,121} = 7.81$, $p < 0.01$ and $F_{1,121} = 5.64$, $p < 0.05$, respectively). The difference between the genotype groups and the combined groups (TT and CT/CC) in the effects of MDMA on heart rate remained significant after Bonferroni correction for multiple testing ($p < 0.04$ and $p < 0.04$, respectively).

Significant main effects of the rs36029 SNP on MDMA-induced elevations of MAP and RPP were found. When we combined the rs36029 AG and GG genotype groups, subjects with the AA genotype presented higher elevations of MAP, heart rate, and RPP than carriers of the G allele ($F_{1,121} = 9.870$, $p < 0.01$; $F_{1,121} = 4.24$, $p < 0.05$; and $F_{1,121} = 6.91$, $p < 0.01$, respectively). The difference in the effects of MDMA on MAP between the genotype groups and difference in the effects of MDMA on MAP and RPP between the combined groups (AA and AG/GG) remained significant after Bonferroni correction for multiple testing ($p < 0.05$, $p < 0.02$, and $p < 0.05$, respectively).

The effects of the rs1861647, rs2242446, and rs36029 SNPs on the peak response to MDMA were similar when the analyses were performed without using MDMA plasma concentrations as covariate in the ANOVAs (Supplementary Table S1). However, none of the SNPs altered the overall cardiovascular response to MDMA as expressed by the AUEC values (Supplementary Table S2) with the exception of the effect of the rs36029 SNP on the MAP.

The rs168924 and rs47958 SNPs did not alter the effects of MDMA. When we applied Bonferroni correction for multiple testing (for five SNPs), none of the statistical findings remained significant in the additive genotype group models (Table 1) with the exception of the effect of the rs2242446 SNP on heart rate, and sex did not alter the influence of the SNPs on the response to MDMA (Supplementary Table S3).

Discussion

The present study investigated the effect of interindividual differences in the *SLC6A2* gene on the cardiovascular and subjective stimulant response to MDMA. None of the investigated SNPs moderated the subjective stimulant effects of MDMA. Three SNPs of the *SLC6A2* gene (rs1861647,

Table 1 Effects of the SLC6A2 SNPs rs168924, rs47958, rs1861647, rs2242446, and rs36029 on the maximum response to MDMA (mean ± SD and statistics)

SNP rs168924	Number of genotypes	AA	AG	GG	F	*p* value	*p* value (Bonferroni corr.)
N (%)		95 (77)	26 (21)	3 (2)			
Female, *N* (%)		52 (55)	12 (46)	0 (0)			
MDMA plasma concentration AUC (ng/ml)	N 95, 26, 3	956 ± 201	957 ± 226	784 ± 126	1.03	NS	NS
Norepinephrine Δplasma concentration at 2 h (pg/ml)	N 68, 14, 2	0.5 ± 0.6	0.8 ± 0.7	0.5 ± 1.0	0.73	NS	NS
Subjective stimulation, ΔE_{max} (%)	N 95, 26, 3	63 ± 33	72 ± 33	41 ± 42	1.07	NS	NS
Mean arterial pressure, ΔE_{max}, (mmHg)	N 95, 26, 3	18 ± 10	20 ± 9	13 ± 15	0.66	NS	NS
Heart rate, ΔE_{max} (bpm)	N 95, 26, 3	19 ± 15	18 ± 15	17 ± 17	0.04	NS	NS
Rate pressure product, ΔE_{max} (mmHg/min)	N 95, 26, 3	4678 ± 2976	4869 ± 3093	3937 ± 3316	0.05	NS	NS
Pupil size, ΔE_{max} (mm)	N 93, 25, 3	0.9 ± 0.5	1.0 ± 0.4	0.7 ± 0.4	0.62	NS	NS
Pupil size after light, ΔE_{max} (mm)	N 93, 25, 3	2.0 ± 0.7	2.1 ± 0.6	1.5 ± 0.6	1.04	NS	NS
SNP rs47958	Number of genotypes	AA	AC	CC	F	*p* value	*p* value (Bonferroni corr.)
N (%)		27 (22)	58 (47)	39 (31)			
Female, *N* (%)		13 (48)	29 (50)	22 (56)			
MDMA plasma concentration AUC (ng/ml)	N 27, 58, 39	941 ± 177	954 ± 234	957 ± 181	0.06	NS	NS
Norepinephrine Δplasma concentration at 2 h (pg/ml)	N 18, 41, 25	0.4 ± 0.8	0.5 ± 0.7	0.6 ± 0.6	0.29	NS	NS
Subjective stimulation, ΔE_{max} (%)	N 27, 58, 39	62 ± 34	64 ± 34	67 ± 32	0.13	NS	NS
Mean arterial pressure, ΔE_{max} (mmHg)	N 27, 58, 39	21 ± 10	17 ± 8	18 ± 11	1.99	NS	NS
Heart rate, ΔE_{max} (bpm)	N 27, 58, 39	21 ± 14	20 ± 16	14 ± 14	2.41	NS	NS
Rate pressure product, ΔE_{max} (mmHg/min)	N 27, 58, 39	5391 ± 2654	4882 ± 3127	3950 ± 2898	2.36	NS	NS
Pupil size, ΔE_{max} (mm)	N 27, 57, 37	0.8 ± 0.3	1.0 ± 0.4	0.9 ± 0.6	1.48	NS	NS
Pupil size after light, ΔE_{max} (mm)	N 27, 57, 37	1.9 ± 0.5	2.1 ± 0.6	1.9 ± 1.0	0.72	NS	NS
SNP rs1861647	Number of genotypes	AA	AG	GG	F	*p* value	*p* value (Bonferroni corr.)
N (%)		12 (10)	55 (45)	56 (46)			
Female, *N* (%)		6 (50)	28 (51)	29 (52)			
MDMA plasma concentration AUC (ng/ml)	N 12, 55, 56	917 ± 172	943 ± 213	966 ± 205	0.36	NS	NS
Norepinephrine Δplasma concentration at 2 h (pg/ml)	N 6, 39, 38	0.3 ± 0.6	0.6 ± 0.6	0.6 ± 0.8	0.52	NS	NS
Subjective stimulation, ΔE_{max} (%)	N 12, 55, 56	73 ± 29	62 ± 32	64 ± 36	0.79	NS	NS
Mean arterial pressure, ΔE_{max} (mmHg)	N 12, 55, 56	17 ± 11	17 ± 9	19 ± 10	0.42	NS	NS
Heart rate, ΔE_{max} (bpm)	N 12, 55, 56	13 ± 11	15 ± 14**	23 ± 15	4.90	0.009	0.045
Rate pressure product, ΔE_{max} (mmHg/min)	N 12, 55, 56	4025 ± 2235	4018 ± 3099*	5527 ± 2851	3.74	0.027	NS
Pupil size, ΔE_{max} (mm)	N 12, 53, 55	0.8 ± 0.3	0.9 ± 0.5	0.9 ± 0.4	0.37	NS	NS
Pupil size after light, ΔE_{max} (mm)	N 12, 53, 55	2.1 ± 0.6	1.9 ± 0.8	2.1 ± 0.6	0.94	NS	NS
SNP rs2242446	Number of genotypes	CC	CT	TT	F	*p* value	*p* value (Bonferroni corr.)
N (%)		15 (12)	50 (40)	59 (48)			
Female, *N* (%)		6 (40)	26 (52)	32 (54)			
MDMA plasma concentration AUC (ng/ml)	N 15, 50, 59	956 ± 174	942 ± 217	960 ± 205	0.09	NS	NS
Norepinephrine Δplasma concentration at 2 h (pg/ml)	N 10, 36, 38	0.3 ± 0.8	0.6 ± 0.7	0.6 ± 0.6	0.55	NS	NS
Subjective stimulation, ΔE_{max} (%)	N 15, 50, 59	72 ± 33	65 ± 31	61 ± 36	0.77	NS	NS
Mean arterial pressure, ΔE_{max} (mmHg)	N 15, 50, 59	24 ± 11	17 ± 8***	17 ± 10***	3.61	0.030	NS
Heart rate, ΔE_{max} (bpm)	N 15, 50, 59	26 ± 18	21 ± 16	14 ± 12***	5.08	0.008	0.038
Rate pressure product, ΔE_{max} (mmHg/min)	N 15, 50, 59	6205 ± 2997	5042 ± 3191	4027 ± 2642***	4.35	0.015	NS

Table 1 (continued)

Pupil size, ΔE_{max} (mm)	N 15, 48, 58	0.7 ± 0.2	0.9 ± 0.4	0.9 ± 0.5	1.81	NS	NS
Pupil size after light, ΔE_{max} (mm)	N 15, 48, 58	1.9 ± 0.5	1.9 ± 0.5	2.1 ± 0.9	0.97	NS	NS
SNP rs36029	Number of genotypes	AA	AG	GG	F	*p* value	*p* value (Bonferroni corr.)
N (%)		46 (37)	56 (45)	22 (18)			
Female, *N* (%)		19 (41)	32 (57)	13 (59)			
MDMA plasma concentration AUC (ng/ml)	N 46, 56, 22	911 ± 180	972 ± 212	989 ± 230	1.58	NS	NS
Norepinephrine Δplasma concentration at 2 h (pg/ml)	N 33, 37, 14	0.7 ± 0.7	0.4 ± 0.7	0.5 ± 0.5	2.57	NS	NS
Subjective stimulation, ΔE_{max} (%)	N 46, 56, 22	65 ± 33	64 ± 33	62 ± 38	0.50	NS	NS
Mean arterial pressure, ΔE_{max} (mmHg)	N 46, 56, 22	20 ± 10	16 ± 10	17 ± 9	4.82	0.010	0.049
Heart rate, ΔE_{max} (bpm)	N 46, 56, 22	21 ± 16	17 ± 15	16 ± 14	2.12	NS	NS
Rate pressure product, ΔE_{max} (mmHg/min)	N 46, 56, 22	5419 ± 2974	4317 ± 3067	4169 ± 2608	3.47	0.034	NS
Pupil size, ΔE_{max} (mm)	N 46, 54, 21	0.9 ± 0.3	0.9 ± 0.4	0.8 ± 0.6	0.62	NS	NS
Pupil size after light, ΔE_{max} (mm)	N 46, 54, 21	1.9 ± 0.5	2.1 ± 0.7	1.9 ± 1.0	1.25	NS	NS

F and *p* values are from ANCOVAs (except for the MDMA concentrations) with MDMA AUC as covariate to account for differences in MDMA concentrations

N number of subjects, *SNP* single nucleotide polymorphism, E_{max} peak effect, *AUC* area under the concentration-time curve from 0 to 6 h, *NS* not significant, *Δ* values are change scores from placebo (mdma-placebo)

*$p < 0.05$; **$p < 0.01$ compared to rs1861647 GG; ***$p < 0.05$ compared to rs2242446 CC

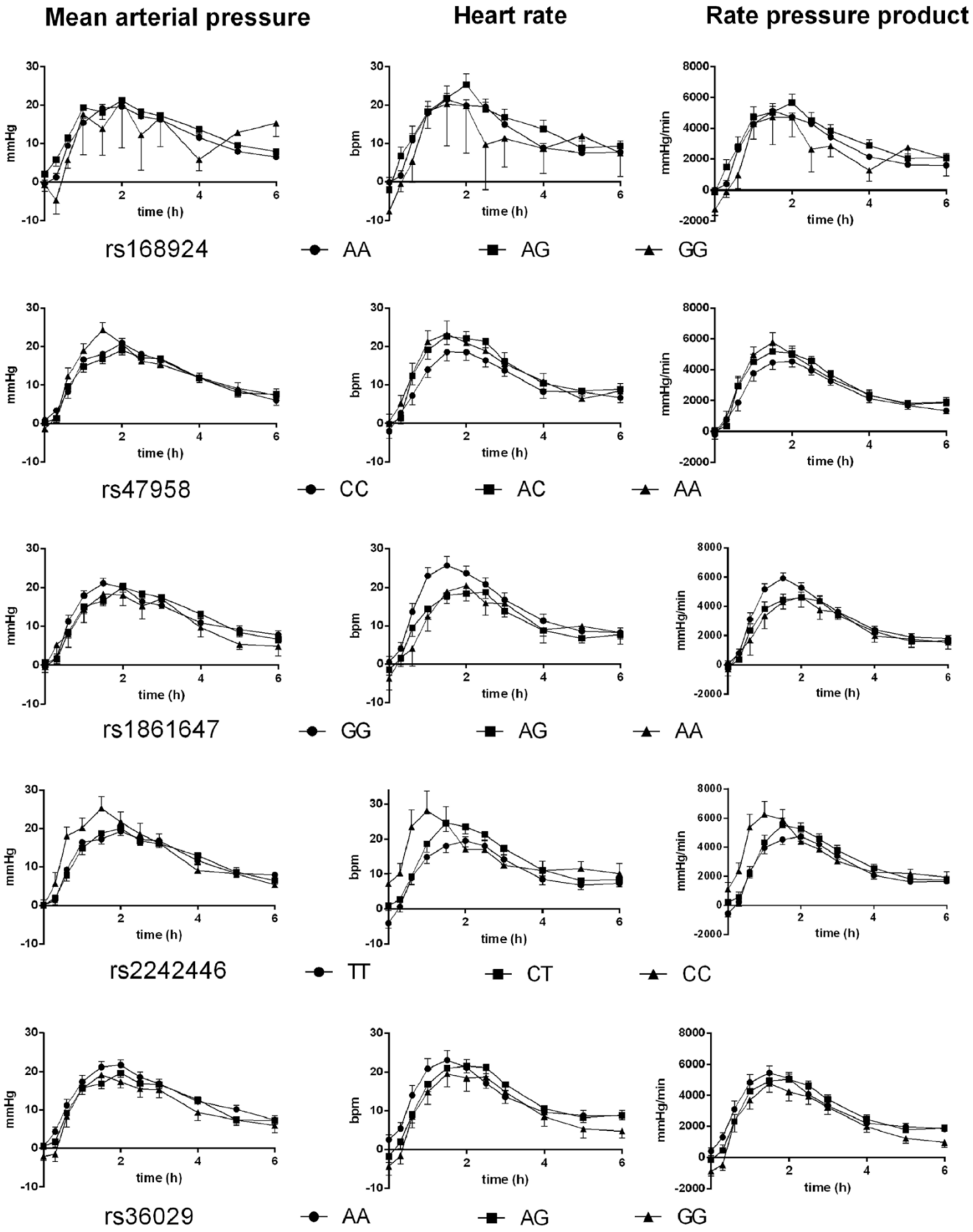
rs2242446, and rs36029) influenced the cardiovascular response to MDMA. However, the effect sizes for these genetic variants were rather small and not very robust. In fact, Bonferroni correction of the data for the five SNPs resulted in the loss of most statistical significance. Thus, although the NET has been implicated in the stimulant-type response to MDMA [7], the genetic variants of the NET gene (*SLC6A2*) that were evaluated herein only minimally influenced the response to MDMA.

To our knowledge, the present study was the first to explore the role of SNPs of the *SLC6A2* gene in the response to MDMA. Thus, no comparisons can be made with other studies that tested MDMA. In a previous study, C-allele carriers of the *SLC6A2* rs2242446 SNP presented higher blood pressure after physical exercise [28], which is consistent with the greater blood pressure response in the present study following the administration of a pharmacological stimulant. Additionally, plasma NE concentrations after exercise differed between different rs2242446 genotypes [28]. In the present study, however, no differences in NE levels after MDMA administration were found between genotype groups. The effects of the *SLC6A2* rs1861647 and rs47958 SNPs on the response to D-amphetamine have previously been reported [26, 27, 39]. Initial studies showed that subjects with the AA genotype of rs1861647 had higher vigor scores after D-amphetamine administration [26]. Additionally, subjects with the CC genotype of rs47958 had higher positive mood scores [27]. However, a subsequent larger replication study found no influence of the different rs1861647 and rs47958 genotypes on the response to D-amphetamine [39] as similarly documented in the present study for the subjective response to MDMA. However, the effects of *SLC6A2* SNPs on the cardiovascular response to D-amphetamine or MDMA have not been studied previously; therefore, the role of the rs1861647 SNP in the cardiovascular stimulant effects of MDMA that was identified in the present study needs further investigation.

Additionally, none of the NET genotypes moderated the MDMA-induced increase in pupil size [21].

The present study has several limitations. First, the sample size was relatively small when considering the mostly small effect sizes for the influence of genetic variants on the MDMA response. Additionally, significant findings in the additive genotype models were mostly lost after Bonferroni correction. Confirmation in studies with larger samples is needed. However, we unlikely missed very large effect sizes for the influence of these genetic variants or possible haplotypes.

Fig. 1 Effects of common genetic variants of the *SLC6A2* gene (rs168924, rs47958, rs1861647, rs2242446, and rs36029) on cardiovascular stimulation after MDMA administration in 124 healthy subjects. Homozygous carriers of *SLC6A2* rs1861647 G allele presented higher elevations of heart rate and rate-pressure product after MDMA than subjects with one G allele. Subjects with the CC genotype of the *SLC6A2* rs2242446 SNP presented higher elevations of heart rate, mean arterial pressure, and rate-pressure product after MDMA administration compared with the TT genotype group. The *SLC6A2* rs168924 and rs47958 SNPs did not significantly alter the response to MDMA. The corresponding maximal effects and statistics are shown in Table 1. The data are expressed as mean ± SEM. MDMA or placebo was administered at time = 0


Mean arterial pressure
Heart rate
Rate pressure product
mmHg
bpm
mmHg/min
time (h)
rs168924
AA
AG
GG
rs47958
CC
AC
AA
rs1861647
GG
AG
AA
rs2242446
TT
CT
CC
rs36029
AA
AG
GG

Second, the study was conducted in mostly young and healthy volunteers. Therefore, the findings cannot necessarily be generalized to people with hypertension or other cardiovascular risk factors. Third, SNPs of the genes of other targets of MDMA, such as the 5-HT transporter [13, 18], may also be involved but were not tested in the present study. However, we considered the moderating effects of known genetic variants that influence the metabolism of MDMA [9, 10] by accounting for interindividual differences in plasma MDMA concentrations.

In conclusion, the present study investigated the influence of genetic polymorphisms of the *SLC6A2* gene on the response to MDMA. Three SNPs of the *SLC6A2* gene (rs2242446, rs1861647, and rs36029) weakly altered the cardiovascular effects of MDMA in healthy subjects. It can be assumed that these genetic polymorphisms may play a minor role in adverse cardiovascular events when MDMA is used recreationally or therapeutically.

Acknowledgements The authors acknowledge the assistance of C. M. Hysek, A. Rickli, Y. Schmid, and P. C. Dolder in conducting the clinical studies; K. Prestin for the assistance with the genotyping; and M. Arends for the text editing.

Author contribution PV analyzed data and wrote the manuscript. HM analyzed the data. MEL conceived the study, obtained funding, analyzed the data, and wrote the manuscript.**Funding information** This study was supported by the Swiss National Science Foundation (grant no. 320030_149493 and 320030_170249).

Compliance with ethical standards

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

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2.6. Role of serotonin transporter and receptor gene variations in the acute effects of MDMA in healthy subjects

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ACS Chemical Neuroscience, 2019, Vol. 10, 3120-3131

ACS Chemical Neuroscience
Cite This: ACS Chem. Neurosci. 2019, 10, 3120−3131
Research Article
pubs.acs.org/chemneuro

Role of Serotonin Transporter and Receptor Gene Variations in the Acute Effects of MDMA in Healthy Subjects

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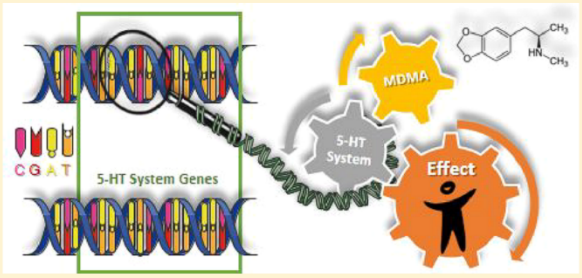
Supporting Information

ABSTRACT: Methylenedioxymethamphetamine (MDMA; ecstasy) is used recreationally and has been investigated as an adjunct to psychotherapy. Most acute effects of MDMA can be attributed to activation of the serotonin (5-hydroxytryptamine [5-HT]) system. Genetic variants, such as single-nucleotide polymorphisms (SNPs) and polymorphic regions in 5-HT system genes, may contribute to interindividual differences in the acute effects of MDMA. We characterized the effects of common genetic variants within selected genes that encode the 5-HT system (*TPH1* [tryptophan 5-hydroxylase 1] rs1800532 and rs1799913, *TPH2* [tryptophan 5-hydroxylase 2] rs7305115, *HTR1A* [5-HT_{1A} receptor] rs6295, *HTR1B* [5-HT_{1B} receptor] rs6296, *HTR2A* [5-HT_{2A} receptor] rs6313, and *SLC6A4* [serotonin transporter] 5-HTTLPR and rs25531) on the physiological and subjective response to 125 mg of MDMA compared with placebo in 124 healthy subjects. Data were pooled from eight randomized, double-blind, placebo-controlled studies that were conducted in the same laboratory. *TPH2* rs7305115, *HTR2A* rs6313, and *SLC6A4* 5-HTTLPR polymorphisms tended to moderately alter some effects of MDMA. However, after correcting for multiple comparisons, none of the tested genetic polymorphisms significantly influenced the response to MDMA. Variations in genes that encode key targets in the 5-HT system did not significantly influence the effects of MDMA in healthy subjects. Interindividual differences in the 5-HT system may thus play a marginal role when MDMA is used recreationally or therapeutically.



KEYWORDS: *MDMA, serotonin system, pharmacogenetics*

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA; molly, ecstasy) is popularly used for its empathic and euphoric effects. Recent research indicates that MDMA may also be useful as an adjunct to psychotherapy in patients with post-traumatic stress disorder (PTSD).[1−3] MDMA mainly acts as a releaser of serotonin (5-hydroxytryptamine [5-HT]) and norepinephrine and to a lesser extent dopamine.[4,5] Compared with amphetamine, typical effects of MDMA can be predominantly attributed to activation of the 5-HT system.[6−16] Key components of the 5-HT system include tryptophan hydroxylase (TPH), the 5-HT transporter (SERT), and 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptors. Manipulations of 5-HT system targets could modulate the effects of MDMA. Pharmacological inhibition of the SERT significantly reduced the psychotropic and most physiological effects of MDMA.[11,13,16,17] Inhibition of the 5-HT_{2A} receptor also attenuated some of the acute effects of MDMA,[12,18,19] whereas $5HT_1$ receptor inhibition had no effect.[20,21]

The role of the 5-HT system in the acute effects of MDMA has been well studied, but little is known about the ways in which interindividual variations of genes that encode targets that are implicated in the mechanism of action of MDMA or its metabolism influence the response to MDMA. For example, genetic variations of the enzymes that are involved in MDMA metabolism (mainly CYP2D6) have been shown to affect plasma levels of MDMA and its metabolites in several clinical studies[22−24] and modulate the pharmacokinetics and some of the pharmacodynamic effects of MDMA. Genetic variants of pharmacological targets of MDMA may also alter its pharmacodynamic effects, but the few studies that have been published to date have reported no or only minimal effects, including potential chance findings.[25−28]

The major target of MDMA in the 5-HT system is the SERT.[4,5,11,29] A common repeat polymorphism in the promoter region of the *SLC6A4* gene (5-HTTLPR), which encodes the SERT, comprises two variants with long (L) and short (S) alleles. Each variant includes a number of SNP variants. However, in the Caucasian population, only the rs25331 SNP is important.[30] In vitro, cells with the LL

Special Issue: Serotonin Research 2018

Received: October 31, 2018
Accepted: December 27, 2018
Published: December 27, 2018

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

polymorphism have approximately double the uptake activity of cells that carry one or two copies of the S allele.[31] In humans, the S allele and L allele with the rs25331 G variant should express an identical low-expressing phenotype.[32−34] Accordingly, three groups were defined: group 1 (LGLG, LGS, and SS) vs. group 2 (LALG, and LAS) vs. group 3 (LALA).[32−34] LG or short 5-HTTLPR allele carriers should present higher levels of serotonin in the synaptic cleft and thus an increase in serotonin signaling compared with homozygous LA carriers. However, MDMA's efficacy crucially depends on activity of the SERT. Individuals with the LG or short 5-HTTLPR variant may present a reduction of MDMA's effects compared with LALA carriers. Kuypers et al. performed a study with 63 polydrug users and found that 75 mg of MDMA produced more anxiety in homozygous L carriers compared with the S group and acutely attenuated self-rated depression in women in the LL group. Pardo-Lozano et al. found higher MDMA-induced cardiovascular effects in L-allele carriers than in SS individuals and more sedation in the SS group than in L-allele carriers.[35] Furthermore, regular ecstasy users who were carriers of the S allele presented a higher risk of mood disorders and emotional and cognitive dysfunction and performed worse on a verbal fluency task.[36−39] Finally, MDMA produced a 2-fold increase in SERT gene expression, and this increase tended to be more pronounced in homozygous L carriers.[40] In contrast, no association was found between the 5-HTTLPR polymorphism and MDMA-induced impairments in memory function or MDMA-induced changes in cortisol levels.[41,42]

MDMA indirectly and partially also directly interacts with 5-HT receptors.[5,10,43] Single-nucleotide polymorphisms of the genes that encode 5-HT receptors could influence the effects of MDMA, but this possibility has not yet been investigated. The rs6295 SNP of the *HTR1A* gene, which encodes the 5-HT_{1A} receptor, may play a role in substance use disorder.[44] Female homozygous carriers of the G allele of the rs6295 who suffered from major depressive disorder benefited more from treatment with a SERT inhibitor than did carriers of the C allele.[45] The rs6296 SNP of *HTR1B*, which encodes the 5-HT_{1B} receptor, was found to influence childhood aggressive behavior. Individuals who were homozygous for the C allele were more aggressive than those who carried the G allele.[46] 5-HT_{2A} receptors are one of the most researched targets of psychoactive drugs. The C allele of the rs6313 SNP of *HTR2A*, which encodes the 5-HT_{2A} receptor, is associated with lower expression and was found to be associated with suicide, a lower ability to adopt the point of view of others, greater anxiety when observing pain, and communication problems.[47−49] However, the rs6313 SNP did not modulate cognitive dysfunction in chronic ecstasy users.[38]

The rate-limiting step in 5-HT biosynthesis is catalyzation by TPH, and MDMA inhibits TPH activity.[50,51] Tryptophan hydroxylase has two isoforms: TPH1 and TPH2. The rs1800532 SNP of TPH1 has been reported to influence gene transcription, and the rare T allele was associated with a decrease in 5-HT synthesis.[52] The T allele has also been associated with SERT inhibitor treatment efficacy and the risk for bipolar disorder and alcohol dependence.[53,54] Additionally, the rs7305115 SNP of *TPH2* has been associated with susceptibility to suicide, in which the A allele was significantly less frequent in suicide attempters than in nonattempters.[55,56]

The present study investigated whether the acute effects of MDMA are influenced by genetic variations within the serotonergic system. We evaluated whether the *TPH1* rs1800532 and rs1799913 SNPs, *TPH2* rs7305115 SNP, *HTR1A* rs6295 SNP, *HTR1B* rs6296 SNP, *HTR2A* rs6313 SNP, and *SLC6A4* 5-HTTLPR and rs25531 polymorphisms influence MDMA-induced subjective, emotional, empathic, cardiovascular, thermogenic, and adverse effects. The goals of the study were to explore contributions from a large number of serotonin system gene variants to responses to MDMA. Thus, the study was mainly exploratory. However, we expected that the results of previous smaller studies that included some of these SNPs would be replicated.[26,35]

RESULTS

Effects of the SNPs on the maximum response (E_{max}) to MDMA are shown in Table 1. Table S1 shows the data for the response to MDMA over time (AUEC). Tables S2 and S3 show the uncorrected statistics for E_{max} and AUEC, respectively. Sex or previous illicit drug experience did not significantly alter the results.

Genotyping. The distribution of the alleles and genotypes did not differ from the distributions that were reported elsewhere in Caucasian cohorts (Ensembl database release 94, October 2018). The minor allele frequencies for rs1800532 and rs1799913, rs7305115, rs6295, rs6296, rs6313, 5-HTTLPR, and rs25531 were T (98 [40%]), A (89 [36%]), G (122 [49%]), G (65 [26%]), A (106 [43%]), S (98 [40%]), and LG+S (106 [43%]), respectively. The tested genetic variants were consistent with Hardy−Weinberg equilibrium ($p > 0.05$).

Subjective Effects. On the tested VASs and AMRSs, MDMA significantly altered the E_{max} values for all reported parameters. With the exception of a decrease in "appetite," all of the parameters were increased by MDMA (Figure 1). The effects of serotonergic system gene polymorphisms on the subjective effects of MDMA are shown in Table 1. Carriers of the *HTR2A* rs6313 A allele had higher ratings of "good drug effect," "trust," AMRS "well-being," "high-mood," and "dreaminess" compared with homozygous G-allele carriers ($F_{1,121} = 6.93$, $p < 0.01$, $F_{1,49} = 6.07$, $p < 0.05$, $F_{1,121} = 5.68$, $p < 0.05$, $F_{1,121} = 6.04$, $p < 0.05$, and $F_{1,121} = 6.95$, $p < 0.01$, respectively). Individuals with the short allele of 5-HTTLPR had higher ratings of "good drug effect," "drug liking," and "closeness to others" and lower ratings of "bad drug effect" compared with the homozygous long allele group ($F_{1,121} = 6.51$, $p < 0.05$, $F_{1,121} = 5.06$, $p < 0.05$, $F_{1,121} = 5.95$, $p < 0.05$, and $F_{1,121} = 4.94$, $p < 0.05$, respectively). Subjects with two long alleles had higher ratings of "fear and depression" on the AMRS compared with short allele carriers ($F_{1,121} = 5.78$, $p < 0.05$). Subjects with the LALA genotype of the *SLC6A4* rs25331 SNP had higher ratings of "fear and depression" on the AMRS and lower ratings of "any drug effect," "good drug effect," and "drug liking" compared with short allele and LG carriers ($F_{1,120} = 4.70$, $p < 0.05$, $F_{1,120} = 4.00$, $p < 0.05$, $F_{1,120} = 5.48$, $p < 0.05$, and $F_{1,120} = 4.51$, $p < 0.05$, respectively). Nyholt correction for multiple comparisons indicated that the genetic polymorphisms had no significant effect on these subjective parameters.

Emotion Recognition. On the FERT, MDMA impaired the recognition of fearful, sad, and angry faces compared with placebo ($F_{1,67} = 47$, $p < 0.001$, $F_{1,67} = 15$, $p < 0.001$, and $F_{1,67} = 17$, $p < 0.001$, respectively). None of the serotonergic system gene variants modulated the effects of MDMA on the FERT.

Empathy. MDMA decreased cognitive empathy for all emotions ($F_{1,67} = 5.0$, $p < 0.05$) and increased explicit

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Table 1. Effects of Polymorphisms in the Serotonergic System on the Maximal Response to 125 mg of MDMA (Mean ± SD and Statistics) Corrected with MDMA AUC_6 (Exclusive Plasma Concentrations)

	TPH1 rs1800532	GG	GT	TT	F	p value	p value[a]	η^2
N (%)		44 (35)	62 (50)	18 (15)				
female, *N* (%)		23 (52)	31 (50)	10 (56)				
MDMA plasma concentration C_{max}, ng/mL	*N*: 44, 62, 18	221 ± 51	228 ± 48	232 ± 42	0.43	NS		0.007
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 44, 62, 18	944 ± 221	949 ± 202	998 ± 200	0.48	NS		0.008
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 44, 62, 18	81 ± 19	73 ± 26	74 ± 26	2.32	NS	NS	0.027
good drug effect	*N*: 44, 62, 18	81 ± 23	72 ± 29	72 ± 28	2.00	NS	NS	0.029
bad drug effect	*N*: 44, 62, 18	14 ± 23	21 ± 29	14 ± 19	1.50	NS	NS	0.023
drug liking	*N*: 44, 62, 18	81 ± 21	74 ± 29	76 ± 29	1.05	NS	NS	0.016
closeness to others	*N*: 44, 62, 18	23 ± 17	21 ± 19	27 ± 19	0.39	NS	NS	0.006
high-mood	*N*: 44, 62, 18	74 ± 32	71 ± 31	71 ± 31	0.37	NS	NS	0.006
talkative	*N*: 44, 62, 18	25 ± 20	19 ± 19	25 ± 17	1.38	NS	NS	0.021
appetite	*N*: 24, 42, 6	−7 ± 33	−6 ± 31	−15 ± 22	0.20	NS	NS	0.006
tired	*N*: 41, 53, 15	21 ± 32	18 ± 33	23 ± 31	0.13	NS	NS	0.002
fear	*N*: 24, 42, 6	4 ± 14	9 ± 20	4 ± 9	0.91	NS	NS	0.026
happy	*N*: 26, 40, 15	28 ± 18	27 ± 20	33 ± 18	0.29	NS	NS	0.007
content	*N*: 26, 40, 15	32 ± 14	29 ± 21	32 ± 18	0.65	NS	NS	0.015
trust	*N*: 20, 20, 12	23 ± 18	23 ± 23	25 ± 26	0.15	NS	NS	0.005
want to be hugged	*N*: 20, 20, 12	11 ± 19	19 ± 20	24 ± 20	0.83	NS	NS	0.028
want to hug	*N*: 20, 20, 12	14 ± 19	19 ± 19	23 ± 19	0.33	NS	NS	0.011
vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 44, 62, 18	26 ± 12	22 ± 13	27 ± 12	1.62	NS	NS	0.024
diastolic blood pressure, mmHg	*N*: 44, 62, 18	14 ± 11	14 ± 9	15 ± 9	0.04	NS	NS	0.001
mean arterial pressure, mmHg	*N*: 44, 62, 18	18 ± 10	17 ± 10	19 ± 9	0.13	NS	NS	0.002
rate pressure product, mmHg/min	*N*: 44, 62, 18	5311 ± 3048	4314 ± 2906	4779 ± 2783	1.61	NS	NS	0.025
body temperature, °C	*N*: 44, 62, 18	0.3 ± 0.5	0.2 ± 0.5	0.4 ± 0.5	1.27	NS	NS	0.021
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	*N*: 44, 62, 18	5.8 ± 5.5	4.9 ± 5.1	4.9 ± 6.5	0.38	NS	NS	0.006
high mood	*N*: 44, 62, 18	3.5 ± 3.1	2.7 ± 3.1	2.9 ± 3.7	0.75	NS	NS	0.012
fear/depression	*N*: 44, 62, 18	0.0 ± 3.4	1.3 ± 3.2	0.6 ± 2.0	2.40	NS	NS	0.038
dreaminess	*N*: 44, 62, 18	3.3 ± 2.6	3.1 ± 3.5	2.5 ± 3.2	0.58	NS	NS	0.009
List of Complaints Δscore								
acute, up to 6 h, *N*	*N*: 44, 62, 18	8.5 ± 7.4	8.6 ± 6.8	8.5 ± 4.8	0.04	NS	NS	0.001
subacute, up to 24 h, *N*	*N*: 44, 62, 18	5.1 ± 5.6	4.7 ± 5.3	3.7 ± 5.4	0.70	NS	NS	0.011
	TPH2 rs7305115	AA	AG	GG	F	p value	p value[a]	η^2
N (%)		14 (11)	61 (49)	49 (40)				
female, N (%)		7 (50)	30 (49)	27 (55)				
MDMA plasma concentration C_{max}, ng/mL	*N*: 14, 61, 49	221 ± 46	225 ± 49	228 ± 49	0.15	NS		0.003
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 14, 61, 49	958 ± 215	947 ± 209	962 ± 207	0.08	NS		0.001
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 14, 61, 49	77 ± 20	75 ± 25	77 ± 24	0.11	NS	NS	0.001
good drug effect	*N*: 14, 61, 49	71 ± 31	74 ± 28	79 ± 24	0.59	NS	NS	0.009
bad drug effect	*N*: 14, 61, 49	17 ± 21	15 ± 29	20 ± 22	0.33	NS	NS	0.005
drug liking	*N*: 14, 61, 49	69 ± 35	76 ± 27	81 ± 23	1.27	NS	NS	0.020
closeness to others	*N*: 14, 61, 49	19 ± 20	22 ± 17	25 ± 19	0.85	NS	NS	0.012
high-mood	*N*: 14, 61, 49	72 ± 29	69 ± 34	75 ± 28	0.49	NS	NS	0.007
talkative	*N*: 14, 61, 49	18 ± 23	21 ± 19	24 ± 17	0.76	NS	NS	0.012
appetite	*N*: 7, 33, 32	−3 ± 32	−9 ± 28	−6 ± 34	0.07	NS	NS	0.002
tired	*N*: 12, 54, 43	13 ± 34	20 ± 31	22 ± 33	0.28	NS	NS	0.005
fear	*N*: 7, 33, 32	−1 ± 12	6 ± 19	9 ± 17	0.86	NS	NS	0.025
happy	*N*: 12, 41, 28	27 ± 21	28 ± 18	29 ± 20	0.05	NS	NS	0.001
content	*N*: 12, 41, 28	27 ± 22	30 ± 17	32 ± 19	0.28	NS	NS	0.007
trust	*N*: 7, 28, 17	14 ± 30	23 ± 20	28 ± 21	1.11	NS	NS	0.036
want to be hugged	*N*: 7, 28, 17	7 ± 8	14 ± 20	26 ± 20	2.29	NS	NS	0.073
want to hug	*N*: 7, 28, 17	7 ± 8	16 ± 19	26 ± 20	2.62	NS	NS	0.080
vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 14, 61, 49	22 ± 13	25 ± 14	23 ± 11	0.92	NS	NS	0.014
diastolic blood pressure, mmHg	*N*: 14, 61, 49	14 ± 9	15 ± 10	14 ± 8	0.28	NS	NS	0.004
mean arterial pressure, mmHg	*N*: 14, 61, 49	16 ± 10	18 ± 10	17 ± 9	0.50	NS	NS	0.008

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Table 1. continued

	TPH2 rs7305115	AA	AG	GG	F	p value	p value[a]	η^2
vital signs parameters ΔE_{max}								
rate pressure product, mmHg/min	*N*: 14, 61, 49	3757 ± 2787	4786 ± 2838	4952 ± 3135	0.95	NS	NS	0.015
body temperature, °C	*N*: 14, 61, 49	−0.1 ± 0.6	0.3 ± 0.5*	0.2 ± 0.4	4.11	0.019	NS	0.064
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	*N*: 14, 61, 49	3.4 ± 4.3	5.3 ± 6.0	5.5 ± 4.9	0.91	NS	NS	0.015
high mood	*N*: 14, 61, 49	2.4 ± 3.1	3.1 ± 3.5	3.0 ± 3.0	0.33	NS	NS	0.005
fear/depression	*N*: 14, 61, 49	−0.5 ± 3.3	0.8 ± 3.6	1.0 ± 2.5	1.19	NS	NS	0.019
dreaminess	*N*: 14, 61, 49	2.9 ± 3.6	2.8 ± 2.9	3.4 ± 3.3	0.51	NS	NS	0.008
List of Complaints Δscore								
acute, up to 6 h, *N*	*N*: 14, 61, 49	7.4 ± 7.1	8.4 ± 6.7	9.0 ± 6.8	0.30	NS	NS	0.005
subacute, up to 24 h, *N*	*N*: 14, 61, 49	2.9 ± 4.2	4.7 ± 5.9	5.2 ± 5.0	0.97	NS	NS	0.015
	5HTR1A rs6295	CC	CG	GG	F	p value	p value[a]	η^2
N (%)		29 (23)	68 (55)	27 (22)				
female, *N* (%)		14 (48)	36 (53)	14 (52)				
MDMA plasma concentration C_{max}, ng/mL	*N*: 29, 68, 27	218 ± 46	233 ± 48	216 ± 50	1.64	NS		0.026
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 29, 68, 27	903 ± 180	1005 ± 216[#]	880 ± 183	4.99	0.008		0.076
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 29, 68, 27	79 ± 20	79 ± 23	66 ± 27	2.06	NS	NS	0.024
good drug effect	*N*: 29, 68, 27	77 ± 23	77 ± 28	71 ± 29	0.37	NS	NS	0.005
bad drug effect	*N*: 29, 68, 27	25 ± 24	17 ± 26	10 ± 24	2.78	NS	NS	0.041
drug liking	*N*: 29, 68, 27	78 ± 23	78 ± 28	73 ± 28	0.23	NS	NS	0.004
closeness to others	*N*: 29, 68, 27	21 ± 20	24 ± 18	21 ± 16	0.08	NS	NS	0.001
high-mood	*N*: 29, 68, 27	70 ± 31	73 ± 33	71 ± 29	0.16	NS	NS	0.002
talkative	*N*: 29, 68, 27	18 ± 18	24 ± 19	21 ± 18	0.46	NS	NS	0.007
appetite	*N*: 18, 38, 16	−13 ± 37	−3 ± 28	−10 ± 30	1.49	NS	NS	0.041
tired	*N*: 26, 58, 25	23 ± 34	22 ± 31	11 ± 33	1.05	NS	NS	0.019
fear	*N*: 18, 38, 16	6 ± 9	9 ± 23	3 ± 8	0.79	NS	NS	0.023
happy	*N*: 16, 48, 17	25 ± 20	28 ± 20	33 ± 16	1.09	NS	NS	0.025
content	*N*: 16, 48, 17	28 ± 19	30 ± 19	33 ± 18	0.57	NS	NS	0.014
trust	*N*: 11, 30, 11	20 ± 24	23 ± 21	27 ± 24	1.10	NS	NS	0.035
want to be hugged	*N*: 11, 30, 11	14 ± 19	18 ± 21	19 ± 19	0.55	NS	NS	0.019
want to hug	*N*: 11, 30, 11	14 ± 19	18 ± 20	22 ± 16	1.23	NS	NS	0.040
vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 29, 68, 27	21 ± 15	25 ± 12	23 ± 12	0.37	NS	NS	0.006
diastolic blood pressure, mmHg	*N*: 29, 68, 27	16 ± 9	14 ± 10	13 ± 9	0.99	NS	NS	0.015
mean arterial pressure, mmHg	*N*: 29, 68, 27	18 ± 10	18 ± 10	16 ± 9	0.38	NS	NS	0.006
rate pressure product, mmHg/min	*N*: 29, 68, 27	4916 ± 3052	4707 ± 3032	4612 ± 2729	0.39	NS	NS	0.006
body temperature, °C	*N*: 29, 68, 27	0.2 ± 0.6	0.2 ± 0.5	0.3 ± 0.4	0.50	NS	NS	0.008
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	*N*: 29, 68, 27	4.9 ± 5.3	5.4 ± 4.9	5.1 ± 6.7	0.07	NS	NS	0.001
high mood	*N*: 29, 68, 27	2.6 ± 3.2	3.0 ± 3.0	3.4 ± 3.9	0.42	NS	NS	0.007
fear/depression	*N*: 29, 68, 27	0.2 ± 3.2	1.3 ± 3.3	0.0 ± 2.7	2.32	NS	NS	0.037
dreaminess	*N*: 29, 68, 27	3.2 ± 3.3	3.4 ± 3.2	2.1 ± 2.7	1.30	NS	NS	0.021
List of Complaints Δscore								
acute, up to 6h, N	*N*: 29, 68, 27	7.7 ± 7.0	9.1 ± 6.4	8.0 ± 7.3	0.09	NS	NS	0.001
subacute, up to 24h, N	*N*: 29, 68, 27	5.7 ± 5.0	4.5 ± 5.8	4.1 ± 4.8	1.22	NS	NS	0.019
	5HTR1B rs6296	CC	CG	GG	F	p value	p value[a]	η^2
N (%)		69 (56)	45 (36)	10 (8)				
Female, *N* (%)		34 (49)	23 (51)	7 (70)				
MDMA plasma concentration C_{max}, ng/mL	*N*: 69, 45, 10	222 ± 47	229 ± 50	241 ± 49	0.87	NS		0.014
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 69, 45, 10	925 ± 203	976 ± 208	1061 ± 209	2.32	NS		0.037
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 69, 45, 10	75 ± 22	77 ± 24	77 ± 32	0.45	NS	NS	0.005
good drug effect	*N*: 69, 45, 10	74 ± 28	79 ± 25	71 ± 28	0.82	NS	NS	0.012
bad drug effect	*N*: 69, 45, 10	18 ± 24	16 ± 29	19 ± 25	0.40	NS	NS	0.006
drug liking	*N*: 69, 45, 10	77 ± 27	79 ± 25	70 ± 30	0.89	NS	NS	0.014
closeness to others	*N*: 69, 45, 10	22 ± 18	23 ± 19	28 ± 18	0.07	NS	NS	0.001
high-mood	*N*: 69, 45, 10	70 ± 31	76 ± 31	66 ± 39	0.87	NS	NS	0.013
talkative	*N*: 69, 45, 10	21 ± 19	22 ± 20	24 ± 16	0.00	NS	NS	0.000
appetite	*N*: 40, 27, 5	−4 ± 31	−14 ± 32	−2 ± 22	0.55	NS	NS	0.015

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Table 1. continued

	5HTR1B rs6296	CC	CG	GG	F	p value	p value[a]	η^2
Visual Analog Scale rating ΔE_{max}								
tired	*N*: 63, 39, 7	22 ± 34	19 ± 31	4 ± 21	1.84	NS	NS	0.032
fear	*N*: 40, 27, 5	7 ± 19	7 ± 16	0 ± 17	0.41	NS	NS	0.012
happy	*N*: 44, 30, 7	28 ± 19	28 ± 20	37 ± 17	0.26	NS	NS	0.006
content	*N*: 44, 30, 7	31 ± 18	28 ± 19	37 ± 17	0.53	NS	NS	0.013
trust	*N*: 29, 18, 5	20 ± 22	23 ± 22	45 ± 7	1.15	NS	NS	0.037
want to be hugged	*N*: 29, 18, 5	14 ± 19	19 ± 21	26 ± 22	0.51	NS	NS	0.018
want to hug	*N*: 29, 18, 5	14 ± 18	21 ± 19	27 ± 21	1.18	NS	NS	0.038
vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 69, 45, 10	23 ± 13	27 ± 12	22 ± 10	1.41	NS	NS	0.021
diastolic blood pressure, mmHg	*N*: 69, 45, 10	14 ± 10	13 ± 9	16 ± 11	0.46	NS	NS	0.007
mean arterial pressure, mmHg	*N*: 69, 45, 10	18 ± 10	18 ± 10	17 ± 10	0.21	NS	NS	0.003
rate pressure product, mmHg/min	*N*: 69, 45, 10	4513 ± 2801	5306 ± 3130	3702 ± 2956	1.91	NS	NS	0.029
body temperature, °C	*N*: 69, 45, 10	0.2 ± 0.5	0.3 ± 0.5	0.2 ± 0.7	0.75	NS	NS	0.012
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	*N*: 69, 45, 10	5.2 ± 5.1	4.7 ± 5.9	7.7 ± 4.9	1.24	NS	NS	0.020
high mood	*N*: 69, 45, 10	2.9 ± 3.1	2.7 ± 3.3	4.7 ± 3.2	1.57	NS	NS	0.026
fear/depression	*N*: 69, 45, 10	1.0 ± 3.0	0.5 ± 3.7	−0.2 ± 1.9	0.82	NS	NS	0.013
dreaminess	*N*: 69, 45, 10	3.0 ± 3.2	3.0 ± 3.2	4.1 ± 2.8	0.37	NS	NS	0.006
List of Complaints Δscore								
acute, up to 6 h, *N*	*N*: 69, 45, 10	8.2 ± 6.8	9.4 ± 6.5	7.0 ± 7.5	0.90	NS	NS	0.014
subacute, up to 24 h, *N*	*N*: 69, 45, 10	5.0 ± 5.5	4.6 ± 5.3	3.2 ± 5.9	1.18	NS	NS	0.018
	5HTR2A rs6313	AA	AG	GG	F	p value	p value[a]	η^2
N (%)		22 (18)	62 (50)	40 (32)				
female, *N* (%)		8 (36)	36 (58)	20 (50)				
MDMA plasma concentration C_{max}, ng/mL	*N*: 22, 62, 40	211 ± 44	238 ± 48	216 ± 47	4.00	0.021		0.062
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 22, 62, 40	885 ± 173	990 ± 206	937 ± 220	2.30	NS		0.037
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 22, 62, 40	69 ± 24	80 ± 23	73 ± 24	0.53	NS	NS	0.006
good drug effect	*N*: 22, 62, 40	76 ± 23	81 ± 26^{+}	66 ± 29	3.46	0.035	NS	0.049
bad drug effect	*N*: 22, 62, 40	13 ± 20	17 ± 27	22 ± 25	1.05	NS	NS	0.016
drug liking	*N*: 22, 62, 40	77 ± 20	82 ± 27	69 ± 27	2.23	NS	NS	0.034
closeness to others	*N*: 22, 62, 40	20 ± 17	26 ± 18	19 ± 19	1.06	NS	NS	0.015
high-mood	*N*: 22, 62, 40	67 ± 31	79 ± 29	64 ± 33	2.45	NS	NS	0.036
talkative	*N*: 22, 62, 40	22 ± 18	24 ± 19	19 ± 19	0.39	NS	NS	0.006
appetite	*N*: 12, 42, 18	−6 ± 29	−8 ± 29	−6 ± 37	0.07	NS	NS	0.002
tired	*N*: 20, 54, 35	22 ± 27	20 ± 34	18 ± 33	0.19	NS	NS	0.003
fear	*N*: 12, 42, 18	0 ± 4	6 ± 13	14 ± 27	2.72	NS	NS	0.074
happy	*N*: 17, 34, 30	29 ± 17	32 ± 18	24 ± 21	0.94	NS	NS	0.021
content	*N*: 17, 34, 30	32 ± 17	32 ± 19	27 ± 19	0.52	NS	NS	0.012
trust	*N*: 10, 20, 22	35 ± 17^{+}	28 ± 20	14 ± 23	3.62	0.034	NS	0.106
want to be hugged	*N*: 10, 20, 22	17 ± 19	23 ± 20	12 ± 20	1.07	NS	NS	0.036
want to hug	*N*: 10, 20, 22	20 ± 17	23 ± 19	12 ± 20	1.09	NS	NS	0.035
vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 22, 62, 40	21 ± 10	25 ± 14	24 ± 12	0.42	NS	NS	0.006
diastolic blood pressure, mmHg	*N*: 22, 62, 40	14 ± 14	14 ± 9	14 ± 7	0.17	NS	NS	0.003
mean arterial pressure, mmHg	*N*: 22, 62, 40	17 ± 12	18 ± 10	18 ± 8	0.02	NS	NS	0.000
rate pressure product, mmHg/min	*N*: 22, 62, 40	4522 ± 2610	4439 ± 2772	5311 ± 3360	1.61	NS	NS	0.025
body temperature, °C	*N*: 22, 62, 40	0.3 ± 0.5	0.2 ± 0.6	0.2 ± 0.5	0.21	NS	NS	0.003
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	*N*: 22, 62, 40	5.1 ± 4.1	6.3 ± 5.1^{+}	3.5 ± 6.1	3.18	0.045	NS	0.050
high mood	*N*: 22, 62, 40	2.8 ± 2.7	3.7 ± 3.0^{++}	2.0 ± 3.6	3.78	0.026	NS	0.059
fear/depression	*N*: 22, 62, 40	0.9 ± 2.9	0.5 ± 3.1	1.1 ± 3.6	0.52	NS	NS	0.009
dreaminess	*N*: 22, 62, 40	2.9 ± 3.8	3.9 ± 3.0^{+}	2.0 ± 2.7	4.02	0.020	NS	0.061
List of Complaints Δscore								
acute, up to 6h, N	*N*: 22, 62, 40	7.3 ± 5.8	8.2 ± 6.6	9.8 ± 7.4	1.27	NS	NS	0.020
subacute, up to 24h, N	*N*: 22, 62, 40	3.9 ± 5.4	4.6 ± 5.0	5.3 ± 6.1	0.57	NS	NS	0.009
	SLC6A4 5-HTTPLR	LL	LS	SS	F	p value	p value[a]	η^2
N (%)		45 (36)	60 (48)	19 (15)				
female, *N* (%)		27 (60)	26 (43)	11 (58)				

DOI: 10.1021/acschemneuro.8b00590
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Table 1. continued

	SLC6A4 5-HTTPLR	LL	LS	SS	F	p value	p value[a]	η^2
MDMA plasma concentration C_{max}, ng/mL	*N*: 45, 60, 19	232 ± 50	222 ± 44	223 ± 57	0.61	NS		0.010
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 45, 60, 19	1000 ± 222	933 ± 187	913 ± 224	1.80	NS		0.029
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 45, 60, 19	74 ± 24	77 ± 24	77 ± 22	2.17	NS	NS	0.026
good drug effect	*N*: 45, 60, 19	70 ± 27	77 ± 26	83 ± 29	3.67	0.028	NS	0.052
bad drug effect	*N*: 45, 60, 19	25 ± 25	13 ± 27	13 ± 17	2.46	NS	NS	0.037
drug liking	*N*: 45, 60, 19	72 ± 27	79 ± 25	83 ± 31	2.79	NS	NS	0.042
closeness to others	*N*: 45, 60, 19	19 ± 20	24 ± 17	26 ± 18	3.07	NS	NS	0.042
high-mood	*N*: 45, 60, 19	68 ± 32	72 ± 32	81 ± 26	2.47	NS	NS	0.036
talkative	*N*: 45, 60, 19	22 ± 18	21 ± 19	23 ± 19	0.24	NS	NS	0.004
appetite	*N*: 20, 41, 11	−8 ± 31	−7 ± 32	−8 ± 30	0.10	NS	NS	0.003
tired	*N*: 39, 54, 16	26 ± 31	19 ± 33	8 ± 31	1.27	NS	NS	0.022
fear	*N*: 20, 41, 11	11 ± 17	6 ± 19	2 ± 6	1.00	NS	NS	0.029
happy	*N*: 33, 36, 12	26 ± 21	29 ± 17	32 ± 20	1.17	NS	NS	0.026
content	*N*: 33, 36, 12	28 ± 19	31 ± 17	33 ± 20	0.84	NS	NS	0.020
trust	*N*: 25, 19, 8	23 ± 23	24 ± 21	24 ± 21	0.35	NS	NS	0.012
want to be hugged	*N*: 25, 19, 8	17 ± 21	18 ± 20	18 ± 19	0.26	NS	NS	0.009
want to hug	*N*: 25, 19, 8	18 ± 20	17 ± 19	20 ± 18	0.15	NS	NS	0.005
vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 45, 60, 19	23 ± 13	25 ± 13	24 ± 11	0.97	NS	NS	0.015
diastolic blood pressure, mmHg	*N*: 45, 60, 19	15 ± 11	13 ± 9	15 ± 9	0.44	NS	NS	0.007
mean arterial pressure, mmHg	*N*: 45, 60, 19	18 ± 11	18 ± 9	18 ± 8	0.21	NS	NS	0.003
rate pressure product, mmHg/min	*N*: 45, 60, 19	4525 ± 2971	4799 ± 3031	5031 ± 2764	0.63	NS	NS	0.010
body temperature, °C	*N*: 45, 60, 19	0.1 ± 0.5	0.3 ± 0.6	0.3 ± 0.4	1.11	NS	NS	0.018
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	*N*: 45, 60, 19	5.4 ± 5.0	4.4 ± 5.4	7.2 ± 5.9	1.93	NS	NS	0.031
high mood	*N*: 45, 60, 19	3.2 ± 3.1	2.5 ± 3.3	4.2 ± 3.1	2.23	NS	NS	0.036
fear/depression	*N*: 45, 60, 19	1.6 ± 3.0	0.1 ± 3.4	0.6 ± 2.7	3.07	NS	NS	0.049
dreaminess	*N*: 45, 60, 19	3.0 ± 3.2	3.1 ± 3.2	3.1 ± 3.2	0.10	NS	NS	0.002
List of Complaints Δscore								
acute, up to 6 h, *N*	*N*: 45, 60, 19	9.7 ± 7.1	7.4 ± 6.8	9.4 ± 5.3	1.28	NS	NS	0.020
subacute, up to 24 h, *N*	*N*: 45, 60, 19	5.3 ± 5.9	4.8 ± 5.2	2.9 ± 4.8	0.97	NS	NS	0.015
	SLC6A4 rs25531	LALA	LALG+LAS	LGLG+LGS +SS	F	p value	p value[a]	η^2
N (%)		42 (34)	56 (46)	25 (20)				
female, *N* (%)		25 (60)	24 (43)	15 (60)				
MDMA plasma concentration C_{max}, ng/mL	*N*: 42, 56, 25	233 ± 51	220 ± 44	230 ± 54	0.97	NS		0.074
MDMA plasma concentration AUC_6, ng·h/mL	*N*: 42, 56, 25	998 ± 225	931 ± 189	945 ± 217	1.32	NS		0.080
Visual Analog Scale rating ΔE_{max}								
any drug effect	*N*: 42, 56, 25	74 ± 26	76 ± 24	82 ± 21	2.52	NS	NS	0.037
good drug effect	*N*: 42, 56, 25	70 ± 28	76 ± 25	85 ± 26	3.69	0.028	NS	0.056
bad drug effect	*N*: 42, 56, 25	24 ± 24	13 ± 28	17 ± 21	1.32	NS	NS	0.053
drug liking	*N*: 42, 56, 25	72 ± 28	78 ± 24	84 ± 29	2.69	NS	NS	0.042
closeness to others	*N*: 42, 56, 25	20 ± 20	22 ± 17	28 ± 17	2.74	NS	NS	0.052
high-mood	*N*: 42, 56, 25	67 ± 34	71 ± 31	84 ± 24	3.44	0.035	NS	0.053
talkative	*N*: 42, 56, 25	23 ± 19	20 ± 19	24 ± 18	0.30	NS	NS	0.006
appetite	*N*: 20, 35, 17	−7 ± 30	−6 ± 31	−9 ± 32	0.13	NS	NS	0.005
tired	*N*: 37, 49, 22	26 ± 31	19 ± 33	12 ± 33	0.97	NS	NS	0.023
fear	*N*: 20, 35, 17	10 ± 17	5 ± 20	6 ± 11	0.56	NS	NS	0.044
happy	*N*: 30, 38, 12	26 ± 21	30 ± 17	32 ± 20	1.13	NS	NS	0.029
content	*N*: 30, 38, 12	28 ± 19	32 ± 17	33 ± 20	0.97	NS	NS	0.026
trust	*N*: 22, 21, 8	22 ± 23	26 ± 21	24 ± 21	0.43	NS	NS	0.021
want to be hugged	*N*: 22, 21, 8	16 ± 20	18 ± 21	18 ± 19	0.18	NS	NS	0.009
want to hug	*N*: 22, 21, 8	17 ± 19	18 ± 20	20 ± 18	0.13	NS	NS	0.007
Vital signs parameters ΔE_{max}								
systolic blood pressure, mmHg	*N*: 42, 56, 25	22 ± 13	25 ± 13	26 ± 11	1.81	NS	NS	0.056
diastolic blood pressure, mmHg	*N*: 42, 56, 25	15 ± 11	13 ± 9	16 ± 8	1.03	NS	NS	0.027
mean arterial pressure, mmHg	*N*: 42, 56, 25	17 ± 11	17 ± 9	20 ± 8	0.84	NS	NS	0.022
rate pressure product, mmHg/min	*N*: 42, 56, 25	4503 ± 2954	4539 ± 2959	5622 ± 2880	1.66	NS	NS	0.044
body temperature, °C	*N*: 42, 56, 25	0.2 ± 0.5	0.2 ± 0.6	0.4 ± 0.4	0.89	NS	NS	0.041

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Table 1. continued

	SLC6A4 rs25531	LALA	LALG+LAS	LGLG+LGS +SS	F	p value	p value[a]	η^2
Adjective Mood Rating Scale rating ΔE_{max}								
well-being	N: 42, 56, 25	5.1 ± 4.9	4.6 ± 5.7	6.4 ± 5.5	0.90	NS	NS	0.056
high mood	N: 42, 56, 25	3.0 ± 3.1	2.5 ± 3.4	3.8 ± 3.0	1.25	NS	NS	0.066
fear/depression	N: 42, 56, 25	1.6 ± 2.8	0.3 ± 3.3	0.3 ± 3.5	2.33	NS	NS	0.050
dreaminess	N: 42, 56, 25	3.1 ± 3.2	3.0 ± 3.1	3.3 ± 3.2	0.08	NS	NS	0.014
List of Complaints Δscore								
acute, up to 6 h, N	N: 42, 56, 25	9.2 ± 7.2	7.9 ± 6.7	9.1 ± 6.2	0.28	NS	NS	0.030
subacute, up to 24 h, N	N: 42, 56, 25	5.2 ± 5.9	4.7 ± 5.2	4.0 ± 5.3	0.22	NS	NS	0.024

[a]N, number of subjects; SD, standard deviation; NS, not significant; D, values are change scores from placebo; ap value additionally corrected for multiple comparisons according to the Nyholt method; η^2, eta square; $^*p < 0.05$ compared to AA; $^{\#}p < 0.05$; $^{+}p < 0.05$; $^{++}p < 0.01$ compared to GG.

emotional empathy for positive emotions ($F_{1,67} = 8.5$, $p < 0.01$) compared with placebo. None of the serotonergic system gene variants altered the effects of MDMA on the MET.

Physiological Effects. MDMA significantly increased the E_{max} values for blood pressure, MAP, RPP, and body temperature. The effects of the polymorphisms on elevations of blood pressure, MAP, RPP, and body temperature in response to MDMA are shown in Table 1. MDMA produced a higher peak body temperature in G-allele carriers of the *TPH2* rs7305115 SNP compared with homozygous A-allele carriers ($F_{1,121} = 4.84$, $p < 0.05$). Nyholt correction for multiple comparisons indicated that the genetic polymorphisms had no significant effect on these physiological parameters.

Adverse Effects of MDMA. MDMA significantly increased LC scores after up to 6 h and up to 24 h (Table 1). Specifically, MDMA increased the acute and subacute scores for "lack of appetite," "nausea," and "dizziness." Individuals with the GG genotype of the *TPH2* rs7305115 SNP suffered more often from acute "lack of appetite" than individuals with the AA genotype (mean ± SD: 0.36 ± 0.50 for AA vs. 0.76 ± 0.43 for GG; $p = 0.017$). Subjects with the A allele of the *HTR2A* rs6313 SNP felt less acute "dizziness" than subjects who were homozygous for the G allele (mean ± SD: 0.25 ± 0.44 for AA/AG vs. 0.55 ± 0.55 for GG; $p = 0.0012$). Nyholt correction for multiple comparisons indicated that the genetic polymorphisms had no significant effect on the adverse effects of MDMA.

Plasma Concentrations of MDMA. MDMA concentrations are shown in Table 1. MDMA concentrations similarly increased across all serotonergic system gene variants (Table 1), with the exception of the rs6295 SNP (MDMA AUC_6; CG vs GG, $p < 0.05$) and rs6313 SNP (MDMA C_{max}). Peak MDMA concentrations and AUC_6 values were (mean ± SD) 226 ± 48 ng/mL and 954 ± 208 ng·h/mL in the total of 124 subjects.

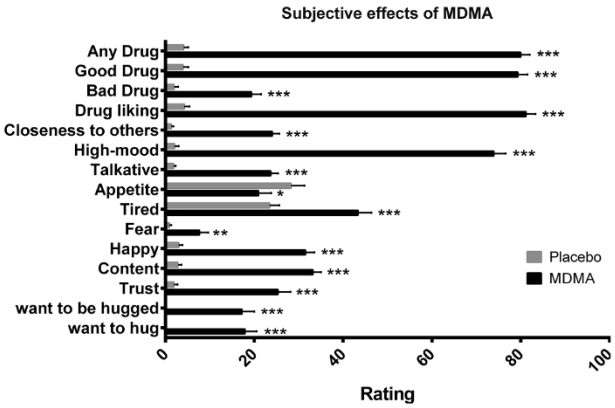

Figure 1. Maximal effects of MDMA on subjective Visual Analog Scale ratings. MDMA significantly altered E_{max} values for all of the reported parameters. With the exception of a decrease in "appetite," all of the parameters were increased by MDMA. Data are expressed as mean ± SEM; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with placebo.

DISCUSSION

The present study investigated the effects of interindividual differences in genes that encode the 5-HT system on MDMA-induced mood, empathogenic, cardiovascular, thermogenic, and adverse effects. Although genetic variants of 5-HT system genes have been implicated in different phenotypes and although the effects of MDMA largely depend on the release of 5-HT, only the *TPH2* rs7305115, *HTR2A* rs6313, and *SLC6A4* 5-HTTLPR polymorphisms tended to moderately alter some effects of MDMA. However, the effect size was limited. After applying Nyholt correction to correct for Type I errors, none of the genetic variants that were evaluated herein significantly influenced the acute subjective or physiological effects of MDMA.

Most of the polymorphisms that were tested in the present study were investigated for the first time in association with the acute effects of controlled administration of MDMA in healthy human subjects. We could not reproduce results from two previous smaller studies on the modulatory role of *SLC6A4* polymorphisms in the acute effects of MDMA. One reason for this could be the correction for multiple testing. In fact, before correcting for multiple testing, our results were consistent with the findings of Kuypers et al. (2018b), which were not corrected for multiple testing to avoid Type II errors. In both studies, homozygous carriers of the L allele felt more anxiety/fear compared with S-allele carriers. However, the exploratory nature of the present study requires a correction method to avoid Type I errors.

Another previous study also suggested that the 5-HTTLPR polymorphism may play an important role in modulating the risk of adverse effects of MDMA, mainly cardiovascular effects.[35] However, MDMA-induced cardiovascular effects were not influenced by 5-HT system gene variations in the present study, which was larger and more methodologically sound than that of Pardo-Lozano et al.

The discrepancies between these studies may be attributable to the different doses of MDMA. In contrast to the 75−100 mg doses of MDMA that were used in Kuypers et al. and Pardo-

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Lozano et al., we used 125 mg of MDMA, which is the dose that is also used in patients.[1−3,57,58] As shown in an earlier study, the 125 mg dose is stronger and produced greater good drug effects compared with the 75 mg dose.[59] 5-HT system genotypes may present more modulatory effects when MDMA is taken at a lower dose and not at higher doses, such as in therapeutic settings.

The present study has limitations. First, although this is the largest uniform cohort with mostly MDMA-naive healthy subjects, confirmation in studies with larger samples is needed, which is the case for all such genetic studies. The sample size was relatively small when considering the mostly small effect sizes for the influence of genetic variants on the MDMA response. Additionally, tests for empathy and emotion recognition were only completed by 69 subjects. However, we unlikely missed very large effect sizes for the influence of the tested genetic variants. Second, the study was conducted in mostly young and healthy volunteers. Therefore, the findings cannot necessarily be generalized to other populations, such as psychiatric patients and elderly subjects. Third, SNPs of genes of other targets of MDMA may also be involved but were not tested in the present study. However, previous studies (e.g., with oxytocin receptor genes) showed minimal to no altering effects.[25,27,28] Nevertheless, we corrected for the modulatory effects of known genetic variants that influence the metabolism of MDMA[22,23] and also unequal proportions of MDMA concentrations between 5-HT genotypes by accounting for interindividual differences in plasma MDMA concentrations.

In light of recent efforts to use MDMA as an adjunct to psychotherapy for PTSD, modulators of the effects of MDMA should be identified. Our results showed that genetic variations of genes that encode the 5-HT system did not markedly influence the effects of MDMA in healthy subjects. The results correspond with previous findings that genetic variations in pharmacodynamic targets of MDMA may play a minor role and do not mirror the robust findings of genetic variations in enzymes influencing the pharmacokinetic of MDMA. Therefore, interactions between MDMA and 5-HT system genotypes are unlikely to be an important factor to consider when MDMA is used recreationally. Whether the same might hold true for patient populations will require further investigation in those populations.

METHODS

Study Design. This was a pooled analysis of eight double-blind, placebo-controlled, crossover studies in healthy subjects that used similar methods.[6,14,60−65] The studies included a total of 136 healthy subjects. Seven studies included 16 subjects each, for a total of 112 subjects, who received 125 mg of MDMA twice, once alone and once after pretreatment with a medication.[6,14,60−65] An additional study included 24 subjects who received 125 mg of MDMA alone, placebo, or other treatments.[6] In the present analysis, only data from the MDMA-alone and placebo sessions were used. In all of the studies, the washout periods between single-dose administrations of MDMA were at least 7 days to exclude possible carry-over effects. The studies were all registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01771874, and NCT01951508). All of the studies were approved by the local ethics committee and Swiss Agency for Therapeutic Products (Swissmedic). The studies were conducted in accordance with the Declaration of Helsinki. MDMA administration in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Informed consent was obtained from all of the participants. All of the subjects were paid for their participation. Detailed pharmacokinetic and safety data from these studies have been reported elsewhere.[22,23,59] Test sessions were conducted in a quiet hospital research ward with no more than two research subjects present per session. The participants were comfortably lying in hospital beds and were mostly listening to music and not engaging in physical activities. MDMA was given without food in the fasting state in the morning at 8:00−9:00 AM. A small standardized lunch was served at 12:00−1:00 PM.

Subjects. A total of 136 healthy subjects of European descent, 18−44 years old (mean ± SD = 24.8 ± 4 years), were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, three participants did not give consent for genotyping, and eight subjects participated twice (only participation that included all outcome measures was used), resulting in a final data set from 124 subjects (64 women). The mean ± SD body weight was 68 ± 10 kg (range: 46−90 kg).

The exclusion criteria included a history of psychiatric disorders, physical illness, a lifetime history of illicit drug use more than five times (with the exception of past cannabis use), illicit drug use within the past 2 months, and illicit drug use during the study, determined by urine tests that were conducted before the test sessions as reported in detail elsewhere.[14,61−63] Forty-three subjects had prior illicit drug experiences (1−5 times), of which 22 subjects had previously used MDMA (1−3 times), seven subjects had previously used amphetamine or methamphetamine (1 time), 10 subjects had previously used cocaine (1−3 times), six subjects had previously used lysergic acid diethylamide (1 time), and 11 subjects had previously used psilocybin (1−4 times).

Study Drug. (±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 mg, prepared as gelatin capsules (Bichsel Laboratories, Interlaken, Switzerland). Similar amounts of MDMA are found in ecstasy pills[66] and have been used in clinical studies in patients.[1,2] The doses were not adjusted for body weight or sex. The dose per body weight (mean ± SD) was 1.9 ± 0.3 mg/kg (1.7 ± 0.2 mg/kg for men and 2.1 ± 0.3 mg/kg for women, range: 1.4−2.7 mg/kg).

Physiological Effects. Blood pressure, heart rate, and body temperature were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Systolic and diastolic blood pressure and heart rate were measured using an automatic oscillometric device (OMRON Healthcare Europe NA, Hoofddorp, Netherlands). The measurements were performed in duplicate at an interval of 1 min and after a resting time of at least 10 min. The averages were calculated for the analysis. Mean arterial pressure (MAP) was calculated as diastolic blood pressure + (systolic blood pressure − diastolic blood pressure)/3. The rate pressure product (RPP) was calculated as systolic blood pressure × heart rate. Core (tympanic) temperature was measured using a Genius 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY). In one study (n = 21), the 2 h time point was not used.

Acute and subacute adverse effects were assessed using the list of complaints (LC).[62,67] The scale consisted of 66 items, yielding a total adverse effects score (nonweighted sum of the item answers) that reliably measures physical and general discomfort. Additionally, serotonin-related adverse effects, such as "dizziness," "nausea," and "lack of appetite," were analyzed separately. Bruxism (item 66, a common side effect of MDMA) was included in the LC that was used in 92 subjects. The LC was administered 3−6 h (acute adverse effects up to 6 h) and 24 h (subacute adverse effects up to 24 h) after MDMA or placebo administration.

Subjective Effects. To assess subjective effects, a Visual Analog Scale (VAS) was presented as a 100 mm horizontal line (0−100%), marked from "not at all" on the left to "extremely" on the right. The VASs for "closeness to others," "happy," "content," "trust," "want to be hugged," and "want to hug," were bidirectional (±50%). "Trust," "want to be hugged," "want to hug," "want to be alone," and "want to be with others" were assessed in 52 subjects. "Appetite" and "fear" were assessed in 72 subjects. "Happy" and "content" were assessed in 81 subjects. "Tired" was assessed in 109 subjects.[7] The scale was administered before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. The 60-item Adjective Mood

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Rating Scale (AMRS)[68] was administered 1 h before and 1.25, 2, and 5 h after drug administration.

Emotion Recognition. To measure emotion recognition, we used the Facial Emotion Recognition Task (FERT), which is sensitive to the effects of MDMA[6,8,64,69,70] and other serotonergic substances.[71] The task included 10 neutral faces and 160 faces that expressed one of four basic emotions (happiness, sadness, anger, and fear), with pictures morphed between 0% (neutral) and 100% in 10% steps. Two female and two male pictures were used for each of the four emotions. The stimuli were presented in random order for 500 ms and then were replaced by the rating screen where the participants had to indicate the correct emotion. The outcome measure was accuracy (proportion correct) and misclassification (emotions that were indicated incorrectly). The FERT was performed 90 min after drug administration. FERT data were available from 68 subjects.

Empathy. The Multifaceted Empathy Test (MET) is a reliable and valid task that assesses the cognitive and emotional aspects of empathy.[72] The MET is sensitive to oxytocin,[73] MDMA,[7,8,20,74] and other psychoactive substances.[71,75] The computer-assisted test consisted of 40 photographs that showed people in emotionally charged situations. To assess cognitive empathy, the participants were required to infer the mental state of the subject in each scene and indicate the correct mental state from a list of four responses. Cognitive empathy was defined as the percentage of correct responses relative to total responses. To measure emotional empathy, the subjects were asked to rate how much they were feeling for an individual in each scene (i.e., explicit emotional empathy) and how much they were aroused by each scene (i.e., implicit emotional empathy) on a 1−9 point scale. The latter rating provides an inherent additional assessment of emotional empathy, which is considered to reduce the likelihood of socially desirable answers. The three aspects of empathy were each tested with 20 stimuli with positive valence and 20 stimuli with negative valence, resulting in a total of 120 trials. The MET was performed 90−180 min after drug administration. MET data were available from 68 subjects.

Plasma Concentrations of MDMA. Plasma levels of MDMA were determined before and 0.5, 1, 1.5, 2, 3, 4, and 6 h after drug administration.[64]

Genotyping. Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. Genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne, Switzerland) and TaqMan Genotyping Master Mix. Fluorescence was detected using the ViiA7 real-time polymerase chain reaction (PCR) system. We assayed the following SNPs: *TPH1* rs1800532 (assay: C_8940793_10) and rs1799913 (assay: C_2645661_10), *TPH2* rs7305115 (assay: C_8376164_10), *HTR1A* rs6295 (assay: C_11904666_10), *HTR1B* rs6296 (C_2523534_20), and *HTR2A* rs6313 (assay: C_3042197_1_). We also used the following method to genotype the *SLC6A4* 5-HTTLPR polymorphism (43 base pair [bp] deletion) and rs25531 SNP. Genotypes were determined by PCR using 0.025 units of PrimeSTAR GXL DNA polymerase (TakaraClontech), PrimeSTAR GXL buffer (1 mM Mg^{2+}), dNTP Mix (2.5 mM each), and primer set 5′-GCCAGCACCTAACCCCTAAT and 5′-GGTTGCAGGGGA-GATCCT (7.5 pmol each) in a total reaction volume of 25 μL. The following temperature profile was applied in a T100 thermal cycler (Bio-Rad, Cressier, Switzerland): initial activation step of 98 °C (5 min) and 45 cycles of 98 °C (10 s), 60 °C (15 s), and 68 °C (30 s), with final extension at 68 °C (5 min). The sizes of the resulting PCR products were assessed by 4% agarose gel electrophoresis. Amplicons of 212 bp were designated as short variant (S), and amplicons of 268 bp were designated as long variant (L). The genotypes of the rs25331 SNP were determined by PCR using 0.5 units of Taq DNA polymerase, recombinant (Thermo Fisher Scientific), 10× PCR Buffer (1 mM Mg^{2+}), dNTP Mix (0.2 mM each), and the primer set 5′-GGACCGCAAGGTGGGCGGGAG-GCTTGGAG and 5′-CTCCTAGGATCGCTCCTGCATC (0.2 pmol each) in a total volume of 25 μL. Polymerase chain reaction was performed with an initial activation step of 95 °C (3 min) and 49 cycles of 95 °C (30 s), 59.7 °C (25 s), and 72 °C (30 s), with final extension at 72 °C (5 min). The PCR products were digested using 10 units of BcnI (NciI, Thermo Fisher Scientific) and the Buffer Tango by incubation overnight at 37 °C using the T100 thermal cycler. The sizes of the resulting fragments were assessed by 3% agarose gel electrophoresis. Long fragments that carried the A allele (244 bp) were distinguished from fragments that carried the G allele (174 bp), and the short PCR products resulted in a fragment size of 201 bp. The genotypes were designated as LALA (244 bp), LALG (244 bp + 174 bp), LGLG (174 bp), LAS (244 bp + 201 bp), LGS (201 bp + 174 bp), and SS (201 bp). The rs25531 genotype could not be determined in one subject. The LG and S alleles should express an identical phenotype.[32−34] Accordingly, three groups were defined: group 1 (LGLG, LGS, and SS) vs group 2 (LALG, and LAS) vs group 3 (LALA). The genotypes of *TPH1* rs1800532 and rs1799913 were in total linkage disequilibrium; therefore, only the results for rs1800532 are reported. Most of the tested genes lay not on the same chromosome and a haplotype approach also leads to very small groups. It was therefore rejected. Combined variants with a polygenetic risk score could improve power. However, there were no specific hypotheses for the present study to support such an approach.

Statistical Analysis. The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK). For repeatedly measured data, peak effects (E_{max}) and areas under the effect-time curve (AUEC) from 0 to 6 h values were determined for MDMA and placebo. Differences in E_{max} and AUEC values (Δ; MDMA-placebo) were then analyzed using one-way analysis of variance (ANOVA), with genotype as the between-group factor, followed by the Tukey post hoc test. To ensure that modulatory effects of genotype over time were not missed, ΔAUEC values were tested accordingly in an additional analysis. The level of significance was set at $p < 0.05$. The Nyholt correction method was used to account for multiple comparisons and displayed separately in all tables.[76] We thereby corrected for the 19 subjective effects (VAS+AMRS), 3 emotions in the FERT and 2 empathies in the MET, 5 vital parameters, and 8 items in the LC which have all been shown sensitive to the effects of MDMA. In addition, this was then corrected for each of the 7 polymorphisms tested, resulting in $19 + 5 + 5 + 8 \times 7 = 259$ variables and an effective number of independent variables (V_{eff}) of 217.48 according to Nyholt. Consequently, this leads to a corrected significance threshold of $p < 0.00023$ to keep Type I error rate at 5%. To account for differences in plasma concentrations of MDMA that were caused by differences in body weight, dosing, or metabolizing enzymes,[22,23] the area under the MDMA plasma concentration−time curve from 0 to 6 h (AUC) was included as a covariate in the ANOVAs, and we report the corrected statistics. Because the time point of the peak plasma concentration (C_{max}) and E_{max} differ, the MDMA plasma concentration AUC was taken for correction. Furthermore, to eliminate biases resulting from differences in the plasma concentration of MDMA, AUC seems to be more reliable as it shows consistent moderation to differences in the metabolism of MDMA.[23] Additionally, modulatory effects of sex or illicit drug experience were explored by adding sex, and illicit drug as a between-subjects factor in the ANOVAs (sex × genotype; drug experience × genotype). E_{max} values were obtained directly from the observed data, and the AUC and AUEC were calculated using the linear-log trapezoidal method in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ). The primary analysis was performed using an additive genotype model for SNPs. Recessive or dominant model analysis was also performed, the results of which are reported only when the additive model was significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.8b00590.

DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

Data for the response to MDMA over time (AUEC) (XLSX)

Uncorrected statistics for E_{max} (XLSX)

Uncorrected statistics for AUEC (XLSX)

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Author Contributions

P.V. analyzed the data and wrote the manuscript. H.E.M.z.S. analyzed the data. M.E.L. conceived the study, obtained funding, analyzed the data, and wrote the manuscript.

Funding

This study was supported by the Swiss National Science Foundation (Grant Nos. 320030_149493 and 320030_170249).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of C. M. Hysek, A. Rickli, Y. Schmid, and P. C. Dolder in conducting the clinical studies, K. Prestin and Janine Hussner for assistance with genotyping, and M. Arends for text editing.

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DOI: 10.1021/acschemneuro.8b00590
ACS Chem. Neurosci. 2019, 10, 3120−3131

2.7. No influence of dopamine system gene variations on acute effects of MDMA

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Frontiers in Psychiatry, 2019, Vol. 10, Article 755

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 24 October 2019
doi: 10.3389/fpsyt.2019.00755

No Influence of Dopamine System Gene Variations on Acute Effects of MDMA

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3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is a recreational substance also investigated as medication for posttraumatic stress disorder. Dopamine (DA) system stimulation likely contributes to the acute mood effects of amphetamines, including MDMA. Genetic variants, such as single-nucleotide polymorphisms (SNPs), and polymorphic regions of the DA system genes may in part explain interindividual differences in the acute responses to MDMA in humans. We characterized the effects of common genetic variants within genes coding for key players in the DA system including the dopamine D2 receptor (DRD2/ANKK1 rs1800497, DRD2 rs6277, and rs107959), the dopamine transporter (DAT1 rs28363170, rs3836790, rs6347, rs11133767, rs11564774, rs460000, and rs463379), and dopamine D4 receptor [DRD4, variable-number tandem repeat (VNTR)] on the subjective and autonomic response to MDMA (125 mg) in pooled data from randomized, placebo-controlled, crossover studies in a total of 149 healthy subjects. Plasma concentrations of MDMA were used as covariate in the analysis to control for individual pharmacokinetic (metabolic and weight) differences. None of the tested genetic polymorphisms within the DA system altered effects of MDMA when adjusting for multiple comparisons. Genetic variations in genes coding for players of the DA system are unlikely to explain interindividual variations in the acute effects of MDMA in humans.

Keywords: dopamine, SCL6A3, DAT1, DRD2, DRD4, MDMA

OPEN ACCESS

Edited by:
Matthew W. Johnson, Johns Hopkins University, United States

Reviewed by:
Sean Dolan, Johns Hopkins University, United States
Zach Walsh, University of British Columbia Okanagan, Canada

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Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *25 June 2019*
Accepted: *19 September 2019*
Published: *24 October 2019*

Citation:
Vizeli P and Liechti ME (2019) No Influence of Dopamine System Gene Variations on Acute Effects of MDMA. Front. Psychiatry 10:755. doi: 10.3389/fpsyt.2019.00755

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is widely used recreationally for its euphoric effects. Additionally, recent investigations are looking into MDMA as a medication to assist psychotherapy in patients with posttraumatic stress disorder (PTSD) (1–3). MDMA acts mainly as a releaser and reuptake inhibitor of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) *via* an interaction with the respective transporter (4–7). The subjective effects of MDMA have been shown to mainly depend on transporter-mediated release of 5-HT and NE (8). In animals, however, the possibility was raised that the importance of interaction with the DA system would increase with the amount of drug taken (9). To what extent DA is mediating the acute effects of MDMA in humans is unclear. For example, the positive effects of MDMA were diminished after pharmacological inhibition of DA receptors with haloperidol (10). In addition, MDMA-induced hyperactivity was reduced in knockout mice without the DA receptor D_2 gene (DRD2) (11). However, in contrast to the strongly diminished effect of MDMA in subjects with a blocked serotonin transporter, preventing the interaction of MDMA with the DA transporter

(DAT) by pretreatment with bupropion or methylphenidate had no effect on the acute mood effects of MDMA in humans (12–15). Studies on the influence of genetic polymorphisms in the DA system could add adjuvant information to this matter and may also explore the role of the DA system in the interindividual differences in the response to MDMA. So far, only genetic variations of the enzymes that are involved in MDMA metabolism (mainly CYP2D6) displayed a robust influence on MDMA plasma levels in several clinical studies (16–18) and also showed a concomitant modulation of the pharmacodynamic effects of MDMA. However, genetic variants of pharmacological targets of MDMA may also alter its pharmacodynamic effects. A few studies explored the role of genetic polymorphisms of the 5-HT, NE, and oxytocin systems and found only minimal influences on acute effects of MDMA (19–23).

The DAT is a key target for many stimulant-type drugs, including cocaine, amphetamine, methylphenidate, and MDMA (6, 24). Additionally, the transporter is involved in various psychiatric disorders and treatment approaches (25–27). Subsequently, genetic polymorphisms within the single copy gene coding for the DAT (DAT1, SLC6A3) were investigated in relation to cocaine dependence and abuse, methamphetamine psychosis, attention-deficit/hyperactivity disorder (ADHD) and treatment, and bipolar disorder (28–36). Two common variable-number tandem repeat (VNTR) polymorphisms were most extensively studied. One, the rs28363170, is located in the 3′ untranslated region (3′UTR) of the DAT1 gene and exhibits 9 or 10 repeats as most common forms (37). Homozygous carriers of the 9-repeat allele were found to be at a higher risk for persistent ADHD, and the 10/10 genotype was associated with ADHD in children (38). Subjects with the 9/9 genotype were less susceptible to the subjective effects of amphetamine (39). However, carriers of at least one 9-repeat allele showed higher ratings of "high," "any drug effect," "anxious," and "stimulated" after cocaine (40). Conversely, homozygous 10-repeat carriers in combination with a 5-repeat allele of the other extensively studied VNTR in the DAT1, the rs3836790, displayed a lower response to "good drug effects," "bad drug effects," "depressed," and "anxious" (40). The rs3836790 VNTR is located in intron 8 of the human DAT1 gene. The most common forms of this VNTR are 5 or 6 repeats (30). A study in a Brazilian sample found a positive association of the 6-repeat allele and cocaine addiction (28). In contrast, another yet smaller case-control study in a Spain sample showed an overrepresentation of the 5/5 genotype in cocaine abusers (33).

MDMA also directly and indirectly interacts with DA receptors (4). Especially the inhibition of the D_2 with haloperidol showed a significant reduction in MDMA positive effects (10). MDMA-unrelated pharmacogenetic studies showed a positive association of the minor allele of the DRD2 single-nucleotide polymorphisms (SNPs) rs1079597 and rs1800497 with heroin dependence (41), rs6277 and rs1800497 with nicotine dependence (42), and rs6277 with alcohol dependence in males (43). The VNTR polymorphism within the gene coding for the subtype 4 of the DA receptors (DRD4) is also frequently studied in relation to psychiatric disorders and personality traits (44–47). DRD4 VNTR variations range from 2 repeats to 10 repeats, with 4 and 7 repeats as the most frequent forms (48). The presence of a 7-repeat allele has been linked with personal traits like high novelty seeking, risky decision making, and broad sexual interest (44, 47). Moreover, children and adolescents suffering from ADHD and carrying the 7-repeat allele had to take higher doses of methylphenidate to reach sufficient efficacy (49). This finding is in line with earlier results from an *in vitro* study showing a reduced sensitivity of the 7-repeat allele toward DA compared with the 2- and 4-repeat allele (50).

The present study is the first to explore the influence of variants within genes coding for the DA system on the acute effects of MDMA in humans. We analyzed DRD2/ANKK1 rs1800497, DRD2 rs6277, and rs107959, DAT1 rs28363170, rs3836790, rs6347, rs11133767, rs11564774, rs460000, and rs463379, DRD4 VNTR and their influence on acute subjective and autonomic effects of MDMA. Given the partially inconclusive pharmacogenetic studies in addition to the unclear degree to which MDMA effects are driven by the interaction with the DA system, we hypothesized that genetic mutations of the DA system would not influence cardiostimulant effects and have only minimal influence on the mood effects of MDMA.

METHODS

Study Design

This was a pooled analysis of nine double-blind, placebo-controlled, crossover studies that used similar methods and were conducted in healthy subjects and in the same laboratory (14, 15, 51–55). The studies included a total of 164 healthy subjects. Seven studies included 16 subjects each, for a total of 112 subjects, who received 125 mg MDMA twice, once alone and once after pretreatment with a medication (14, 15, 51–54). Two additional studies included 24 and 28 subjects who received 125 mg MDMA alone, placebo, or other treatments (55; Holze et al., unpublished). In the present analysis, only data from the MDMA-alone and placebo sessions were used. In all of the studies, the washout periods between single-dose administrations of MDMA were at least 7 days to exclude possible carryover effects. The studies were all registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01465685, NCT01771874, NCT01951508, and NCT03019822). All of the studies were approved by the local ethics committee and, if necessary, Swiss Agency for Therapeutic Products (Swissmedic). The studies were conducted in accordance with the Declaration of Helsinki. MDMA administration in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Written informed consent was obtained from all of the participants. All of the subjects were paid for their participation. Detailed pharmacokinetic and safety data from these studies have been reported elsewhere (17, 18, 56). Test sessions were conducted in a quiet hospital research ward with no more than two research subjects present per session. The participants were comfortably lying in hospital beds and were mostly listening to music and not engaging in physical

activities. MDMA was given without food in the fasting state in the morning at 8:00–9:00 AM. A small standardized lunch was served at 12:00–1:00 PM.

Subjects

A total of 164 healthy subjects of European descent, 18–45 years old (mean ± SD = 25.3 ± 4 years), were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, three participants did not give consent for genotyping, and 11 subjects participated twice (only one participation that included all outcome measures was used), resulting in a final data set of 149 subjects (76 women). The mean ± SD body weight was 69 ± 11 kg (range: 46–97 kg).

The exclusion criteria included a history of psychiatric disorders, physical illness, a lifetime history of illicit drug use more than 10 times (with the exception of past cannabis use), illicit drug use within the past 2 months, and illicit drug use during the study, as determined by urine tests that were conducted before the test sessions, as reported in detail elsewhere (52–54). Fifty-five subjects had prior illicit drug experiences (1–8 times), of which 27 subjects had previously used MDMA (1–5 times), 14 subjects had previously used amphetamine or methamphetamine (1–2 times), 11 subjects had previously used cocaine (1–4 times), eight subjects had previously used lysergic acid diethylamide (1–2 times), and 11 subjects had previously used psilocybin (1–4 times).

Study Drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 mg prepared as gelatin capsules (25 and 100 mg). Similar amounts of MDMA are found in ecstasy pills (57) and have been used in clinical trials in patients (1, 2). The doses were not adjusted for body weight or sex. The dose per body weight (mean ± SD) was 1.9 ± 0.3 mg/kg (range: 1.3–2.7 mg/kg).

Physiological Effects

Blood pressure, heart rate, and body temperature were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Systolic and diastolic blood pressures and heart rate were measured using an automatic oscillometric device (OMRON Healthcare Europe NA, Hoofddorp, Netherlands). The measurements were performed in duplicate at an interval of 1 min and after a resting time of at least 10 min. The averages were calculated for the analysis. Mean arterial pressure (MAP) was calculated as diastolic blood pressure + (systolic blood pressure – diastolic blood pressure)/3. The rate pressure product (RPP) was calculated as systolic blood pressure × heart rate. Core (tympanic) temperature was measured using a Genius 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA). In two studies (N = 46), the 2-h time point was not used.

Pharmacodynamic Measures

Visual Analog Scales (VASs) were repeatedly used to assess subjective effects over time (58). The VASs included for instance "any drug effect," "good drug effect," and "stimulated." The VASs were presented as 100 mm horizontal lines (0–100%), marked from "not at all" on the left to "extremely" on the right. Subjective effects like "concentration," "appetite," "tired," "want to be hugged," "want to hug," and "talkative" were bidirectional (±50 mm). Not all VAS components were presented in all studies. Exact numbers of subjects per genotype group are reported in **Tables 1–3**. The VASs were applied before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. In two studies (N = 46), the 2-h time point is missing; additionally, in one study (N = 21), the 2.5-h time point is also missing.

The 60-item Likert-type scale of the short version of the Adjective Mood Rating Scale (AMRS) (59) was administered before and 1.25, 2, and 5 h after MDMA or placebo administration. The AMRS contains subscales for activity, well-being, and anxiety–depression.

Genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. SNP genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne, Switzerland). We assayed the following SNPs: DRD2/ankyrin repeat and kinase domain containing 1 (ANKK1) SNPs rs1800497 (assay: C___7486676_10), DRD2 rs6277 (assay: C__11339240_10), and rs1079597 (assay: C___2278884_10), and DAT1 SNPs rs6347 (assay: C___8769902_10) and rs11133767 (assay: C___3024834_10) and rs11564774 (assay: C__25761679_10) and rs460000 (assay: C___3284837_10) and rs463379 (assay: C___3284827_10). We also used the following method to genotype the polymorphisms in DRD4 exon III VNTR, DAT1 3'UTR VNTR rs28363170, and DAT1 Intron 8 (5/6) VNTR rs3836790. Genotypes were determined by polymerase chain reaction (PCR) using 2.5, 1.25, and 1.25 units of HotStarTaq DNA polymerase (QIAGEN Instruments AG, Hombrechtikon, Switzerland), respectively; 1.5 µl PCR Buffer 10x each (15 mM Mg^{2+}; QIAGEN Instruments AG, Hombrechtikon, Switzerland); 0.25, 1, and 1 µl dNTP Mix (40 mM), respectively; and primer set *5'-GCGACTACGTGGTCTACTCG* and *5'-AGGACCCTCATGGCCTTG, 5'-TGTGGTGTAGGGAACG GCCTGAG* and *5'-CTTCCTGGAGGTCACGGCTCAAGG*, and *5'-G CATGTGGATGTGTTCTTGCA* and *5'-TCATCCCAGGGACATCT GCTA* (both 1 µl, both 0.5 µl, and both 0.5 µl, respectively) in a total reaction volume of 15 µl each. The following temperature profile was applied in a T100 thermal cycler (Bio-Rad, Cressier, Switzerland): for DRD4 (Exon III VNTR): initial activation step of 95°C (15 min) and 30 cycles of 98°C (60 s), 67.5°C (60 s), and 72°C (60 s), with final extension at 72°C (5 min); for DAT1 (3'UTR VNTR) rs28363170 and (intron8 5/6 VNTR) rs3836790: initial activation step of 95°C (15 min) and 30 cycles of 98°C (25 s), 95°C (35 s), and 72°C (45 s), with final extension at 72°C (5 min). The sizes of the resulting PCR products were assessed by 3.5% (for DRD4 exon III VNTR) and 2.5% (for DAT1 3'UTR VNTR rs28363170 and Intron 8 VNTR rs3836790) agarose gel electrophoresis. Amplicons of the DRD4 (Exon III VNTR in chromosome 11) of 379 bp were designated as 2 repeats (2R), and amplicons of every additional 48 bp were designated as 2+x times 48 bp variants [up to 9R (with 379 bp + 7 × 48 bp = 715 bp)]. Four and 7-repeat amplicons were the

TABLE 1 | Effects of polymorphisms in the dopamine receptor D2 gene on the maximal response to 125 mg MDMA (mean ± SD (N) and statistics) corrected with MDMA AUC_6 (exclusive plasma concentrations).

DRD2/ANKK1 rs1800497	**AA**	**AG**	**GG**	**F**	**p value**	**p value[a]**	**η^2**
N	2	46	101				
Female, N [%]	1 (50)	30 [65]	45 [45]				
Drug experience, N [%]	2 (100)	17 [37]	36 [36]				
MDMA plasma concentration Cmax, ng/ml	236 ± 76 (2)	239 ± 47 (46)	223 ± 49 (101)	1.71	NS	NS	0.023
MDMA plasma concentration AUC_6, ng*h/ml	964 ± 235 (2)	994 ± 199 (46)	944 ± 205 (101)	0.97	NS	NS	0.013
Visual Analog Scale rating ΔE_{max}							
Any drug effect	84 ± 18 (2)	77 ± 23 (46)	70 ± 28 (101)	0.74	NS	NS	0.008
Good drug effect	94 ± 9 (2)	78 ± 26 (46)	70 ± 30 (101)	1.31	NS	NS	0.016
Bad drug effect	24 ± 35 (2)	21 ± 25 (46)	14 ± 24 (101)	1.14	NS	NS	0.015
Drug liking	96 ± 6 (2)	78 ± 28 (46)	72 ± 29 (101)	1.03	NS	NS	0.013
Stimulated	91 ± 13 (2)	68 ± 32 (46)	59 ± 35 (101)	1.44	NS	NS	0.018
High mood	96 ± 6 (2)	73 ± 30 (46)	66 ± 34 (101)	0.98	NS	NS	0.012
Concentration	28 ± 31 (2)	6.2 ± 14 (46)	9.2 ± 16 (101)	2.09	NS	NS	0.028
Talkative	48 ± 4 (2)	18 ± 20 (46)	22 ± 18 (101)	3.12	0.047*	NS	0.040
Appetite	7.5 ± 9.2 (2)	-5.3 ± 39 (23)	-8.9 ± 27 (47)	0.43	NS	NS	0.012
Tired	24 ± 8 (2)	19 ± 34 (33)	20 ± 32 (74)	0.07	NS	NS	0.001
Fear	7.0 ± 9.9 (2)	7.3 ± 15 (31)	5.9 ± 17 (64)	0.06	NS	NS	0.001
Happy	50 (1)	26 ± 19 (32)	27 ± 19 (73)	0.63	NS	NS	0.011
Want to be hugged	NA (0)	13 ± 18 (23)	13 ± 19 (54)	0.12	NS	NS	0.001
Want to hug	NA (0)	14 ± 17 (23)	13 ± 18 (54)	0.02	NS	NS	0.000
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	21 ± 31 (2)	25 ± 11 (46)	23 ± 13 (101)	0.32	NS	NS	0.004
Diastolic blood pressure, mmHg	13 ± 11 (2)	15 ± 10 (46)	13 ± 9 (101)	0.78	NS	NS	0.010
Mean arterial pressure, mmHg	14 ± 22 (2)	19 ± 10 (46)	16 ± 9 (101)	0.91	NS	NS	0.011
Heat rate beat/min	31 ± 33 (2)	20 ± 15 (46)	16 ± 13 (101)	2.07	NS	NS	0.027
Rate pressure product, mmHg/min	6,343 ± 6,658 (2)	4,967 ± 2,855 (46)	4,203 ± 2,776 (101)	1.26	NS	NS	0.017
Body temperature, °C	0.5 ± 0.1 (2)	0.2 ± 0.4 (46)	0.2 ± 0.5 (101)	0.34	NS	NS	0.005
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	10 ± 8 (2)	2.1 ± 4.2 (46)	2.4 ± 5.3 (101)	2.40	0.09	NS	0.032
High mood	7.5 ± 0.7 (2)	2.2 ± 2.8 (46)	3.0 ± 3.2 (101)	3.49	0.033*	NS	0.046
Fear/depression	-1.5 ± 2.1 (2)	1.2 ± 3.3 (46)	1.2 ± 3.4 (101)	0.63	NS	NS	0.009
DRD2 rs6277	**AA**	**AG**	**GG**	**F**	**p value**	**p value[a]**	**η^2**
N	50	73	26				
Female, N [%]	25 [50]	39 [53]	12 [46]				
Drug experience, N [%]	18 [36]	29 [40]	8 [31]				
MDMA plasma concentration Cmax, ng/ml	225 ± 52 (50)	233 ± 47 (73)	221 ± 47 (26)	0.70	NS	NS	0.009
MDMA plasma concentration AUC_6, ng*h/ml	949 ± 213 (50)	974 ± 203 (73)	939 ± 186 (26)	0.38	NS	NS	0.005
Visual Analog Scale rating ΔE_{max}							
Any drug effect	70 ± 31 (50)	73 ± 25 (73)	77 ± 21 (26)	0.95	NS	NS	0.011
Good drug effect	72 ± 31 (50)	74 ± 26 (73)	71 ± 31 (26)	0.04	NS	NS	0.000
Bad drug effect	11 ± 21 (50)	16 ± 24 (73)	25 ± 30 (26)	3.42	0.036*	NS	0.043
Drug liking	74 ± 30 (50)	74 ± 26 (73)	73 ± 34 (26)	0.01	NS	NS	0.000
Stimulated	57 ± 37 (50)	63 ± 33 (73)	71 ± 32 (26)	1.63	NS	NS	0.020
High mood	67 ± 33 (50)	70 ± 32 (73)	67 ± 36 (26)	0.09	NS	NS	0.001
Concentration	10 ± 16 (50)	6.3 ± 15 (73)	12 ± 18 (26)	1.59	NS	NS	0.021
Talkative	22 ± 18 (50)	20 ± 19 (73)	22 ± 20 (26)	0.25	NS	NS	0.003
Appetite	-17 ± 32 (26)	-0.8 ± 30 (35)	-4.5 ± 25 (11)	2.26	NS	NS	0.061
Tired	23 ± 33 (36)	20 ± 33 (55)	14 ± 29 (18)	0.44	NS	NS	0.008
Fear	4.5 ± 10 (35)	5.7 ± 14 (47)	13 ± 29 (15)	1.64	NS	NS	0.034
Happy	29 ± 18 (32)	26 ± 20 (53)	27 ± 18 (21)	0.30	NS	NS	0.005
Want to be hugged	13 ± 18 (24)	13 ± 19 (38)	12 ± 17 (15)	0.08	NS	NS	0.002
Want to hug	12 ± 17 (24)	14 ± 19 (38)	13 ± 16 (15)	0.01	NS	NS	0.000
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	24 ± 13 (50)	23 ± 13 (73)	24 ± 11 (26)	0.10	NS	NS	0.001
Diastolic blood pressure, mmHg	13 ± 9 (50)	14 ± 8 (73)	14 ± 13 (26)	0.08	NS	NS	0.001
Mean arterial pressure, mmHg	17 ± 10 (50)	17 ± 9 (73)	18 ± 11 (26)	0.16	NS	NS	0.002
Heat rate beat/min	19 ± 14 (50)	16 ± 15 (73)	20 ± 14 (26)	1.57	NS	NS	0.021
Rate pressure product, mmHg/min	4,635 ± 2,630 (50)	4,211 ± 3,111 (73)	4,867 ± 2,541 (26)	0.85	NS	NS	0.011
Body temperature, °C	0.3 ± 0.6 (50)	0.2 ± 0.5 (73)	0.2 ± 0.4 (26)	0.42	NS	NS	0.006
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	2.6 ± 5.7 (50)	2.2 ± 4.9 (73)	2.7 ± 4.5 (26)	0.15	NS	NS	0.002

(Continued)

TABLE 1 | Continued

DRD2/ANKK1 rs1800497	AA	AG	GG	F	p value	p value[a]	η^2
High mood	3.1 ± 3.2 (50)	2.9 ± 3.2 (73)	2.1 ± 3.0 (26)	0.84	NS	NS	0.012
Fear/depression	0.7 ± 3 (50)	1.3 ± 3.1 (73)	1.5 ± 4.5 (26)	0.79	NS	NS	0.011
DRD2 rs1079597	**CC**	**CT**	**TT**	**F**	**p value**	**p value[a]**	**η^2**
N	111	37	1				
Female, N [%]	53 [48]	22 [59]	1 [100]				
Drug experience, N [%]	40 [36]	14 [38]	1 [100]				
MDMA plasma concentration Cmax, ng/ml	226 ± 49 (111)	234 ± 49 (37)	290 (1)	1.19	NS	NS	0.016
MDMA plasma concentration AUC_6, ng*h/ml	949 ± 202 (111)	985 ± 208 (37)	1130 (1)	0.78	NS	NS	0.011
Visual Analog Scale rating ΔE_{max}							
Any drug effect	71 ± 28 (111)	76 ± 21 (37)	96 (1)	0.44	NS	NS	0.005
Good drug effect	71 ± 30 (111)	76 ± 25 (37)	100 (1)	0.44	NS	NS	0.006
Bad drug effect	13 ± 24 (111)	25 ± 26 (37)	0 (1)	3.09	0.049*	NS	0.039
Drug liking	73 ± 29 (111)	77 ± 28 (37)	100 (1)	0.40	NS	NS	0.005
Stimulated	59 ± 35 (111)	71 ± 31 (37)	100 (1)	1.64	NS	NS	0.020
High mood	68 ± 33 (111)	70 ± 32 (37)	100 (1)	0.28	NS	NS	0.004
Concentration	8.9 ± 16 (111)	6.3 ± 15 (37)	50 (1)	3.92	0.022*	NS	0.051
Talkative	22 ± 18 (111)	19 ± 21 (37)	50 (1)	1.58	NS	NS	0.020
Appetite	-11 ± 29 (53)	2.4 ± 35 (18)	1.0 (1)	1.35	NS	NS	0.037
Tired	21 ± 32 (80)	18 ± 34 (28)	30 (1)	0.14	NS	NS	0.002
Fear	5.5 ± 16 (73)	9.5 ± 17 (23)	0 (1)	0.61	NS	NS	0.013
Happy	27 ± 19 (78)	26 ± 19 (27)	50 (1)	0.62	NS	NS	0.011
Want to be hugged	13 ± 18 (58)	14 ± 19 (19)	NA (0)	0.01	NS	NS	0.000
Want to hug	13 ± 18 (58)	15 ± 18 (19)	NA (0)	0.03	NS	NS	0.000
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	23 ± 13 (111)	26 ± 11 (37)	43 (1)	1.52	NS	NS	0.019
Diastolic blood pressure, mmHg	13 ± 9 (111)	15 ± 11 (37)	20 (1)	0.65	NS	NS	0.008
Mean arterial pressure, mmHg	16 ± 9 (111)	19 ± 10 (37)	29 (1)	1.33	NS	NS	0.017
Heat rate beat/min	16 ± 14 (111)	20 ± 14 (37)	54 (1)	3.89	0.023*	NS	0.050
Rate pressure product, mmHg/min	4,240 ± 2,838 (111)	4,972 ± 2,700 (37)	11,050 (1)	3.24	0.042*	NS	0.041
Body temperature, °C	0.2 ± 0.5 (111)	0.3 ± 0.4 (37)	0.6 (1)	0.65	NS	NS	0.009
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	2.4 ± 5.3 (111)	2.0 ± 3.9 (37)	16 (1)	3.74	0.026*	NS	0.049
High mood	3 ± 3.2 (111)	2.3 ± 2.8 (37)	8.0 (1)	1.92	NS	NS	0.026
Fear/depression	1.1 ± 3.2 (111)	1.2 ± 3.7 (37)	0 (1)	0.07	NS	NS	0.001

*N, number of subjects; SD, standard deviation; NS, not significant; Δ, values are change scores from placebo; [a]p value additionally corrected for multiple comparisons according to the Nyholt method; η^2, eta square; *, uncorrected $p < 0.05$.*

most common forms. Complete genotype and allele distributions are depicted in **Supplementary Table S1**. For the analysis, groups were made with cumulative ≤8 repeats or cumulative >8 repeats in both alleles. Amplicons of the DAT1 (3'UTR VNTR) rs28363170 of 448 bp were designated as 9 repeats (9R), and amplicons of 488 bp were designated as 10R. Individuals possessing other repeats were excluded from the analysis. Amplicons of the DAT1 (intron 8 5/6 VNTR) rs3836790 of 295 bp were designated as 5 repeats (5R), and amplicons of 325 bp were designated as 6 repeats (6R). The pairwise linkage disequilibrium (LD) and relative physical location of the determined SNPs on chromosome 11 (DRD2) and 5 (DAT1) are shown in Supplementary Figure 1. The tested genetic variants were consistent with Hardy–Weinberg equilibrium ($p > 0.05$).

Statistical Analysis

The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). For repeatedly measured data, peak effects (E_{max}) and areas under the effect-time curve (AUEC) from 0- to 6-h values were determined for MDMA and placebo. Differences in E_{max} (Δ; MDMA-placebo) were then analyzed using one-way analysis of variance (ANOVA), with genotype as the between-group factor. The level of significance was set at $p < 0.05$. The Nyholt correction method was used to account for multiple comparisons and displayed separately in all tables (60). We thereby corrected for 17 subjective effect ratings (VAS+AMRS), and six vital parameters. In addition, this was then corrected for each of the 11 polymorphisms tested, resulting in $(17 + 6) \times 11 = 253$ variables and an effective number of independent variables (V_{eff}) of 183.6 according to Nyholt. Consequently, this led to a corrected significance threshold of $p < 0.00027$ to keep Type I error rate at 5%. To account for differences in plasma concentrations of MDMA that were caused by differences in body weight, dosing, or metabolizing enzymes (17, 18), the area under the MDMA plasma concentration–time curve from 0 to 6 h (AUC) was included as a covariate in the ANOVAs, and we report the corrected statistics. Additionally, modulatory effects of sex were explored by adding sex as a between-subjects factor in the ANOVAs (sex × genotype). E_{max} values were obtained directly from the observed data. AUC and AUEC values were calculated using the linear-log trapezoidal method in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA). The primary analysis was performed using an additive genotype model approach for SNPs. Recessive or dominant model analysis

TABLE 2 | Effects of polymorphisms in the dopamine transporter 1 gene on the maximal response to 125 mg MDMA (mean ± SD (N) and statistics) corrected with MDMA AUC_6 (exclusive plasma concentrations).

DAT1 3'-UTR rs28363170	**99**	**910**	**1010**	**F**	**p value**	**p value[a]**	**η^2**
N	8	56	79				
Female, N [%]	2 [25]	29 [52]	41 [52]				
Drug experience, N [%]	4 [50]	18 [32]	31 [39]				
MDMA plasma concentration Cmax, ng/ml	221 ± 44 (8)	227 ± 46 (56)	230 ± 50 (79)	0.14	NS	NS	0.002
MDMA plasma concentration AUC_6, ng*h/ml	939 ± 183 (8)	958 ± 180 (56)	961 ± 214 (79)	0.04	NS	NS	0.001
Visual Analog Scale rating ΔE_{max}							
Any drug effect	78 ± 20 (8)	73 ± 26 (56)	71 ± 28 (79)	0.48	NS	NS	0.006
Good drug effect	84 ± 21 (8)	73 ± 28 (56)	71 ± 30 (79)	0.97	NS	NS	0.013
Bad drug effect	11 ± 21 (8)	15 ± 29 (56)	16 ± 22 (79)	0.14	NS	NS	0.002
Drug liking	83 ± 22 (8)	77 ± 26 (56)	70 ± 31 (79)	1.26	NS	NS	0.017
Stimulated	63 ± 35 (8)	60 ± 35 (56)	63 ± 35 (79)	0.09	NS	NS	0.001
High mood	79 ± 32 (8)	68 ± 34 (56)	67 ± 33 (79)	0.57	NS	NS	0.008
Concentration	14 ± 22 (8)	7.3 ± 16 (56)	9.3 ± 16 (79)	0.76	NS	NS	0.011
Talkative	23 ± 16 (8)	19 ± 18 (56)	22 ± 19 (79)	0.56	NS	NS	0.008
Appetite	-20 ± 25 (7)	-2.6 ± 37 (28)	-7 ± 26 (35)	1.14	NS	NS	0.032
Tired	6.9 ± 37 (7)	17 ± 31 (44)	25 ± 33 (53)	1.38	NS	NS	0.026
Fear	6.3 ± 21 (7)	4.1 ± 9.5 (33)	8 ± 19 (55)	0.59	NS	NS	0.013
Happy	15 ± 16 (3)	27 ± 20 (40)	27 ± 18 (59)	0.37	NS	NS	0.007
Want to be hugged	0 (1)	16 ± 20 (28)	10 ± 16 (44)	1.16	NS	NS	0.030
Want to hug	0 (1)	16 ± 19 (28)	11 ± 16 (44)	1.01	NS	NS	0.027
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	27 ± 15 (8)	25 ± 11 (56)	22 ± 14 (79)	1.06	NS	NS	0.014
Diastolic blood pressure, mmHg	21 ± 18 (8)	14 ± 8 (56)	12 ± 9 (79)	3.84	0.024*	NS	0.049
Mean arterial pressure, mmHg	25 ± 14 (8)	18 ± 8 (56)	16 ± 10 (79)	3.79	0.025*	NS	0.048
Heat rate beat/min	16 ± 8 (8)	18 ± 15 (56)	16 ± 15 (79)	0.28	NS	NS	0.004
Rate pressure product, mmHg/min	4,623 ± 2,214 (8)	4,684 ± 2,835 (56)	4,194 ± 2,970 (79)	0.54	NS	NS	0.007
Body temperature, °C	0 ± 0.3 (8)	0.3 ± 0.5 (56)	0.2 ± 0.5 (79)	1.34	NS	NS	0.019
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	4.0 ± 5.2 (8)	2.0 ± 4.9 (56)	2.3 ± 5.2 (79)	0.53	NS	NS	0.008
High mood	2.6 ± 1.5 (8)	3.0 ± 3.2 (56)	2.7 ± 3.3 (79)	0.16	NS	NS	0.002
Fear/depression	0.5 ± 2 (8)	1.5 ± 3.6 (56)	1 ± 3 (79)	0.64	NS	NS	0.009
DAT1 Intron 8 rs3836790	**55**	**56**	**66**	**F**	**p value**	**p value[a]**	**η^2**
N	7	54	85				
Female, N [%]	3 [43]	25 [46]	46 [54]				
Drug experience, N [%]	4 [57]	21 [39]	29 [34]				
MDMA plasma concentration Cmax, ng/ml	218 ± 43 (7)	225 ± 54 (54)	231 ± 45 (85)	0.48	NS	NS	0.007
MDMA plasma concentration AUC_6, ng*h/ml	894 ± 201 (7)	945 ± 211 (54)	972 ± 196 (85)	0.65	NS	NS	0.009
Visual Analog Scale rating ΔE_{max}							
Any drug effect	66 ± 20 (7)	69 ± 29 (54)	75 ± 26 (85)	0.59	NS	NS	0.007
Good drug effect	69 ± 26 (7)	72 ± 30 (54)	74 ± 28 (85)	0.05	NS	NS	0.001
Bad drug effect	-0.7 ± 19 (7)	18 ± 29 (54)	16 ± 21 (85)	1.57	NS	NS	0.020
Drug liking	79 ± 20 (7)	73 ± 29 (54)	74 ± 30 (85)	0.24	NS	NS	0.003
Stimulated	58 ± 33 (7)	58 ± 35 (54)	66 ± 34 (85)	0.70	NS	NS	0.009
High mood	65 ± 27 (7)	66 ± 36 (54)	71 ± 31 (85)	0.19	NS	NS	0.003
Concentration	-0.6 ± 5 (7)	9.2 ± 18 (54)	8.5 ± 15 (85)	1.18	NS	NS	0.016
Talkative	15 ± 15 (7)	20 ± 19 (54)	22 ± 19 (85)	0.37	NS	NS	0.005
Appetite	-21 ± 29 (3)	-9.8 ± 34 (25)	-4.9 ± 30 (43)	0.49	NS	NS	0.014
Tired	-10 ± 28 (5)	20 ± 33 (41)	22 ± 31 (61)	1.82	NS	NS	0.032
Fear	1.7 ± 2.9 (3)	4.7 ± 14 (35)	7.7 ± 18 (58)	0.48	NS	NS	0.010
Happy	20 ± 16 (4)	26 ± 19 (42)	29 ± 19 (57)	0.28	NS	NS	0.005
Want to be hugged	9.8 ± 20 (4)	13 ± 19 (29)	14 ± 18 (42)	0.05	NS	NS	0.001
Want to hug	8.5 ± 17 (4)	14 ± 19 (29)	14 ± 18 (42)	0.06	NS	NS	0.001
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	31 ± 7 (7)	24 ± 12 (54)	23 ± 14 (85)	2.00	NS	NS	0.026
Diastolic blood pressure, mmHg	18 ± 6 (7)	15 ± 11 (54)	12 ± 8 (85)	2.99	0.05	NS	0.038
Mean arterial pressure, mmHg	23 ± 6 (7)	18 ± 10 (54)	16 ± 10 (85)	3.67	0.028*	NS	0.046
Heat rate beat/min	16 ± 5 (7)	19 ± 15 (54)	17 ± 14 (85)	0.38	NS	NS	0.005
Rate pressure product, mmHg/min	4,394 ± 1,421 (7)	4,878 ± 2,775 (54)	4,264 ± 2,999 (85)	0.96	NS	NS	0.013
Body temperature, °C	0.6 ± 0.6 (7)	0.3 ± 0.5 (54)	0.2 ± 0.5 (85)	2.59	0.08	NS	0.035
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	1.7 ± 3.1 (7)	2.2 ± 5.9 (54)	2.4 ± 4.7 (85)	0.08	NS	NS	0.001
High mood	2.3 ± 1.9 (7)	2.7 ± 3.3 (54)	3.0 ± 3.2 (85)	0.29	NS	NS	0.004
Hear/depression	-1.1 ± 2.8 (7)	1.4 ± 3.2 (54)	1.2 ± 3.5 (85)	1.80	NS	NS	0.025

(Continued)

TABLE 2 | Continued

DAT1 rs6347	CC	CT	TT	F	p value	p value[a]	η^2
N	12	60	77				
Female, N [%]	6 [50]	29 [48]	41 [53]				
Drug experience, N [%]	5 [42]	23 [38]	27 [35]				
MDMA plasma concentration Cmax, ng/ml	225 ± 46 (12)	224 ± 50 (60)	232 ± 48 (77)	0.45	NS	NS	0.006
MDMA plasma concentration AUC_6, ng*h/ml	933 ± 221 (12)	947 ± 192 (60)	973 ± 210 (77)	0.39	NS	NS	0.005
Visual Analog Scale rating ΔE_{max}							
Any drug effect	68 ± 24 (12)	70 ± 29 (60)	76 ± 25 (77)	0.73	NS	NS	0.008
Good drug effect	70 ± 26 (12)	71 ± 30 (60)	74 ± 28 (77)	0.12	NS	NS	0.002
Bad drug effect	3.9 ± 18 (12)	17 ± 29 (60)	17 ± 21 (77)	1.47	NS	NS	0.019
Drug liking	77 ± 23 (12)	72 ± 30 (60)	75 ± 29 (77)	0.16	NS	NS	0.002
Stimulated	65 ± 29 (12)	56 ± 36 (60)	67 ± 33 (77)	1.37	NS	NS	0.017
High mood	68 ± 28 (12)	66 ± 36 (60)	71 ± 31 (77)	0.16	NS	NS	0.002
Concentration	3.7 ± 10 (12)	8.4 ± 18 (60)	9.4 ± 15 (77)	0.69	NS	NS	0.009
Talkative	22 ± 15 (12)	19 ± 20 (60)	22 ± 18 (77)	0.29	NS	NS	0.004
Appetite	-4.1 ± 25 (7)	-13 ± 33 (27)	-3.8 ± 30 (38)	0.71	NS	NS	0.020
Tired	12 ± 36 (10)	20 ± 31 (42)	22 ± 33 (57)	0.28	NS	NS	0.005
Fear	1.0 ± 1.9 (7)	4.6 ± 13 (40)	8.5 ± 19 (50)	1.09	NS	NS	0.023
Happy	25 ± 15 (7)	25 ± 20 (45)	29 ± 19 (54)	0.36	NS	NS	0.007
Want to be hugged	11 ± 17 (5)	11 ± 18 (33)	15 ± 19 (39)	0.30	NS	NS	0.008
Want to hug	10 ± 15 (5)	10 ± 17 (33)	16 ± 18 (39)	0.70	NS	NS	0.017
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	29 ± 8 (12)	24 ± 13 (60)	23 ± 13 (77)	1.87	NS	NS	0.024
Diastolic blood pressure, mmHg	17 ± 7 (12)	14 ± 11 (60)	12 ± 8 (77)	2.19	NS	NS	0.027
Mean arterial pressure, mmHg	22 ± 6 (12)	18 ± 10 (60)	16 ± 10 (77)	2.87	0.06	NS	0.035
Heat rate beat/min	16 ± 12 (12)	17 ± 15 (60)	18 ± 14 (77)	0.03	NS	NS	0.000
Rate pressure product, mmHg/min	4,592 ± 2,500 (12)	4,550 ± 2,840 (60)	4,384 ± 2,950 (77)	0.14	NS	NS	0.002
Body temperature, °C	0.4 ± 0.6 (12)	0.2 ± 0.5 (60)	0.2 ± 0.5 (77)	1.13	NS	NS	0.015
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	3.3 ± 5.1 (12)	2.3 ± 5.7 (60)	2.4 ± 4.6 (77)	0.23	NS	NS	0.003
High mood	3.7 ± 2.6 (12)	2.6 ± 3.2 (60)	2.9 ± 3.2 (77)	0.66	NS	NS	0.009
Fear/depression	-0.8 ± 2 (12)	1.2 ± 3.3 (60)	1.4 ± 3.4 (77)	2.28	NS	NS	0.030
DAT1 rs11133767	**CC**	**CT**	**TT**	**F**	**p value**	**p value[a]**	**η^2**
N	62	66	20				
Female, N [%]	33 [53]	35 [53]	7 [35]				
Drug experience, N [%]	22 [35]	18 [27]	14 [70]				
MDMA plasma concentration Cmax, ng/ml	230 ± 48 (62)	230 ± 48 (66)	213 ± 50 (20)	1.05	NS	NS	0.014
MDMA plasma concentration AUC_6, ng*h/ml	965 ± 210 (62)	977 ± 200 (66)	876 ± 179 (20)	1.98	NS	NS	0.027
Visual Analog Scale rating ΔE_{max}							
Any drug effect	75 ± 25 (62)	69 ± 28 (66)	74 ± 23 (20)	1.70	NS	NS	0.019
Good drug effect	74 ± 28 (62)	70 ± 30 (66)	77 ± 26 (20)	1.38	NS	NS	0.018
Bad drug effect	18 ± 23 (62)	14 ± 25 (66)	16 ± 29 (20)	0.73	NS	NS	0.009
Drug liking	74 ± 31 (62)	72 ± 29 (66)	82 ± 23 (20)	1.72	NS	NS	0.022
Stimulated	68 ± 32 (62)	57 ± 36 (66)	63 ± 35 (20)	2.37	0.10	NS	0.029
High mood	71 ± 32 (62)	65 ± 34 (66)	72 ± 34 (20)	1.40	NS	NS	0.018
Concentration	9.5 ± 16 (62)	7.8 ± 16 (66)	8.6 ± 16 (20)	0.19	NS	NS	0.003
Talkative	23 ± 19 (62)	19 ± 19 (66)	21 ± 17 (20)	0.61	NS	NS	0.008
Appetite	-2.9 ± 29 (32)	-8.6 ± 30 (27)	-15 ± 35 (13)	1.21	NS	NS	0.034
Tired	25 ± 34 (45)	19 ± 28 (47)	10 ± 37 (17)	1.13	NS	NS	0.020
Fear	8.9 ± 20 (44)	1.8 ± 4.9 (37)	11 ± 20 (15)	2.57	0.08	NS	0.053
Happy	27 ± 19 (42)	26 ± 19 (52)	30 ± 17 (11)	0.87	NS	NS	0.016
Want to be hugged	10 ± 16 (30)	15 ± 19 (39)	14 ± 22 (7)	0.44	NS	NS	0.011
Want to hug	11 ± 16 (30)	15 ± 18 (39)	14 ± 22 (7)	0.38	NS	NS	0.010
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	22 ± 13 (62)	25 ± 13 (66)	23 ± 12 (20)	1.43	NS	NS	0.018
Diastolic blood pressure, mmHg	12 ± 8 (62)	14 ± 9 (66)	15 ± 12 (20)	1.83	NS	NS	0.023
Mean arterial pressure, mmHg	16 ± 9 (62)	18 ± 9 (66)	19 ± 11 (20)	1.86	NS	NS	0.023
Heat rate beat/min	17 ± 15 (62)	18 ± 15 (66)	18 ± 12 (20)	0.05	NS	NS	0.001
Rate pressure product, mmHg/min	4,337 ± 3,024 (62)	4,600 ± 2,817 (66)	4,551 ± 2,573 (20)	0.23	NS	NS	0.003
Body temperature, °C	0.2 ± 0.5 (62)	0.3 ± 0.5 (66)	0.3 ± 0.5 (20)	0.72	NS	NS	0.010
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	3.2 ± 4.9 (62)	2 ± 5.7 (66)	1.4 ± 2.9 (20)	1.23	NS	NS	0.017
High mood	3.1 ± 3.3 (62)	2.7 ± 3.3 (66)	2.4 ± 2.1 (20)	0.42	NS	NS	0.006
Fear/depression	1.0 ± 3.6 (62)	1.4 ± 3.3 (66)	0.6 ± 2 (20)	0.42	NS	NS	0.006

(Continued)

TABLE 2 | Continued

DAT1 rs11564774	**CC**	**CG**	**GG**	**F**	**p value**	**p value[a]**	**η^2**
N	81	58	10				
Female, N [%]	43 [53]	32 [55]	1 [10]				
Drug experience, N [%]	31 [38]	17 [29]	7 [70]				
MDMA plasma concentration Cmax, ng/ml	230 ± 49 (81)	230 ± 49 (58)	199 ± 35 (10)	1.96	NS	NS	0.026
MDMA plasma concentration AUC_6, ng*h/ml	967 ± 211 (81)	967 ± 196 (58)	851 ± 155 (10)	1.55	NS	NS	0.021
Visual Analog Scale rating ΔE_{max}							
Any drug effect	72 ± 27 (81)	73 ± 27 (58)	74 ± 23 (10)	0.60	NS	NS	0.007
Good drug effect	71 ± 29 (81)	75 ± 28 (58)	75 ± 27 (10)	0.59	NS	NS	0.007
Bad drug effect	17 ± 22 (81)	16 ± 26 (58)	13 ± 36 (10)	0.03	NS	NS	0.000
Drug liking	71 ± 31 (81)	77 ± 27 (58)	78 ± 21 (10)	0.95	NS	NS	0.012
Stimulated	64 ± 34 (81)	62 ± 36 (58)	51 ± 30 (10)	0.29	NS	NS	0.004
High mood	69 ± 32 (81)	68 ± 35 (58)	73 ± 29 (10)	0.40	NS	NS	0.005
Concentration	9.0 ± 15 (81)	7.4 ± 16 (58)	11 ± 20 (10)	0.29	NS	NS	0.004
Talkative	23 ± 19 (81)	19 ± 19 (58)	20 ± 11 (10)	0.55	NS	NS	0.007
Appetite	-4.8 ± 28 (36)	-6.4 ± 34 (29)	-24 ± 27 (7)	1.51	NS	NS	0.041
Tired	24 ± 33 (54)	16 ± 31 (46)	12 ± 29 (9)	1.00	NS	NS	0.018
Fear	8 ± 19 (55)	2.5 ± 5.8 (35)	13 ± 25 (7)	1.86	NS	NS	0.038
Happy	27 ± 18 (60)	28 ± 20 (41)	21 ± 14 (5)	0.05	NS	NS	0.001
Want to be hugged	10 ± 16 (45)	19 ± 21 (29)	0 ± 0 (3)	2.36	NS	NS	0.056
Want to hug	11 ± 16 (45)	19 ± 20 (29)	0 ± 0 (3)	2.26	NS	NS	0.053
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	23 ± 14 (81)	25 ± 11 (58)	24 ± 12 (10)	0.49	NS	NS	0.006
Diastolic blood pressure, mmHg	13 ± 9 (81)	14 ± 8 (58)	18 ± 17 (10)	2.97	0.05	NS	0.037
Mean arterial pressure, mmHg	16 ± 10 (81)	18 ± 8 (58)	22 ± 13 (10)	2.90	0.06	NS	0.036
Heat rate beat/min	16 ± 15 (81)	19 ± 15 (58)	16 ± 7 (10)	0.66	NS	NS	0.009
Rate pressure product, mmHg/min	4,219 ± 2,944 (81)	4,902 ± 2,877 (58)	3,963 ± 1,624 (10)	1.03	NS	NS	0.013
Body temperature, °C	0.2 ± 0.5 (81)	0.3 ± 0.4 (58)	0.3 ± 0.6 (10)	0.73	NS	NS	0.010
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	2.5 ± 5.2 (81)	2.4 ± 5.3 (58)	2.0 ± 2.7 (10)	0.02	NS	NS	0.000
High mood	2.8 ± 3.3 (81)	3.0 ± 3.2 (58)	2.2 ± 1.3 (10)	0.26	NS	NS	0.004
Fear/depression	0.9 ± 3 (81)	1.4 ± 3.7 (58)	1.2 ± 1.5 (10)	0.46	NS	NS	0.006
DAT1 rs460000	**GG**	**GT**	**TT**	**F**	**p value**	**p value[a]**	**η^2**
N	94	48	7				
Female, N [%]	52 [55]	22 [46]	2 [29]				
Drug experience, N [%]	33 [35]	20 [42]	2 [29]				
MDMA plasma concentration Cmax, ng/ml	232 ± 50 (94)	221 ± 47 (48)	221 ± 37 (7)	0.98	NS	NS	0.013
MDMA plasma concentration AUC_6, ng*h/ml	971 ± 200 (94)	937 ± 214 (48)	958 ± 185 (7)	0.46	NS	NS	0.006
Visual Analog Scale rating ΔE_{max}							
Any drug effect	73 ± 26 (94)	71 ± 28 (48)	72 ± 20 (7)	0.01	NS	NS	0.000
Good drug effect	75 ± 28 (94)	69 ± 31 (48)	67 ± 26 (7)	0.68	NS	NS	0.009
Bad drug effect	18 ± 26 (94)	14 ± 21 (48)	9.1 ± 17 (7)	0.57	NS	NS	0.007
Drug liking	77 ± 27 (94)	69 ± 33 (48)	65 ± 27 (7)	1.27	NS	NS	0.016
Stimulated	61 ± 35 (94)	66 ± 33 (48)	57 ± 37 (7)	0.71	NS	NS	0.009
High mood	70 ± 33 (94)	67 ± 32 (48)	64 ± 33 (7)	0.14	NS	NS	0.002
Concentration	9.1 ± 17 (94)	7.9 ± 15 (48)	5.4 ± 11 (7)	0.23	NS	NS	0.003
Talkative	23 ± 19 (94)	18 ± 18 (48)	17 ± 20 (7)	0.86	NS	NS	0.011
Appetite	-6.8 ± 33 (53)	-6.7 ± 26 (15)	-15 ± 16 (4)	0.11	NS	NS	0.003
Tired	19 ± 32 (72)	22 ± 34 (32)	19 ± 23 (5)	0.23	NS	NS	0.004
Fear	6.4 ± 18 (66)	5 ± 9.5 (27)	14 ± 10 (4)	0.56	NS	NS	0.012
Happy	28 ± 19 (65)	25 ± 19 (38)	24 ± 25 (3)	0.32	NS	NS	0.006
Want to be hugged	16 ± 20 (41)	8.6 ± 15 (33)	20 ± 26 (3)	1.85	NS	NS	0.044
Want to hug	16 ± 19 (41)	8.8 ± 14 (33)	22 ± 26 (3)	2.12	NS	NS	0.050
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	23 ± 13 (94)	24 ± 13 (48)	26 ± 15 (7)	0.35	NS	NS	0.005
Diastolic blood pressure, mmHg	14 ± 10 (94)	13 ± 9 (48)	12 ± 6 (7)	0.16	NS	NS	0.002
Mean arterial pressure, mmHg	17 ± 10 (94)	17 ± 9 (48)	16 ± 7 (7)	0.11	NS	NS	0.001
Heat rate beat/min	17 ± 14 (94)	17 ± 13 (48)	22 ± 21 (7)	0.32	NS	NS	0.004
Rate pressure product, mmHg/min	4,500 ± 2,891 (94)	4,239 ± 2,512 (48)	5,607 ± 4,506 (7)	0.66	NS	NS	0.009
Body temperature, °C	0.2 ± 0.5 (94)	0.3 ± 0.5 (48)	0.2 ± 0.6 (7)	0.84	NS	NS	0.011
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	2.4 ± 5.7 (94)	2.1 ± 4.0 (48)	4.0 ± 2.4 (7)	0.42	NS	NS	0.006
High mood	3 ± 3.1 (94)	2.5 ± 3.3 (48)	3.7 ± 3.5 (7)	0.63	NS	NS	0.009
Fear/depression	1.2 ± 3.2 (94)	1.4 ± 3.6 (48)	-1.4 ± 3.6 (7)	2.25	NS	NS	0.030

(Continued)

TABLE 2 | Continued

DAT1 rs463379	CC	CG	GG	F	p value	p value[a]	η^2
N	7	47	93				
Female, N [%]	2 [29]	21 [45]	51 [55]				
Drug experience, N [%]	2 [29]	20 [43]	32 [34]				
MDMA plasma concentration Cmax, ng/ml	221 ± 37 (7)	221 ± 47 (47)	232 ± 50 (93)	0.86	NS	NS	0.012
MDMA plasma concentration AUC_6, ng*h/ml	958 ± 185 (7)	934 ± 215 (47)	970 ± 200 (93)	0.49	NS	NS	0.007
Visual Analog Scale rating ΔE_{max}							
Any drug effect	72 ± 20 (7)	71 ± 28 (47)	73 ± 26 (93)	0.00	NS	NS	0.000
Good drug effect	67 ± 26 (7)	69 ± 31 (47)	75 ± 28 (93)	0.60	NS	NS	0.008
Bad drug effect	9.1 ± 17 (7)	13 ± 21 (47)	18 ± 27 (93)	0.62	NS	NS	0.008
Drug liking	65 ± 27 (7)	69 ± 33 (47)	77 ± 27 (93)	1.19	NS	NS	0.016
Stimulated	57 ± 37 (7)	65 ± 33 (47)	61 ± 35 (93)	0.68	NS	NS	0.009
High mood	64 ± 33 (7)	67 ± 32 (47)	70 ± 33 (93)	0.10	NS	NS	0.001
Concentration	5.4 ± 11 (7)	8.1 ± 15 (47)	9.2 ± 17 (93)	0.23	NS	NS	0.003
Talkative	17 ± 20 (7)	19 ± 18 (47)	23 ± 19 (93)	0.64	NS	NS	0.009
Appetite	-15 ± 16 (4)	-6.7 ± 26 (15)	-6.8 ± 33 (53)	0.11	NS	NS	0.003
Tired	19 ± 23 (5)	22 ± 34 (32)	19 ± 32 (72)	0.23	NS	NS	0.004
Fear	14 ± 10 (4)	5 ± 9.5 (27)	6.4 ± 18 (66)	0.56	NS	NS	0.012
Happy	24 ± 25 (3)	25 ± 19 (37)	28 ± 18 (64)	0.23	NS	NS	0.004
Want to be hugged	20 ± 26 (3)	7.7 ± 14 (32)	15 ± 19 (40)	2.02	NS	NS	0.050
Want to hug	22 ± 26 (3)	8.1 ± 14 (32)	15 ± 19 (40)	2.22	NS	NS	0.054
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	26 ± 15 (7)	24 ± 14 (47)	23 ± 13 (93)	0.27	NS	NS	0.004
Diastolic blood pressure, mmHg	12 ± 6 (7)	13 ± 9 (47)	14 ± 10 (93)	0.15	NS	NS	0.002
Mean arterial pressure, mmHg	16 ± 7 (7)	17 ± 9 (47)	17 ± 10 (93)	0.09	NS	NS	0.001
Heat rate beat/min	22 ± 21 (7)	17 ± 13 (47)	17 ± 14 (93)	0.30	NS	NS	0.004
Rate pressure product, mmHg/min	5,607 ± 4,506 (7)	4,245 ± 2,539 (47)	4,514 ± 2,903 (93)	0.64	NS	NS	0.009
Body temperature, °C	0.2 ± 0.6 (7)	0.3 ± 0.5 (47)	0.2 ± 0.5 (93)	0.67	NS	NS	0.009
Adjective Mood Rating Scale rating ΔE_{max}							
Activity	4.0 ± 2.4 (7)	2.1 ± 4.1 (47)	2.4 ± 5.7 (93)	0.41	NS	NS	0.006
High mood	3.7 ± 3.5 (7)	2.5 ± 3.3 (47)	2.9 ± 3.1 (93)	0.57	NS	NS	0.008
Fear/depression	-1.4 ± 3.6 (7)	1.4 ± 3.6 (47)	1.2 ± 3.2 (93)	2.27	NS	NS	0.031

*N, number of subjects; SD, standard deviation; NS, not significant; Δ, values are change scores from placebo; [a]p value additionally corrected for multiple comparisons according to the Nyholt method; η^2, eta square; *, uncorrected p < 0.05.*

was also performed, the results of which are reported only when the additive model was initially significant.

RESULTS

MDMA significantly altered all tested VAS and AMRS E_{max} values. Subjects did not significantly differ in MDMA plasma concentration or previous drug experience across genotype groups, with the exception of DAT1 rs11133767. Participants carrying two T-alleles showed disproportionately more illicit drug experiences than carriers of the C-allele (70% vs. 31%, respectively; $\chi^2 = 11.2$, $p < 0.001$).

The influence of polymorphisms within genes coding for the DRD2, DAT1, and DRD4 on the maximal acute subjective and autonomic effects of MDMA is shown in **Tables 1–3**, respectively. **Supplementary Table S2** shows the data for the total response to MDMA over time (AUEC). **Supplementary Tables S3** and **S4** show the uncorrected statistics for E_{max} and AUEC, respectively. Homozygous A-allele carriers of the DRD2 rs1800497 showed a higher score in VASs "talkative" ($F_{1,147} = 4.23$, $p < 0.05$) and in AMRSs "activity" and "high mood" ($F_{1,147} = 4.62$, $p < 0.05$ and $F_{1,147} = 4.50$, $p < 0.05$, respectively) compared to carriers of the G-allele. Subjects with two 9R-alleles of the DAT1 rs28363170 had a higher MDMA-induced increase in diastolic blood pressure and MAP compared to subjects with a 10R-allele ($F_{1,141} = 7.12$, $p < 0.01$ and $F_{1,141} = 6.56$, $p < 0.05$, respectively). Regarding the DAT1 rs3836790, MDMA produced a higher increase in MAP in individuals homozygous for the 5R-allele compared to 6R-allele carriers ($F_{1,144} = 4.31$, $p < 0.05$).

Nyholt correction for multiple comparisons yielded statistics indicating that the genetic polymorphisms had no significant effect on the subjective and autonomic parameters. Sex did not significantly modulate the results.

DISCUSSION

The current study expands previous research on whether the acute effects of MDMA are modulated by common genetic polymorphisms in pharmacological targets of MDMA. So far, the focus lied on the role of the NE and 5-HT system genetics in the acute effects of MDMA (22, 23). This is the first study to concentrate on a selection of genetic polymorphisms within the human DA system (namely, D_2, D_4, and DAT).

Action on the DA system is thought to be crucial for the effects of most psychostimulant substances (6, 24, 61), and pharmacogenetic studies demonstrated that different phenotypes are affected by various DA genotypes. As for MDMA, however, none of the herein investigated genetic polymorphisms significantly altered the acute effects after consideration of Type I error correction.

TABLE 3 | Effects of the variable-number tandem repeat polymorphism in the dopamine receptor D4 gene on the maximal response to 125 mg MDMA (mean ± SD (N) and statistics) corrected with MDMA AUC_6 (exclusive plasma concentrations).

DRD4 VNTR	≤8 Repeats	>8 Repeats	F	p value	p value[a]	η^2
N	87	59				
Female, N [%]	44 [51]	31 [53]				
Drug experience, N [%]	31 [36]	22 [37]				
MDMA plasma concentration Cmax, ng/ml	229 ± 44 (87)	226 ± 55 (59)	0.16	NS	NS	0.001
MDMA plasma concentration AUC_6, ng*h/ml	965 ± 189 (87)	948 ± 221 (59)	0.25	NS	NS	0.002
Visual Analog Scale rating ΔE_{max}						
Any drug effect	74 ± 26 (87)	71 ± 26 (59)	0.35	NS	NS	0.002
Good drug effect	73 ± 30 (87)	73 ± 26 (59)	0.01	NS	NS	0.000
Bad drug effect	17 ± 23 (87)	15 ± 27 (59)	0.08	NS	NS	0.001
Drug liking	74 ± 31 (87)	75 ± 25 (59)	0.12	NS	NS	0.001
Stimulated	63 ± 35 (87)	63 ± 34 (59)	0.07	NS	NS	0.000
High mood	68 ± 34 (87)	71 ± 31 (59)	0.51	NS	NS	0.003
Concentration	8.2 ± 17 (87)	9.0 ± 15 (59)	0.09	NS	NS	0.001
Talkative	20 ± 19 (87)	23 ± 19 (59)	1.27	NS	NS	0.008
Appetite	-5.8 ± 33 (47)	-10 ± 26 (25)	0.41	NS	NS	0.006
Tired	24 ± 32 (68)	13 ± 32 (41)	2.63	NS	NS	0.023
Fear	6.6 ± 18 (56)	5.6 ± 14 (38)	0.08	NS	NS	0.001
Happy	26 ± 20 (59)	30 ± 17 (44)	1.33	NS	NS	0.012
Want to be hugged	13 ± 19 (40)	13 ± 18 (34)	0.01	NS	NS	0.000
Want to hug	14 ± 19 (40)	13 ± 17 (34)	0.02	NS	NS	0.000
Vital signs parameters ΔE_{max}						
Systolic blood pressure, mmHg	25 ± 12 (87)	22 ± 13 (59)	1.24	NS	NS	0.008
Diastolic blood pressure, mmHg	14 ± 9 (87)	13 ± 10 (59)	0.11	NS	NS	0.001
Mean arterial pressure, mmHg	17 ± 9 (87)	17 ± 10 (59)	0.11	NS	NS	0.001
Heat rate beat/min	18 ± 15 (87)	17 ± 14 (59)	0.03	NS	NS	0.000
Rate pressure product, mmHg/min	4,561 ± 2,967 (87)	4,393 ± 2,746 (59)	0.06	NS	NS	0.000
Body temperature, °C	0.3 ± 0.5 (87)	0.2 ± 0.5 (59)	0.19	NS	NS	0.001
Adjective Mood Rating Scale rating ΔE_{max}						
Activity	2.3 ± 5.2 (87)	2.7 ± 4.9 (59)	0.26	NS	NS	0.002
High mood	2.8 ± 3.3 (87)	3.0 ± 3.0 (59)	0.18	NS	NS	0.001
Fear/depression	1.1 ± 3.7 (87)	0.9 ± 3 (59)	0.10	NS	NS	0.001

N, number of subjects; SD, standard deviation; NS, not significant; Δ, values are change scores from placebo; [a]p value additionally corrected for multiple comparisons according to the Nyholt method; η^2, eta square.

Nevertheless, this missing link between DA genetic variations and MDMA-related phenotypes might not solely be caused by a lack of genetic influence on the MDMA effects but rather the potentially minor role of DA in MDMA effects. Although MDMA is an amphetamine, it acts mainly on the 5-HT system and therefore leads to its classification as an entactogen (7, 62).

The present study has limitations. Although this analysis was done using the largest sample of healthy human subjects who received MDMA in placebo-controlled studies, the sample size is still relatively small when considering the partially small rare allele groups and mostly weak effect sizes for the influence of genetic variants on the MDMA response. This is especially influencing spurious, uncorrected effects (i.e., the AA carrier group for the SNP DRD2/ANKK1 rs1800497 with N = 2). Larger cohorts might show a more balanced sample distribution, which might lead to different results. Additionally, the study was conducted in healthy volunteers with a single dose of 125 mg MDMA. Therefore, the findings may not be applied to other populations and situations, such as psychiatric patients and the use of higher doses of MDMA. Furthermore, SNPs in genes of other targets of MDMA may also be involved. However, we corrected for the modulatory effects of known genetic variants that influence the metabolism of MDMA (17, 18) by taking interindividual differences in plasma MDMA concentrations into account. We also might have missed some relevant genetic polymorphisms. A novel potentially functional SNP within the DAT1 has been described in recent research. However, the SNP showed no significant alteration in the inhibition of DA uptake by MDMA in human embryonic kidney 293 cells (63). We have also not tested for rare haplotypes because a haplotype approach may lead to very small groups and more potential statistical artifacts. However, a haplotype suggested by Brewer et al., which consists of rs28363170 10/10 genotype and at least one rs3836790 5R-allele carriers, showed a reduced subjective response to cocaine compared to others (40). The same haplotype showed no effect in the present study. In fact, uncorrected results even implied opposite and incoherent effects, with 10R carriers showing lower MDMA-induced MAP changes and 5/5 carriers showing higher MAP changes than subjects with the 9/9 genotype or a 6R-allele, respectively. This incoherency may be attributable to the different substances used (cocaine vs. MDMA) and different cohorts (80% males of African descent vs. the sex-balanced sample of European descent) (40). Additionally, MDMA may interact with a different binding site on the DAT compared to other stimulants like cocaine (64). Finally, previous drug experiences were not equally distributed among DAT1 rs11133767 genotype groups, and effects might slightly depend on previous substance use experiences. Because of the involvement of DA in addiction, subjects carrying a TT genotype may be more prone to illicit substance use (65). Apart from this finding, given that our cohort included mostly

drug-naive subjects with limited drug use experience, some alleles associated with increased drug use might even be underrepresented. However, the tested variants were consistent with the Hardy–Weinberg equilibrium and comparable with frequencies found in European genome databases.

We conclude that the present findings align with previous studies in that variations in genes coding for players of the monoaminergic systems are unlikely to explain interindividual variations in the acute effects of MDMA in humans.

DATA AVAILABILITY STATEMENT

The datasets for this manuscript are not publicly available because the individual genotyping consent did not include storing in public repository. Requests to access the datasets should be directed to Matthias Liechti, Matthias.liechti@usb.ch.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission Nordwest- und Zentralschweiz (EKNZ). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PV analyzed the data and wrote the manuscript. ML conceived the study, obtained funding, and wrote the manuscript.

FUNDING

This study was supported by the Swiss National Science Foundation (grant no. 320030_170249).

ACKNOWLEDGMENTS

The authors acknowledge the assistance of C.M. Hysek, A. Rickli, Y. Schmid, P.C. Dolder, and F. Holze in conducting the clinical studies, H. Meyer zu Schwabedissen, K. Prestin, and Janine J. Hussner for assistance with genotyping, and M. Arends for text editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2019.00755/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DISCUSSION, CONCLUSION & OUTLOOK

The scope of present thesis describes comprehensive safety pharmacology and pharmacogenetics of MDMA in healthy human subjects. The study findings are detailed in the published papers above. Here is a brief discussion of the whole work, a conclusion and an outlook.

Safety pharmacology of MDMA

MDMA effected predominantly acute positive subjective sensations. The administration of MDMA at doses of 75 and 125 mg was overall safe and well tolerated in healthy subjects. Unpleasant subjective drug effects and adverse effects were more pronounced in women. Lack of appetite, dry mouth, cold feet, sweating, restlessness and palpitations were the most frequent acute adverse events reported, indicating moderate sympathomimetic toxicity. Transient hypertension (systolic blood pressure >160 mmHg) and tachycardia (heart rate >100 beats/min) were observed in one third of the participants who received 125 mg MDMA. 5 % of the subjects reached transient systolic blood pressure peaks over 180 mmHg, which is considered as severe hypertension. However, no clinical symptoms of hypertensive crises were observed and the results were comparable with cardiovascular stimulation in other studies with MDMA or other stimulants such as D-amphetamine, methylphenidate, and methamphetamine (Kirkpatrick et al., 2012; Wardle et al., 2012; Hysek et al., 2014b; Bershad et al., 2015). Special attention was paid to the thermogenic reaction to MDMA, since hyperpyrexia is considered as the most important life-threatening complication of MDMA use (Liechti et al., 2005; Halpern et al., 2011; Liechti, 2014). Body temperature increased up to 39.1 °C in our studies, which is consistent with other controlled trials (Freedman et al., 2005; Kolbrich et al., 2008). However, the results should be cautiously compared to emergencies, since there are often additional risk factors such as high ambient temperature in recreational settings (Dafters, 1995). Levels of liver enzymes and kidney function did not differ before and one month after MDMA administration. Although we did not hypothesize hepatotoxic effects at the doses used, idiosyncratic hepatotoxicity is reported in rare cases (Henry et al., 1992; Ellis et al., 1996; Antolino-Lobo et al., 2011; Atayan et al., 2015; Maharaj et al., 2015). This type of hepatotoxicity is observed with many marketed medications (Krähenbühl and J.Pichler, 2017).

Due to the aforementioned results, administration of a fix dose of 100 mg MDMA for women and 125 mg dose for men is suggested. In line with our recommendation, phase 3 trials use 80 mg of MDMA with the option to escalate the dose up to 120 mg (Mithoefer et al., 2019). Our data did not raise any safety concerns related to further studies of MDMA in controlled medical environments, including as an adjunct to psychotherapy. However, these results should be interpreted with caution, as only healthy subjects were included, and sympathomimetic stimulation has been observed. To assess this matter, further research should be conducted with patients suffering from (somatic) comorbidities, and those at risk for sympathomimetic toxicity.

Pharmacogenetic profiles of MDMA

MDMA is a stimulant and an entactogen (Nichols, 1986; Bershad et al., 2016a). Subjects taking MDMA display distinct subjective, prosocial, and autonomic effects (Hysek et al., 2014a; Schmid et al., 2014). However, there is individual variation in the effects of MDMA. Some of this variance may be explained by different genetic predispositions of the pharmacokinetic and pharmacodynamic players involved in the response to MDMA in humans.

For example, CYP2D6 plays a major role in the metabolism of MDMA (de la Torre et al., 2012; Rietjens et al., 2012; Yubero-Lahoz et al., 2012). We confirmed in a meaningful sample size the critical involvement of CYP2D6 genotypes and underlined the results with phenotype data. In addition, we showed for the first time the influence of polymorphism in CYP1A2, CYP2B6, and CYP2C19 on the pharmacokinetic and pharmacodynamic response to MDMA in humans. Results display that subjects with CYP2D6 poor metabolizer status had a disabled major metabolic pathway and therefore exhibited higher MDMA and MDA, and lower HMMA blood plasma concentrations compared with subjects identified as extensive metabolizer (normal). CYP2D6 PMs also showed a more rapid increase of the subjective and autonomic drug effects in the beginning of the acute MDMA effects. These findings are consistent with the previously observed mechanism-based inhibition of CYP2D6 that turns all subjects into functional PMs within 1-2 h (Yang et al., 2006; O'Mathuna et al., 2008). When CYP2D6 function decreases over time, other CYPs might become more important. We showed, consistent with in vitro studies (Kreth et al., 2000; Meyer et al., 2008), that CYP2B6, CYP1A2, and CYP2C19 contributed to the conversion of MDMA to MDA in humans. In line with the auto-inhibition of CYP2D6, the influence of polymorphisms in the CYP2B6 became more apparent 3-4 h after MDMA administration. Furthermore, we found higher MDA levels in subjects who smoke 6-10 cigarettes a day and possess the inducible genotype of the CYP1A2 compared with subjects who smoke less and/or have the non-inducible polymorphism. However, we tested only very moderate smokers and only 4 had the inducible type. If this result remains significant in study cohorts with more and heavier smokers is still to investigate. So far, the consequences of this enhanced conversion from MDMA to MDA are unclear. Since the effect and toxicity of MDA is similar to MDMA, this may not be clinically relevant (Molliver et al., 1986; Esteban et al., 2001; Monks et al., 2004; Baggott et al., 2019).

In a second part of this thesis, we focused on the pharmacodynamic effect of MDMA and looked into pharmacological targets of the MDMA mechanism. MDMA acts primarily through the release and reuptake inhibition of 5-HT, NE, and DA, but also increases oxytocin levels (Thompson et al., 2007; Dumont et al., 2009; Hysek et al., 2012d; Simmler et al., 2013; Hysek et al., 2014a; Kirkpatrick et al., 2014). Polymorphisms in genes coding for components of those monoamine and neuroendocrine systems were investigated for their influence on the response to MDMA. Regarding the polymorphisms in the OXTR (3 SNPs) we suspected alterations in the

prosocial effects, since a clinical study indicated different sociability after MDMA (Bershad et al., 2016b). Additionally, oxytocin is known to increase similar empathogenic effects (Di Simplicio et al., 2009; Hurlemann et al., 2010). In fact, we observed moderating effects for MDMA-induced feelings of trust and desire for company, but between genotype groups of another SNP (rs1042778) than was indicated by the previous study (rs53576).

As for the investigations on the monoamine system genes and their influence on MDMA effects, this thesis was the first research conducted for most of the tested polymorphisms. Therefore, results had to be carefully corrected for statistical errors and tested predominantly for plausible effects (e.g. NET gene [SLC6A2] for cardiovascular effects). Most of the tested genetic polymorphisms in the 5-HT (7 SNPs and 1 repeat polymorphism), NE (5 SNPs), and DA (10 SNPs and 1 repeat polymorphism) systems did not alter the effects of MDMA when adjusting for multiple comparisons. Despite rigorous correction to avoid Type I errors, some polymorphisms in the NE gene SLC6A2 (rs1861647, rs2242446, rs36029) slightly, but significantly, moderated the acute MDMA-induced cardiovascular response. Furthermore, variations in genes that encode key targets in the 5-HT system (rs7305115, rs6313, 5-HTTLPR) tended to moderate some MDMA effects such as “good drug effect”, “drug liking”, or “closeness to others”. Before correction for multiple testing the results from the present study reproduced the non-corrected results from a recent study by Kuypers et al. (Kuypers et al., 2018). In both studies, carriers of the short allele (5-HTTLPR) felt less MDMA-induced anxiety/fear than carriers of two long alleles. However, an influence by 5-HT system gene variations on cardiovascular effects of MDMA were not observed. Subsequently, this thesis is failing to replicate findings from another study showing greater cardiovascular effects in long allele carriers of the 5-HTTLPR compared to exclusive short allele carriers (Pardo-Lozano et al., 2012). Nevertheless, the present thesis was conducted with a higher sample size and a more methodologically sound analysis. Furthermore, results from pharmacogenetic studies with d-amphetamine and polymorphisms within SLC6A2 and SLC6A3 could also not be replicated for MDMA (Dlugos et al., 2007; Dlugos et al., 2011), but were also contradicted by the same group in a follow-up study (Hart et al., 2013). Altogether, genetic polymorphisms in the monoamine systems may play a marginal role in acute MDMA effects and are unlikely to explain the whole spectrum of interindividual variations.

Limitations & Outlook

While the data provide valuable insight into safety pharmacology and the pharmacogenetics of MDMA in humans, some limitations should be kept in mind.

First of all, we examined only healthy and mostly young volunteers. Translating results from phase I studies to psychiatric patients or people with special health conditions and probable co-medication is limited and needs further investigation. A second limitation but also strength of this cohort is its uniformity. Our participants are predominantly of European descent. Thus, the relevance and reproducibility of the results may vary between ethnicity. For example, CYP2D6

genotype frequencies differ considerably across the major ethnic groups (Gaedigk et al., 2017). Furthermore, although this is the largest standardized MDMA cohort to date, some rare mutations and especially rare haplotypes might be missed.

Another clear limitation is that additional players involved in MDMA effects feature genetic variations as well. For instance, COMT gene contains a main functional SNP (rs4680) that results in an amino acid exchange from Val to Met. The COMT Val-allele is associated with a high, and the Met-allele with a low activity of the enzyme (Maria et al., 2012). As mentioned before (see section 1.3.), COMT is an important enzyme in the metabolism of MDMA. Therefore, the COMT polymorphism might have an impact on the acute effects of MDMA. In fact, a study has shown influence on the cardiovascular effects, while others associated low COMT activity with a higher increase in plasma cortisol concentration and lower plasma sodium after MDMA administration (Aitchison et al., 2012; Pardo-Lozano et al., 2012; Wolff et al., 2012). Another target worth investigating might be the monoamino oxidase A (MOA-A). It catalyzes oxidative deamination of several monoamines including www5-HT, NE, and DA. Steuer et al. showed relevant inhibition (>30%) of MDA and MDMA toward MAO-A catalyzed deamination of DA and 5-HT (Steuer et al., 2016). There is a known variable number tandem repeat (VNTR) promoter polymorphism and an SNP (rs6323) that modifies the MAO-A expression (Sabol et al., 1998; Verma et al., 2014). Further studies should examine the impact of those polymorphism on clinical pharmacology of MDMA. Moreover, individual differences in the UCP3 gene may modulate the hyperthermic effects of MDMA since UCP3 is involved in thermogenesis through uncoupling of mitochondrial oxidative phosphorylation in skeletal muscle (Brand and Esteves, 2005). Interestingly, in knock-out mice deficient in UCP3 the thermogenic response to MDMA was diminished (Mills et al., 2003). The hyperthermic reaction to MDMA is one of the most dangerous effects, and genetic factors that might be involved should be discussed separately in the scope of a future publication. Also worth mentioning are mutations in genes coding for the trace amine-associated receptor (TAAR1) or the arginine vasopressin receptor 1A (AVPR1A) that could alter some effects of MDMA. TAAR1 is involved in the triggering of the phosphorylation of DAT, NET, and SERT, which reverse the transport from the cytosol into the synaptic cleft (Miller, 2011; Simmler et al., 2016). AVPR1A gene variations could alter possibly the risk of hyponatremia and the prosocial effects of MDMA similarly to the findings of the OXTR genotypes (Knafo et al., 2008; Avinun et al., 2011).

Certainly, there are several additional targets with genetic variations involved in MDMA effects that should be investigated in the future. However, not all mutations are in fact functional and pharmacogenetic studies are mostly designed retrospectively and thus observational. Prospective studies would be needed to confirm the here seen results. Additionally, to identify risk genetic polymorphisms for MDMA intoxications a database from a network like the European Drug Emergencies Network (Euro-DEN Plus) should be developed that also includes genetic information. However, linking genetics to MDMA response variability is not the only way to predict individual drug responses. An ongoing investigation in our study sample, displays

different predictors of the response to MDMA including character traits, mood before drug intake, and previous drug experience, as it has been showed for psilocybin (Studerus et al., 2012).

In the coming years, phase 3 studies will likely reveal further details about the clinical safety of MDMA in a broader population. Results from the present studies may pave the way for future studies with MDMA in larger sample sizes and help to evaluate what is important for individually different responses to MDMA intake and just as much what is not.

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