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REPEATED ADMINISTRATION OF MDMA CAUSES TRANSIENT DOWN-REGULATION OF SEROTONIN 5-HT$_2$ RECEPTORS

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Summary—The present study examined short- and long-term effects of MDMA (3,4-methylenedioxymethamphetamine) on serotonin (5-HT$_2$ and 5-HT$_{1c}$) receptors in the brain of the rat. *N*1-Methyl-2-[^{125}I]lysergic acid diethylamide ([^{125}I]MIL) was used to label these receptors *in vitro* and *in vivo*. The usefulness of [^{125}I]MIL for *in vivo* detection of changes in 5-HT$_2$ receptors was confirmed in preliminary experiments in which rats were treated chronically with mianserin (5 mg/kg, once daily for 10 days). Decreases in specific *in vivo* binding of [^{125}I]MIL, after treatment with mianserin were found to be of the same magnitude as those determined by others, using *in vitro* methods. The MDMA (8 doses; 5–20 mg/kg each) was administered to rats over a period of 4 days. At various times after administration of the last dose of MDMA, the binding of [^{125}I]MIL was measured. Acutely, treatment with MDMA (20 mg/kg) reduced specific *in vivo* binding of [^{125}I]MIL in all regions of brain studied. For example, in the frontal cortex, specific binding of [^{125}I]MIL was decreased by 80% at 6 hr and by 62% at 24 hr, after cessation of treatment with MDMA. Twenty-one days after administration of MDMA however, the number of binding sites for [^{125}I]MIL was back to control levels. Reductions in *in vivo* binding of [^{125}I]MIL in frontal cortex were dependent on the dose of MDMA injected and were associated with decreases in the number of binding sites for [^{125}I]MIL (B_{max} values) in tissue homogenates of the same area. Autoradiographic studies of MDMA-treated rats confirmed the decreased density of 5-HT$_2$ receptors and also suggested that the 5-HT$_{1c}$ receptor of the choroid plexus was not affected. These results indicate that repeated administration of MDMA caused transient down-regulation of 5-HT$_2$ receptors in the brain of the rat. Further, they demonstrated that [^{125}I]MIL is a suitable radioligand for labeling 5-HT$_2$ receptors, both *in vitro* and *in vivo*. Once labeled with an appropriate radionuclide for SPECT (single photon emission computed tomography) or PET (positron emission tomography), MIL should prove useful for monitoring changes in the density of serotonin receptors in the living mammalian brain.

Key words—*N*1-methyl-2-[^{125}I]LSD, [^{125}I]MIL, *in vivo* binding, 3,4-methylenedioxymethamphetamine (MDMA), 5-HT$_2$ and 5-HT$_{1c}$ receptors.

The psychotropic, ring-substituted amphetamine, 3,4-methylenedioxymethamphetamine (MDMA) has been used illicitly for recreational purposes (Peroutka, 1987) and clinically as an adjunct to psychotherapy (Greer and Tolbert, 1990). It has been postulated that MDMA exerts its acute behavioral and physiological effects by releasing serotonin (5-hydroxytryptamine; 5-HT) (Nichols, Lloyd, Hoffman, Nichols and Yim, 1982; Schmidt, Wu and Lovenberg, 1986). Also, MDMA has been shown to be neurotoxic in several animal species, with long-term effects including severe decreases in the concentrations of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) and in the activity of tryptophan hydroxylase (TPH) (Commins, Vosmer, Virus, Woolverton, Schuster and Seiden, 1987; Johnson, Hanson and Gibb, 1988; Mokler, Robinson and Rosecrans, 1987; Schmidt, 1987; Stone, Merchant, Hanson and Gibb, 1987). In addition, repeated systemic administration of MDMA has been reported to cause long-term decrements in the number of uptake sites for 5-HT (Battaglia, Yeh and DeSouza, 1988; Scheffel and Ricaurte, 1990). Widespread damage of serotonergic axon terminals has been demonstrated in both rats and primates (O'Hearn, Battaglia, DeSouza, Kuhar and Molliver, 1988; Ricaurte, DeLanney, Irwin and Langston, 1988a; Ricaurte, Forno, Wilson, DeLanney, Irwin, Molliver and Langston, 1988b; Wilson, Ricaurte and Molliver, 1989). While the effects of treatment with MDMA on 5-HT nerve fibers and terminals have been studied extensively, little is known about its effects on post-synaptic serotonin receptors. The purpose of this study was to investigate the consequences of acute and chronic treatment with MDMA on central post-synaptic 5-HT$_2$ and 5-HT$_{1c}$ receptors in the brain of the rat, using the radioligand *N*1-methyl-2-[^{125}I]lysergic acid diethylamide ([^{125}I]MIL).

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METHODS

Chemicals

(±)Methylenedioxymethamphetamine hydrochloride and D-(+)-lysergic acid diethylamide bitartrate, were obtained from the National Institute of Drug Abuse, Research Technology Branch, Rockville, Maryland. Mianserin hydrochloride, spiperone, domperidone and ketanserin tartrate were purchased from Research Biochemicals Inc., Natick, Massachusetts.

Animals

Male Sprague–Dawley rats, weighing 180–230 g (Charles River, Wilmington, Massachusetts), were used. They were given free access to standard rat chow and water and housed in plastic cages in a temperature controlled room (22°C) on a 12 hr light/12 hr dark schedule.

Treatment with drugs

Acute treatment with MDMA. Groups of 4 rats were injected subcutaneously (s.c.) with (±)MDMA hydrochloride (20 mg/kg in 0.5 ml saline). Controls received 0.5 ml saline (s.c.).

Chronic treatment with MDMA. (±)MDMA hydrochloride (5, 10 or 20 mg/kg; in 0.5 ml saline) was administered subcutaneously to groups of 5–6 rats, 8 times (at 8:30 a.m. and 5:00 p.m. for 4 consecutive days). During treatment with MDMA, the animals were kept singly in standard plastic rat cages; 12 hr afterwards, they were transferred to their regular holding cages (3–4 animals per cage). Control rats were injected with 0.5 ml saline at the same times the experimental animals received MDMA.

Treatment with mianserin. Mianserin hydrochloride (5 mg/kg in 0.5 ml saline) was injected subcutaneously once daily for 10 days to 6 rats; controls received injections of saline (0.5 ml, s.c.).

Preparation of [^{125}I]MIL

The [^{125}I]MIL was prepared as previously described (Hoffman, Scheffel, Lever, Karpa and Hartig, 1987). The radioligand was isolated with >99% radiochemical purity by reverse phase HPLC in 40% over-all radiochemical yield. Standard samples of D-(+)-*N*1-methyl-2-Br-LSD (Lever, Dannals, Wilson, Ravert, Scheffel, Hoffman, Hartig, Wong and Wagner, 1989) were used for HPLC estimation of [^{125}I]MIL specific activity (1175–1668 mCi/μmol), using u.v. detection (254 nm). Specific activity, calculated by this method, agreed within 5–10% of the specific activity quoted for the batches of sodium [^{125}I]iodide used.

Receptor studies

In vivo binding of [^{125}I]MIL. The [^{125}I]MIL (approx 10 μCi) was injected intravenously through the tail vein into unanesthetized rats. For the time–activity study, the rats were killed by guillotine at 15, 30 and 60 min after the injection of trace. Rats treated with mianserin, received [^{125}I]MIL 4 days after the last dose of drug and were sacrificed 1 hr after [^{125}I]MIL. Rats treated with MDMA (20 mg/kg), were injected with [^{125}I]MIL at 6 hr, 1, 3, 7 or 21 days after the last injection of MDMA. Regional binding of [^{125}I]MIL in the CNS was determined 1 hr later. For the dose–response study (5–20 mg/kg MDMA), [^{125}I]MIL was injected 24 hr after the last injection of MDMA and 1 hr before sacrifice. Rats were killed by decapitation, the brains were removed, placed on ice and dissected as described elsewhere (Scheffel and Ricaurte, 1990). Samples of brain were weighed and counted for their [^{125}I]radioactivity, in an automated gamma scintillation counter (Packard, Downers Grove, Illinois). Aliquots of the [^{125}I]MIL injectate were counted along with the samples and served as standards. Specific regional accumulation of [^{125}I]MIL was calculated by subtracting concentrations of [^{125}I]MIL in the cerebellum (representing nonspecific binding) from total concentrations of [^{125}I] in each of the respective tissues.

The half-life of recovery of 5-HT$_2$ receptors in the frontal cortex was determined from percentage specific binding data for [^{125}I]MIL, and plotted against time (6 hr, 1, 3, and 7 days) after administration of the last dose of MDMA. A method described by Mauger, Sladeczek and Bockaert (1982) was employed to calculate the $T_{1/2}$.

In vitro binding of [^{125}I]MIL. Studies of the binding of [^{125}I]MIL were performed using the method described by Hoffman *et al.* (1987) with minor modifications. Control rats (injections of saline) and rats treated with MDMA, were killed by guillotine 6 and 24 hr after the last dose of MDMA was administered. Pre-frontal cortices were dissected on ice, pooled, placed immediately into 20 volumes of ice-cold 50 mM Tris buffer (pH 7.4 at 37°C) and homogenized for 15 sec (Brinkmann Polytron, setting 6). The homogenates were centrifuged for 10 min at 50,000 *g* and 2–4°C, the supernatants were then discarded, the tissue pellets resuspended in fresh buffer and the homogenates recentrifuged. The wash-procedure was repeated a total of 3 times to remove potential traces of residual MDMA from the homogenates, as efficiently as possible. After the final resuspension, 40 mg aliquots of the homogenate (in a volume of 1.5 ml) were stored at −70°C until the day of the assay.

For the receptor assay, the tissue concentrate was diluted to 6 mg/ml and homogenized once more by Polytron (15 sec, setting 6). Duplicate samples of twelve decreasing concentrations of [^{125}I]MIL (0.7–0.01 nM), in 50 mM Tris buffer containing 0.01% bovine serum albumin (BSA), were incubated in a final volume of 0.15 ml with 0.3 mg pre-frontal cortex. Nonspecific binding was determined in the presence of 1 μM ketanserin. The incubation was carried out for 20 min at 37°C. The tissue samples were then filtered through Whatman GF/B filters, pretreated with 3% polyethyleneimine, using a

Brandel automatic harvester and washed three times with ice-cold Tris buffer. The radioactivity on the filters was counted in an automated gamma scintillation counter. Scatchard analysis of the binding of [^{125}I]MIL was carried out by using the computer program "LIGAND" (McPherson, 1985). The protein content was determined using a commercially available BCA protein assay kit (Pierce, Rockford, Illinois) and BSA as standard. Inhibition of the binding of [^{125}I]MIL by MDMA (K_i value) was determined in 2 assays by incubating triplicate samples of tissue homogenates and [^{125}I]MIL (0.35 nM) with 12 different concentrations of MDMA, ranging from 10^{-4} to 10^{-11} M. Filtration and counting of the samples were performed as described above. The results of this study are presented in the text.

Autoradiographic assays. The topographic distribution of binding of [^{125}I]MIL to 5-HT$_2$ and 5-HT$_{1c}$ receptors was assessed by film autoradiography, using minor modifications of previously reported procedures (Kuhar and Unnerstall, 1990; Blue, Yagaloff, Mamounas, Hartig and Molliver, 1988; Hoffman *et al.*, 1987). Briefly, the brains of control and MDMA-treated rats were removed from the skulls, rapidly frozen in isopentane at −30°C and stored for less than 48 hr at −78°C. After equilibration to −20°C, coronal sections of 20 μm thickness were prepared with a microtome cryostat (Hacker Bright Model OTF/AS), thaw-mounted onto subbed microscope slides and then stored for 2 days at −20°C. The tissue sections were incubated for 60 min at room temperature with 0.15 nM [^{125}I]MIL in 50 mM Tris buffer, containing 0.02% BSA and 50 nM domperidone (pH 7.5). Domperidone was used to preclude any secondary binding of the radioligand to dopamine D$_2$ receptors. Nonspecific binding was defined by incubation of adjacent sections in a bath which was identical, except for the inclusion of 500 nM ketanserin tartrate to block 5-HT$_2$ receptors and the majority of 5-HT$_{1c}$ receptors. Sections were washed repeatedly with ice-cold Tris buffer and then dipped rapidly in ice-cold distilled water. After drying, the slides were apposed, along with [^{125}I]-Microscale standards, to Amersham Hyperfilm-^{3}H in X-ray cassettes. After 18 hr the film was developed and fixed. The optical densities of the film in various regions of tissue were converted to radioactivity concentrations in tissue using the standards for radioactivity per mg tissue, the specific activity of the radioligand and computer assisted image analysis (MCID Image Analyzer System, Imaging Research, Inc.). Film exposure times were chosen such that the optical densities spanned less than 0.8 optic density units and were bracketed by the values of the microscale standards.

Statistics

The data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's *post-hoc* tests for comparing control to treatment means. Differences were considered significant when $P < 0.05$.

RESULTS

In vivo labeling of 5-HT$_2$ receptors in brain of rat with [^{125}I]MIL

As in the mouse (Hoffman *et al.*, 1987), [^{125}I]MIL labeled central 5-HT$_2$ receptors in the rat. After

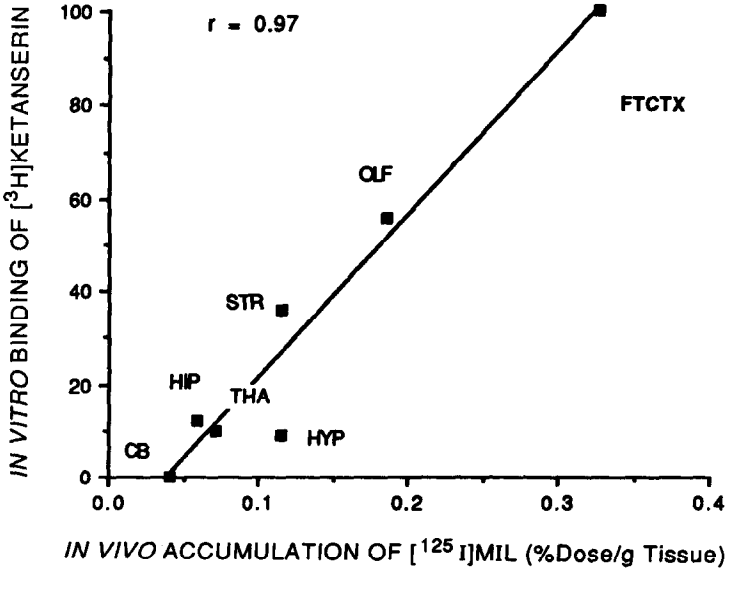

Fig. 1. Correlation ($r = 0.97$) of *in vivo* accumulation of [^{125}I]MIL (% injected dose/g tissue) in different regions of the brain of the rat at 1 hr after intravenous injection of the radioligand and *in vitro* specific binding of [^{3}H]ketanserin (from Leysen, 1985) to 5-HT$_2$ receptors, in the same regions (binding to pre-frontal cortex is set at 100%). CB = cerebellum, HIP = hippocampus, THA = thalamus, STR = striatum, HYP = hypothalamus, OLF = olfactory tubercles, FTCTX = pre-frontal cortex.

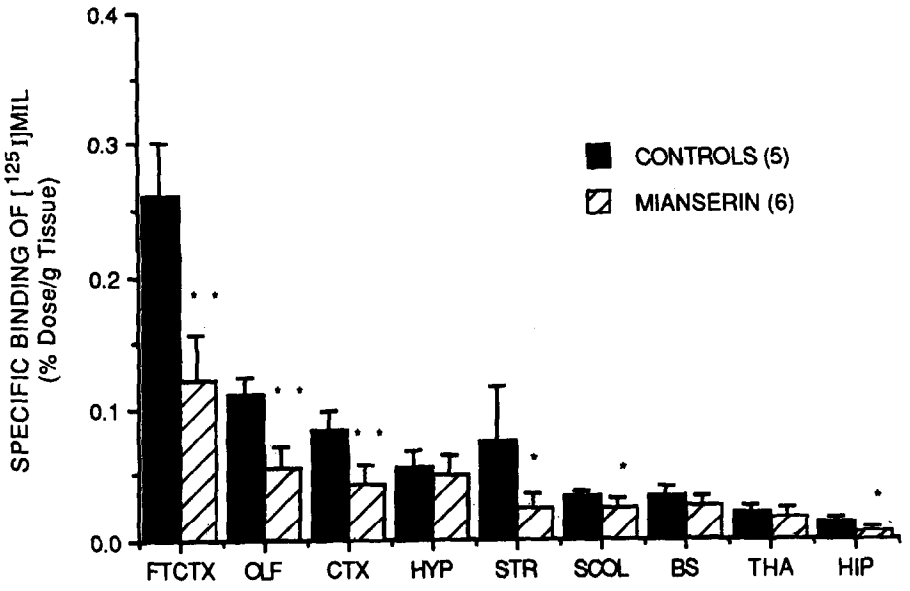

Fig. 2. Reduction of specific *in vivo* binding of [^{125}I]MIL in different regions of the brain of the rat 4 days after treatment of the animals with mianserin (5 mg/kg, s.c., once daily for 10 days). Specific binding = total minus nonspecific binding (= % injected dose (I.D.)/g tissue minus % I.D./g cerebellum). Data are presented as means ± 1 SD. FTCTX = pre-frontal cortex, OLF = olfactory tubercles, CTX = rest of the cerebellar cortex, HYP = hypothalamus, STR = striatum, SCOL = superior colliculi, BS = brain stem, THA = thalamus, HIP = hippocampus. Comparisons were made by ANOVA, followed by two-sided Dunnett's test; significantly different from controls: $^{**}P < 0.01$; $^{*}P < 0.05$.

intravenous injection of [^{125}I]MIL, the greatest accumulation occurred in the pre-frontal cortex and smallest in the cerebellum. One hour after intravenous injection, the ratio of pre-frontal cortex to cerebellum reached 8.0 ± 1.2 (mean ± SD; $n = 3$). At that time, an excellent correlation ($r = 0.97$) was noted between the regional distribution of concentration of [^{125}I] in the 7 regions studied and the distribution of binding sites for [^{3}H]ketanserin, as determined by an *in vitro* assay (Leysen, 1985) (Fig. 1). Blocking studies, carried out by pre-injecting ketanserin (5 mg/kg) intravenously, 5 min before intravenous administration of the radioligand, showed >95% inhibition of specific *in vivo* [^{125}I]MIL binding

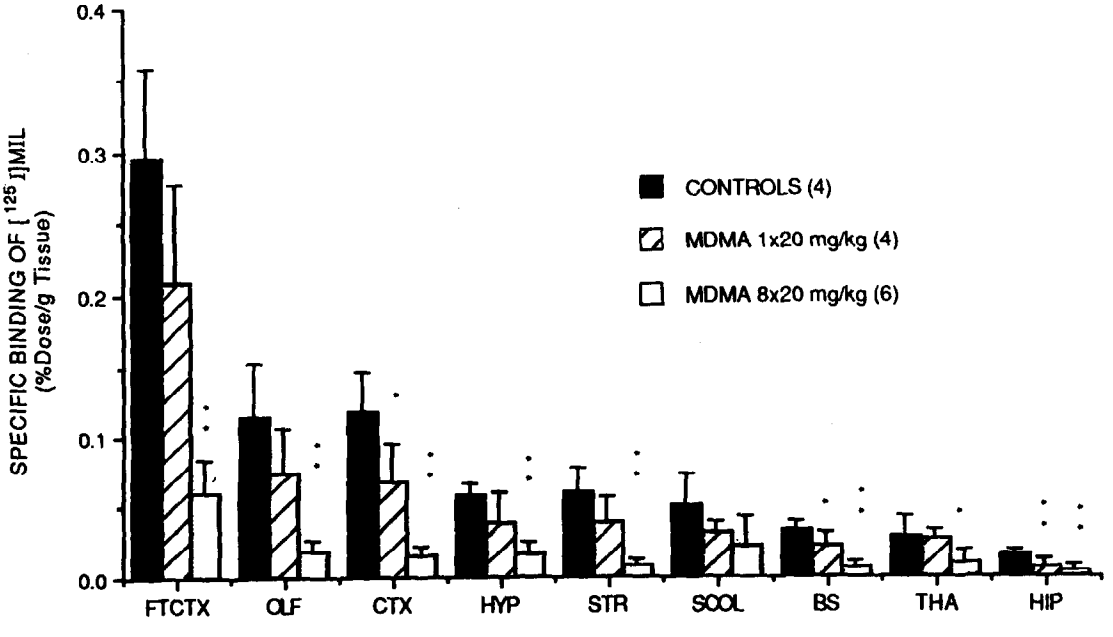

Fig. 3(A) *Caption on facing page.*

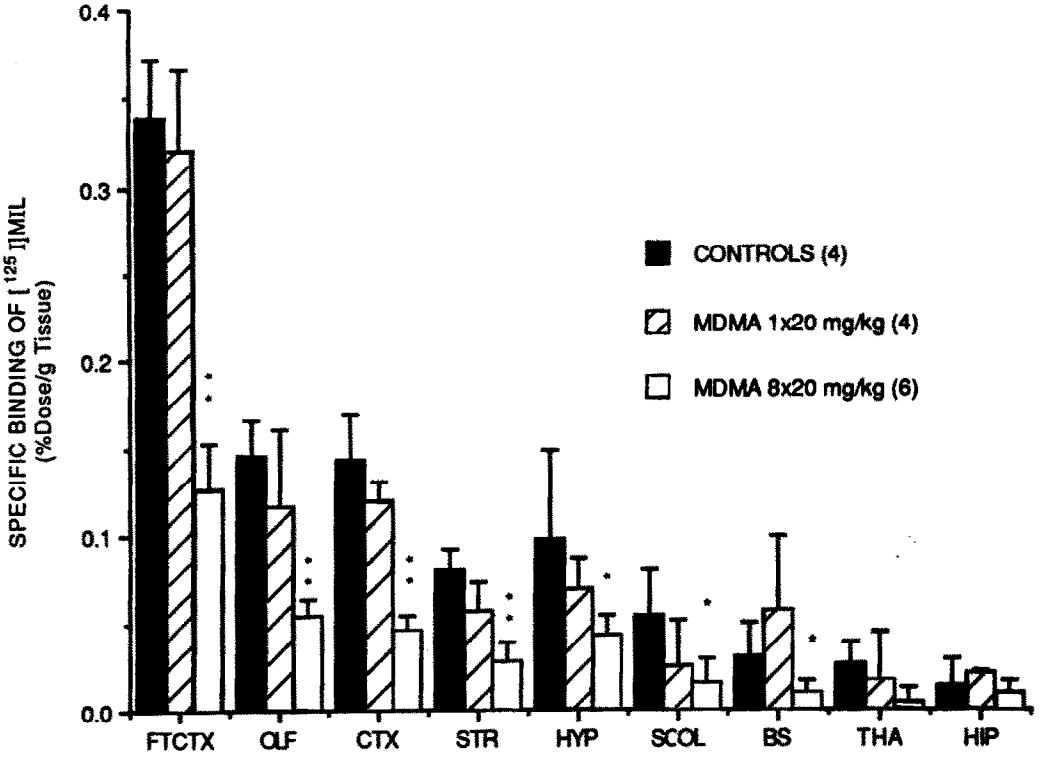

Fig. 3(B)

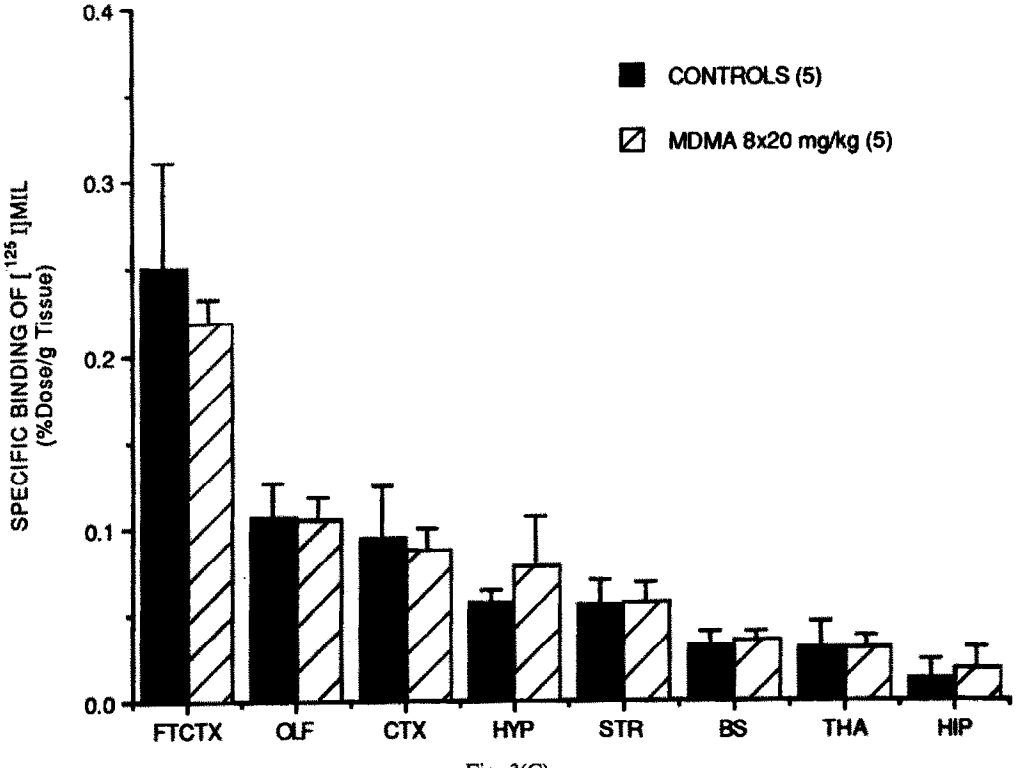

Fig. 3(C)

Fig. 3. (A) Effects of acute and chronic treatment with MDMA on specific *in vivo* binding of [^{125}I]MIL in the brain of the rat, 6 hr after administration of the last dose of MDMA. Data are means ± 1 SD. Regions of the brain are the same as in Fig. 1. Statistical analysis as in Fig. 2: $^{**}P < 0.01$; $^{*}P < 0.05$. (B) Effects of acute and chronic treatment with MDMA on specific *in vivo* binding of [^{125}I]MIL in the brain of the rat 24 hr after administration of the last dose of MDMA. (C) Effect of treatment with MDMA (8 times, 20 mg/kg, administered over 4 consecutive days) on specific *in vivo* binding of [^{125}I]MIL in the brain of the rat, 7 days after administration of the last dose of MDMA.

in all regions examined (data not shown). In contrast, nonspecific (cerebellar) binding of [^{125}I]MIL was not affected by ketanserin.

In vivo binding of [^{125}I]MIL after chronic treatment with mianserin

In order to validate the measurement of specific *in vivo* binding of [^{125}I]MIL for determining changes in the number of 5-HT$_2$ receptors, rats were treated with the antagonist mianserin under a protocol shown to be effective in down-regulating the receptor to a significant degree (Sanders-Bush, Breeding and Roznoski, 1987). Chronic treatment of rats with mianserin resulted in significant decreases in *in vivo* binding of [^{125}I]MIL in regions rich in 5-HT$_2$ receptors indicating down-regulation of 5-HT$_2$ receptors. Rats treated chronically with mianserin were injected intravenously with [^{125}I]MIL, 4 days after the last dose of drug was administered. As seen in Fig. 2, the specific binding of [^{125}I]MIL was significantly reduced in the pre-frontal cortex (−53%), the cortex (−49%), in the olfactory tubercles (−51%) and in the striatum (−68%). Smaller reductions were observed in the brain stem (−21%) and the superior colliculi (−30%), whereas concentrations of activity in the hypothalamus and thalamus remained unaffected by the treatment with drug. Decreases in *in vivo* specific binding of [^{125}I]MIL in the pre-frontal cortex, after chronic treatment with mianserin were of the same magnitude as those observed by Sanders-Bush *et al.* (1987) in *in vitro* assays (−52% in frontal cortex of rat).

In vivo binding of [^{125}I]MIL after acute treatment with MDMA

A single dose of MDMA (20 mg/kg) had no effect on specific *in vivo* binding of [^{125}I]MIL to 5-HT$_2$ receptors (Figs 3A and B). Although concentrations of [^{125}I]MIL were not statistically different from controls, there was a tendency for reduced binding of [^{125}I]MIL to be noticeable 6 hr after administration of MDMA (Fig. 3A).

In vivo binding of [^{125}I]MIL after chronic treatment with MDMA

Chronically-administered MDMA (20 mg/kg, injected twice a day for 4 days), had significant effects on 5-HT$_2$ receptor binding sites. Decreases in specific binding of [^{125}I]MIL ranged between 70–90% at 6 hr (Fig. 3A) and between 55–80% at 24 hr after cessation of treatment with MDMA (Fig. 3B). At 7 days (Fig. 3C) and at 21 days (data not shown), after chronic treatment with MDMA was discontinued, no significant changes from controls in specific accumulation of [^{125}I]MIL could be observed in any of the regions of the brain.

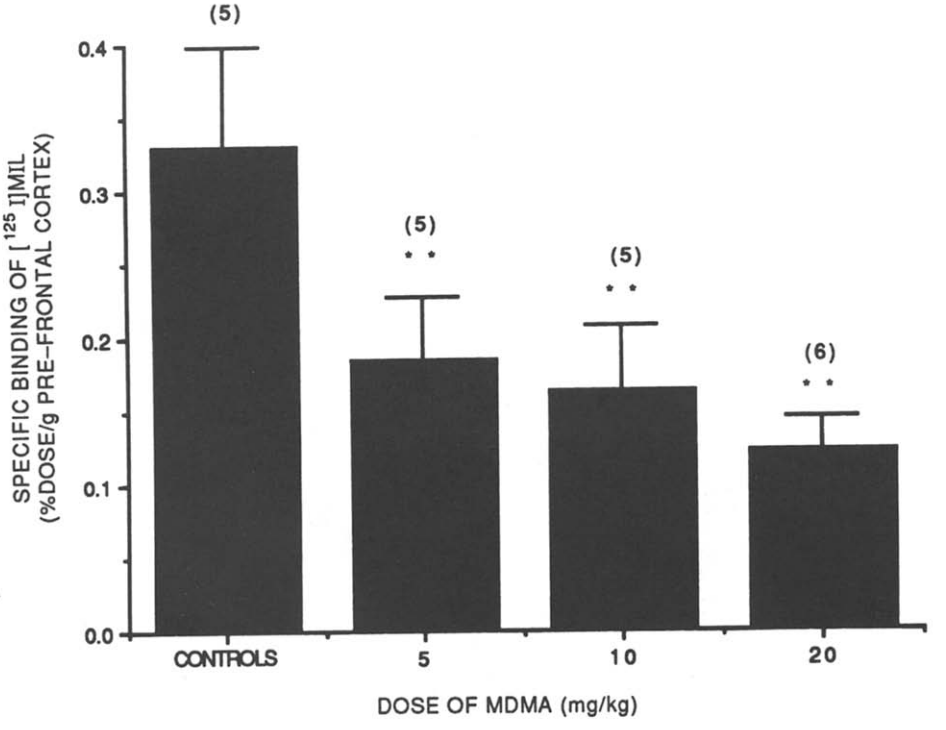

Fig. 4. Effect of dose of MDMA on specific *in vivo* binding of [^{125}I]MIL in the pre-frontal cortex. Rats were treated with either MDMA (5, 10 or 20 mg/kg doses) or saline (controls), twice daily for 4 consecutive days. The [^{125}I]MIL was injected 24 hr after cessation of treatment with MDMA and distribution of [^{125}I]MIL determined after sacrifice of the animals 1 hr later. (n) = Number of animals, statistical analysis as in Fig. 2. Although there was a dose-dependent decline in mean binding of [^{125}I]MIL in the pre-frontal cortex, the differences in binding between the three dose levels of MDMA were statistically not significant.

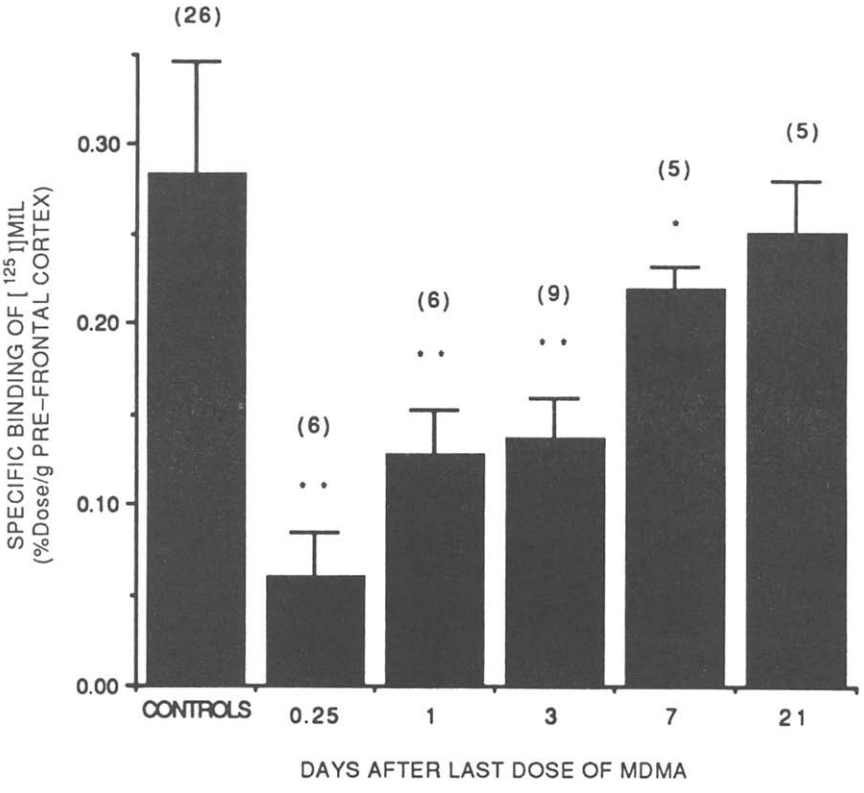

Fig. 5. Time-course of recovery of binding of [^{125}I]MIL in pre-frontal cortex of rat, measured *in vivo* by following accumulation of [^{125}I]MIL in this region after intravenous injection of the radioligand at different times (6 hr to 21 days) after cessation of chronic treatment with MDMA. (n) = Number of animals. Data of specific binding of [^{125}I]MIL in pre-frontal cortex at 0.25, 1 and 7 days are taken from Fig. 3 (A, B and C).

In vivo binding of [^{125}I]MIL after chronic administration of different doses of MDMA

As shown in Fig. 4, decreases in specific binding of [^{125}I]MIL were dependent on the dose of MDMA administered. At 24 hr after cessation of treatment with MDMA (8 doses over a 4-day period), specific binding of [^{125}I]MIL in the prefrontal cortex was reduced by 44.1, 50.8 and 63.1%, resulting from treatment with doses of MDMA of 5, 10 or 20 mg/kg, respectively.

Half-life of recovery of 5-HT$_2$ binding sites

After treatment with MDMA was discontinued, recovery of 5-HT$_2$ binding sites was noted. The time-dependent changes in specific accumulation of [^{125}I]MIL, observed in the pre-frontal cortex after cessation of chronic application of MDMA (8 × 20 mg/kg MDMA, administered over a 4-day period) are shown in Fig. 5. The binding of [^{125}I]MIL was significantly decreased up to three days; however, at 7 days, the decrease was less than 25% and by 21 days, binding of [^{125}I]MIL was back to control levels. From these data, a half life ($T_{\frac{1}{2}}$) of approximately 4 days was calculated for recovery of the 5-HT$_2$ receptors.

In vitro binding of [^{125}I]MIL after treatment with MDMA

The maximum number of binding sites (B_{max} values) of the binding of [^{125}I]MIL to 5-HT$_2$ receptors in pre-frontal cortex was significantly reduced 6 and 24 hr after chronic treatment with MDMA (8 doses of 20 mg/kg, administered over 4 days) was discontinued (Table 1). *In vitro* binding of [^{125}I]MIL to tissue homogenates of frontal cortex from control and MDMA-treated rats was studied for comparison with

Table 1. *In vitro* binding of [^{125}I]MIL to 5-HT$_2$ receptors in pre-frontal cortex of rat

	(N)	K_D (nm)	B_{max} (fmol/mg protein)
Controls	(8)	0.64 ± 0.04	617 ± 41
MDMA (6 hr)	(6)	0.74 ± 0.05	217 ± 9**
MDMA (24 hr)	(8)	0.62 ± 0.06	333 ± 18**

(N) = Number of determinations. Data are presented as means ± SEM.

**Significantly different from controls ($P < 0.001$).

Dissociation constants (K_D's) and maximum number of binding sites (B_{max} values) of [^{125}I]MIL binding to 5-HT$_2$ receptors in frontal cortex of saline injected control rats and rats treated with MDMA (8 × 20 mg/kg over a 4 day period). Animals were sacrificed either 6 or 24 hr after the last MDMA dose was administered.

the previously measured *in vivo* binding of [^{125}I]MIL. Six and 24 hr after the last dose of MDMA was administered, the binding affinity constants (K_D = 0.64 nM) were not significantly different from controls. The number of 5-HT$_2$ receptors, however, was reduced by 65 and 46% (i.e. from 617 to 217 and 333 fmol/mg protein), respectively.

To investigate the possibility that decreases in *in vitro* binding of [^{125}I]MIL were the result of competition of residual MDMA with [^{125}I]MIL for binding to the 5-HT$_2$ receptor sites, (±)MDMA was added to homogenates of the pre-frontal cortex of the rat and incubated with the tissue for 30 min at 37°C. The tissue homogenates were then washed and binding of [^{125}I]MIL studied, as described in Methods. Incubation of 10^{-7} M (±)MDMA with the homogenates did not lead to significant changes in either K_D or B_{max}; even relatively large concentrations of MDMA (10^{-5} M) did not decrease the binding of [^{125}I]MIL in *in vitro* assays (data not shown).

Autoradiographic studies with [^{125}I]MIL

Down-regulation of binding sites for [^{125}I]MIL after chronic treatment with MDMA in eight 20 mg/kg doses, was also shown using quantitative autoradiography (Fig. 6). In the control brain of rat (Fig. 6A), [^{125}I]MIL labeled 5-HT$_2$ sites in cortex, striatum and claustrum. The 5-HT$_{1c}$ sites of the choroid plexus were labeled, as previously shown (Yagaloff, Lozano, Van Dyke, Levine and Hartig, 1986; Blue *et al.*, 1988; Hoffman *et al.*, 1987). Binding of [^{125}I]MIL to these sites was displaceable by ketanserin (Fig. 6B). Labeling of 5-HT$_2$, but not 5-HT$_{1c}$ sites, was prevented by incubation in the presence of 200 nM spiperone (data not shown). Treatment with MDMA significantly reduced the binding of [^{125}I]MIL in all regions, except in the 5-HT$_{1c}$ rich choroid plexus (Fig. 6C), although nonspecific binding in this and all other regions was equivalent to the control (Fig. 6D).

Quantitative evaluation of the autoradiographic data confirmed the previous results. Total binding of [^{125}I]MIL was significantly reduced by treatment with MDMA. Decreases in binding were observed in the claustrum, as well as in striatal and cortical areas ($P < 0.01$), but not in the choroid plexus ($P > 0.05$), at 6 hr after cessation of treatment with MDMA. Nonspecific binding of [^{125}I]MIL was low in all areas, ranging from 10–15% of total binding and was unaffected by the treatment with drug. Similarly, specific radioligand binding as assessed by subtracting nonspecific from total binding, in paired adjacent sections (Fig. 7), was decreased by 67% in the frontal cortex, by 50% in the parietal cortex somatosensory

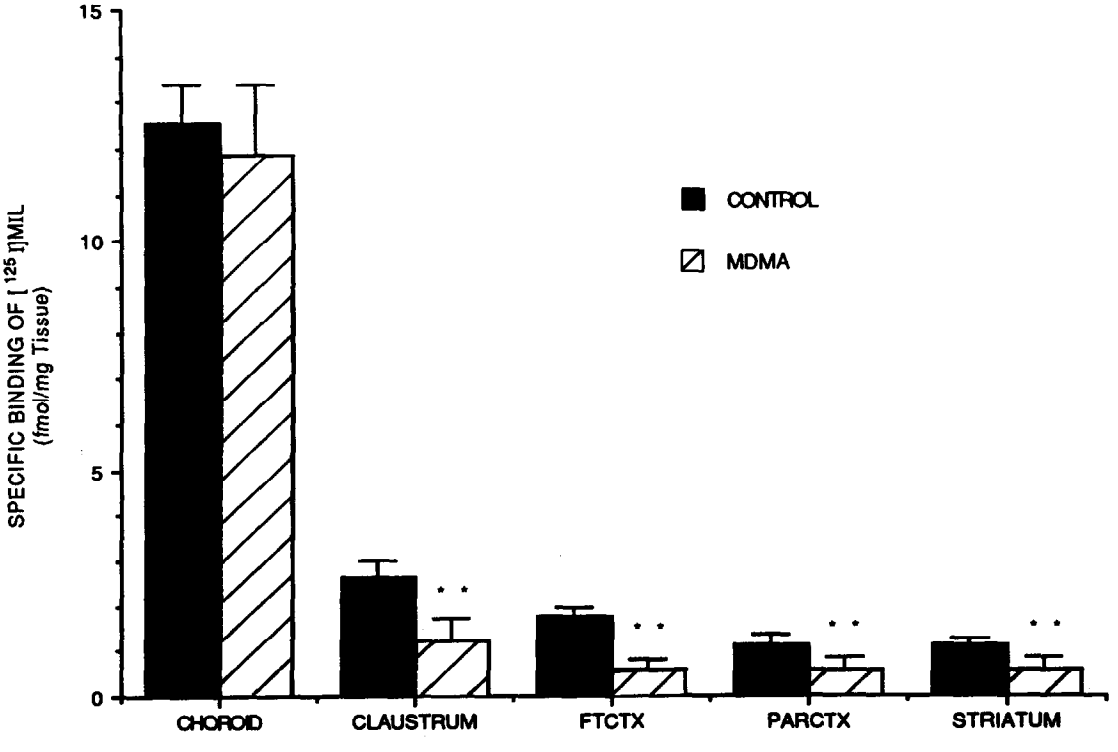

Fig. 7. Specific binding of [^{125}I]MIL in selected regions of brain from control and MDMA-treated rats, measured by quantitative autoradiography in sections similar to those shown in Fig. 6. Values are means ± 1 SD for specific binding of [^{125}I]MIL, calculated as the difference between total and nonspecific binding in 5–7 pairs of adjacent coronal sections. Statistics as in Fig. 2; $^{**}P < 0.01$. CHOROID = choroid plexus, FTCTX = pre-frontal cortex, PARCTX = parietal cortex.

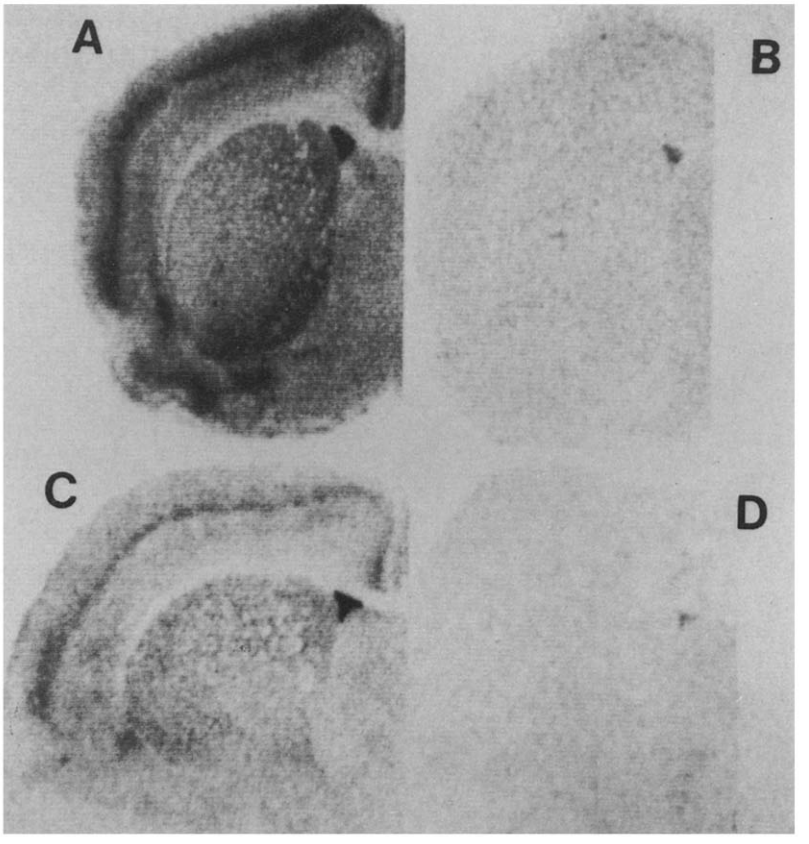

Fig. 6. Autoradiographic localization of binding of [^{125}I]MIL in brain of a control rat (A and B) and brain from a MDMA-treated animal, 6 hr after cessation of chronic administration of MDMA (C and D). Coronal sections were taken approximately 9.0 mm from the interaural plane, according to Paxinos and Watson (1986). (A) and (C) Are images of total binding, in slices incubated with 0.15 nM [^{125}I]MIL and 50 nM domperidone. (B) and (D) Represent nonspecific binding, as defined by binding of [^{125}I]MIL (0.15 nM) in the presence of 500 nM ketanserin.

area, by 49% in the striatum, and by 54% in the claustrum ($P < 0.01$ for all 4 areas).

Although the greatest concentrations of bound [^{125}I]MIL were found in the choroid plexus, the decrease in specific binding in this region amounted to only 5% ($P > 0.05$), suggesting little or no effect of MDMA on binding of [^{125}I]MIL to 5-HT$_{1c}$ receptors.

DISCUSSION

The results of this investigation indicated that chronic treatment with MDMA (8 × 20 mg/kg over 4 days) caused a transient reduction in post-synaptic 5-HT$_2$ receptors in the CNS of the rat. *In vivo* data showed specific accumulation of [^{125}I]MIL to be significantly reduced in all areas of the brain of the rat (Figs 3A and B). The reductions were greatest 6 hr after the last dose of MDMA was administered. Recovery of [^{125}I]MIL receptor binding sites occurred over a period of 1 week. The half-life of recovery of binding sites for [^{125}I]MIL in the pre-frontal cortex was estimated to be 4 days, which was very similar to the half-life of receptor regeneration of between 3.0 and 5.5 days, calculated by Leysen, Janssen and Niemegeers (1989) on the basis of *ex vivo* binding assays with [^{3}H]ketanserin.

Decreases in *in vivo* accumulation of [^{125}I]MIL in the pre-frontal cortex of the rat, following chronic treatment with MDMA were dependent on the dose of MDMA administered (0–20 mg/kg) and correlated well with reductions in B_{max} values of binding of [^{125}I]MIL, determined *in vitro*. Six and 24 hr after the last dose of MDMA was administered, the *in vivo* specific binding of [^{125}I]MIL was reduced by 80 and 62%, respectively, whereas the maximum number of binding sites (B_{max}), at these times was reduced by 65 and 46%, respectively. Similarly, an *ex vivo* autoradiographic study, performed 6 hr after cessation of treatment with MDMA, showed a 67% reduction in specific binding of [^{125}I]MIL in the frontal cortical area of the brain of the rat. These findings demonstrated that the ligand MIL could be successfully used to quantitatively measure changes in serotonin receptors *in vivo*.

Further, the results showed that effects of MDMA on post-synaptic serotonin receptors could only be measured for a limited period of time after cessation of treatment with drug. Investigations on the effects of another serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), have repeatedly shown that this agent does not elicit changes in the density of 5-HT$_2$ receptors (Barbaccia, Gandolfi, Chuang De-Maw and Costa, 1983; Conn and Sanders-Bush, 1986; Eison, Eison, Yocca and Gianutsos, 1989; Fischette, Nock and Renner, 1987; Stockmeier and Kellar, 1986). However, 5-HT$_2$ receptors were generally measured 1 week or later, after injection of 5,7-DHT. Other lesioning studies on 5-HT neurons yielded similar results, with one exception (Frazer, Offord and Lucki, 1988). In that study, the density of 5-HT$_2$ receptors was measured relatively early, i.e. 4 days after bisection of the frontal cortex (Leysen, Geerts, Gommeren, Verwimp and Van Gompel, 1982). Thus, the time of observation appears to be of critical importance. Since 5-HT$_2$ receptor binding recovers fairly rapidly, it seems reasonable to expect that long-term changes in the density of 5-HT$_2$ receptors after lesioning of 5-HT neurons are absent, but short-term changes, not investigated so far, may still be present.

The mechanism by which chronic treatment with MDMA caused transient down-regulation of 5-HT$_2$ receptors can only be speculated upon. Although the kinetics and concentrations of MDMA in the brain of the rat are unknown, direct competition of the drug for binding at the post-synaptic receptor is unlikely, since (±)MDMA possesses a very low affinity for this site (Lyon, Glennon and Titeler, 1986). *In vitro*, (±)MDMA inhibited binding of [^{125}I]MIL at the micromolar level ($K_i = 5.3\ \mu$M; as determined in this study). Generally, MDMA is thought to act directly on the pre-synaptic serotonin nerve terminal and not on post-synaptic sites, since its neurotoxic damage can be prevented by pre-administration of specific blockers of the uptake of serotonin, such as citalopram or fluoxetine but not by 5-HT$_2$ receptor agents, such as mianserin (Sanders-Bush *et al.*, 1987). The decrease in 5-HT$_2$ receptors may, however, be the result of secondary action by MDMA, i.e. the release of 5-HT into the synaptic cleft. Similar to MDMA, fenfluramine, another potent neurotoxin with a 5-HT-releasing action, has also been found to cause reductions in 5-HT$_2$ receptors (Koshikawa, Koshikawa and Stephenson, 1985). This observation was made 3 hr after injection of the drug, again underscoring the importance of early measurement of the transient phenomenon of down-regulation of 5-HT$_2$ receptors.

The radioligand [^{125}I]MIL was shown to be an excellent agent for labeling post-synaptic serotonin receptors, both *in vivo* and *in vitro*. Previous studies with [^{125}I]MIL (Blue *et al.*, 1988; Hoffman *et al.*, 1987) and with [^{125}I]LSD (Yagaloff *et al.*, 1986) have shown that these radioligands selectively label 5-HT$_2$ receptors, without significant labeling of 5-HT$_{1c}$ sites in striatal and cortical regions. However, these radioiodinated ergots do bind with high affinity to the 5-HT$_{1c}$ receptor, which apparently is expressed to the exclusion of the 5-HT$_2$ site in the choroid plexus. Since *in vitro* studies in homogenates of frontal cortex have shown that only a small portion (< 10%) of the binding of [^{125}I]MIL in this region is to 5-HT$_{1c}$ receptors, such cross-reactivity is not problematic for the homogenate assays, discussed here. In the brain of the rat, 5-HT$_{1c}$ receptors are reported to be widespread (Molineaux, Jessell, Axel and Julius, 1989) but the proportion of 5-HT$_{1c}$ receptors is generally assumed to be small, compared to that of 5-HT$_2$ sites, for areas other than choroid plexus. Since the exact proportion of densities of 5-HT$_2$ to 5-HT$_{1c}$ receptors is

not known with certainty, the possibility cannot be excluded of co-labeling 5-HT_2 and 5-HT_{1c} receptors with [^{125}I]MIL in certain regions of the brain of the rat, examined *in vivo* during the course of the present study.

The autoradiographic studies presented here, confirmed the significant down-regulation of cortical 5-HT_2 sites, observed *in vitro* and *in vivo*. The data suggest that the 5-HT_{1c} receptors of the choroid plexus are only minimally influenced by chronic treatment with MDMA. The reasons for this lack of responsiveness are not clear. However, if serotonin released by MDMA causes down-regulation of receptors, the possibility exists that elevated levels of serotonin, generated near the choroid plexus receptor, may not be as effective as at other sites because of its more rapid removal through the ventricle.

In summary, the results of this investigation indicate that chronic treatment with MDMA causes down-regulation of 5-HT_2 receptors in the brain of the rat but has little or no effect on the 5-HT_{1c} receptors of the choroid plexus. Since [^{125}I]MIL can be successfully employed to measure changes in 5-HT_2 receptors *in vivo*, appropriately radiolabeled versions of MIL or close analogs, should be useful for detecting alterations in densities of serotonin receptors in living brain, by tomographic imaging. In this regard, one analog of MIL, *N*1-([^{11}C]-methyl)-2-Br-LSD has recently been synthesized (Lever *et al.*, 1989) and used for mapping densities of 5-HT_2 receptor in normal human brain (Wong, Lever, Hartig, Dannals, Villemagne, Hoffman, Wilson, Ravert, Links, Scheffel and Wagner, 1987). In addition, it has recently been determined that another analog, *N*1-ethyl-2-I-LSD (EIL), labels serotonin receptors *in vivo* with high specificity and is suitable for SPECT studies of serotonin receptors, when radiolabeled with iodine-123 (Lever, Scheffel, Musachio, Stathis and Wagner, 1991).

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