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# MDMA use is associated with increased spatial BOLD fMRI visual cortex activation in human MDMA users

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**Abstract**

Previous animal studies have demonstrated that 3,4-methylenedioxymethamphetamine (MDMA) exposure causes serotonin axotomy that is greatest in occipital cortex (including primary visual cortex) where serotonergic axons innervate neurons and blood vessels. Human MDMA users have altered serotonergic function and reduced gray matter density in occipital cortex. The fMRI BOLD method is potentially sensitive to both the neuronal and vascular consequences of MDMA-induced serotonin toxicity. To test the hypothesis that MDMA users have altered visual system function, we used the fMRI BOLD technique to assay visual cortical activation after photic stimulation in a group of adult MDMA users. Because MDMA users worldwide are polydrug users and therefore difficult to match to comparison groups in terms of polydrug exposure, we conducted a primary within-group analysis examining the correlation between lifetime episodes of MDMA exposure and measures of visual cortical activation. The within-group correlational analysis in the MDMA user group revealed that the degree of prior MDMA exposure was significantly positively correlated with the number of activated pixels for photic stimulation ($r=0.582$, $p=0.007$). A secondary between-group comparison of MDMA users with non-MDMA users found overall greater levels of polydrug exposure in the MDMA user cohort but no significant differences in visual cortical activation measures between the two groups. Additional research is needed to clarify the origin and significance of the current findings.


*Keywords:* 3,4-Methylenedioxymethamphetamine; MDMA; Ecstasy; Neurotoxicity; Neuroimaging; Functional magnetic resonance imaging; fMRI; Serotonin

## 1. Introduction

The recreational drug 3,4-methylenedioxymethamphetamine (MDMA) is sold under the street name of Ecstasy. While many published reports refer to MDMA and Ecstasy as synonymous, it should be made clear Ecstasy tablets are of variable purity, thus potentially confounding attempts to examine MDMA effects in human users. Given that the degree to which recreational Ecstasy use correlates with MDMA use, for clarity, this report will refer to humans who consume Ecstasy with the intent of consuming MDMA as "MDMA users." MDMA has been associated with serotonergic axonal toxicity in primate and non-primate animal models (Molliver et al., 1990; Ricaurte et al., 1988a,b, 1992; Insel et al., 1989; Schmidt, 1987; O'Hearn et al., 1988). Whether or not MDMA produces long-lasting or permanent axon death in human users remains unknown. However, indirect evidence assessing serotonergic function in human MDMA users has revealed differences between MDMA users and control subjects on a variety of assays of serotonergic function. In comparison to controls, MDMA users have reduced serotonin transporter density (McCann et al., 1998, 2005; Semple et al., 1999), reduced cerebrospinal fluid (CSF) serotonin metabolites (Ricaurte et al., 1990; McCann et al., 1994), and altered EEG measures known to correlate with

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serotonergic tone (Croft et al., 2001). Further, various cognitive studies and other forms of neuroimaging cited below (e.g., Cowan et al., 2003; McCann et al., 1998) have suggested that MDMA users display differences in brain structure or function when compared to control subjects.

Because serotonin is ubiquitously distributed in the human brain, many different neural systems are potentially affected by MDMA-induced serotonergic toxicity. However, the existing animal data suggests that MDMA-induced axotomy is not uniform. Rather, it appears that axons most distal to the brainstem cell bodies of origin of the serotonergic axons (namely those in occipital cortex) suffer the greatest toxicity following MDMA exposure (Molliver et al., 1990; Hatzidimitriou et al., 1999). If the same pattern is replicated in human MDMA users, this finding suggests that occipital cortical regions, including visual cortex, would be most strongly affected in terms of direct serotonergic axon loss. In accord with this prediction, several lines of evidence suggest that occipital cortex shows the greatest structural evidence of serotonergic axon loss in humans self-exposed to MDMA. For example, positron emission tomography (PET) and single photon emission tomography (SPECT) studies of serotonin transporters (an indirect marker for serotonergic axon integrity) have revealed significant reductions in serotonin transporter binding, most pronounced in occipital cortex (McCann et al., 1998, 2005; Semple et al., 1999). Similarly, a report using SPECT to assay postsynaptic 5-$HT_{2A}$ receptor found changes in occipital cortex with reduced receptor levels in recent MDMA users and increased receptor levels in abstinent MDMA users (Reneman et al., 2002a). Chang et al. (1999) (but not others, employing similar methods, e.g., Reneman et al., 2002b) reported increased levels of myoinositol (MI) in midline occipital cortex. MI is a putative glial marker and Chang et al. (1999) speculated that MI might be increased due to glial activation in response to degenerating serotonergic axons. Using structural magnetic resonance imaging (MRI), we demonstrated that MDMA users exhibited bilateral reductions in gray matter density in Brodmann area 18 (BA 18) in occipital cortex (Cowan et al., 2003). This reduction in gray matter density is consistent with reduced neuron or glial cell volume but may have alternative explanations (Cowan et al., 2003).

As occipital cortical structure and function is altered in MDMA users, additional studies of this region are warranted with the aim of determining if such changes could serve as markers not only of MDMA exposure, but to track brain recovery after treatment or cessation of use. In this regard, studies of the primary and secondary visual cortical subregions of occipital cortex seem to offer promise. Serotonin is present at all levels of the visual system (including visual cortex) and plays an important role in visual function (e.g., Waterhouse et al., 1990). Existing evidence suggests that visual system function might be a useful assay for examining the effects of drug-induced monoamine toxicity. Renshaw et al. (1994) previously used the fMRI BOLD method to demonstrate that medicated patients with schizophrenia (a psychiatric illness strongly linked to altered monoaminergic function) showed altered occipital cortical activation following retinal stimulation with red light. We chose to employ a similar BOLD fMRI paradigm to that used in prior studies (Cowan et al., 2000; Renshaw et al., 1994) to compare occipital cortical activation in MDMA users and controls. Because serotonergic axons (Reinhard et al., 1979; Marco et al., 1999) innervate and regulate the cerebral microvasculature and because the fMRI BOLD method is dependent on vascular reactivity, we predicted that any alterations in occipital cortical activation in MDMA users would result from some combination of neurophysiological and/or neurovascular differences. Thus, if serotonergic axons innervating cerebral microvessels and supplying serotonin as a neurotransmitter are lost or reduced in MDMA users, altered vascular reactivity might lead to BOLD signal alterations independently of changes in cortical neurophysiology.

As MDMA users worldwide are polydrug users and therefore difficult to match with regard to polydrug exposure in case-control study designs we conducted a primary analysis examining the correlation between prior MDMA exposure and fMRI measures of BOLD occipital cortical activation. A secondary analysis included a case-control comparison between MDMA users and non-MDMA users. To better understand the mechanism of MDMA effects, we examined two measures of regional brain activation: percent BOLD signal change and number of activated pixels. While these two measures are related and to some degree interdependent, percent BOLD signal change reflects the degree or amplitude of regional brain activation whereas the number of activated pixels is a measure of the spatial extent of regional brain activation.

## 2. Methods

### 2.1. Participants

The human subjects' research protocol was approved by the McLean Hospital Institutional Review Board and conformed to principles of the World Medical Association's Declaration of Helsinki. We enrolled participants ages 18–35 who answered advertisements for an MRI study of MDMA or other drug use or who were referred by other study subjects. Participants were recruited as part of a larger study examining structural, functional, and spectroscopic effects of MDMA use. Most participants participated in a previously published structural MRI study (Cowan et al., 2003). Participants were excluded if they had a current Axis I psychiatric diagnosis, general medical illness, contraindications to MR scanning, or if they had a positive urine drug screen (Triage, Drugs of Abuse Panel, includes: amphetamines/methamphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates (non-methadone), phencyclidine, propoxyphene, tetrahydrocannabinol, tricyclic antidepressants) or positive alcohol breath test on the day of the scan. We divided the participants into two groups: those having a minimum of 4 episodes of prior MDMA use (MDMA users) and those not having any prior MDMA use (non-MDMA users). Subjects filled out drug use, medical, and psychiatric questionnaires prior to neuroimaging and had a face-to-face interview with a psychiatrist to rule out substance dependence (nicotine, caffeine, and MDMA dependence were permitted) or

psychiatric disorders according to DSM-IV criteria. Subjects were asked to abstain from MDMA use for at least 3 weeks prior to study enrollment. We assessed prior lifetime drug use information for alcohol, amphetamine, cannabis, cocaine, hallucinogens (non-MDMA), heroin, opiates (non-heroin), and PCP. Because we wanted to assess for toxicity at lower MDMA exposure levels, and because range reporting for drug use events is more accurate than continuous exposure assessment (Matt et al., 2003), we used a drug use history questionnaire containing a categorical scale to collect lifetime drug use history data in terms of episodes of drug use. These categories were 1–4; 5–10; 11–39; and 40+ episodes of use. An episode of use was defined as a 24-h period starting at the onset of drug ingestion. This method of assessment thus produced a frequency estimate of number of days of exposure to a given drug.

### 2.2. Imaging

We collected echoplanar images on a 1.5-T General Electric (Milwaukee, WI) Signa whole body magnetic resonance scanner in an oblique plane parallel to the calcarine fissure to assess photic stimulation-induced BOLD signal changes. Acquisition parameters were TR = 3 S, flip angle = 90°, matrix = 64 × 64 pixels, FOV = 20 × 20 cm, 3 × 3 mm in-plane resolution. A total of 256 images were obtained at each location using a 5-in. receive-only surface coil. Photic stimulation was delivered simultaneously to both eyes using specially constructed fiber optic goggles that delivered light perceived as red (660 nm peak wavelength; 13 lux) using light emitting diodes. Subjects also underwent stimulation with blue light, with the order of blue or red stimulation randomized, as an exploratory analysis. Because analysis of blue light findings suggested a similar trend as for red light, and because red light paradigms have been widely used in the literature, results from blue photic stimulation are not further considered here. Stimulus intensities were the same for all subjects. The stimulus appeared as a nearly confluent (6 × 8 fiber) grid of individual fiber optic fibers subtending approximately two-thirds of visual space and flashing at a temporal frequency of 8 Hz. Stimulus intensities were chosen (based on pilot data using the same stimulus and analysis methods) to produce reliable suprathreshold but not maximal activation in occipital cortex. Stimuli were delivered using a balanced block design with 30 s stimulus periods alternating with 30 s rest periods, with four stimulus and four rest periods per scanner run.

### 2.3. Image analysis

We analyzed the data using Brain Voyager 4.0 (Brain Innovation, Maastricht, The Netherlands) software, employing the general linear model (GLM) to construct statistical parametric maps (SPMs) (Friston et al., 1996) to examine regional brain activation in response to photic stimulation. We preprocessed the images prior to constructing SPMs by first performing two-dimensional motion correction using a decoupled automated rotation and translation algorithm (DART, Maas et al., 1997). Data trials having motion greater than 2 mm in the translational direction or 0.5° in the rotational direction were excluded from subsequent analysis. We then performed slice scan time correction to the images to compensate for acquisition order, spatially smoothed the images using a 4 mm full width half maximum (FWHM) Gaussian kernel, and temporally smoothed the images by employing linear trend removal and 3 Hz high pass filtering (Skudlarski et al., 1999).

Using a threshold of $p \leq 0.05$ (Bonferroni corrected for the number of multiple pixel-wise comparisons within the ROI), we convolved the photic stimulation time course paradigm with Brain Voyager's default (delta = 2.5, tau = 1.25) gamma function hemodynamic response function (HRF) to construct SPMs (Boynton et al., 1996). We constructed SPMs as contrasts comparing photic stimulation to no stimulation (darkness) as a baseline separately from each subject run. A 10 × 15 pixel region of interest (ROI) approximately circumscribing primary (V1) and secondary (V2) visual cortex (Cowan et al., 2000) was drawn on the image with the overlayed SPM map in the right and left occipital cortex of each slice (Fig. 1).

This method permitted the creation of an ROI of identical size and dimension for each subject. The guidelines for drawing the ROI were that the long axis of the rectangular ROI was parallel to the interhemispheric fissure. The medial edge of the ROI was at least 1 voxel width from the midline, and the posterior edge of the ROI was at least 1 voxel from the posterior edge of the brain. We used the time course BOLD signal data from the significantly *activated pixels* within each ROI to calculate the percent BOLD signal change in response to photic stimulation. The number of pixels within the ROI reaching significance threshold (number of activated pixels) was used as a measure of spatial extent of activation.

### 2.4. Statistical analysis

Statistical analyses were carried out using SPSS, version 13.0.1 (SPSS Inc., Chicago IL 60606). Comparisons of demographic and background characteristics between cases and controls were conducted using independent samples *t*-tests

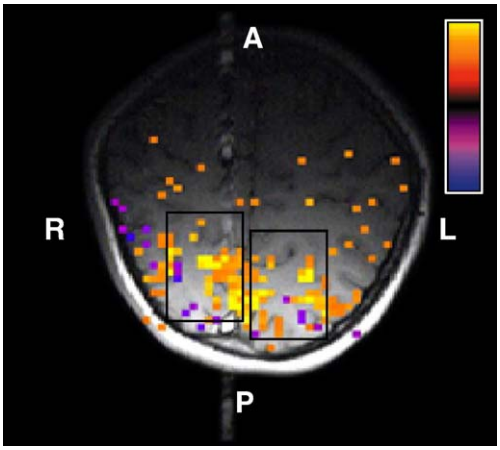


Fig. 1. Photic activation in visual cortex. This figure shows a representative activation map of visual cortex with superimposed regions of interest. The image is an oblique surface coil slice. A is anterior, P is posterior, R is right, L is left. Activation map calibration bar shows increasing activation from top to bottom with blue the most negative and bright yellow the most positively correlated.

Table 1
Sample characteristics

| | MDMA users ($N$=20) | Non-MDMA users ($N$=23) |
|---|---|---|
| Age * | 20.8±2.0 years | 25.3±3.9 years |
| Sex ** | Female ($N$=12) | Female ($N$=10) |
| | Male ($N$=8) | Male ($N$=13) |
| Handedness ** | Right ($N$=15) | Right ($N$=22) |
| | Left ($N$=4) | Left ($N$=1) |
| | Unknown ($N$=1) | |
| Race | Caucasian ($N$=19) | Caucasian ($N$=21) |
| | Asian ($N$=1) | Asian ($N$=1) |
| | | Black ($N$=1) |

* Significantly different, $p$=0.006.
** Not significantly different at $p \leq 0.05$.

(years of age), Chi-squared tests of independence (gender, handedness, categorical drug use), and Mann-Whitney $U$ tests (ordinal drug use assessments and non-parametric group comparisons).

The primary outcome measures included the number of pixels having a $p$-value above threshold (number of activated pixels) and the average percent BOLD signal change among activated pixels (percent BOLD signal change). The primary analyses of main effects of MDMA use on the number of activated pixels and percent BOLD signal change were assessed using mixed model analysis of variance procedures. Secondary assessments of the association between lifetime drug use history and the primary outcome measures were conducted using Spearman correlations. Additional analyses comprised of correlational procedures and tests of mean differences were conducted to examine associations between primary outcome variables and potential confounding variables. Partial correlations were used to examine the unique associations between variables of interest with statistically significant confounding associations partialled out.

## 3. Results

### 3.1. Sample characteristics

We studied 20 MDMA users and 23 non-MDMA users with an age range of 18–35 years. Sample characteristics are summarized in Table 1. The MDMA users were significantly younger than the MDMA non-users (age 20.8±2.0 years versus 25.3±3.9 years; $p$=0.006). There were 10 (43.5%) females and 13 males in the non-MDMA group versus 12 (60.0%) females and 8 males in the MDMA users group. This difference in sex distribution between the groups was not statistically significant ($p$=0.280). A majority of both groups were right-handed (non-MDMA: 95.7%, $N$=22 versus MDMA: 78.9%, $N$=15). Handedness was not recorded for one MDMA-using subject. While the percentage of left-handed subjects in the MDMA using group was greater than that in the non-MDMA group, this difference in the pattern of handedness distribution was not significant ($p$=0.096). With regard to ethnicity, all subjects were Caucasian with the exception of one Asian subject in the MDMA user group and one Asian and one Black subject in the non-MDMA users group.

### 3.2. Brain activation associations

#### 3.2.1. Within-users comparison

In the primary within-group comparison of 20 MDMA users a rather strong and statistically significant correlation was seen between the amount of reported lifetime MDMA use and number of activated pixels ($r$=0.582, $p$=0.007). Conversely, no statistically significant associations were seen between MDMA usage and percent BOLD signal change ($r$=0.029, $p$=0.902) (Fig. 2).

Within the MDMA user group, several variables that covaried with MDMA usage were found to be associated with brain activation at a statistically significant level, primarily with percent BOLD signal change. Lifetime alcohol ($r$=−0.466, $p$=0.002), hallucinogens ($r$=−0.361, $p$=0.020), marijuana ($r$=−0.612, $p$=0.004), and sedatives ($r$=−0.734, p<0.001) use were all *inversely* associated with percent BOLD signal change but not with the number of activated pixels. There were no significant correlations of prior amphetamine, cocaine, opiate (non-heroin), or PCP use with either outcome variable. No subjects in the MDMA user group reported heroin exposure.

#### 3.2.2. Between-group comparison

Compared to non-MDMA users, MDMA users had statistically significantly higher levels of prior lifetime alcohol ($p$=0.038), amphetamine ($p$<0.001), cannabis ($p$<0.001),

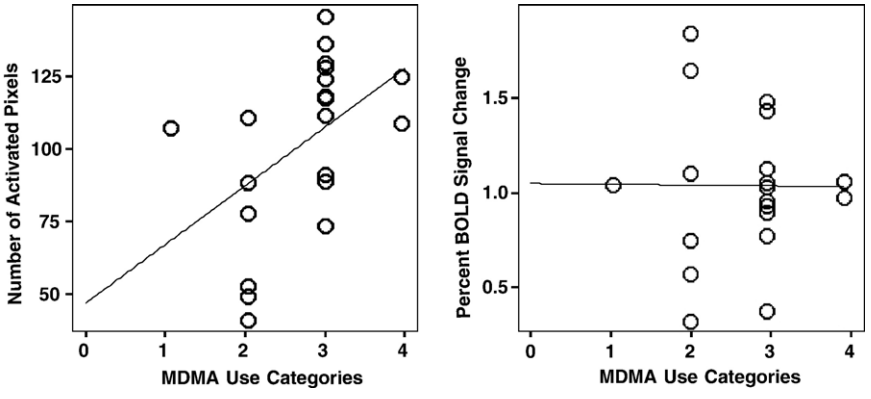


Fig. 2. MDMA use versus visual cortical activation. Scatter plots with linear regression line depicting average visual cortical number of activated pixels or percent BOLD signal change versus lifetime episodes of MDMA use. Data are from MDMA user group only.

cocaine ($p<0.001$), hallucinogens ($p<0.001$), opiates ($p=0.003$), sedatives ($p=0.001$), and phencyclidine (PCP) ($p=0.007$) (see Table 2). No subjects in either group reported heroin use.

For the outcome variables *of* number of activated pixels and percent BOLD signal change, there were no differences in the mean value for each outcome measure ($p=0.37$ for number of activated pixels comparison; $p=0.30$ for percent BOLD signal change comparison) between non-MDMA users and MDMA users (Table 3).

As described above, while the MDMA user within-group analysis showed a clear positive correlation between lifetime MDMA exposure and photic activation for the number of activated pixels, the overall mean number of activated pixels did not differ for this measure between MDMA users and the non-MDMA user comparison group. Given the finding that other drug use was negatively correlated with the percent BOLD signal change for several drugs that were used more heavily in the MDMA group than in the non-MDMA user group, we reasoned that a portion of this difference might be accounted for by the association with polydrug exposure. Namely, if polydrug exposure is negatively correlated with the percent BOLD signal change, by reducing the signal to noise ratio for a given pixel, polydrug effects might be predicted to reduce the number of activated pixels. Therefore, it would seem that the associations of these drugs might have opposite effects on the primary outcome measure leading to the presence of a positive correlation between MDMA exposure and the number of activated pixels but an overall mean that is equal between the MDMA user and non-MDMA user groups (the latter having less polydrug exposure than the MDMA user group). To address this conundrum, we first divided the MDMA user group into a "high" exposure and a "low" exposure group based on the graphical patterns of the number of activated pixels by aggregating data from the two highest exposure groups and comparing it to data from the two lowest exposure groups (Table 3). This analysis revealed a mean number of activated pixels in the low exposure MDMA group that was significantly less than that of the high MDMA exposure group ($p=0.004$). Given the strong positive correlation between prior MDMA exposure and outcome, this was not an unexpected finding. In contrast, comparison of the percent BOLD signal change in low versus high MDMA-exposed groups revealed no significant differences ($p=0.782$).

Table 2
Drug use episode frequencies in non-MDMA and MDMA users

| | Past drug use episodes | | | | | | |
|---|---|---|---|---|---|---|---|
| Drug | None % (*N*) | 1–4 % (*N*) | 5–10 % (*N*) | 11–39 % (*N*) | ≥40 % (*N*) | *z* | *p* |
| *Alcohol* | | | | | | | |
| Non-MDMA | 4.3 (1) | 8.7 (2) | 4.3 (1) | 52.2 (12) | 30.4 (7) | 2.074 | 0.038 |
| MDMA | 0.0 (0) | 0.0 (0) | 5.0 (1) | 35.0 (7) | 60.0 (12) | | |
| *Amphetamines* | | | | | | | |
| Non-MDMA | 95.2 (20) | 0.0 (0) | 0.0 (0) | 4.8 (1) | 0.0 (0) | 3.595 | <0.001 |
| MDMA | 40.0 ( 8) | 15.0 (3) | 15.0 (3) | 25.0 (5) | 5.0 (1) | | |
| *Cannabis* | | | | | | | |
| Non-MDMA | 52.2 (12) | 26.1 (6) | 4.3 (1) | 17.4 (4) | 0.0 (0) | 5.182 | <0.001 |
| MDMA | 0.0 (0) | 0.0 (0) | 5.0 (1) | 50.0 (10) | 45.0 (9) | | |
| *Cocaine* | | | | | | | |
| Non-MDMA | 95.7 (22) | 4.3 (1) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 3.922 | <0.001 |
| MDMA | 40.0 (8) | 40.0 (8) | 10.0 (2) | 10.0 (2) | 0.0 (0) | | |
| *Hallucinogens* | | | | | | | |
| Non-MDMA | 95.2 (20) | 4.8 (1) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 4.806 | <0.001 |
| MDMA | 20.0 (4) | 20.0 (4) | 35.0 (7) | 20.0 (4) | 5.0 (1) | | |
| *MDMA* | | | | | | | |
| Non-MDMA | 100.0 (23) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 6.155 | <0.001 |
| MDMA | 0.0 (0) | 5.0 (1) | 30.0 (6) | 55.0 (11) | 10.0 (2) | | |
| *Opiates* | | | | | | | |
| Non-MDMA | 100.0 (21) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 2.927 | 0.003 |
| MDMA | 65.0 (13) | 20.0 (4) | 5.0 (1) | 10.0 (2) | 0.0 (0) | | |
| *PCP* | | | | | | | |
| Non-MDMA | 100.0 (21) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 2.683 | 0.007 |
| MDMA | 70.0 (14) | 30.0 (6) | 0.0 (0) | 0.0 (0) | 0.0 (0) | | |
| *Sedatives* | | | | | | | |
| Non-MDMA | 90.5 (19) | 9.5 (2) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 3.281 | 0.001 |
| MDMA | 45.0 (9) | 15.0 (3) | 30.0 (6) | 10.0 (2) | 0.0 (0) | | |

Table 3
Activation measures

| Group | N | Number of activated pixels mean (±SD) | Percent bold signal change mean (±SD) |
|---|---|---|---|
| Non-MDMA | 23 | 111.4 (19.9) | 1.19 (0.4) |
| MDMA (all) | 20 | 101.4 (29.9) | 1.04 (0.4) |
| MDMA (low) | 7 | 75.6 (28.2) * | 1.04 (0.5) |
| MDMA (high) | 13 | 112.8 (19.8) | 1.04 (0.3) |

* Mean is significantly lower versus non-MDMA ($p=0.01$) and MDMA high ($p=0.004$).

Next, we compared the number of activated pixels and percent BOLD signal change of the high MDMA exposure group with the non-MDMA user group. Mean activations did not differ between the two groups for either the number of activated pixels ($p=0.52$) or the percent BOLD signal change ($p=0.40$).

Finally, we compared the means of the low MDMA exposure group with the non-MDMA user group (Table 3). Similar to the comparisons between the MDMA users and the control group, the low MDMA exposure group had a mean number of activated pixels significantly lower than the control group ($p=0.01$) while the average percent BOLD signal change was similar between the two groups ($p=0.39$).

#### 3.2.3. Non-MDMA users

For the non-MDMA group, there were no significant correlations of amphetamines, cocaine, hallucinogens, marijuana, nicotine (as average daily use), or sedatives with either the percent BOLD signal change or number of activated pixels. Alcohol use showed a non-significant trend for negative correlation ($p=0.065$, $r=-0.391$) with percent BOLD signal change in the non-MDMA group and showed a significant ($p=0.007$, $r=-0.549$) negative correlation with the number of activated pixels. The non-MDMA users group did not report exposure to heroin, opiates, or PCP.

#### 3.2.4. Age and sex effects

Across both groups (non-MDMA user and MDMA user combined) age did not have a statistically significant association with the activation variables ($p>0.05$). There was a statistically significant main effect of sex on percent BOLD signal change ($p\leq 0.001$) with females having lower percent BOLD signal change (mean 0.93%) than males (mean 1.32%), but there was no effect of sex on the number of activated pixels ($p>0.05$). There was no statistically significant difference between the distributions of males and females in the MDMA usage groups nor were there statistically significant interaction effects of gender and MDMA use ($p>0.05$).

## 4. Discussion

This BOLD fMRI study of occipital cortical red photic activation indicates that the degree of prior MDMA exposure positively correlates with the number of activated pixels in occipital cortex following red light stimulation. In contrast, there were no clear associations between the degree of MDMA use and percent BOLD signal change. However, multiple other drugs of abuse showed negative correlations for at least one measure of occipital cortical activation. Potential mechanisms for this finding include (singly or in combination): (1) brain differences present prior to MDMA use, (2) non-specific neuronal alterations due to MDMA or polydrug exposure, (3) MDMA-induced neuronal alterations due to reduced serotonergic neurotransmission, (4) polydrug-induced changes in neuronal function, and (5) MDMA or polydrug-induced changes in brain vasculature.

### 4.1. MDMA and occipital cortex

Converging evidence from animal and human studies of MDMA effects suggests that occipital cortex is altered by systemically administered MDMA. Some layer IV and other visual cortical serotonin axons appear to be permanently lost in animal models of MDMA toxicity (e.g., Hatzidimitriou et al., 1999). PET studies using the serotonin transporter ligand [$^{11}$C] McN5652 or [$^{11}$C] DASB to label terminal axons found statistically reduced levels of serotonin transporter binding in the occipital cortex (among other regions) of MDMA users (McCann et al., 1998, 2005). Receptors of the serotonin 2A subtype (5-HT$_{2A}$) are up-regulated in the occipital cortex of human MDMA users (Reneman et al., 2002a), a finding potentially reflecting a compensatory response to loss of serotonergic neurotransmission. Chang et al. (1999) found increased myoinositol (MI) in the occipital cortex of MDMA users. However, this finding was not replicated by Reneman et al. (2002b) despite the use of similar methods and voxel locations. However, in both studies (Chang et al., 1999; Reneman et al., 2002b) occipital *N*-acetylaspartate (NAA), a neuronal marker, was not altered. Oliveri and Calvo (2003) reported that MDMA users displayed evidence for reduced visual cortical excitability threshold when transcranial magnetic stimulation (TMS) was employed to elicit visual phosphenes. Using voxel-based morphometry (VBM), we recently reported that MDMA polydrug users have lower gray matter concentration bilaterally in occipital cortex than controls (Cowan et al., 2003). Of note, most of the subjects participating in the current report also provided structural brain imaging data to the VBM study (Cowan et al., 2003). In the VBM study, the area of reduced gray matter concentration was slightly lateral to the midline, comprising Brodmann area 18 of occipital cortex. Therefore, the occipital regions studied in the prior and current report do not exactly overlap. The present report thus supplements the extant basic and clinical literature in suggesting functional differences in the occipital cortex of MDMA users. To date, we are not aware of studies specifically examining retinal and LGN toxicity in an MDMA-exposed cohort.

### 4.2. Number of activated pixels and percent BOLD signal change as measures of brain activation

The existing evidence does not permit a definitive conclusion regarding the relationship between the number of activated pixels and percent BOLD signal change. In general,

detection of physiologically relevant activation in BOLD paradigms is dependent upon the signal to noise ratio (SNR). Therefore, factors that enhance the SNR will lead to an increased number of activated pixels (Parrish et al., 2000). As such, until 100% of the potentially relevant (i.e., stimulus-coupled) pixels are activated, an increase in percent BOLD signal change for a given stimulus predicts the detection of increased numbers of physiologically coupled activated pixels due to an increase in the SNR (increased signal). In studying drugs of abuse, particularly with regard to drugs affecting monoamine function, there exists the potential for altered vascular tone, altered numbers of activated neurons, and altered receptive field size or shape. Given this complexity, and without additional experiments aimed at isolating specific processes, it is not possible to reach conclusions about the underlying mechanisms producing the current results.

### 4.3. Serotonin toxicity and photic activation

Serotonin, along with other monoamines, influences the encoding of sensory stimuli in visual system and other regions (Hurley et al., 2004) where serotonin generally decreases SNR and norepinephrine increases SNR via effects on spontaneous or stimulus-coupled neuronal activity. However, this general description of serotonin action may not hold for a given neuron or neuronal subset because serotonin has complex postsynaptic actions that are dependent upon postsynaptic receptor subtype (e.g., Bonasera and Tecott, 2000) and interactions with other locally released transmitters (Jacobs and Fornal, 1993; Jacobs and Azmitia, 1992). Because serotonin alters the firing activity of both excitatory and inhibitory neurons in occipital cortex, the physiological implications of changes in measures of activation are not intuitive. Because visual cortical activation is frequency dependent (roughly maximum at 8 Hz stimulation under normal conditions; Fox and Raichle, 1984; Kwong et al., 1992), altered activation measures in occipital cortex could also result from shifts in visual system sensitivity to a particular frequency. Because we used only 8 Hz stimulation in this study, we cannot address this issue. Since serotonin is present at all levels of primary visual processing including the retina, the LGN and the primary visual cortex, it is possible that altered serotonin function at any site along the visual pathway could produce changes in BOLD signal at primary visual cortex. Serotonergic axons densely innervate primary visual cortex, particularly layer IV, where many LGN axons project (Foote and Morrison, 1984). While the 5-$HT_{2A}$ receptor is generally considered excitatory (Van Oekelen et al., 2003) it is unclear what increased occipital cortical 5-$HT_{2A}$ receptor numbers reported by others (Reneman et al., 2002a) imply in the context of potential reductions in agonist signaling. The positive association reported here between lifetime MDMA exposure and number of activated pixels, if considered as a measure of spatial activation, is consistent with loss of a serotonergic inhibitory role, as is the evidence of lowered stimulus thresholds in the TMS study cited above (Oliveri and Calvo, 2003). However, it is difficult to explain the lack of effect on the percent BOLD signal change measure unless loss of serotonin innervation specifically influences spatial activation parameters, such as center-surround inhibition. In fMRI BOLD paradigms, the observed extent of spatial activation is in part influenced by the number of averaged images (Saad et al., 2003), suggesting that increased spatial extent could also result from increased signal to noise ratio in a group of voxels.

### 4.4. Polydrug use and photic activation

In regard to the neurovascular mechanism giving rise to the correlation between MDMA dose and pixel number, it is notable that we did not detect an overall increase in the number of activated pixels in the MDMA group compared to the non-MDMA users. However, this comparison is compounded by the fact that the control group had lower levels of exposure to multiple drugs of abuse, a problem that faces studies of MDMA users worldwide. For this reason, our primary comparison was a within-group analysis of MDMA users by level of prior exposure. Use of other drugs in the MDMA user group may be a partial explanation for these findings because other drugs of abuse were significantly correlated with reductions in the percent BOLD signal change, suggesting that the overall effect might result from divergent effects of MDMA and non-MDMA recreational drugs on activation measures.

These results are consistent with differential effects of MDMA exposure and polydrug exposure on BOLD fMRI activation measures in visual occipital cortex during photic stimulation at 8 Hz with red light. However, it should be noted that association between the exposure variables and the outcome measures, even when an exposure-response correlation can be shown is not sufficient to conclude that drug exposure caused the observed effects.

### 4.5. Blood vessels and photic activation

Because the BOLD signal is dependent on intact neurovascular coupling of increased regional neuronal activity to increased regional blood flow (Ogawa et al., 1990; Kwong et al., 1992), factors affecting the vasculature could account for some or all of the observed findings (Stephan et al., 2004). Serotonergic axons innervate cerebral microvessels where they appear to have mixed actions, but a primarily vasoconstrictive role (Cohen et al., 1996). Serotonergic neurons in both the median and dorsal raphe nuclei appear to contribute axons to microvessels (Reinhard et al., 1979; Marco et al., 1999), but the precise functional role of serotonin in regulating the microcirculation is complex (Cohen et al., 1996). For human occipital arteries (but not necessarily other brain regions) serotonin appears to serve as a vasoconstrictor at low concentrations, but serves as a vasodilator at higher concentrations (Verheggen et al., 2004). Using 133-xenon SPECT, Chang et al. (2000) studied MDMA users abstinent for at least 2 weeks from MDMA to determine if MDMA users showed regional cerebral blood flow (rCBF) differences. Overall, abstinent MDMA users had mild but non-significant reductions in rCBF versus controls. A subset of MDMA users in the

Chang et al. (2000) study who received an oral MDMA dose and were restudied between 2 and 3 weeks post-drug had significantly decreased rCBF in multiple brain regions.

### 4.6. MDMA and functional neuroimaging studies

Existing functional neuroimaging studies in MDMA users have focused on cognitive tasks including measures of attention, working, and episodic memory. Overall, these studies reveal no strongly consistent pattern of regional brain alterations in MDMA users but suggest that increased BOLD activation is a common finding. For measures of attention, there were no apparent effects of MDMA exposure on [$H_2^{15}0$] PET-assayed regional cerebral blood flow during a continuous performance task (Gamma et al., 2001). In various working memory tasks, adolescent MDMA users had higher levels of hippocampal activation versus controls during a verbal working memory task (Jacobsen et al., 2004). Daumann and colleagues (Daumann et al., 2003a,b, 2004) conducted a series of studies examining working memory in MDMA users and concluded that overall, their group of studies suggested increased parietal activation in MDMA users in the face of relatively intact cognitive performance. Moeller et al. (2004) reported greater BOLD signal increases in the prefrontal cortex, hippocampus, thalamus, and basal ganglia of MDMA users versus controls during a working memory task. To date, we are unaware of published functional neuroimaging studies of primary sensory system function in MDMA users.

### 4.7. Clinical implications

Current findings from MRS (Chang et al., 1999), VBM (Cowan et al., 2003), TMS (Oliveri and Calvo, 2003) and fMRI (in the present report) suggest that occipital cortex in human MDMA users shows altered structure and function when experimentally probed in a laboratory setting. To date, we are aware of only one other report (Oliveri and Calvo, 2003) that has directly examined visual cortical function in human MDMA users. This report used transcranial magnetic stimulation to examine thresholds for generation of perceivable visual activity in MDMA users, who were found to have increased visual cortical excitability. Because human MDMA users, to our knowledge, do not report visual system perturbations (aside from those occurring during drug intoxication), the clinical implications of the existing findings in visual cortex remain unclear. Further research, using additional methods of assaying visual function and correlating findings in visual cortex with findings from other brain regions and from cognitive testing, will be necessary before the significance of the current report and similar findings can be determined.

### 4.8. Limitations

This study is a cross-sectional study and therefore does not have the ability to segregate pre-existing from acquired effects that might be permitted employing a longitudinal design in current MDMA users. However, our finding of an association between degree of MDMA exposure and spatial extent of brain activation raises the concern that MDMA exposure may produce dose-associated alterations in brain function. As in most published reports of MDMA users, polydrug exposure was higher in MDMA users than in the non-MDMA group. However, since MDMA users and controls did not show group differences with regard to primary outcome measures, the impact of this difference is minimized. While we hypothesized that MDMA-induced serotonergic axon toxicity might cause altered brain activation, the study design did not allow us to obtain evidence for altered serotonin function in this cohort. In support of a potential serotonergic role for the present findings include results from other investigators finding indirect evidence for occipital cortical serotonergic denervation including reduced serotonin transporter binding and increased 5-$HT_{2A}$ receptor density, albeit at higher exposure levels than in our study. Sex differences are unlikely to account for the present results because the MDMA and non-MDMA groups did not differ significantly by sexual composition and female sex was significantly associated only with percent BOLD signal change with no detectable effects of sex on the number of activated pixels.

### 4.9. Significance

While other reports have suggested that occipital cortex and its visual subregions might be altered in MDMA-exposed individuals, this report specifically indicates that MDMA exposure is associated in a dose-response manner with increased spatial extent of regional brain activation. This finding, when coupled to evidence for lowered cortical excitability in MDMA users (Oliveri and Calvo, 2003), suggests a potentially specific effect of MDMA axonopathy on threshold for or spatial spread of cortical activity. However, the implications of altered BOLD activation should be interpreted only in the context of much converging data. While the relationship between altered BOLD signal and altered neuronal function is unclear, increased BOLD activation has been reported in response to photic stimulation in patients with schizophrenia (Renshaw et al., 1994) and in individuals having increased risk for Alzheimer's disease (Bondi et al., 2005).

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