Reversibility of ecstasy-induced reduction in serotonin transporter availability in polydrug ecstasy users

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Abstract. *Purpose:* Animal data suggest that the synthetic drug ecstasy may damage brain serotonin neurons. Previously we reported protracted reductions in the availability of the serotonin transporter (SERT), an index of integrity of the axon terminals of brain serotonergic neurons, in SERT-rich brain regions in current human ecstasy users. Comparison of current ecstasy users and former ecstasy users yielded some evidence that this reduction might be reversible. However, participant selection effects could not be ruled out. Therefore, follow-up examinations were performed in these subjects to test the following a priori hypothesis in a prospective longitudinal design that eliminates participant selection effects to a large extent: availability of the SERT increases towards normal levels when ecstasy use is stopped, and remains unchanged or is further decreased if use is continued.
Methods: Two follow-up positron emission tomography measurements using the SERT ligand [^{11}C](+)McN5652 were completed by 15 current and nine former ecstasy users. All subjects used illicit drugs other than ecstasy, too. The time interval between repeated measurements was about 1 year. The time course of the availability of the SERT was analysed in the following SERT-rich regions: mesencephalon, putamen, caudate and thalamus.
Results: Current ecstasy users showed a consistent increase in the availability of the SERT in the mesencephalon during the study (Friedman test: p=0.010), which most likely was caused by a decrease in the intensity of ecstasy consumption (Spearman correlation coefficient −0.725, p=0.002). Former ecstasy users showed a consistent increase in SERT availability in the thalamus (Friedman test: p=0.006).
Conclusion: Ecstasy-induced protracted alterations in the availability of the SERT might be reversible.

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Introduction

There is increasing evidence that the use of the popular recreational synthetic drug ecstasy causes alterations of the serotonergic system [1]. Functional imaging using positron emission tomography (PET) or single-photon emission computed tomography (SPECT) revealed decreased availability of the presynaptic serotonin transporter (SERT) in different brain regions of ecstasy users [2, 3].

Longitudinal studies in a baboon [4], positive partial correlation of relative uptake of the SPECT SERT ligand [^{123}I]β-CIT and the interval since the last ecstasy intake [3], and comparison of current ecstasy users and former ecstasy users have all suggested that ecstasy-induced reductions in SERT availability may be reversible [5–8]. However, the hypothesis of reversibility has not yet been tested in a longitudinal study of the SERT in humans, which would be less prone to participant selection effects than comparison of different groups. Therefore, the aim of the present study was to analyse the time course of SERT availability in both current and former ecstasy users who where examined by PET three times with a time interval of about 1 year between each examination. To our knowledge, this is the first longitudinal study of SERT availability in human ecstasy users.

Results of the PET measurements at baseline have been reported previously [7–9]. A volume of interest (VOI)-based analysis revealed that SERT availability in the SERT-rich regions mesencephalon, putamen, caudate and thalamus was reduced by the order of 10–30% in current ecstasy users as compared with drug-naïve controls and polydrug controls (statistically significant in

mesencephalon and thalamus) [7]. In the present paper, we focus on the results from the follow-up studies. The studies complied with the current German laws inclusive of ethics approval.

Materials and methods

Sample at baseline

At baseline, 117 healthy subjects assigned to four different groups were investigated: 30 current ecstasy users, 29 former ecstasy users, 29 subjects without a history of illicit drug abuse (drug-naives) and 29 subjects with abuse of psychoactive agents other than ecstasy (polydrug users). Exclusion criteria were acute major illness, age less than 18 years, pregnancy or breast feeding, epilepsy, lifetime diagnoses of major depression or schizophrenia and alcohol, opiate or benzodiazepine dependence according to DSM-IV criteria [10]. Liver diseases, for example, were excluded by blood analysis (glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, γ-glutamyl transpeptidase, alkaline phosphatase). Subjects suffering from any psychotic or mood disorder (except dysthymia) according to DSM-IV criteria that was not substance related were also excluded. Substance-related disorders, except alcohol-, opiate- or benzodiazepine-related disorders, were permitted. A detailed description of further inclusion/exclusion criteria as well as demographics, ecstasy use and exposure to drugs other than ecstasy at baseline has been given previously [7–9].

Positron emission tomography

Dynamic PET with the highly selective SERT ligand [^{11}C](+)McN5652 was performed on a full-ring system ECAT EXACT 921/47 (Siemens/CTI, Knoxville, TN, USA) in 2D-mode [11–15]. After the transmission scan for attenuation correction, 400–600 MBq [^{11}C](+)McN5652, dissolved in 40 ml 0.9% NaCl, was injected through a vein of the left hand at a flow rate of 600 ml/h. Thus, the total infusion time was 4 min. At the beginning of tracer injection a dynamic scan protocol was initiated including 35 frames with a total acquisition time of 90 min (9×20, 6×30, 4×60, 5×120, 8×300 and 3×600 s). Forty-seven transaxial slices with 64×64 voxels were reconstructed using an iterative method (ira-1.25 software, H. Fricke, Medical University, Hannover, Germany) (OSEM: 24 subsets, 3 iterations). The voxel size was 3.4×3.4×3.4 mm^3, and in-plane spatial resolution was about 9 mm full-width at half-maximum (FWHM). No scatter correction was performed. Reconstructed images were realigned and stereotactically normalised to an [^{11}C](+)McN5652 template [7] using the SPM99 software package (Welcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, UK) [16]. In order to quantify the availability of the SERT for the tracer used, local distribution volume ratios (DVRs) were obtained by application of the multilinear reference tissue method for reversible binding [17, 18]. Grey matter of the cerebellum was chosen as an (almost) SERT-free reference region [19, 20]. The starting point of the multilinear regression analysis was fixed at 12 min. The following SERT-rich brain regions, in which the availability of the SERT can be measured reliably with the [^{11}C](+)McN5652 probe [12, 13], were selected for evaluation using standardised VOIs predefined in the template: mesencephalon, putamen, caudate and thalamus. The cerebellum was partly out of the field of view in about 5% of the PET measurements. The cerebellum VOI was adjusted manually in these cases. Further details of the injected tracer dose, scan schedule, image reconstruction, stereotactic normalisation, definition of VOIs and application of the multilinear reference tissue method have been given previously [7].

Follow-up

Two repeat PET measurements were completed by 16 current ecstasy users and 13 former ecstasy users. One of these current ecstasy users was excluded retrospectively because drug history data ascertained by a standardised questionnaire in a semi-structured interview were not in agreement with the drug analysis of hair samples. Four of the former ecstasy users were excluded from the further analyses because they restarted ecstasy consumption after baseline PET (more than one ecstasy tablet in the last 6 months before either of the repeat PETs). Drug history data of the remaining current ecstasy users [n=15: ten males, age at baseline 24.0±3.3 (19–30) years, and five females, age at baseline 23.2±4.7 (19–30) years] and the remaining former ecstasy users [n=9: six males, age at baseline 28.0±4.9 (21–36) years, and three females, age at baseline 20.7±1.5 (19–22) years] are given in Table 1. Plausibility of the drug history data was verified by testing of hair samples. Hair samples were analysed (gas chromatography/mass spectrometry) for amphetamines, metamphetamines and the following methylenedioxyamphetamines: methylenedioxymethamphetamine (MDMA, main psychoactive constituent of ecstasy, which, therefore, is identified with ecstasy throughout the present paper), methylenedioxyamphetamine (MDA), methylenedioxy-*N*-ethylamphetamine (MDEA) and methylbenzodioxolyl-butamine (MBDB). Drug hair data are given in Table 2. The self-reports of ecstasy use or abstinence for the past 6 months before the investigation were compatible with the hair analyses in all cases included in the analyses.

The time interval between the 1st repeat PET (T2) and the baseline PET (T1), and between the 2nd repeat PET (T3) and the 1st repeat PET was 14.1±2.5 and 10.8±1.4 months in the group of current ecstasy users, and 13.2±1.7 and 11.3±2.8 months in the group of former ecstasy users (mean±1 standard deviation).

Participants abstained from use of all psychoactive drugs (including alcohol) except for nicotine and caffeine for at least 3 days before each PET measurement. Abstention in this period was verified by urine or blood screening on the day of the PET. In most subjects an additional urine test had been performed 2–3 days before PET so that abstention periods up to 6 days could be verified by urine screening.

DSM-IV-axis-I diagnoses in both current ecstasy users and former ecstasy users at all time points are given in Table 3. The prevalence of disorders not related to substance abuse appeared to be well within the range of the normal population [21].

The study was approved by the local ethics committee and the national radiation protection authority. All participants gave their written informed consent.

Statistical analyses

Repeated measurements of both DVR and drug consumption characteristics were first compared by non-parametric two-way analysis on ranks (Friedman test) in both groups of subjects, i.e. current ecstasy users and former ecstasy users, separately. Whenever the Friedman test revealed a significant difference between the three time points ($p \leq 0.05$), post hoc comparisons of pairs (T2 vs T1, T3 vs T2 and T3 vs T1) were performed by application of the non-parametric Wilcoxon signed-ranks test for matched pairs. An effect was considered statistically significant when the test had reached the significance level α=0.05.

Table 1. Time course of ecstasy use and exposure to drugs other than ecstasy in current ecstasy users (*CU*, *n*=15) and former ecstasy users (*FU*, *n*=9) who completed three PET measurements with a time interval of about 1 year between each measurement

	Group	Baseline (T1)[a]	1st repeat (T2)[a]	2nd repeat (T3)[a]	Friedman test		Wilcoxon test					
					df=2		T2–T1		T3–T2		T3–T1	
					χ^2	*p**	*Z*	*p***	*Z*	*p***	*Z*	*p***
Time since last ecstasy ingestion (days)	CU	21 (18, 35), 24.3±13.6	32 (14, 120), 73.1±96.7	32 (7, 272), 130.3±172.6	2.136	0.358						
	FU	229 (120, 383), 340±400	460 (314, 683), 550±359	800 (412, 1032), 760±455	9.556	0.006	−2.429	0.015	−2.192	0.028	−2.547	0.011
Ecstasy dose in last 6 months (tablets)	CU	25 (13, 52.5), 38.7±37.3	5 (2, 21), 24.3±42.5	3.5 (0, 46), 22.2±31.9	6.933	0.030	−2.556	0.011	−0.739	0.460	−1.789	0.074
	FU	0 (0, 1.5), 1.5±3.3	0 (0, 0), 0.1±0.3	0 (0, 0), 0.1±0.3	4.625	0.123						
Cannabis dose in last 6 months (g)	CU	4.2 (0.0, 15.5), 27.5±63.3	3.0 (0.0, 14.0), 17.6±35.0	0.6 (0.0, 15.0), 22.8±61.1	2.390	0.330						
	FU	2.0 (0.3, 275), 148±235	0.3 (0.0, 115), 86±146	0.5 (0.0, 141), 83±151	9.152	0.007	−1.955	0.051	−0.524	0.600	−1.955	0.051
Cocaine dose in last 6 months (g)	CU	0.6 (0.2, 1.0), 2.1±5.2	0.0 (0.0, 1.5), 1.9±4.9	0.4 (0.0, 2.2), 2.6±5.6	3.962	0.143						
	FU	0.0 (0.0, 1.0), 0.7±1.3	0.0 (0.0, 6.0), 2.8±5.5	0.0 (0.0, 0.5), 3.4±9.8	0.353	0.907						
Amphetamine dose in last 6 months (g)	CU	1.0 (0.0, 4.9), 6.5±14.3	0.0 (0.0, 1.0), 3.7±11.5	0.0 (0.0, 9.0), 11.3±30.6	3.818	0.152						
	FU	0.0 (0.0, 0.13), 2.3±6.7	0.0 (0.0, 0.0), 0.0±0.0	0.0 (0.0, 0.0), 0.01±0.03	2.000	0.778						
LSD dose in last 6 months (μg)	CU	0.0 (0.0, 12.5), 16.7±45.7	0.0 (0.0, 0.0), 13.3±51.6	0.0 (0.0, 0.0), 3.3±12.9	4.769	0.074						
	FU	0.0 (0.0, 0.0), 5.6±16.7	0.0 (0.0, 0.0), 0.0±0.0	0.0 (0.0, 0.0), 0.0±0.0	2.000	1.000						
Typical alcohol dose in last 6 months (g/week)	CU	70.0 (27.5, 112.2), 85.1±77.2	72.4 (54.1, 88.0), 122.4±180.4	80.6 (40.0, 212.2), 122.4±113.9	0.255	0.925						
	FU	118.4 (29.9, 335.5), 163.6±150.5	71.5 (10.0, 275.4), 125.7±151.9	62.1 (2.4, 145.7), 80.1±92.9	2.387	0.352						
Typical nicotine dose in last 6 months (cigarettes/week)	CU	57 (0, 140), 62.3±68.2	20 (0, 100), 56.5±70.7	70 (0, 105), 66.4±68.1	1.378	0.525						
	FU	127 (83, 178), 135±82	133 (93, 181), 126±65	125 (23, 185), 111±80	0.897	0.686						

Data were ascertained by a standardised questionnaire in a semi-structured interview. None of the subjects reported having consumed methadone, heroin or other opiates in the last 6 months before any of the PET measurements. The same held true for mescaline. Two current ecstasy users and two former ecstasy users reported having consumed psilocybin (2.0–7.5 mushrooms). Two current ecstasy users reported having consumed laughing gas (3–18 balloons). Poppers (inhaled nitrites) and γ-hydroxybutyrate had been used by one current ecstasy user each (2–5 nasal inhalations and 10 ml, respectively)

[a] Data are given as median (25th percentile, 75th percentile), mean±1 standard deviation

*Exact significance *p*

**Asymptotic significance *p*

Table 2. Drug hair analysis in current ecstasy users (*CU*, *n*=15) and former ecstasy users (*FU*, *n*=9) who completed three PET measurements with a time interval of about 1 year between each measurement

	Group	Baseline (T1)[a,b]	1st repeat (T2)[a]	2nd repeat (T3)[a]
Hair length (cm)	CU	5.0 (2.8, 6.0), 4.7±2.4	4.0 (2.5, 12.0), 7.8±7.4	3.5 (2.0, 12.0), 8.0±8.2
	FU	5.0 (3.3, 6.0), 5.3±2.9	5.0 (2.3, 19.0), 11.1±15.0	3.0 (2.3, 15.8), 8.8±11.4
Amount of hair (mg)	CU	40.0 (24.0, 62.0), 41.9±22.5	52.0 (19.0, 61.0), 43.3±23.1	52.0 (30.0, 59.0), 45.9±16.5
	FU	57.0 (49.0, 65.5), 56.4±9.7	64.0 (41.5, 67.0), 55.3±19.9	47.0 (25.0, 51.0), 38.8±17.4
Amphetamines (μg/g hair)	CU	0.00 (0.00, 0.00), 0.44±1.45	0.00 (0.00, 0.00), 0.55±1.75	0.00 (0.00, 0.00), 0.68±2.49
	FU	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00
MDMA (μg/g hair)	CU	2.63 (0.16, 9.38), 7.51±13.5	0.64 (0.00, 2.26), 3.20±6.83	1.43 (0.00, 4.29), 3.27±6.77
	FU	0.00 (0.00, 0.05), 0.06±0.14	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.13±0.39
MDA (μg/g hair)	CU	0.00 (0.00, 0.71), 0.33±0.49	0.00 (0.00, 0.06), 0.07±0.22	0.00 (0.00, 0.00), 0.07±0.22
	FU	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00
MDEA (μg/g hair)	CU	0.28 (0.00, 1.32), 0.71±1.01	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00
	FU	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00	0.00 (0.00, 0.00), 0.00±0.00

Metamphetamines were detected in only one hair sample (0.20 μg/g in a current ecstasy user at the second repeat PET). None of the hair samples contained MBDB

[a] Data are given as median (25th percentile, 75th percentile), mean±1 standard deviation

[b] In two current ecstasy users, drug hair data at baseline were no longer available at the time of writing

The time course of the SERT availability in current ecstasy users was further evaluated by parametric univariate analysis of variance of repeated measurements. Sex was taken into account as a fixed between-subject factor. The significance of within-subject effects was computed either assuming sphericity or using the Greenhouse-Geisser F-correction depending on the result of the Mauchly sphericity test. Normality of the distributions of SERT availability at the different time points T1, T2 and T3 was tested by the Kolmogorov-Smirnov method.

Table 3. DSM-IV-axis-I diagnoses in current ecstasy users (*n*=15) and former ecstasy users (*n*=9) who completed three PET measurements with a time interval of about 1 year between each measurement

Diagnosis	Baseline (T1)		1st repeat PET (T2)		2nd repeat PET (T3)	
	Current users	Former users	Current users	Former users	Current users	Former users
Disorders not related to substance use						
Dysthymia 300.4	0	0	0	0	0	0
Depressive disorder NOS 311	0	1 (a), 1 (f)	0	2 (f)	0	1 (a), 1 (f)
General anxiety disorder 300.02	0	1 (a)	0	1 (f)	0	1 (f)
Agoraphobia 300.22	0	1 (f)	1 (f)	1 (p)	1 (f)	1 (f)
Social phobia 300.23	1 (a), 1 (f)	1 (p)	2 (f)	1 (f)	2 (f)	1 (f)
Specific phobia 300.29	0	1 (a)	0	1 (f)	0	1 (f)
Adjustment disorder 309.xx	1 (p), 3 (f)	1 (f)	1 (p), 3 (f)	1 (f)	1 (p), 3 (f)	1 (f)
Anorexia 307.1	1 (f)	0	1 (f)	0	1 (f)	0
Eating disorder NOS 307.50	1 (a), 1 (p)	0	2 (f)	0	2 (f)	0
Substance-related disorders						
Ecstasy-induced anxiety disorder 292.89	1 (a)	1 (f)	1 (f)	1 (f)	1 (f)	1 (f)
Ecstasy-induced affective disorder 292.84	3 (a), 1 (p)	1 (f)	1 (a), 2 (p), 1(f)	1 (f)	1 (p), 3 (f)	1 (f)
Cannabis-induced anxiety disorder 292.89	1 (p)	0	1 (f)	0	1 (f)	0
Cannabis-induced affective disorder 292.84	1 (f)	0	1 (f)	0	1 (f)	0
Cocaine-induced anxiety disorder 292.89	0	0	0	0	0	0
LSD-induced anxiety disorder 292.89	0	0	0	0	0	0
Multiple substance-induced anxiety disorder 292.89	1 (f)	0	1 (f)	0	1 (f)	0
Multiple substance-induced affective disorder 292.84	0	2 (a), 1 (p)	0	1 (a), 1 (p), 1(f)	0	1 (a), 2 (f)

a acute, *p* in partial remission, *f* in full remission

Table 4. Time course and statistical analysis of repeated measurements of DVR in current ecstasy users (*CU*, *n*=15) and former ecstasy users (*FU*, *n*=9) who completed three PET measurements with a time interval of about 1 year between each measurement

	Group	Baseline (T1)[a]	1st repeat (T2)[a]	2nd repeat (T3)[a]	Friedman test		Wilcoxon test					
					df=2		T2–T1		*df*=2		T2–T1	
					χ^2	*p**	χ^2	*p***	χ^2	*p***	χ^2	*p***
DVR mesencephalon	CU	1.137 (1.111, 1.205), 1.147±0.050	1.169 (1.142, 1.199), 1.171±0.029	1.207 (1.188, 1.255), 1.213±0.043	8.933	0.010	−1.449	0.147	−2.556	0.011	−2.726	0.006
	FU	1.196 (1.177, 1.300), 1.215±0.084	1.220 (1.187, 1.298), 1.238±0.059	1.231 (1.168, 1.253), 1.217±0.070	2.000	0.398						
DVR putamen	CU	1.322 (1.246, 1.374), 1.323±0.091	1.335 (1.289, 1.404), 1.338±0.083	1.349 (1.286, 1.429), 1.352±0.098	0.933	0.711						
	FU	1.394 (1.285, 1.431), 1.372±0.095	1.376 (1.323, 1.468), 1.387±0.095	1.432 (1.337, 1.461), 1.402±0.078	3.556	0.187						
DVR caudate	CU	1.177 (1.087, 1.245), 1.172±0.091	1.196 (1.146, 1.240), 1.190±0.098	1.177 (1.147, 1.216), 1.185±0.074	1.600	0.513						
	FU	1.267 (1.172, 1.317), 1.245±0.081	1.227 (1.176, 1.312), 1.236±0.081	1.217 (1.205, 1.281), 1.237±0.072	0.889	0.685						
DVR thalamus	CU	1.341 (1.260, 1.386), 1.336±0.085	1.358 (1.280, 1.413), 1.359±0.091	1.377 (1.319, 1.506), 1.400±0.093	4.933	0.096						
	FU	1.396 (1.297, 1.453), 1.378±0.095	1.408 (1.253, 1.514), 1.393±0.126	1.432 (1.326, 1.506), 1.424±0.089	9.556	0.006	−1.125	0.260	−1.362	0.173	−2.666	0.008

[a] Data are given as median (25th percentile, 75th percentile), mean±1 standard deviation

*Exact significance *p*

**Asymptotic significance *p*

In order to evaluate putative interactions of the time course of SERT availability and the time course of the intensity of ecstasy consumption in the group of current ecstasy users, a variable ΔI was defined to classify changes in the intensity of ecstasy consumption according to ΔI=−1 ("decreased intensity") if the number of ecstasy tablets taken in the last 6 months before the PET was smaller at the 2nd repeat PET than at baseline (n=11), and ΔI=1 ("equal or increased intensity") otherwise (n=4). Then the univariate analysis of variance was repeated with ΔI as a second fixed between-subject factor in addition to sex.

Finally, to quantify a putative correlation of the change in the SERT availability and the change in the intensity of ecstasy consumption, a straight line was fitted to the three data points {x,y}={1, DVR(T1)}, {2, DVR(T2)} and {3, DVR(T3)} of the time course of SERT availability (DVR) in each individual subject. The slope dDVR of the fit was used as a measure of the change in the SERT availability. A second straight line was fitted to the time course of the number of ecstasy tablets taken in the last 6 months before the PET (XTCD6), analogously. The slope dXTCD6 of this fit was used as a measure of the change in the intensity of ecstasy consumption. The correlation of dDVR and dXTCD6 in the group of all current ecstasy users was evaluated by the non-parametric Spearman-rho test.

All statistic computations were performed using SPSS 10.0.7 for MS Windows. All tests of significance were two-tailed. No Bonferroni adjustment for the number of VOIs was performed.

Results

Current ecstasy users

DVR in current ecstasy users showed a consistent increase over the course of time (Table 4). The difference between the mean DVR at the three time points was statistically significant according to the Friedman test in the mesencephalon (Table 4, Fig. 1). Post hoc testing revealed that the difference between the 2nd repeat PET (T3) and the 1st repeat PET (T2) was significant. The difference between the 1st repeat PET and baseline PET (T1) failed to reach the significance level.

The effect in the mesencephalon was confirmed by the univariate analysis of variance: the within-subject effect of time was significant (F=9.315, df=2, p=0.001) as was the within-subject contrast T3 versus T2 (F=17.116, df=1, p=0.001), while the contrast T2 versus T1 was again not significant (F=0.936, df=1, p=0.351). In addition to the results of non-parametric testing, a significant linear within-subject time contrast was revealed by the univariate analysis of variance (F=11.724, df=1, p=0.005). The within-subject effect of time×sex was not significant (F=2.193, df=2, p=0.132). The distributions of the DVR in the mesencephalon at the different time points were consistent with normality according to the Kolmogorov-Smirnov test (T1: Z=0.711, n=15, p=0.693, T2: Z=0.653, n=15, p=0.788, T3: Z=0.491, n=15, p=0.969), and sphericity could be assumed according to the Mauchly test (χ^2=3.427, df=2, p=0.180).

Concerning the time course of the use of ecstasy as well as other illicit drugs, and the use of alcohol and nicotine in the group of current ecstasy users, among the tested variables only the ecstasy dose in the last 6 months before PET showed a significant effect (Table 1). There was a consistent decrease in the ecstasy dose, statistically significant between baseline and the 1st repeat PET (Fig. 2).

Univariate analysis of variance with the variable ΔI as a fixed between-subject factor in current ecstasy users revealed a significant within-subject interaction of the time course of the DVR in the mesencephalon and ΔI (F=7.060, df=1, p=0.004). SERT availability in the mesencephalon tended to increase in subjects whose intensity of ecstasy consumption decreased in the time course of the study (ΔI=−1), while it tended to remain unchanged in subjects with constant or increasing intensity of ecstasy consumption (ΔI=0) (Fig. 3). This relation was confirmed by the correlation analysis. There was a strong, highly significant negative correlation between the change in SERT availability in the mesencephalon (dDVR) and the change in the intensity of ecstasy consumption (dXTCD6) (Spearman correlation coefficient=−0.725, n=15, p=0.002), i.e. the more pronounced the increase in SERT availability, the stronger was the reduction in the intensity of ecstasy consumption (Fig. 4). Correlation analysis also revealed negative correlation coefficients between the

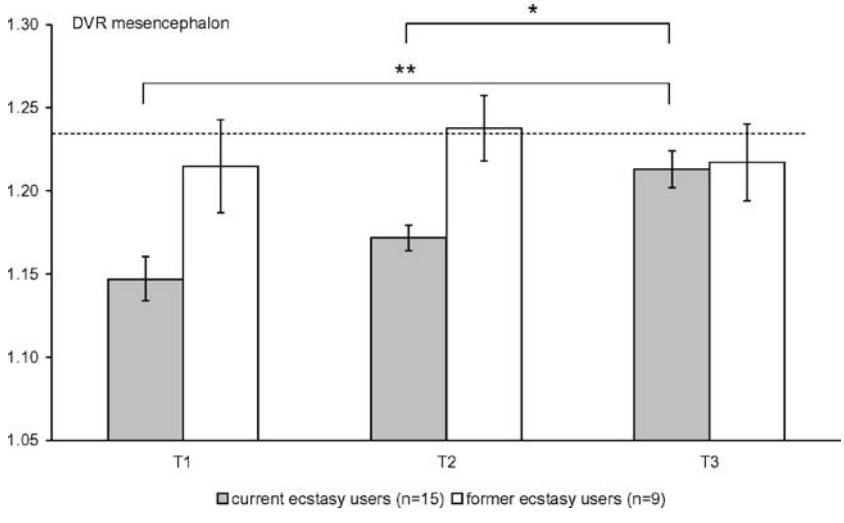

Fig. 1. Time course of SERT availability (DVR) in the mesencephalon in current ecstasy users (n=15) and former ecstasy users (n=9). Data are given as mean ± standard error of the mean. The *dashed line* represents the mean DVR in the mesencephalon in polydrug controls at baseline [7]. *p≤0.05, **p≤0.01 according to Wilcoxon signed-ranks test for matched pairs

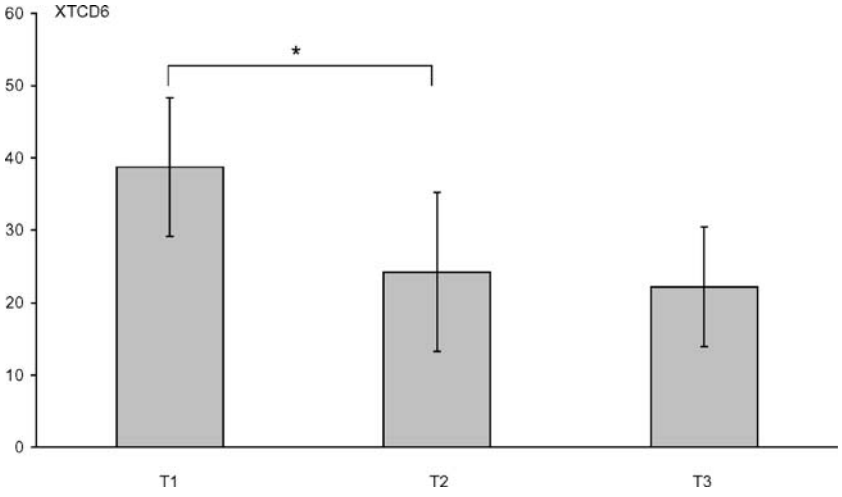

Fig. 2. Time course of the ecstasy dose taken in the last 6 months before PET (XTCD6) in current ecstasy users (n=15) (mean±standard error of the mean). *$p \leq 0.05$ according to Wilcoxon signed-ranks test for matched pairs

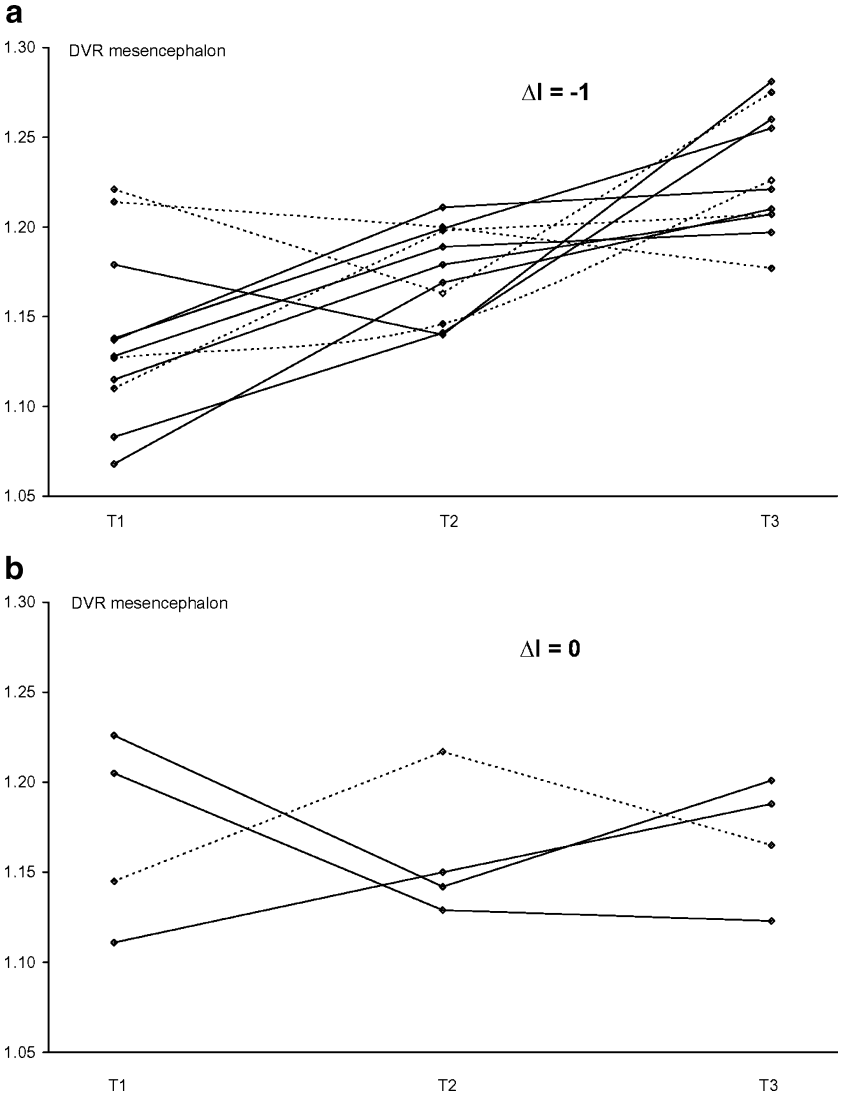

Fig. 3. Time course of the SERT availability (DVR) in the mesencephalon of current ecstasy users (n=15), subdivided into those subjects who showed a decrease in the intensity of ecstasy consumption in the time course (n=11, $\Delta I=-1$, **a**), and those subjects who showed constant or increasing intensity of ecstasy consumption in the time course (n=4, $\Delta I=0$, **b**). *Dotted line* females, *solid line* males

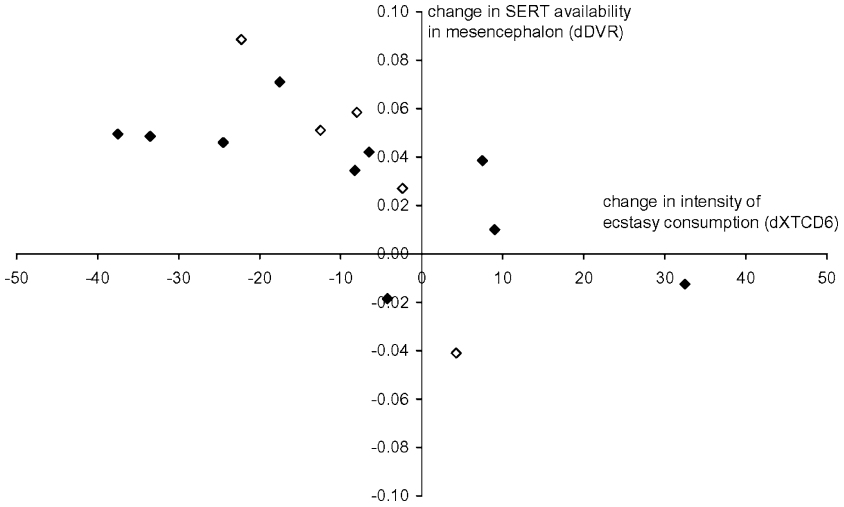

Fig. 4. Change in SERT availability (dDVR) in the mesencephalon in current ecstasy users (n=15) versus change in the intensity of ecstasy consumption, i.e. change in the number of ecstasy tablets taken in the last 6 months before the PET (dXTCD6). *Open rhombus* females, *closed rhombus* males)

change in the intensity of ecstasy consumption (dXTCD6) and the change in SERT availability in the putamen, caudate and thalamus (dDVR), the correlation being statistically significant in the putamen (Spearman correlation coefficient=−0.536, −0.368 and −0.511, p=0.039, 0.177 and 0.052, in the putamen, caudate and thalamus, respectively (n=15)).

Former ecstasy users

In former ecstasy users the DVR in the thalamus showed a consistent increase over the course of time which was statistically significant according to the Friedman test (p=0.006) (Table 4). Post hoc testing revealed that the difference between the 2nd repeat PET (T3) and the baseline PET (T1) was significant.

Discussion

In the present study the time course of SERT availability was investigated in ecstasy users in a prospective longitudinal design by performing two follow-up PET measurements with a time interval of about 1 year between the measurements.

Current ecstasy users, i.e. subjects who at baseline used ecstasy on a regular basis, showed a consistent, statistically significant increase in SERT availability in the mesencephalon over the time course of the study. At the end of the study, SERT availability was close to normal values (Fig. 1). This most likely is explained by a corresponding decrease in the intensity of ecstasy consumption of the current ecstasy users during the period of the study. This hypothesis was supported by the following facts: (a) among the tested drug consumption variables (including alcohol and nicotine), the number of ecstasy tablets taken in the last 6 months before PET was the only one that changed (decreased) significantly during the study, and (b) there was a significant interaction and correlation between the time course of SERT availability and the change in the intensity of ecstasy consumption. Due to the sequential performance of PET scans, these results provide relatively direct evidence for the reversibility of ecstasy-induced alterations in SERT availability in the mesencephalon as measured by PET.

Concerning region specificity, SERT availability in the putamen, caudate and thalamus in current ecstasy users also showed an increase over time, similar to that in the mesencephalon. Although the increase in these regions was not statistically significant, in the case of the putamen there was a significant negative correlation between the change in SERT availability and the change in the intensity of ecstasy consumption. These results appear compatible with the hypothesis that the recovery of SERT availability in the mesencephalon in current ecstasy users is not specific for the mesencephalon, but reflects recovery throughout SERT-rich regions or even throughout the whole brain [4, 22]. However, it should be acknowledged that the present study does not provide any information on cerebral cortical brain regions.

The fact that our results do not suggest the recovery of SERT availability to be strongly region specific retrospectively justifies our decision not to consider the different VOIs as independent and, therefore, not to perform Bonferroni adjustment for the number of VOIs. However, it is noteworthy that both the time effect and the correlation between the change in the intensity of ecstasy consumption and the change in SERT availability in the mesencephalon would have been statistically significant even after Bonferroni adjustment for the number of VOIs (corrected significance level α=0.05/4=0.013).

Mean time since last ecstasy ingestion in current ecstasy users at baseline PET was 24 days (Table 1). The mean time interval between the repeat PET measurements was about 12 months. Thus, our findings in current ecstasy users suggest that recovery of SERT availability takes place over a period of several months to a few years. This might explain why there was no reduction in SERT availability in former ecstasy users at baseline [7, 8], because mean time since last ecstasy ingestion in former ecstasy users at baseline was 340 days (Table 1).

Affective and anxiety disorders may be related to the observed alterations in SERT availability. In fact, acute ecstasy-induced affective disorder was found in three current ecstasy users at baseline (Table 3). At the end of the study, i.e. at the time point of the second repeat PET, the ecstasy-induced affective disorder had either partially (n=1) or fully remitted (n=2) in all of these subjects. Similarly, the ecstasy-induced anxiety disorder which at baseline was diagnosed as acute in one current ecstasy user had fully remitted at the end of the study. This appears to be in accordance with the consistent increase towards normal values of SERT availability in the current ecstasy users. However, a detailed analysis of correlations between SERT availability and substance-induced disorders as well as behavioural measures will be given elsewhere.

There is strong evidence that the availability of the SERT as measured by SPECT or PET is age related. Yamamoto et al. reported a decline in SERT availability in SERT-rich brain regions, measured by PET with [^{11}C](+) McN5652, of about 10% per decade [23]. In the present study the interval between baseline PET and first follow-up PET and between first and second follow-up PET was about 1 year in each case. Therefore, according to the results of Yamamoto et al., an age-related decrease in SERT availability of about 1% would have been expected between each of the PET scans. However, the Pearson correlation analysis performed by Yamamoto et al. included subjects aged between 20 and 79 years. Yamamoto et al. did not perform correlation analysis for different age ranges separately. Thus, it is not clear whether the reported age-related decline also holds true in the age range (18–36 years) of the subjects included in the present study. Figure 2 in [24] suggests that the SERT availability might be constant until the age of about 40 and declines only after this age. Partial correlation analysis controlling for sex in the group of drug-naïve controls studied at baseline in the present study (n=29, age 18–33 years [7]) also did not reveal a significant correlation between age and SERT availability. Facing this lack of clear evidence of an age-related decline in SERT availability during the present study, we decided not to correct the measured DVRs for age. This is a conservative approach, since correction for age would increase the DVR at the first follow-up PET relative to the baseline PET and further increase the DVR at the second follow-up PET relative to the first follow-up PET. This would add to the observed consistent increase in SERT availability during the study.

Volkow et al. [25], using PET and the dopamine transporter (DAT) ligand [^{11}C]*d-threo*-methylphenidate, reported recovery from loss of DAT in abstinent methamphetamine users, in close analogy to the present findings. Striatal DAT availability in five methamphetamine users significantly increased towards normal values at 3–14 months of abstinence. The changes in DAT in the putamen were negatively correlated with the mean daily dose of methamphetamine used prior to the baseline PET. However, neuropsychological tests did not improve to the same extent. The authors suggested that this might reflect increased arborisation of viable terminals that is insufficient to compensate for the effects of dopamine terminal loss on dopamine-mediated cerebral function. Axonal regeneration may also lead to abnormal, dysfunctional circuitry. Analysis of the time course of neuropsychological performance in the present study is not yet completed. However, preliminary results indicate that ecstasy users do not recover from deficits in verbal recall performance with sustained ecstasy abstinence [9].

In *former* ecstasy users, the DVR in the thalamus showed a consistent, statistically significant increase over time, which, at first sight, might appear rather surprising. There had been no significant difference in the thalamus between former ecstasy users and any of the control groups at baseline [7]. This result might suggest some kind of overshoot of SERT availability above normal levels over the course of 1 to several years of abstinence from heavy ecstasy use (cf. [4, 26]). The DVR in the thalamus in former ecstasy users at the 2nd repeat PET was indeed slightly increased compared with both control groups at baseline [7]. However, the difference was not statistically significant.

There was a significant time effect (decrease) in the intensity of cannabis use in the group of former ecstasy users during the time course of the study (Table 1). However, the change in DVR in the thalamus did not correlate with the change in intensity in cannabis consumption (data not shown).

Limitations

It was intended to study typical ecstasy users representative of the local ecstasy user scene. Therefore, the study was designed as a pure field study. In particular, we did not prompt the ecstasy users to stop consumption of ecstasy, although this would have made the analysis of the data more straightforward. The disadvantage of this would have been that only participants who agreed to stop using ecstasy would have been included. It appears likely that these would have been the subjects who suffered most from the process of putative ecstasy neurotoxicity. In order to avoid this participant selection effect we decided in favour of a pure field study.

It should be noted that all ecstasy users enrolled in this study were in fact polydrug ecstasy users, i.e. all subjects used or had used other illicit drugs in addition to ecstasy. However, this is in agreement with a recent review of Cole and Sumnall, who state that the use of the term ‘ecstasy user’ does not imply the exclusive use of ecstasy on a regular basis in a similar fashion to the implication that a heroin-dependent population uses only heroin, because epidemiological studies have so far failed to identify a group of users who use ecstasy exclusively [27].

Hair analysis at baseline detected MDMA in the hair of only two of nine *former* ecstasy users. However, the median length of the hair sampled in former ecstasy users at baseline was 5.0 cm (Table 2) so that detection of drug use was restricted to at most the period of about the last 6 months before PET. Rate of hair growth varies individually

from 0.6 to 3.4 cm/month [28]. The median of the self-reported time since last ecstasy ingestion was about 7.5 months in former ecstasy users at baseline (Table 1). Therefore, the fact that at baseline MDMA was detected in the hair of only two former ecstasy users does not contradict the self-reports of previous chronic use of ecstasy in this group. On the other hand, drug hair data also did not provide forensic evidence for previous chronic use of ecstasy in the remaining seven subjects in this group. However, drug hair data did provide evidence that the former ecstasy users did not use ecstasy chronically between the PET measurements. Only a single probe (at the second repeat PET) contained a small amount of MDMA. The detected amount of MDMA was compatible with one ecstasy tablet in the last 6 months before PET. Therefore, this subject was not excluded from the study.

It is noteworthy that hair analysis proved that the *current* ecstasy users actually consumed ecstasy. Therefore, the present results on the reversibility of ecstasy-induced reduction in SERT availability are not affected by the 6-month time window of hair analysis.

Participants abstained from use of all psychoactive drugs except for nicotine and caffeine for at least 3 days before each PET measurement. One might be concerned that this minimum abstention period is too short to exclude (partial) inhibition of the binding of [^{11}C](+)McN5652 to the SERT by the presence of ecstasy in the brain. To our knowledge competition assays to measure the competition of ecstasy and [^{11}C](+)McN5652 for the binding to the SERT have not yet been performed. However, Mas et al. reported that after oral administration of ecstasy in recreational ecstasy users, plasma levels of ecstasy declined following a mono-exponential model with mean elimination half-life of about 8 h [29]. Animal data suggest that clearance of ecstasy from brain tissue parallels blood clearance [30]. Thus, within 3 days (72 h) brain levels of ecstasy are expected to drop to about 0.2% of the peak level. Now, a typical recreational dose of three tablets [31] each containing 100 mg ecstasy [32] is expected to result in an ecstasy peak concentration in the extracellular space of the brain tissue of the order of 10 μ*M* [30]. Within 72 h this is reduced to about 0.02 μ*M*, which, in terms of the affinity of ecstasy for the SERT, is a very small concentration (K_i=0.61 μ*M* [33]). Therefore, according to the classical theory of pharmacology [34], the availability of the SERT for [^{11}C](+)McN5652 binding should not be affected significantly by the presence of ecstasy a minimum of 72 h after ingestion of ecstasy ([ecstasy]/Ki≈0.02 μ*M*/0.61 μ*M*≈0.03<1). In addition, it should be noted that actual abstinence periods were significantly longer than the minimum of 3 days required (Table 1).

Test–retest data for the DVR of the SERT ligand [^{11}C](+)McN5652 in humans, which might be useful to assess the reliability of the present results, are not available. However, animal data for [^{11}C](+)McN5652 and another SERT ligand, [^{11}C]DASB [4, 14, 35], in combination with test–retest data for [^{11}C]DASB in humans [36], suggest that the test–retest variability (standard deviation/mean value) of the [^{11}C](+)McN5652-DVR in SERT-rich regions, e.g. the mesencephalon, in healthy human subjects is below 5%. The coefficient of variation (standard deviation/mean value) of the DVR in the mesencephalon in the group of drug-naïve controls at baseline, which was 3.8% [7], also indicates rather good test–retest stability.

Animal studies indicate that serotonergic axon terminals are damaged by ecstasy while cell bodies are spared from neurotoxic effects [37]. Thus, it seems that ecstasy does not affect the brain serotonin neuron number but rather the serotonin innervation pattern, i.e. the local density of serotonergic axon terminals. Since the SERT is mostly located presynaptically [38], it appears to be a useful target for the detection of alterations in the density of serotonergic axon terminals by in vivo functional imaging in the human brain. However, neuroimaging cannot differentiate between changes in the SERT within intact neurons and changes (sprouting etc.) in the number of axon terminals. In addition, functional brain imaging measures the "availability" of the SERT for the ligand used, which is not only a function of SERT density, but depends also on the actual affinity of the SERT for the ligand [39, 40] and, possibly, the synaptic transmitter concentration [41, 42] and transmitter occupancy of the SERT. Thus, in the outcome measures of functional brain imaging, alterations in SERT density might be superimposed by other effects. This might explain the seeming discrepancy between the results of the present study (recovery of SERT availability) and the results of Hatzidimitriou et al., who, upon histological examination of the brain serotonin innervation pattern in squirrel monkeys, found a reduced density of serotonergic axons in the forebrain as long as 7 years after ecstasy treatment, although the deficits were less severe than at 18 months after the treatment [43]. Regional differences (subcortical regions in the present study vs cortex in the study of Hatzidimitriou et al.) as well as drug dose and pattern of exposure might also be reasons for the seeming discrepancy.

Conclusion

In conclusion, our findings yield evidence for the reversibility of ecstasy-induced protracted alterations in SERT availability as measured by PET. However, it should be noted that the SERT is a dynamic component of the serotonergic system and is modified by multiple mechanisms. Therefore, reversibility of SERT binding does not imply full reversibility of potential neurotoxic effects. In order to further elucidate this question, additional follow-up examinations of ecstasy users are required.

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