Psychopharmacology (1991) 105:113–118

Psychopharmacology

Alterations in hippocampal function following repeated exposure to the amphetamine derivative methylenedioxymethamphetamine ("Ecstasy")

John Sharkey, Douglas E. McBean, and Paul A.T. Kelly

University of Edinburgh, Department of Clinical Neurosciences, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

Received September 19, 1990 / Final version February 27, 1991

Abstract. The effect of the psychomotor stimulant, 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"), upon integrated cerebral function was measured in rats using the quantitative [^{14}C]deoxyglucose autoradiographic technique. Animals were injected with MDMA (20 mg/kg sc) twice daily for 4 days. Fourteen days after the final administration, [^{3}H]-paroxetine binding to 5HT uptake sites was reduced by 89% in membranes prepared from tissue samples of frontal cortex. In the same rats [^{3}H]-paroxetine binding autoradiography revealed heterogeneity in the regional distribution of 5–HT uptake site depletion within neocortex (0–92%) and hippocampus (30–95%). Despite these profound reductions in 5–HT uptake sites no significant alterations were found in glucose utilisation in any area of neocortex examined. However, significant increases in glucose use were found in subregions of the hippocampus, most notably within the pyramidal cell layer of CA2 and CA3 (25–35%). This study provides direct evidence that the loss of 5–HT innervation caused by exposure to MDMA results in lasting functional changes in hippocampus.

Key words: 3,4-Methylenedioxymethamphetamine (MDMA) – Neurotoxicity – 5-Hydroxytryptamine – Paroxetine – [^{14}C]2-Deoxyglucose – Rat

Methylenedioxymethamphetamine (MDMA, "Ecstasy") is becoming a widely used recreational drug of abuse (Siegel 1986; Peroutka 1987). The structural similarities of MDMA to the psychomotor stimulant amphetamine, and the prototypic hallucinogen mescaline, confer upon this drug both stimulant and psychotomimetic activity in man (Grinspoon and Bakalar 1986; Shulgin 1986). In experimental animals, the acute administration of MDMA provokes stereotyped behaviours similar to that produced by other psychomotor stimulants such as cocaine, amphetamine and phencyclidine (Glennon and Young 1984; Wilkerson and London 1989).

Offprint requests to: J. Sharkey

Like other amphetamine-related drugs of abuse, MDMA possesses potent neurotoxic properties (Schmidt 1987) which appear to be selective for 5–HT neurones (Battaglia et al. 1987a, 1988). Exposure to MDMA results in profound decreases in the concentrations of 5–HT and its metabolites in the brain (Schmidt et al. 1986; Stone et al. 1986; Battaglia et al. 1987; Commins et al. 1987; Gibb et al. 1987), the depletion of 5–HT uptake sites (Battaglia et al. 1987, 1988a) and a marked reduction in the density of 5–HT immunoreactive axon terminals (Battaglia et al. 1987b; Commins et al. 1987; O'Hearn et al. 1987). These deficits in 5–HT markers can persist for at least 6 months after exposure to MDMA (Battaglia et al. 1988b). At the anatomical level, the effects of MDMA are manifest in the terminal fields of serotonergic neurones whilst the cell bodies of the dorsal and median raphe nuclei remain relatively unaffected (Battaglia et al. 1987; Molliver 1987; O'Hearn et al 1987). However, within some terminal fields, most notably neocortex and hippocampus, the loss of 5–HT fibres is not homogeneous, with some subregions of these structures being profoundly affected whilst others are relatively spared (O'Hearn et al. 1987).

Whilst the anatomical and biochemical sequelae of MDMA exposure are well documented, it is not known whether the selective loss of serotonergic neurones has any persistent consequences for the functional organization of the brain. Within neocortex and hippocampus in particular, where the loss of 5–HT is not homogeneous it is impossible to predict what the functional consequences might be. In the present study we have examined the effects of repeated administration of MDMA upon neocortical and hippocampal function, as it is reflected in altered rates of local cerebral glucose ultilization (Sokoloff 1977), and have related these to the pattern of depletion in [^{3}H]paroxetine-labelled 5–HT uptake sites within these areas.

Material and methods

Male Sprague-Dawley rats (150–175 g) were injected with MDMA (20 mg/kg SC, $n = 5$) or saline vehicle (0.2 ml, $n = 5$) twice daily for

4 consecutive days. This treatment regime has previously been shown to produce a loss of serotonergic fibres in a variety of terminal fields (Battaglia et al. 1987; Commins et al. 1987; O'Hearn et al. 1987).

Local cerebral glucose utilization (lCGU) was measured 14 days after the last injection of MDMA using the fully quantitative [^{14}C]-2-deoxyglucose autoradiographic technique (Sokoloff et al. 1977). On the day of study, the rats were anaesthetized with halothane and polyethylene cannulae inserted into both femoral arteries (for the subsequent sampling of arterial blood and monitoring of blood pressure) and veins (for the intravenous administration of radiolabelled tracers). Incision sites were infiltrated with local anaesthetic and sutured closed. A loose-fitting plaster cast was applied around the pelvis and lower abdomen and taped to a block. Thus restrained and supported, the anaesthetic was discontinued and the rats allowed to recover from the anaesthetic for at least 2 h before any subsequent procedures were initiated. Arterial blood pressure and rectal temperature were monitored throughout the recovery and subsequent measurement periods.

The measurement of lCGU was initiated with a 30-s intravenous pulse injection of 2-deoxy-D-[1-^{14}C]-glucose (4.625 mBq/kg in 0.7 ml saline). Over the subsequent 45 min, 14 timed arterial blood samples (80 µl) were withdrawn for the determination of plasma [^{14}C] (liquid scintillation analysis) and glucose concentrations (semiautomated glucose oxidase assay). At the end of the measurement period, the animals were killed by an intravenous injection of pentobarbitone, the brain excised intact and frozen to −45° C within 2–3 min of death.

To assess the efficacy of the lesion, samples of frontal cortex were taken for in vitro saturation analysis of [^{3}H]paroxetine binding. The remainder of the brain was sectioned in a cryostat (20 µm) and processed for [^{14}C]2-deoxyglucose autoradiography as previously described (Sokoloff et al. 1977). Sections adjacent to those used for 2-deoxyglucose autoradiography were thaw-mounted onto gelatin coated glass slides for subsequent [^{3}H]paroxetine autoradiographic binding.

Saturation analysis of [^{3}H]-paroxetine (specific activity, 23.1 Ci/mmol) binding to tissue samples of frontal cortex was performed as described previously (Battaglia et al. 1987). In preliminary experiments we found that the washing procedures used in the preparation of tissue homogenates was sufficient to reduce tissue radioactivity from approximately 1100 dpm/mg tissue to background levels on both ^{14}C or ^{3}H channels (approximately 35 dpm and 31 dpm, respectively). Membranes were incubated for 2 h (at 22° C) in six concentrations (2–1000 pM) of [^{3}H]-paroxetine with non-specific binding determined by the addition of citalopram (4 µM) to the incubation medium. Samples were rapidly filtered through polyethyleneimine (0.05%) presoaked Whatman GF/C and washed with three 5 ml aliquots of cold buffer. Filters were equilibrated in 10 ml scintillation cocktail (Picofluor-40) overnight before analysis by liquid scintillation spectrometry (Tri-Carb 1600CA; Canberra Packard). The data from these radioligand binding studies were analyzed using the non-linear, least squares, curve fitting programme, LIGAND (Munson and Rodbard 1980).

The preparation of slide-mounted sections for [^{3}H]-paroxetine autoradiography was according to the protocol described previously (De Souza and Kuyatt 1987) but with the addition of two 5-min prewashes in buffer (50 mM TRIS–HCl 120 mM NaCl and 5 mM KCl, pH = 7.7 at 23° C) to remove residual [^{14}C] label from the tissue (Chalmers and McCulloch 1989). Sections were then incubated in the same buffer containing a saturating concentration (250 pM) of [^{3}H]paroxetine. Non-specific binding was defined in adjacent sections by [^{3}H]paroxetine binding in the presence of 4 µM citalopram. Following incubation, the sections were washed in buffer, dipped in deionized water and rapidly dried under a stream of cold air. The sections, together with a series of ^{3}H-standards were apposed to X-ray film in a light-tight cassette and stored at −70° C for 4–6 weeks.

Analysis of the [^{14}C]2-deoxyglucose and [^{3}H]paroxetine binding autoradiograms was performed using a computer-based image analysis system (Quantimet-970; Cambridge Instruments, UK). For each brain region, optical density measurements were made bilaterally from six autoradiograms in which the area of interest could be anatomically defined (Paxinos and Watson 1986). Local tissue tracer concentrations were derived by comparing the optical densities of the appropriate standards with the mean optical density value of the region of interest. Local rates of glucose utilisation were calculated from the tissue [^{14}C]-tracer concentrations and the plasma history of the [^{14}C] and glucose using the operational equation of the method (Sokoloff et al. 1977). Statistical analysis was performed using Student's *t*-test, with acceptable levels of significance set at 5%.

Results

Analysis of [^{3}H]-paroxetine binding isotherms derived from homogenates of frontal cortex revealed an apparently homogenous population of binding sites in saline-treated control rats ($n = 5$) with affinity (K_D) = 12.8 ± 0.7 pM and density (B_{max}) of 17.2 ± 1.1 fmol/mg tissue (Fig. 1).

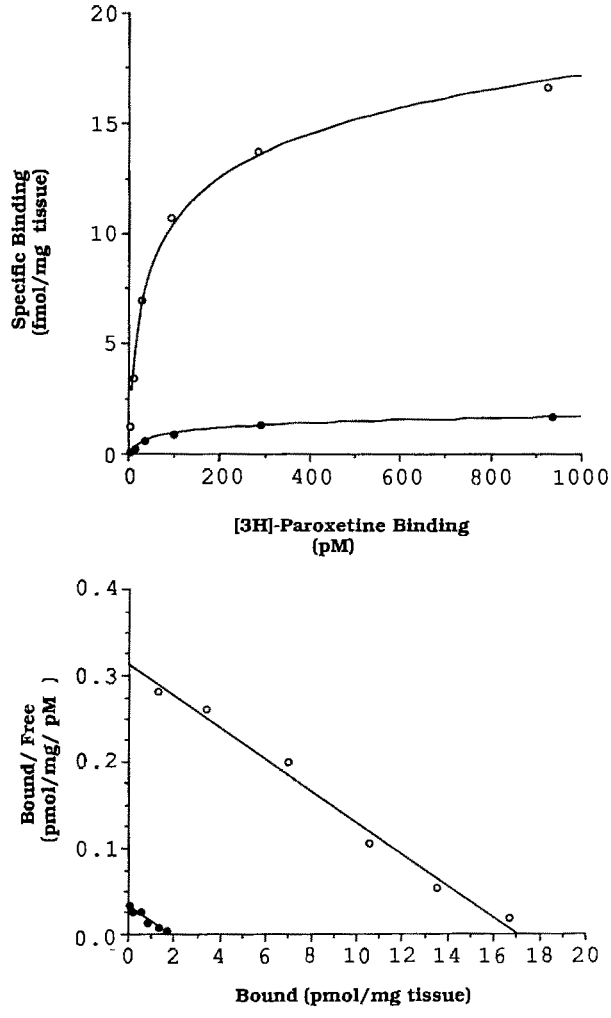

Fig. 1. Saturation isotherms *(top)* and Scatchard plots *(bottom)* of [^{3}H]-paroxetine binding to neocortical cell membranes prepared from representative rats which had been pretreated with either MDMA (●) (20 mg/kg, twice daily for 4 days) or saline vehicle (○). The animals were killed 14 days after the final injection and membranes prepared as described by Battaglia et al. (1987a). Each data point represents the mean specific binding of triplicate determinations performed on tissue from a single animal. Data were analysed using the "LIGAND" curve fitting programme

Table 1. Effects of MDMA exposure upon local cerebral glucose utilisation and [^{3}H]-paroxetine labelled 5HT uptake sites in rat brain

Brain region		5HT uptake sites		Local glucose use	
		Saline	MDMA	Saline	MDMA
Hippocampus					
Subiculum		181±10	13± 7[b]	63±4	70±3
Ammon's horn (pyramidal cell layer)					
Dorsal	CA1	69± 7	22± 6[b]	49±3	52±2
	CA2	146± 9	33± 6[b]	68±5	88±6[b]
	CA3	149±12	26± 7[b]	65±5	88±6[b]
Ventral	CA1	75± 8	19± 9[b]	47±5	54±2
	CA2	85±14	12± 6[b]	62±5	82±5[b]
	CA3	94±12	19± 8[b]	68±5	85±5[b]
Dentage gyrus					
Molecular layer		98±10	47±13[b]	73±2	80±1[b]
Granular layer		49±12	34± 4	50±2	54±2
Neocortex					
Parietal (area 1)					
	layers I–II	165±19	32± 6[b]	94±3	94±6
	layer IV	203±18	46±14[b]	109±2	106±4
	layer VI	47±13	19± 7	83±2	85±4
Frontal (area 1)					
	layers I–II	94± 5	17± 6[b]	95±6	93±6
	layer IV	64± 4	5± 2[b]	110±7	110±5
	layer VI	56± 4	11± 4[b]	85±3	88±5
Cingulate (area 1)					
	layers I–II	124± 8	19± 3[b]	82±4	84±3
	layer IV	39± 6	25± 9	104±6	106±6
	layer VI	34± 7	8± 4[b]	86±4	96±7
Cingulate (area 2) layers I–II		139±10	19± 3[b]	109±5	101±3
Occipital (area 2)					
	layers I–II	101± 9	17±10[b]	91±3	93±6
	layer IV	48± 9	47± 9	103±3	111±5
	layer VI	71± 6	5± 2[b]	89±4	92±4
Entorhinal (lateral)					
Superficial layers		199±12	10± 3[b]	71±4	73±3
Deep layers		59± 7	19± 7[b]	57±4	58±2
Striate (primary visual area)[a]		93± 9	30± 8[b]	110±4	115±3
Temporal (primary auditory area)[a]		35± 4	36±14	160±4	151±5
Raphe					
Dorsal nuclei		278± 3	283±20	74±2	68±2
Medial nuclei		169± 8	173± 9	78±3	77±3
Caudate nucleus (dorsal)		93± 8	17± 6[b]	84±2	86±2

Data are presented as mean binding density (fmol/mg tissue) or mean glucose use (μmol/100 g/min)±SEM for saline ($n=5$) and MDMA ($n=5$) pretreated animals

[a] Denotes measurements made in cortical layer IV

[b] $P<0.05$

In contrast, the repeated exposure to MDMA resulted in an 89% reduction in the density of [^{3}H]-paroxetine binding sites ($B_{max}=1.9\pm0.4$ fmol/mg tissue, $P<0.05$; $n=5$) (Fig. 1). An apparent 67% decrease in binding affinity ($K_D=4.2\pm0.6$ pM) probably reflects methodological limitations in determining K_D when receptor densities are so low.

The distribution of [^{3}H]-paroxetine-labelled 5–HT uptake sites, determined by quantitative autoradiography, is shown in Table 1. [^{3}H]-Paroxetine binding was heterogeneously distributed throughout the neuroanatomical components of the hippocampal formation with the highest densities of radiolabel observed within the subiculum (181±10 fmol/mg tissue equivalents). In the dorsal hippocampus, a band of enhanced labelling was observed in the outer blade of CA2 and CA3 fields of Ammon's horn and in the molecular layer of the dentate gyrus.

Within neocortex the pattern of [^{3}H]-paroxetine binding was heterogeneous, not only between cortical regions but across the different cortical layers. At the level of the caudate nucleus bands of elevated [^{3}H]-paroxetine binding were observed within cortical layers I and IV–V of primary somatosensory cortex (parietal cortex: area 1). By contrast, only the superficial band of high binding density was evident in the adjacent primary motor cortex (frontal cortex: area 1). This laminar pattern, with the highest binding densities observed in the superficial layers of cortex, was also observed in other areas with the exception of occipital cortex which showed a band of reduced [^{3}H]-paroxetine binding in layer IV.

Repeated administration of MDMA resulted in pro-

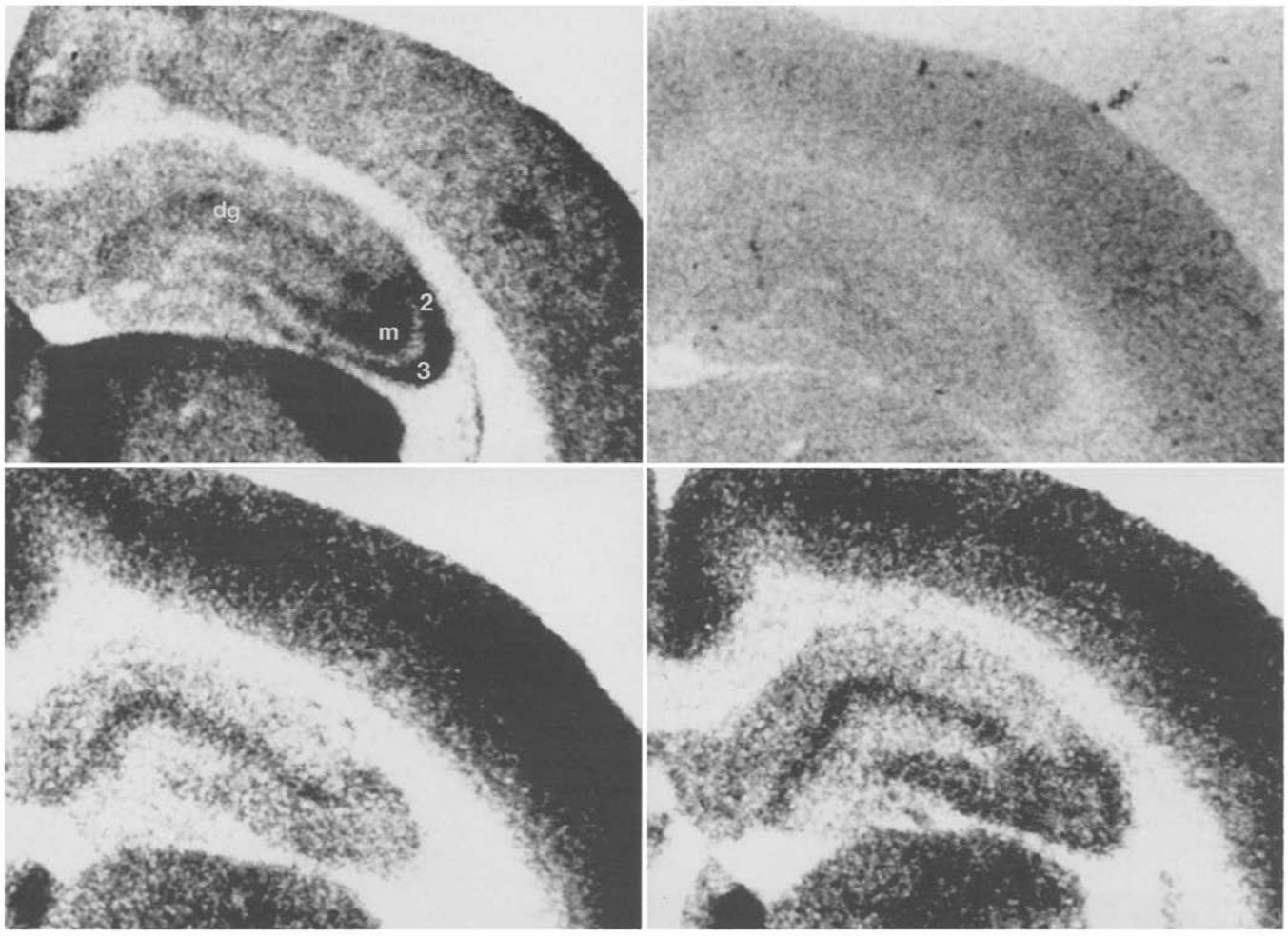

Fig. 2. Coronal sections of rat brain at the level of the habenula showing the dorsal hippocampus. *Upper:* Distribution of [^{3}H]-paroxetine-labelled 5–HT uptake sites 14 days after exposure to MDMA (20 mg/kg twice daily for 4 days: *right*) or saline vehicle *(left)*. Note the contrast between the high binding density within the molecular layer of the dentate gyrus (*dg*) and the molecular (*m*) and outer layers of the CA2 (*2*) and CA3 (*3*) fields of Ammon's horn in the saline-treated animal with the relatively uniform distribution of [^{3}H]-paroxetine binding in the MDMA treated animal. *Lower:* [^{14}C]-2-deoxyglucose autoradiograms of brain sections adjacent to those used for [^{3}H]-paroxetine binding autoradiography. MDMA pretreatment *(right)* resulted in higher rates of metabolic activity in the molecular layer of the dentate gyrus and in the outer layers of CA2 and CA3 when compared to saline control *(left)*

found, but regionally selective reductions in [^{3}H]-paroxetine binding within both neocortex and hippocampus. There were no significant changes in the density of 5–HT uptake sites within layer VI of parietal cortex, and layer IV of auditory and occipital cortex, but profound reductions in binding density (65–92%) in all other cortical areas examined. Similarly, with the exception of the granular layer of the dentate gyrus, exposure to MDMA produced significant reductions (52–95%) in all hippocampal fields examined. No changes in binding densities were detected in either the dorsal or median raphe nuclei.

In neither the raphe nuclei, which provide the source of forebrain serotonergic innervation, nor in many areas receiving serotonergic input (e.g. neocortex, caudate nucleus) were any significant changes in lCGU measured despite the fact that there were widespread reductions in 5–HT uptake sites within these projection areas following MDMA treatment. Similarly, in subiculum and CA1, where [^{3}H]-paroxetine binding was reduced by 95% and 75% respectively, there were no significant changes in lCGU. Only in hippocampal fields CA2 and CA3 and in the molecular layer of the dentate gyrus were profound losses of [^{3}H]-paroxetine-labelled uptake sites accompanied by significant increases in local cerebral glucose utilisation. Although moderate in dentate gyrus (+10%), the changes in glucose use within CA2 and CA3 (25–35%) were clearly evident from visual inspection of the autoradiograms (Fig. 2).

Discussion

In the present study we have shown that the MDMA treatment protocol which we adopted produced an 87% decrease in 5–HT uptake sites in homogenates of frontal cortex 14 days after treatment. This degree of depletion is comparable to that which has been reported previously

(Battaglia et al. 1987, 1988a, b). However, the greater spatial resolution afforded by the autoradiographic analysis of [^{3}H]paroxetine binding, which we also used in this study, revealed a degree of heterogeneity in the pattern of 5–HT uptake site depletion within neocortex and the hippocampal formation.

Although a gross depletion in 5–HT markers following exposure to MDMA has been reported within neocortex and the hippocampal formation (Stone et al. 1986; Battaglia et al. 1987; Commins et al. 1987), immunocytochemical investigations reveal a subpopulation of 5–HT fibres which are apparently resistant to the neurotoxic actions of the drug (O'Hearn et al. 1988). Thus, the population of large beaded fibres emanating from the median raphe nuclei, which predominate in the molecular layer of the hippocampal CA1 field and in portions of the granular cell layer of the dentate gyrus, have been reported to be relatively spared following exposure to MDMA. In contrast, the finer axons of dorsal raphe efferents, which innervate the molecular layer of dentate gyrus and layers of CA1 other than the molecular layer, are substantially depleted by MDMA (O'Hearn et al. 1987). In neocortex, there is evidence of selective sparing in a minority of 5–HT-like immunoreactive fibres (e.g. layer VI of parietal cortex). However, even in these areas of neocortex, it is the finer 5–HT fibres of the dorsal raphe which predominate, with the result that following MDMA treatment there are widespread reductions in 5–HT immunoreactive fibres throughout neocortex (Molliver 1987; O'Hearn et al. 1987). Whether selective vulnerability of subpopulations of 5–HT fibres provides the mechanism underlying the observed heterogeneous depletion of [^{3}H]paroxetine binding in hippocampus and neocortex is unclear, as the spatial resolution afforded by the autoradiographic techniques used in this study do not allow us to characterize the morphological characteristics of the individual fibres within each area.

The neuroanatomical pathways involved in the initiation and expression of MDMA-induced behaviours, in response to acute treatment, have previously been examined in the rat using the unique metabolic mapping properties of the 2-deoxyglucose quantitative autoradiographic technique (Wilkerson and London 1989; London et al. 1990). The pattern of alterations in local cerebral glucose utilization elicited by MDMA was similar to that produced by other psychomotor stimulants with pronounced increases in glucose use within components of the extrapyramidal motor system (Wilkerson and London 1989). However in the present study, performed 14 days after sub-chronic treatment with MDMA, the pattern of alterations in local cerebral glucose utilization was both qualitatively and quantitatively different from those observed following acute administration.

Despite reduction in [^{3}H]paroxetine-labelled binding sites of up to 93%, functional activity in neocortex was unchanged. This observation is in keeping with previous reports in which local cerebral glucose utilization was unchanged by either electrolytic or 5,7–DHT induced lesions of the raphe nuclei (Cudennec et al. 1988a). Taken together with data from electrical stimulation studies, these previous results have been take to suggest that ascending serotenergic projections may exert a phasic, rather than tonic influence upon the integrated functional activity of the mammalian brain (Cudennec et al. 1988b). However, our analysis of subfields of the hippocampus clearly shows that in CA2 and CA3 fields of Ammon's horn, marked increases (25–33%) in function-related glucose utilization accompanied reductions in 5–HT uptake sites despite the fact that these decreases were no more profound than those found in neocortex. Thus the functional integrity of hippocampal fields CA2 and CA3 would appear to be particularly vulnerable to the loss of 5–HT terminals which results from exposure to MDMA, since in the other hippocampal subfields examined, reduction in the density of [^{3}H]paroxetine-labelled binding sites of equal magnitude had little effect on glucose use.

The previous perception of MDMA as a safe recreational drug and potential psychotherapeutic agent (Dowling 1986) has been revised in the light of recent observations that the drug possesses potent neurotoxic properties (Ricaurte et al. 1985; Battaglia et al. 1987; O'Hearn et al. 1988). However, there has been no evidence that MDMA produces any lasting fuctional deficit in the human brain. Similarly, in sub-human primates, no changes in spontaneous behaviour have been detected following pretreatment with MDMA, despite 50% reductions in forebrain 5–HT levels, nor have any deficits been found in rats when tested across a range of behavioural paradigms (Slikker et al. 1989).

From the data presented here, it would appear that the lasting effects of MDMA on brain function are not widespread, although subregions of the hippocampus certainly do appear to be susceptible to functional disruption. This selective vulnerability of hippocampus to MDMA exposure may simply not be sufficient to become manifest as detectable deficits in memory and learning processes. However, hippocampal 5–HT systems may be involved in clinical depression (Treiser et al. 1981; Meltzer 1989; Welner et al. 1989), and if the dysfunction which we have found in rat hippocampus is also to be found in man, the abuse of MDMA may possibly result in a subsequent predisposition to psychiatric disturbance.

Acknowledgements. This study was supported by the Medical Research Council. J. Sharkey is funded by the Wellcome Trust. D.E. McBean was funded by the James A. Kennedy Bequest (University of Edinburgh).

References

Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ, DeSouza EB (1987) 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^{3}H]-paroxetine-labeled serotonin uptake sites. J Pharmacol Exp Ther 242:911–916

Battaglia G, Sharkey J, Kuhar MJ, DeSouza EB (1988a) Neuroanatomical specificity of MDA and MDMA-induced degeneration of serotonin neurones in rat brain. Soc Neurosci Abstr 14:222.5

Battaglia G, Yeh SY, DeSouza EB (1988b) MDMA-induced neu-

rotoxicity: parameters of degeneration and recovery of brain serotonin systems. Pharmacol Biochem Behav 29:269–274

Chalmers DT, McCulloch J (1989) Excitatory amino acid receptor sites and glucose use after acute orbital enucleation J Cereb Blood Flow Metab 9[Suppl. 1]:s299

Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987) Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 241:338–345

Cudennec A, Duverger D, Nishikawa T, McRae-Degueurce A, MacKenzie ET, Scatton B (1988a) Influence of ascending serotonergic pathways on glucose use in the conscious rat brain. II. Effects of electrolytic or neurotoxic lesions of the dorsal and/or median raphe nucleus. Brain Res 444:214–226

Cudennec A, Duverger D, Serrano A, Scatton B, MacKenzie ET (1988b) Influence of ascending serotonergic pathways on glucose use in the conscious rat brain. II. Effects of electrical stimulation of the rostral raphe nuclei. Brain Res 444:227–246

De Souza EB, Kuyatt BL (1987) Autoradiographic localization of 3H-paroxetine labeled serotonin uptake sites in rat brain. Synapse 1:488–496

Dowling J (1986) The psychological and physiological effects of MDMA on normal volunteers. J Psychoactive Drugs 18:335–340

Gibb JW, Stone DM, Stahl DC, Hanson GR (1987) The effect of amphetamine-like designer drugs on monoaminergic systems in rat brain. Natl Inst Drug Abuse Res Monogr Ser 76:316–321

Glennon RA, Young R (1984) MDA: an agent that produces stimulus effects similar to those of 3,4–DMA, LSD and cocaine. Eur J Pharmacol 99:249–250

Grinspoon L, Bakalar JB (1986) Can drugs be used to enhance the psychotherapeutic process? Am J Psychother 40:393–404

Habert E, Graham D, Tahraoui L, Claustre Y, Langer SZ (1985) Characterization of [^{3}H]paroxetine binding to rat cortical membranes. Eur J Pharmacol 118:107–114

Kosofsky BE, Molliver ME (1987) The serotonergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. Synapse 1:153–168

London ED, Wilkerson G, Ori C, Kimes AS (1990) Central action of psychomotor stimulants on glucose utilisation in extrapyramidal motor areas of the rat brain. Brain Res 512:155–158

Meltzer H (1989) Serotonergic dysfunction in depression. Br J Psychiatry Suppl Dec [8]:25–31

Molliver ME (1987) Serotonergic neuronal systems: what their anatomical organization tells us about function. J Clin Psychopharmacol 7:3s–23s

Munson P, Rodbard D (1980) LIGAND: a versatile computerised approach for the characterisation of ligand binding systems. Anal Biochem 107:220–239

O'Hearn E, Battaglia G, DeSouza EB, Kuhar MJ, Molliver ME (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 8:2788–2803

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, New York

Peroutka SJ (1987) Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") on an undergraduate campus. N Engl J Med 317:1542–1543

Ricaurte GA, Forno LS, Wilson MA, De Lanney LE, Irwin I, Molliver ME, Langston JW (1988) (±)3,4-methylenedioxymethamphetamine selectively damages central serotonergic neurons in non-human primates. JAMA 260:51–55

Schmidt CJ, Taylor, VL (1988) Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons. Eur J Pharmacol 156:121–131

Shulgin AT (1986) The background and chemistry of MDMA. J Psychoactive Drugs 18:291–300

Siegel RK (1986) MDMA. Nonmedical use and intoxication. J Psychoactive Drugs 18:349–354

Slikker W, Holson RR, Ali SF, Kolta MG, Paule MG, Scallet AC, McMillan DE, Bailey JR, Hong JS, Scalzo FM (1989) Behavioral and neurochemical effects of orally administered MDMA in the rodent and non-human primate. Neurotoxicology 10:529–542

Sokoloff L (1977) Relationship between physiological function and energy metabolism in the central nervous system. J Neurochem 29:13–26

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [^{14}C]-deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916

Stone DM, Stahl DC, Hanson GR, Gibb JW (1986) The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur J Pharmacol 128:41–48

Treiser SL, Cascio GS, O'Donohue TL, Thoa NB, Jacobowitz DM, Keller KJ (1981) Lithium increases serotonin release and decreases serotonin receptors in hippocampus. Science 213:1529–1531

Welner SA, De Montigny C, Desroches J, Desjardins P, Suranyi-Cadotte BE (1989) Autoradiographic quantification of serotonin 1A receptors in rat brain following antidepressant drug treatment. Synapse 4:347–352

Wilkerson G, London ED (1989) Effects of methylenedioxymethamphetamine on local cerebral glucose utilization in the rat. Neuropharmacology 28:1129–1138