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Pre-treatment with 3,4-methylenedioxymethamphetamine (MDMA) causes long-lasting changes in 5-HT_{2A} receptor-mediated glucose utilization in the rat brain

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Abstract

The current study examined the long-term effect of brief exposure to 3,4-methylenedioxymethamphetamine (MDMA) on local cerebral glucose utilization (LCGU) in specific brain regions immediately following administration of the 5-$HT_{2A/2C}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Wistar rats (post-natal day (PND) 28, n = 24) were administered MDMA (5 mg/kg, i.p.) or saline (1 ml/kg, i.p.) four times daily for 2 consecutive days and core body temperature was recorded. Fifty-five days later and 10 min following injection of DOI (1 mg/kg, i.p.) or saline, LCGU was measured using the [^{14}C]2-deoxyglucose (2-DG) technique. In the 4 hours following the initial injection (PND 28), MDMA-treated rats exhibited significant hyperthermia compared with saline-treated controls ($p < 0.05$–0.01). Eight weeks later, immediately following DOI challenge, LCGU was significantly elevated (an increase of 47%, $p < 0.05$) in the nucleus accumbens of MDMA/DOI pre-treated rats, compared with that in MDMA/saline pre-treated controls. A similar trend was observed in other areas such as the lateral habenula, somatosensory cortex and hippocampal regions (percentage changes of 27–41%), but these did not reach significance. Blood glucose levels were significantly elevated in both groups of DOI-treated rats ($p < 0.05$–0.01). Thus, brief exposure of young rats to an MDMA regimen previously shown to cause anxiety-like behaviour and modest serotonergic neurotoxicity (Bull *et al.*, 2004) increased DOI-induced energy metabolism in the nucleus accumbens and tended to increase metabolism in other brain regions, including the hippocampus, consistent with the induction of long-term brain region specific changes in synaptic plasticity.

Keywords

[^{14}C]2-deoxyglucose autoradiography, 5-hydroxytryptamine (5-HT, serotonin), 5-HT_{2A} receptor, local cerebral glucose utilization (LCGU), 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), nucleus accumbens

Introduction

Recreational use of the psychoactive amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), is becoming increasingly popular among young people, yet the long-term consequences of drug exposure are unclear. In previous studies, pre-treatment with an MDMA dosage regimen that caused modest serotonergic neurotoxicity, resulted in a long-lasting reduction in social behaviour in the rat (Fone *et al.*, 2002; Gurtman *et al.*, 2002; Bull *et al.*, 2003; McGregor *et al.*, 2003) and an alteration in the behavioural response evoked by the 5-$HT_{2A/2C}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; Bull *et al.*, 2004) but not that evoked by the 5-HT_{2C} receptor agonist, *m*-chlorophenylpiperazine (mCPP; Bull *et al.*, 2003). To further evaluate whether MDMA pre-treatment caused persistent alterations in 5-HT_{2A} receptor function, the current study investigated whether prolonged abstinence from MDMA was associated with any modifications in 5-HT_{2A} receptor agonist-induced changes in local cerebral glucose utilization (LCGU) in discrete brain areas.

The 5-HT_{2A} receptor has been previously implicated in the psychological effects of MDMA. In man, MDMA-induced perceptual and emotional changes are diminished following administration of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin (Liechti *et al.*, 2000). Furthermore, it has been proposed that over-stimulation of the 5-

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HT_{2A} receptor may be involved in the actions of hallucinogenic drugs (Titeler *et al.*, 1988; Sadzot *et al.*, 1989). In rats, a neurotoxic regimen of MDMA caused a decrease followed by a time-dependent recovery of cortical 5-HT_{2A} receptor density that was positively correlated with the extent of 5-HT depletion (Reneman *et al.*, 2002). Human data from the same group, using [^{123}I]R91150 and single photon emission computed tomography (SPECT), show that recent MDMA users had significantly lower 5-HT_{2A} receptor density in the frontal, parietal and occipital cortices compared with MDMA-naive controls, whilst ex-MDMA users had a higher receptor density in the occipital cortex, possibly reflecting a compensatory up-regulation of post-synaptic receptors (Reneman *et al.*, 2002). Of particular relevance to the current study, recent animal data, utilizing an identical MDMA treatment regimen to the one employed herein (4×5 mg/kg for 2 consecutive days), showed that the long-term anxiogenic effects of MDMA in the social interaction and emergence paradigms were associated with profound decreases in cortical, striatal and hypothalamic 5-$HT_{2A/2C}$ receptor densities (McGregor *et al.*, 2003) measured using [^{125}I]DOI and autoradiography. In the rat, MDMA pre-treatment also appears to impair long-term cognitive function. For instance, in a delayed non-match to place (DNMTP) procedure, MDMA-treated rats developed a selective, delay-dependent, deficit in performance, measured up to 18 days following MDMA administration (Marston *et al.*, 1999). Furthermore, 3 months following an identical dosage regimen to that employed herein, MDMA pre-treated rats showed impaired memory relative to vehicle-treated controls in a novel object recognition task (Morley *et al.*, 2001).

The [^{14}C]2-deoxyglucose ([^{14}C]2-DG) utilization technique, used to identify brain region specific metabolic changes, was first developed by Sokoloff and colleagues, and has previously been used to measure the acute effects of MDMA (Sokoloff *et al.*, 1977). Following a single dose of MDMA (5–30 mg/kg, i.p.), glucose utilization was stimulated in components of the extrapyramidal system (Wilkerson and London 1989). A recent study revealed that acute exposure of Dark Agouti rats to a single dose of MDMA (15 mg/kg, i.p.) elicited significant increases in local cerebral glucose utilization that were most pronounced in the motor system (globus pallidus +82% and medial striatum +71%; $p < 0.05$ vs controls; Quate *et al*., 2004) and which were uncoupled with acute changes in local cerebral blood flow, suggesting that MDMA might cause acute cerebrovascular dysfunction. In a longer-term study, in which 2 weeks elapsed following MDMA pre-treatment (20 mg/kg, twice daily for 4 consecutive days) significant elevations of LCGU were found in the CA2 and CA3 sub-regions of the hippocampus and the dentate gyrus (Sharkey *et al.*, 1991). Human studies have also been undertaken to determine whether a history of illicit MDMA use impacts on brain region energy metabolism. Positron emission tomography (PET) studies with 2-[^{18}F]-fluro-2-deoxyglucose (FDG) showed a global reduction in FDG uptake sites in MDMA users (between 3 days and 96 months after the last ingestion of ecstasy), with depletions being most pronounced in the striatum (Buchert *et al.*, 2001; Obrocki *et al.*, 2002). Furthermore, this study identified a positive link between the onset of MDMA abuse and the severity of FDG uptake site depletion.

A growing body of evidence suggests that 5-HT_{2A} receptor function may change following exposure to MDMA (Bull *et al.*, 2004). The current study therefore examined the long-term effect of MDMA on basal and 5-HT_2 agonist-induced brain glucose utilization to examine whether MDMA produces long-term brain region specific metabolic changes that could reflect altered synaptic plasticity and contribute to the enduring behavioural changes seen following MDMA administration in the rat.

Methods and materials

Animals and housing

Male Wistar rats (Charles River, UK, 167 ± 9 g (mean ± SEM), n = 24) were housed in groups of four in a temperature-controlled environment (21 ± 2°C) under a 12 h light/dark cycle (lights on 07.00–19.00 h). Rats had access to food and water *ad libitum*. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 using a blind drug treatment protocol.

Starting at 09.00–10.00h on post-natal day (PND) 28–29, rats were injected with either racemic 3,4-methylenedioxymethamphetamine (MDMA hydrochloride, Sigma, UK, 5 mg/kg, i.p.) or vehicle (0.154 M NaCl, 1 ml/kg, i.p.) four times daily at hourly intervals and returned to their home cage directly following each injection. Rectal temperature was recorded immediately before the first injection and thereafter at hourly intervals over a 4 h period on both days of drug administration, using a thermocouple probe with a digital readout (Portec Instrumentation Ltd, P9005), inserted 6–8 cm into the rectum whilst using light restraint. Rats were then left undisturbed in the home cage for a period of 8 weeks, during which time no further drug administration occurred, handling was kept to a minimum and bedding was changed once per week.

Surgical procedure

Following the 55 day abstinence period, on PND 84, rats were anaesthetized using isoflurane (99.9% w/w, Abbott) and the tail vein and one tail artery cannulated with Portex tubing (i.d. 0.58 mm, o.d. 0.96 mm). Cannulae were pre-filled with heparinized saline (100 I.U./ml in 0.154 M NaCl) to prevent blood clotting. When rats showed the first indication of the righting reflex they were placed into a restraining apparatus (clear Perspex tubes with adjustable metal restraining bars, Merck Sharp and Dohme, in house), where they recovered fully from the anaesthesia for a period of at least 2 h, during which time arterial blood samples, of approximately 75 µl, were taken to assess blood glucose (Glucometer, Bayer) and blood gas (pO_2, pCO_2 and pH) levels (Rapidlab 248, Bayer). Blood pressure and heart rate were also recorded throughout the procedure by connecting a strain gauge transducer to the tail artery cannula (Graphtec). To enable blood samples to be taken rats remained under restraint throughout the course of the procedure, during which time their heart rate and blood pressure were assessed constantly and behavioural indicators of stress, such as vocalizations, were also monitored subjectively.

Measurement of [^{14}C]2-DG utilization following acute challenge with DOI

Following full recovery from the anaesthesia (at least 2 h), rats were administered either the 5-HT$_{2A}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, RBI, UK, 1 mg/kg i.p.) or saline (0.154 M NaCl, Steriflex, 1 ml/kg i.p.) and any changes in physiological variables recorded. This dose of DOI and route of administration was selected to enable direct comparison with a previous behavioural study where a change in 5-HT$_{2A}$ receptor responsivity was reported following MDMA pre-treatment (Bull *et al.*, 2004). Routinely 7 min after drug injection, a blood sample was analysed for glucose content. Ten minutes after drug injection, 10 µCi of [^{14}C]2-DG (Amersham, 61 mCi/mM) was injected intravenously (in a volume of 0.5 ml over 30 s) *via* the tail vein. Seventy-five µl samples of blood were taken at 0, 15, 30 and 45 s after the start of the [^{14}C]2-DG injection and then at 1, 2, 3, 7.5, 10, 15, 25, 35 and 45 min after injection. The health of the rat, with regard to blood pressure, heart rate and behavioural indicators of stress, was continually monitored throughout the 45 min procedure. At 45 min the final blood sample was taken and the rat humanely killed by intravenous injection of pentobarbitone (120 mg/kg, Euthatal, Rhône Mérieux). The animal was immediately decapitated and the brain removed and frozen, by immersion in liquid isopentane (BDH), held on dry ice at −45 ºC, and stored at −80 ºC until sectioning.

Blood sampling

Blood samples were taken into small eppendorf tubes containing 5 µl heparin (5000 I.U./ml, Multiparin) to minimize blood clotting. For each sampling time-point, blood glucose levels (as required for the Sokoloff calculation) were determined from a 10 µl sample (Glucometer, Bayer). The remaining blood was then centrifuged at 1600 G for 5 min and two 20 µl plasma samples per time-point drawn off into vials and left overnight in scintillation fluid (Hydrofluor, National Diagnostics) for subsequent counting on a scintillation counter (Beckman LS-600TA).

Brain sectioning

Immediately prior to sectioning, the frozen brain (−80 °C) was mounted onto a pre-cooled cryostat chuck, coated with pre-cooled embedding matrix (Lipshaw MJG Co., USA) and allowed to equilibrate to −15 ºC in the cryostat (Leica, Germany). Three consecutive sections (20 µm) were taken onto a coverslip (BDH) and placed on a heating plate at 60 ºC to rapidly dry out the tissue and minimize diffusion of the radiolabel. The next 10 sections were then discarded and the process continued throughout the whole brain. Mounted sections were placed in a film cassette with [^{14}C] standard microscales (Amersham, UK) and left for 18 days apposed with Biomax MS film (Kodak). Films were developed under safe illumination by immersion in Phenisol developer (Ilford) and transferred to a stop solution (Ilfostop, Ilford) followed by a fixing solution (Hypam, Ilford).

Statistical analysis

[^{14}C]2-DG autoradiograms were analysed using a computer-based image analysis system (MCID, Imaging Research, Canada). Tissue isotope concentrations were measured from autoradiographic images of brain tissue, relative to [^{14}C] standard microscales (Amersham, UK) and LCGU calculated using operational equations for the technique (Sokoloff *et al.*, 1977). Densities of specific brain structures were measured at matched levels throughout the brain (Paxinos and Watson 1997) reading six sections (left- and right-hand side) per brain structure, thus giving 12 readings per structure per rat. Data were examined for normality using the Kolmogorov-Smirnov (KS) test and met the criteria for the parametric analysis performed. All data are presented as mean ± SEM and were analysed using either a Student's *t*-test or 1- and 2-way ANOVA with *post hoc* analysis, where appropriate, using Tukey's Multiple Comparison, Fischer's PLSD and Bonferroni tests. In all cases, data were considered to be significant when $p \leq 0.05$.

Results

Acute effect of MDMA on core body temperature

On the first day of drug administration, core body temperature was significantly elevated in MDMA-treated compared with saline-treated rats ($p < 0.05$–0.01, Student's *t*-test, Fig. 1a). This hyperthermia only reached significance 2 h after the initial injection and was maintained for at least 1 h after the fourth injection. In marked contrast, the hyperthermic effect of MDMA was not evident on day 2 using an identical dosage regimen, but instead the temperature of both treatment groups remained comparable for the duration of the study (Fig. 1b).

Effect of MDMA on physiological parameters following acute challenge with DOI

At both 35 and 55 min after DOI (1 mg/kg, i.p.) administration, the blood glucose level was significantly elevated compared with that in saline controls, irrespective of whether rats had received MDMA pre-treatment [Fisher's PLSD test following ANOVA, $F_{(3,20)} = 9.1$, $p < 0.001$ and $F_{(3,19)} = 5.32$, $p < 0.01$ for 35 and 55 min, respectively, Fig. 2]. At the point of administration of DOI (0 min), ANOVA revealed a main effect of drug treatment on blood pressure [ANOVA, $F_{(3,20)} = 3.12$, $p < 0.05$] which was significantly elevated in MDMA/DOI-treated rats compared with that in both saline/DOI and MDMA/saline-treated controls [$p < 0.05$, Fisher's PLSD, Figure 3]. There were no statistically significant effects of DOI or MDMA and no interaction between DOI and MDMA on heart rate, blood gases or pH at any time of analysis during the [^{14}C]2-DG procedure (data not shown).

Effect of MDMA on LCGU following acute challenge with DOI

The possible interaction between MDMA and DOI-induced changes in LCGU was examined in 30 brain regions. Previous

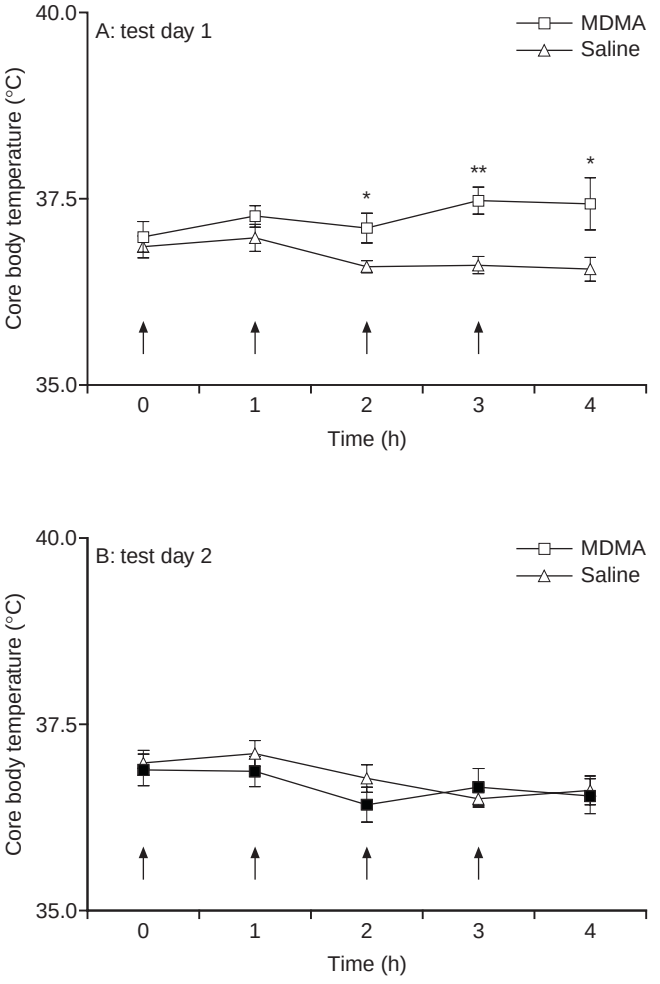

Figure 1 The effect of MDMA (4×5 mg/kg over 4 h on 2 consecutive days) or saline (1 ml/kg) on core body temperature recorded at 1 h intervals on consecutive days of drug administration. Data expressed as mean ± SEM (n = 12 for each treatment group), arrows denote injection points.

*p < 0.05 and **p < 0.01 from saline-treated controls (Student's unpaired t-test).

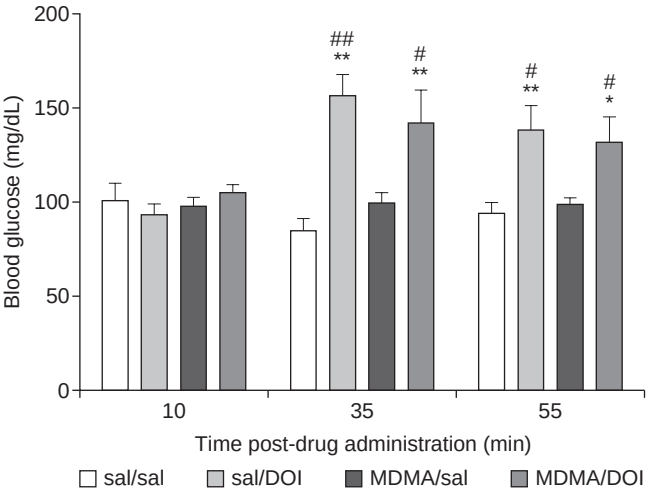

Figure 2 The effect of DOI or saline on blood glucose (mg/dl, mean ± SEM, n = 6 per group) measured during the [^{14}C]2-DG procedure, at 10, 35 and 55 min post-injection, 56 days after pre-treatment with either saline or MDMA (4×5 mg/kg over 4 h on 2 consecutive days), as indicated above.

*p < 0.05, **p < 0.01 and ***p < 0.001 from the saline/saline pre-treated group and #p < 0.05 and ##p < 0.01 from the MDMA/saline pre-treated group (Fisher's PLSD test following ANOVA).

exposure to MDMA did not cause any significant change in LCGU compared with that in saline pre-treated rats in any brain region examined (Table 1). The percentage changes in LCGU in the MDMA/DOI group, relative to those in both the saline/saline and MDMA/saline treatment groups, are also presented in Table 1. From these values it can be seen that LCGU levels were consistently higher in MDMA/DOI treated rats than other groups in most brain regions, reaching 47% above that in MDMA/saline in the nucleus accumbens, 41% in the lateral habenular and 31–34% in the lateral hypothalamus, somatosensory cortex and several hippocampal areas. However, statistical analysis showed that the increase in LCGU only reached significance in the nucleus accumbens where there was significant interaction between MDMA and DOI [$F_{(3,23)} = 3.06$, $p = 0.05$, Table 1], a main effect of DOI [$F_{(1,18)} = 4.67$, $p = 0.044$], and a significant difference between MDMA/saline and MDMA/DOI ($p < 0.05$). LCGU in hippocampal regions followed a similar trend but this failed to reach significance (even in the dentate gyrus where this was closest [$F_{(3,23)} = 1.49$, $p = 0.2476$]). Further analysis of data failed to reveal any significant change in any other brain region, with the exception of the lateral hypothalamus where there was a main effect of MDMA [$F_{(1,19)} = 4.65$, $p = 0.044$] but no significant difference between any of the four treatment groups.

Discussion

Previous investigation by our group has shown that 12 to 20 days abstinence from a short period of MDMA administration (7.5 or 15 mg/kg i.p. twice daily for 3 days) produces a long-lasting reduction in social interaction in the rat (Fone *et al.*, 2002; Bull *et al.*, 2003). Furthermore, this was accompanied by a marked alteration in some of the behaviours elicited by acute injection of the 5-HT$_{2A/2B/2C}$ receptor agonist, DOI (Bull *et al.*, 2004). The current study supports the suggestion that 5-HT$_{2A}$ receptor function and/or expression may undergo long-term alteration following MDMA. Specifically, DOI produced significantly greater LCGU in the

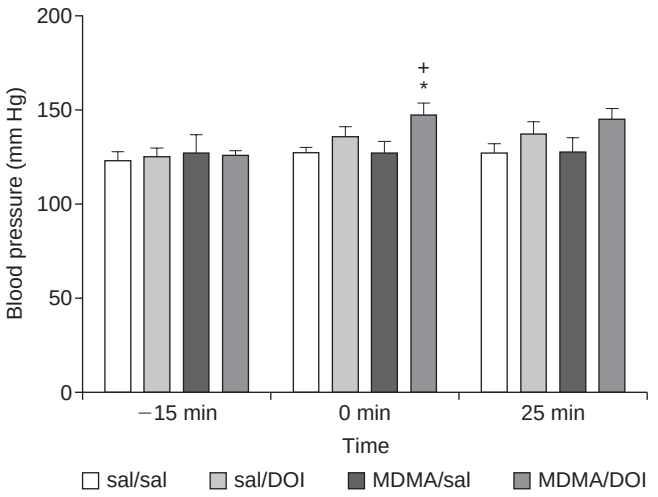

Figure 3 The effect of DOI or saline on mean arterial blood pressure (mm Hg, mean ± SEM, n = 6 for each treatment group) recorded during the [^{14}C]2-DG procedure, at −15, 0 and 25 min post-injection, 55 days after pre-treatment with either saline or MDMA (4×5 mg/kg, over 4 h on 2 consecutive days), as indicated above.

*$p < 0.05$ from the saline/saline pre-treated group and +$p < 0.05$ from the MDMA/saline pre-treated group (Fisher's PLSD test following ANOVA).

nucleus accumbens (and similar changes in several hippocampal and cortical areas, but these were not significant) compatible with the hypothesis that compensatory changes in 5-HT$_{2A}$ receptor function, possibly due to changes in synaptic plasticity, occur following MDMA treatment.

Acute administration of MDMA to rats on the first injection day in this study produced a hyperthermic response lasting several hours that became more pronounced with cumulative dosing, consistent with previous findings at ambient temperatures above 19°C (Nash *et al.*, 1988; Gordon *et al.*, 1991; Dafters 1994; Dafters and Lynch 1998). A recent study has suggested that this hyperthermia is ultimately mediated by increased dopamine release and the resultant stimulation of D_1 dopamine receptors (Mechan *et al.*, 2002) secondary to 5-HT release. In our previous study, using an identical dosage regimen and conducted at the same ambient temperature (Bull *et al.*, 2004) the hyperthermia was preceded by hypothermia, not evident in the current study. A critical difference, however, is that in the current study rats were returned to their home cage group between injections (rather than isolated in an activity chamber). The effect of this change in protocol is consistent with the observation that MDMA inhibits normal heat loss mechanisms (Mechan *et al.*, 2002) and produces a greater effect at higher ambient temperatures (Dafters and Lynch, 1998; Mechan *et al.*, 2001) and when animals are allowed to aggregate (Green *et al.*, 2004). Of particular note is the complete absence of hyperthermia on the second day of MDMA administration. Several previous studies have shown that doses of MDMA that cause serotonergic neurotoxicity produce a long-lasting compromise in thermoregulation such as the response to a change in ambient temperature (Dafters and Lynch, 1998; Mechan *et al.*, 2001). Furthermore, Shankaran and Gudelsky showed that a neurotoxic regimen of MDMA (10 mg/kg, i.p. 4× over a 8 h period) diminished the magnitude of the thermogenic response to subsequent challenge with MDMA 1 week later, possibly due to reduced 5-HT receptor sensitivity (Shankaran and Gudelsky 1999). However, the attenuation of the thermogenic response to MDMA only 24 h after the first injection, as observed herein, is more likely to relate to the extensive 5-HT depletion that occurs within 24 h of drug administration (Schmidt *et al.*, 1987) or inhibition of the rate limiting biosynthetic enzyme, tryptophan hydroxylase (Garcia-Osta *et al.*, 2004) rather than degeneration of 5-HT nerve terminals that would require a longer interval. Alternatively, acute depletion of dopamine as reported with three consecutive injections of methamphetamine during a day using microdialysis in the striatum (Baldwin *et al.*, 1993) may also occur with MDMA and would also be expected to attenuate the hyperthermic response.

In the present study the 5-HT$_{2A/2B/2C}$ receptor agonist, DOI, significantly elevated blood glucose levels between 30–60 min post-injection. DOI-induced hyperglycaemia in rats (Chaouloff *et al.*, 1990) has previously been shown to be inhibited by ketanserin and ritanserin, consistent with it being induced by 5-HT$_{2A}$ receptor activation. The normoglycaemic range in the rat is defined as 7–8 mM (corresponding to 126–144 mg/dl) with hyperglycaemia defined as 28–31 mM (464–558 mg/dl). Clearly the glucose levels recorded in DOI-treated rats fall well below hyperglycaemic levels and as such are unlikely to have any impact on the lumped constant of the deoxyglucose method and consequently, LCGU values (Schuier *et al.*, 1990).

The procedure employed herein used restraint of the animal during the Sokoloff procedure, which is a well-known stressor. No vocalizations were audible or seen during the procedure, but no quantitative measurement of ultrasonic vocalizations was made to establish whether MDMA-treated rats found the procedure more stressful than controls. Blood pressure and heart rate measurements were taken throughout the experiment, and these remained constant, both before, during and 25 min after drug administration in the restraint apparatus in all groups regardless of pre-treatment, suggesting that there was no marked group difference in sympathetic activation associated with the technique. However, it can not be excluded that if MDMA pre-treatment alters the response of the animal to a stressful environment, this also may contribute to a change in the response seen to administration of an anxiogenic drug, such as DOI, to these animals.

Interestingly, of the 30 brain regions studied, the elevation of LCGU selectively induced by DOI in MDMA but not saline pre-treated rats only reached significance in the nucleus accumbens. A similar pattern was observed in several hippocampal regions and areas such as the lateral habenula and lateral hypothalamus, which may also have reached significance if a larger group size had been possible. Interestingly, 5-HT depletion in the nucleus accumbens occurs 2 and 4 weeks after cessation of a high MDMA dosage regimen (20 mg/kg daily for 10 consecutive days, Mayerhofer *et*

Table 1 The effect of previous exposure to MDMA (5 mg/kg, 4×daily for 2 consecutive days) or saline (1 ml/kg, i.p.) on LCGU (μmol/100 g/min) in selected brain regions recorded using the [^{14}C]2-DG procedure, commenced 10 min following administration of DOI (1 mg/kg) or saline (Sal, 1 ml/kg) (data expressed as mean±SEM, n=6 for each treatment group). The right hand pair of columns shows the percentage change in LCGU in the MDMA/DOI group relative to those in the saline/saline and MDMA/saline groups, as indicated.

Brain region		Local cerebral glucose utilization (μmol/100 g/min)				(MDMA/DOI)% change	
		sal/sal	sal/DOI	MDMA/sal	MDMA/DOI	vs sal/sal	vs MDMA/sal
Corpus callosum		25.5±5.5	27.1±5.3	30.7±9.5	28.5±4.8	12	−7
Caudate putamen		84.0±7.2	93.2±4.5	80.2±13.6	101.5±13.9	21	27
Nucleus accumbens		51.9±2.0	48.5±1.2	45.0±6.2	**66.0±8.6***	**27**	47
Medial septum		45.4±3.5	53.4±2.6	51.0±7.4	60.0±9.7	32	18
Auditory cortex	layers 1–6	71.3±6.5	73.7±5.2	75.7±13.8	77.7±13.7	9	3
	layers 1–2	85.6±6.9	80.4±5.4	79.5±15.0	101.2±12.8	18	27
	layers 3–6	82.3±6.7	80.6±5.0	75.6±13.1	104.0±13.6	26	38
Cingulate cortex	layers 1–6	78.8±6.2	86.1±3.4	73.4±12.9	94.0±13.7	19	28
	layers 1–2	81.9±7.0	89.7±4.0	80.8±14.6	100.5±15.7	23	24
	layers 3–6	76.9±5.9	83.6±3.4	72.4±8.9	88.8±11.9	16	23
Occipital cortex	layers 1–6	73.0±5.6	71.4±4.4	72.8±11.1	90.7±11.8	24	25
	layers 1–2	72.7±5.2	70.2±4.7	71.3±10.4	90.6±11.9	25	27
	layers 3–6	73.3±5.9	72.3±4.3	73.9±11.7	90.2±11.3	23	22
Retrosplenial cortex	layers 1–6	82.3±7.4	72.3±3.3	74.7±11.3	91.9±15.0	12	23
	layers 1–2	86.8±8.3	77.4±3.4	82.3±13.5	100.5±17.1	16	22
	layers 3–6	79.0±6.8	68.7±3.3	69.0±10.2	84.6±12.5	7	23
Somatosensory cortex	layers 1–6	75.9±5.0	79.0±3.7	69.9±10.8	93.7±10.6	24	34
	layers 1–2	80.8±5.2	80.1±4.4	72.9±11.2	97.4±10.8	21	34
	layers 3–6	72.8±5.0	78.4±3.4	67.9±10.7	91.5±10.8	26	35
Hippocampus	Dentate gyrus	48.5±2.2	46.3±2.9	42.0±6.3	56.4±7.2	16	34
	CA1	51.4±3.6	49.2±2.5	44.5±6.4	58.2±8.7	13	31
	CA2	50.8±3.6	46.6±2.5	45.6±6.8	57.7±9.2	13	27
	CA3	51.38±3.2	48.2±1.8	44.7±6.0	59.1±9.6	15	32
Lateral hypothalamus		46.7±3.5	56.4±2.7	45.6±8.2	62.4±8.5	34	37
Lateral habenular		74.2±6.8	77.7±4.3	63.9±8.8	90.1±13.7	21	41
Inferior colliculi		166.0±13.3	123.8±9.1	142.3±25.1	178.6±37.0	8	26
DMPAG		52.8±4.2	48.5±1.8	48.0±6.3	62.7±9.5	19	31
Dorsal raphé		55.69±5.0	70.7±4.5	61.0±8.8	71.0±15.9	28	16
Medial raphé		63.9±6.6	72.6±3.2	67.3±5.9	76.6±10.1	20	14
Rostral periolivary nuclei		57.6±15.6	41.2±2.5	41.3±3.8	52.6±6.1	−9	27

*$p<0.05$ from the MDMA/saline pre-treated group (Tukey's Multiple Comparison test following ANOVA)

al., 2001). Drugs of abuse such as cocaine also exact long-lasting changes at excitatory synapses in the nucleus accumbens and ventral tegmental area due to induction of long-term potentiation and long-term depression therein (Thomas and Malenka, 2003), leading to the development of neuronal plasticity in forebrain dopaminergic and glutaminergic transmission during drug withdrawal (Pierce and Kalivas, 1997). It is conceivable that a similar adaptive mechanism is induced following withdrawal from a neurotoxic MDMA dosage regimen, resulting in changes in neuronal plasticity. Previous studies have shown that acute injection of cocaine and amphetamine, at doses which produce hyperlocomotion and reinforcement, also selectively increases glucose utilization in the nucleus accumbens and medial prefrontal cortex (Porrino *et al.*, 1984; Porrino *et al.*, 1988; Porrino, 1993). Further-

more, acute intravenous methamphetamine (0.5–2.5 mg/kg, i.v.) also increases glucose utilization in the nucleus accumbens (Pontieri *et al.*, 1990). Yet in one of the few long-term studies, LCGU was significantly reduced in the nucleus accumbens, 7 days following repeated exposure to amphetamine (10 mg/kg once daily for 14 days, Tsai *et al.*, 1995). The reason for this apparent discrepancy is currently unclear.

The nucleus accumbens receives a dense input of serotonergic fibres from the raphé nuclei (Moore *et al.*, 1978), glutamatergic projections from the prefrontal cortex and the hippocampus and is a dopaminergic region strongly associated with the rewarding properties of cocaine and amphetamine (Koob, 1992). There is evidence that MDMA may increase vulnerability to the rewarding actions of cocaine in rats, since pre-treatment facilitates the acquisition of cocaine self-administration (Fletcher *et al.*, 2001) and enhances the conditioned place preference response to cocaine (Horan *et al.*, 2000; Fone *et al.*, 2002). *In vivo* microdialysis studies have shown that the cocaine-induced increase in extracellular dopamine in the nucleus accumbens is significantly greater in rats pre-treated with MDMA (20 mg/kg twice daily for 4 consecutive days, Morgan *et al.*, 1997), further substantiating a link between MDMA and dopaminergic neurotransmission in this region. Acute MDMA administration increases extracellular dopamine and 5-HT in the nucleus accumbens (Yamamoto and Spanos, 1988; White *et al.*, 1994) and the inhibitory effect of MDMA on neuronal firing in the nucleus accumbens is partially blocked by the dopamine D_1 receptor antagonist, SCH 39166, consistent with the involvement of dopamine in this response (White *et al.*, 1994). In addition, 6-hydroxydopamine-induced lesions of the nucleus accumbens attenuate the hyperlocomotor response to MDMA (Gold *et al.*, 1989). Yet the role of dopamine in MDMA-induced neurotoxicity remains controversial and is species-dependent. In the rat, MDMA spares dopaminergic neurones and is selectively neurotoxic to serotonergic neurones, (Stone *et al.*, 1986; Battaglia *et al.*, 1987; Schmidt and Kehne, 1990; Lew *et al.*, 1996; Sabol *et al.*, 1996), making an indirect mechanism, in which the long-term serotonergic effect of MDMA subsequently impacts on dopaminergic systems, more likely. Multiple 5-HT receptors are located in the nucleus accumbens, including the 5-HT$_{2A}$ receptor (Pazos *et al.*, 1985; Mengod *et al.*, 1990; Pompeiano *et al.*, 1994). Previous studies with DOI have shown it to inhibit glutamate-evoked firing of accumbal cells, probably mediated by DOI-induced dopamine release from pre-synaptic terminals (Obradovic *et al.*, 1996). In further support of this, activation of 5-HT$_{2A/2C}$ receptors within the nucleus accumbens increases local dopaminergic neurotransmission (Yan *et al.*, 2000). Indeed, activation of 5-HT$_{2A}$ receptors may play a permissive role in MDMA-induced dopamine release in the nucleus accumbens (Bankson and Cunningham, 2001), accounting for the observed elevation in DOI-evoked LCGU after MDMA in this brain region.

Overall, these data suggest that brief exposure of young rats to a MDMA regimen that causes modest serotonergic neurotoxicity and a persistent 'anxiety-like' behaviour (Fone *et al.*, 2002; Gurtman *et al.*, 2002; Bull *et al.*, 2003; McGregor *et al.*, 2003; Bull *et al.*, 2004) also elicits a brain region dependent increase in energy metabolism most notably in the nucleus accumbens following pharmacological challenge with DOI that may result from compensatory changes in 5-HT$_{2A}$ receptor function. At this point, it is uncertain whether this increased 5-HT$_{2A}$ receptor-mediated glucose utilization reflects an increase in neuronal activity or is the consequence of longer-term changes in synaptic plasticity, and future investigations should aim to define the underlying molecular mechanisms.

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