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

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Abstract

Background: 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.

Methods: 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 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.

Results: *TPH2* rs7305115, *HTR2A* rs6313, and *SLC6A4* 5-HTTLPR polymorphisms tended to moderately alter some effects of MDMA. However, after correcting for multiple testing, none of the tested genetic polymorphisms significantly influenced the response to MDMA.

Conclusions: Genetic variations of 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.

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 posttraumatic 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 polymorphism have approximately double the uptake activity of cells that carry one or two copies of the S allele [31]. In humans, individuals with the L allele and G variant of rs25331 present the same low-expressing phenotypes as S-allele carriers [32-34]. Consequently, 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. (2018b) performed a study with 63 polydrug users and found that 75 mg 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. (2012) 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 two-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 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 non-attempters [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. 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. Supplementary Table S1 shows the data for the response to MDMA over time (AUEC). Supplementary Tables S2 and S3 show the uncorrected statistics for E_{max} and AUEC, respectively. Sex did not significantly alter the results.

Table 1. Effects of polymorphisms in the serotonergic system on the maximal response to 125 mg MDMA (mean±SD and statistics) corrected with MDMA AUC_6 (exclusive plasma concentrations)

	TPH1 rs1800532	**GG**	**GT**	**TT**	**F**	**p value**	**p value[a]**
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	
MDMA plasma concentration AUC_6, ng*h/ml	N: 44, 62, 18	944±221	949±202	998±200	0.48	NS	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 44, 62, 18	81±19	73±26	74±26	2.32	NS	NS
Good drug effect	N: 44, 62, 18	81±23	72±29	72±28	2.00	NS	NS
Bad drug effect	N: 44, 62, 18	14±23	21±29	14±19	1.50	NS	NS
Drug liking	N: 44, 62, 18	81±21	74±29	76±29	1.05	NS	NS
Closeness to others	N: 44, 62, 18	23±17	21±19	27±19	0.39	NS	NS
High-mood	N: 44, 62, 18	74±32	71±31	71±31	0.37	NS	NS
Talkative	N: 44, 62, 18	25±20	19±19	25±17	1.38	NS	NS
Appetite	N: 24, 42, 6	-7±33	-6±31	-15±22	0.20	NS	NS
Tired	N: 41, 53, 15	21±32	18±33	23±31	0.13	NS	NS
Fear	N: 24, 42, 6	4±14	9±20	4±9	0.91	NS	NS
Happy	N: 26, 40, 15	28±18	27±20	33±18	0.29	NS	NS
Content	N: 26, 40, 15	32±14	29±21	32±18	0.65	NS	NS
Trust	N: 20, 20, 12	23±18	23±23	25±26	0.15	NS	NS
want to be hugged	N: 20, 20, 12	11±19	19±20	24±20	0.83	NS	NS
want to hug	N: 20, 20, 12	14±19	19±19	23±19	0.33	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 44, 62, 18	26±12	22±13	27±12	1.62	NS	NS
Diastolic blood pressure, mmHg	N: 44, 62, 18	14±11	14±9	15±9	0.04	NS	NS
Mean arterial pressure, mmHg	N: 44, 62, 18	18±10	17±10	19±9	0.13	NS	NS
Rate pressure product, mmHg/min	N: 44, 62, 18	5311±3048	4314±2906	4779±2783	1.61	NS	NS
Body temperature, °C	N: 44, 62, 18	0.3±0.5	0.2±0.5	0.4±0.5	1.27	NS	NS
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
high mood	N: 44, 62, 18	3.5±3.1	2.7±3.1	2.9±3.7	0.75	NS	NS
fear/depression	N: 44, 62, 18	0.0±3.4	1.3±3.2	0.6±2.0	2.40	NS	NS
dreaminess	N: 44, 62, 18	3.3±2.6	3.1±3.5	2.5±3.2	0.58	NS	NS
List of Complaints Δscore							
acute, up to 6h, N	N: 44, 62, 18	8.5±7.4	8.6±6.8	8.5±4.8	0.04	NS	NS
subacute, up to 24h, N	N: 44, 62, 18	5.1±5.6	4.7±5.3	3.7±5.4	0.70	NS	NS
	TPH2 rs7305115	**AA**	**AG**	**GG**	**F**	**p value**	**p value[a]**
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	
MDMA plasma concentration AUC_6, ng*h/ml	N: 14, 61, 49	958±215	947±209	962±207	0.08	NS	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 14, 61, 49	77±20	75±25	77±24	0.11	NS	NS
Good drug effect	N: 14, 61, 49	71±31	74±28	79±24	0.59	NS	NS
Bad drug effect	N: 14, 61, 49	17±21	15±29	20±22	0.33	NS	NS
Drug liking	N: 14, 61, 49	69±35	76±27	81±23	1.27	NS	NS
Closeness to others	N: 14, 61, 49	19±20	22±17	25±19	0.85	NS	NS
High-mood	N: 14, 61, 49	72±29	69±34	75±28	0.49	NS	NS

Talkative	N: 14, 61, 49	18±23	21±19	24±17	0.76	NS	NS
Appetite	N: 7, 33, 32	-3±32	-9±28	-6±34	0.07	NS	NS
Tired	N: 12, 54, 43	13±34	20±31	22±33	0.28	NS	NS
Fear	N: 7, 33, 32	-1±12	6±19	9±17	0.86	NS	NS
Happy	N: 12, 41, 28	27±21	28±18	29±20	0.05	NS	NS
Content	N: 12, 41, 28	27±22	30±17	32±19	0.28	NS	NS
Trust	N: 7, 28, 17	14±30	23±20	28±21	1.11	NS	NS
want to be hugged	N: 7, 28, 17	7±8	14±20	26±20	2.29	NS	NS
want to hug	N: 7, 28, 17	7±8	16±19	26±20	2.62	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 14, 61, 49	22±13	25±14	23±11	0.92	NS	NS
Diastolic blood pressure, mmHg	N: 14, 61, 49	14±9	15±10	14±8	0.28	NS	NS
Mean arterial pressure, mmHg	N: 14, 61, 49	16±10	18±10	17±9	0.50	NS	NS
Rate pressure product, mmHg/min	N: 14, 61, 49	3757±2787	4786±2838	4952±3135	0.95	NS	NS
Body temperature, °C	N: 14, 61, 49	-0.1±0.6	0.3±0.5*	0.2±0.4	4.11	0.019	NS
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
high mood	N: 14, 61, 49	2.4±3.1	3.1±3.5	3.0±3.0	0.33	NS	NS
fear/depression	N: 14, 61, 49	-0.5±3.3	0.8±3.6	1.0±2.5	1.19	NS	NS
dreaminess	N: 14, 61, 49	2.9±3.6	2.8±2.9	3.4±3.3	0.51	NS	NS
List of Complaints Δscore							
acute, up to 6h, N	N: 14, 61, 49	7.4±7.1	8.4±6.7	9.0±6.8	0.30	NS	NS
subacute, up to 24h, N	N: 14, 61, 49	2.9±4.2	4.7±5.9	5.2±5.0	0.97	NS	NS

	5HTR1A rs6295	**CC**	**CG**	**GG**	**F**	**p value**	**p value[a]**
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	
MDMA plasma concentration AUC_6, ng*h/ml	N: 29, 68, 27	903±180	1005±216[#]	880±183	4.99	0.008	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 29, 68, 27	79±20	79±23	66±27	2.06	NS	NS
Good drug effect	N: 29, 68, 27	77±23	77±28	71±29	0.37	NS	NS
Bad drug effect	N: 29, 68, 27	25±24	17±26	10±24	2.78	NS	NS
Drug liking	N: 29, 68, 27	78±23	78±28	73±28	0.23	NS	NS
Closeness to others	N: 29, 68, 27	21±20	24±18	21±16	0.08	NS	NS
High-mood	N: 29, 68, 27	70±31	73±33	71±29	0.16	NS	NS
Talkative	N: 29, 68, 27	18±18	24±19	21±18	0.46	NS	NS
Appetite	N: 18, 38, 16	-13±37	-3±28	-10±30	1.49	NS	NS
Tired	N: 26, 58, 25	23±34	22±31	11±33	1.05	NS	NS
Fear	N: 18, 38, 16	6±9	9±23	3±8	0.79	NS	NS
Happy	N: 16, 48, 17	25±20	28±20	33±16	1.09	NS	NS
Content	N: 16, 48, 17	28±19	30±19	33±18	0.57	NS	NS
Trust	N: 11, 30, 11	20±24	23±21	27±24	1.10	NS	NS
want to be hugged	N: 11, 30, 11	14±19	18±21	19±19	0.55	NS	NS
want to hug	N: 11, 30, 11	14±19	18±20	22±16	1.23	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 29, 68, 27	21±15	25±12	23±12	0.37	NS	NS
Diastolic blood pressure, mmHg	N: 29, 68, 27	16±9	14±10	13±9	0.99	NS	NS
Mean arterial pressure, mmHg	N: 29, 68, 27	18±10	18±10	16±9	0.38	NS	NS
Rate pressure product, mmHg/min	N: 29, 68, 27	4916±3052	4707±3032	4612±2729	0.39	NS	NS
Body temperature, °C	N: 29, 68, 27	0.2±0.6	0.2±0.5	0.3±0.4	0.50	NS	NS
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
high mood	N: 29, 68, 27	2.6±3.2	3.0±3.0	3.4±3.9	0.42	NS	NS
fear/depression	N: 29, 68, 27	0.2±3.2	1.3±3.3	0.0±2.7	2.32	NS	NS
dreaminess	N: 29, 68, 27	3.2±3.3	3.4±3.2	2.1±2.7	1.30	NS	NS
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
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

	5HTR1B rs6296	**CC**	**CG**	**GG**	**F**	**p value**	**p value[a]**
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	

MDMA plasma concentration AUC_6, ng*h/ml	N: 69, 45, 10	925±203	976±208	1061±209	2.32	NS	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 69, 45, 10	75±22	77±24	77±32	0.45	NS	NS
Good drug effect	N: 69, 45, 10	74±28	79±25	71±28	0.82	NS	NS
Bad drug effect	N: 69, 45, 10	18±24	16±29	19±25	0.40	NS	NS
Drug liking	N: 69, 45, 10	77±27	79±25	70±30	0.89	NS	NS
Closeness to others	N: 69, 45, 10	22±18	23±19	28±18	0.07	NS	NS
High-mood	N: 69, 45, 10	70±31	76±31	66±39	0.87	NS	NS
Talkative	N: 69, 45, 10	21±19	22±20	24±16	0.00	NS	NS
Appetite	N: 40, 27, 5	-4±31	-14±32	-2±22	0.55	NS	NS
Tired	N: 63, 39, 7	22±34	19±31	4±21	1.84	NS	NS
Fear	N: 40, 27, 5	7±19	7±16	0±17	0.41	NS	NS
Happy	N: 44, 30, 7	28±19	28±20	37±17	0.26	NS	NS
Content	N: 44, 30, 7	31±18	28±19	37±17	0.53	NS	NS
Trust	N: 29, 18, 5	20±22	23±22	45±7	1.15	NS	NS
want to be hugged	N: 29, 18, 5	14±19	19±21	26±22	0.51	NS	NS
want to hug	N: 29, 18, 5	14±18	21±19	27±21	1.18	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 69, 45, 10	23±13	27±12	22±10	1.41	NS	NS
Diastolic blood pressure, mmHg	N: 69, 45, 10	14±10	13±9	16±11	0.46	NS	NS
Mean arterial pressure, mmHg	N: 69, 45, 10	18±10	18±10	17±10	0.21	NS	NS
Rate pressure product, mmHg/min	N: 69, 45, 10	4513±2801	5306±3130	3702±2956	1.91	NS	NS
Body temperature, °C	N: 69, 45, 10	0.2±0.5	0.3±0.5	0.2±0.7	0.75	NS	NS
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
high mood	N: 69, 45, 10	2.9±3.1	2.7±3.3	4.7±3.2	1.57	NS	NS
fear/depression	N: 69, 45, 10	1.0±3.0	0.5±3.7	-0.2±1.9	0.82	NS	NS
dreaminess	N: 69, 45, 10	3.0±3.2	3.0±3.2	4.1±2.8	0.37	NS	NS
List of Complaints Δscore							
acute, up to 6h, N	N: 69, 45, 10	8.2±6.8	9.4±6.5	7.0±7.5	0.90	NS	NS
subacute, up to 24h, N	N: 69, 45, 10	5.0±5.5	4.6±5.3	3.2±5.9	1.18	NS	NS

	5HTR2A rs6313	**AA**	**AG**	**GG**	**F**	**p value**	**p value[a]**
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	
MDMA plasma concentration AUC_6, ng*h/ml	N: 22, 62, 40	885±173	990±206	937±220	2.30	NS	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 22, 62, 40	69±24	80±23	73±24	0.53	NS	NS
Good drug effect	N: 22, 62, 40	76±23	81±26^{+}	66±29	3.46	0.035	NS
Bad drug effect	N: 22, 62, 40	13±20	17±27	22±25	1.05	NS	NS
Drug liking	N: 22, 62, 40	77±20	82±27	69±27	2.23	NS	NS
Closeness to others	N: 22, 62, 40	20±17	26±18	19±19	1.06	NS	NS
High-mood	N: 22, 62, 40	67±31	79±29	64±33	2.45	NS	NS
Talkative	N: 22, 62, 40	22±18	24±19	19±19	0.39	NS	NS
Appetite	N: 12, 42, 18	-6±29	-8±29	-6±37	0.07	NS	NS
Tired	N: 20, 54, 35	22±27	20±34	18±33	0.19	NS	NS
Fear	N: 12, 42, 18	0±4	6±13	14±27	2.72	NS	NS
Happy	N: 17, 34, 30	29±17	32±18	24±21	0.94	NS	NS
Content	N: 17, 34, 30	32±17	32±19	27±19	0.52	NS	NS
Trust	N: 10, 20, 22	35±17^{+}	28±20	14±23	3.62	0.034	NS
want to be hugged	N: 10, 20, 22	17±19	23±20	12±20	1.07	NS	NS
want to hug	N: 10, 20, 22	20±17	23±19	12±20	1.09	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 22, 62, 40	21±10	25±14	24±12	0.42	NS	NS
Diastolic blood pressure, mmHg	N: 22, 62, 40	14±14	14±9	14±7	0.17	NS	NS
Mean arterial pressure, mmHg	N: 22, 62, 40	17±12	18±10	18±8	0.02	NS	NS
Rate pressure product, mmHg/min	N: 22, 62, 40	4522±2610	4439±2772	5311±3360	1.61	NS	NS
Body temperature, °C	N: 22, 62, 40	0.3±0.5	0.2±0.6	0.2±0.5	0.21	NS	NS
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
high mood	N: 22, 62, 40	2.8±2.7	3.7±3.0^{++}	2.0±3.6	3.78	0.026	NS
fear/depression	N: 22, 62, 40	0.9±2.9	0.5±3.1	1.1±3.6	0.52	NS	NS
dreaminess	N: 22, 62, 40	2.9±3.8	3.9±3.0^{+}	2.0±2.7	4.02	0.020	NS
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

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

	SLC6A4 5-HTTPLR	**LL**	**LS**	**SS**	**F**	**p value**	**p value[a]**
N (%)		45 (36)	60 (48)	19 (15)			
Female, N (%)		27 (60)	26 (43)	11 (58)			
MDMA plasma concentration C_{max}, ng/ml	N: 45, 60, 19	232±50	222±44	223±57	0.61	NS	
MDMA plasma concentration AUC_6, ng*h/ml	N: 45, 60, 19	1000±222	933±187	913±224	1.80	NS	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 45, 60, 19	74±24	77±24	77±22	2.17	NS	NS
Good drug effect	N: 45, 60, 19	70±27	77±26	83±29	3.67	0.028	NS
Bad drug effect	N: 45, 60, 19	25±25	13±27	13±17	2.46	NS	NS
Drug liking	N: 45, 60, 19	72±27	79±25	83±31	2.79	NS	NS
Closeness to others	N: 45, 60, 19	19±20	24±17	26±18	3.07	NS	NS
High-mood	N: 45, 60, 19	68±32	72±32	81±26	2.47	NS	NS
Talkative	N: 45, 60, 19	22±18	21±19	23±19	0.24	NS	NS
Appetite	N: 20, 41, 11	-8±31	-7±32	-8±30	0.10	NS	NS
Tired	N: 39, 54, 16	26±31	19±33	8±31	1.27	NS	NS
Fear	N: 20, 41, 11	11±17	6±19	2±6	1.00	NS	NS
Happy	N: 33, 36, 12	26±21	29±17	32±20	1.17	NS	NS
Content	N: 33, 36, 12	28±19	31±17	33±20	0.84	NS	NS
Trust	N: 25, 19, 8	23±23	24±21	24±21	0.35	NS	NS
want to be hugged	N: 25, 19, 8	17±21	18±20	18±19	0.26	NS	NS
want to hug	N: 25, 19, 8	18±20	17±19	20±18	0.15	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 45, 60, 19	23±13	25±13	24±11	0.97	NS	NS
Diastolic blood pressure, mmHg	N: 45, 60, 19	15±11	13±9	15±9	0.44	NS	NS
Mean arterial pressure, mmHg	N: 45, 60, 19	18±11	18±9	18±8	0.21	NS	NS
Rate pressure product, mmHg/min	N: 45, 60, 19	4525±2971	4799±3031	5031±2764	0.63	NS	NS
Body temperature, °C	N: 45, 60, 19	0.1±0.5	0.3±0.6	0.3±0.4	1.11	NS	NS
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
high mood	N: 45, 60, 19	3.2±3.1	2.5±3.3	4.2±3.1	2.23	NS	NS
fear/depression	N: 45, 60, 19	1.6±3.0	0.1±3.4	0.6±2.7	3.07	NS	NS
dreaminess	N: 45, 60, 19	3.0±3.2	3.1±3.2	3.1±3.2	0.10	NS	NS
List of Complaints Δscore							
acute, up to 6h, N	N: 45, 60, 19	9.7±7.1	7.4±6.8	9.4±5.3	1.28	NS	NS
subacute, up to 24h, N	N: 45, 60, 19	5.3±5.9	4.8±5.2	2.9±4.8	0.97	NS	NS

	SLC6A4 rs25531	**LALA**	**LALG+LAS**	**LGLG+LGS+SS**	**F**	**p value**	**p value[a]**
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	
MDMA plasma concentration AUC_6, ng*h/ml	N: 42, 56, 25	998±225	931±189	945±217	1.32	NS	
Visual Analog Scale rating ΔE_{max}							
Any drug effect	N: 42, 56, 25	74±26	76±24	82±21	2.52	NS	NS
Good drug effect	N: 42, 56, 25	70±28	76±25	85±26	3.69	0.028	NS
Bad drug effect	N: 42, 56, 25	24±24	13±28	17±21	1.32	NS	NS
Drug liking	N: 42, 56, 25	72±28	78±24	84±29	2.69	NS	NS
Closeness to others	N: 42, 56, 25	20±20	22±17	28±17	2.74	NS	NS
High-mood	N: 42, 56, 25	67±34	71±31	84±24	3.44	0.035	NS
Talkative	N: 42, 56, 25	23±19	20±19	24±18	0.30	NS	NS
Appetite	N: 20, 35, 17	-7±30	-6±31	-9±32	0.13	NS	NS
Tired	N: 37, 49, 22	26±31	19±33	12±33	0.97	NS	NS
Fear	N: 20, 35, 17	10±17	5±20	6±11	0.56	NS	NS
Happy	N: 30, 38, 12	26±21	30±17	32±20	1.13	NS	NS
Content	N: 30, 38, 12	28±19	32±17	33±20	0.97	NS	NS
Trust	N: 22, 21, 8	22±23	26±21	24±21	0.43	NS	NS
want to be hugged	N: 22, 21, 8	16±20	18±21	18±19	0.18	NS	NS
want to hug	N: 22, 21, 8	17±19	18±20	20±18	0.13	NS	NS
Vital signs parameters ΔE_{max}							
Systolic blood pressure, mmHg	N: 42, 56, 25	22±13	25±13	26±11	1.81	NS	NS
Diastolic blood pressure, mmHg	N: 42, 56, 25	15±11	13±9	16±8	1.03	NS	NS
Mean arterial pressure, mmHg	N: 42, 56, 25	17±11	17±9	20±8	0.84	NS	NS
Rate pressure product, mmHg/min	N: 42, 56, 25	4503±2954	4539±2959	5622±2880	1.66	NS	NS

Body temperature, °C	N: 42, 56, 25	0.2±0.5	0.2±0.6	0.4±0.4	0.89	NS	NS
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
high mood	N: 42, 56, 25	3.0±3.1	2.5±3.4	3.8±3.0	1.25	NS	NS
fear/depression	N: 42, 56, 25	1.6±2.8	0.3±3.3	0.3±3.5	2.33	NS	NS
dreaminess	N: 42, 56, 25	3.1±3.2	3.0±3.1	3.3±3.2	0.08	NS	NS
List of Complaints Δscore							
acute, up to 6h, N	N: 42, 56, 25	9.2±7.2	7.9±6.7	9.1±6.2	0.28	NS	NS
subacute, up to 24h, N	N: 42, 56, 25	5.2±5.9	4.7±5.2	4.0±5.3	0.22	NS	NS

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

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 (Fig 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.

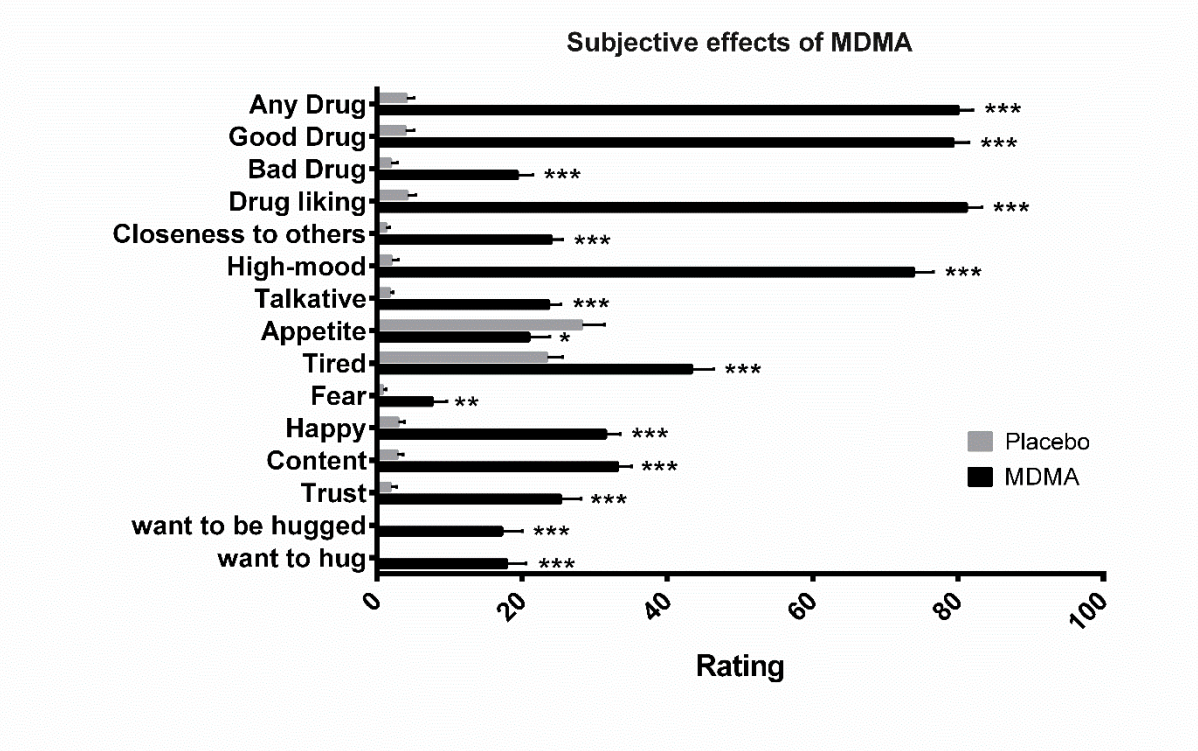

Figure 1. Maximal effects of MDMA on subjective Visual Analog Scale ratings. MDMA significantly altered E_{max} values for all of the reported parameters. With the exception of a decrease in "appetite," all of the parameters were increased by MDMA. The data are expressed as mean ± SEM. *$p < 0.05$, **$p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

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 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.

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 Pardo-Lozano et al. (2012).

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. (2018b) and Pardo-Lozano et al. (2012), we used 125 mg 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, 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. Therefore, interactions between MDMA and 5-HT system genotypes may not be an important factor to consider when MDMA is used therapeutically or recreationally.

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 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 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 dataset 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-two 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, USA). 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 (non-weighted 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 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'-GGTTGCAGGGGAGATCCT (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'-GGACCGCAAGGTGGGCGGGAGGCTTGGAG 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.

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 0-6 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 × 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 0-6 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, and the AUC and AUEC 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 for SNPs. Recessive or dominant model analysis was also performed, the results of which are reported only when the additive model was significant.

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Contributors

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

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Supplementary Tables

Supplementary Table S1. Effects of polymorphisms in the serotonergic system on the response to 125mg MDMA (mean±SD and statistics) corrected with MDMA AUC_0

Visual Analog Scale rating ΔAUC_0	TPH1 rs1800532	GG	GT	TT	F	p value	p value*	TPH2 rs7305115	AA	AG	GG	F	p value	p value*	SHTR1A rs6295	CC	CG	GG	F	p value	p value*	SHTR1B rs6296	CC	CG	GG	F	p value	p value*	SHTR2A rs6313	AA	AG	GG	F	p value	p value*	SLC6A4 5-HTTLPR	LL
Any drug effect	N: 44, 62, 18	203±91	189±99	197±84	0.59	NS	NS	N: 14, 61, 49	205±75	192±99	197±92	0.09	NS	NS	N: 29, 68, 27	193±81	208±99	165±87	0.54	NS	NS	N: 69, 45, 10	190±92	199±98	214±88	0.07	NS	NS	N: 22, 62, 40	184±90	214±95	173±89	1.60	NS	NS	N: 45, 60, 19	210±107
Good drug effect	N: 44, 62, 18	240±118	218±119	207±106	1.17	NS	NS	N: 14, 61, 49	218±107	230±128	221±106	0.20	NS	NS	N: 29, 68, 27	217±106	237±124	202±107	0.10	NS	NS	N: 69, 45, 10	218±119	232±119	232±95	0.10	NS	NS	N: 22, 62, 40	219±119	248±122	191±117	2.32	NS	NS	N: 45, 60, 19	223±124
Bad drug effect	N: 44, 62, 18	15±46	23±46	16±28	0.65	NS	NS	N: 14, 61, 49	21±31	16±54	22±31	0.22	NS	NS	N: 29, 68, 27	27±38	23±41	3±53	2.18	NS	NS	N: 69, 45, 10	19±45	18±38	30±50	0.18	NS	NS	N: 22, 62, 40	17±46	19±41	20±48	0.07	NS	NS	N: 45, 60, 19	33±60
Drug liking	N: 44, 62, 18	257±118	231±119	232±132	0.99	NS	NS	N: 14, 61, 49	228±116	249±131	234±108	0.46	NS	NS	N: 29, 68, 27	230±111	248±125	232±119	0.06	NS	NS	N: 69, 45, 10	234±120	253±120	226±100	0.63	NS	NS	N: 22, 62, 40	232±121	266±131	205±125	2.65	NS	NS	N: 45, 60, 19	239±130
Closeness to others	N: 44, 62, 18	38±59	33±59	63±68	1.43	NS	NS	N: 14, 61, 49	18±65	16±61	48±58	1.53	NS	NS	N: 29, 68, 27	16±61	39±68	41±59	0.52	NS	NS	N: 69, 45, 10	40±63	41±57	22±61	1.20	NS	NS	N: 22, 62, 40	33±58	47±62	29±60	0.64	NS	NS	N: 45, 60, 19	40±69
High-mood	N: 44, 62, 18	180±123	170±107	168±117	0.29	NS	NS	N: 14, 61, 49	167±103	170±123	178±105	0.07	NS	NS	N: 29, 68, 27	161±100	186±124	153±96	0.09	NS	NS	N: 69, 45, 10	173±117	176±110	159±113	0.64	NS	NS	N: 22, 62, 40	161±121	192±107	151±116	1.07	NS	NS	N: 45, 60, 19	186±126
Talkative	N: 44, 62, 18	29±70	7±71	45±100	2.11	NS	NS	N: 14, 61, 49	3±87	17±81	29±67	0.72	NS	NS	N: 29, 68, 27	3±76	24±81	31±63	1.02	NS	NS	N: 69, 45, 10	21±78	24±65	-11±105	1.18	NS	NS	N: 22, 62, 40	25±73	19±79	20±74	0.12	NS	NS	N: 45, 60, 19	27±87
Appetite	N: 24, 42, 6	-44±94	-10±75	-33±59	1.21	NS	NS	N: 7, 33, 12	6±66	-28±91	-25±74	0.47	NS	NS	N: 18, 38, 16	-31±80	-11±79	-43±88	1.64	NS	NS	N: 40, 27, 5	-8±54	-43±109	-40±75	1.25	NS	NS	N: 12, 42, 18	-23±54	-30±81	-7±97	0.59	NS	NS	N: 20, 41, 11	-15±82
Tired	N: 41, 53, 15	29±100	35±85	20±69	0.36	NS	NS	N: 12, 54, 43	39±77	28±90	31±92	0.11	NS	NS	N: 26, 58, 25	41±90	41±85	-5±91	2.15	NS	NS	N: 63, 39, 7	36±81	28±103	0±73	1.12	NS	NS	N: 20, 54, 35	32±55	34±94	26±97	0.10	NS	NS	N: 39, 54, 16	36±90
Fear	N: 24, 42, 6	3±12	10±33	1±2	0.61	NS	NS	N: 7, 33, 12	0±4	9±37	7±13	0.28	NS	NS	N: 18, 38, 16	3±5	11±36	1±3	1.25	NS	NS	N: 40, 27, 5	7±34	7±14	1±8	0.12	NS	NS	N: 12, 42, 18	-1±3	4±9	18±50	2.46	NS	NS	N: 20, 41, 11	9±16
Happy	N: 26, 40, 15	61±62	56±86	77±57	0.33	NS	NS	N: 12, 41, 28	48±68	62±79	66±62	0.26	NS	NS	N: 16, 48, 17	62±85	59±80	68±42	0.48	NS	NS	N: 44, 30, 7	56±80	62±68	91±61	0.30	NS	NS	N: 17, 34, 30	68±71	74±64	44±85	1.10	NS	NS	N: 33, 36, 12	58±84
Content	N: 26, 40, 15	70±60	67±90	73±48	0.14	NS	NS	N: 12, 41, 28	57±98	71±76	72±61	0.22	NS	NS	N: 16, 48, 17	73±90	65±78	76±43	0.75	NS	NS	N: 44, 30, 7	63±80	69±66	107±62	0.51	NS	NS	N: 17, 34, 30	73±75	85±64	49±82	1.56	NS	NS	N: 33, 36, 12	67±83
Trust	N: 20, 20, 12	50±59	54±85	68±71	0.04	NS	NS	N: 7, 28, 17	18±95	56±68	71±65	1.58	NS	NS	N: 11, 30, 11	40±94	55±64	74±68	1.36	NS	NS	N: 29, 18, 5	46±78	56±66	109±28	0.64	NS	NS	N: 10, 20, 22	79±60	82±70**	22±66	4.21	0.021	NS	N: 25, 19, 8	45±81
want to be hugged	N: 20, 20, 12	-4±66	41±70	68±88	2.97	NS	NS	N: 7, 28, 17	-1±39	25±87	52±69	1.08	NS	NS	N: 11, 30, 11	37±64	36±82	8±77	0.30	NS	NS	N: 29, 18, 5	29±82	34±78	21±57	0.71	NS	NS	N: 10, 20, 22	15±94	44±73	24±74	0.42	NS	NS	N: 25, 19, 8	35±94
want to hug	N: 20, 20, 12	2±62	39±68	54±65	2.07	NS	NS	N: 7, 28, 17	20±71	21±66	44±69	0.36	NS	NS	N: 11, 30, 11	38±61	32±69	8±69	0.42	NS	NS	N: 29, 18, 5	31±73	28±64	16±51	0.74	NS	NS	N: 10, 20, 22	3±70	39±68	31±66	1.03	NS	NS	N: 25, 19, 8	31±79
Vital signs parameters ΔAUC_0																																					
Systolic blood pressure, mmHg	N: 44, 62, 18	98±42	91±41	115±44	1.65	NS	NS	N: 14, 61, 49	101±47	100±46	95±36	0.25	NS	NS	N: 29, 68, 27	89±41	102±43	102±38	0.68	NS	NS	N: 69, 45, 10	97±42	102±44	98±36	0.09	NS	NS	N: 22, 62, 40	92±43	102±46	95±34	0.36	NS	NS	N: 45, 60, 19	98±46
Diastolic blood pressure, mmHg	N: 44, 62, 18	58±38	65±29	69±26	0.73	NS	NS	N: 14, 61, 49	69±38	62±34	63±28	0.25	NS	NS	N: 29, 68, 27	63±29	63±32	62±38	0.26	NS	NS	N: 69, 45, 10	68±31	54±34	70±28	3.46	0.034	NS	N: 22, 62, 40	54±34	67±35	62±26	0.76	NS	NS	N: 45, 60, 19	63±31
Mean arterial pressure, mmHg	N: 44, 62, 18	71±36	74±30	84±26	0.74	NS	NS	N: 14, 61, 49	79±34	75±34	74±28	0.21	NS	NS	N: 29, 68, 27	72±31	76±31	75±35	0.22	NS	NS	N: 69, 45, 10	78±31	69±33	80±27	1.57	NS	NS	N: 22, 62, 40	67±31	79±36	73±25	0.56	NS	NS	N: 45, 60, 19	75±32
Rate-pressure product, mmHg/min	N: 44, 62, 18	18572±8375	16677±9444	19299±8468	0.82	NS	NS	N: 14, 61, 49	18061±9152	17823±8399	17520±9676	0.04	NS	NS	N: 29, 68, 27	17749±8487	17854±9543	17399±8078	0.08	NS	NS	N: 69, 45, 10	17342±8481	18943±9287	14948±10432	1.17	NS	NS	N: 22, 62, 40	17977±9134	16643±8299	19280±9734	1.59	NS	NS	N: 45, 60, 19	17614±10248
Body temperature, °C	N: 44, 62, 18	1.3±2.9	0.9±2.6	1.5±3.4	0.53	NS	NS	N: 14, 61, 49	-0.4±3.3	1.7±2.9*	0.9±2.4	3.53	0.032	NS	N: 29, 68, 27	0.7±2.9	1.2±3.0	1.3±2.3	0.39	NS	NS	N: 69, 45, 10	0.8±2.9	1.6±2.7	1.0±2.9	1.16	NS	NS	N: 22, 62, 40	1.8±2.9	1.1±2.7	0.7±3.0	0.92	NS	NS	N: 45, 60, 19	0.8±2.6
Adjective Mood Rating Scale rating ΔAUC_0																																					
well-being	N: 44, 62, 18	14.9±23.1	11.4±22.8	9.5±30.1	0.39	NS	NS	N: 14, 61, 49	11.2±20.7	11.3±27.4	14.0±20.6	0.20	NS	NS	N: 29, 68, 27	12.5±24.2	11.1±20.6	15.2±31.6	0.23	NS	NS	N: 69, 45, 10	12.6±24.3	9.7±25.1	22.0±17.1	1.33	NS	NS	N: 22, 62, 40	14.6±17.5	17.1±21.7*	3.7±28.4	4.23	0.017	NS	N: 45, 60, 19	12.0±19.7
high mood	N: 44, 62, 18	9.4±13.5	6.5±12.9	6.7±16.0	0.62	NS	NS	N: 14, 61, 49	7.5±11.3	7.3±15.2	8.0±12.2	0.04	NS	NS	N: 29, 68, 27	6.7±11.3	6.7±11.8	10.7±17.5	0.97	NS	NS	N: 69, 45, 10	7.6±14.1	6.4±13.2	12.5±10.9	0.81	NS	NS	N: 22, 62, 40	8.0±10.9	10.7±12.5**	2.6±15.2	4.56	0.012	NS	N: 45, 60, 19	8.1±11.6
fear/depression	N: 44, 62, 18	-3.8±12.6	1.0±10.5	0.6±6.5	2.71	NS	NS	N: 14, 61, 49	-2.4±11.7	-0.4±12.3	-0.8±9.0	0.18	NS	NS	N: 29, 68, 27	-2.8±11.2	0.8±11.1	-2.6±10.3	2.08	NS	NS	N: 69, 45, 10	0.7±9.3	-2.5±13.4	-3.3±8.6	1.32	NS	NS	N: 22, 62, 40	1.2±8.2	-2.9±11.1	1.5±11.7	2.35	NS	NS	N: 45, 60, 19	1.9±9.4
dreaminess	N: 44, 62, 18	9.2±9.2	8.6±10.7	6.3±12.4	0.64	NS	NS	N: 14, 61, 49	8.1±11.0	8.0±10.4	9.2±10.5	0.16	NS	NS	N: 29, 68, 27	8.8±11.2	9.3±10.7	6.0±8.8	0.62	NS	NS	N: 69, 45, 10	7.9±10.4	8.7±10.9	11.1±8.5	0.21	NS	NS	N: 22, 62, 40	8.7±12.4	10.5±9.5*	5.1±10.1	3.13	0.047	NS	N: 45, 60, 19	8.5±11.7

N, number of subjects; AUEC, area under the effect-time curve; SD, standard deviation; NS, not significant; Δ, values are change scores from placebo; *p value additionally corrected for multiple comparisons according to the Nyholt correction; * p < 0.05 compared to AA; ** p < 0.05, *** p < 0.01 compared to GG; # p < 0.05 compared to SS; ‡ p < 0.05 compared to LgSa/LgSa.

Supplementary Table S2. Effects of polymorphisms in the serotonergic system on the response to 125mg MDMA (mean±SD and statistics)

Visual Analog Scale rating ΔE_{max}	TPH1 rs1800532	GG	GT	TT	F	p value	p value*	TPH2 rs7305115	AA	AG	GG	F	p value	p value*	SHTR1A rs6295	CC	CG	GG	F	p value	p value*	SHTR1B rs6296	CC	CG	GG	F	p value	p value*	SHTR2A rs6313	AA	AG	GG	F	p value	p value*	SLC6A4 5-HTTLPR	LL
Any drug effect	N: 44, 62, 18	81±19	73±26	74±26	1.30	NS	NS	N: 14, 61, 49	77±20	75±25	77±24	0.18	NS	NS	N: 29, 68, 27	70±20	79±23	66±27	2.92	NS	NS	N: 69, 45, 10	75±22	77±24	77±32	0.05	NS	NS	N: 22, 62, 40	69±24	80±23	73±24	1.92	NS	NS	N: 45, 60, 19	74±24
Good drug effect	N: 44, 62, 18	81±23	72±29	72±28	1.52	NS	NS	N: 14, 61, 49	71±31	74±28	79±24	0.63	NS	NS	N: 29, 68, 27	77±23	77±28	71±29	0.56	NS	NS	N: 69, 45, 10	74±28	79±25	71±28	0.62	NS	NS	N: 22, 62, 40	76±23	81±26*	66±29	4.00	0.021	NS	N: 45, 60, 19	70±27
Bad drug effect	N: 44, 62, 18	14±23	21±29	14±19	1.22	NS	NS	N: 14, 61, 49	17±21	15±29	20±22	0.39	NS	NS	N: 29, 68, 27	25±24	17±26	10±24	2.49	NS	NS	N: 69, 45, 10	18±24	16±29	19±25	0.11	NS	NS	N: 22, 62, 40	13±20	17±27	22±25	0.95	NS	NS	N: 45, 60, 19	25±25
Drug liking	N: 44, 62, 18	81±21	74±29	70±29	0.86	NS	NS	N: 14, 61, 49	69±35	76±27	81±23	1.28	NS	NS	N: 29, 68, 27	78±23	78±28	73±28	0.36	NS	NS	N: 69, 45, 10	77±27	79±25	70±30	0.49	NS	NS	N: 22, 62, 40	77±20	82±27	69±27	2.71	NS	NS	N: 45, 60, 19	72±27
Closeness to others	N: 44, 62, 18	23±17	21±19	27±19	0.62	NS	NS	N: 14, 61, 49	19±20	22±17	25±19	0.85	NS	NS	N: 29, 68, 27	21±20	24±18	21±16	0.31	NS	NS	N: 69, 45, 10	22±18	23±19	28±18	0.52	NS	NS	N: 22, 62, 40	20±17	26±18	19±19	2.01	NS	NS	N: 45, 60, 19	19±20
High-mood	N: 44, 62, 18	74±32	71±31	71±31	0.21	NS	NS	N: 14, 61, 49	72±29	69±34	75±28	0.57	NS	NS	N: 29, 68, 27	70±31	73±33	71±29	0.13	NS	NS	N: 69, 45, 10	70±31	76±31	66±39	0.59	NS	NS	N: 22, 62, 40	67±31	79±29	64±33	3.46	0.035	NS	N: 45, 60, 19	68±32
Talkative	N: 44, 62, 18	25±20	19±19	25±17	1.42	NS	NS	N: 14, 61, 49	18±23	21±19	24±17	0.79	NS	NS	N: 29, 68, 27	18±18	24±19	21±18	1.07	NS	NS	N: 69, 45, 10	21±19	22±20	24±16	0.09	NS	NS	N: 22, 62, 40	22±18	24±19	19±19	0.57	NS	NS	N: 45, 60, 19	22±18
Appetite	N: 24, 42, 6	-7±33	-6±31	-15±22	0.22	NS	NS	N: 7, 33, 12	-3±32	-9±28	-6±34	0.14	NS	NS	N: 18, 38, 16	-13±37	-3±28	-10±30	0.78	NS	NS	N: 40, 27, 5	-4±31	-14±32	-2±22	0.95	NS	NS	N: 12, 42, 18	-6±29	-8±29	-6±37	0.06	NS	NS	N: 20, 41, 11	-8±31
Tired	N: 41, 53, 15	21±32	18±33	23±31	0.19	NS	NS	N: 12, 54, 43	13±34	20±31	22±33	0.34	NS	NS	N: 26, 58, 25	23±34	22±31	11±33	1.33	NS	NS	N: 63, 39, 7	22±34	19±31	4±21	1.10	NS	NS	N: 20, 54, 35	22±27	20±34	18±33	0.11	NS	NS	N: 39, 54, 16	26±31
Fear	N: 24, 42, 6	4±14	9±20	4±9	0.87	NS	NS	N: 7, 33, 12	-1±12	6±19	9±17	0.86	NS	NS	N: 18, 38, 16	6±9	9±23	3±8	0.82	NS	NS	N: 40, 27, 5	7±19	7±16	0±17	0.43	NS	NS	N: 12, 42, 18	0±4	6±13	14±27	2.73	NS	NS	N: 20, 41, 11	11±17
Happy	N: 26, 40, 15	28±18	27±20	33±18	0.45	NS	NS	N: 12, 41, 28	27±21	28±18	29±20	0.05	NS	NS	N: 16, 48, 17	25±20	28±20	33±16	0.67	NS	NS	N: 44, 30, 7	28±19	28±20	37±17	0.76	NS	NS	N: 17, 34, 30	29±17	32±18	24±21	1.49	NS	NS	N: 33, 36, 12	26±21
Content	N: 26, 40, 15	32±14	29±21	32±18	0.36	NS	NS	N: 12, 41, 28	27±22	30±17	32±19	0.29	NS	NS	N: 16, 48, 17	28±19	30±19	33±18	0.27	NS	NS	N: 44, 30, 7	31±18	28±19	37±17	0.71	NS	NS	N: 17, 34, 30	32±17	32±19	27±19	0.61	NS	NS	N: 33, 36, 12	28±19
Trust	N: 20, 20, 12	21±18	21±23	25±26	0.02	NS	NS	N: 7, 28, 17	14±30	23±20	28±21	1.06	NS	NS	N: 11, 30, 11	20±24	23±21	27±24	0.29	NS	NS	N: 29, 18, 5	20±22	23±22	45±7	2.91	NS	NS	N: 10, 20, 22	35±17	28±20	14±23	4.35	0.022	NS	N: 25, 19, 8	23±23
want to be hugged	N: 20, 20, 12	11±19	19±20	24±20	1.64	NS	NS	N: 7, 28, 17	7±8	14±20	26±20	2.85	NS	NS	N: 11, 30, 11	14±19	18±21	19±19	0.17	NS	NS	N: 29, 18, 5	14±19	19±21	26±22	0.84	NS	NS	N: 10, 20, 22	17±19	23±20	12±20	1.65	NS	NS	N: 25, 19, 8	17±21
want to hug	N: 20, 20, 12	14±19	19±19	23±19	0.99	NS	NS	N: 7, 28, 17	7±8	16±19	26±20	3.06	NS	NS	N: 11, 30, 11	14±19	18±20	22±16	0.42	NS	NS	N: 29, 18, 5	14±18	21±19	27±21	1.38	NS	NS	N: 10, 20, 22	20±17	23±19	12±20	1.71	NS	NS	N: 25, 19, 8	18±20
Vital signs parameters ΔE_{max}																																					
Systolic blood pressure, mmHg	N: 44, 62, 18	26±12	22±13	27±12	1.75	NS	NS	N: 14, 61, 49	22±13	25±14	23±11	0.73	NS	NS	N: 29, 68, 27	21±15	25±12	23±12	1.01	NS	NS	N: 69, 45, 10	23±13	27±12	22±10	1.41	NS	NS	N: 22, 62, 40	21±10	25±14	24±12	0.95	NS	NS	N: 45, 60, 19	23±13
Diastolic blood pressure, mmHg	N: 44, 62, 18	14±11	14±9	15±9	0.10	NS	NS	N: 14, 61, 49	14±9	15±10	14±8	0.19	NS	NS	N: 29, 68, 27	16±9	14±10	13±9	0.59	NS	NS	N: 69, 45, 10	14±10	13±9	16±11	0.39	NS	NS	N: 22, 62, 40	14±14	14±9	14±7	0.01	NS	NS	N: 45, 60, 19	15±11
Mean arterial pressure, mmHg	N: 44, 62, 18	18±10	17±10	19±9	0.25	NS	NS	N: 14, 61, 49	16±10	18±10	17±9	0.38	NS	NS	N: 29, 68, 27	18±10	18±10	16±9	0.37	NS	NS	N: 69, 45, 10	18±10	18±10	17±10	0.03	NS	NS	N: 22, 62, 40	17±12	18±10	18±8	0.21	NS	NS	N: 45, 60, 19	18±11
Rate-pressure product, mmHg/min	N: 44, 62, 18	5311±1048	4314±2906	4779±2783	1.48	NS	NS	N: 14, 61, 49	3757±2787	4786±2838	4952±3135	0.91	NS	NS	N: 29, 68, 27	4916±1052	4707±3032	4612±2729	0.08	NS	NS	N: 69, 45, 10	4511±2801	5308±3130	3702±2956	1.67	NS	NS	N: 22, 62, 40	4522±2610	4439±2772	5311±3360	1.13	NS	NS	N: 45, 60, 19	4525±2971
Body temperature, °C	N: 44, 62, 18	0.3±0.5	0.2±0.5	0.4±0.5	1.24	NS	NS	N: 14, 61, 49	-0.1±0.6	0.3±0.5*	0.2±0.4	4.17	0.018	NS	N: 29, 68, 27	0.2±0.6	0.2±0.5	0.3±0.4	0.58	NS	NS	N: 69, 45, 10	0.2±0.5	0.3±0.5	0.2±0.7	0.72	NS	NS	N: 22, 62, 40	0.3±0.5	0.2±0.6	0.2±0.5	0.24	NS	NS	N: 45, 60, 19	0.3±0.5
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	0.37	NS	NS	N: 14, 61, 49	3.4±4.3	5.3±6.0	5.5±4.9	0.92	NS	NS	N: 29, 68, 27	4.9±5.3	5.4±4.9	5.1±6.7	0.10	NS	NS	N: 69, 45, 10	5.2±5.1	4.7±5.9	7.7±4.9	1.30	NS	NS	N: 22, 62, 40	5.1±4.1	6.3±5.1*	3.5±6.1	3.27	0.041	NS	N: 45, 60, 19	5.4±5.0
high mood	N: 44, 62, 18	3.5±5.1	2.7±3.1	2.9±3.7	0.75	NS	NS	N: 14, 61, 49	2.4±3.1	3.1±3.5	3.0±3.0	0.33	NS	NS	N: 29, 68, 27	2.6±3.2	3.0±3.0	3.4±3.9	0.41	NS	NS	N: 69, 45, 10	2.9±3.1	2.7±3.3	4.7±3.2	1.61	NS	NS	N: 22, 62, 40	2.8±2.7	3.7±3.0*	2.0±3.6	3.82	0.025	NS	N: 45, 60, 19	3.2±3.1
fear/depression	N: 44, 62, 18	0.0±3.4	1.3±3.2	0.6±2.0	2.42	NS	NS	N: 14, 61, 49	-0.5±3.3	0.8±3.6	1.0±2.5	1.20	NS	NS	N: 29, 68, 27	0.2±3.2	1.3±3.3	0.0±2.7	2.15	NS	NS	N: 69, 45, 10	1.0±3.0	0.5±3.7	-0.2±1.9	0.80	NS	NS	N: 22, 62, 40	0.9±2.9	0.5±3.1	1.1±3.6	0.52	NS	NS	N: 45, 60, 19	1.6±3.0
dreaminess	N: 44, 62, 18	3.3±2.6	3.1±3.5	2.5±3.2	0.43	NS	NS	N: 14, 61, 49	2.9±3.6	2.8±2.9	3.4±3.3	0.56	NS	NS	N: 29, 68, 27	3.2±3.3	3.4±3.2	2.1±2.7	1.83	NS	NS	N: 69, 45, 10	3.0±3.2	3.0±3.2	4.1±2.8	0.57	NS	NS	N: 22, 62, 40	2.9±3.8	3.9±3.0**	2.0±2.7	4.55	0.012	NS	N: 45, 60, 19	3.0±3.2
List of Complaints Ascore																																					
acute, up to 6h, N	N: 44, 62, 18	8.5±7.4	8.6±6.8	8.5±4.8	0.01	NS	NS	N: 14, 61, 49	7.4±7.1	8.4±6.7	9.0±6.8	0.31	NS	NS	N: 29, 68, 27	7.7±7.0	9.1±6.4	8.0±7.3	0.51	NS	NS	N: 69, 45, 10	8.2±6.8	9.4±6.5	7.0±7.5	0.71	NS	NS	N: 22, 62, 40	7.3±5.8	8.2±6.6	9.8±7.4	1.15	NS	NS	N: 45, 60, 19	9.7±7.1
subacute, up to 24h, N	N: 44, 62, 18	5.1±5.6	4.7±5.3	3.7±5.4	0.43	NS	NS	N: 14, 61, 49	2.9±4.2	4.7±5.9	5.2±5.0	0.94	NS	NS	N: 29, 68, 27	5.7±5.0	4.5±5.8	4.1±4.8	0.83	NS	NS	N: 69, 45, 10	5.0±5.5	4.6±5.3	3.2±5.9	0.47	NS	NS	N: 22, 62, 40	3.9±5.4	4.6±5.0	5.3±6.1	0.52	NS	NS	N: 45, 60, 19	5.3±5.9

N, number of subjects; SD, standard deviation; NS, not significant; Δ, values are change scores from placebo; *p value additionally corrected for multiple comparisons according to the Nyholt correction; * p < 0.05 compared to AA; ** p < 0.05, *** p < 0.01 compared to GG; # p < 0.05 compared to SS.

Supplementary Table S3. Effects of polymorphisms in the serotonergic system on the response to 125mg MDMA (mean±SD and statistics)

Visual Analog Scale rating ΔAUEC$_0$	TPH1 rs1800532	GG	GT	TT	F	p value	p value^a	TPH2 rs7305115	AA	AG	GG	F	p value	p value^a	SHTR1A rs6295	CC	CG	GG	F	p value	p value^a	SHTR1B rs6296	CC	CG	GG	F	p value	p value^a	SHTR2A rs6313	AA	AG	GG	F	p value	p value^a	SLC6A4 5-HTTLPR	LL
Any drug effect	N: 44, 62, 18	203±93	189±99	197±84	0.28	NS	NS	N: 14, 61, 49	205±75	192±96	197±92	0.13	NS	NS	N: 29, 68, 27	193±81	208±99	185±87	2.14	NS	NS	N: 69, 45, 10	190±92	199±96	214±88	0.34	NS	NS	N: 22, 62, 40	184±90	214±95	173±89	2.54	NS	NS	N: 45, 60, 19	210±107
Good drug effect	N: 44, 62, 18	240±118	218±119	207±106	0.69	NS	NS	N: 14, 61, 49	218±107	230±128	221±106	0.11	NS	NS	N: 29, 68, 27	217±106	237±124	202±107	0.99	NS	NS	N: 69, 45, 10	218±119	232±119	232±95	0.22	NS	NS	N: 22, 62, 40	219±119	248±112	191±117	2.99	NS	NS	N: 45, 60, 19	223±124
Bad drug effect	N: 44, 62, 18	15±46	23±46	16±28	0.56	NS	NS	N: 14, 61, 49	21±31	16±54	22±31	0.27	NS	NS	N: 29, 68, 27	27±38	23±41	3±53	2.69	NS	NS	N: 69, 45, 10	19±45	18±38	30±59	0.33	NS	NS	N: 22, 62, 40	17±46	19±41	20±48	0.03	NS	NS	N: 45, 60, 19	33±60
Drug liking	N: 44, 62, 18	257±118	231±119	232±132	0.67	NS	NS	N: 14, 61, 49	228±116	249±131	234±108	0.30	NS	NS	N: 29, 68, 27	230±111	248±125	232±119	0.31	NS	NS	N: 69, 45, 10	234±123	253±120	226±100	0.43	NS	NS	N: 22, 62, 40	232±121	266±111	205±125	3.44	0.035	NS	N: 45, 60, 19	239±130
Closeness to others	N: 44, 62, 18	38±59	33±59	63±68	1.80	NS	NS	N: 14, 61, 49	18±65	36±61	48±58	1.48	NS	NS	N: 29, 68, 27	36±61	39±68	41±59	0.06	NS	NS	N: 69, 45, 10	40±63	41±57	22±61	0.44	NS	NS	N: 22, 62, 40	33±58	47±62	29±60	1.17	NS	NS	N: 45, 60, 19	40±69
High-mood	N: 44, 62, 18	180±123	170±107	168±117	0.11	NS	NS	N: 14, 61, 49	167±103	170±123	178±105	0.09	NS	NS	N: 29, 68, 27	161±100	186±124	153±96	1.05	NS	NS	N: 69, 45, 10	173±117	176±110	159±113	0.10	NS	NS	N: 22, 62, 40	161±121	192±107	151±116	1.80	NS	NS	N: 45, 60, 19	186±126
Talkative	N: 44, 62, 18	29±70	7±71	45±100	2.23	NS	NS	N: 14, 61, 49	3±87	17±81	29±67	0.74	NS	NS	N: 29, 68, 27	9±76	24±81	31±63	1.06	NS	NS	N: 69, 45, 10	23±78	24±65	-11±105	0.91	NS	NS	N: 22, 62, 40	25±73	19±79	20±74	0.04	NS	NS	N: 45, 60, 19	27±87
Appetite	N: 24, 42, 6	-4±94	-10±75	-1±59	1.41	NS	NS	N: 7, 33, 32	6±66	-28±91	-25±74	0.51	NS	NS	N: 18, 38, 16	-11±80	-11±79	-43±88	1.02	NS	NS	N: 40, 27, 5	-8±54	-41±109	-40±75	1.61	NS	NS	N: 12, 42, 18	-23±54	-30±81	-7±97	0.53	NS	NS	N: 20, 41, 11	-25±82
Tired	N: 41, 53, 15	28±100	35±85	20±69	0.19	NS	NS	N: 12, 54, 43	39±77	28±90	31±92	0.07	NS	NS	N: 26, 58, 25	41±90	41±85	-5±91	2.69	NS	NS	N: 63, 39, 7	36±81	28±103	0±73	0.52	NS	NS	N: 20, 54, 35	32±55	34±94	26±97	0.09	NS	NS	N: 39, 54, 16	36±90
Fear	N: 24, 42, 6	3±12	10±33	1±2	0.64	NS	NS	N: 7, 33, 32	0±4	9±37	7±13	0.26	NS	NS	N: 18, 38, 16	3±5	11±36	1±3	1.07	NS	NS	N: 40, 27, 5	7±34	7±14	1±8	0.12	NS	NS	N: 12, 42, 18	-1±3	4±9	18±50	2.34	NS	NS	N: 20, 41, 11	9±16
Happy	N: 26, 40, 15	61±62	56±86	77±57	0.43	NS	NS	N: 12, 41, 28	48±88	62±79	66±62	0.25	NS	NS	N: 16, 48, 17	62±85	59±80	68±42	0.09	NS	NS	N: 44, 30, 7	56±80	62±68	91±61	0.70	NS	NS	N: 17, 34, 30	68±71	74±64	44±85	1.41	NS	NS	N: 33, 36, 12	58±84
Content	N: 26, 40, 15	70±60	67±90	73±48	0.05	NS	NS	N: 12, 41, 28	57±98	71±76	72±61	0.20	NS	NS	N: 16, 48, 17	73±90	65±78	76±43	0.15	NS	NS	N: 44, 30, 7	63±80	69±66	107±62	1.05	NS	NS	N: 17, 34, 30	73±75	85±64	49±82	2.04	NS	NS	N: 33, 36, 12	67±83
Trust	N: 20, 20, 12	50±59	54±85	68±71	0.25	NS	NS	N: 7, 28, 17	18±95	56±68	71±65	1.43	NS	NS	N: 11, 30, 11	40±94	55±64	74±68	0.62	NS	NS	N: 29, 18, 5	46±78	56±66	109±28	1.69	NS	NS	N: 10, 20, 22	78±60	82±70**	22±66	5.02	0.010	NS	N: 25, 19, 8	45±81
want to be hugged	N: 20, 20, 12	-4±66*	41±70	68±88	3.98	0.025	NS	N: 7, 28, 17	-1±39	25±87	52±69	1.34	NS	NS	N: 11, 30, 11	37±64	36±82	8±77	0.56	NS	NS	N: 29, 18, 5	29±82	34±78	21±57	0.05	NS	NS	N: 10, 20, 22	15±94	44±73	24±74	0.57	NS	NS	N: 25, 19, 8	35±94
want to hug	N: 20, 20, 12	2±82	39±68	54±65	2.84	NS	NS	N: 7, 28, 17	20±71	21±66	44±69	0.70	NS	NS	N: 11, 30, 11	38±61	32±69	8±93	0.63	NS	NS	N: 29, 18, 5	31±73	28±64	16±51	0.10	NS	NS	N: 10, 20, 22	3±70	39±68	31±66	0.99	NS	NS	N: 25, 19, 8	31±79
Vital signs parameters ΔAUEC$_0$																																					
Systolic blood pressure, mmHg	N: 44, 62, 18	98±42	91±41	115±44	1.97	NS	NS	N: 14, 61, 49	101±47	100±46	95±36	0.19	NS	NS	N: 29, 68, 27	89±43	101±43	100±38	0.92	NS	NS	N: 69, 45, 10	97±42	100±44	98±36	0.06	NS	NS	N: 22, 62, 40	92±43	102±46	95±34	0.54	NS	NS	N: 45, 60, 19	98±46
Diastolic blood pressure, mmHg	N: 44, 62, 18	58±38	65±29	69±26	0.89	NS	NS	N: 14, 61, 49	69±38	62±34	63±28	0.27	NS	NS	N: 29, 68, 27	63±29	63±32	62±38	0.01	NS	NS	N: 69, 45, 10	68±31	54±34	70±28	2.68	NS	NS	N: 22, 62, 40	54±34	67±35	62±26	1.46	NS	NS	N: 45, 60, 19	63±31
Mean arterial pressure, mmHg	N: 44, 62, 18	71±36	74±30	84±26	1.03	NS	NS	N: 14, 61, 49	79±34	75±34	74±28	0.18	NS	NS	N: 29, 68, 27	72±31	76±31	75±35	0.14	NS	NS	N: 69, 45, 10	78±31	69±33	80±27	1.02	NS	NS	N: 22, 62, 40	67±31	79±36	73±25	1.26	NS	NS	N: 45, 60, 19	75±32
Rate pressure product, mmHg/min	N: 44, 62, 18	18572±8375	16677±9444	19299±8468	0.90	NS	NS	N: 14, 61, 49	18081±9152	17823±8199	17520±9676	0.03	NS	NS	N: 29, 68, 27	17749±8487	17854±9543	17399±8078	0.02	NS	NS	N: 69, 45, 10	17342±8481	18943±9287	14948±10432	0.96	NS	NS	N: 22, 62, 40	17977±9134	16643±8299	19280±9734	1.07	NS	NS	N: 45, 60, 19	17614±10248
Body temperature, °C	N: 44, 62, 18	1.3±2.9	0.9±2.6	1.5±3.4	0.51	NS	NS	N: 14, 61, 49	-0.4±3.3	1.7±2.9*	0.9±2.4	3.59	0.031	NS	N: 29, 68, 27	0.7±2.9	1.2±3.0	1.3±2.3	0.36	NS	NS	N: 69, 45, 10	0.8±2.9	1.6±2.7	1.0±2.9	1.07	NS	NS	N: 22, 62, 40	1.8±2.9	1.1±2.7	0.7±3.0	0.97	NS	NS	N: 45, 60, 19	0.8±2.8
Adjective Mood Rating Scale rating ΔAUEC$_0$																																					
well-being	N: 44, 62, 18	14.9±23.3	11.4±22.8	9.5±30.3	0.41162987	NS	NS	N: 14, 61, 49	11.2±20.7	11.3±27.4	14.0±20.6	0.19299241	NS	NS	N: 29, 68, 27	12.5±24.2	11.1±20.6	15.2±31.6	0.28315767	NS	NS	N: 69, 45, 10	12.6±24.1	9.7±25.1	22.0±17.1	1.07540335	NS	NS	N: 22, 62, 40	14.6±17.5	17.1±21.7**	3.7±28.4	4.11754622	0.019	NS	N: 45, 60, 19	12.0±19.7
high mood	N: 44, 62, 18	9.4±11.5	6.5±12.9	6.7±16.0	0.62143618	NS	NS	N: 14, 61, 49	7.5±11.3	7.3±15.2	8.0±12.2	0.03719787	NS	NS	N: 29, 68, 27	6.7±13.3	6.7±11.8	10.7±17.5	0.93884665	NS	NS	N: 69, 45, 10	7.6±14.1	6.4±13.2	12.5±10.9	0.81509688	NS	NS	N: 22, 62, 40	8.0±10.9	10.7±12.5**	2.6±15.2	4.55269053	0.012	NS	N: 45, 60, 19	8.1±11.6
fear/depression	N: 44, 62, 18	-3.8±12.6	1.0±10.5	0.6±6.5	2.67714633	NS	NS	N: 14, 61, 49	-2.4±11.7	-0.4±12.3	-1.8±9.0	0.18956566	NS	NS	N: 29, 68, 27	-2.8±11.2	0.8±11.1	-2.6±10.3	1.61655088	NS	NS	N: 69, 45, 10	0.7±9.3	-2.5±13.4	-3.3±8.6	1.46398903	NS	NS	N: 22, 62, 40	1.2±8.2	-2.9±11.1	1.5±11.7	2.51683099	NS	NS	N: 45, 60, 19	1.9±9.4
dreaminess	N: 44, 62, 18	9.2±9.2	8.6±10.7	6.3±12.4	0.49383894	NS	NS	N: 14, 61, 49	8.1±11.0	8.0±10.4	9.2±10.5	0.18410361	NS	NS	N: 29, 68, 27	8.8±11.2	9.3±10.7	6.0±8.8	0.9582068	NS	NS	N: 69, 45, 10	7.9±10.4	8.7±10.9	11.1±8.5	0.41381799	NS	NS	N: 22, 62, 40	8.7±12.4	10.5±9.5*	5.1±10.1	3.4603452	0.035	NS	N: 45, 60, 19	8.5±11.7

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; * p < 0.05 compared to TT; * p < 0.05 compared to AA; * p < 0.05, ** p < 0.01 compared to GG; * p < 0.05 compared to SS; * s < 0.05 compared to lgSA/lgSA.