The effects of acutely administered MDMA on spontaneous brain function measured with arterial spin labelling and BOLD resting-state functional connectivity

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Abstract (words =250)

3,4-methylenedioxymethamphetamine (MDMA) is a potent monoamine releaser that produces an acute euphoria in most individuals. Here MDMA was orally administered to 25 healthy individuals in a double-blind, placebo controlled, balanced order study. Arterial spin labelling (ASL) and seed-based resting state functional connectivity (RSFC) were used to generate spatial maps of changes in cerebral blood flow (CBF) and RSFC after MDMA. Participants underwent two ASL scans and two BOLD scans in a scanning session and there were two such sessions (MDMA and placebo), separated by one week. Only decreased CBF was observed after MDMA and this was localised to the right medial temporal lobe (MTL), the thalamus, the inferior visual cortex and the somatosensory cortex. Decreases in right amygdala and hippocampal CBF correlated with subjective ratings of the intensity of MDMA's effects. The RSFC results complemented the CBF results: using a ventromedial prefrontal cortex (vmPFC) seed, decreased vmPFC-posterior cingulate cortex (PCC) RSFC was observed under MDMA; using a hippocampal seed, decreased hippocampal-mPFC RSFC was found and the magnitude of this effect correlated positively with ratings of both the intensity and euphoric nature of the MDMA experience; and using an amygdala seed, increased amygdala-hippocampal RSFC was found and the magnitude of this correlated positively with the intensity ratings. Thus, together, the MTLs appear to be specifically implicated in the mechanism of action of MDMA. These outcomes are discussed in the context of recent efforts to evaluate the potential of MDMA as an adjunct to psychotherapy for post-traumatic stress disorder (PTSD).

Introduction

MDMA is a serotonin (5-HT) dopamine (DA) and noradrenaline (NA) releaser (Rothman et al., 2001). It is also a popular recreational drug that is valued by users for to its acute pro-social and euphoriant properties (Holland, 2001). It has been administered in human research on a number of occasions (de la Torre et al., 2000; Harris et al., 2002; Vollenweider et al., 1998) but few studies have investigated its acute effects on brain function using fMRI (Bedi et al., 2009; Kuypers et al., 2011; Ramaekers et al., 2009) or other neuroimaging modalities (Frei et al., 2001; Gamma et al., 2000; Lansbergen et al., 2011).

MDMA has a relatively unique profile of subjective effects; described as a hybrid between a stimulant and psychedelic (Gouzoulis-Mayfrank et al., 1996). MDMA acts at DA, NA and 5-HT transporters to inhibit reuptake and stimulate release; however, its greater action at the serotonin transporter differentiates it from most other stimulants (Bradbury et al., 2013) and accounts for much (but not all) of its euphoriant effects (Liechti and Vollenweider, 2001; van Wel et al., 2012). Although the pharmacology of MDMA is reasonably well understood, relatively little is known about its systems-level effects on brain activity, e.g. in terms of changes in brain network dynamics. MDMA has recently been investigated as a potential adjunct to psychotherapy in the treatment of PTSD with positive, albeit preliminary, outcomes (Mithoefer et al., 2011; Mithoefer et al., 2013).

Despite significant developments in resting-state fMRI in recent years (Fox and Raichle, 2007) there have been no resting-state fMRI studies on the acute effects of MDMA. Here we combined ASL and RSFC to address this knowledge gap. ASL provides a quantitative measure of CBF or perfusion (Detre and Alsop, 1999) and RSFC measures functional coupling between spatially distributed brain regions via spontaneous fluctuations in the BOLD signal (Biswal et al., 1995). Combining these complementary techniques can yield

important new information about how a drug alters brain activity to produce its characteristic subjective effects (Carhart-Harris et al., 2012). Given MDMA's recognised acute pro-social and positive mood effects, we predicted changes in CBF and RSFC in brain systems implicated in social and affective processing, namely limbic structures and midline cortical regions, such as the medial prefrontal cortex (mPFC). CBF changes were assessed at a whole-brain level and the choice of RSFC seeds was informed by the CBF results and aforementioned hypotheses.

Methods

Design

This was a within-subjects, double-blind, randomised, placebo-controlled study. Participants were scanned twice, 7 days apart, once after MDMA and once after placebo. The study was approved by NRES West London Research Ethics Committee, Imperial College London's Joint Compliance and Research Office (JCRO), Imperial College's Research Ethics Committee (ICREC), IMANOVA Centre for Imaging Science and Imperial College London's Faculty of Medicine, and was conducted in accordance with Good Clinical Practice guidelines. A Home Office Licence was obtained for the storage and handling of a Schedule 1 drug and Imperial College London sponsored the research.

Participants

The study involved 25 healthy participants (mean age 34 ± 11, 7 females) with at least 1 previous experience with MDMA. None of the participants had used MDMA for at least 7 days and other drugs for at least 48 hours, and this was confirmed by a urine screen. An alcohol breathalyser test confirmed that none of the participants had recently consumed alcohol. Participants had used MDMA an average of 35 (±51) times before (range = 1 to 200) and the mean time since last use was 1400 (±2351) days (range = 7 to 7300 days).

Participants were screened for general health, MR-compatibility and present mental health. Screening involved routine blood tests, electrocardiogram, heart rate, blood pressure and a brief neurological exam. Other drug use parameters were as follows (values are means ± SD (range)): Alcohol weekly units 12.8 ±10.2 (0-35), daily cigarettes 1.7 ±4.6 (0-20), cannabis lifetime uses 267.4 ±323 (0-1000+), LSD lifetime uses 27.3 ±100 (0-500), psilocybin lifetime uses 9.5 ±20 (0-100), ketamine lifetime uses 21.6 ±46.7 (0-200), mephedrone lifetime uses 3.6 ¬7.4 (0-30), amphetamine lifetime uses 17.8 ±36.9 (0-150), cocaine lifetime uses 49.6 ±152 (0-750). Participants had mean Beck Depression scores of 3.9 ±4.8 (0-18) and Spielberger Trait Anxiety scores of 31.7 ± 5.9 (20-46). All subjects were deemed physically and mentally healthy at the time of study entry and none had any history of drug or alcohol dependence.

Scanning parameters

MR images were acquired on a 3T Siemens Tim Trio (Siemens Healthcare, Erlangen, Germany) using a 32-channel phased array head coil. Anatomical reference images were acquired using the ADNI-GO recommended MPRAGE parameters (1mm isotropic voxels, TR = 2300ms, TE = 2.98ms, 160 sagittal slices, 256x256 in-plane resolution, flip angle = 9 degrees, bandwidth = 240Hz/pixel, GRAPPA acceleration = 2). T2*-weighted echo-planar (EPI) images were acquired for the resting state functional scan using 3mm isotropic voxels in a 192mm in-plane FOV, TR=2s, echo time = 31ms, 80 degree flip angle, 36 axial slices in each TR, bandwidth = 2298 Hz/pixel, and a GRAPPA acceleration of 2. 180 volumes were acquired during the functional imaging paradigm which took 6 minutes to complete and there were 2 of these resting state scans.

ASL parameters

ASL flow maps were acquired using the Q2TIPS pulsed ASL preparation with a 2D EPI readout (Luh et al., 1999). At each inflow time, 16 5-mm slices were imaged in a 240mm FOV, 14ms TE, 64x64 matrix, 2500ms TR, and 2232 Hz/pixel bandwidth, giving 3.8mm in-plane resolution. Thirteen alternating tag-control pairs plus an initial M0 calibration volume were acquired in one minute and 15 seconds for each inflow time. Seven inflow times from 750ms to 1650ms in steps of 150ms were taken.

Drug and dosing parameters

There were 2 ASL and 2 BOLD resting-state scans during each 60 minute functional scanning session. The first ASL scan began 50 minutes after oral administration of 100mg encapsulated MDMA-HCl and on a separate occasion, placebo (100mg MDMA-.HCl encapsulated ascorbic acid/vitamin-C) and the second ASL scan began 103 minutes after capsule ingestion. Participants simply relaxed with their eyes closed during the ASL and BOLD resting state scans. The first resting-state BOLD scan took place 60 minutes after capsule ingestion and the second resting-state BOLD scan occurred 113 minutes after capsule ingestion. Peak subjective effects were reported 100 minutes post administration of MDMA, generally consistent with the plasma t-max of MDMA (Kolbrich et al., 2008). The order of MDMA and placebo administration was counterbalanced. Note: behavioural paradigms not relevant to the present report were performed in the (~40 minute) period between the first and second pair of ASL and BOLD resting-state scans.

Insert figure 1 here

Subjective ratings

Subjects gave ratings of the intensity of the subjective effects of MDMA using a simple visual analogue scale (VAS) completed via button press in the scanner. There was a bottom

anchor of ‘no effects’ (or 0%) and a top anchor of ‘extremely intense effects’ (100%). The rating bar defaulted on ‘no effects’ and could be moved up in increments of 5% via button press (middle finger) and down in 5% increments via button press with the index finger. Subjects rated the intensity of the subjective effects before and after each resting state scan.

Subjects also completed a more extensive list of VAS-style items to assess more specific subjective effects. This was completed 4.5 hours after capsule ingestion, when most of the subjective effects of MDMA had subsided. Some items were tailored to refer to commonly reported subjective effects of MDMA, expressed in colloquial terms (e.g. ‘I felt loved up’), and others were items used in previous research with psilocybin (Carhart-Harris et al., 2012), selected in order to assist comparisons with this classic ‘psychedelic’ state. The VAS scales had a bottom anchor of ‘no, not more than usually’ and a top anchor of ‘yes, much more than usually’ with the exception of a control item that read ‘I felt entirely normal’. In this case, the bottom anchor read ‘No, I experienced a different state altogether and the top anchor read ‘Yes, just as I usually feel’. There were 32 items in total in this questionnaire and its basic format was based on the APZ questionnaire for altered states of consciousness (Dittrich, 1998).

Correlation analyses

Correlations between changes in CBF and RSFC and subjective ratings of effects intensity and positive affect were performed on data from anatomically defined ROIs. Pearson’s *r* was used to calculate the statistical significance of correlations and statistical thresholds were corrected for multiple comparisons using Bonferonni correction. To look at positive affect, we collapsed five items related to positive mood effects into one single factor, taking the mean for these items for each subject. These items were: ‘I felt amazing’, ‘I felt loved-up’, ‘I felt energised and enthusiastic’, ‘I felt a profound inner peace’, ‘I felt an inner warmth’.

Tests were corrected for multiple comparisons (.05/10 for the ASL analyses because correlations with 5 regions and 2 different subjective rating parameters were explored, and .05/2 for the RSFC analyses because the 3 seed-based analyses were independent but 2 different subjective rating parameters were explored for each). For the correlations using positive affect ratings, all data points were included in the analyses; however, five participants gave ratings of zero for effects intensity while on MDMA in the scanner, despite reporting noticeable subjective effects on exiting it, and so these were considered null and removed.

ASL analysis

The ASL time series for each inflow time scan was motion corrected using rigid body translation/rotation and then registered to each other using AFNI tools (http://afni.nimh.nih.gov/afni). For each inflow time scan, the tag and control time series were interpolated to the TR, subtracted and averaged. CBF and arterial arrival times were quantified by fitting a general kinetic model (Buxton et al., 1998) to the resulting multi-inflow time data using a non-linear fitting routine. The M0 of blood was estimated from the white matter signal in the M0 calibration scans assuming a ratio of the proton density of blood to that in white matter R=1.06 (Wong et al., 1998). Other parameters used in the model were infow time blood=1.7, T*2 blood=0.1 s (Silvennoinen et al., 2003), T*2 white-matter=0.047s (Wansapura et al., 1999) and q=1 (Wong et al., 1998). The quantified CBF maps were registered to each individual's structural scan using transformations calculated from the original EPI data and were converted into standard MNI space using FLIRT within the FMRIB Software Library (FSL: http://www.fmrib.ox.ac.uk/fsl). The free software 'MRIcron' was used to produce the images shown in figure 3.

Functional connectivity analysis

Three separate 'seed'-based functional connectivity analyses were performed using three a priori defined regions: the ventromedial prefrontal cortex (vmPFC), bilateral hippocampal and bilateral amygdala seeds. The vmPFC mask was the same as one previously used in an analysis involving psilocybin (Carhart-Harris et al., 2012) to assist comparisons with this drug. The hippocampal mask was constructed by combining the right and left hippocampal structures and parahippocampal gyrus (anterior and posterior divisions) and thresholding this mask by 50% using the Harvard-Oxford probabilistic atlas. The same approach was taken to construct the bilateral amygdala mask, combining the left and right amygdala and setting a threshold of 50% probability. Mean time series were derived from these masks for each subjects' 4 scans (i.e. placebo rest 1 and 2 and MDMA rest 1 and 2). Time series from spheres placed in the white matter (WM) and cerebrospinal fluid (CSF) were acquired, as was the average gray matter (GM) signal from a whole brain GM mask.

General linear modelling (GLM) was used to assess between-condition (i.e. drug versus placebo) differences in functional connectivity. At the single subject level, a high-pass filter of 100 seconds was used, each volume was spatially smoothed (5mm FWHM) and motion regressors were added to the model. Each model contained 4 explanatory variables: the time series from the region of interest (ROI) or seed (e.g. the vmPFC, hippocampus or amygdala) plus the GM, WM and CSF time series. The vmPFC was chosen based on it being implicated in depression (Mayberg et al., 2005) and in order for us to make a comparison with a previous psilocybin study which used this seed (Carhart-Harris et al., 2012), and the hippocampus and amygdala were chosen based on the results of the ASL analysis. The first temporal derivative was included in the GLM and first level contrasts were set-up to display effects in clusters where there was positive coupling to the ROI. The functional images were registered to each individual's T1 anatomical scan and then to a standard brain.

For the higher-level analyses, maps were produced to reveal where in the brain was significantly coupled to each ROI. Each subject's two placebo resting state scans were included in the GLM and a mixed-effects ordinary least squares analysis was run to produce maps using a default cluster threshold of $Z > 2.3$, $p < 0.05$. To compare the MDMA and placebo conditions, all of the subject's scans were included as inputs to the model and the MDMA condition was simply contrasted with the placebo condition using the same thresholds as above. Of potentially 100 resting-state scans, only 2 could not be analysed due to excessive movement. MRIcron was used to produce the displayed maps.

Results

Basic subjective and physiological effects

The self-rated intensity of MDMA's subjective effects was variable across subjects. Five subjects failed to notice any subjective effects during the scanning period, whereas three gave maximal ratings indicating 'extremely intense effects'. Peak drug effects were reported 100 minutes after ingestion of MDMA (the intensity was rated at 52% ± 32, range = 0-100%, 0% = 'no effects' and 100% = 'extremely intense effects') coinciding with the beginning of the second ASL scan (beginning 103 minutes after capsule ingestion). However, the average intensity remained relatively consistent throughout the scanning period, i.e. intensity was rated at 44% ± 35 at the end of the first ASL scan and 43% ± 32 at the end of the second BOLD scan. Most volunteers reported positive mood effects after MDMA and items pertaining to positive mood were among the highest scored (e.g. the item 'I felt amazing' was the highest rated item under MDMA, fig. 2).

ASL results

Subtracting the two ASL scans post MDMA administration from the two ASL scans post placebo revealed robust decreases in CBF after MDMA. The images shown in figure 3 were

produced using cluster-correction (2590 voxels) to adjust for multiple comparisons and a whole-brain corrected statistical threshold of $p < 0.05$. At this threshold, only decreases in CBF were observed and these were localised to the regions shown in figure 3. Increases in CBF could only be observed at an unacceptable statistical threshold of $p < 0.3$ uncorrected.

When contrasts were split so that the effect of MDMA in the first and second ASL scans could be observed separately, consistent maps were revealed, with only decreases in CBF after MDMA. The decreases were slightly more marked and of a greater spatial extent in the second ASL scan than the first. These maps can be viewed in the supplementary material (figure S1).

Correlations between CBF effects and subjective ratings

Regions showing the most marked reductions in CBF under MDMA included the visual cortex, thalamus, somatosensory cortex, right hippocampus and right amygdala. Thus, correlational analyses were restricted to these ROIs. Masks were derived from an anatomical atlas and CBF changes in the relevant regions were correlated with self-ratings of the intensity of MDMA's subjective effects. Significant positive correlations were observed between the magnitude of the CBF decreases in the right amygdala ($p = .002$) and right hippocampus ($p = .004$) under MDMA and the subjective intensity of the drug effects (figure 4). Correcting for multiple comparisons gave a revised statistical threshold of $p < .005$ (.05/10) and thus these correlations survived this threshold. Since the amygdala and hippocampus are limbic structures known to be involved in affective processing, we also examined correlations between the CBF changes and ratings of positive affect after MDMA and although correlations were in the predicted direction, no significant relationships were found.

Potential relationships between the visual cortex CBF decreases and ratings of altered visual perception were also examined, as was somatosensory cortex CBF decreases versus ratings of unusual bodily sensations, but no significant relationships were found.

vmPFC BOLD resting state functional connectivity results

Regions positively coupled (warm colours) to the vmPFC seed (red region) at baseline can be seen in the top row of figure 5. When coupling under MDMA (scans 1 & 2) was contrasted against coupling under placebo (scans 1 & 2), significant increases (warm colours) and decreases (cool colours) under MDMA were observed.

Hippocampal BOLD resting state functional connectivity results

Regions positively coupled (warm colours) to the bilateral hippocampal seed (red regions) at baseline (placebo resting-state scans 1 & 2) can be seen in the top row of figure 6. Increases (warm colours) and decreases (cool colours) in hippocampal coupling can be seen in the rows below.

Amygdala BOLD resting state functional connectivity results

Regions positively coupled (warm colours) to the bilateral amygdalae seed (red regions) baseline can be seen in the top row of figure 7. Significant increases (warm colours) and decreases (cool colours) in amygdalal coupling can be seen in the rows below.

Correlations between functional connectivity effects and subjective ratings

Correlational analyses for the RSFC analyses were restricted to regions that showed the most marked changes in coupling in each of the three seed-based analyses – and selection was further constrained by specific questions informed by previous literature on functional relationships between coupling parameters and psychological phenomena (Andrews-Hanna

et al., 2010; Berman et al., 2011). Specifically, we looked at vmPFC-PCC coupling (where significant decreases in RSFC were observed under MDMA), hippocampal-vmPFC coupling (decreased RSFC under MDMA), and amygdala-hippocampal coupling (increased RSFC under MDMA). Correcting for multiple comparisons (05/2 = .025), significant correlations were found for changes in coupling strength between the bilateral hippocampi seeds and the vmPFC and ratings of elevated mood after MDMA ($p = .011$) and intense subjective effects ($p = .0097$) i.e., the greater the decreases in coupling under MDMA, the more intense and positive were the subjective effects (fig 8). A significant relationship was also found for increases in amygdala-hippocampal coupling under MDMA and ratings of intensity of effects ($p = .020$). The relationships between changes in vmPFC-PCC coupling and subjective intensity and positive mood after MDMA were all in the predicted direction but non-significant.

Examining the influence of subject motion

In addition to including motion parameter time courses as confound variables in the first level general linear models, we also explored between-subject differences in motion to rule this out as a possible explanation of the connectivity results. There was significantly more movement in the MDMA than placebo scans (t-test, p = .003). However, the magnitude of this difference was very small, i.e. the mean relative movement per volume in the RSFC scans was .072±.04mm under placebo and 0.099±.08mm under MDMA. Crucially, Pearson correlation did not reveal any significant relationships between subject motion and vmPFC-PCC, hippocampus-vmPFC or amygdala-hippocampus RSFC. Finally, the significant correlations between subjective ratings and connectivity measures were unchanged when variance associated with motion was partialled out.

Discussion

This is the first resting state fMRI study on the acute effects of MDMA on human brain function. The MTLs were particularly implicated in the drug's effects; decreased CBF was seen in the amygdala and hippocampus which correlated with the intensity of the drug's subjective effects, and decreased hippocampal-vmPFC and increased amygdala-hippocampal RSFC was also found. The magnitude of the former correlated with both the intensity and euphoric nature of MDMA's effects, whereas the magnitude of the latter correlated only with intensity ratings.

The CBF decreases after MDMA were localised to the sub-calcarine visual cortex, pre-SMA, somatosensory cortex, superior frontal gyrus, midbrain/brainstem, thalamus, hippocampus/parahippocampus and amygdala. It was inferred that the visual cortex effects may have been related to the mild visual-perceptual effects of MDMA but there were no correlations with ratings of relevant subjective items. Nevertheless, it is interesting to note that the 5-HT_{1B}R is especially densely expressed in the sub-calcarine domain of the visual cortex (Varnas et al., 2011). Thus, MDMA-released endogenous 5-HT may have stimulated this particular 5-HT receptor in this region to have produced the relevant CBF decreases.

The decreases in CBF in the MTLs were one of the most intriguing results of this study, particularly since the magnitude of these decreases correlated positively with subjective intensity ratings (fig 4). The MTL structures receive a dense serotonergic innervation (Wilson and Molliver, 1991) and 5-HT is found in higher concentrations in the hippocampus than DA and NA (Reader et al., 1989). The hippocampus (Kohler et al., 1986) and amygdala (Costes et al., 2005) express post-synaptic 5-HT_{1A} receptors in high concentrations, endogenous 5-HT has a relatively high affinity for 5-HT_{1A} receptors (Paterson et al., 2013) and the effect of 5-HT stimulation of 5-HT_{1A}Rs is hyper-polarisation and a decrease in cell firing rate (Andrade, 2011). Thus, while other 5-HT receptors are expressed in the

hippocampus, amygdala and parahippocampus e.g. the 5-HT_7R and 5-HT_{2A}R (Hoyer et al., 1986; Varnas et al., 2004), it is reasonable to infer that the marked decreases in MTL CBF were due to an effect of 5-HT on inhibitory post-synaptic 5-HT_{1A}Rs.

Elevated limbic activity is a reliable characteristic of anxiety states (Engel et al., 2009). Serotonergic anxiolytic medications such as selective serotonin reuptake inhibitors and the direct 5-HT_{1A}R agonist buspirone, are thought to achieve their therapeutic action via stimulation of inhibitory post-synaptic 5-HT_{1A}R receptors, thereby normalising limbic hyperactivity (Gordon and Hen, 2004). MDMA also appears to have anxiolytic properties, as illustrated by the subjective ratings shown in figure 2 and previous reports of pro-social behaviours under the drug (Bedi et al., 2010; Holland, 2001; Hysek et al., 2012c). Thus, given MDMA's potent serotonin releasing properties (Rothman et al., 2001), it can be inferred that the reduced limbic CBF observed here, is related, at least in part, to the drug's positive mood effects. Contradicting the role of 5-HT_{1A} receptors in MDMA's mechanism of action however, is the negative finding that pindolol pre-treatment failed to significantly attenuate the drug's subjective effects (van Wel et al., 2012); however, pindolol is a partial agonist that may not provide effective blockade of post-synaptic 5-HT_{1A} receptors (Artigas et al., 2001; Romero et al., 1996). Pre-treatment studies with potent and selective antagonists are required to elucidate the specific receptor subtypes mediating the decreases in CBF.

In addition to the ASL outcomes, the RSFC analyses also yielded robust results. For example, the decreases in vmPFC-PCC coupling under MDMA are of interest given recent evidence that vmPFC-PCC coupling is positively associated with rumination in depression (Berman et al., 2011). On this basis, we had predicted that the decreases in vmPFC-PCC coupling would correlate with the drug's positive mood effects but although there was a trend in this direction, it was not significant. Decreased vmPFC-PCC RSFC has also been

found with psilocybin (Carhart-Harris et al., 2012), a non-selective 5-HT_{2A} receptor agonist with potent consciousness-altering properties. Psilocybin promotes an unconstrained style of cognition that is the inverse of ruminative thinking in depression. Participants described a similar liberation of cognition and imagination under MDMA (fig 2) and this may be related to the decrease in vmPFC-PCC coupling. In future research with MDMA, it would be interesting to incorporate pre-treatment with a selective 5-HT_{2A}R antagonist to test the involvement of this specific receptor on the drug's effects. The 5-HT_{2A}R is highly expressed in both the mPFC and PCC (Erritzoe et al., 2009) and 5-HT_{2A}R blockade was found to significantly attenuate the positive mood effects of both MDMA (van Wel et al., 2012) and psilocybin (Kometer et al., 2012).

Regarding other circuitry implicated in MDMA's action, decreased mPFC-hippocampal RSFC was observed (Figs 5 & 6). The uncinate fasciculus connects the vmPFC and MTL structures (Gutman et al., 2009) and other indirect connections (e.g. via the retrosplenial cortex and ventral PCC) likely account for the substantial baseline functional connectivity between these regions (top rows in Figs. 5 & 6). Research in rodents has shown that the mPFC exerts a top-down inhibitory influence on limbic activity (Hariri et al., 2000), often observed in the context of emotional control (Rosenkranz et al., 2003). Interestingly, these regions have also been implicated in the pathophysiology of PTSD. For example, patients with pronounced dissociative symptoms exhibit elevated mPFC and reduced limbic responses to trauma cues (Lanius et al., 2010) presumably due to an exaggerated influence of the mPFC on the MTLs (Lanius et al., 2012). MDMA has recently begun to be formally investigated as an adjunct to psychotherapy for PTSD (Mithoefer et al., 2011; Mithoefer et al., 2013). It is claimed that MDMA makes it easier for patients to cope with the distress of reliving their trauma when required to do so in psychotherapy (Mithoefer et al., 2013). Like limbic hyperactivity, increased coupling between the mPFC and hippocampus is a marker of

anxiety states (Adhikari et al., 2010). Here we found a significant positive correlation between the magnitude of the decreases in mPFC-hippocampal coupling under MDMA and ratings of positive mood/euphoria (Fig 8). Thus, MDMA may achieve both its euphoric and potentially therapeutic effects, at least in part, by decreasing mPFC-hippocampal coupling.

While many aspects of the RSFC results are interesting, we have focused on those effects that were especially marked and relevant to previous work. Thus, the final effect deserving of special attention is the increased coupling between the amygdalae and the hippocampi under MDMA (Fig 7). Like the decrease in mPFC-hippocampal RSFC, the magnitude of the increases in amygdala-hippocampal RSFC correlated with the subjective intensity of MDMA's effects. In an important recent study, decreased amygdala-hippocampal RSFC was found in patients with PTSD, relative to non-PTSD combat veterans (Sripada et al., 2012). The authors speculated that this may relate to an impaired ability to contextualise affective information in the patients. This lends support to the idea that MDMA may be useful in PTSD treatment because it implies that the drug may *reverse* this particular biomarker of PTSD. Although speculative, the increase in amygdala-hippocampal coupling under MDMA may allow patients to add contextual richness to their traumatic memories, ensuring that raw, under-processed memories (e.g. encoded in the amygdala) are not left isolated from a narrative (conferred by the hippocampus). Moreover, the combination of MDMA with supportive psychotherapy may work to prolong any potential benefit from increased amygdala-hippocampal communication. Further work is required test both the safety and efficacy of MDMA in PTSD and the specific mechanisms by which it may be effective. However, the results of the present study indicate that the limbic system may be specifically implicated in any therapeutic action of the drug.

There have been no previous resting state fMRI studies on MDMA but a steady state positron emission tomography (PET) study measured CBF after 119mg/70kg MDMA in 16 healthy volunteers (Gamma et al., 2000). Since the conditions were not equivalent to the present study's (e.g. participants performed a low level cognitive task during many of the scans), it is difficult to make a comparison. Some decreases in CBF were observed in the thalamus, amygdala and somatosensory cortex but increases in CBF (in the orbitofrontal cortex, visual cortex and cerebellum) were also observed. In another PET study of a pro-serotonergic agent, intravenous d-fenfluramine was administered during steady-state cognition and increased frontal cortical and decreased thalamic CBF was observed (Meyer et al., 1996). In terms of consistencies with the present study's outcomes, both of these previous studies found decreased thalamic CBF post-administration of serotonin releasers. However, given the methodological differences, only cautious comparisons can be made.

Although this was the largest and most advanced acute MDMA human imaging study to date, it has some limitations. We did not incorporate 'retroicor' to correct for the physiological variance (Glover et al., 2000). However, this process had a negligible effect on the results when previously applied to psilocybin fMRI data (Carhart-Harris et al., 2012). Similarly, the breath-hold paradigm incorporated into the psilocybin fMRI design to test for drug-vascular interactions did not indicate any modulatory influence with this serotonergic agent. Even so, given the hemodynamic nature of the ASL and BOLD signals, it remains at least theoretically possible that some of the effects observed in the present study were the product of a direct vascular action of MDMA or (released) serotonin, and the design would have benefited from the inclusion of retroicor and/or a breath-hold paradigm. However, contradicting a direct vascular action of the drug is the fact that the effects observed were localised to functionally meaningful brain regions (e.g. the MTLs) rather than being global in extent or proximal to regions with a high vascular input. That the imaging outcomes

correlated with the subjective ratings also further reduces the likelihood that they are mere artefacts of a direct vascular action.

Another limitation is the lack of a pharmacological pre-treatment component to our design. There have now been several pre-treatment studies with MDMA in humans (Hysek et al., 2012a; Hysek et al., 2012b; Hysek et al., 2013; Hysek and Liechti, 2012; Hysek et al., 2011; Hysek et al., 2012d; Hysek et al., 2010; Liechti et al., 2001; Liechti et al., 2000) and the next stage would be to look at the influence of specific receptor blockade on the imaging outcomes reported in this paper. Only by doing this, will we be able to make confident inferences about the pharmacology underlying the effects presented in this paper.

To conclude, the present study was the first to use fMRI to directly address the question of how MDMA works on the human brain to elicit its characteristic subjective effects. The results revealed decreased CBF in MTL regions, decreased RSFC between the mPFC and hippocampus and increased RSFC between the amygdala and hippocampus, all of which correlated with the drug's subjective effects. Thus, MTL regions are specifically implicated in the mechanism of action of MDMA. Further research with MDMA and related drugs are required to extend what has been learned here.

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Conflict of interest

The authors declare no conflict of interest.

Figure 1. Schematic showing scanning protocol: Placebo (vitamin-C) or MDMA-HCl (100mg) was ingested at time zero and the first ASL scan occurred 50 minutes after. This was a repeated measures design and the two scans (placebo and MDMA) occurred one week apart and the scan order was counterbalanced so that half of the volunteers received MDMA the first scan and half in the second.

Figure 2. Subjective effects of MDMA: Items were rated were rated 4 hours after drug administration. Participants were instructed to complete them with reference to the peak drug effects (where applicable). The items marked with an asterisk were rated significantly higher after MDMA than placebo ($p < 0.001$, Bonferonni correction for multiple comparisons). Shown are the mean ratings for 25 participants plus the positive standard errors from the mean.

Figure 3. MDMA decreases CBF in the right hippocampus and amygdala, the visual cortex, pre-SMA, superior frontal gyrus and primary somatosensory cortex. Displayed are regions of significantly less CBF under MDMA (scans 1 + 2) versus placebo (scans 1 + 2). These images are cluster-corrected giving a whole brain corrected statistical threshold of $p < 0.05$. The blue lines intersecting a single coronal and sagittal slice indicate the planar position of all of the slices displayed in the figure. Slices were chosen that revealed significant effects.

Figure 4. Decreased right amygdalal and hippocampal CBF predicts intense subjective effects after MDMA. Values on the y-axis were derived from subtracting the CBF from the first and second ASL scans under MDMA from the first and second ASL scans under

placebo. Values on the x-axis are ratings from the first and second ASL scans under MDMA. Correlations were performed using Pearson's r and a corrected p value of < 0.005 was used. Note that the decreases in CBF under MDMA versus placebo increase in magnitude from left to right. Thus, the greater the decreases in CBF in the amygdalae and hippocampi under MDMA, the more intense were the drug's subjective effects.

Figure 5. Changes in vmPFC RSFC under MDMA. The image at the top reveals positive coupling (warm colours) with the seed region (red) at baseline (the average of the 2 placebo scans). The images below reveal areas where there were significant increases (warm colours) and decreases (cool colours) in the MDMA RS scans versus the placebo RS scans. Images are cluster corrected at a threshold of $z > 2.3$, $p < 0.05$. The blue lines intersecting the axial and sagittal sections at the far right of two of the rows indicate the planar position of the presented slices. Slices were selected based on the presence of significant effects.

Figure 6. Changes in hippocampal/parahippocampal RSFC under MDMA. The image at the top reveals positive coupling (warm colours) with the seed region (red) at baseline (the average of the 2 placebo scans). The images below reveal areas where there was a significant increase (warm colours) and decreases (cool colours) in the MDMA RS scans versus the placebo RS scans. Images are cluster corrected at a threshold of $z > 2.3$, $p < 0.05$. The blue lines intersecting the axial and sagittal sections at the far right of two of the rows indicate the planar position of the presented slices. Slices were selected based on the presence of significant effects.

Figure 7. Changes in amygdala RSFC under MDMA. The image at the top reveals positive coupling (warm colours) with the seed region (red) at baseline (the average of the 2 placebo scans). The images below reveal areas where there was a significant increase (warm

colours) and decreases (cool colours) in the MDMA RS scans versus the placebo RS scans. Images are cluster corrected at a threshold of $z > 2.3$, $p < 0.05$. The blue lines intersecting the axial and sagittal sections at the far right of two of the rows indicate the planar position of the presented slices. Slices were selected based on the presence of significant effects.

Figure 8. Decreased hippocampal-vmPFC coupling predicts intense and euphoric effects after MDMA. The x-axis of the top chart expresses the change in bilateral hippocampal (red) coupling with the vmPFC (cool colour) after MDMA versus placebo and the y-axis expresses each subjects' rating of the intensity of MDMA's subjective effects rated in the scanner after their first and second BOLD RS scan. The x-axis of the bottom chart expresses the change in hippocampal-vmPFC coupling as described above and the y-axis expresses each subjects' average score for 5 items referring to the positive mood effects of MDMA ('I felt amazing', 'I felt loved-up', 'I felt energised and enthusiastic', 'I felt a profound inner peace' and 'I felt an inner warmth'). Both correlations were significant after correction for multiple comparisons ($p < 0.02$). Note that the decreases in coupling under MDMA relative to placebo increase in magnitude from left to right. Thus, the greater the decrease in hippocampal-vmPFC coupling under MDMA, the more intense and euphoric were its subjective effects.

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