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Review

Meta-analysis of molecular imaging of serotonin transporters in ecstasy/polydrug users

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ABSTRACT

We conducted a meta-analysis on the available data from studies investigating SERTs in ecstasy users and polydrug using controls. From 7 studies we compared data from 157 ecstasy users and 148 controls across 14 brain regions. The main effect suggested ecstasy/MDMA related SERT reductions (SMD = 0.52, 95% CIs [0.40, 0.65]; $Z = 8.36$, $p < .01$, $I^2 = 89\%$). A significant effect of subgroups ($X^2 = 37.41$, df = 13, $p < .01$, $I^2 = 65.3\%$) suggested differential effects across brain ROIs. Ecstasy users showed significant SERT reductions in 11 out of the 14 regions, including every neocortical and limbic region analysed. Greatest effects were observed in the occipital cortex (SMD = 1.09, 95% CIs [0.70, 1.48]). No group effects were observed in subcortical areas of the caudate, putamen and midbrain. Literature on Postsynaptic $5HT_{2A}$ receptor imaging was synthesised with these results. We conclude that, in line with preclinical data, serotonin axons with the longest projections from the raphe nuclei appear to be most affected by ecstasy/MDMA use.

Contents

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1. Introduction

Ecstasy (3,4-methylenedioxymethamphetamine) remains one of the most popular illicit recreational drugs in the UK, with a lifetime prevalence of 9.3% (EMCDDA, European Drug Report 2015), and presents a major public health concern given recent increases in reports of MDMA-related deaths and increasing strength of tablets (Global Drugs Survey 2015 report tablets containing upwards of 200 mg of MDMA).

Acute effects of ecstasy/MDMA administration include increases in energy and euphoria (Dumont and Verkes, 2006), amongst other psychological and physiological alterations that are primarily mediated by serotonin and norepinephrine release (Hysek et al., 2011; Hysek et al., 2012). The increase in serotonin neurotransmission following MDMA administration is understood to be produced via action at the serotonin transporter (SERT) (Verrico et al., 2007). Long lasting toxic effects of MDMA on central serotonin neurons have been observed in primates (Ricaurte et al., 1988) and animal research has demonstrated evidence of lasting reductions in markers of central serotonin axons and axon terminals (Ricaurte and McCann, 2001). Moreover, evidence from preclinical anatomical studies suggests that there is an association between axon length and vulnerability to neurotoxic effects of MDMA (Molliver et al., 1990), whereby the longest axons extending from 5-HT neurons are more susceptible to damage from MDMA. Serotonin neurons project from the raphe nuclei which are situated near the midline of the brainstem (Hornung, 2003). As such those neurons with the longest axons are those innervating the cortex, and are thus more susceptible to MDMA related neuroadaptations than those shorter axons innervating subcortical structures (Urban et al., 2012).

It is difficult to make direct comparisons between the selective serotonergic neurotoxicity observed in animal studies using ecstasy/MDMA and those using human participants. Moreover, direct measurement of the effect of ecstasy on human brain serotonin axons is not possible *in vivo*. However molecular imaging of markers for presynaptic SERT and postsynaptic $5HT_{2A}$ receptors is possible in humans using Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT).

Using radio-ligand neuroimaging has enabled the progression of the study of potential ecstasy/MDMA related serotonin axon neuroadaptations. However, there has been much variation in the reported results from molecular imaging studies with currently abstinent (usually for at least 2 weeks prior to testing) ecstasy users and control groups. Inconsistencies can be observed between studies in magnitude of, and regional extent of effect. It would therefore be beneficial to the field to be able to draw definitive conclusions from the gamut of molecular imaging studies conducted in this research area.

Different findings have been observed between studies regarding susceptibility to SERT reductions in the striatum (caudate and putamen) of ecstasy users, for example McCann et al. (2005) report no differences between users and MDMA-naïve polydrug users in these areas using 2 different tracers, and several other studies observe little effect on SERT in these structures (Semple et al., 1999; Kish et al., 2010; Urban et al., 2012). However, Buchert et al. (2003) report reduced cortical SERT Distribution Volume Ratio (DVR) s in ecstasy users relative to polydrug controls. The thalamus has also been reported to show no reduction in SERT in ecstasy users in some cases (Semple et al., 1999; Kish et al., 2010), and significantly reduced SERT relative to controls in others (Buchert et al., 2003; McCann et al., 2005).

Cortical regions are more consistently affected, with ecstasy users regularly showing SERT reductions in the occipital cortex (McCann et al., 1998; McCann et al., 2005; Kish et al., 2010; Urban et al., 2012) as well as reports of increased postsynaptic $5\text{-}HT_{2A}$ receptor binding (Reneman et al., 2000). However not all regions show this consistency. Furthermore, some analyses report significant reductions in global SERT (McCann et al., 1998) for ecstasy users compared to controls, whereas others report more selective regional SERT reductions (McCann et al., 2005; Kish et al., 2010), potentially due to a highly heterogeneous distribution of SERT in the human brain (Kish et al., 2005).

Most studies in these populations suggest that ecstasy use does have some effect on serotonin. However, the regional distribution and magnitude of these effects remain unknown. It is understood that differences in radioligands may contribute to some of the inconsistencies in the literature. For example [^{123}I]ßCIT—is not SERT specific and [^{11}C]McN5652 has been criticised for having a modest specific to non-specific binding ratio (Heinz and Jones, 2000; Kish et al., 2010; Kuikka and Ahonen, 1999). Equally, differences in drug use history will always vary from study to study and are likely to influence findings, particularly as dose is inversely correlated with SERT (McCann et al., 2005). However, potentially the biggest problem for reporting consistent effects in this area is due to many molecular imaging studies having small samples.

Therefore, the aim of the current study was to meta-analyse the available data from molecular imaging studies on the central serotonin markers of presynaptic SERT and postsynaptic $5\text{-}HT_{2A}$ receptor availability, in current (but abstinent at time of testing) ecstasy users and non-user controls. The overall aim was to observe

whether ecstasy/MDMA use has an effect on SERT and 5-HT_{2A} receptor availability, and to provide a comprehensive and consistent understanding and synthesis of the available literature on the effects of MDMA/ecstasy on human brain serotonin function.

2. Methods

2.1. Eligibility criteria

2.1.1. Participants

We included studies that conducted molecular imaging of serotonin transporters (using PET or SPECT) in human ecstasy/MDMA using participants aged over 18 years, who did not have a history of major psychiatric or neurological problems and were abstinent from drug use at the time of testing.

2.1.2. Studies

Studies that conducted molecular imaging of serotonin transporters (using PET or SPECT) and reported molecular imaging outcomes relating to cerebral serotonin transporter binding (presynaptic SERT) and/or cerebral serotonin receptor binding (postsynaptic $5HT_{2A}$ receptors), using Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) were eligible for inclusion in the meta-analysis.

2.1.3. Comparator groups and study design

To be eligible for inclusion, all studies were required to have an ecstasy/MDMA using condition (current users) which was compared to at least one form of control/comparison group, in a between subjects design.

2.1.4. Outcome measure

SPECT and PET studies investigating SERT and $5HT_{2A}$ receptor binding can have a range of outcome measures. These include the simple ratio of binding in a particular region of interest in comparison to a non-binding reference region (Specific Uptake Ratio—SUR). Binding potential (BP_{ND}) may also be calculated when dynamic emission sequences are available, which gives the reader information about specific binding relative to reference tissue. V_3" refers to the specific to non-displaceable equilibrium partition and DVR refers to an outcome that is associated with the equilibrium of the tracer in the brain, which is reported when arterial input function is measured (Gryglewski et al., 2014).

2.2. Data search and extraction

2.2.1. Information sources and search strategy

For the formal search strategy, 3 electronic databases were searched during April 2015. These were: PsychINFO, Scopus and Web of Science. Systematic searches used the key terms 'Ecstasy' OR 'MDMA' AND, 'PET' OR 'SPECT' OR 'molecular imaging'. Manual searches of the reference sections of identified articles and relevant sources were also conducted to supplement the formal electronic search. Further to this, supplementary electronic searches of the 3 databases previously used were undertaken prior to publication with no further articles identified.

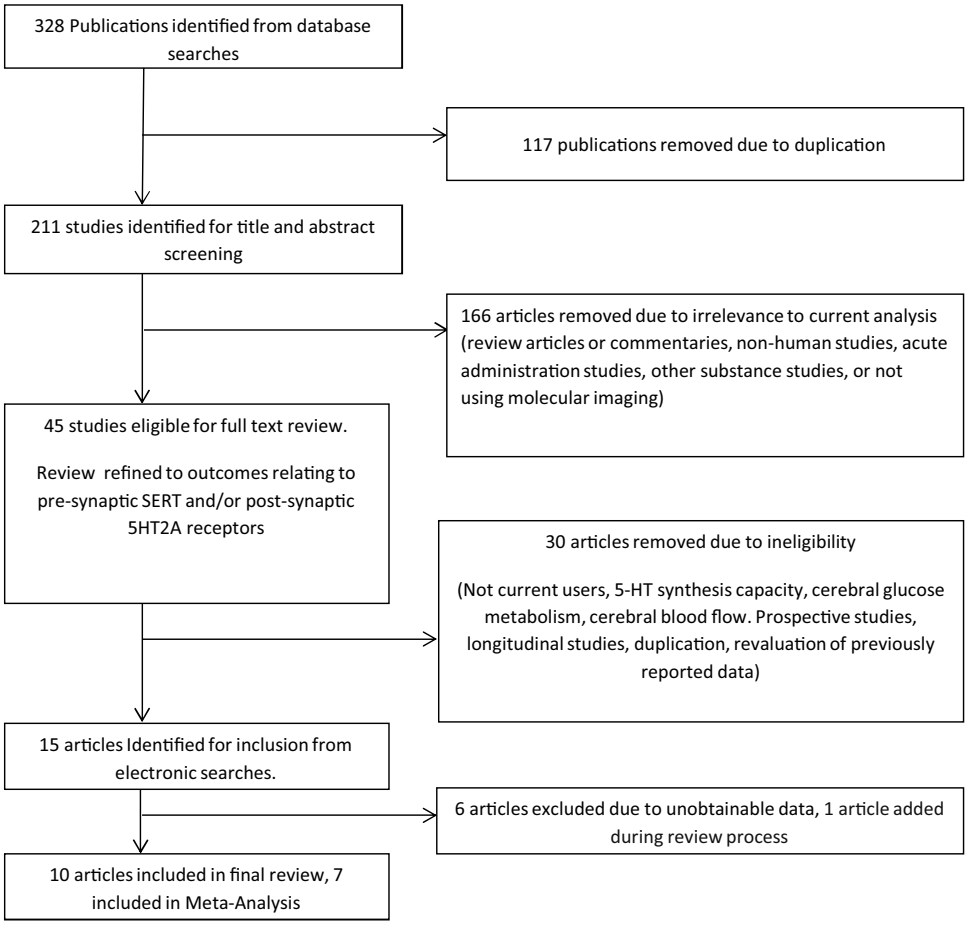

Fig. 1. Meta-analysis search results and flow chart.

2.2.2. Article selection and extraction of data

Independent searches of the electronic databases were carried out by two authors (CR and CM). Both authors were responsible for assessment of articles for inclusion in analysis, with any disagreements resolved by discussion. One author (CR) extracted the relevant data from the studies, which was cross-checked by a second author (CM). In cases where a study met the inclusion criteria but insufficient information was provided to compute the effect size, data was requested from the corresponding author of the paper. Data requests were not met for 6 articles (Reneman et al., 2001a,b; Thomasius et al., 2003; Buchert et al., 2004; de Win et al., 2004, 2008). Therefore, analyses were conducted on data from 7 publications (Fig. 1).

2.2.3. Additional handling of data

Means and Standard Deviations (SDs) were estimated from the figure presented in McCann et al. (1998), using Web Plot Digitizer 3.8 (Rohatgi, 2015). Data presented in Semple et al. (1999) provided separate means and SDs for the left and right side of each brain region measured; for inclusion of this data in the current analysis the authors transformed this data into a mean value for each brain region by combining the mean values for left and right and dividing by 2. The same procedure was used for transforming the SDs.

2.2.4. Data items extracted for individual studies

The following data were extracted from the published articles for analysis separately for each group of participants: outcome for each region reported, age, estimated lifetime dose of ecstasy, time since last use, type of tracer, type of outcome measure, number of participants in group and gender split (Table 1). These variables were recorded in the form they were originally presented. In cases where mean abstinence duration from ecstasy was not reported, minimum abstinence required for inclusion in the study was recorded. In cases where mean lifetime doses of ecstasy use were not reported, an estimate was calculated if possible from the data available, for example McCann et al., 2005 report usual dose and lifetime exposures, in cases such as this an estimate lifetime dose was calculated by multiplying these values. Ecstasy user groups could broadly be defined by two categories (current users, former users). Control groups would also generally fall within 3 categories, for example "polydrug controls" refers to groups that were MDMA naïve but were recruited due to them having some degree of matching for use of other drugs. 'Controls with some polydrug use' refers to groups who were not recruited explicitly due to them having used illicit drugs, yet use of illicit drugs had not resulted in exclusion of participants. Drug naïve controls generally allowed for use of legally available drugs such as nicotine and alcohol.

2.3. Statistical and subgroup analysis

Standardised Mean Difference (SMD) and Standard Error (SE) of the SMD between experimental conditions were calculated separately for reported brain regions in each study. Individual SMDs were synthesised by meta-analysis using the method of generic inverse variance (random effects assumed) in the software package RevMan 5.2 (The Nordic Cochrane Centre, Copenhagen). The magnitude of effects of SMDs can be interpreted thus: 0.2 = a small effect, 0.5 = a moderate effect and 0.8 = a large effect (Higgins and Green, 2011) SMDs were employed due to the main outcomes of interest in PET and SPECT studies on SERT binding and serotonin receptor binding having several possible outcome measures.

SMD allows for variation in outcome measures, by estimating the differences between 2 experimental conditions on an outcome variable, and dividing the difference by the pooled SDs of the outcome variable.

Table 1
Summary of studies included in the meta-analysis.

Author (Year)	Method	Tracer	Outcome measure	Controls				Ecstasy users				
				Control type	*n*	F%	Age	*n*	F%	Age	Lifetime dose (tablets)	Time since last use
McCann et al. (1998)	PET	[11C]McN5652	DVR	Controls with some poly	15	40	28.3	14	33.33	26.6	880.8	19 weeks
Semple et al. (1999)	SPECT	[123I]ß-CIT	SUR	Polydrug controls	10	0	24.2	10	0	25.5	672	18 days
Buchert et al. (2003)	PET	[11C]McN5652	DVR	Polydrug controls	29	48.28	24.4	30	50	24.5	827	24 days
McCann et al. (2005)	PET	[11C]DASB	DV spec	Controls with some poly	19	57.89	26	23	43.48	22.04	173.56	4.74 months
Kish et al. (2010)	PET	[11c]DASB	BP_{ND}	Controls with some poly	50	50	26	49	42.86	25.9	206	14 days min
Urban et al. (2012)	PET	[11c]DASB	BP_{ND}	Controls with some poly	13	38.46	27.3	13	38.46	30.8	–	5.7 weeks
Frojaer et al. (2014)	PET	[11c]DASB	BP_{ND}	Controls with some poly	32	21.88	35.3	18	11.11	24.5	–	11 days min

Table 2
Meta-analysis summary table by brain region.

Region	Studies	Total *n*	SMD	*p* from Meta-Analysis
Frontal Cortex	3	148 (73 ecstasy users)	0.54	0.002
Midbrain	5	246 (117 ecstasy users)	0.13	0.36
Parietal Cortex	4	196 (99 ecstasy users)	0.97	0.0003
Temporal Cortex	4	196 (99 ecstasy users)	0.94	<0.00001
Occipital Cortex	5	216 (109 ecstasy users)	1.09	<0.00001
Caudate	6	275 (139 ecstasy users)	0.19	0.15
Putamen	6	275 (139 ecstasy users)	0.22	0.11
Thalamus	6	275 (139 ecstasy users)	0.31	0.002
Anterior Cingulate	4	187 (95 ecstasy users)	0.48	0.0001
Hippocampus	3	167 (85 ecstasy users)	0.57	0.006
DLPFC	2	68 (36 ecstasy users)	0.83	0.02
Posterior Cingulate	2	62 (33 ecstasy users)	0.80	0.03
Amygdala	2	68 (36 ecstasy users)	0.35	0.01
Insula	2	125 (63 ecstasy users)	0.62	0.06

2.3.1. Analytic strategy

The meta-analysis was conducted by separating effect sizes from each study into brain regions, as specified in individual studies. We examined the main effect and also formal subgroup analyses, whereby each brain region appraised in 2 or more studies was considered a sub-group. This main analysis was conducted on the 7 articles that investigated SERT availability and was a comparison of current ecstasy users binding vs polydrug controls/controls with some polydrug use. One study (Urban et al., 2012) reported both presynaptic SERT binding and postsynaptic $5HT_{2A}$ receptor binding, as such the 10 papers yielded 7 that were suitable for SERT binding comparison and 4 that were eligible for postsynaptic $5HT_{2A}$ receptor binding assessment. Due to the low number of articles eligible for postsynaptic $5HT_{2A}$ receptor analysis, and differences in brain regions investigated, we provide a narrative synthesis rather than formal analysis of these studies.

One publication (McCann et al., 2005) conducted SERT binding analysis using 2 different tracers, within the same sample—as such, only data using the tracer [^{11}C]DASB is included in the meta-analysis. This choice is based upon [11C]DASB having important advantages over [^{11}C]McN5652 in detecting SERT density, namely, a greater specific-to-nonspecific equilibrium activity ratio (McCann et al., 2005). A high correlation between the two tracers ($r = 0.97$) justified inclusion of only [^{11}C]DASB. Most studies included 2 conditions and as such potentially contributed one comparison per brain region to main analysis. Moreover, from the 7 studies deemed acceptable for analysis of SERTs, it was noted that only one of these (Buchert et al., 2003) employed a drug naïve control group (and also a former ecstasy user group). However, this study also employed a polydrug control group. Therefore, it was decided, that analysis should focus on control groups that had some drug use (polydrug controls and controls with some polydrug use). Consequently, all between group comparisons in the main meta-analysis have some degree of matching for use of other drugs. This controls for concomitant use of other drugs, which is regularly reported in the literature in this area. We conducted random effects models due to high heterogeneity across studies.

3. Results

3.1. Study selection

Initial literature searches yielded 80 papers in the Scopus database, 41 papers in PsychINFO and 207 papers from Web of Science. After removing duplicated papers a total of 211 papers remained. Following a brief review of the remaining articles' titles and abstracts, a further 166 were excluded as they were not relevant to the current analysis. Excluded papers included review articles or commentaries (32), non-human studies (68), acute administration studies (10), other substance/not serotonin studies (16), no use of molecular imaging (24), non-substance populations (12) and case studies (4). Following this 45 full articles were reviewed. Studies were removed at this stage if they did not have a control group or did not have a current user group, investigated serotonin synthesis capacity or cerebral glucose metabolism and cerebral blood flow. Prospective studies on novice ecstasy users, as well as longitudinal studies using a within groups design were also excluded at this stage. Following adherence to these exclusion criteria, 15 studies remained. Due to unavailable data in 6 studies, and a further eligible study identified during the review process final assessment was conducted on data from 10 publications (7 contributing data to the meta-analysis).

3.2. Overview

3.2.1. Participant characteristics

Individual study information including sample sizes and participant characteristics are given in Table 1.

3.3. Pre synaptic SERT meta-analysis

Data from 7 published studies (Table 1) were included in analyses of presynaptic SERT binding. From these there was a total of 157 ecstasy/MDMA users (7 studies), 19 polydrug controls (2 studies), 129 controls with some polydrug use (5 studies) and 29 drug naïve controls (1 study). One study also included a former ecstasy/MDMA sample, which provided a total of 29 participants.

3.3.1. Meta-analyses

Included studies examining presynaptic SERT binding in ecstasy users vs polydrug/controls with some polydrug use had investigated various ROIs. Each brain region that was investigated by 2 or more studies was identified as a subgroup for meta-analysis. The test for overall effect was significant (SMD = 0.52, 95% CIs [0.40, 0.65]; $Z = 8.36$, $p < .01$, $I^2 = 89\%$) which demonstrated significant SERT reductions ecstasy users versus polydrug using controls. There was also a significant effect of subgroups ($X^2 = 37.41$, df = 13, $p < .01$, $I^2 = 65.3\%$) suggesting differential effects across brain ROI (Fig. 2 and Table 2). Individual analyses are reported below.

3.3.2. Frontal cortex

Three studies with a total of 73 ecstasy users and 75 controls assessed SERT binding in the frontal cortex. A significant difference was observed between the two comparison groups (SMD = 0.54, 95% CIs [0.19, 0.89]; $Z = 3.04$, $p < .01$; $I^2 = 71\%$), suggesting SERT reductions in ecstasy users relative to controls.

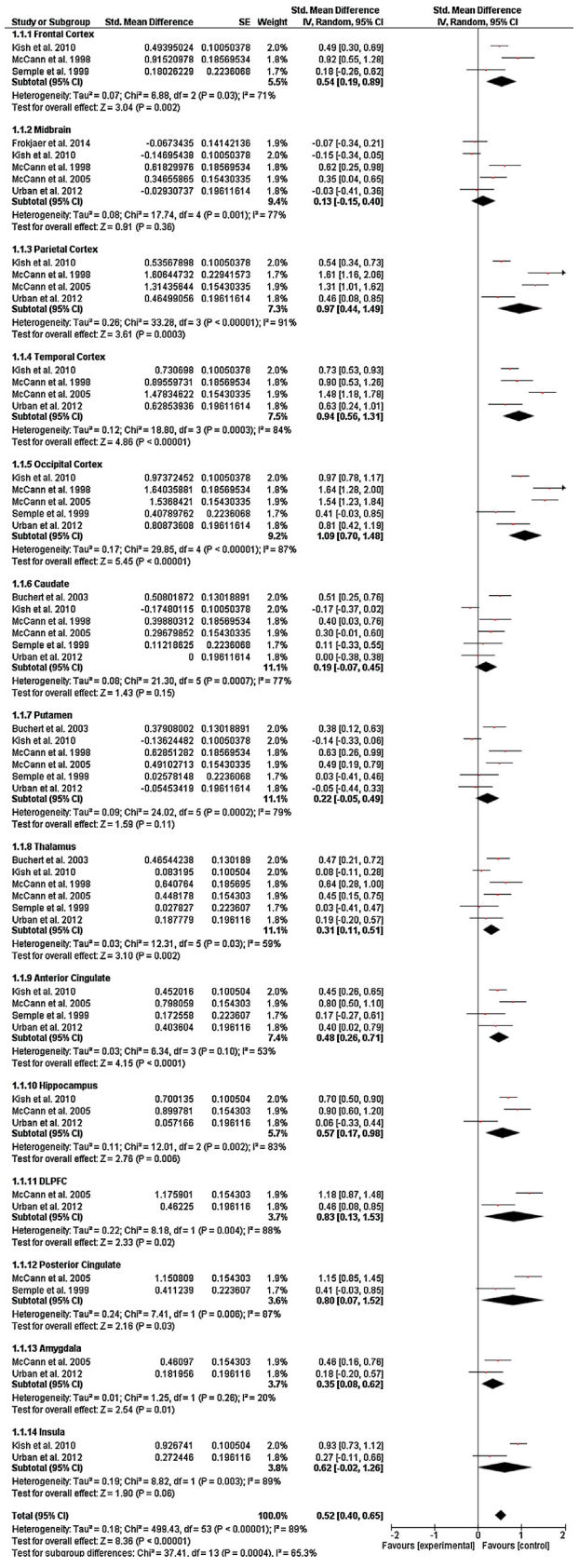

Fig. 2. Forest plot of studies assessing SERT by brain region. I^2 is an indicator of heterogeneity between comparisons. Inverse variance meta-analysis using standardized mean differences.

3.3.3. Midbrain

There were 5 studies that assessed SERT in the midbrain, with a total of 117 ecstasy users and 129 controls. No significant difference in SERT was observed between groups in this brain region (SMD = 0.13, 95% CIs [−0.15, 0.40]; $Z = 0.91$, $p > .05$; $I^2 = 77\%$).

3.3.4. Parietal cortex

Four studies reported SERT values in the parietal cortex, there were 99 ecstasy users in total and 97 controls. There was a significant between group difference in this region (SMD = 0.97, 95% CIs [0.44, 1.49]; $Z = 3.61$, $p < .01$; $I^2 = 91\%$). Ecstasy users display reduced SERT relative to controls here.

3.3.5. Temporal cortex

Four studies assessed SERT in the temporal cortex, with a total of 99 ecstasy users compared to 97 controls. Analysis in this area showed a significant difference (SMD = 0.94, 95% CIs [0.56, 1.31]; $Z = 4.86$, $p < .01$; $I^2 = 84\%$), with ecstasy users displaying reduced SERT relative to controls.

3.3.6. Occipital cortex

Five studies gave SERT values in the occipital cortex. One hundred and nine ecstasy users were compared to 107 controls. Again, there were significant differences between the two groups (SMD = 1.09, 95% CIs [0.70, 1.48]; $Z = 5.45$, $p < .01$; $I^2 = 87\%$) which reflect reduced SERT in ecstasy users compared to controls.

3.3.7. Caudate

Six studies investigated SERT in the caudate, providing a total of 139 ecstasy users and 136 controls. The difference between groups in this region was non-significant (SMD = 0.19, 95% CIs [−0.07, 0.45]; $Z = 1.43$, $p > .05$; $I^2 = 77\%$).

3.3.8. Putamen

Six studies assessed SERT in the putamen (139 ecstasy users vs 136 controls), and again no differences were observed between groups in the meta-analysis (SMD = 0.22, 95% CIs [−0.05, 0.49]; $Z = 1.59$, $p > .05$; $I^2 = 79\%$).

3.3.9. Thalamus

Six studies were included in analysis of SERT in the thalamus, giving a total of 139 ecstasy users and 136 controls. Analysis showed that there were significant between group differences in the thalamus (SMD = 0.31, 95% CIs [0.11, 0.51]; $Z = 3.10$, $p < .01$; $I^2 = 59\%$), whereby ecstasy users have reduced SERT relative to controls.

3.3.10. Anterior cingulate

A total of 4 studies assessed SERT in the anterior cingulate, totalling 95 ecstasy users versus 92 controls. The difference between these groups was statistically significant (SMD = 0.48, 95% CIs [0.26, 0.71]; $Z = 4.15$, $p < .01$; $I^2 = 53\%$), again ecstasy users have reduced SERT relative to controls.

3.3.11. Hippocampus

Three studies reported SERT comparisons in the hippocampus (total $n = 85$ ecstasy users, 82 controls). Differences in SERT were significant (SMD = 0.57, 95% CIs [0.17, 0.98]; $Z = 2.76$, $p < .01$; $I^2 = 83\%$) with ecstasy users displaying reduced SERT here compared to controls.

3.3.12. DLPFC

Two studies were included in analysis of DLPFC SERT, with a total of 36 ecstasy users and 32 controls. The difference in SERT in the DLPFC between users and controls was statistically significant (SMD = 0.83, 95% CIs [0.13, 1.53]; $Z = 2.33$, $p < .01$; $I^2 = 88\%$)

whereby ecstasy users show reduced SERT.

3.3.13. Posterior cingulate

Two studies were used to evaluate SERT in the posterior cingulate, providing a total of 33 ecstasy users and 29 controls. The meta-analysis revealed a significant between group difference here (SMD = 0.80, 95% CIs [0.07, 1.52]; $Z = 2.16$, $p < .05$; $I^2 = 87\%$) again ecstasy users display reduced SERT compared to controls here.

3.3.14. Amygdala

Two studies were entered into the meta-analysis for comparison of SERT in the amygdala, providing a total of 36 ecstasy users and 32 controls. This analysis showed a significant difference between the two groups (SMD = 0.35, 95% CIs [0.08, 0.62]; $Z = 2.54$, $p = .01$; $I^2 = 20\%$). Ecstasy users show reduced SERT in the amygdala.

3.3.15. Insula

Two studies had available data for analysis of SERT in the insula, these provided a total of 62 participants in the ecstasy user group and 63 participants in the control group. The difference between these two groups was approaching significance (SMD = 0.62, 95% CIs [−0.02, 1.26]; $Z = 1.90$, $p = .06$; $I^2 = 89\%$), again ecstasy users show reduced SERT in the insula relative to controls.

3.4. Evidence of publication bias

Visual examination of a funnel plot suggested asymmetry in study effects. Funnel plot asymmetry is often associated with publication bias (Light and Pillemer, 1984), which is something often reported as problematic in research into ecstasy related harm (Cole, 2014). However, interpretation of asymmetry may be difficult due to a number of issues including small sample sizes (Stern and Harbord, 2004), such as those observed in many types of imaging studies, including those involved in the current analysis and high heterogeneity.

In order to further examine any potential publication bias and small study effects we performed a test for an excess of significant findings (Ioannidis and Trikalonis, 2007). We estimated the average statistical power for all the effect sizes used in our analysis based on the fixed-effects point estimate separately for each region (overall statistical power = 46.48%). Based on this level of power we would expect 25.09 of the 54 effect sizes in the meta-analysis to be significant, yet the observed number of effect sizes that were significant was 34. The difference between observed and expected was beyond chance ($p = 0.08$, two-sided), thus there was an excess of observed significant findings in the meta-analysis which may be attributable to publication bias.

3.5. Post-synaptic $5HT_{2A}$ receptor binding

The systematic literature search process yielded 4 papers for analysis of $5HT_{2A}$ receptor binding. One of these assessed both presynaptic SERT and post-synaptic $5HT_{2A}$ binding, using PET and so was assessed in the main meta-analysis above. However, as there were only 4 papers assessing postsynaptic binding matching our review criteria, two studies using a drug naïve control group (Reneman et al., 2000, 2002), one using a control group with some polydrug use (Urban et al., 2012) and one assessing a polydrug comparison group (Di Iorio et al., 2012), there was little scope for comparison groups. Adding to this complication is that there was little consistency between studies in brain regions identified for comparison. As such a formal meta-analysis was inappropriate for ecstasy users versus controls in postsynaptic $5HT_{2A}$ receptor binding. Nevertheless, the data from these studies is reviewed here and synthesised with the findings from the meta-analysis. For regional distribution volumes of SERT and 5-HT_{2A} receptors see Table 3.

Table 3
Ranked (from highest to lowest) regional distribution volumes of SERT (as per Parsey et al., 2006) and 5-HT_{2A} receptors (as per Adams et al., 2004).

SERT	5-HT_{2A}
Midbrain	Superior medial frontal cortex
Ventral striatum	Occipital cortex
Thalamus	Dorsolateral prefrontal cortex
Dorsal putamen	Ventral lateral prefrontal cortex
Dorsal caudate	Parietal cortex
Insula	Superior temporal cortex
Hippocampus	Orbito-frontal cortex
Cingulate	Medial inferior temporal cortex
Anterior cingulate	Sensory motor cortex
Temporal lobe	Anterior cingulate gyrus
Medial prefrontal cortex	Insula
Occipital lobe	Putamen/pilladus
Orbital prefrontal cortex	Thalamus
Dorsolateral prefrontal cortex	Caudate nucleus
Cerebellar grey matter	Amygdala/hippocampus

Reneman et al. (2000) studied cortical 5-HT_{2A} receptor densities in five currently abstinent MDMA users compared to 9 healthy age and education matched control subjects. It was observed that overall binding ratios were higher in the MDMA group than controls and this difference was statistically significant in the occipital cortex. The authors suggest that this increase in receptors is a result of 5-HT depletion, as this leads to upregulation of 5-HT_{2A} receptors in animal studies in an attempt to maintain homeostasis. They also cite their finding in the occipital cortex to be in line with work showing severe MDMA related 5-HT depletion in the occipital cortex in primates (Scheffel et al., 1998). This finding is intriguing, given that in the current meta-analysis ecstasy/MDMA related reductions in SERT showed the greatest effect in the occipital cortex (SMD = 1.09). So this area is potentially particularly sensitive to ecstasy/MDMA induced neurotoxicity. However it is acknowledged by Reneman and colleagues that their study has a small sample and thus should be treated as pilot data.

More recently Urban et al. (2012) investigated neocortical post-synaptic 5-HT_{2A} receptor integrity in 13 current and recently detoxified MDMA users and 13 age, ethnicity and gender matched healthy controls. Individual region analysis revealed significant increase in 5-HT_{2A} receptor binding in ecstasy users compared to controls in the DLPFC and in the parietal cortex. However these did not survive correction for multiple comparisons. There were no observed correlations between 5-HT_{2A} receptor availability and clinical parameters of ecstasy use history (e.g. time since last use). As in the former studies, the authors suggest that 5-HT_{2A} receptor upregulation is a possible response to sustained 5-HT depletion. It is observed that higher binding was observed in the neocortex, where SERT density is lower in this study (SERTs were also analysed by Urban et al. and this data is included in the current meta-analysis). Indeed cortical regions display consistent SERT reductions in the current meta-analysis in ecstasy users compared to controls.

Finally, Di Iorio et al. (2012) assessed 5-HT_{2A} receptor binding in 14 female MDMA users, who had a minimum of 2 weeks drug abstinence prior to scanning and 10 female non-MDMA exposed controls who had some cannabis and alcohol exposure. The greatest difference was reported in the temporal cluster, whereby MDMA users had 20.5% higher binding than controls. A further interesting finding was that lifetime MDMA dose was positively and significantly correlated with receptor binding in 4 cortical clusters (frontoparietal, occipitotemporal, frontolimbic and frontal regions). Di Iorio et al. (2012) suggest their results extend those of Reneman et al. (2000) to multiple cortical regions and show a lack of recovery with extended abstinence and a dose-response effect. However this is a female only cohort, and is another study that has a small sample size.

In line with a lack of recovery, Reneman et al. (2002) observed significantly higher 5-HT_{2A} binding in 7 ex MDMA users (min

of 2 months abstinence), compared to 11 drug naïve controls in the occipital cortex using [^{123}I]R91150 SPECT. Moreover, a significant correlation was observed between cortical 5-HT$_{2A}$ receptor binding and duration of abstinence, but not with lifetime dose of MDMA. Conversely, recent MDMA users (n = 17 min abstinence 1 week) had lower [^{123}I]R91150 binding ratios than controls in all cortical regions examined (frontal, parietal and occipital cortices). Reneman et al. (2002) suggest that MDMA use results in a sub-chronic down regulation of 5-HT$_{2A}$ receptors, and a chronic up-regulation compensating for low synaptic 5-HT. In the context of the other work reported here on post synaptic 5-HT$_{2A}$ receptor binding, these findings suggest that 5-HT$_{2A}$ receptor adaptations in ecstasy users are very complex and thus interpretation of findings requires caution.

The overall findings from the post-synaptic 5-HT$_{2A}$ receptor binding can be synthesised with the findings from the meta-analysis to suggest that MDMA may have an effect on serotonin system function. Indeed, postsynaptic receptor levels seem to show less of an effect in subcortical regions. This is also analogous to subcortical regions (putamen, caudate, midbrain) showing no difference between users and controls in the meta-analysis. Many of the studies on receptor binding have small numbers and so may be criticised for being unreliable. However the studies reviewed here, appear to corroborate the findings of MDMA related adaptations to cortical SERT in ecstasy users subject to meta-analysis.

4. Discussion

The results from this meta-analysis demonstrated significant reductions in pre-synaptic SERT availability in ecstasy/MDMA users versus controls. Our subgroup analysis on different ROIs demonstrated significant reductions in brain regions including: the frontal cortex, parietal cortex, temporal cortex, occipital cortex and dorsolateral prefrontal cortex, anterior cingulate, posterior cingulate, hippocampus, amygdala, insula and the thalamus. The overall effect size was moderate with large heterogeneity. However the subgroup analysis was also significant, suggesting that differences in effect size between groups varied by subgroup (brain region), which suggests that not all regions that were analysed showed the effect of ecstasy related SERT reduction.

The systematic literature search produced 7 published articles, with available data, for inclusion in a meta-analysis of presynaptic SERT availability in ecstasy users compared to an MDMA, but not drug naïve control sample. Data from a total of 157 ecstasy users, and 148 controls was included in analysis, suggesting mean group sizes of 22 and 21 respectively per imaging study. Although these are typical molecular imaging study sample sizes, it is understandable that detection of small group differences has been unreliable in the literature (Gryglewski et al., 2014). To this end this meta-analysis has successfully synthesised results from studies assessing SERT availability and the results are therefore more conclusive than individual reports.

Each brain region included in the analysis that could be described as being in the neocortex produced a significant difference between groups, whereby ecstasy users displayed reduced SERT compared to controls (frontal cortex, parietal cortex, temporal cortex, occipital cortex and dorsolateral prefrontal cortex). The greatest effect was observed in the occipital cortex, which is unsurprising as this has regularly been reported as an area of significance for reduced SERT in ecstasy users(McCann et al., 1998, 2005; Kish et al., 2010; Urban et al., 2012) as well as postsynaptic 5-HT$_{2A}$ receptor binding (Reneman et al., 2000). It is worthy of note that 4 of these neocortical areas (occipital cortex, parietal cortex, temporal cortex and DLPFC) are the 4 regions showing the greatest effect sizes in the whole analysis. This is consistent with the preclinical research suggesting that axon length is associated with greater vulnerability to neuroadaptations with MDMA (Molliver et al., 1990). Moreover it is interesting that studies that isolated the DLPFC have a greater effect size than those that considered the frontal cortex as a region of interest, as this is in line with findings of Urban et al. (2012) who suggest that even within cortical regions, differences can be observed with dorsal regions, which are associated with longer axon lengths showing greater effects than medial regions. Neuroadaptation in the DLPFC is also consistent with behavioural data, suggesting that executive functioning is impaired in ecstasy users relative to controls (e.g. Montgomery et al., 2005) and recent functional Near-Infrared Spectroscopy research suggesting alteration to blood flow in the DLPFC of ecstasy users relative to controls (Roberts and Montgomery, 2015).

Similarly, all regions analysed that could be categorised as making up part of the limbic system showed significant differences between groups in the current analysis (anterior cingulate, posterior cingulate, hippocampus, amygdala, insula). These regions are not covered in detail in the postsynaptic 5-HT$_{2A}$ receptor imaging literature as they are only examined in Urban et al. (2012), who observed no significant differences between users and controls. However the same pattern of results was observed in the same paper's SERT data, which is included in the meta-analysis. Furthermore, 5 of the 6 papers that data were not available for, did not examine any limbic areas (Reneman et al., 2001a,b; Thomasius et al., 2003; de Win et al., 2004, 2008), whereas one paper (Buchert et al., 2004) observed MDMA related SERT reductions in the posterior cingulate and the hippocampus, which is consistent with the findings of the meta-analysis.

Conversely, to the neocortex and the limbic areas, the thalamus was the only subcortical region that showed significant between group differences. No differences were observed between ecstasy users and controls in the caudate, putamen (striatal cortex) or the midbrain. These, findings from the meta-analysis are interesting if they are placed alongside the papers that were omitted from analysis due to unavailable data. Indeed Buchert et al. (2004) suggest reductions in the left caudate in current ecstasy users relative to controls and Thomasius et al. (2003) also observed reduced SERT availability in the caudate nucleus of current ecstasy users. These findings suggest that the results on subcortical areas may not be as decisive as the meta-analysis concludes. However data from these studies was not made available for inclusion. Nevertheless the current authors assume the findings from the current analysis to be robust given the amount of studies included. Moreover, there is a good theoretical basis to expect that SERT rich areas such as the striatum may not show large reductions relative to controls. As mentioned previously preclinical data suggest that longer axon projections from serotonin neurons may be more susceptible to MDMA related damage than shorter projections (Molliver et al., 1990). Therefore, because the caudate, putamen and midbrain are relatively close to the raphe nuclei, they may be more robust to the damaging effects of MDMA on their axons. This is supported by animal models of MDMA related neurotoxicity, whereby serotonergic neural regeneration is selective for centrally located neuronal structures (Fischer et al., 1995).

While this meta-analysis shows clear evidence of neuroadaptation following ecstasy use, it cannot provide confirmation regarding regeneration or reversibility of MDMA related effects on central serotonin axons. PET scans using [11C]DASB were conducted in former MDMA users with at least 1 year abstinence (Selvaraj et al., 2009) to observe whether SERT availability could normalise over time. It was reported that former users showed normal SERT as compared to polydrug and drug naïve controls. However, this study did not incorporate a current user group into analysis, and therefore the results need to be treated with caution. Perhaps well designed

longitudinal studies would be better able to answer questions about the reversibility of the effects captured in our meta-analysis.

There are several limitations to the current analysis. First of all, as we were limited to results from 7 studies, it is of course possible that data that was unavailable to us at the time of analysis could alter the current findings. The studies identified generally had low statistical power (see above) and together provided a small number of estimates for some ROIs. Therefore, well powered studies examining all ROIs are required to confirm the findings reported. Furthermore, due to the limited number of studies, it was not appropriate to conduct a meta-regression, in which correlations between drug use (lifetime dose, recency of use, frequency of use) and SERTs could be observed. However there are several reports of SERT binding correlating significantly with lifetime dose and years of use (Thomasius et al., 2003; Buchert et al., 2004; McCann et al., 2005; Kish et al., 2010).

Concomitant use of other drugs is regularly identified as a confounding factor in research in ecstasy users. It is often argued that use of other drugs is at least partly responsible for effects seen in ecstasy using populations. It is for this reason that the decision was taken to conduct analysis on ecstasy users compared to controls that are at least matched in some way for use of other drugs. Furthermore, radiotracers used in studies included in this meta-analysis, should have a reasonable selectivity for SERTs. Other co-used drugs are not known for their effects on 5-HT, for example alcohol use is not associated with SERT alterations in humans (Martinez et al., 2009) and cannabis and cocaine are not known for toxic effects on 5-HT axons. Moreover polydrug users have often been observed to show no differences in SERT binding compared to drug naïve controls (Buchert et al., 2004; Selvaraj et al., 2009).

Many of the studies included in the current analysis used small samples, which is potentially a reason for the asymmetrical funnel plot observed with the data. However small samples can also lead to concerns over the reliability of the data. Reliability concerns and variability in effects of ecstasy/MDMA on SERT and 5-HT_{2A} receptors from molecular imaging studies suggested that a meta-analysis was necessary, using all of the available data, so to draw more concrete conclusions about ecstasy/MDMA related serotonergic neuroadaptations.

Finally, concerns have been raised about the reliability of β-CIT-SPECT and [11C]McN-5652 for detecting neocortical SERT density (Heinz and Jones, 2000; Parsey et al., 2000). These areas have comparatively low densities of SERT relative to subcortical regions and thus have a worse signal-to-noise ratio. Moreover, these two radio tracers, whilst showing SERT specific binding in the brainstem and thalamus, do not show SERT specific binding in the cerebral cortex. As such, the reliability of some of the early work in this area using these radio-ligands need interpreting with caution. Moreover, 3 original articles included in the current analysis used these less reliable radio-ligands, and is therefore a limitation for interpretation of the current analysis.

To conclude, our meta-analytic investigation suggests that SERT availability is reduced significantly in current ecstasy users relative to polydrug controls/controls with some polydrug use in all areas of the neocortex and limbic system, with areas requiring longer axon projection being most vulnerable to toxicity. The meta-analysis also suggests that SERT rich regions of the subcortex (putamen, caudate and midbrain) are unaffected by ecstasy use. Review of the post-synaptic 5-HT_{2A} receptor data suggests that there may also be some compensatory upregulation of receptors in neocortical areas. The clinical significance of these findings is speculative, however it is conceivable that the observed effects of SERT and 5-HT_{2A} receptors contribute to mood disorders associated with ecstasy/MDMA use, as well as other psychobiological sequelae, such as altered neuroendocrine function. Furthermore the observed effects on the serotonin system inferred from the current analysis, may underpin the many neurocognitive deficits observed in ecstasy users.

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