Original Paper

Serotonergic neurotransmission in emotional processing: New evidence from long-term recreational poly-drug ecstasy use

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Abstract

The brain's serotonergic system plays a crucial role in the processing of emotional stimuli, and several studies have shown that a reduced serotonergic neurotransmission is associated with an increase in amygdala activity during emotional face processing. Prolonged recreational use of ecstasy (3,4-methylene-dioxymethamphetamine [MDMA]) induces alterations in serotonergic neurotransmission that are comparable to those observed in a depleted state. In this functional magnetic resonance imaging (fMRI) study, we investigated the responsiveness of the amygdala to emotional face stimuli in recreational ecstasy users as a model of long-term serotonin depletion. Fourteen ecstasy users and 12 non-using controls underwent fMRI to measure the regional neural activity elicited in the amygdala by male or female faces expressing anger, disgust, fear, sadness, or no emotion. During fMRI, participants made a sex judgement on each face stimulus. Positron emission tomography with ^{11}C-DASB was additionally performed to assess serotonin transporter (SERT) binding in the brain. In the ecstasy users, SERT binding correlated negatively with amygdala activity, and accumulated lifetime intake of ecstasy tablets was associated with an increase in amygdala activity during angry face processing. Conversely, time since the last ecstasy intake was associated with a trend toward a decrease in amygdala activity during angry and sad face processing. These results indicate that the effects of long-term serotonin depletion resulting from ecstasy use are dose-dependent, affecting the functional neural basis of emotional face processing.

Keywords

MDMA, ecstasy, serotonin, fMRI, emotion, amygdala

Introduction

Serotonin (5-hydroxytryptamine [5-HT]) plays a crucial role in emotional processes. A considerable number of imaging studies involving pictures of emotional faces show that changes in serotonergic function are associated with changes in amygdala reactivity when viewing negative facial expressions: Acute tryptophan depletion, which reduces central serotonin synthesis, leads to higher amygdala activity when processing negative face expressions (Cools et al., 2005; Van der Veen et al., 2007). Several studies have found that acute/subacute SSRI intervention leads to a decrease in amygdala activation (Anderson et al., 2007; Arce et al., 2008; Del-Ben et al., 2005; Maron et al., 2016). Further, when the cerebral serotonin level is pharmacologically enhanced by a three-week intervention with a selective serotonin reuptake inhibitor (SSRI), the ensuing decreased cerebral [11C]SB207145-PET binding in response to pharmacologically increased brain serotonin levels is associated with lower threat-related amygdala reactivity (Fisher et al., 2015). Whereas it is relatively clear that induction of acute and subacute changes in serotonin neurotransmission leads to changes in emotional processing, less is known about how the neural processing of emotional information is affected by chronic cerebral serotonin depletion.

Ecstasy, or 3,4-methylene-dioxymethamphetamine (MDMA) is a widely used recreational drug that has immediate effects in

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terms of improved mood and feelings of empathy (Carhart-Harris et al., 2014; Hysek et al., 2014). In this paper, we will use the term "ecstasy" when referring to the recreational human, and "MDMA" when referring to experimental human/animal studies. MDMA exerts its primary effects on the serotonin neurotransmitter system, in particular by reversing normal serotonin transporter (SERT) function and hence releasing serotonin from the storage vesicles into the synaptic cleft (Rudnick and Wall, 1992). Animal studies (Axt et al., 1992; Battaglia et al., 1988, 1991; Fischer et al., 1995; Hatzidimitriou et al., 1999; Lew et al., 1996; Molliver et al., 1990; Scanzello et al., 1993; Scheffel et al., 1998) show that repeated exposure to moderate and high doses of MDMA is associated with a reduction in cerebral serotonin levels and a decreased number of SERT binding sites. In humans, prolonged recreational use of ecstasy is also associated with reductions in SERT in both cortical and subcortical brain areas (Buchert et al., 2003; De Win et al., 2008; Erritzoe et al., 2011; Kish et al., 2010; McCann et al., 2005; Reneman et al., 2001; Urban et al., 2012). Most (Buchert et al., 2004; De Win et al., 2008; Erritzoe et al., 2011; Kish et al., 2010; McCann et al., 2005; Reneman et al., 2001), although not all (Selvaraj et al., 2009; Semple et al., 1999), molecular imaging studies show that the accumulated lifetime intake of ecstasy correlates negatively with SERT binding, supporting a dose-dependent relationship between recreational ecstasy use and reductions in SERT binding. Thus, it also seems plausible that in humans, an ecstasy-associated reduction in SERT is associated with reduced cerebral serotonin levels, and that the SERT changes may even result from chronically reduced serotonin levels. Additional support for serotonin depletion in recreational ecstasy use comes from the finding of increased levels of the post-synaptic serotonin 2A receptor in most (Reneman et al., 2002; Urban et al., 2012; Watkins, 2012), although not all (Erritzoe et al., 2011), studies where ecstasy users have had their serotonin 2A receptors measured. Importantly, lowering brain serotonin levels in preclinical models leads to *low* SERT combined with *high* serotonin 2A receptor levels, and low SERT has also been found to be associated with high serotonin 2A receptor levels in humans (Erritzoe et al., 2010). The reduction in subcortical SERT binding in ecstasy users seems to be reversible, since a positive correlation with time of abstinence from ecstasy intake has been observed (Buchert et al., 2004; Erritzoe et al., 2011; McCann et al., 2005; Semple et al., 1999). Taken together, data from these preclinical and clinical studies indicate that there is a causal relationship between ecstasy intake and effects on the serotonergic system. Reduced SERT (human and animal studies) and serotonin (animal studies) after MDMA/ecstasy exposure in combination with the observed correlation between serotonin depletion and reduced SERT binding in animal studies (Rattray et al., 1996; Rothman et al., 2003) makes it plausible that SERT binding can be regarded as a representation of the extent of chronic—but most likely reversible—serotonin depletion in long-term ecstasy users. With MDMA being an interesting and promising candidate as adjunct to psychotherapy for treatment of post-traumatic stress disorder (Mithoefer et al., 2013) and possibly other conditions as well (Sessa and Johnson, 2015), it is important to explore the possible long-term impact of this drug on serotonergic neurotransmission, as well as the functional consequences of this. Although the multiple and not always pure MDMA doses used recreationally differ from the only few—and pure—doses employed in therapy, recreational use of MDMA/ecstasy can serve as a model for the long-term effect of repeated doses.

In the present study, we used functional magnetic resonance imaging (fMRI) to investigate the functional effects of long-term recreational ecstasy use, representing a model of long-term serotonin depletion, on the neural basis of emotional responses in the amygdala. We hypothesized that amygdala engagement to aversive stimuli would show (1) a positive correlation with accumulated lifetime ecstasy use; (2) a negative correlation with SERT binding, as assessed with positron emission tomography (PET) imaging, in the amygdala; and (3) a negative correlation with time since last use of ecstasy, suggesting recovery of amygdala response.

Materials and methods

Design

This is a cross-sectional case-control study with a single fMRI (emotional faces paradigm) and PET (serotonin transporter, SERT, binding measurement) scan acquired for each participant. Amygdala fMRI-BOLD response to emotional faces and this measure's correlations with amygdala SERT binding, accumulated use of MDMA, as well as with time elapsed since last MDMA use served as the main study outcome measures.

Participants

Fourteen users of ecstasy (M_{age}=25.5 years; two females) and 12 non-using control subjects (M_{age}=23.4 years; two females) were recruited by flyers, advertisements posted on relevant websites, and word of mouth. Potential candidates were invited to a face-to-face screening that involved assessment of history of alcohol, tobacco, and illicit drug use (using the Copenhagen Substance Screening Questionnaire available from the authors on request and modified Danish versions of the Customary Drinking and Drug Use Record (Brown et al., 1998) and Lifetime Drinking History (Skinner and Sheu, 1982)), as well as screening of current and previous psychiatric symptoms using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) interview (Wing et al., 1990). All participants included in the present study, which focuses on fMRI investigations not presented previously, constitute of individuals who took part in a simultaneously conducted study with PET measurements of serotonergic markers—the PET results obtained in the larger sample have been published previously (Erritzoe et al., 2011). To avoid acute drug effects in the ecstasy users, use of drugs was not allowed seven days before the PET and MRI scans, which were conducted on separate days at the Neurobiology Research Unit at Rigshospitalet and at the Danish Research Centre for Magnetic Resonance at Hvidovre Hospital, respectively. Abstinence was confirmed by urine screen on the PET and MRI days. Control individuals were excluded if they reported more than 15 lifetime exposures to cannabis (due to potential effects on the serotonergic system; Hill et al., 2006) or had any history of use of other illegal drugs, and urine screen on the day of the scan was carried out. Demographic and drug data are presented in Table 1. No present or prior neurological or psychiatric disorders (ICD-10 or DSM IV Axis I diagnostic criteria for obsessive-compulsive

Table 1. Demographic and drug data.

	MDMA users (*n*=14)		Controls (*n*=12)		*p*-Value (*t*-test)
	Mean (*SD*)	Range (IQR)	Mean (*SD*)	Range (IQR)	
Age, years	25.5 (4.8)	22.1–27.9	23.4 (2.9)	21.0–24.4	NS
Sex					
Male	11		10		
Female	2		2		
Education, total years	14.1 (2.5)	12.0–17.0	16.0 (1.5)	13.0–17.0	NS
Alcohol					
Age at first use, years	13.5 (1.5)	13.0–14.8	13.4 (0.9)	13.0–14.0	NS
Tobacco					
Age at first use, years	14.2 (3.3)	12.3–16.8	14.0 (2.0)	13.0–15.0	NS
Ecstasy					
No. of subjects who have ever used	14				
Age at first use, years	19.7 (3.7)	18.0–20.8			
Lifetime ecstasy sessions, No.	189.0 (219.8)	19.5–213.8			
Calculated lifetime tablets used, *n*	980.1 (1722.4)	73.1–988.8			
Time since last use, days	51.8 (26.8)	30.0–69.8			
Hallucinogen					
No. of subjects who have ever used	11				
Age at first use, years	18.8 (2.4)	18–20			
Lifetime sessions with use, *n*	41.6 (62.2)	10.5–32.5			
Time since last use, days	448.9 (830.2)	31–336			
Cannabis					
No. of subjects who have ever used	14				
Age at first use, years	15.8 (3.2)	13.5–17.0			
Lifetime sessions with use, *n*	566.0 (685.1)	107.5–844.0			
Time since last use, days	341.4 (1042.8)	16.3–73.3			
Amphetamine					
No. of subjects who have ever used	13				
Age at first use, years	18.9 (4.2)	16.0–19.0			
Lifetime sessions with use, *n*	128.1 (154.3)	21.3–191.8			
Time since last use, days	359.3 (516.9)	33.0–710.0			
Cocaine					
No. of subjects who have ever used	13				
Age at first use, years	20.5 (3.8)	19.0–21.0			
Lifetime sessions with use, *n*	56.4 (120.9)	1.5–48.5			
Time since last use, days	226.6 (285.0)	37.0–347.0			

MDMA: 3,4-methylene-dioxymethamphetamine; SD: standard deviation; IQR: interquartile range.

disorder, anxiety, major depression, bipolar disorder/mania, or schizophrenia) were allowed for any of the subjects, and all subjects had a normal neurological examination and were lifetime naïve to antidepressants and antipsychotics.

The study was approved by the local Ethics Committee, Copenhagen and Frederiksberg, Denmark ((KF)01-124/04 with amendment (KF)11-283038), and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Functional MRI face paradigm. Participants were presented with pictures of faces from the Pictures of Facial Affect Series (Ekman and Friesen, 1976). The following types of affect were presented: neutral (A), anger (B), disgust (C), fear (D) and sadness (E). Using a blocked design, eight pictures of each emotion were presented for three seconds, with an interval of 0.75 seconds between stimuli presentations and emotion blocks. Sixteen blocks were presented in an ABACADAE design, each letter designating a block. Each block included eight trials and lasted 30 seconds. Subjects were asked to identify the sex of the faces by using a response box. The responses were registered during an interval starting 50 ms and ending 3750 ms from each stimuli presentation.

Behavioral data analysis. Regression analysis was used to investigate possible correlations between reaction times (RT) and $\log_2$ to the lifetime intake of ecstasy tablets or $\log_2$ to the number of days since the last use of ecstasy. Due to our previous findings that the effects of cumulative ecstasy lifetime intake and time since last use of ecstasy on SERT binding follow a $\log_2$

distribution, we expected a $\log_2$-linear relationship between RT and lifetime intake and time since last use (for calculation of number of ecstasy tablets, please see Erritzoe et al., 2011). Analysis and results that include lifetime intake and/or number of days since last use of ecstasy (and the same is the case for amphetamine) will in this manuscript refer to "$\log_2$ to" the number of accumulated lifetime doses/time since last use, but in order to improve readability, in the Results and Discussion sections, we have left out "$\log_2$ to" from the text. Differences in RT were assessed using a repeated measures analysis of variance model, with *group* as between-subject factor (ecstasy users and control subjects) and *emotion* as within-subject factor (the five facial expressions). The significance level was set to $\alpha = 0.05$. Post hoc *t*-tests were carried out in order to test paired comparisons between facial expressions. The Greenhouse–Geisser method was used to correct for non-sphericity.

MDMA use and SERT binding

The degree of serotonin depletion as reflected by cerebral SERT binding was assessed with PET and the selective SERT radioligand, 11C-DASB, as described in more detail by Erritzoe et al. (2011). In short, PET data were acquired on a GE-Advance scanner (GE, Milwaukee, WI) as a dynamic 90-minute emission recording after intravenous injection of the radiotracer, 11C-DASB. There was maximum of 1.6 months between the 11C-DASB PET and the fMRI experiment ($M \pm SD = 0.4 \pm 0.5$); approximately half of the group had PET before MRI, and vice versa. The vast majority was scanned within one to two weeks; only two were scanned with an interval of more than a month. For each participant, a mean SERT binding value from the amygdala was calculated.

To confirm previous results obtained in the overlapping study sample (Erritzoe et al., 2011), we used linear regression analysis to test whether the $\log_2$ to the accumulated lifetime intake of ecstasy tablets correlated with SERT binding from the amygdala. Since effects of lifetime ecstasy intake on SERT become smaller with increasing abstinence from ecstasy (Erritzoe et al., 2011), we controlled for this by also including the $\log_2$ to the number of days since the last use of ecstasy in the analysis. Also using a linear regression analysis, we tested whether the $\log_2$ to the number of days since the last use of ecstasy correlated with SERT binding from the amygdala (controlling for the $\log_2$ to the accumulated lifetime intake of ecstasy tablets). The possible difference between ecstasy users and controls in amygdala SERT binding was tested with an independent samples *t*-test.

Functional image processing

Image acquisition. Subjects were scanned using blood-oxygen-level dependent (BOLD) fMRI in a 3T MR scanner (Magnetom Trio; Siemens, Erlangen, Germany). To allow for high-resolution sampling of the amygdala, we acquired data in an oblique slab with a gradient-echo echo-planar imaging (EPI) sequence (repetition time (TR)=2.5 seconds, echo time (TE)=30 ms, matrix=64 × 64, voxel size=3 mm × 3 mm × 2 mm, volumes=192, slices=38, flip angle 90°, with an interleaved slice acquisition order). Field of view excluded dorsal parietal cortex, prefrontal cortex, and ventroposterior occipital cortex.

Functional imaging analysis. Functional data were preprocessed and analyzed using the statistical parametric mapping software package (SPM8; www.fil.ion.ucl.ac.uk). Preprocessing included spatial realignment, co-registration to the anatomical image, segmentation, and normalization to the standard Montreal Neurological Institute (MNI) template, and smoothing using a symmetric 6-mm Gaussian kernel.

Subject-level models were constructed using five emotional face regressors (neutral, angry, fearful, disgusted, and sad faces), together with regressors modulating the events by their RT values. In addition, the subject-level models included a regressor for incorrect sex discrimination answers, and 24 nuisance regressors to correct for movement artifacts, including first- and second-order movement parameters and spin history effects (Friston et al., 1996).

T-contrasts comparing BOLD responses to emotional and neutral images were created for each participant. These maps were used in group-level models assessing: (1) the main effects of emotional face processing across all participants, (2) differences in BOLD responses between ecstasy users and control subjects, (3) correlations between BOLD responses and the $\log_2$ to the accumulated lifetime ecstasy intake (controlling for the $\log_2$ to the number of days since the last use of ecstasy) in ecstasy users, (4) correlations between brain activity and regional SERT binding in the amygdala (volume weighted mean of left and right amygdala) in ecstasy users and control subjects, and (5) correlations between BOLD responses and the $\log_2$ to the number of days since the last use of ecstasy (controlling for the $\log_2$ to the accumulated lifetime ecstasy intake) in ecstasy users. Drug use data used for correlations with MRI data and PET data were acquired in MRI and PET scan days, respectively. In (3) and (5), one subject was excluded due to lack of precise information about the number of days of abstinence prior to the MRI investigation day, but the person was eligible for other analysis due to a negative urine sample.

Our a priori region of interest (ROI) was the amygdala. At first, we therefore restricted the correction for multiple comparisons to the amygdala ROI, as defined by the SPM anatomy toolbox (Eickhoff et al., 2005); *p*-values are provided as $p_{(svc)}$ (small-volume correction). We set the significance level for activated voxels at $p<0.05$ corrected for multiple comparisons using the family-wise error correction (FWE). The entry threshold was set to $p<0.001$ uncorrected with an extent threshold of five contiguous voxels. Second, a whole-brain analysis was performed. The significance level for activated voxels was set at $p<0.05$ corrected for multiple comparisons (FWE). The threshold was the same as in the ROI analysis. All activations are reported at peak level and are in standard MNI stereotactic space.

Mediation analysis. To test whether associations between ecstasy usage and BOLD response were mediated by SERT, a path analysis was used to decompose the total effect of MDMA usage into direct and indirect effects. The direct effect of ecstasy exposure (logarithm of the accumulated lifetime intake of MDMA tablets) on the mean BOLD response across the amygdala (as defined in the SPM anatomy toolbox; Eickhoff et al., 2005) is the conditional effect adjusting for SERT binding. The indirect effect of MDMA on the mean BOLD response is the difference in the ecstasy effect between a model, where SERT BP is controlled for compared to when it is not. This difference in

effect is equivalent to the product between the effect that ecstasy has on SERT and the effect that SERT has on BOLD response. Linearity assumptions were assessed graphically. Standard errors of the indirect effect were calculated by the delta method and were validated by comparison with 95% quantiles from a parametric bootstrap.

Structural image processing. A T1-weighted anatomical MRI was acquired for each subject (matrix=192×256×256, voxel size=1 mm × 1 mm × 1 mm, FOV=256 mm, repetition time (TR)=1540 ms, echo time (TE)=3.93 ms, inversion time (TI)=800 ms, flip angle=90°). All acquired images were visually inspected to ensure sufficient quality.

Structural data were preprocessed and analyzed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) in SPM8. All structural images were corrected for spatial distortions owing to non-linearities in the gradient system of the scanner (Jovicich et al., 2006). The resulting tissue maps were warped (DARTEL), modulated (non-linear only), and smoothed (8 mm Gaussian).

An independent two-samples *t*-test was carried out in order to investigate differences in gray-matter volume between ecstasy users and non-using controls. In order to make sure that fMRI and SERT binding levels outcomes were not biased by differences in brain volume, we investigated the brain volume by applying a linear regression analysis within the 12 ecstasy users with the two variables: $\log_2$ to the accumulated lifetime intake of ecstasy tablets, and $\log_2$ to the number of days since the last use of ecstasy as covariates.

Results

One subject (male ecstasy user, 26.8 years old) was excluded from all analyses because of a high degree of wrong answers (22%) in the sex discrimination task (see 2.2), which was interpreted as a lack of attention to the visual stimuli (percentage of correct answers for ecstasy users: $M\pm SD$=92±3; percentage of correct answers for non-using control subjects: $M\pm SD$=90±3).

Behavioral results

There was a main effect of facial expression on RT in the sex discrimination task (F[3, 69]=6, p=0.001) across all participants. Post hoc *t*-tests showed that this effect was driven by slower RT when viewing angry (t[24]=4.4, p<0.001) and fearful (t[24]=3.1, p=0.005) compared with neutral faces (Figure 1). There were no significant differences in RT between ecstasy users and control subjects (F[1, 23]=0.3, p=0.598), and no correlation between RT and lifetime intake of ecstasy tablets (R^2=0.093, p=0.310) or time since last use of ecstasy (R^2=0.037, p=0.548).

MDMA use and SERT binding

There was a significant negative correlation between the accumulated lifetime intake of ecstasy tablets and regional SERT binding in the amygdala (R^2=0.460). A doubling in the consumption of tablets corresponded to a mean decrease in SERT binding of 0.074 in the amygdala (95% confidence interval [CI] –0.134 to –0.013; p=0.022). The positive correlation between the number of days since the last use of ecstasy and SERT binding from the amygdala observed in Erritzoe et al. (2011) was not significant in our subset sample (p=0.298), but a significant difference between ecstasy users and controls in amygdala SERT binding (t[24]=3.9, p<0.001; ecstasy: 1.14±0.28, controls: 1.54±0.25) was still found.

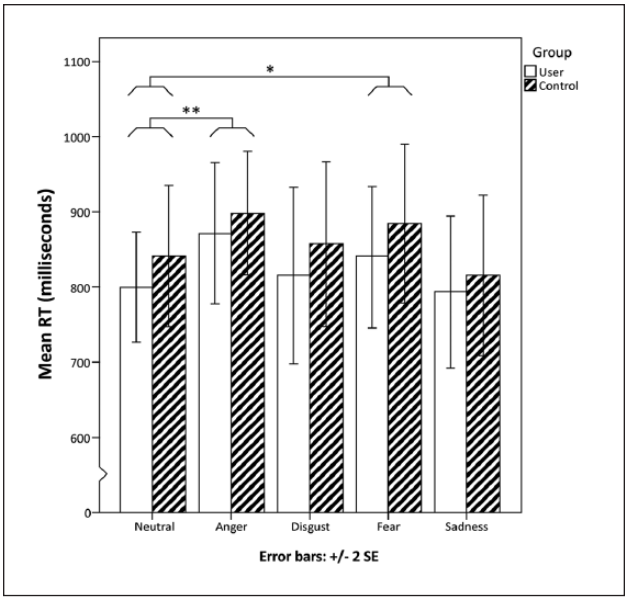

Figure 1. Reaction times in the sex discrimination task. Error bars±2 SE.
*p=0.005; **p=0.0002.

fMRI results

Main effects of emotional versus neutral faces. There were no significant differences in BOLD response between ecstasy users and non-using control subjects. Main effects of emotional face processing across all participants were in bilateral amygdala (xyz=[–22 –6 –18], z=4.9, $p_{(svc)}$<0.001; xyz=[22 –2 –22], z=4.6, $p_{(svc)}$=0.001; Figure 2). When exploring the four emotions individually against the neutral faces baseline, fearful face processing was associated with bilateral amygdala activity (xyz=[–22 –4 –18], z=4.4, $p_{(svc)}$=0.004; xyz=[22 –6 –14], z=4.3, $p_{(svc)}$=0.007), and angry face processing with right amygdala activity (xyz=[22 –6 –12], z=3.7, $p_{(svc)}$=0.035).

Correlation between accumulated lifetime drug use and effects of emotional versus neutral face processing. During angry (compared with neutral) face processing, there was a positive relationship between the accumulated lifetime intake of ecstasy tablets and left amygdala activity (xyz=[–24 0 –24], z=4.9, $p_{(svc)}$<0.001; Figure 3(a); non-significant activation in the right amygdala: xyz=[34 –2 –26], z=3.5, $p_{(svc)}$=0.125). Extracted first principal eigenvariates from bilateral amygdala ROI, as defined by the SPM anatomy toolbox (30), also revealed a positive correlation with the accumulated lifetime intake of ecstasy tablets (R^2=0.825; Figure 3(b); calculated in SPSS). Lifetime ecstasy use was positively correlated with lifetime use of amphetamine (R^2=0.867, p<0.001) and cocaine (R^2=0.627, p=0.001) but

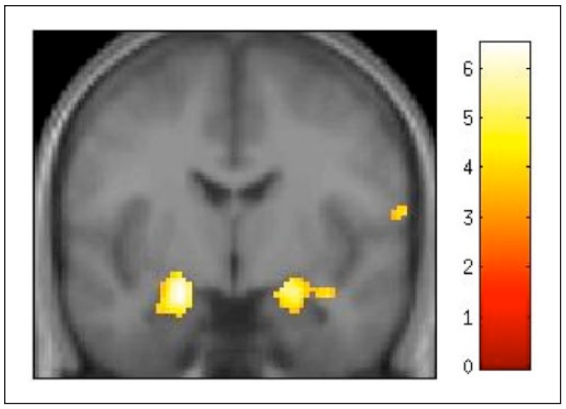

Figure 2. Main effects of aversive face processing in all 25 participants (contrast: angry+disgusted+fearful+sad faces>neutral faces). Activations were centered at *xyz*=(−22 −6 −18)], *z*=4.9, $p_{(svc)}$=0.0004, no. of voxels=225 in the left amygdala and *xyz*=(22 −2 −22), *z*=4.6, $p_{(svc)}$=0.001, no. of voxels=194 in the right amygdala. Activations are superimposed on the mean T1 template image from the whole sample.

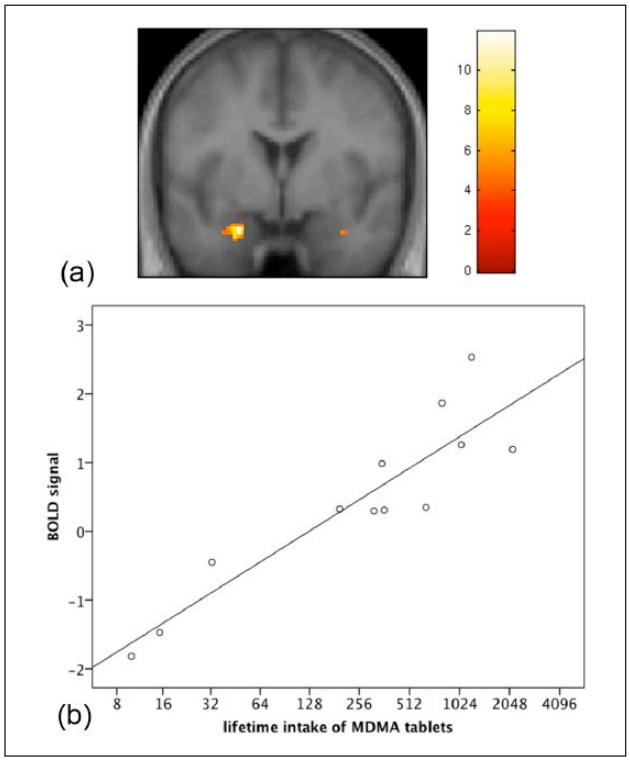

Figure 3. (a) Statistical parametric map showing a positive correlation between left amygdala activity and the $\log_2$ to the accumulated lifetime intake of MDMA tablets during angry face processing compared with neutral face processing. Activation was centered at *xyz*=(−24 0 −24)], *z*=4.9, $p_{(svc)}$=0.0004, no. of voxels=68. (b) Scatterplot of lifetime intake of MDMA tablets and left amygdala activation (R^2=0.825, *p*=0.0004).

not with hallucinogens (R^2=0.039, *p*=0.518) and cannabis (R^2=0.047, *p*=0.478). Inclusion of these potential confounding factors (lifetime intake of drugs other than ecstasy) in the model

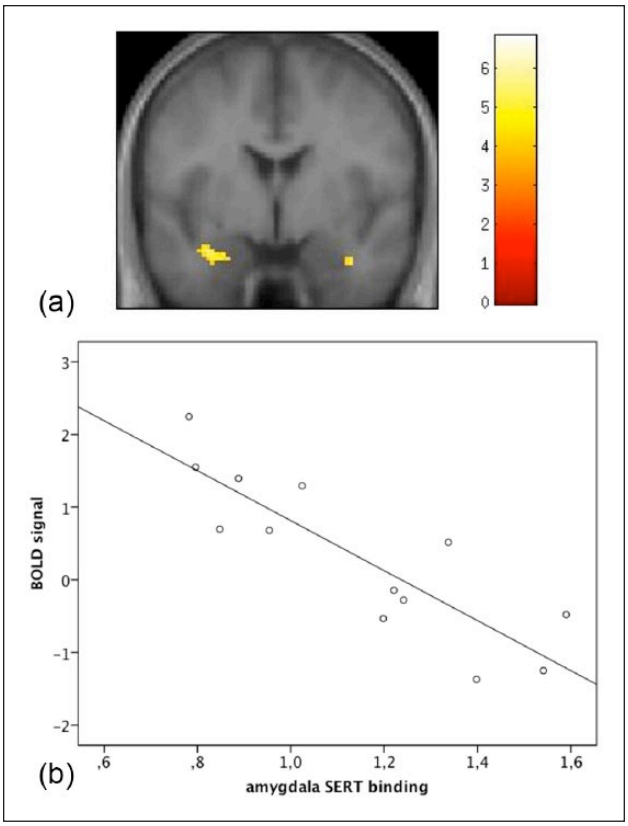

Figure 4. (a) Statistical parametric map showing a negative correlation between amygdala activity and SERT binding from the amygdala during angry face processing, compared to neutral face processing. Activation was centered at *xyz*=(−34 −2 −22), *z*=3.8, $p_{(svc)}$=0.043, no. of voxels-37. (b) Scatterplot of amygdala SERT binding and left amygdala activation (R^2=0.741, *p*=0.0002).

did not change this result, except from the inclusion of the accumulated lifetime amphetamine use. When including this covariate, the effect of the lifetime ecstasy use on amygdala activity was no longer present. A model with only the lifetime amphetamine use (controlling for days of abstinence from amphetamine) did, however, not show any significant correlation between the lifetime amphetamine use and amygdala activity (*xyz*=[–22 –2 –26], *z*=3.49, $p_{(svc)}$=0.144).

Correlation between amygdala SERT binding and effects of emotional versus neutral faces. In ecstasy users, there was a negative relationship between SERT binding and the relative increase in amygdala activity for angry compared with neutral faces (*xyz*=[–34 –2 –22], *z*=3.8, $p_{(svc)}$=0.043; Figure 4(a)). The extracted first principal eigenvariates from the amygdala ROI confirmed a significant inverse correlation with SERT binding (R^2=0.741; Figure 4(b)). A similar trend toward a correlation between SERT binding and reactivity to angry versus neutral faces was also observed for the right amygdala (*xyz*=[36 –4 –26], *z*=3.7, $p_{(svc)}$=0.064). In controls, there was no significant correlation between SERT binding and amygdala activity. There were no significant differences in amygdala (ROI) response to angry versus neutral faces between ecstasy users and controls (*p*>0.05; ecstasy: 1.16±0. 92; controls: 0.68±0.72).

Correlations between time since the last use of ecstasy and effects of emotional versus neutral faces. There was a trend toward a negative correlation between the number of days since the last use of ecstasy and activity in the left amygdala (*xyz*=[–22 –6 –30], z=3.8, $p_{(svc)}$=0.067; right amygdala: xyz=[30 4 –30], z=3.3, $p_{(svc)}$=0.204) for angry relative to neutral faces, and in the right amygdala for sad relative to neutral faces (*xyz*=[30 0 –16], z=3.9, $p_{(svc)}$=0.053).

Mediation analysis. The direct effect of the accumulated lifetime intake of ecstasy tablets on amygdala activity during angry face processing, that is, the conditional effect adjusting for SERT binding, was significant (p=0.037; 95% CI 0.01–0.39), whereas the indirect effect was not (p=0.52; 95% CI –0.10 to 0.21; Figure S1) (Supplementary information is available on the *Journal of Psychopharmacology*'s website). Hence, there was no support for the hypothesis that the effect of accumulated lifetime ecstasy use on BOLD responses in the amygdala was mediated by SERT. The total effect, that is, the marginal effect of the accumulated lifetime intake of ecstasy tablets, was significant (p=0.002; 95% CI 0.09–0.39; Figure S1).

Structural MRI results

Ecstasy users and control subjects did not show any significant differences in amygdala gray-matter volume. VBM analysis yielded neither any significant correlations between the accumulated lifetime ecstasy intake and gray-matter volume in the amygdala, nor any significant correlations between the number of days since the last use of ecstasy and amygdala gray-matter volume.

Discussion

To our knowledge, this is the first study to examine the effects of long-term ecstasy use on the neural responses to emotional face expressions. Relative to neutral face stimuli, main effects of emotional processing were found bilaterally in the amygdala, showing increased neural activity, especially in response to fearful and angry faces. This concurs well with a number of studies showing that viewing emotional faces, fearful faces in particular, activates the amygdala (Fusar-Poli et al., 2009). While there was no ecstasy effect on task performance, ecstasy users did, as hypothesized, show higher amygdala activity with increased lifetime ecstasy use during angry face processing; that is, the more ecstasy tablets the ecstasy users had taken during their lifetime, the more activation they displayed in amygdala when watching angry faces. In the ecstasy user group, SERT binding correlated negatively with amygdala activity in response to angry faces. Nonsignificant statistical trends for activity during processing of angry and sad face processing suggested that amygdala activity waned with increasing time since the last intake of ecstasy. Neither the analyses of emotional expressions other than anger nor the whole-brain analysis revealed any significant results.

Thus, our results support the hypothesis that long-term ecstasy use alters the neural basis of emotional face processing. This effect is dose-dependently related to lifetime consumption of ecstasy and appears to be reduced with increased time since last use. Interestingly, the linear relationship was consistently expressed for angry faces but not for other aversive facial expressions. This observation is in line with the results of Bedi et al. (2009) who found that acute MDMA intake alters the amygdala response to angry, but not fearful, facial expressions. The limited sample size of this study does, however, not allow us to conclude that there is not an effect of lifetime ecstasy intake on processing of other aversive facial expressions.

While acute MDMA intake has been shown to diminish amygdala activation (Bedi et al., 2009), we found the opposite effect in long-term ecstasy users. This supports our hypothesis that long-term ecstasy users are in a chronic, albeit potentially reversible, serotonin-depleted state and therefore in accordance with studies showing that serotonin depletion, as induced by acute tryptophan depletion, leads to elevated amygdala activity when processing negative facial expressions (Cools et al., 2005; Van der Veen et al., 2007). When including the lifetime amphetamine use in the model, the effect of the lifetime intake of ecstasy tablets on amygdala activity was no longer significant. This may be due to high correlation between ecstasy and amphetamine use (R^2=0.867). Since the lifetime amphetamine use in itself did not have a significant effect on amygdala activity during angry face processing, our interpretation of the results is that the effect of ecstasy use on emotional processing would be present also in the absence of amphetamine use.

The present study was carried out on a subsample of our previous study sample of chronic ecstasy users (Erritzoe et al., 2011), and we confirmed a negative correlation between SERT binding and accumulated ecstasy use. Hence, it could be speculated that our present fMRI results, showing a positive correlation between lifetime use of ecstasy tablets and left amygdala activity, was mediated by SERT density; that is, a larger lifetime intake of ecstasy tablets was associated with lower SERT binding levels (reflecting serotonin depletion), possibly leading to a higher degree of amygdala activation during angry face processing. In the ecstasy user group, SERT binding was indeed negatively correlated with amygdala reactivity to angry faces, which is in line with Rhodes et al. (2007), showing a negative correlation in the left amygdala between SERT density and activity during emotional face processing. Post hoc mediation analysis did, however, not support the mediation hypothesis, although these results need to be interpreted with caution given the small sample size and hence the low statistical power. In short, our study suggests that there are functional consequences of a chronically depleted serotonin system as indexed by lowered SERT. Of note, an augmented amygdala response to angry faces has also been observed in mood disorders (Stuhrmann et al., 2011) and could within a population with reduced serotonergic tone represent a subclinical vulnerability marker for such conditions.

In line with several other studies (Buchert et al., 2004; McCann et al., 2005; Selvaraj et al., 2009; Semple et al., 1999), we have recently reported (Erritzoe et al., 2011) that recovery of subcortical—but not cortical—SERT availability takes place after termination of ecstasy use. Importantly, here, we found trends showing that days of abstinence from ecstasy correlated negatively with left amygdala activity during angry face processing and with right amygdala activity during sad face processing. Since lifetime use of ecstasy tablets correlated positively with amygdala activity during angry face processing, the trend toward a negative correlation between days of abstinence from ecstasy and amygdala activity during angry face processing might be a potential sign of functional reversibility.

There are limitations to the current study. Because of the cross-sectional nature of our study, it cannot be ruled out that the exaggerated amygdala response to angry faces and/or the low cerebral SERT among heavy ecstasy users represents pre-existing traits associated with an increased preference for the use of ecstasy. We consider this less likely because, as discussed already, interventional animal studies (Axt et al., 1992; Battaglia et al., 1988, 1991; Fischer et al., 1995; Hatzidimitriou et al., 1999; Lew et al., 1996; Molliver et al., 1990; Scanzello et al., 1993; Scheffel et al., 1998) have shown that administration of MDMA lowers cerebral SERT levels, and data from our group (Erritzoe et al., 2011) and others support the presence of an ecstasy dose–response relationship (Buchert et al., 2004; De Win et al., 2008; Kish et al., 2010; McCann et al., 2005; Reneman et al., 2001) and recovery of SERT binding with abstinence from ecstasy (Buchert et al., 2004; McCann et al., 2005; Semple et al., 1999). As for all investigations of the long-term consequences of illicit drug use, especially the use of ecstasy, there will be uncertainties about the precision of the users' reporting of drug use and actual content of substance taken. As explained in more details elsewhere (Erritzoe et al., 2011), hair analysis for MDMA, use of systematic semi-structured questionnaires, and access to systematically acquired data on the content of Danish ecstasy pills in the period of data collection was employed to minimize these factors.

MDMA has several effects on the serotonergic system, such as inhibiting tryptophan hydroxylase, the rate-limiting enzyme for serotonin synthesis, and serotonin degradation by monoamine oxidase B (Gamma et al., 2000). It is possible that it is not the specific effect of MDMA on SERT, but other effects of prolonged MDMA use on the serotonergic neurotransmitter system that mediate the effect of MDMA use on brain responses to emotional faces. MDMA also has noradrenergic and dopaminergic effects (Green et al., 2003) that could affect amygdala activation. An additional limitation of our study is that we did not record hormonal contraception or menstrual-cycle phase for the two females in each group. These factors have been shown to affect face processing (Derntl et al., 2008; Gingnell et al., 2012). However, we do not have any reason to suspect differences in contraceptive use or cycle phase between groups, why the lack of this information is considered as added noise, potentially reducing the power of the study.

In conclusion, these results emphasize the important role of serotonergic neurotransmission in the amygdala for processing angry face expressions. We show that long-term ecstasy use has a dose-dependent effect on the amygdala response to angry faces. Importantly, on the basis of earlier work on amygdala responses to emotional face stimuli after manipulation of serotonin levels, this finding is in support of the hypothesis that recreational use of ecstasy can cause serotonin depletion. The decreased SERT binding among ecstasy users in the current as well as in several previous samples further supports this notion. The fact that changes observed in the current study showed signs, although at a trend-level, of reversibility with sustained abstinence is also in agreement with previous PET/SPECT imaging studies. With the recent focus on MDMA as a potential therapeutic tool in psychiatry (Sessa, 2007), it is important to emphasize that heavy use of the same substance in a recreational setting is associated with functional and molecular—possibly reversible—changes related to serotonergic neurotransmission.

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