

RESEARCH PAPER

MDMA 'ecstasy' increases cerebral cortical perfusion determined by bolus-tracking arterial spin labelling (btASL) MRI

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BACKGROUND AND PURPOSE

The purpose of this study was to assess cerebral perfusion changes following systemic administration of the recreational drug 3,4-methylenedioxymethamphetamine (MDMA 'ecstasy') to rats.

EXPERIMENTAL APPROACH

Cerebral perfusion was quantified using bolus-tracking arterial spin labelling (btASL) MRI. Rats received MDMA (20 mg·kg⁻¹; i.p.) and were assessed 1, 3 or 24 h later. Rats received MDMA (5 or 20 mg·kg⁻¹; i.p.) and were assessed 3 h later. In addition, rats received MDMA (5 or 10 mg·kg⁻¹; i.p.) or saline four times daily over 2 consecutive days and were assessed 8 weeks later. Perfusion-weighted images were generated in a 7 tesla (7T) MRI scanner and experimental data was fitted to a quantitative model of cerebral perfusion to generate mean transit time (MTT), capillary transit time (CTT) and signal amplitude.

KEY RESULTS

MDMA reduces MTT and CTT and increases amplitude in somatosensory and motor cortex 1 and 3 h following administration, indicative of an increase in perfusion. Prior exposure to MDMA provoked a long-term reduction in cortical 5-HT concentration, but did not produce a sustained effect on cerebral cortical perfusion. The response to acute MDMA challenge (20 mg·kg⁻¹; i.p.) was attenuated in these animals indicating adaptation in response to prior MDMA exposure.

CONCLUSIONS AND IMPLICATIONS

MDMA provokes changes in cortical perfusion, which are quantifiable by btASL MRI, a neuroimaging tool with translational potential. Future studies are directed towards elucidation of the mechanisms involved and correlating changes in cerebrovascular function with potential behavioural deficits associated with drug use.

Abbreviations

5-HIAA, 5-hydroxyindole acetic acid; 7T, 7 tesla; BBB, blood-brain barrier; btASL, bolus-tracking arterial spin labelling; CBF, cerebral blood flow; CTT, capillary transit time; CVA, cerebrovascular accidents; EB, Evans blue; FLASH, snapshot fast low-angle shot; FOV, field of view; IDL, Interactive Data Language; MDMA, 3,4-methylenedioxymethamphetamine; MTT, mean transit time; pCO₂, partial pressure of carbon dioxide; RARE, rapid acquisition with relaxation enhancement; rCBV, regional cerebral blood volume; ROI, region of interest; SERT, 5-HT transporter; TE, echo time; TR, repetition time

Introduction

To date, there have been reports of both increased and decreased cerebral blood flow (CBF) following 3,4-methylenedioxymethamphetamine (MDMA) administration in humans using PET (Vollenweider *et al.*, 1998; Gamma *et al.*, 2000) and single photon emission computerized tomography co-registered with MRI (Chang *et al.*, 2000). The findings suggested a sub-chronic persistent vasoconstrictive effect of MDMA administration leading investigators to speculate that long-lasting changes to 5-HT function might underlie the observed effects (Chang *et al.*, 2000; Finsterer *et al.*, 2003).

de Win *et al.* (2007) prospectively studied sustained effects (>2 weeks abstinence) of a low dose of ecstasy (1.8 ± 1.3 tablets) on the brain in ecstasy-naïve volunteers using perfusion-weighted MRI for the determination of relative regional cerebral blood volume (rCBV). A sustained decrease in rCBV in the thalamus, dorsolateral frontal cortex and superior parietal cortex was observed in new low-dose ecstasy users. In line with previous investigations, the authors propose that decreases in CBV may indicate that even low-ecstasy doses can induce prolonged vasoconstriction in some brain areas due to sustained MDMA-mediated serotonergic effects.

There is a growing awareness of the incidence of cerebrovascular accidents (CVA) in young people with a history of exposure to recreational drugs in general and MDMA in particular (Kaku and Lowenstein, 1990; Gledhill *et al.*, 1993; Hanyu *et al.*, 1995; Petitti *et al.*, 1998; Perez *et al.*, 1999; Agaba *et al.*, 2002; Auer *et al.*, 2002; Miranda and O'Neill, 2002; Ferrington *et al.*, 2006). In this regard, acute or long-term changes to cerebral blood perfusion may contribute to such events in vulnerable drug users. A role for 5-HT is implicated on account of the acute effects of MDMA on 5-HT release from serotonergic nerve endings and the proposed involvement of 5-HT in regulating the brain microcirculation (Parsons, 1991; Cohen *et al.*, 1996).

In order to gain greater insight and clarity with regard to the acute, subacute and chronic effects of MDMA, animal studies investigating CBF and CBV changes following MDMA administration are of importance. In this regard, it has been previously shown in laboratory animal investigations that MDMA-induced 5-HT dysfunction alters cerebrovascular control mechanisms in a manner that is consistent with the known vasoconstrictor properties of 5-HT (Quate *et al.*, 2004; Ferrington *et al.*, 2006).

MDMA administration leads to the long-term depletion of central 5-HT and 5-hydroxyindole acetic acid (5-HIAA) in rats (Colado *et al.*, 1993; Aguirre *et al.*, 1998; O'Shea *et al.*, 1998; Shankaran and Gudelsky, 1998; Wallace *et al.*, 2001; Green *et al.*, 2003; Thompson *et al.*, 2004), long-term reductions in 5-HT immunoreactive axons in non-human primates (Hatzidimitriou *et al.*, 1999) and ligand binding to the 5-HT transporter (SERT), a hallmark of 5-HT nerve terminal integrity, in a number of rodent studies (Scanzello *et al.*, 1993; Colado *et al.*, 1995; O'Shea *et al.*, 1998) and a study in Göttingen minipigs (Cumming *et al.*, 2007). Gramsbergen and Cumming (2007) reported that serotonin mediates changes in striatal glucose and lactate metabolism following systemic MDMA administration in rats. With regard to functional con-

sequences for cerebral perfusion, van Donkelaar *et al.* (2010) reported increases in CBF in prefrontal cortex, lateral amygdala, substantia nigra and locus coeruleus 3 weeks after a 4 day repeated regimen of MDMA in rats when compared with vehicle-treated controls. McBean and colleagues also showed that 6–9 weeks after treatment with methylenedioxyamphetamine, a demethylated form of MDMA and also a 5-HT-specific neurotoxin, rats showed focal increases in CBF in excess of metabolic demand (McBean *et al.*, 1991).

Recently, a new quantitative bolus-tracking arterial spin labelling (btASL) MRI technique was developed and described by Kelly *et al.* (2009; 2010) for the measurement of perfusion state in the rodent brain. The objective of this investigation was to use the btASL technique to determine regional-, time- and dose-dependent changes on cerebral perfusion in the rat following single acute administration of MDMA. As MDMA is a recreational drug that is taken repeatedly by recreational users over short periods and provokes a long-term reduction in central 5-HT concentration, the effect of repeated exposure to MDMA with subsequent long-term central 5-HT loss on cerebral cortical perfusion and CBV were assessed. Moreover, it was of interest to determine if prior exposure to MDMA and subsequent long-term cortical 5-HT loss would influence the response to acute challenge with MDMA.

Methods

Animals

Male Wistar rats (175–250 g) were obtained from the Bioresearch Unit, Trinity College Dublin and housed, four per cage, in medium-sized, hard-bottomed polypropylene cages under standard housing, at a constant temperature ($20 \pm 2^\circ\text{C}$) and lighting conditions (12 h light : 12 h dark cycle). Food and water were freely available. All experiments were in compliance with the European Council Directive, 1986 (86/806/EEC). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Drug preparation

MDMA HCl (National Institute of Drug Abuse, USA) was dissolved in 0.89% saline and administered through i.p. injection at a volume of $1 \text{ mL}\cdot\text{kg}^{-1}$.

Anaesthesia and animal preparation

Rats were anaesthetized with ketamine ($100 \text{ mg}\cdot\text{mL}^{-1}$, $0.5 \text{ mL}\cdot\text{kg}^{-1}$ bodyweight) and xylazine ($20 \text{ mg}\cdot\text{mL}^{-1}$, $0.5 \text{ mL}\cdot\text{kg}^{-1}$). In some experiments, the right femoral vein was catheterized and used as a portal for blood sampling and administration of contrast agent. Animals were placed onto a custom-built fibreglass cradle and a mechanical ventilator (Ugo Basile, Comerio, VA, Italy) delivered adequate inflowing gas to the facemask and the respiration signal was monitored using custom hardware and software (SA Instruments Inc., Stony Brook, NY, USA). Animals were placed into the 7 tesla (7T), 30 cm bore animal MR system (Bruker Biospin 70/30 magnet system; Bruker Biospin, Ettlingen, Germany). Anaesthetic depth was controlled by maintaining respiration rate at

Table 1

Physiological measurements assessed following acute MDMA administration

	Treatment Group	
	Vehicle	MDMA
Body temperature increase (°C)	0.38 ± 0.18	2.01 ± 0.16
pCO ₂ (mm Hg)	46.64 ± 1.57	44.86 ± 1.39
pH	7.39 ± 0.05	7.3 ± 0.01

Body temperature was measured over a 3 h period prior to animals being anaesthetized and placed into the MRI scanner. pCO₂ and pH were measured using a blood sample obtained from the catheterized femoral vein, using a calibrated transcutaneous blood gas analyser. Data are expressed as mean ± SEM ($n = 16$). ** $P < 0.01$ versus vehicle-treated animals (Student's t -test).

60–85 breaths per min. The partial pressure of carbon dioxide (pCO₂) and pH were measured, in a blood sample obtained from the catheterized femoral vein, using a calibrated transcutaneous blood gas analyser (TCM4, Radiometer Copenhagen, Willich, Germany) before commencing the scanning (Table 1). In the repeated administration experiment, a hyperthermic response was observed on the first day of drug administration, which was not present on the second day. Eight weeks following MDMA administration, acute MDMA challenge provoked a maximum body temperature increase of $3.84 \pm 0.21^\circ\text{C}$ in comparison with $0.53 \pm 0.22^\circ\text{C}$ in vehicle-treated controls.

MRI

btASL MRI assesses cerebral perfusion through the calculation of two transit times: mean transit time (MTT), which represents time taken for labelled arterial blood to traverse the vasculature and reach the imaging plane, and capillary transit time (CTT), which represents time taken for the blood to disperse at the imaging plane. MTT is inversely proportional to CBF, while CTT is inversely proportional to CBF squared. A third quantifiable output is the btASL signal amplitude, which is derived from the area under the signal-time ASL curve and is proportional to CBV (see Kelly *et al.*, 2009; 2010). Changes in MTT or CTT should match changes in flow or perfusion, that is when the transit time increases then flow or perfusion decreases. If a change in flow is postulated then it should be supported by a proportional change in either MTT or CTT or both. Where a reduction in MTT is observed, this is ordinarily accompanied by a reduction in CTT and, taken together, is indicative of an increase in perfusion. Readers may consult these reports for a comprehensive and detailed discussion on these perfusion parameters.

A high-resolution anatomical scan [T_2 -weighted rapid acquisition with relaxation enhancement; (RARE)] was generated using the following parameters: slice thickness = 1.5 mm, repetition time (TR) = 3134.511 ms, echo time (TE) = 12 ms, RARE factor = 8, RF flip angle = $90^\circ/180^\circ$, field of view (FOV) = 3×3 cm, image matrix = 128×128 and total scan time = 50 s. A continuous ASL sequence was subse-

quently applied, as previously described (Kelly *et al.*, 2009). Briefly, the sequence consisted of a 5 s preparation interval, which contained the inversion pulse followed by snapshot fast low-angle shot (FLASH) acquisition. The sequence was used to provide signal-time curves of the passage of a 3 s bolus through the primary motor cortex region using the following parameters: slice thickness = 2 mm, TR = 6.938 ms, TE = 2.63 ms, RF flip angle = 30° , FOV = 3×3 cm and image matrix = 128×64 . MTT, CTT and signal amplitude values were generated by fitting the non-compartmental model of cerebral perfusion to the experimental data (Kelly *et al.*, 2009).

An increase in blood–brain barrier (BBB) permeability has been reported following MDMA administration in rats (Sharma and Ali, 2008). Consideration, therefore, should be given to a potential role for BBB disruption as a contributing factor in mediating MDMA-induced changes in CBF or CBV. Evans blue (EB) dye extravasation was assessed in separate groups of animals treated identically to those undergoing MRI as previously described (Sharma and Ali, 2008). In addition, a contrast-enhanced MRI approach was adopted for assessment of BBB integrity. Previously, Pirkko and Johnson (2008) assessed BBB permeability changes in an animal model of multiple sclerosis using contrast agent (gadolinium)-enhanced MRI. Gadolinium (Magnevist) (1 mL; i.v.) was infused via the portal secured to the femoral vein 5 min following completion of btASL. A FLASH scan sequence was employed to assess gadolinium distribution throughout the cerebrovasculature using the following parameters: slice thickness = 1.2 mm, TR = 312.5 ms, TE = 2.53 ms, RF flip angle = 30° , FOV = 3×3 cm, image matrix = 128×128 and total scan time = 60 min. Figure 1A shows the appearance of contrast agent in a coronal brain slice in time series pre- and post-i.v. administration of gadolinium generated following subtraction of background obtained prior to the gadolinium infusion. A region of interest (ROI) within the frontal cortex was chosen to assess BBB integrity (Figure 1B and C); and density of contrast change was measured, prior to and at intervals, following gadolinium administration.

Data were analysed using data acquisition and analysis software, Paravision (Bruker Biospin), and scripts written in Interactive Data Language (IDL; ITT Visual Information Systems, Boulder, CO, USA) software version 7.0. The Coyote IDL Library (Fanning Software Consulting, Fort Collins, CO, USA; downloaded from <http://www.dfanning.com>) was used to generate CBV maps. Changes in CBV are represented on a colour scale found adjacent to the CBV maps (Figures 2D, 3D and 5D). A bright colour on the CBV map indicates an area with high signal intensity and as signal intensity directly relates to CBV, ROIs with high signal intensity and CBV are represented as the brightest areas. ImageJ (Rasband, National Institute of Mental Health, Bethesda, MD, USA) software was used to select ROIs for analysis. ROIs were drawn in the spatially normalized high-resolution anatomical brain image obtained. Two brain sections at different levels along the coronal plane were analysed. The first coronal section comprised motor, somatosensory and insular cortex in addition to striatum; and the second coronal section comprised visual, auditory and parietal association cortex as well as thalamus and hippocampus. These regions were chosen due to the fact that they are highly innervated by 5-HT (Cohen *et al.*, 1996). The curve-fitting routine in Mathematica (Wolfram Research

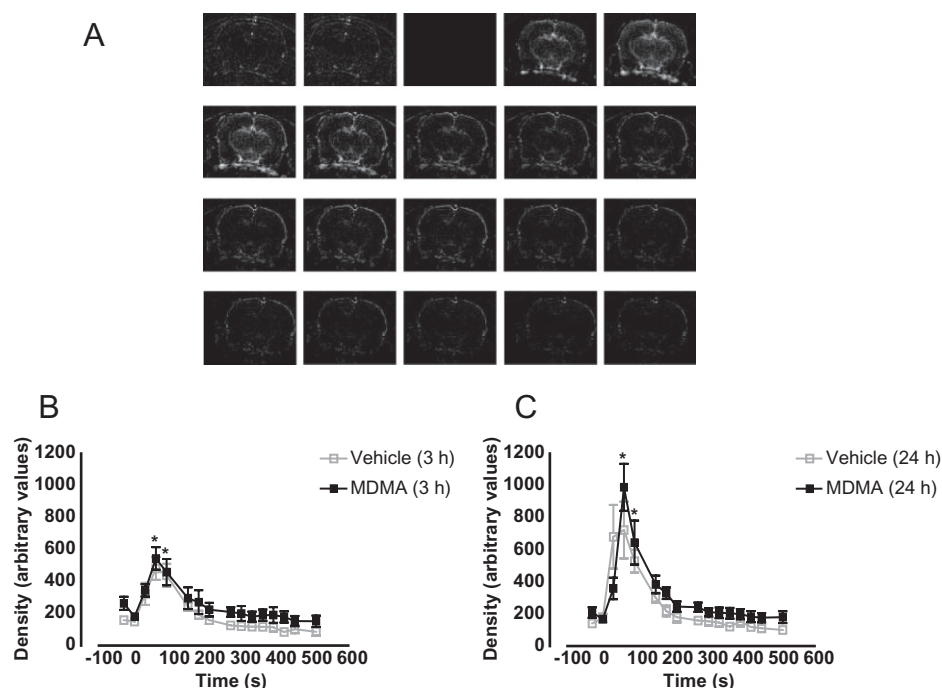


Figure 1

MR images representing the appearance and clearance of gadolinium contrast agent over time following i.v. administration. Images show the appearance of gadolinium in a 30 s time series pre- and post-i.v. administration of gadolinium. The images (A) were generated by subtraction of background (time 0) prior to and following infusion. Analysis of change in density showed an increase in contrast that peaks after 1 min followed by a return to baseline 3 min later. No differences in contrast change were found between MDMA and vehicle-treated animals either (B) 3 or (C) 24 h after drug administration. Data are expressed as mean density $\pm$ SEM ($n = 8$).

Inc., Version 5.1, Champaign, IL, USA) was used to calculate MTT, CTT and signal amplitude from the btASL signal-time curves as previously described (Kelly *et al.*, 2010).

Determination of regional 5-HT and 5-HIAA concentration

Immediately following scanning, animals were decapitated and the brain was removed and dissected quickly on ice. Brains were dissected as previously described (Harkin *et al.*, 2001). Frontal cortex and striatum were harvested for determination of neurotransmitter concentration. 5-HT and 5-HIAA concentrations were determined by high-performance LC coupled to electrochemical detection as previously described (Durkin *et al.*, 2008).

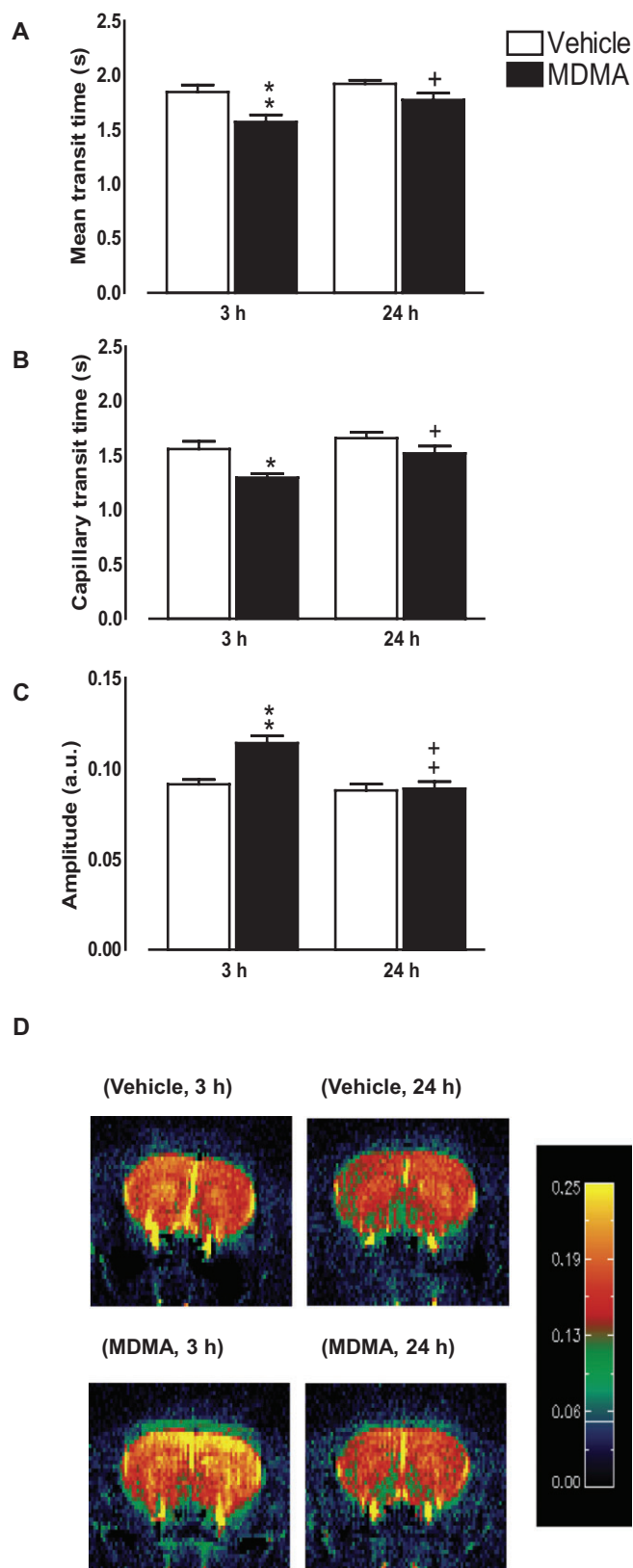
Experimental design

Study 1. MDMA-induced changes in CBF and CBV were examined over time. Animals received a single administration of MDMA (20 mg·kg⁻¹; i.p.) or vehicle (saline) and were placed into the MRI scanner 3 or 24 h later. BBB integrity was assessed *in vivo* and *ex vivo* in the 3 and 24 h treatment groups as described earlier. In a separate experiment where the femoral vein was not prepared for gadolinium infusion, animals received MDMA (20 mg·kg⁻¹; i.p.) or vehicle (saline) and were placed into the MRI scanner 1 h later. This was carried out to capture the effects of MDMA at an earlier time following drug administration.

Study 2. Dose-dependent changes in cerebral perfusion following MDMA administration were examined. Animals received a single administration of MDMA (5 or 20 mg·kg⁻¹; i.p.) and were placed into the scanner 3 h later.

Study 3. MDMA (5 or 10 mg·kg⁻¹; i.p.) or saline (0.89%) was administered four times per day over 2 consecutive days. Animals remained in their home cages for a further 8 weeks. On the day of testing, animals received a single administration of MDMA (20 mg·kg⁻¹; i.p.) or vehicle (saline). Animals were anaesthetized and placed into the MRI scanner 3 h later.

Initially, a lower dose of MDMA (5 mg·kg⁻¹; i.p.) administered four times daily over 2 days was tested. After 8 weeks, a long-term 5-HT loss was not observed in the cortex. Subsequently, a higher dose of MDMA as reported was used. The treatment regimen and recovery period has been employed previously to assess long-term effects of MDMA associated with sustained 5-HT loss (Durkin *et al.*, 2008). Perfusion parameters were measured 8 weeks following MDMA administration to allow time for sustained 5-HT loss to be established. There were no fatalities over the repeated dose regime. The animals showed mild symptoms of the 5-HT syndrome including hyperthermia with evidence of pharmacological tolerance on the second day as reported. Animals gained weight as normal for the 8 weeks following dosing and prior to the MRI scans. Additional behavioural testing was not carried over this period.

**Figure 2**

MDMA provokes a time-dependent decrease in MTT and CTT with a corresponding increase in signal amplitude in somatosensory cortex. MDMA (20 mg·kg⁻¹) provokes a decrease in (A) MTT and (B) CTT 3 and 24 h following drug administration, and increases (C) signal amplitude 3 h following drug administration when compared with vehicle-treated controls in somatosensory cortex. ANOVA of MTT and CTT showed a time × MDMA interaction [$F_{(1,28)} = 8.35$, $P < 0.01$] and [$F_{(1,28)} = 4.59$, $P < 0.05$], respectively. *Post hoc* comparisons revealed that MTT and CTT were decreased in MDMA-treated animals 3 h ($P < 0.01$) following drug administration when compared with vehicle-treated controls. There was an increase in (A) MTT and (B) CTT over time when MDMA-treated animals were compared 3 and 24 h ($P < 0.01$) following drug administration. ANOVA of signal amplitude showed a time × MDMA interaction [$F_{(1,28)} = 11.622$, $P < 0.01$]. *Post hoc* comparisons revealed that signal amplitude was increased in MDMA-treated animals 3 h ($P < 0.01$), but not 24 h, following drug administration when compared with vehicle-treated controls. There was a decrease in signal amplitude over time in MDMA-treated animals when compared 3 and 24 h ($P < 0.01$) following drug administration (C). (D) CBV maps depicting MDMA-induced increases in CBV 3 h following drug administration in a representative coronal brain section. Colour change depicting changes to CBV had largely returned to baseline, being indistinguishable from vehicle control, 24 h following drug administration. Data are expressed as mean ± SEM ($n = 8$). * $P < 0.05$; ** $P < 0.01$ versus vehicle at corresponding time point. ⁺ $P < 0.05$; ⁺⁺ $P < 0.01$ versus MDMA (3 h) (Student–Newman–Keuls *post hoc* test).

Statistical analysis

Statistical analysis was carried out using GBStat v.10 (Dynamic Microsystems, Inc., Silver Spring, MD, USA). Student's *t*-test, one-way, two-way and repeated measures ANOVA were performed where appropriate. If significant changes were observed, a Dunnett's or Student–Newman–Keuls *post hoc* test was carried out. Data were deemed significant when $P < 0.05$.

The drug/molecular target nomenclature conform to the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al.*, 2011).

Results

Study 1: MDMA provokes a time-dependent decrease in MTT and CTT and an increase in signal amplitude in somatosensory, primary and secondary motor cortex

In the present investigation, a decrease in MTT and CTT (Figure 2A,B) with a corresponding increase in signal amplitude (Figure 2C) were measured in somatosensory cortex following MDMA administration. These reductions in MTT and CTT may be taken as evidence for increased CBF in response to MDMA while an increase in signal amplitude reflects an increase in CBV in line with the increased perfusion. CBV maps depict the increase in CBV following acute MDMA administration that returns to normal 24 h later (Figure 2D).

Changes observed 1 h following drug administration. Drug effects were also determined in the somatosensory cortex 1 h following administration in animals, which were not sub-

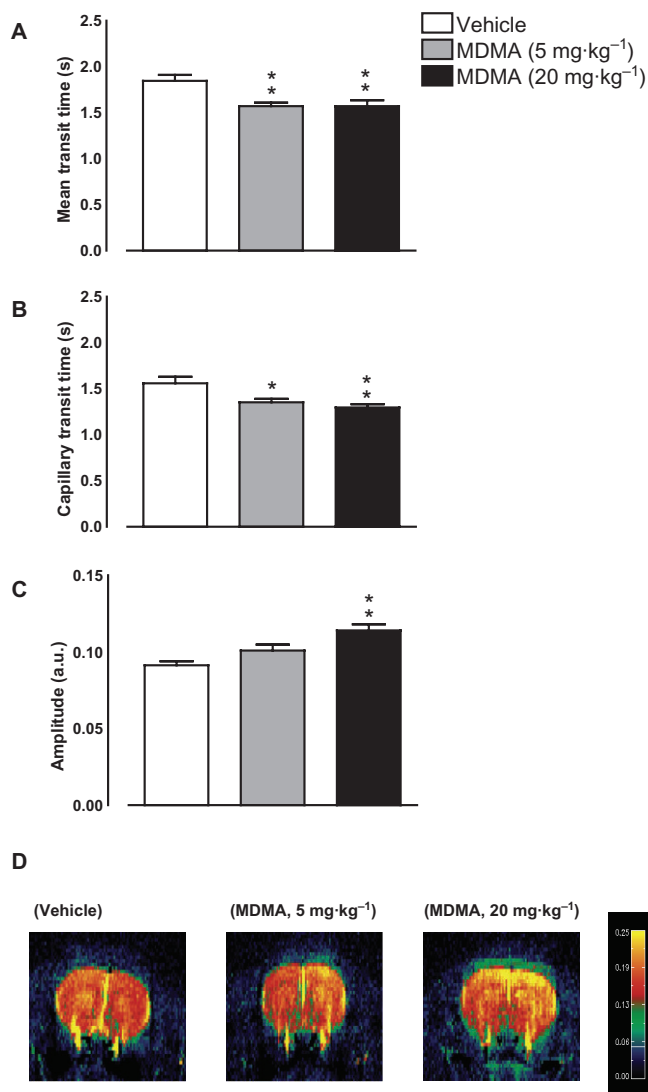


Figure 3

MDMA provokes a dose-related decrease in MTT and CTT and increase in signal amplitude in somatosensory cortex. MDMA (5 and 20 mg·kg⁻¹) induced a decrease in (A) MTT and corresponding increase in (C) signal amplitude in somatosensory cortex. MDMA (20 mg·kg⁻¹) induced a decrease in (B) CTT. ANOVA of MTT and CTT showed an effect of MDMA [$F_{(2,21)} = 7.733$, $P < 0.01$] and [$F_{(2,21)} = 7.62$, $P < 0.01$], respectively. *Post hoc* comparisons revealed that MTT ($P < 0.01$) and CTT ($P < 0.05$ and $P < 0.01$, respectively) were decreased 3 h following MDMA (5 and 20 mg·kg⁻¹) administration when compared with vehicle-treated controls. The magnitude of change in MTT was similar with both doses of MDMA (A) and (B). ANOVA of signal amplitude showed an effect of MDMA [$F_{(2,21)} = 10.29$, $P < 0.001$]. *Post hoc* comparisons revealed a dose-related increase in amplitude 3 h following MDMA (20 mg·kg⁻¹) administration only when compared with vehicle-treated controls ($P < 0.01$) (C). (D) CBV maps depicting dose-dependent MDMA-induced increases in CBV 3 h following drug administration in a representative coronal brain section. Increased CBV is evident in cortical regions following administration of MDMA (5 and 20 mg·kg⁻¹) in comparison with a vehicle-treated control animal. Data are expressed as mean $\pm$ SEM ($n = 8$). * $P < 0.05$; ** $P < 0.01$ versus vehicle (Dunnett's *post hoc* test).

jected to femoral vein catheterization. Student's *t*-test revealed a significant reduction in MTT ($P < 0.001$; 1.69 ± 0.04 and 1.42 ± 0.04) and CTT ($P < 0.01$; 1.52 ± 0.07 and 1.24 ± 0.03) and a significant increase in signal amplitude ($P < 0.01$; 0.098 ± 0.003 and 0.12 ± 0.005 , respectively) when compared with vehicle-treated controls.

Other regional effects. Similar changes to those observed in the somatosensory cortex were obtained in the primary and secondary motor cortex (Table 2).

A reduction in MTT and CTT and increase in signal amplitude ($P < 0.05$; Student's *t*-test) was observed in the ventral striatum 1 h following MDMA administration when compared with vehicle treated controls (1.66 ± 0.03 and 1.57 ± 0.02 , 1.39 ± 0.04 and 1.31 ± 0.01 , and 0.098 ± 0.002 and 0.104 ± 0.002 , respectively); however, no effects of MDMA were observed in this region 3 or 24 h following drug administration.

An increase in signal amplitude in the absence of significant changes in transit times was observed in the auditory cortex ($P < 0.001$; 0.076 ± 0.001 and 0.095 ± 0.0024), parietal association cortex ($P < 0.01$; 0.073 ± 0.003 and 0.099 ± 0.004) and thalamus ($P < 0.05$; 0.084 ± 0.002 and 0.097 ± 0.004) 3 h, but at no other time, following MDMA administration in comparison with vehicle-treated controls.

No further changes in MTT, CTT or signal amplitude were observed in dorsal striatum, retrosplenial cortex, visual cortex or hippocampus following MDMA administration at any of the times assessed (data not shown).

Cortical 5-HT concentrations were reduced following MDMA 3 and 24 h following drug administration when compared with vehicle-treated controls (Figure 4A). There was a decrease in 5-HIAA concentrations also 3 h, but not 24 h, following drug administration (Figure 4B). By contrast to the cortex, MDMA failed to influence 5-HT concentrations in the striatum (Figure 4E). A reduction in striatal 5-HIAA concentrations was evident 3 h following drug administration when compared with vehicle controls, which had returned to control levels 24 h later (Figure 4F). Student's *t*-test revealed that cortical 5-HT concentration was significantly reduced ($P < 0.05$) 1 h following MDMA administration in comparison with vehicle-treated controls (509.5 ± 37.44 ng·g⁻¹ and 334.7 ± 62.11 ng·g⁻¹, respectively). There was no change in 5-HIAA concentrations 1 h following MDMA administration. By contrast to the cortex, MDMA failed to influence 5-HT concentration in the striatum. Student's *t*-test revealed that 5-HIAA was significantly reduced ($P < 0.05$) 1 h following MDMA administration in comparison with vehicle-treated controls (813.8 ± 17.6 ng·g⁻¹ and 733.4 ± 27.55 ng·g⁻¹, respectively).

Study 2: MDMA provokes a dose-related decrease in MTT and CTT and increase in signal amplitude in somatosensory, primary and secondary motor cortex

MDMA, at low dose (5 mg·kg⁻¹ i.p.) and high dose (20 mg·kg⁻¹ i.p.), decreases MTT and CTT (Figure 3A,B) with a concomitant increase in signal amplitude (Figure 3C) indicative of an increase in CBF and CBV, respectively. CBV maps depict a dose-related increase in CBV (Figure 3D).

Table 2

MDMA provokes a time-dependent decrease in MTT and CTT and increase in signal amplitude in motor cortex

		3 h			24 h		
		MTT (s)	CTT (s)	Amplitude (a.u.)	MTT (s)	CTT (s)	Amplitude (a.u.)
Primary motor	Vehicle	2.00 ± 0.04	1.63 ± 0.07	0.087 ± 0.002	2.13 ± 0.06	1.83 ± 0.06	0.084 ± 0.003
	MDMA	1.73 ± 0.05**	1.36 ± 0.03*	0.103 ± 0.002**	1.93 ± 0.07 ⁺	1.61 ± 0.07 ⁺⁺	0.085 ± 0.004 ⁺⁺
Secondary motor	Vehicle	2.15 ± 0.06	1.77 ± 0.08	0.08 ± 0.002	2.19 ± 0.06	1.82 ± 0.07	0.08 ± 0.004
	MDMA	1.84 ± 0.04*	1.41 ± 0.02*	0.1 ± 0.003**	2.08 ± 0.07	1.72 ± 0.09 ⁺	0.08 ± 0.005 ⁺

ANOVA of MTT and CTT in the primary motor cortex showed an effect of time [$F_{(1,28)} = 9.68$, $P < 0.05$ and $F_{(1,28)} = 15.32$, $P < 0.001$] and an effect of MDMA [$F_{(1,28)} = 17.413$, $P < 0.001$ and $F_{(1,28)} = 17.143$, $P < 0.001$], respectively. *Post hoc* comparisons revealed that MTT ($P < 0.01$) and CTT ($P < 0.05$) were reduced in MDMA-treated animals 3 and 24 h following drug administration when compared with vehicle-treated controls. There was an increase in MTT and CTT over time when MDMA-treated animals were compared 3 and 24 h following drug administration ($P < 0.05$). ANOVA of signal amplitude in the primary motor cortex showed an effect of time [$F_{(1,28)} = 12.08$, $P < 0.01$] and an effect of MDMA [$F_{(1,28)} = 10.69$, $P < 0.01$]. *Post hoc* comparisons revealed that signal amplitude was increased in MDMA-treated animals 3 h ($P < 0.01$), but not 24 h, following drug administration when compared with vehicle-treated controls. There was a decrease in signal amplitude over time in MDMA-treated animals when compared 3 and 24 h following drug administration ($P < 0.01$).

ANOVA of MTT in the secondary motor cortex showed an effect of MDMA [$F_{(1,28)} = 7.86$, $P < 0.01$]. *Post hoc* comparisons revealed that MTT was decreased in MDMA-treated animals 3 h ($P < 0.05$) following drug administration when compared with vehicle-treated controls. ANOVA of CTT in the secondary motor cortex showed an effect of time [$F_{(1,28)} = 5.57$, $P < 0.05$] and an effect of MDMA [$F_{(1,28)} = 9.55$, $P < 0.01$]. *Post hoc* comparisons revealed that CTT was decreased in MDMA-treated animals 3 h ($P < 0.05$) following drug administration when compared with vehicle-treated controls. There was an increase in CTT over time when MDMA-treated animals were compared 3 and 24 h following drug administration ($P < 0.05$). ANOVA of signal amplitude in the secondary motor cortex showed an effect of time [$F_{(1,28)} = 6.76$, $P < 0.05$] and an effect of MDMA [$F_{(1,28)} = 6.31$, $P < 0.05$]. *Post hoc* comparisons revealed that signal amplitude was increased in MDMA-treated animals 3 h ($P < 0.01$), but not 24 h, following drug administration when compared with vehicle-treated controls. There was a decrease in signal amplitude over time in MDMA-treated animals when compared 3 and 24 h ($P < 0.05$) following drug administration. Data are expressed as mean ± SEM ($n = 8$). * $P < 0.05$ and ** $P < 0.01$ versus vehicle at corresponding time point. ⁺ $P < 0.05$ and ⁺⁺ $P < 0.01$ versus MDMA (3 h) (Student–Newman–Keuls *post hoc* test).

Other regional effects. Similar changes to those observed in the somatosensory cortex were obtained in the primary and secondary motor cortex (Table 3).

Increases in signal amplitude ($P < 0.05$) were observed in auditory cortex [$F_{(2,21)} = 18.29$, $P < 0.001$] (0.076 ± 0.004 and 0.095 ± 0.011) and thalamus [$F_{(2,21)} = 6.77$, $P < 0.01$] (0.084 ± 0.005 and 0.097 ± 0.011) following MDMA administration (20 mg·kg⁻¹) and in the parietal association cortex [$F_{(2,21)} = 14.82$, $P < 0.001$] (0.073 ± 0.003 and 0.089 ± 0.002 and 0.073 ± 0.003 and 0.099 ± 0.004 , respectively) following MDMA administration (5 and 20 mg·kg⁻¹). No changes in MTT, CTT or signal amplitude were observed in insular cortex, dorsal or ventral striatum, retrosplenial cortex, visual cortex or hippocampus at either of the doses assessed (data not shown).

MDMA (5 and 20 mg·kg⁻¹) produced a dose-related reduction in cortical 5-HT (Figure 4C) and 5-HIAA (Figure 4D) concentration 3 h following drug administration. Striatal 5-HIAA concentration (Figure 4H) was reduced following MDMA (20, but not 5 mg·kg⁻¹) when compared with vehicle-treated controls. In a similar fashion to the time course experiment, MDMA failed to influence striatal 5-HT concentration (Figure 4G).

Study 3: Prior exposure to MDMA attenuates increased cerebral cortical perfusion induced by acute MDMA challenge

As previously found MDMA, (20 mg·kg⁻¹, i.p.) induced a reduction in MTT and CTT (Figure 5A,B), and an increase in

signal amplitude (Figure 5C) in the somatosensory cortex in comparison with vehicle-treated controls. Prior exposure to MDMA (10, but not 5 mg·kg⁻¹, four times daily for 2 days followed by 8 weeks) had no effect alone but attenuated the acute response to MDMA-induced perfusion changes (Figure 5). CBV maps depict the attenuated response to MDMA challenge in CBV following prior MDMA exposure (Figure 5D).

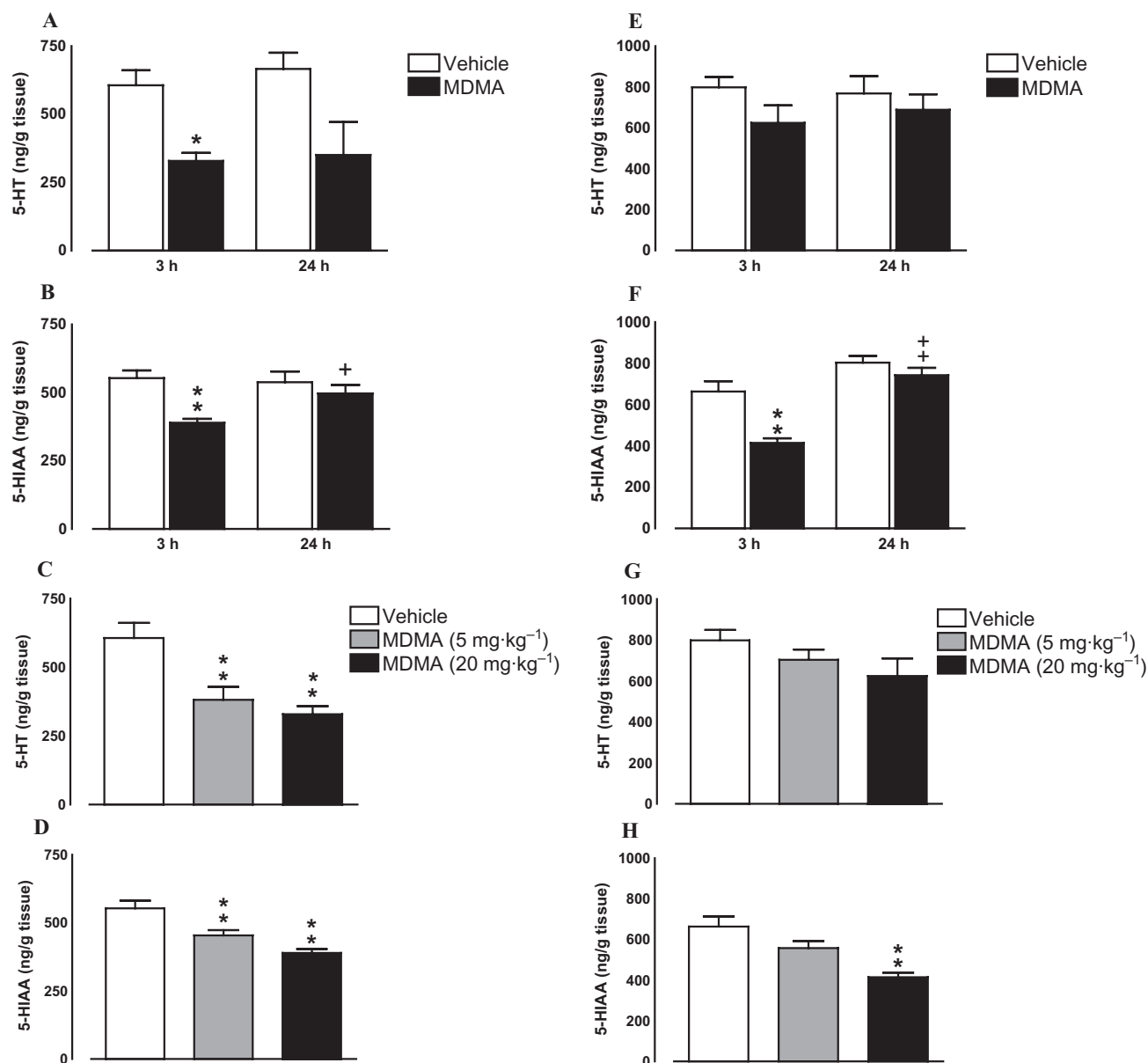
Prior exposure to MDMA (10, but not 5 mg·kg⁻¹) provoked a long-term (8 weeks) reduction in cortical 5-HT concentrations (Figure 6). MDMA did not provoke a change in striatal 5-HT concentrations in the time course and dose–response experiments (Figure 4E,G). Moreover, the results from these experiments indicated that perfusion changes were confined to the cortex (Figure 4A,C). For these reasons, measurements of 5-HT in the long-term experiment were restricted to the frontal cortex.

Other regional effects. Similar effects to those reported in the somatosensory cortex were found in the primary and secondary motor cortex; and the results may be found in Table 4.

ANOVA of signal amplitude in the auditory cortex showed an effect of MDMA pretreatment [$F_{(1,30)} = 8.03$, $P < 0.01$]. *Post hoc* comparisons revealed that acute MDMA challenge produced an increase in signal amplitude (0.088 ± 0.003), in the absence of any change in MTT or CTT, 3 h following administration in comparison with vehicle-treated control animals (0.078 ± 0.003). No changes in MTT, CTT or amplitude were observed in insular, visual, parietal association or

Frontal cortex

Striatum

**Figure 4**

MDMA provokes a time- and dose-dependent decrease in 5-HT and 5-HIAA concentration. Animals received MDMA (5 or 20 mg·kg⁻¹); and bTASL was performed 3 or 24 h after drug administration. Cortical and striatal 5-HT and 5-HIAA concentration were subsequently determined post-mortem. ANOVA of cortical 5-HT concentration showed an effect of MDMA [$F_{(1,28)} = 15.87$, $P < 0.001$]. *Post hoc* comparisons revealed a decrease in 5-HT concentration 3 and 24 h following drug administration when compared with vehicle-treated controls (A). ANOVA of 5-HIAA concentration showed a time $\times$ MDMA interaction [$F_{(1,28)} = 4.25$, $P < 0.05$]. *Post hoc* comparisons revealed a decrease in cortical 5-HIAA concentration 3 h, but not 24 h, following drug administration when compared with vehicle-treated controls (B). By contrast to the cortex, MDMA failed to influence 5-HT (E) concentration in the striatum. ANOVA of striatal 5-HIAA concentration (F) showed a time $\times$ MDMA interaction [$F_{(1,28)} = 6.57$, $P < 0.05$]. *Post hoc* comparisons revealed a reduction in striatal 5-HIAA concentration 3 h following drug administration when compared with vehicle-treated controls, which had returned to control levels 24 h later. MDMA (5 and 20 mg·kg⁻¹) produced a dose-related reduction in cortical 5-HT (C) and 5-HIAA (D) concentration 3 h following drug administration. ANOVA of 5-HT and 5-HIAA concentration showed an effect of MDMA [$F_{(2,21)} = 10.6$, $P < 0.001$] and [$F_{(2,21)} = 15.28$, $P < 0.001$], respectively. ANOVA of striatal 5-HIAA concentration showed an effect of MDMA [$F_{(2,21)} = 11.04$, $P < 0.001$]. Striatal 5-HIAA concentration (H) was reduced following MDMA (20 but not 5 mg·kg⁻¹) when compared with vehicle-treated controls. In a similar fashion to the time course experiment, MDMA failed to influence striatal 5-HT concentration (G). Data are expressed as mean $\pm$ SEM ($n = 8$). * $P < 0.05$; ** $P < 0.01$ versus vehicle at corresponding time point. + $P < 0.05$ versus MDMA (3 h.) (Student–Newman–Keuls or Dunnett’s *post hoc* test).

Table 3

MDMA provokes a dose-related decrease in MTT and CTT and increase in signal amplitude in motor cortex

		MTT (s)	CTT (s)	Amplitude (a.u.)
Primary motor	Vehicle	2.00 ± 0.04	1.63 ± 0.08	0.087 ± 0.002
	MDMA (5 mg·kg ⁻¹)	1.72 ± 0.06**	1.47 ± 0.06	0.096 ± 0.003*
	MDMA (20 mg·kg ⁻¹)	1.73 ± 0.05**	1.36 ± 0.03**	0.103 ± 0.002**
Secondary motor	Vehicle	2.08 ± 0.09	1.72 ± 0.09	0.086 ± 0.003
	MDMA (5 mg·kg ⁻¹)	1.89 ± 0.07	1.6 ± 0.08	0.092 ± 0.003
	MDMA (20 mg·kg ⁻¹)	1.85 ± 0.04	1.41 ± 0.02**	0.102 ± 0.003**

ANOVA of MTT in the primary motor cortex showed an effect of MDMA [$F_{(2,21)} = 10.4$, $P < 0.001$]. *Post hoc* comparisons revealed that MTT was decreased 3 h following MDMA (5 and 20 mg·kg⁻¹) administration when compared with vehicle-treated controls ($P < 0.01$). The magnitude of change in MTT was similar with both doses of MDMA. ANOVA of CTT in the primary motor cortex showed an effect of MDMA [$F_{(2,21)} = 5.38$, $P < 0.05$]. *Post hoc* comparisons revealed dose-related effects where CTT was reduced 3 h following MDMA (20 mg·kg⁻¹) administration only when compared with vehicle-treated controls ($P < 0.05$). ANOVA of signal amplitude showed an effect of MDMA [$F_{(2,21)} = 10.32$, $P < 0.001$]. *Post hoc* comparisons revealed a dose-dependent increase in signal amplitude 3 h following MDMA administration (5 and 20 mg·kg⁻¹) when compared with vehicle treated controls ($P < 0.05$; $P < 0.01$, respectively). The magnitude of change in amplitude was greater with the higher dose of MDMA.

ANOVA of MTT in the secondary motor cortex showed no effect of MDMA [$F_{(2,21)} = 2.93$, $P = 0.07$] albeit approaching significance. ANOVA of CTT in the secondary motor cortex showed an effect of MDMA [$F_{(2,21)} = 4.63$, $P < 0.05$]. *Post hoc* comparisons revealed dose-dependent effects where CTT was reduced 3 h following MDMA (20 mg·kg⁻¹) administration only when compared with vehicle-treated controls ($P < 0.01$). ANOVA of signal amplitude in the secondary motor cortex showed an effect of MDMA [$F_{(2,21)} = 6.93$, $P < 0.01$]. *Post hoc* comparisons revealed a dose-dependent increase in amplitude 3 h following MDMA (20 mg·kg⁻¹) administration only when compared with vehicle-treated controls ($P < 0.01$). Data are expressed as mean ± SEM ($n = 8$). * $P < 0.05$, ** $P < 0.01$ versus vehicle (Dunnett's *post hoc* test).

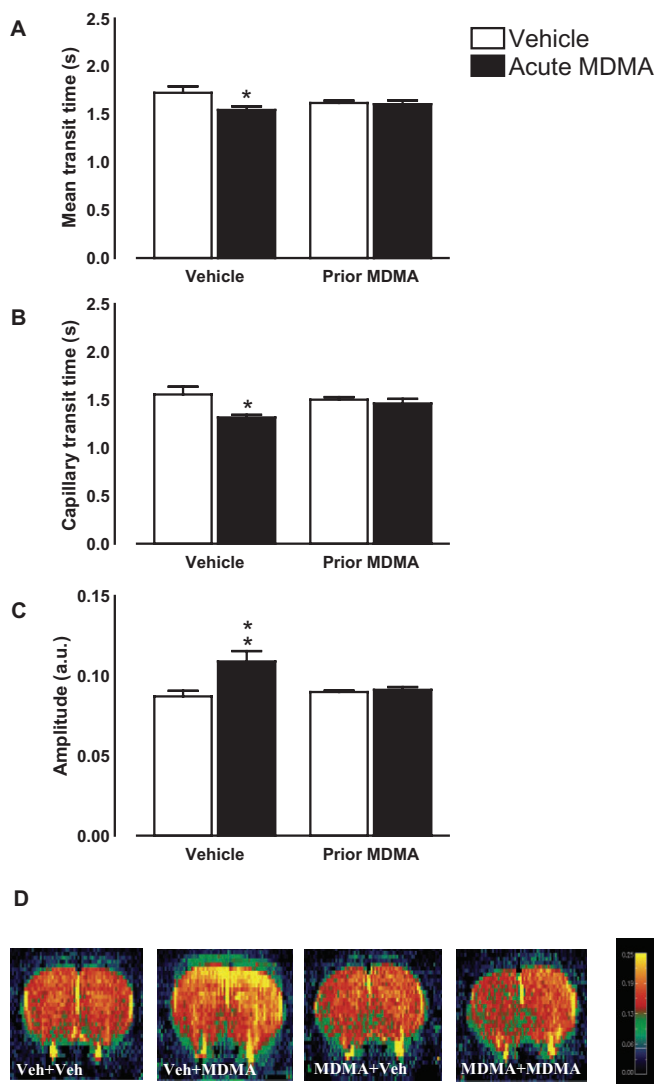
retrosplenial cortex, dorsal or ventral striatum, hippocampus or thalamus (data not shown).

Discussion and conclusions

In the present investigation, MDMA provoked a time- and dose-related decrease in MTT and CTT coupled with an increase in signal amplitude when compared with vehicle-treated controls. Reductions in MTT and CTT may be taken as evidence for increased CBF in response to MDMA. Related to this, increases in signal amplitude reflect an increase in CBV in line with increased perfusion (Kelly *et al.*, 2010). The effects of MTT, CTT and signal amplitude were regionally dependent with changes significant in frontal cortical regions, including the somatosensory and motor cortex, but not subcortical or posterior cortical regions. MDMA-induced reductions in transit times were most notable 1 and 3 h following drug administration with effects significantly less apparent after 24 h. In tandem, changes to signal amplitude and CBV were observed 1 and 3 h, but absent 24 h, following drug administration. When taken together, the results indicate that MDMA provokes acute dose-related increases in CBF and CBV in cortical regions that persist for several hours, but are reduced or absent 24 h after drug administration. No differences in clearance of gadolinium (Figure 1A–C) or extravasation of EB into parenchyma (including cortex, caudate, septum, hippocampus, thalamus, brain stem and cerebellum; data not shown) between drug-treated and vehicle-treated groups were observed. Thus, changes in CBF and CBV occurred independently of any observable loss of integrity to the BBB.

Hyperthermia does not appear to have a role to play in the MDMA-induced increases in perfusion. A separate set of experiments conducted, using the 5-HT releaser fenfluramine, mimicked the responses obtained with MDMA, that is reduced MTT and CTT with a corresponding increase in signal amplitude; yet, fenfluramine produced a hypothermic response at the dose administered when compared with vehicle-treated controls. Thus, increased perfusion is obtained following administration of these substituted amphetamines despite opposing effects on core body temperature. In the repeated administration experiment, hyperthermia was produced on the first day of drug administration, which was not present on day 2. Eight weeks following repeated MDMA administration, acute challenge with MDMA once again provoked a hyperthermic response; yet, despite a raised core body temperature no increase in cortical perfusion was observed. Increased temperature peaks 1 h following drug administration and re-approaches baseline 3 h following drug administration, a time when increased cerebral perfusion is observed. Coupled with the observations following fenfluramine administration, it is, therefore, unlikely that acute changes in core body temperature are what mediate the changes observed in cerebral perfusion. Brain temperature was not assessed in the current investigation. It has previously been reported that MDMA (9 mg·kg⁻¹) may raise brain temperature provoking lethality in rats; but this is under warm ambient conditions (29°C) where heat dissipation mechanisms are impaired (see Kiyatkin, 2010).

As expected, MDMA was associated with a significant reduction in cortical 5-HT and 5-HIAA concentration 3 h following drug administration. While the reduction in 5-HT



persists for 24 h, cortical 5-HIAA concentration returns to control levels over this time indicating a recovery of 5-HT metabolism following MDMA administration. By contrast, MDMA failed to produce a reduction in striatal 5-HT concentration although striatal 5-HIAA concentrations were reduced 3 h following drug administration. Overall, the effects obtained are consistent with numerous previous reports where the 5-HT depleting action of MDMA is most notable in cortical areas (Wang *et al.*, 2004; Baumann *et al.*, 2007). Unlike the majority of amphetamine studies (Rosa-Neto *et al.*, 2004, for review), we found no activation by MDMA of CBF in the basal ganglia, which leads us to suggest that the net effects of MDMA on the release of 5-HT and/or dopamine in the rat striatum did not alter local afferent activity sufficiently to perturb CBF. Our findings are also consistent with Rosa-Neto *et al.* (2004) who reported that MDMA failed to influence CBF in the pig striatum yet drug effects were apparent in the frontal cortex. Such observations in relation to the regional specificity of the effects of MDMA are also in line with the findings of some clinical investigations where PET and structural brain imaging have shown that cerebral SERT

Figure 5

Prior exposure to MDMA attenuates increased cerebral cortical perfusion induced by acute MDMA challenge in somatosensory cortex. Acute MDMA administration produces a decrease in (A) MTT and (B) CTT with a corresponding increase in (C) signal amplitude in somatosensory cortex. Prior MDMA administration attenuates these acute MDMA-induced changes in all three cortical regions. ANOVA of MTT and CTT showed an effect of acute MDMA challenge [$F_{(1,28)} = 4.05$, $P < 0.05$] and [$F_{(1,28)} = 7.51$, $P < 0.05$], respectively. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in MTT and CTT 3 h following administration in comparison with vehicle-treated control animals ($P < 0.05$). Prior MDMA did not influence MTT or CTT when compared with controls. In addition, acute MDMA failed to provoke a decrease in MTT or CTT in the prior MDMA-exposed group (A) and (B). ANOVA of signal amplitude showed an MDMA pre-treatment $\times$ acute MDMA challenge interaction [$F_{(1,28)} = 7.1$, $P < 0.05$]. *Post hoc* comparisons revealed that acute MDMA challenge produced an increase in signal amplitude 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence amplitude when compared with controls. In addition, acute MDMA challenge failed to provoke an increase in signal amplitude in the prior MDMA-exposed group (C). (D) Representative CBV maps depicting the ability of prior MDMA exposure attenuate the increase in CBV induced by acute MDMA challenge. Data are expressed as mean $\pm$ SEM ($n = 8$). * $P < 0.05$; ** $P < 0.01$ versus corresponding vehicle-treated control animals (Student–Newman–Keuls *post hoc* test).

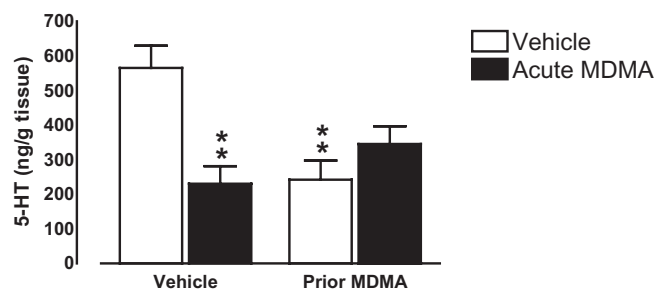


Figure 6

Cortical 5-HT concentration in response to prior MDMA exposure and acute MDMA challenge. Acute MDMA administration reduces 5-HT concentration in comparison with vehicle-treated control animals. Long-term 5-HT depletion is evident 8 weeks following repeated MDMA administration in comparison with vehicle-treated controls. ANOVA of cortical 5-HT concentration following repeated MDMA administration (10 mg·kg⁻¹; i.p., four times daily over 2 days) showed an MDMA pre-treatment $\times$ acute MDMA interaction [$F_{(1,28)} = 8.32$, $P < 0.01$]. *Post hoc* comparisons revealed that acute MDMA challenge produced a decrease in 5-HT concentration 3 h following administration when compared with vehicle-treated controls. In addition, 5-HT concentration was reduced in the prior MDMA-exposed groups, indicative of long-term 5-HT loss, in comparison with vehicle-treated controls (Figure 6). Striatal 5-HT concentration was not assessed in this study due to the fact that there was no change in concentration evident in the acute time or dose-related studies. Data are expressed as mean $\pm$ SEM ($n = 8$). ** $P < 0.01$ versus Vehicle-treated control animals (Student–Newman–Keuls *post hoc* test).

Table 4

Prior exposure to MDMA attenuates increased cerebral cortical perfusion induced by acute MDMA challenge in cortex

		Vehicle			Acute MDMA		
		MTT (s)	CTT (s)	Amplitude (a.u.)	MTT (s)	CTT (s)	Amplitude (a.u.)
Primary motor	Vehicle	1.98 ± 0.02	1.64 ± 0.07	0.080 ± 0.004	1.75 ± 0.04**	1.46 ± 0.06*	0.096 ± 0.006*
	Prior MDMA	1.85 ± 0.05	1.73 ± 0.04	0.082 ± 0.002	1.89 ± 0.04 ⁺	1.68 ± 0.05 ⁺	0.080 ± 0.002 ⁺
Secondary motor	Vehicle	1.94 ± 0.09	1.70 ± 0.09	0.080 ± 0.004	1.69 ± 0.07*	1.43 ± 0.05**	0.094 ± 0.006
	Prior MDMA	1.92 ± 0.03	1.74 ± 0.02	0.083 ± 0.002	1.89 ± 0.05 ⁺	1.63 ± 0.05 ⁺	0.078 ± 0.002

ANOVA of MTT in the primary motor cortex showed an effect of acute MDMA challenge [$F_{(1,28)} = 6.3$, $P < 0.05$] and an MDMA pretreatment × acute MDMA challenge interaction [$F_{(1,28)} = 11.99$, $P < 0.01$]. MDMA produced a decrease in MTT 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence MTT when compared with controls. In addition, acute MDMA challenge failed to provoke a decrease in MTT in the prior MDMA-exposed group. ANOVA of CTT in the primary motor cortex showed an effect of MDMA pretreatment [$F_{(1,28)} = 7.7$, $P < 0.01$] and an effect of acute MDMA challenge [$F_{(1,28)} = 4.13$, $P = 0.05$]. MDMA produced a decrease in CTT 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence CTT when compared with controls. In addition, acute MDMA challenge failed to provoke a decrease in CTT in the prior MDMA-exposed group. ANOVA of signal amplitude in the primary motor cortex showed an effect of MDMA pretreatment × acute MDMA challenge interaction [$F_{(1,28)} = 5.05$, $P < 0.05$]. MDMA produced an increase in signal amplitude 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence signal amplitude when compared with controls. In addition, acute MDMA challenge failed to provoke an increase in signal amplitude in the prior MDMA-exposed group.

ANOVA of MTT in the secondary motor cortex showed an effect of acute MDMA challenge [$F_{(1,28)} = 5.26$, $P < 0.05$]. MDMA produced a decrease in MTT 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence MTT when compared with controls. In addition, acute MDMA challenge failed to provoke a decrease in MTT in the prior MDMA-exposed group. ANOVA of CTT in the secondary motor cortex showed an effect of MDMA pretreatment [$F_{(1,28)} = 5.27$, $P < 0.05$] and an effect of acute MDMA challenge [$F_{(1,28)} = 11.63$, $P < 0.01$]. MDMA produced a decrease in CTT 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence CTT when compared with controls. In addition, acute MDMA challenge failed to provoke a decrease in CTT in the prior MDMA-exposed group. ANOVA of signal amplitude in the secondary motor cortex showed an effect of MDMA pretreatment × acute MDMA challenge interaction [$F_{(1,28)} = 5.13$, $P < 0.05$]. MDMA did not produce a significant increase in signal amplitude 3 h following administration in comparison with vehicle-treated control animals. Prior MDMA did not influence signal amplitude when compared with controls. In addition, acute MDMA challenge failed to provoke an increase in signal amplitude in the prior MDMA-exposed group. Data are expressed as mean ± SEM ($n = 8$). * $P < 0.05$ and ** $P < 0.01$ versus vehicle-treated group. ⁺ $P < 0.05$ versus vehicle acute MDMA group (Student–Newman–Keuls *post hoc* test).

binding is affected in cortical regions in abstinent ecstasy users leading investigators to propose that behavioural problems during abstinence might be related to changes in blood perfusion limited to cortical regions (Kish *et al.*, 2010).

Data from the time and dose–response experiments presented suggest that MDMA-induced increases in CBF and CBV are more localized to frontal cortical regions, a finding that is consistent with the results of Kelly *et al.* (1994) and Quate *et al.* (2004). Quate *et al.* (2004) reported 19% increases in CBF in frontal cortex using an autoradiographic technique 25 min after MDMA (15 mg·kg⁻¹; i.p.) administration in rats. The results are consistent with other reports (Rosa-Neto *et al.*, 2004; Ferrington *et al.*, 2006) of increased CBF in cortical fields associated with acute MDMA administration. It is of interest that the increase in cortical CBF reported occurred in the absence of any change in local cerebral glucose utilization suggesting a direct cerebrovascular response to MDMA, independent of changes in metabolic demand. It is tempting to speculate that 5-HT may be an important mediator given that MDMA provokes 5-HT release, 5-HT fibres have been identified innervating cerebral arteries, arterioles and veins, and 5-HT possesses potent vasoactive properties (Steinbusch, 1981; Cohen *et al.*, 1996).

While many of the effects of MDMA on neuronal function may be explained by the release of 5-HT, this action may

not account for acute CBF and CBV changes observed. 5-HT is known to have predominant constrictive actions on blood vessels (Cohen *et al.*, 1996), which would reduce CBF, actions, which are not consistent with the effects observed. Some investigators have put this mechanism forward to account for decreased rCBF consistent with acute vasoconstriction associated with MDMA-mediated serotonergic effects (Chang *et al.*, 2000; Reneman *et al.*, 2000). MDMA, however, following an initial increase in 5-HT release over a time course of hours, results in an acute 5-HT-depleted state. Such a depletion, with reduced perivascular 5-HT release and subsequent loss of 5-HT-mediated constrictor tone, may then lead to vasodilatation and prevailing increased CBF. In the present investigation, changes observed in cortical perfusion were not well related with alterations in 5-HT and 5-HIAA concentrations; and any prediction of CVA based on 5-HT concentrations is speculative. Moreover, experiments employing central 5-HT depletion, inhibition of SERT or antagonism of 5-HT receptors have not, to date, provided evidence in support of a role for 5-HT in mediating the cerebral perfusion changes provoked by MDMA (data not shown). Further work is required to clarify the mechanisms mediating cortical perfusion changes associated with MDMA.

Repeated administration of MDMA (10 mg·kg⁻¹; i.p., four times daily over 2 days) produced a long-term depletion of

cortical 5-HT concentration yet failed to produce a long-term change in MTT, CTT or signal amplitude in somatosensory or motor cortex, 8 weeks following drug exposure when compared with vehicle-treated controls. Long-term 5-HT loss alone may not influence tone of the microvasculature or metabolic demand at baseline and, consequently, cerebral perfusion remains unchanged. Prior MDMA exposure and 5-HT loss, however, attenuate the response to acute MDMA challenge. The results have implications in relation to long-term deficits in the regulation of cerebral perfusion associated with prior MDMA exposure, not basally apparent, but, which appear in the event of pharmacological challenge.

Prior exposure to MDMA with associated long-term 5-HT loss has been reported to diminish the behavioural, physiological and neurochemical response to subsequent MDMA challenge including the 5-HT behavioural syndrome (Shankaran and Gudelsky, 1999; Shankaran *et al.*, 2001), the core body temperature response (Green *et al.*, 2004) and the ability of MDMA to increase extracellular 5-HT concentration (Shankaran *et al.*, 2001; Amato *et al.*, 2007). Ferrington and co-workers (2006) reported reductions in CBF in 12 brain regions analysed, including posterior cingulate and piriform cortex, associated with acute MDMA challenge to Dark Agouti rats following prior MDMA exposure 3 weeks earlier. van Donkelaar *et al.* (2010) reported a global increase in CBF relative to cerebral metabolism following acute tryptophan depletion in rats exposed to a repeated regimen of MDMA 3 weeks earlier. Both investigations used quantitative autoradiographic techniques to determine CBF and cerebral metabolism in tandem and confirmed significant reductions in SERT binding. These observations may be regarded as consistent with the findings of the present investigation where prior MDMA exposure attenuated cortical perfusion change associated with acute serotonergic challenge.

In conclusion, this study provides important evidence regarding cortical hyperperfusion following acute and repeated administration of MDMA in a rodent model. Taken together, with the ability of MDMA to produce sustained cardiovascular effects and hypertension (Vollenweider *et al.*, 1998; Gamma *et al.*, 2000; Ferrington *et al.*, 2006), such changes may have important implications in relation to increased risk of CVA in recreational ecstasy users. In this regard, MDMA may contribute to pre-existing conditions or vulnerabilities such as congenital abnormalities in vascular structure or function. Concerns have been raised that a proportion of those who use MDMA may suffer infarcts leading to cognitive decline stemming from a vascular rather than purely neuronal pathology (Ferrington *et al.*, 2006). Despite a growing awareness that long-term ecstasy use may predispose to impairments in cognition (Gouzoulis-Mayfrank *et al.*, 2000; 2003; Daumann *et al.*, 2005; Kalechstein *et al.*, 2007; Rodsiri *et al.*, 2011), to date, there have been no investigations to determine if such changes may be associated with changes in cerebral perfusion. Future clinical studies of MDMA users are likely to be directed towards correlating cognitive decline with loss of 5-HT nerve terminals in addition to cerebrovascular function. In this regard bTASL MRI will be a useful investigational tool with translational potential for assessment of the long-term effects of MDMA 'ecstasy' on cerebral blood perfusion.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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