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Effects of Repeated Administration of 3,4-Methylenedioxymethamphetamine on 5-Hydroxytryptamine Neuronal Activity and Release in the Rat Brain *in Vivo*

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ABSTRACT

In experimental animals, administration of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) leads to extensive, but incomplete, loss of 5-hydroxytryptamine (5-HT) innervation in the brain. Here, we report the effects of MDMA on 5-HT neuronal function measured in the rat *in vivo* using electrophysiological and microdialysis techniques. Two weeks after administration of an established neurotoxic regimen of MDMA (20 mg/kg s.c., twice daily for 4 days) we found; 1) no change in either the density or the firing activity of 5-HT neurons in the dorsal raphé nucleus; 2) no change in basal extracellular 5-HT in either the frontal cortex or the hippocampus, although extracellular 5-hydroxyindoleacetic acid was reduced by about 50% in both regions; and 3) no change in the amount of 5-HT released in the hippocampus in response to electrical stimulation (5 Hz) of either the dorsal or median raphé nucleus, but a marked reduction in the amount of 5-HT released in the frontal cortex after electrical stimulation of the dorsal raphé nucleus. In summary, although MDMA causes marked 5-HT neurotoxicity, our data suggest that 5-HT cell firing is unchanged and, furthermore, that 5-HT release is maintained in some (but not all) forebrain regions even in response to physiological levels of stimulation.

The substituted amphetamine MDMA (Ecstasy) is in widespread use as a recreational drug. Evidence from animal studies indicates that MDMA is toxic to central 5-HT neurons when administered repeatedly or in a single high dose. For example, biochemical studies in the rat consistently find that repeated MDMA causes a long-lasting decrease in markers of 5-HT neurons (*e.g.*, 5-HT, 5-HIAA and 5-HT uptake sites) in the brain (Stone *et al.*, 1986; Schmidt, 1987; Commins *et al.*, 1987; Battaglia *et al.*, 1987). Furthermore, immunohistochemical studies have confirmed that 5-HT axons and terminals suffer structural damage and degenerate after MDMA (Commins *et al.*, 1987; O'Hearn *et al.*, 1988). However, although this loss of 5-HT innervation to the forebrain is extensive, it is incomplete. Moreover, most, if not all, 5-HT cell bodies are not destroyed by MDMA (O'Hearn *et al.*, 1988).

Although repeated MDMA administration causes marked 5-HT neurotoxicity, many neurophysiological functions in which central 5-HT systems are known to play a role (*e.g.*, locomotor behavior, sexual function and feeding) are unaltered in MDMA-treated animals (Slikker *et al.*, 1989; Poland, 1990; Ricaurte *et al.*, 1993; Robinson *et al.*, 1993). For instance, Ricaurte *et al.* (1993) found that rats administered neurotoxic doses of MDMA had no impairment in memory function, although a memory deficit was detected in rats administered the 5-HT neurotoxin, 5,7-DHT. In humans, many neuropsychological measures are reportedly normal in users of MDMA, although subtle disturbances in sleep architecture, memory function and behavior have been noted (Krystal *et al.*, 1992; Allen *et al.*, 1993; McCann *et al.*, 1994). There are, however, several case reports of episodes of severe psychiatric illness which have been ascribed to the repeated use of MDMA (Hayner and McKinney, 1986; McCann and Ricaurte, 1991; McGuire and Fahy, 1991; Pallanti and Mazzi, 1992).

The maintenance of 5-HT-dependent behaviors in animals exposed to MDMA might be explained by compensatory increases in the function of those 5-HT neurons spared by the lesion. In a recent microdialysis study we found normal basal extracellular 5-HT levels in the cortex of rats treated with MDMA, although 5-HT released by a releasing agent (*d*-fenfluramine) was decreased markedly (Series *et al.*, 1994). Similar results were obtained in rats partially lesioned with 5,7-DHT (Kirby *et al.*, 1995). These studies concord with previous biochemical studies finding increases in the levels of tryptophan hydroxylase mRNA and in the activity of this

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5,7-DHT, 5,7-dihydroxytryptamine; DRN, dorsal raphé nucleus; MRN, median raphé nucleus; HPLC, high-performance liquid chromatography.

enzyme in rats with partial 5-HT lesions (Stachowiak *et al.*, 1986; Bendotti *et al.*, 1990).

In parallel studies in animals with partial dopamine lesions, increases in the turnover and release of dopamine in terminal regions have been observed (Agid *et al.*, 1973; Zigmond *et al.*, 1990). In addition, the electrical activity of the remaining dopaminergic neurons is reported to increase (Hollerman and Grace, 1990). The electrical activity of noradrenergic neurons also increases in animals with incomplete noradrenaline lesions (Chiodo *et al.*, 1983), but it is not known whether 5-HT neuronal activity responds in this way to a partial 5-HT lesion.

Here, we have examined the effects of MDMA on two measures of 5-HT neuronal function in the rat *in vivo*: 1) the electrical activity of 5-HT neurons in the DRN and 2) extracellular 5-HT in two forebrain regions (frontal cortex and ventral hippocampus) monitored under basal conditions and in response to electrical stimulation of the midbrain raphé nuclei.

Methods

MDMA treatment. Male Sprague-Dawley rats (Harlan-Olac, Bicester, UK) were housed under controlled conditions of temperature and humidity in a 12-hr light/dark cycle (lights on 8:00 A.M.). Groups of rats (180–200 g) received twice-daily injections of MDMA (20 mg/kg s.c.) or saline (1 ml/kg s.c.) for 4 days. During the treatment, animals were housed singly or in pairs, but were returned to group cages (≤6 per cage) 24 hr after the last injection. This MDMA regime has been shown previously to decrease markedly biochemical markers of 5-HT neurons and numbers of immunocytochemically identified 5-HT axon terminals, while having minimal effects on other monoamine neurotransmitters (Battaglia *et al.*, 1987; Commins *et al.*, 1987; O'Hearn *et al.*, 1988).

Experiments were carried out 14 to 16 days after the start of the MDMA (or saline) treatment. Rats treated at the same time were allocated for use in either a microdialysis or electrophysiological experiment.

General surgical procedures. Animals were anesthetized with chloral hydrate (500 mg/kg i.p., plus supplementary doses as necessary) and placed in a stereotaxic frame (Kopf, Tujunga, CA) with the incisor bar set at −3.3 mm (flat skull position). The skull was exposed and burr holes were drilled to allow implantation of recording electrodes, stimulating electrodes and microdialysis probes as appropriate. Stereotaxic coordinates, taken from the atlas of Paxinos and Watson (1982), are given in millimeters from bregma and the dura surface.

Electrophysiological recording. Extracellular recordings were made from the DRN as described previously (Gartside *et al.*, 1995; Hajós *et al.*, 1995a). Briefly, electrodes were prepared from glass micropipettes and filled with 2 M NaCl saturated with Pontamine sky blue (3–8 megohm impedance *in vitro*). The extracellular signal was amplified (×1000) using a DC amplifier, and was filtered (300–3000 Hz band-pass) and then displayed on an oscilloscope and chart recorder. The signal was also recorded on digital audio tape for off-line analysis.

The experimenter was blind to the treatment. In each animal, the recording electrode was positioned initially just above the DRN (AP, −7.7; ML, 0; DV, −4.5). The electrode was then lowered through the DRN, to a maximum depth of 6.0 mm, using a hydraulic microdriver. Further descents were made, each time the electrode being raised 4.5 mm and then moved 100 or 200 μm in a lateral and/or anterior/posterior direction before commencing the next descent.

During each electrode descent, baseline recordings (usually at least 3 min duration) were made from all spontaneously active 5-HT neurons encountered. These neurons fired single, broad (1–2 msec) action potentials in a slow (0.2–3 Hz) and regular pattern, which is characteristic of immunohistologically identified 5-HT neurons (Aghajanian and VanderMaelen, 1982).

Recordings were also collected from another type of neuron encountered in the DRN. These neurons were indistinguishable from classical 5-HT neurons with the exception that they sometimes fired brief bursts (two, three or even four spikes with an interspike interval of 3–12 msec) in place of the expected single spike. We have recently described these burst-firing neurons in more detail and presented evidence which suggests that they are also 5-HT neurons (Hajós *et al.*, 1995b; Hajós and Sharp, 1996). All neurons of this type encountered were recorded.

Seven to 10 neurons were recorded from each animal. At the end of each experiment, the position of the last recorded neuron was marked by iontophoretic ejection of a small amount of Pontamine sky blue. The brain was removed and postfixed in 4% paraformaldehyde, and the final electrode position was verified by inspection of cresyl violet-stained sections.

Microdialysis procedure. A concentric microdialysis probe (Hospal AN69 membrane, 300 μm diameter, 4 mm window) was implanted into either the ventral hippocampus (AP, −4.0; ML, −4.6; DV, −9.0) or the frontal cortex (AP, +3.2; ML, −3.0; DV, −5.5), and cemented in place using dental acrylic. A coaxial bipolar stainless steel stimulating electrode (NE-100, Clark Electromedical Instruments, Reading, UK) was then implanted into the DRN (AP, −7.6; ML, 0; DV, −6.0). The stimulating electrode was connected *via* a constant current unit (Grass PS1U6, Quincy, MA) to a stimulator (Grass S48) and an oscilloscope.

The microdialysis probe was perfused (2 μl/min) with artificial cerebrospinal fluid (composition in millimolar: NaCl, 140; KCl, 3; $CaCl_2$, 2.4; $MgCl_2$, 1.0; Na_2HPO_4, 1.2; NaH_2PO_4, 0.27; and glucose, 7.2, pH 7.4) containing the 5-HT reuptake inhibitor, citalopram (1 μM). Samples of the dialysate were collected every 20 min.

Once levels of 5-HT in the dialysates had stabilized (2–3 hr after probe implantation), a 20-min electrical stimulation (1 msec square pulse, 300 μA current, 5 Hz) was applied to the DRN. In the animals with a microdialysis probe implanted in the hippocampus, immediately after the DRN stimulation the electrode was lowered (2 mm) into the MRN and, 60 min later, a 20-min electrical stimulation was applied to the MRN. Animals with a microdialysis probe implanted in the frontal cortex received a stimulation of the DRN only. The rationale for the stimulations was based on the known raphé inputs to the ventral hippocampus and frontal cortex (see McQuade and Sharp, 1995 for further details).

After each experiment, the brain was removed and postfixed in 4% paraformaldehyde. The correct (final) location of the stimulating electrode was verified by inspection of slide-mounted sections. In some animals the stimulating electrode was found to be located outside the DRN or MRN. Data from these animals are included in analysis of basal 5-HT and 5-HIAA levels only.

HPLC-electrochemical detection. Immediately after collection, dialysates were analyzed for 5-HT and 5-HIAA by HPLC with electrochemical detection. The indoles were separated on a Microsorb column (C_{18}, Rainin, Woburn, MA) with a mobile phase (methanol, 12.5%; disodium EDTA, 0.03%; NaH_2PO_4, 127 mM; and octane sulfonic acid, 15 μM, pH 4.0) pumped at 1.2 ml/min using an HPLC pump (LKB, Rockville, MD). Detection was by a BAS (W. Lafayette, IN) (LC-4) potentiometer with a working electrode (glassy carbon) set at +0.7 V.

Data analysis and statistics. For the electrophysiological studies, data were analyzed off-line by feeding discriminator output pulses to the Spike 2 analog program for Macintosh (Cambridge Electronic Design, Cambridge, UK). The firing rate (spikes/10 sec) and regularity (percentage of coefficient of variation) of the interspike interval) of each neuron were determined from the base-line recording (usually 3 min duration). In the case of burst-firing neurons, only the first spike of each burst was used in calculating the regularity. For the microdialysis experiments, data are expressed as

absolute amounts of 5-HT and 5-HIAA (femtomole and picomole, respectively) per 20 min sample or change from base line.

Differences in the distribution of firing rates were assessed by one-way analysis of variance (F test). Comparisons of group means were made using paired and unpaired Student's t test, as appropriate. Data are expressed as mean ± S.E.; n = sample number. Significance at the 95% levels is reported; n.s. denotes not significant.

Results

Effect of MDMA on neuronal activity in the DRN. Extracellular recordings were made from a total of 104 neurons in the DRN of saline (n = 6)- or MDMA (n = 7)-treated rats. Of these neurons, 60 had the electrophysiological characteristics of classical 5-HT neurons (Aghajanian and VanderMaelen, 1982), whereas 35 had 5-HT-like characteristics but were burst-firing (Hajós *et al.*, 1995b; Hajós and Sharp, 1996). A further nine cells were found, on subsequent analysis, to have significantly different firing patterns from either of the above, and were excluded from the study.

Classical 5-HT neurons. The number of classical 5-HT neurons encountered per electrode descent through the DRN in MDMA-treated rats was not different to that found in the saline-treated group (table 1). Neither the mean firing rate nor the regularity of firing of the classical 5-HT neurons differed significantly between the MDMA- and saline-treated groups (table 1), nor was the distribution of the firing rates significantly different (F = 1.0, n.s., F test) (fig. 1A). Classical 5-HT neurons in saline- and MDMA-treated animals were indistinguishable with respect to the characteristics of their action potentials (size, shape and duration: data not shown).

Burst-firing, presumed 5-HT neurons. The number of burst-firing neurons encountered per electrode descent through the DRN of rats treated with MDMA or saline was similar (table 1). In addition, burst-firing DRN neurons in rats treated with MDMA or saline were similar with respect to their mean firing rate and regularity of firing (table 1). Analysis of the distribution of the firing rates of burst-firing neurons revealed no significant difference between the saline- and MDMA-treated groups (F = .003, n.s., F test) (fig. 1B). For these neurons, the proportion of spikes occurring within a burst and the proportion occurring as a single spike was calculated. The mean percentage of spikes occurring in a burst did not differ between the two treatment groups [15.9 ± 3.7 (15) *vs.* 15.6 ± 3.3 % (20); saline *vs* MDMA: n.s., unpaired t test]. In MDMA-treated rats, 36 ± 10% (7) of the total DRN neurons recorded in each rat were burst-firing, a value which was similar to that found in the control group [32 ± 8% (6)] (n.s., unpaired t test).

Effect of MDMA on 5-HT and 5-HIAA in Dialysates from Frontal Cortex and Hippocampus

Basal 5-HT and 5-HIAA. In MDMA-treated rats, basal levels of 5-HT in dialysates of the frontal cortex were not significantly different from those in saline-treated rats (n.s., unpaired t test), whereas levels of 5-HIAA were reduced by approximately 52% ($P < .01$, unpaired t test) (fig. 2). Similarly, in dialysates of the hippocampus, basal 5-HT levels were not different in MDMA- and saline-treated groups (n.s., unpaired t test), whereas basal levels of 5-HIAA were approximately 45% lower in MDMA than in saline-treated animals ($P < .01$, unpaired t test) (fig. 2).

Stimulation-Evoked 5-HT

Frontal cortex. Electrical stimulation of the DRN (5 Hz, 20 min) evoked about a 3-fold increase in dialysate 5-HT in the frontal cortex of saline-treated rats (fig. 3). This response was markedly smaller in rats treated with MDMA (amount of 5-HT released [peak minus baseline], 105 ± 27 (7) *vs.* 35 ± 4 (6) fmol/20 min sample; saline *vs.* MDMA: $P < .05$, unpaired t test).

Ventral hippocampus. As shown in figure 4A, electrical stimulation of the DRN (5 Hz, 20 min) also evoked a large (approximately 3-fold) increase in 5-HT in dialysates of the hippocampus. Surprisingly, the amount of 5-HT released by electrical stimulation of the DRN in rats treated with MDMA was not different from the saline group (amount of 5-HT released: 94 ± 29 (5) *vs.* 114 ± 6 (4) fmol/20 min sample; saline *vs.* MDMA: n.s., unpaired t test).

In these same rats, stimulation of the MRN (5 Hz, 20 min) caused a marked increase in 5-HT levels in dialysates of the hippocampus in both the saline and the MDMA-treated groups (fig. 4B). The amount of 5-HT released by stimulation of the MRN was not altered by MDMA treatment (110 ± 53 (5) *vs.* 92 ± 18 (4) fmol/20 min sample; saline *vs.* MDMA: n.s., unpaired t test).

Discussion

There is much evidence from biochemical, histological and immunocytochemical studies that, on repeated administration, MDMA is a 5-HT neurotoxin. However, despite its 5-HT neurotoxicity, MDMA appears not to induce gross alterations in behaviors known to involve 5-HT (see Introductory section). In the present study, we have used a combination of electrophysiological and microdialysis experiments to examine whether MDMA, administered in a standard neurotoxic regimen (Battaglia *et al.*, 1987; Commins *et al.*, 1987; Mol-

TABLE 1

Summary of the electrophysiological characteristics of classical 5-HT and burst-firing neurons recorded from the DRN of saline- and MDMA-treated animals

Data are mean ± S.E. (n = sample number). There are no significant differences in any of the parameters between the saline and MDMA groups (unpaired Student's t test). CV, coefficient of variation.

	Classical 5-HT			Burst-Firing		
	Neurons/descent	Firing rate (spikes/10 sec)	Regularity (% CV)	Neurons/descent	Firing rate (spikes/10 sec)	Regularity (% CV)
Saline	1.20 ± 0.26	13.7 ± 1.1	31.0 ± 1.4	0.6 ± 0.12	11.6 ± 1.3	33.3 ± 2.1
(n = 6)	(25)	(31)	(31)	(25)	(15)	(15)
MDMA	1.16 ± 0.16	11.9 ± 1.4	30.6 ± 1.1	0.8 ± 0.18	11.8 ± 1.4	30.9 ± 1.5
(n = 7)	(25)	(29)	(29)	(25)	(20)	(20)

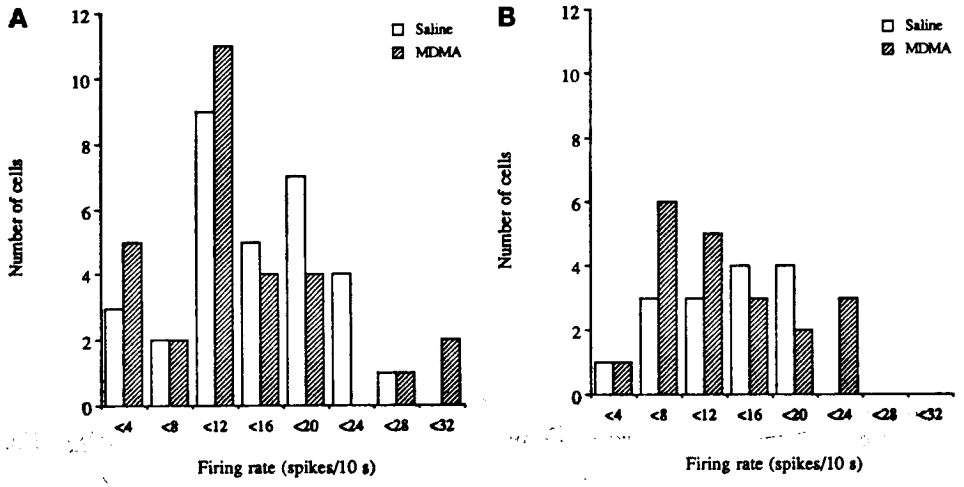

Fig. 1. Distribution of firing rates of classical 5-HT (A) and burst-firing neurons (B) recorded from the DRN of saline- and MDMA-treated rats.

liver *et al.*, 1990), leads to a disruption of 5-HT neuronal function in the rat *in vivo*. Two weeks after MDMA treatment, we found: 1) no change in either the density or firing activity of 5-HT neurons in the DRN; 2) no change in basal extracellular 5-HT in either the frontal cortex or ventral hippocampus, but a marked reduction in extracellular 5-HIAA in both regions; and 3) no change in the amount of 5-HT released in the hippocampus by electrical stimulation of the DRN or MRN, but a marked reduction in that released in the frontal cortex in response to stimulation of the DRN.

5-HT neuronal activity. By using extracellular recording techniques, we examined the effects of MDMA on both the density (number of neurons encountered per electrode descent) and firing characteristics (firing rate, regularity, shape of action potential) of two types of neurons in the DRN; one type with electrophysiological characteristics typical of 5-HT-containing neurons (Aghajanian and VanderMaelen, 1982) and another type, which has many of the features of classical 5-HT neurons, but which displays burst-firing activity (Hajós *et al.*, 1995b). We have accumulated evidence that these burst-firing neurons are also 5-HT neurons (Hajós *et al.*, 1995b; Hajós and Sharp, 1996). MDMA treatment did not alter the density or firing characteristics of either type of neuron.

These electrophysiological data support the notion that 5-HT cell bodies in the DRN are "spared" from the neurotoxic effects of MDMA. Previous studies have shown that 5-HT-like immunoreactivity and the density of 5-HT uptake sites in the DRN are unaffected by MDMA treatment despite a loss of these markers in the forebrain (O'Hearn *et al.*, 1988; Battaglia *et al.*, 1991). The fact that MDMA spares 5-HT cell bodies distinguishes it from the classical neurotoxin 5,7-DHT, which destroys both 5-HT cell bodies and their axons (Baumgarten *et al.*, 1982). Furthermore, electrophysiological studies show that, in rats treated with 5,7-DHT, there is a large reduction in the incidence of both classical and burst-firing presumed 5-HT neurons in the DRN (Aghajanian *et al.*, 1978; Hajós and Sharp, 1996).

The lack of change in the firing rate or pattern of either classical 5-HT neurons or burst-firing, presumed 5-HT neurons, in rats treated with MDMA indicates that the electrical activity of these 5-HT neurons does not adapt to compensate for the loss of their axons. This result contrasts with findings that, in rats lesioned with the catecholamine neurotoxin, 6-hydroxydopamine, spared dopaminergic and noradrenergic neurons increase their electrical activity (Chiodo *et al.*, 1983; Hollerman and Grace, 1990). The difference might be explained by the fact that 6-hydroxydopamine destroys not only axons, but also cell bodies, and that the catecholamine lesion is likely to be more complete than the 5-HT neurotoxicity studied here. However, in a recent study of rats lesioned with 5,7-DHT, we did not find evidence of increased electrical activity of those few 5-HT neurons spared by the lesion (Hajós and Sharp, 1996). This might suggest that there are fundamental differences in the way that 5-HT and catecholamine neurons respond to a partial lesion.

Microdialysis measurements of 5-HT and 5-HIAA. Here we found that basal extracellular levels of 5-HT in dialysates from the frontal cortex and ventral hippocampus were unchanged by MDMA treatment, but that levels of 5-HIAA in the same samples were much reduced. Many previous studies have reported that the regime of MDMA

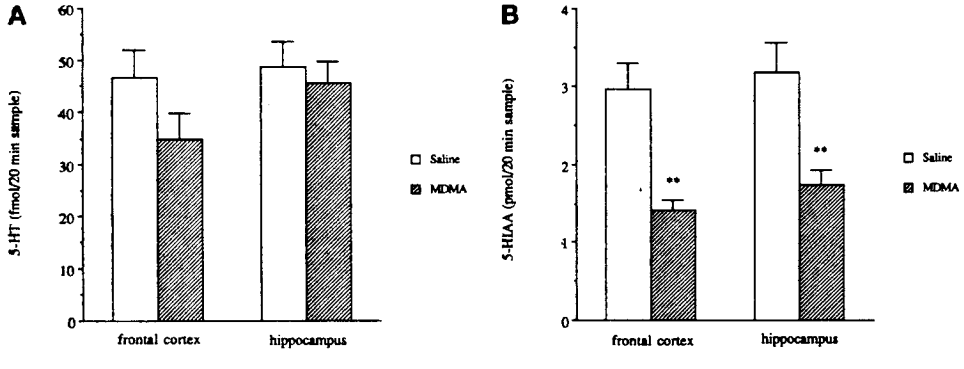

Fig. 2. Basal extracellular levels of 5-HT (A) and 5-HIAA (B) in dialysates of the frontal cortex and hippocampus in saline- and MDMA-treated animals. Data are mean ± S.E., $n = 8–11$ animals per group. **$P < .01$ (unpaired t test).

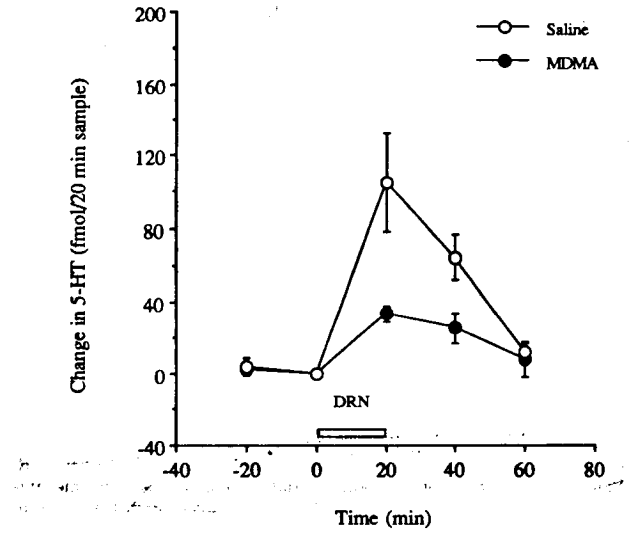

Fig. 3. Change in 5-HT in dialysates of the frontal cortex in response to electrical stimulation of the DRN in saline- and MDMA-treated animals. An electrical stimulation (20 min, 5 Hz) was applied to the DRN as indicated by the bar. Data are mean ± S.E., n = 6–7 animals per group. See text for statistical analysis.

treatment used here both decreases biochemical markers of 5-HT neurons (including tissue levels of 5-HIAA) and causes degeneration of 5-HT axon terminals (Battaglia *et al.*, 1987; Commins *et al.*, 1987; O'Hearn *et al.*, 1988). The present data confirm the findings of our recent study (Series *et al.*, 1994), in which we reported normal 5-HT, but low 5-HIAA, in cortical dialysates collected from rats treated with neurotoxic doses of MDMA, as well as in those treated with *p*-chloroamphetamine and *d*-fenfluramine. Furthermore, although early microdialysis studies reported decreased basal levels of 5-HT (as well as 5-HIAA) in rats lesioned with 5,7-DHT (Kalén *et al.*, 1988; Sharp and Foster, 1991), it was reported recently that basal extracellular 5-HT could be maintained until tissue levels of 5-HT are depleted by more than 95% (Kirby *et al.*, 1995). Taken together, these data indicate that extracellular levels of 5-HT in the brain may be preserved in the presence of a 5-HT lesion, but that they will eventually fall as the 5-HT lesion becomes near complete.

Under basal conditions, extracellular 5-HT is dependent largely on nerve impulse flow (see Sharp *et al.*, 1989, 1990). Because the firing rate of 5-HT cells in the anesthetized rat is around 1 Hz (see fig. 1), our finding of unchanged basal 5-HT in MDMA-treated rats suggests that 5-HT terminals in the frontal cortex (and hippocampus) can maintain 5-HT release at low levels of 5-HT neuronal activity. However, when release was evoked by electrical stimulation of the DRN, the amount of 5-HT released in the frontal cortex was much reduced in MDMA-treated animals compared to controls. The stimulation frequency used, 5 Hz, is within the normal range of firing activity of 5-HT neurons in nonanesthetized animals (0.5–6 Hz) (Jacobs and Azmitia, 1992). Taken together, our data suggest that, after MDMA, although cortical 5-HT nerve terminals can maintain 5-HT release at low frequency of cell firing (see above), they are unable to do so when the frequency of cell firing increases, albeit within the physiological range. Evidence that cortical 5-HT systems are unable to fully compensate for the lesion induced by MDMA was also apparent in our recent study in which we found that the amount of cortical 5-HT released in response to a pharmacological stimulus (*d*-fenfluramine) was diminished in rats pretreated with MDMA, even though basal 5-HT levels were unchanged (Series *et al.*, 1994).

In the hippocampus, even though 5-HIAA levels were much decreased, both basal levels of 5-HT and the 5-HT response to DRN stimulation, were unaffected by MDMA treatment. These findings are evidence that 5-HT release in hippocampus is maintained in MDMA-treated rats, even under conditions of increased 5-HT neuronal activity (*i.e.*, when neurons are stimulated at 5 Hz). Because the 5-HT measurements were made with a 5-HT reuptake inhibitor present in the perfusion medium, the possibility that the loss of 5-HT reuptake sites associated with the loss of 5-HT axon terminals simply makes more 5-HT available outside of the neuron, does not seem likely. A more plausible explanation is that, in the hippocampus of MDMA-treated rats, the amount of 5-HT released per 5-HT nerve terminal is enhanced, possibly by an increase in the size of the releasable pool in the undamaged 5-HT terminals. The likelihood of this coming about through changes in the electrical activity of the spared 5-HT neurons can be discounted on the basis of the present study (see above). There is, however, evidence of increased 5-HT synthesis in forebrain regions of rats with partial 5-HT lesions (Stachowiak *et al.*, 1986), which might serve to maintain 5-HT release. An increase in dopamine synthesis has been postulated to explain the apparently normal release of

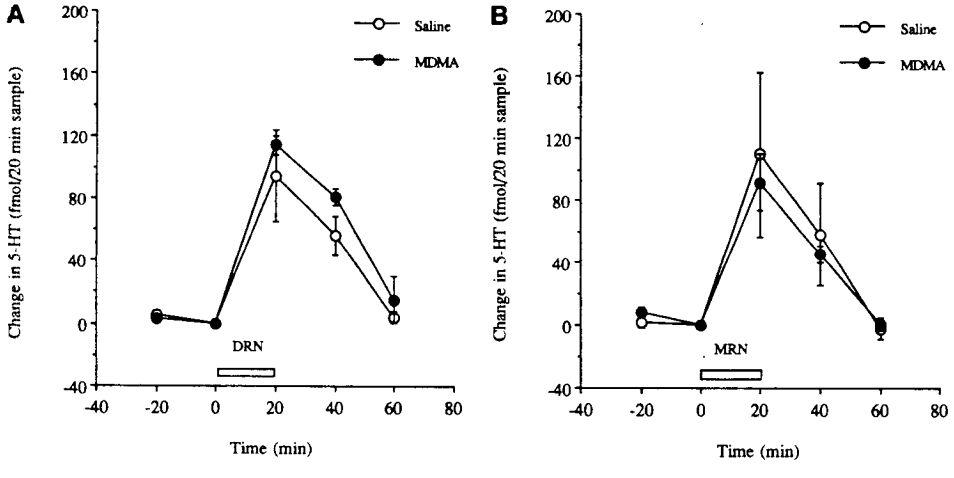

Fig. 4. Change in 5-HT in dialysates of the hippocampus in response to electrical stimulation of the DRN (A) and the MRN (B) in saline- and MDMA-treated animals. Electrical stimulations (20 min, 5 Hz) were applied as indicated by the bars. Data are mean ± S.E., n = 4–5 animals per group. See text for statistical analysis.

dopamine in rats with incomplete dopamine denervations (Zigmond *et al.*, 1990).

Whatever the mechanism which accounts for the apparent maintenance of 5-HT release in hippocampus of rats treated with MDMA, this mechanism may be lacking in the frontal cortex. 5-HT release in the frontal cortex might be more affected by MDMA if MDMA induced a relatively greater loss of 5-HT axons and terminals in this region. However, in rats treated with MDMA, [^{3}H]paroxetine binding and whole tissue 5-HT reportedly decrease to the same degree the frontal cortex and hippocampus (Commins *et al.*, 1987; Battaglia *et al.*, 1991). Certainly our present finding that 5-HIAA decreased by the same amount in the frontal cortex and hippocampus suggests that these regions are equally vulnerable to MDMA-induced neurotoxicity.

On the basis of immunohistochemical evidence, it has been suggested previously that MDMA and other neurotoxic amphetamines selectively lesion 5-HT axons originating from the DRN, leaving those of the MRN intact (Mamounas and Molliver, 1988; Mamounas *et al.*, 1991). The present finding that the 5-HT response in frontal cortex to DRN stimulation was attenuated in MDMA-treated rats, is in keeping with this hypothesis. However, the 5-HT response in the hippocampus to DRN stimulation remained intact. Furthermore, whereas the 5-HT response in the hippocampus to MRN stimulation was intact in MDMA-treated rats, it is diminished markedly in rats treated with *p*-chloroamphetamine (McQuade and Sharp, 1995). These inconsistencies not only question whether in functional terms the neurotoxic amphetamines are DRN-selective in their actions, but also emphasize the need to complement immunohistochemical and biochemical measures of 5-HT neuronal loss with *in vivo* measures of 5-HT function.

In conclusion, our data suggest that, in the rat, 2 weeks after exposure to a neurotoxic regimen of MDMA, the spontaneous electrical activity of 5-HT cell bodies (in the DRN) is normal. At the level of the 5-HT nerve terminal, basal 5-HT release appears unchanged in both the hippocampus and the frontal cortex. Furthermore, 5-HT release in the hippocampus is normal, even when 5-HT neurons are physiologically activated (by raphé stimulation), but under these conditions a deficit in 5-HT release in the frontal cortex is clearly apparent. In a recent behavioral study (Series *et al.*, 1995), we could not detect adaptive changes in postsynaptic 5-HT receptor function in MDMA-treated rats. Although MDMA causes marked 5-HT neurotoxicity, maintenance of 5-HT function, at least in some brain regions, may help explain the lack of disruption to behavior detected in earlier studies (see Introductory section). However, the effects of MDMA studied here were in the short-term and deficits in 5-HT neuronal function may be more marked in the long-term, and the behavioral consequences could be more severe.

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