Accepted Manuscript

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PII: S0006-8993(09)01474-7
DOI: doi:[10.1016/j.brainres.2009.07.040](https://doi.org/10.1016/j.brainres.2009.07.040)
Reference: BRES 39441

To appear in: *Brain Research*

Received date: 23 March 2009
Revised date: 2 July 2009
Accepted date: 15 July 2009

Please cite this article as: Gloria M.P. Roberts, Liam Nestor, Hugh Garavan, Learning and memory deficits in ecstasy users and their neural correlates during a face-learning task, *Brain Research* (2009), doi:[10.1016/j.brainres.2009.07.040](https://doi.org/10.1016/j.brainres.2009.07.040)

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Title

Learning and memory deficits in ecstasy users and their neural correlates during a face-learning task

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Number of text pages of whole manuscript: 19 + references

Number of figures: 2

Number of tables: 4

Abstract

It has been consistently shown that ecstasy users display impairments in learning and memory performance. In addition, working memory processing in ecstasy users has been shown to be associated with neural alterations in hippocampal and/or cortical regions as measured by functional magnetic resonance imaging (fMRI). Using functional imaging and a face-learning task, we investigated neural correlates of encoding and recalling face-name associations in 20 recreational drug users whose predominant drug use was ecstasy and 20 controls. To address the potential confounding effects of the cannabis use of the ecstasy using group, a second analysis included 14 previously tested cannabis users (Nestor et al., 2008). Ecstasy users performed significantly worse in learning and memory compared to controls and cannabis users. A conjunction analysis of the encode and recall phases of the task revealed ecstasy-specific hyperactivity in bilateral frontal regions, left temporal, right parietal, bilateral temporal, and bilateral occipital brain regions. Ecstasy-specific hypoactivity was evident in the right dorsal anterior cingulate cortex (ACC) and left posterior cingulate cortex. In both ecstasy and cannabis groups brain activation was decreased in the right medial frontal gyrus, left parahippocampal gyrus, left dorsal cingulate gyrus, and left caudate. These results elucidated ecstasy-related deficits, only some of which might be attributed to cannabis use. These ecstasy-specific effects may be related to the vulnerability of isocortical and allocortical regions to the neurotoxic effects of ecstasy.

Section: Cognitive and Behavioral Neuroscience

Keywords: fMRI, ecstasy, cannabis, memory, face-name associations.

1. Introduction

The most pronounced deficits in ecstasy users appear in learning and memory abilities (Bolla et al. 1998; Parrott and Lasky 1998; Morgan et al., 2000; Rodgers et al., 2000; Bhattachary and Powell 2001; Zakzanis and Young 2001; Gouzoulis-Mayfrank et al., 2003; McCardle et al. 2004) and evidence suggests that a neurotoxic effect of ecstasy on widespread systems may be responsible for these cognitive impairments (Hatzidimitriou et al., 1999; McCann et al., 2000; Reneman et al., 2001; Giorgi et al., 2005; Tamburini et al., 2006; Frenzilli et al., 2007; Buscetti et al., 2008). Studies using functional magnetic resonance imaging (fMRI) confirmed speculations proposed by Gouzoulis-Mayfrank et al. (2003) that the memory dysfunction of ecstasy users might be related to hippocampal dysfunction given that the hippocampus plays a fundamental role in forming new associations or episodic memories (Sperling et al., 2001; Crane and Milner, 2002; Zeineh et al., 2003). For example, Daumann et al. (2005) provided evidence of abnormal hippocampal functioning in ecstasy users during face-profession associations. Jacobsen et al. (2004) reported that adolescent ecstasy users had significantly prolonged reaction times during tests of selective and divided attention and failed to deactivate the left hippocampus during high verbal working memory load compared to matched ecstasy-naive controls.

Polydrug use typically makes it difficult to attribute impairments unambiguously to the ecstasy use of human users. Daumann and colleagues (Daumann et al., 2003a) reported that both heavy and moderate polydrug ecstasy users showed stronger

activations than control participants in the parietal cortex during a working memory task, and heavy users had a weaker fMRI signal than moderate users and control participants in the temporopolar area and to a lesser extent in frontal regions. In a subsequent study, with a group of ecstasy users without significant concomitant use of other illicit drugs and two matched groups of polydrug ecstasy users and nonusers (Daumann et al., 2003b), pure ecstasy users showed a stronger fMRI signal in the parietal cortex compared to both control groups when performing a working memory n-back task. Furthermore, pure ecstasy users revealed lower activations than control participants and/or polyvalent users most notably in inferior temporal regions, the angular gyrus, and the striate cortex, whereas polyvalent users did not differ from controls. Combined, these studies suggest that altered brain activation patterns during cognitive processing in ecstasy users may be associated with prior ecstasy use rather than other concomitantly used drugs (Daumann et al., 2003a; Daumann et al., 2003b). Furthermore, in a longitudinal study Daumann et al. (2004a) reported that these ecstasy-related brain alterations were dose dependent. In another fMRI study Moeller et al. (2004) found that ecstasy users showed greater abnormalities in parietal regions, prefrontal cortex, and thalamic regions compared with non-drug-using controls during performance of an immediate and delayed working memory task. In some of these studies (Daumann et al., 2003a; Daumann et al., 2003b; Daumann et al., 2004a; Daumann et al., 2005) the absence of performance deficits suggests that altered brain activation might be a more sensitive or earlier index of ecstasy-related neurotoxicity than task performance. In sum, little is known about the neuroanatomical basis of memory deficits in ecstasy users despite the potential linkage between memory impairments and neuroanatomical (Jager et al., 2007) and functional (Hales et al., 2008) abnormalities.

In the current study we investigated memory functioning in recreational drug users who predominantly used ecstasy, recreational drug users whose main recreation drug of choice was cannabis and drug-naïve controls. The cannabis using group was included given that cannabis intake is prevalent among ecstasy users (Smart and Ogborne, 2000) and a growing number of studies report memory deficits in both ecstasy and cannabis users (Gouzoulois-Mayfrank et al., 2000; Laws and Kokkalis, 2007). Using the same face-matching task employed in the present study, Nestor et al. (2008) reported reduced activity in frontal and temporal cortices, and relatively increased brain activation in the parahippocampus of heavy cannabis users during fMRI. As animal and human functional imaging evidence of hippocampal and cortical dysregulation is also emerging in both ecstasy using (Landfield et al., 1988; Scallet et al., 1991; Ricaurte et al., 1992; Fischer et al., 1995; Matochik et al., 2005; Hatzidimitriou et al., 1999) and cannabis using (Collins et al., 1995; Gessa et al., 1997; Carta et al., 1998; Nava et al., 2001) populations there remains a need to explore cortical and hippocampal-dependent learning and memory in chronic users of these illicit drugs in humans, thereby disentangling the long-term effects of combining ecstasy and cannabis.

This experiment compared brain activity, under fMRI conditions, between recreational drug users who use ecstasy as their predominant drug of choice and drug-naïve controls using a modified version of the face-name task proposed by Zeineh et al. (2003). Based on previous reports of reduced hippocampal activity in ecstasy users, we predicted memory-related hippocampal dysfunction in ecstasy users versus controls. Given frequent reports of ecstasy-related neurotoxicity in animal studies it

was expected that ecstasy users would show dysfunctional BOLD activation in isocortical regions in addition to the hippocampus. As our sample of ecstasy users were concomitant cannabis users, and as this task has previously been tested in cannabis users (Nestor et al., 2008), these previously tested cannabis users were incorporated into the analysis. As a number of studies that have investigated sex differences provide evidence that chronic ecstasy use in females may lead to a greater cognitive vulnerability (Bolla et al 1998; Topp et al 1999; Lynch et al 2002; Von Gersau et al 2004) an additional goal of the present study was to investigate interactions between ecstasy use and sex.

2. Results

2.1 Behavioural results

Face-number performance for the 3 groups is shown in Figure 2. A 3 (group) by 6 (trial) repeated measures analysis of variance (ANOVA) found a significant main effect of trial ($F=84.4$, $df= 5,133$, $p<0.001$), reflecting an increase in memory performance over the first 6 trials. There was a significant group effect ($F=5.8$, $df=2,51$, $p<0.05$) with *post hoc* tests showing ecstasy users had lower overall levels of recall performance when compared to controls. There were no differences between controls and cannabis users, and between ecstasy users and cannabis users. Analyses showed no trial by group interaction ($F=1.68$, $df=10,133$ $p= p>0.05$), suggesting there was no difference between groups in the gradient of the learning curves. In a separate analysis on ecstasy users and controls there were no gender effects nor interactions involving gender.

2.2 Neuroimaging Results

The t-test versus zero activation maps ($p \leq 0.005$) are shown in Figure 3. The encoding and recall maps were quite similar with both activating bilateral PFC and bilateral hippocampus. The activation patterns of ecstasy users were also similar to controls as demonstrated, as noted earlier, by the lack of significant group differences on the between-groups voxelwise t-tests.

Table 2 summarizes the results of the conjunction analysis that was conducted for ecstasy users, cannabis users, and controls. Three patterns were evident from the 18 clusters that were revealed (Figure 4). Ecstasy-specific increased brain activation (ecstasy > cannabis = controls) was found in the left superior frontal gyrus, bilateral middle frontal gyrus, left middle temporal gyrus, right tuber/declive, right inferior parietal lobule, left inferior occipital gyrus, right inferior temporal gyrus, right middle occipital gyrus, and right cuneus. Of these regions BOLD activation was greater during encoding compared to recall in the left superior frontal gyrus, right tuber/declive, right inferior parietal lobule, right inferior temporal gyrus, and right cuneus. The left inferior frontal gyrus was the only region to show an interaction that was driven by increased BOLD activation for ecstasy users compared to controls during encoding face-number associations. For the second pattern, ecstasy-specific decreased brain activation (ecstasy < cannabis = controls) was evident in a cluster that included the left parahippocampal gyrus and the cingulate and in a second cluster that fell in the right dorsal anterior cingulate. For these regions there were neither task phase effects nor group X task phase interactions. For the third pattern both ecstasy and cannabis groups showed decreased activation relative to controls in the right

medial frontal gyrus, left parahippocampal gyrus, left dorsal cingulate gyrus, and left caudate. BOLD brain activation was greater during encoding compared to the recall task in the left dorsal cingulate gyrus. In the separate analysis restricted to ecstasy users and controls there were no gender effects nor interactions between gender and group.

In ecstasy users the total number of lifetime ecstasy tablets consumed was positively correlated with activation in the right middle temporal gyrus (51, 1, -19) during encoding ($r = 0.48$, $p \leq 0.05$), with the right tuber/declive (51, -61, -22) during recall ($r = 0.49$, $p \leq 0.05$), and with the left inferior occipital gyrus (-38, -84, -1) during recall ($r = 0.53$, $p \leq 0.05$).

3. Discussion

In this hippocampal-dependant face-number paradigm ecstasy users had altered memory performance compared to cannabis users and drug-naïve controls. The fMRI results provide evidence that this compromised performance may be related to neural alterations in a range of cortical and sub-cortical structures. This result may reflect the widespread toxic effects of ecstasy on brain 5-HT neurons (Ricaurte et al., 1992a; Fischer et al., 1995; Hatzidimitriou et al., 1999; Giorgi et al., 2005; Tamburini et al., 2006; Frenzilli et al., 2007; Buscetti et al., 2008) and complements previous reports of functional brain abnormalities during performance of memory tasks in ecstasy users (Daumann et al., 2003a; Daumann et al., 2003b; Daumann et al., 2004a; Jacobsen et al., 2004; Moeller et al., 2004; Daumann et al., 2005). Furthermore, the inclusion of a cannabis group enabled us to separate ecstasy-specific effects (or effects that arise from using both ecstasy and cannabis) from cannabis-specific effects. The results identified a number of hypoactive brain regions, some of which were shared by the cannabis and ecstasy groups and some that were unique to the ecstasy group.

Consistent with previous studies using a similar task, prominent bilateral prefrontal and hippocampal activations were evident during the encoding and recalling of task-number associations (Zeineh et al., 2003; Morgan et al., 1999; Gouzoulis-Mayfrank et al., 2000). Ecstasy-specific dysregulations were found in the left superior frontal gyrus, bilateral middle frontal gyrus, left middle temporal gyrus, right tuber/declive, right inferior parietal lobule, left inferior occipital gyrus, right inferior temporal gyrus, left parahippocampal gyrus, left ACC, right middle occipital gyrus, and right cuneus. In both ecstasy and cannabis groups brain activation was altered in the right medial frontal gyrus, left parahippocampal gyrus, left dorsal cingulate gyrus, and left caudate.

Despite reports suggesting that females are particularly vulnerable to the chronic effects of ecstasy (Bolla et al 1998; Topp et al 1999; Lynch et al 2002; Von Gersau et al 2004) none of the ecstasy-related effects observed here were modulated by sex, a null result that should be tempered by noting that there were just ten male and ten female ecstasy users in the current sample where the drug use variables did not differ between the sexes.

Both ecstasy and cannabis users demonstrated hypoactivity in the left parahippocampal gyrus, bilateral dorsal ACC, and left caudate during the learning and recall phases, when compared to control participants. Historically, the basal ganglia have been implicated in higher-order motor control (Graybiel et al., 1995) but there have been a number of demonstrations of a role for the caudate in learning and memory (Graybiel et al., 1995; Packard et al., 1990, 1994). The parahippocampus has previously been demonstrated to be involved in the formation of face-name associations (Zeineh et al. 2003; Kirwin and Stark 2004) while the dorsal ACC has a broad role in on-line monitoring of behaviour in cognitive tasks (Carter et al., 1998; Mango et al., 2006) and, indeed, the dorsal ACC has been shown to activate with the hippocampus in learning from one's errors (Hester et al. 2008). Consequently, hypoactivity in this network of areas may result in compromised memory abilities: moreover, there is growing evidence that cannabis (Yucel et al. 2008) and ecstasy (Cowan et al., 2003; Daumann et al., 2004b) induce structural hippocampal abnormalities. The lack of memory performance impairments in the cannabis group is surprising given previous results (Nestor et al. 2008) but may reflect sampling characteristics, and may also indicate that dysregulated functional brain activity might be a more sensitive measure of cannabis's effects in accord with the findings of Jager

et al. (2007). Hippocampal hypoactivity in ecstasy users is consistent with two previous fMRI studies (Jacobsen et al., 2004; Daumann et al., 1995) where reduced hippocampal activity during working memory tasks was reported, although this deficit was apparent in the absence of performance differences in the latter.

Ecstasy-specific effects were associated with left superior frontal, bilateral middle frontal, right inferior parietal lobule, bilateral temporal, and bilateral occipital hyperactivity. Ecstasy related hyperactivity is suggestive that ecstasy users are placing greater demands in these brain regions by working harder to perform the task, suggesting pathological effects within these areas. Consistent with the frontal hyperactivity reported here, an fMRI study found that ecstasy users showed greater activation in PFC compared with non-drug-using controls during performance of an immediate and delayed working memory task (Moeller et al., 2004). Furthermore, there is a substantial body of work associating ecstasy with serotonergic frontal deficits (O'Hearn et al., 1988; Wilson et al., 1989; Fischer et al., 1995; Hatzidimitriou et al., 1999). The prefrontal cortex has been implicated in retrieval effort or success (Buckner et al., 1998; Zeineth et al., 2003), and although we did not find any associations between memory performance and the PFC, frontal dysregulation in ecstasy users may underlie their worse performance. Giorgi et al. (2005) demonstrated the occurrence of metabolic hippocampal hyperexcitability measured by 2-deoxyglucose uptake using experimental models of MDMA administration. Although hippocampal hyperactivation was not evident, the pattern of ecstasy-related hyperactivity is in accord with our findings.

Ecstasy-specific hyperactivity in the right inferior parietal lobule and bilateral temporal regions is also consistent with reports of these regions being affected by chronic ecstasy use during working memory in two previous studies (Daumann et al., 2003a; Daumann et al., 2003b). Activity in the left middle temporal gyrus (BA 21) was correlated with total lifetime number of ecstasy tablets consumed and this same left region was found to have reduced cortical grey matter concentrations in ecstasy consumers compared to drug-naïve controls (Cowan et al., 2003). The ecstasy-specific occipital cortex hyperactivity fell within secondary visual cortex known to be involved in forming visual associations and processing visual imagery and memory (Sperling et al., 2001; Stock et al., 2008). Activity in the bilateral occipital gyrus was correlated with total lifetime number of ecstasy tablets consumed which coincides with this same bilateral BA18 region being previously reported to have reduced cortical grey matter concentrations in ecstasy users compared to controls (Cowan et al., 2003). Although we did not find an inverse relationship between the regions that showed hyperactive frontal activations and the regions that revealed hypoactivity, we cannot rule out the possibility of a neural compensatory mechanism, whereby widespread hyperactivity is compensating for the ecstasy-related lack of hippocampal involvement in memory formation. There are reports of widespread neurotoxicity of ecstasy in both isocortical and allocortical brain regions, with a major emphasis placed on serotonergic neurotoxicity (Fischer et al., 1885; Ricaurte et al., 1985; Schmidt 1987a; O Hearn 1988; Ricaurte et al., 1992; Mc Cann et al., 1994 ;Hatzidimitriou et al., 1999; Giorgi et al., 2005; Tamburini et al., 2006; Frenzilli et al., 2007; Buscetti et al., 2008). Given this widespread neurotoxicity and evidence that the serotonergic system plays a significant role in learning and memory that is mediated primarily via

the hippocampal and frontal cortex (Buhot et al. 2000) we speculate that serotonergic toxicity may be at least partly attributable to ecstasy-related deficits.

It should be noted that rather than scanning abstinent ecstasy users in whom persistent neurotoxic effects of ecstasy might be evaluated, the present scans are of recreational drug users who primarily use ecstasy and who have differing periods of abstinence from ecstasy and other illicit drugs. A notable characteristic of this sample is that a large proportion of ecstasy participants tested positive for cannabis and some had also used ecstasy within two-three days prior to fMRI scanning. Negative subacute effects of MDMA on cognition and mood have been previously characterized (Parrott and Lasky, 1998; Parrott, 2001; Parrott, 2004)., Amphetamines have been reported to cause toxicity (Berman et al., 2009) and some of the participants also used amphetamines up to a total of 15 times in their lifetime. A large proportion of ecstasy users were nicotine smokers, and a moderate proportion of the controls reported nicotine use, whereas none of the cannabis users were nicotine users. We did not record if participants smoked nicotine on the day of testing but as participants were not requested to abstain from nicotine prior to testing the potential influence of nicotine as a confounding factor cannot be eliminated. Consequently, the current results might best be interpreted as showing the neurocognitive functioning of current polydrug users, albeit users who vary in their primary drug of use, rather than necessarily demonstrating persistent neurotoxic effects of those drugs.

Despite methodological limitations, the findings of this study demonstrate alterations in a network of brain regions that are related to ecstasy use or to the combined use of ecstasy and cannabis. The inclusion of a cannabis-using group helps to delineate

which effects might be attributed to which drug and suggest that there are a number of regional deficits unique to the ecstasy group. From a systems-level neuroscience perspective, the results indicate that ecstasy users showed unique cingulate and left parahippocampal activity decreases and, we speculate, a compensatory hyperactivity in mainly fronto-parietal cortical areas. Their performance was significantly impaired suggesting that this is a sub-optimal functional neuroanatomy. Whether these results preceded or arose from their ecstasy use will require longitudinal investigations.

4. Experimental Procedure

4.1 Participants

The ecstasy group included 20 chronic users of ecstasy and the drug-naïve group was comprised of 20 participants with no history of illicit drug use. Participants were recruited by poster recruitment and by the snowballing method. Participants in the drug-naïve group were required to have never used any illicit substance. Participants in the ecstasy group were required to be current users of ecstasy and to have consumed at least 40 ecstasy tablets over a period of a year, but not necessarily over the immediately preceding year. With the exception of cannabis, participants in the ecstasy group were excluded if they used any other illicit drugs on more than ten occasions in their lifetime (or more than fifteen times if the substance had not been used in the five years that preceded the study) and were required to be abstinent of these drugs for a minimum period of 10 weeks prior to testing. Participants in both

groups were also excluded if they had reported either past or present neurological or psychiatric problems. Given the fact that daily smoking of cannabis is part of the lifestyle of most club drug users (Daumann et al 2003), cannabis users who were also ecstasy users were not excluded from the study or required to abstain from smoking cannabis prior to participation. All ecstasy participants who reported cannabis use (last use 0.5-12 days since last use) with the exception of one participant who had reported use 3 years prior to study participation tested positive for cannabis. Ecstasy users were requested to abstain from ecstasy for at least 48 hours prior to study participation. Given this abstinence period, all participants provided a negative urine sample for ecstasy. Additional urine analysis screening for methadone, benzodiazepines, cocaine, opiates, barbiturates and tricyclic antidepressants (Cozart Rapiscan, UK) revealed negative results in both groups. All participants gave informed consent and the study was approved by the School of Psychology in Trinity College Dublin. In addition to an ecstasy and control group the cannabis group from Nestor et al. (2008) were incorporated into our analysis.

4.2 Demographics and drug use

Table 1 shows the group demographics and drug use histories for the ecstasy users, cannabis users and controls. ANOVAs' revealed that the groups did not differ in terms of verbal IQ as assessed by the National Adult Reading Test (NART), age, years of education, alcohol or other illicit drugs with the expected exception of ecstasy and cannabis as specified in the selection criteria. As specified in the selection criteria the control group did not use cannabis and the ecstasy users were polydrug users, but the ecstasy group had significantly fewer cannabis joints in their lifetime ($p < 0.05$)

compared to cannabis users. The heavier cannabis use of the cannabis group is not an ideal match for the cannabis use of the ecstasy users but does provide a strong test of any ecstasy-user group effects being ecstasy-specific (or arising from an interaction of combined ecstasy and cannabis use). The ecstasy and cannabis groups self-reported higher BDI scores compared to controls (p 's = 0.05) and the two drug-using groups did not differ from one another.

4.3 Stimuli and Behavioural Protocol

The face-number task used for the imaging procedure was adapted from that used by Zeineh et al. (2003); associations were made between faces and two-digit numbers which could be entered on a keypad during recall thereby avoiding possible head motion associated with verbal responses. The task was programmed using *E-Prime version 1.1* (Psychology Software Tools, Pittsburgh, USA). The task consisted of two runs of three blocks, with each block containing rest, learning, distraction and recall phases (see Figure 1). The beginning of each block commenced with a 30 second rest period. During the learning phase, participants were required to learn eight serially presented face-number pairs that were presented for three seconds each. The numbers paired with each face were randomly selected from the set: 11, 12, 13, 14, 21, 22, 23 and 24. During recall, these numbers were selected using two handheld response keypads (left keypad contained the one and two keys, right keypad contained the three and four keys). Following the presentation of each face-number pair, a fixation crosshair was presented for between one to seven seconds. The face-number association remained consistent throughout each learning phase, with each learning phase being 60 seconds in duration. Eight distracter trials appeared between each learning and recall phase and lasted a total of 22 seconds. The distracter task required

participants to press the three key on the right hand-held keypad each time a black star appeared in the center of a centrally displayed empty circle (see Figure 1). The eight distracter trials were separated by variable delay intervals between three to five seconds. During each recall trial, in random order participants were presented with one of the eight 'learning phase' faces for three seconds, and required to respond using the key-pad with the correct number for each face. A fixation crosshair of variable duration between one to seven seconds followed the presentation of each face. Each recall phase lasted 60 seconds, with a different presentation order of faces administered during each of the six recall blocks. Each run of the face-memory task was 570 seconds in duration. Dependent measures for the behavioural task were the learning curve (percentage correct recall on each of the six recall trials).

4.5 Imaging parameters

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror that reflected a 640 x 480 pixel display, projected on a panel placed behind the subject's head outside the magnet. The mirror was mounted on the head coil in the participants' line of vision. Imaging started with 31.5 seconds of standard scout images to adjust head positioning, followed by a reference scan to resolve sensitivity variations. Imaging used a parallel SENSitivity Encoding (SENSE) approach (Pruessmaan et al. 1999) with reduction factor 2. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV 230 mm, thickness 0.9 mm, voxel size 0.9 x 0.9 x 0.9) were then acquired (total duration 6 minutes), to allow subsequent activation localization and spatial normalization. Thirty-two non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain

were collected using a T2* weighted echo-planar imaging sequence (TE = 35 ms, TR = 2000 ms, FOV 224 mm, 64 x 64 mm matrix size in Fourier space).

4.6 Time-series analyses

The fMRI data were analysed using the AFNI software package (Cox, 1996). Time-series data were motion-corrected using 3D volume registration (least-squares alignment of three translational and three rotational parameters). Activation outside the brain was removed using edge detection algorithms. A block analysis was conducted to estimate the activation for the learning and recall periods separately. These ON-OFF block regressors were convolved with a standard haemodynamic response to accommodate the lag time of the blood oxygen level-dependent (BOLD) response. Multiple regression analyses were then used to derive the average level of block activation as a percentage change relative to the distracter periods over the two runs of the paradigm.

Activation maps were warped into a standard stereotaxic space (Talairach et al., 1998) and spatially blurred with a 4.2-mm full-width at half-maximum isotropic Gaussian filter after performing a second edge detection on the skull stripped brain. Encode and recall activation maps for both ecstasy users and drug-naïve controls were determined with one-sample t tests against the null hypothesis of zero activation changes (i.e., no change relative to distracter-task activity). Significant voxels passed a voxelwise statistical threshold ($t = 8.94$, $P \leq 0.005$) and were required to be part of a larger 286 μl cluster of contiguous significant voxels. Thresholding was determined through Monte

Carlo simulations and resulted in a 5% probability of a cluster surviving due to chance (Figure 3). Voxelwise independent-samples t-tests comparing ecstasy users and drug-naïve controls were performed separately for both encode and recall activations using similar thresholding to the previous t-tests.

No clusters survived the voxelwise between-group comparison. In addition, very similar brain activation patterns were evident for both encoding and recall phases of the task (see Figure 3). Consequently, in order to increase the statistical power of the between-group comparisons, we performed a conjunction analysis that combined the between-group comparisons of the encoding and recall periods. For this conjunction analysis significant voxels from the between-groups t-tests passed a voxelwise statistical threshold ($t = 2.25$, $P \leq 0.003$ (derived from the square root of 0.001)) for both the learning and recall periods and were required to be part of a larger 141 μl cluster of contiguous significant voxels. Thresholding was determined through Monte Carlo simulations and resulted in a 1% probability of a cluster surviving due to chance. The mean activation for each separate cluster of the conjunction map was calculated for all three groups (note that cannabis group is included here but this group did not contribute to the construction of the conjunction map) for both recall and encode task phases, and cluster level statistics were performed in SPSS (SPSS Inc).

4.7 Statistical analysis

Behavioural data are expressed as mean $\pm$ SEM and analyzed with the computerized package SPSS (version 12). On the first six trials of the face-number task trial effects, group effects and interactions were calculated using a 3 (group) X 6 (trial 1-6)

repeated measures analysis of variance (ANOVA) followed by Student-Newman-Keuls *post hoc* tests. As the cannabis group consisted of only 2 females it was excluded from a separate 2 (group) X 6 (trial 1-6) X 2 (gender) repeated measures ANOVA which was carried out to investigate gender effects and gender interactions.

For the conjunction analysis a 3 (Group: ecstasy, cannabis, and controls) X 2 (encode, recall) repeated measures analysis of variance (ANOVA) was performed for each cluster of activation. Within the ecstasy group Pearson's correlations' were carried out to investigate relationships between behavioural performance, drug use and brain activation. A separate 2 (group) X 2 (encode/recall) X 2 (gender) repeated measures ANOVA was carried out to investigate gender effects and gender interactions for each cluster.

Acknowledgements

NIDA. Grant number DA14100.

HRB. Grant number 8AA H01188

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Table 1. Mean and Range for ecstasy, cannabis and control groups on demographics and drug use history. * = $p < 0.05$ Vs control group, + = $p < 0.05$ versus ecstasy group. # = on the day of testing one participant from the control group reported using 1 line of cocaine on 4 occasions (last use was 2 years prior to testing). Within the ecstasy group ecstasy and cannabis use measures did not differ between males and females ($p > 0.05$). The n given in parentheses refers to the number of subjects reporting a non-zero value while the mean and ranges are for the entire group.

Table 2 Cerebral Foci for conjunction analysis of face-number task. Phase X group interaction driven by increased activation for ecstasy users during encoding ($p < 0.05$), ** = $p < 0.01$, *** = $p < 0.001$. E+C↓ = ecstasy +cannabis decreased brain activation. ↑ = increased activation for Encode condition, ↓ = decreased activation for recall condition. Analysis was conducted on ecstasy, cannabis, and control groups. + = Increased activation for males (as only two females were tested in the cannabis group, this group was eliminated from the gender analysis).

Fig. 1 Face-number protocol. Face memory task administered during fMRI data collection. Participants were required to learn and recall face-number pairs over a series of 6 blocks.

Fig. 2 Face-number performance. Mean performance on trials 1-6 in controls, ecstasy users and cannabis users (means and SEM). Ecstasy users had poorer performance compared to controls and cannabis users.

Fig. 3 Sagittal sections showing regions involved in the face-number task. Examination of t -tests ($p=0.005$) for encoding (left column) and recalling (right column) between control participants (upper row) and ecstasy using participants (bottom row) demonstrate considerable overlap between the two conditions and qualitatively similar activations for both groups.

Fig. 4 Cerebral Foci from conjunction analysis of face-number task. Green=Ecstasy-specific increased brain activation, orange=ecstasy-specific decreased brain activation, red=ecstasy and cannabis decreased brain activation.

	Ecstasy (n=20)	Cannabis (n=14)	Controls (n=20)
Age	22.4 (18.1 28.4)	22.4 (18.3 32.0)	22.5 (18.0 29.0)
Years of education	15.8 (11.0 19.0)	16.0 (12.0 19.0)	16.9 (11.0 21.0)
Verbal intelligence score (NART)	122.1 (114.8 129.7)	122.9 (119.2 128.3)	123.3 (114.8 129.4)
Beck Depression Inventory II score	5.8 (0 20)*	6.1 (0 14)*	3.2 (0 10)
Females/males	10/10	2/12	10/10
Ecstasy use in the last month (no. times)	2.3 (1.0 8.0)	0	0
Pills in last month (number)	10.7 (3.0 50.0)	0	0
Last ecstasy use (days)	16.1 (2.4 32.3)	960 (na 1835) (n=8)	0
Lifetime pills (number)	406.5 (50.0 1500.0)	18.8 (0 40) (n=8)	0
Pills in last year (number)	109 (15 600)	0	0
Years of cannabis use	6.8 (0 12.3) (n=15)	7.2 (2.5 16.0)	0
Days of use in last month (number)	16.1 (0 30.0) (n=15)	19.1 (7.0 30.0)	0
Joints in last month (number)	43.2 (0 180.0) (n=15)	82.8 (10.5 210.0)	0
Last cannabis use (days)	102.5 (na 1500.3) (n=15)	3.6 (0.1 4.5)	0
Lifetime joints (number)	2479 (0 10000.0) (n=15)	7925.9 (1000 33653)	0
Years of alcohol use	7.6 (3.6 13.3)	9.0 (4.5 18.0)	6.32 (2.0 10.5)
Alcohol use in the last month (no. days)	9 (0 20)	2.9±0.3 (0 20.0)	6.1 (2.0 15.0)
Average units of alcohol per week	14.4 (1 35.5)	12.5± (5.0 20.0)	10.6 (5.0 21.0)
Years of nicotine use	5 (0 10) (n=14)	0	5.3± (0 9.0) (n= 6)
Years of amphetamine use	3.1 (0 8.0) (n=11)	1.5 (0 2.5) (n=7)	0
Last amphetamine use (days)	362.1± (na 1500.0) (n=11)	415 (na 2520) (n=7)	0
Amphetamine use (no. times)	6.3±1.7 (0 15.0) (n=11)	10.4 (0 10) (n=7)	0
Years of cocaine use	3.0 (0 6.1) (n=17)	0.5 (0 3) (n=9)	#
Last cocaine use (days)	152.3 (na 720.0) (n=17)	408 (na 450) (n=9)	0
Cocaine use (no. times)	9.8± (0 15) (n=17)	6.0 (0 40) (n=9)	0
Years of hallucinogenic use	1.4 (0 7.5) (n=7)	0.6 (0 3) (n=10)	0
Last hallucinogenic use (days)	640.7 (na 2200.0) (n=7)	450 (na 1440) (n=10)	0
Hallucinogenic use (no. times)	2.2 (1 15) (n=7)	10.4 (0 30) (n=10)	0

Table. 1

Table 2

Structure	Broadmann area	Hemisphere	Volume (ul)	Centre of mass			Effects		
				X	Y	Z	Encode > recall	Phase X Group	Pattern
<u>Frontal lobe</u>									
Superior frontal gyrus	6, 9	L	2083	-46	4	28	***	**	E?
Middle frontal gyrus +	6	L	428	-36	-5	47	ns	ns	E?
Middle frontal gyrus	11	R	281	42	45	-11	ns	ns	E?
Middle frontal gyrus	10, 11	L	207	-41	55	-10	ns	ns	E?
Superior frontal gyrus	6	L	192	-12	16	69	ns	ns	E?
<u>Parietal lobe</u>									
Tuber/declive	7	R	458	51	-61	-22	**	ns	E?
Inferior parietal lobule	40	R	442	40	-53	52	***	ns	E?
<u>Temporal lobe</u>									
Middle temporal gyrus	21	L	576	51	1	-19	ns	ns	E?
Inferior temporal gyrus	20	R	309	61	-44	-18	**	ns	E?
<u>Occipital lobe</u>									
Inferior occipital gyrus	18	L	409	-38	-84	-1	ns	ns	E?
Middle occipital gyrus	18	R	285	44	-78	2	ns	ns	E?
<u>Subcortical</u>									
Cuneus	19	R	244	30	-83	29	**	ns	E?
<u>Frontal lobe</u>									
Dorsal anterior cingulate	32	R	240	6	40	4	ns	ns	E?
<u>Subcortical</u>									
Parahippocampal gyrus/cingulate	30	L	303	-17	-44	8	ns	ns	E?
<u>Frontal lobe</u>									
Medial frontal gyrus	8, 32	R	574	13	32	34	ns	ns	E+C?
Dorsal cingulate gyrus	32	L	305	-7	24	38	**	ns	E+C?
<u>Subcortical</u>									
Parahippocampal gyrus		L	368	-28	-16	-16	ns	ns	E+C?
Caudate		L	284	-20	15	19	ns	ns	E+C?

Fig. 1

Fig. 2

Fig 3

Fig. 4