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ORIGINAL RESEARCH ARTICLE

(+) 3,4-Methylenedioxymethamphetamine ('Ecstasy') transiently increases striatal 5-HT_{1B} binding sites without altering 5-HT_{1B} mRNA in rat brain

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(+) 3,4-Methylenedioxymethamphetamine (MDMA) is a psychedelic drug of abuse that causes selective degeneration of serotonergic fibers of dorsal raphe neurons that project throughout the forebrain. Previous studies using pharmacological and behavioral approaches suggested that MDMA treatment leads to desensitization of 5-HT_{1B} receptors. We proposed to test whether this occurs by downregulation of 5-HT_{1B} messenger RNA in dorsal raphe, striatum or CA1 hippocampal neurons and/or 5-HT_{1B} binding site density in hippocampus and basal ganglia. In Experiment I, rats were treated with MDMA using several dosing protocols (2.5 or 10 mg kg^{-1} day^{-1} s.c. given a single time or twice daily for 4 days). The animals were killed 24 h after the last dose. [^{3}H]-citalopram binding to serotonin transporters in hippocampus was reduced in the high dose protocol, indicating degeneration of forebrain serotonergic fibers. Despite the extensive reduction in serotonergic content, 5-HT_{1B} mRNA did not change from control levels in any region when measured by *in situ* hybridization. [^{125}I]-Iodocyanopindolol binding to 5-HT_{1B} sites in hippocampus was also not changed. In Experiment II, high dose MDMA had no effect on 5-HT_{1B} mRNA in any brain region either 1 or 14 days after treatment. However, [^{125}I]-iodocyanopindolol binding more than doubled in striatum 1 day after MDMA treatment but returned to control levels by 14 days. This may have been a transient compensation to early neuronal damage caused by MDMA exposure. These results suggest that previously described changes in 5-HT_{1B} function following MDMA treatment involve only posttranscriptional changes in receptor regulation and do not alter 5-HT_{1B} mRNA levels.

Keywords: MDMA; neurotoxicity; *in situ* hybridization; iodocyanopindolol; citalopram; serotonin; striatum

Introduction

MDMA is a psychedelic drug that is widely abused in the United States and Europe despite its association with neurotoxicity, cardiovascular toxicity, and chronic psychiatric illness.[1] In the brain, MDMA appears to be toxic to a subset of serotonin terminals termed fine fibers (primarily emanating from the dorsal raphe nucleus) while the other beaded fibers (primarily from median the raphe nucleus) are left intact.[2] The mechanism for this neurotoxicity is not known; however, it appears that MDMA enters serotonergic axon terminals via the serotonin transporter inducing an acute, excessive release of serotonin in both types of fibers.[3,4] There are a more complex set of intracellular toxic events which may involve active metabolites of MDMA.[1] At sufficiently high doses, long-term degeneration of fine serotonergic processes can be seen within a few days in both rodents and nonhuman primates with doses for nonhuman primates approaching those of human recreational use.[5,6] In rats there is some degree of axonal regrowth evident after 4 months with substantial recovery in most cases within 1 year.[7,8] Recovery in nonhuman primates, however, appears to occur to a much lesser degree if at all.[9,10]

Geyer and collaborators have shown that the acute release of serotonin caused by MDMA induces motor hyperactivity via postsynaptic 5-HT_{1B} receptors.[11] They also found functional desensitization of 5-HT_{1B} mediated motor behavior following MDMA treatment of rats using drug treatment and cross tolerance approaches.[12] These results suggested that some of the psychotropic and behavioral effects of MDMA may be mediated by altered 5-HT_{1B} receptor function, but the particular neurons expressing these 5-HT_{1B} receptors was not clear from these experiments. In addition, 5-HT_{1B} receptor knockout mice show increased cocaine and ethanol self-administration,[13,14] suggesting that this receptor participates in some manner in the reinforcing or aversive properties of drugs of abuse. Thus, it is also possible that 5-HT_{1B} receptors mediate both acute and chronic effects of ecstasy in addition to motor hyperactivity. A closer evaluation of the effect of MDMA on 5-HT_{1B} receptor regulation seems indicated.

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Throughout the forebrain, the 5-HT$_{1B}$ receptor functions as a terminal heteroreceptor in a variety of nonserotonergic neurons,[15] and in serotonergic neurons it acts as a terminal autoreceptor to inhibit serotonin release.[16,17] In either case the 5-HT$_{1B}$ receptor is transported to axon terminals projecting to many brain regions far from the originating cell body including motor control areas such as basal ganglia. Thus, 5-HT$_{1B}$ binding sites can be readily detected in many brain regions, but the cellular origin of these sites is mixed because of 5-HT$_{1B}$ receptor localization to terminals of heterogeneous projections. In other words, typical binding assays alone cannot determine what cell type is expressing the 5-HT$_{1B}$ binding site even in discrete brain regions. *In situ* hybridization offers the advantage of detecting 5-HT$_{1B}$ mRNA in the cell bodies of discrete brain nuclei that express the receptor, although it does not directly reflect levels of mature receptor protein. While receptor levels may be rapidly regulated by posttranscriptional mechanisms, mRNA levels may reflect longer term adaptive changes following drug exposure as might be expected from the extended period of neurotoxicity of MDMA treatment in rats.

It was our hypothesis that MDMA will induce downregulation of postsynaptic 5-HT$_{1B}$ mRNA and receptors because excessive serotonin is released during the acute phase of MDMA administration, and this would tend to desensitize and downregulate postsynaptic receptors. Since it is difficult to predict which neurons are involved in MDMA-induced motor activity, we decided to examine the raphe, hippocampal, and striatal 5-HT$_{1B}$ receptors because these regions are involved in locomotion and navigation and express 5-HT$_{1B}$ receptors prominently. For comparison, we combined *in situ* hybridization with radioligand binding to detect the 5-HT$_{1B}$ mRNA and receptors. This allowed us to assess two indices of 5-HT$_{1B}$ receptor regulation simultaneously. Each approach has advantages and limitations; together they offer a more complete illustration of 5-HT$_{1B}$ receptor levels following MDMA exposure. Since chronic psychiatric conditions are associated with both mild and heavy levels of MDMA abuse in humans,[18,19] we chose to use several MDMA dosing strategies to determine if doses that do not produce long-term serotonergic degeneration might still alter 5-HT$_{1B}$ levels.

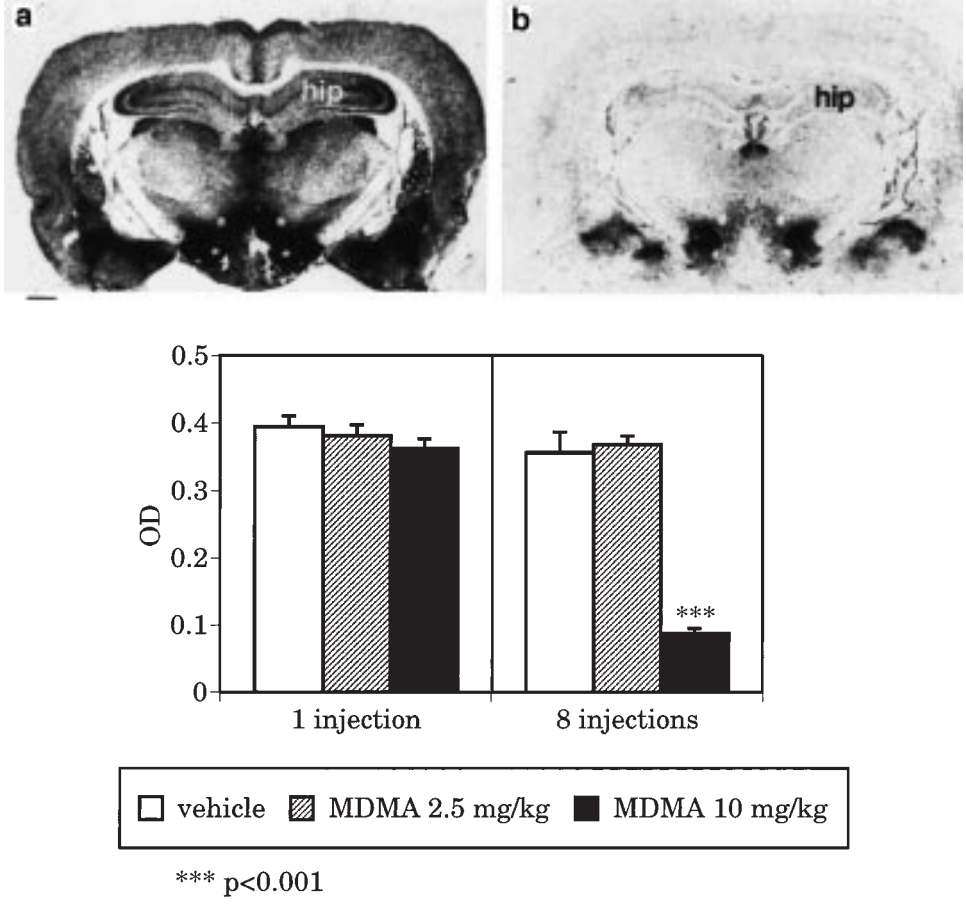

Figure 1 [^{3}H]-Citalopram binding density in hippocampus following MDMA treatment. [^{3}H]-Citalopram binding was used as an index of serotonergic fiber destruction in rat brain 1 day after the final MDMA injection. Typical sections from vehicle and MDMA-treated brains are shown in (a) and (b), respectively. OD was measured from film autoradiographs of tissue sections incubated with 2 nM [^{3}H]-citalopram as previously described. Columns show the mean ± sem for each treatment group (n = 4–5 animals per group). Treatment conditions are indicated below the graph. ***F = 52.4 (5, 21), P<0.001, statistically different from the other conditions (verified with Fisher's PSLD test). The calibration bar = 1 mm.

Methods

Male Sprague–Dawley rats (180–200 g) were housed and utilized in accordance with our institutional and NIH animal care policies. (+) MDMA was provided by NIDA (Bethesda, MD, USA), and was dissolved in saline at 20 mg ml^{-1}. Rats received MDMA injections of 2.5 or 10 mg kg^{-1} s.c. a total of either once only or eight times in a twice daily schedule. Control rats received saline vehicle injections with the same schedule and volumes. Rats were maintained in 20°C rooms and body temperature was not specifically manipulated during the drug treatment. Animals were killed either 1 or 14 days following the last injection and brains were rapidly removed and frozen on dry ice. Four alternating sets of sequential tissue sections (20 μM) were prepared with a cryostat using landmarks for the striatum, anterior hippocampus, and rostral dorsal raphe nucleus. 5-HT$_{1B}$ mRNA was detected in tissue sections by *in situ* hybridization using [^{33}P]-labelled oligonucleotide probes as previously described.[20] The hybridization signal was detected using either film autoradiography (4-day exposure) in Experiment I or phosphor imaging with a Packard Cyclone phosphoscanner (Meriden, CT, USA) (10–24 h exposure) in Experiment II.[21] Serotonergic fiber density was assessed indirectly with [^{3}H]-citalopram binding to serotonin transporter in hippocampal tissue sections as previously described.[22] 5-HT$_{1B}$ binding sites were detected using [^{125}I]-iodocyanopindolol.[23] Briefly, frozen brain sections were thawed and equilibrated in wash buffer (50 mM Tris, pH 7.4, 4 mM $CaCl_2$, 0.01% ascorbic acid) at room temperature for 15 min. The slides were then incubated for 90 min at room temperature with 0.5 nM [^{125}I]-iodocyanopindolol in wash buffer containing pargyline (10 μM) to inhibit monoamine oxidase degradation of the radioligand, DPAT (10 μM) to block 5-HT$_{1A}$ receptors, and isoproterenol (30 μM) to block β receptors. Sections were washed twice in cold wash buffer for 10 min each and briefly rinsed in ice cold water, then dried under a cool air stream. Binding sites were detected by film autoradiography (Experiment I) or phosphor imaging (Experiment II).

Data analysis

All measurements were carried out in a blinded fashion. Quantitative densitometry was completed using MCID (Imaging Research, St Catherines, ON, Canada). The optical density (OD) or digital light units (DLU) from films or phosphoscans, respectively, of two anatomically matched tissue sections from each brain was measured, tissue background was substracted, and the averages were used to calculate treatment group averages. For striatum, an oval target was sampled within the striatum region. Dorsal raphe was sampled with a diamond-shaped target that encompassed the medial region of the nucleus. Globus pallidus and all hippocampal subfields were sampled using the software's edge finding autoscan feature. Statistical comparisons of mRNA and binding site densities were performed using ANOVA followed by Fisher's LSD post-hoc test.

Results

Experiment I

In this experiment we examined several different MDMA dose strategies to determine whether changes

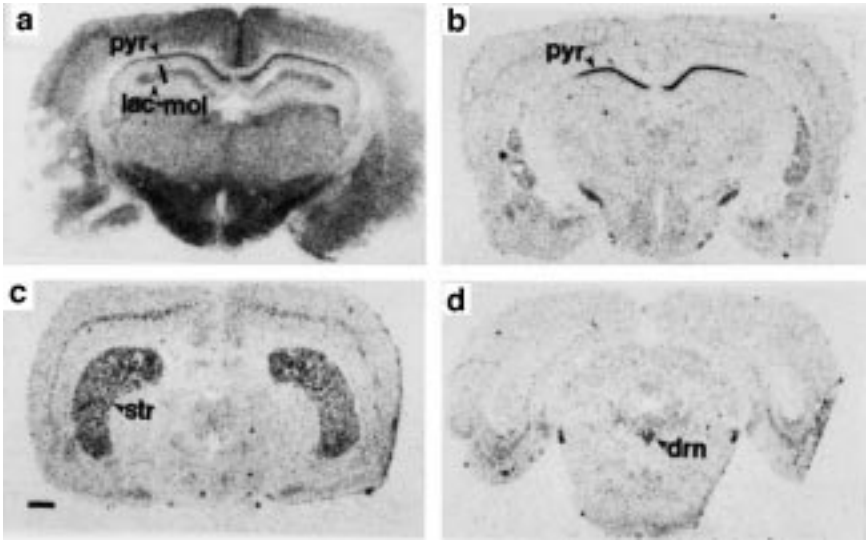

Figure 2 Representative autoradiographs used for quantitation of 5-HT$_{1B}$ mRNA and binding sites. Panel (a) shows [^{125}I]-iodocyanopindolol binding to a hippocampal section; binding over the strata pyramidale of CA1 (pyr) and lacunosum-moleculare of CA3 (lac-mol) were measured densitometrically. Notice that 5-HT$_{1B}$ binding site localization is more complex than 5-HT$_{1B}$ mRNA because these receptors are translocated to axon terminals, often emanating from cell bodies located elsewhere in the brain. Panels (b–d) show *in situ* hybridization using [^{33}P]-dATP labeled oligonucleotide probes specific for 5-HT$_{1B}$ mRNA as previously described[22] at the level of the hippocampus (hip, b), striatum (str, c), and dorsal raphe nucleus (drn, d). See also Figure 5a for [^{125}I]-iodocyanopindolol binding in basal ganglia for comparison to panel (c). The calibration bar = 1 mm.

in 5-HT$_{1B}$ expression paralleled the extent of serotonergic axon degeneration. Animals were killed 24 h after the last MDMA dose to correspond with previous behavioral studies.[12,24] Serotonergic terminal degeneration was assessed by measuring [^{3}H]-citalopram binding to serotonin transporter in hippocampus, which is exclusively contained within serotonergic axons in the forebrain. Treatment with MDMA led to neurotoxicity only in the high dose, 8-injection treatment group, which showed a 75% reduction in [^{3}H]-citalopram binding density in the hippocampus (Figure 1). 5-HT$_{1B}$ expression was assessed by measuring 5-HT$_{1B}$ mRNA and binding site densities. 5-HT$_{1B}$ mRNA was measured densitometrically from autoradiographs (Figure 2). There were no changes in the density of 5-HT$_{1B}$ mRNA in striatum, CA1 stratum pyramidale of hippocampus or the dorsal raphe nucleus at any dose (Table 1). Since 5-HT$_{1B}$ receptor density may also be regulated by posttranslational mechanisms, we then measured [^{125}I]-iodocyanopindolol binding to 5-HT$_{1B}$ sites in hippocampal sections adjacent to those used for 5-HT$_{1B}$ *in situ* hybridization (Figure 3). Binding density of [^{125}I]-iodocyanopindolol in the CA3 stratum lacunosum moleculare showed no change after any of the doses of MDMA. Although in CA1 stratum pyramidale there was a trend toward increased [^{125}I]-iodocyanopindolol binding in the high dose 8-injection treatment group, it was not statistically significant.

Table 1 Regional effects of several MDMA dosing protocols on 5-HT$_{1B}$ mRNA hybridization signals in rat brain 1 day after last MDMA injection

	5-HT$_{1B}$ mRNA hybridization signal (OD ± sem)	*n*
Dorsal Raphe Nucleus		
One injection		
Vehicle	0.131 ± 0.015	5
MDMA 2.5 mg kg^{-1}	0.165 ± 0.008	4
MDMA 10 mg kg^{-1}	0.153 ± 0.019	4
Eight injections		
Vehicle	0.154 ± 0.018	4
MDMA 2.5 mg kg^{-1}	0.161 ± 0.020	5
MDMA 10 mg kg^{-1}	0.163 ± 0.038	4
Hippocampus CA1 s. pyramidale		
One injection		
Vehicle	0.381 ± 0.038	5
MDMA 2.5 mg kg^{-1}	0.457 ± 0.042	4
MDMA 10 mg kg^{-1}	0.486 ± 0.069	4
Eight injections		
Vehicle	0.346 ± 0.047	4
MDMA 2.5 mg kg^{-1}	0.395 ± 0.035	5
MDMA 10 mg kg^{-1}	0.377 ± 0.049	4
Striatum		
One injection		
Vehicle	0.087 ± 0.008	5
MDMA 2.5 mg kg^{-1}	0.111 ± 0.019	4
MDMA 10 mg kg^{-1}	0.119 ± 0.014	4
Eight injections		
Vehicle	0.090 ± 0.008	4
MDMA 2.5 mg kg^{-1}	0.0978 ± 0.011	5
MDMA 10 mg kg^{-1}	0.100 ± 0.014	4

Experiment II

In the second experiment, we tested whether the high dose MDMA injection protocol (10 mg kg^{-1} s.c. twice daily for 4 days) might lead to altered 5-HT$_{1B}$ mRNA or binding levels at a later time point than that used in Experiment I. Animals were killed either 1 or 14 days after the last MDMA injection. The 14-day time point was selected for comparison to a similar study in which chemical axotomy with 5,7-dihydroxytryptamine (5,7-DHT) (which spared dorsal raphe neuron perikarya) caused selective presynaptic reduction in 5-HT$_{1B}$ mRNA at 14 days.[22] [^{3}H]-Citalopram binding,

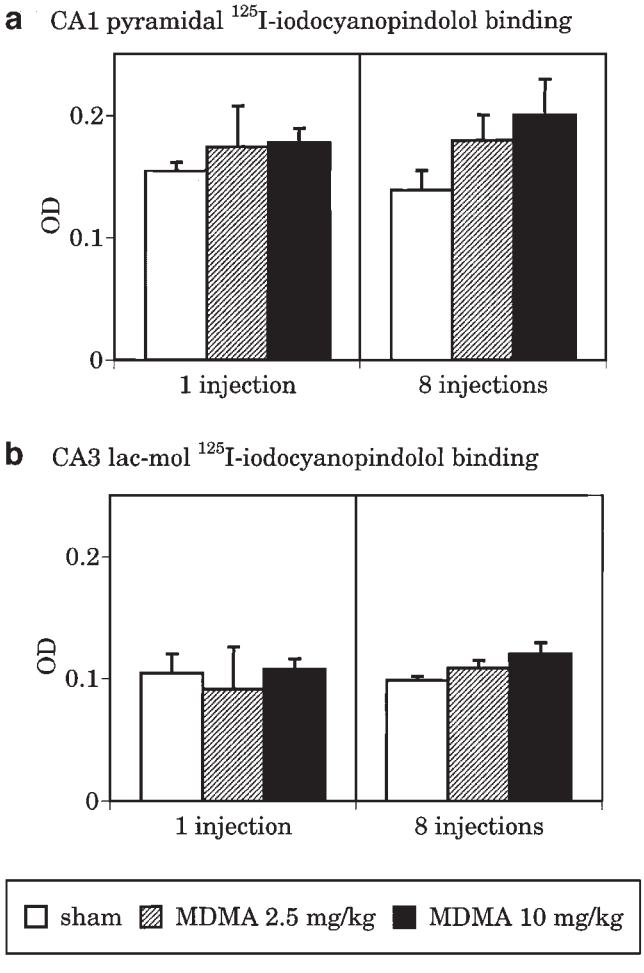

Figure 3 [^{125}I]-Iodocyanopindolol binding density in hippocampus 1 day following MDMA treatment. 5-HT$_{1B}$ binding sites were detected with [^{125}I]-iodocyanopindolol binding, and OD was measured from film autoradiographs of tissue sections in the areas indicated (see Figure 2a). Columns show the means ± sem for each treatment group (n = 4–5). Treatment conditions are indicated below the graphs.

5-HT$_{1B}$ mRNA levels, and [^{125}I]-iodocyanopindolol binding were assessed in adjacent tissue sections in striatum and hippocampus. In dorsal raphe 5-HT$_{1B}$ mRNA alone was measured. [^{3}H]-Citalopram was not measured in dorsal raphe because axon degeneration cannot be assessed from dorsal raphe [^{3}H]-citalopram binding since serotonin transporters are also localized on the soma and dendrites in this nucleus. Similarly, we did not measure [^{125}I]-cyanopindolol binding in dorsal raphe because the axons are degenerated after MDMA treatment, and any residual 5-HT$_{1B}$ binding would not reflect binding in functional axonal terminals.

Treatment with MDMA reduced [^{3}H]-citalopram binding in both striatum and hippocampus by 50–70% (Figure 4). [^{3}H]-Citalopram binding did not return to control levels after 14 days, indicating the persistence of disruption of fine fiber projections from dorsal raphe nucleus to forebrain. We did not detect any significant changes in 5-HT$_{1B}$ receptor mRNA in striatum, hippocampus or dorsal raphe following either 1 or 14 days of recovery from the MDMA treatments (Table 2). [^{125}I]-Iodocyanopindolol binding to forebrain 5-HT$_{1B}$ receptors was also measured densitometrically to assess the levels of mature receptor protein (Figures 5 and 6). While MDMA had no effect on hippocampal or pallidal 5-HT$_{1B}$ binding site density (Figure 6), [^{125}I]-iodocyanopindolol binding was transiently increased by 113% ($P<0.01$) in striatum but returned to control levels at 14 days after MDMA treatment. There was a similar pattern of binding changes in globus pallidus, but they did not reach statistical significance.

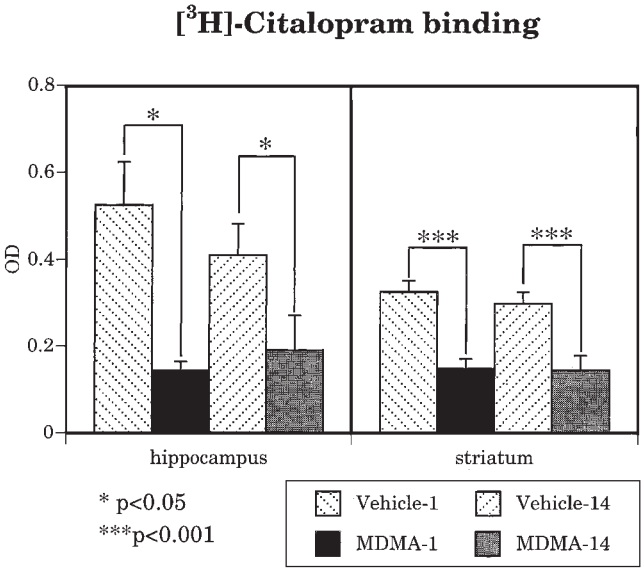

Figure 4 [^{3}H]-citalopram binding density 1 or 14 days following MDMA treatment. MDMA (10 mg kg^{-1}) or vehicle was injected s.c. for a total of eight injections as described in the Methods section. [^{3}H]-Citalopram binding site density (OD) was measured from autoradiographs in whole hippocampus and striatum. Columns show the means ± sem for each treatment group (n = 4–8). Treatment conditions are indicated below the graphs. *F = 4.76 (3, 15), $P<0.05$; ***F = 10.40 (3, 16), $P<0.001$; the statistically different comparisons, validated with Fisher's PSLD test, are indicated by lines over the columns. MDMA induced a similar magnitude of serotonin depletion in hippocampus and striatum and this did not recover after 14 days.

Table 2 Regional effects of MDMA on 5-HT$_{1B}$ mRNA hybridization signals 1 and 14 days after high dose MDMA

	5-HT$_{1B}$ mRNA hybridization signal (DLU ± sem)	*n*
Dorsal Raphe Nucleus		
1 day		
Vehicle	15479 ± 1734	5
MDMA	15395 ± 484	4
14 days		
Vehicle	15441 ± 886	5
MDMA	14042 ± 1047	8
Hippocampus CA1 pyramidale		
1 day		
Vehicle	23192 ± 2311	5
MDMA	25777 ± 2085	4
14 days		
Vehicle	32398 ± 6553	4
MDMA	26854 ± 1381	8
Striatum		
1 day		
Vehicle	8605 ± 1238	5
MDMA	7619 ± 1039	4
14 days		
Vehicle	7797 ± 1811	4
MDMA	6001 ± 553	8

Discussion

We expected MDMA treatment to reduce 5-HT$_{1B}$ mRNA and binding in one or more brain regions, which would have agreed with previous compelling reports of functional desensitization of 5-HT$_{1B}$-induced motor hyperactivity following MDMA-induced serotonin release.[12] However, we found the 5-HT$_{1B}$ mRNA did not change in any of the MDMA treatment protocols, and there was an unexpected but temporary increase in [^{125}I]-iodocyanopindolol binding in striatum.

Since 5-HT$_{1B}$ receptor expression is prominent in striatal neurons and this region participates in motor control, altered 5-HT$_{1B}$ activity in these neurons might be involved in the hyperactivity previously described after acute MDMA treatment. The increase in [^{125}I]-iodocyanopindolol binding runs contrary to what the behavioral data would predict; however, the anatomy of 5-HT$_{1B}$ binding sites is complex. For example, striatum would be predicted to contain at least three sources of 5-HT$_{1B}$ receptors in various axonal terminals: striatal neurons (which have intrastriatal connections and project to globus pallidus and elsewhere),

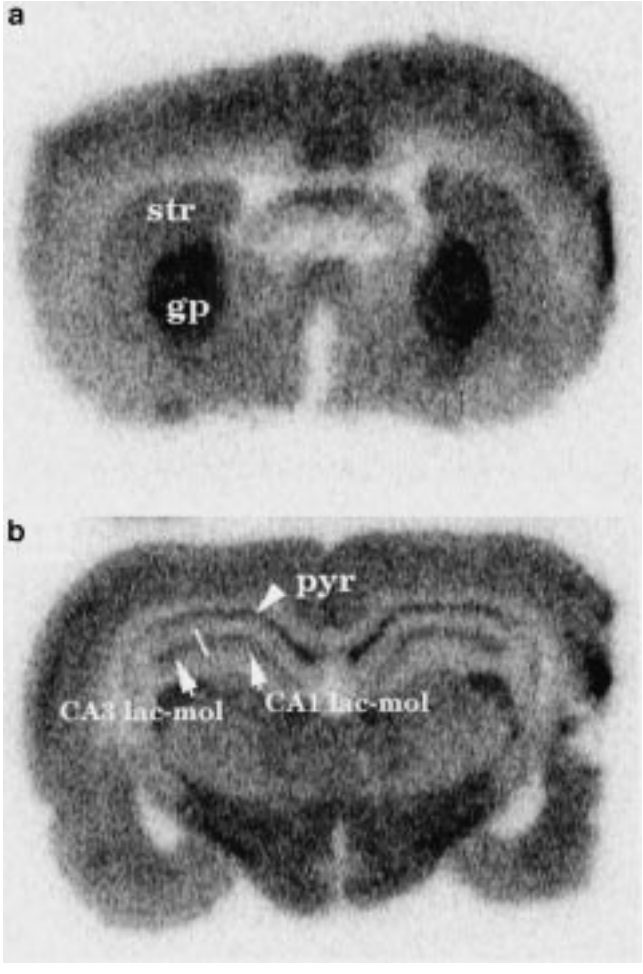

Figure 5 Representative phosphoscans of [^{125}I]-iodocyanopindolol binding in basal ganglia and hippocampus. Radioligand binding in Experiment II was analyzed by phosphoscanning autoradiography. Panel (a) shows [^{125}I]-iodocyanopindolol binding at the level of basal ganglia. There is moderate binding over the striatum (str) and heavy binding over the globus pallidus (gp). Panel (b) shows hippocampal [^{125}I]-iodocyanopindolol binding, which was analyzed in pyramidal (pyr, CA1) and lacunosum-moleculare (lacmol, CA1 and CA3) layers. Phosphoscanned images appear more pixelated than film autoradiographs (compare Figure 5b with 2a), but are quantitatively more linear and accurate over a far greater range of intensities than film autoradiographs, leading to more accurate densitometric analysis.[21]

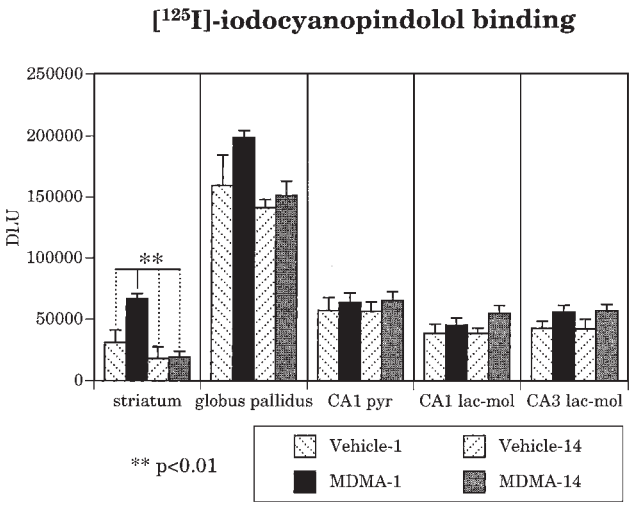

Figure 6 [^{125}I]-Iodocyanopindolol binding 1 or 14 days following MDMA treatment. [^{125}I]-Iodocyanopindolol binding sites were measured from phosphor imaged scans from anatomically matched sections such as those shown in Figure 5. There were no significant changes in any subregion of hippocampus. In basal ganglia, there was a large change in striatal binding 1 day after MDMA treatment was completed as compared to vehicle or MDMA-treated animals after 14 days (**F = 8.33 (3, 16), P = 0.0015). Solid lines over columns are compared to bars indicated with dashed lines. There was a similar trend in globus pallidus, but it was not significant.

extrinsic projections to striatum (eg subthalamic nucleus, cortex) and serotonergic fibers from dorsal raphe nucleus. Since [^{3}H]-citalopram binding to serotonin transporter was significantly reduced, indicating rapid degeneration of presynaptic terminals, it is unlikely that the increase in striatal [^{125}I]-iodocyanopindolol binding to 5-HT$_{1B}$ sites could be on presynaptic terminals from serotonergic neurons. There was a trend to increased globus pallidus [^{125}I]-iodocyanopindolol binding 1 day after MDMA (Figure 6), so it is possible that increased 5-HT$_{1B}$ binding in striatum reflects new synthesis of 5-HT$_{1B}$ receptors in striatal neurons. A possible increase in 5-HT$_{1B}$ synthesis could result from increased striatal 5-HT$_{1B}$ mRNA, but increased striatal 5-HT$_{1B}$ mRNA was not observed (Table 2). If striatal 5-HT$_{1B}$ neurons are responsible for this greater 5-HT$_{1B}$ receptor density, then it did not involve increased gene transcription or stability of messenger RNA. Furthermore, the specific type and distribution of striatal neurons that express 5-HT$_{1B}$ receptors is not known. Perhaps there is a brief compensatory increase in the synthesis or stability of 5-HT$_{1B}$ receptors in some neurons that project to basal ganglia. We did not examine all possible contributors to striatal and pallidal 5-HT$_{1B}$ binding in this study.

Our finding of increased 5-HT$_{1B}$ binding sites in striatum at 1 day but not 14 days after MDMA treatment is interesting in light of a previous study showing elevated [^{125}I]-cyanopindolol binding in rat caudate-putamen homogenate 21 days after 5,7-DHT treatment.[25] We found only a transient increase in 5-HT$_{1B}$ binding in this study. Furthermore, we did not observe reduced 5-HT$_{1B}$ mRNA in dorsal raphe, as we previously did after 5,7-DHT treatment.[22] MDMA toxicity is complex, however, and involves inhibition of tryptophan hydroxylase, inhibition of synaptic membrane and vesicular serotonin transport, as well as terminal degeneration—effects that may be separable.[1] Perhaps 5,7-DHT affects 5-HT$_{1B}$ mRNA synthesis by a mechanism that is distinct from terminal degeneration. Thus, the presynaptic and postsynaptic effects of these two neurotoxins may be similar but not identical.

Our detection of a mismatch in the expression of 5-HT$_{1B}$ mRNA and binding levels in at least one case—the striatum—emphasizes the importance of taking

transcriptional and posttranscriptional levels of regulation into account in considering how these receptors are regulated. The complex distribution of these receptors on axon terminals makes it difficult to determine which populations of neurons bearing 5-HT$_{1B}$ receptors are responsible for drug-induced behavior changes (such as motor hyperactivity). However, *in situ* hybridization and radioligand binding together show that posttranscriptional mechanisms are important in regulation of postsynaptic 5-HT$_{1B}$ receptors in nonserotonergic neurons.

We did not detect reduced 5-HT$_{1B}$ mRNA or binding sites as might have been predicted by behavioral data.[12] There are at least two explanations for this discrepancy. It is possible that 5-HT$_{1B}$ receptors, in a different brain region that we did not examine, participate in the induction of motor hyperactivity and are down-regulated after MDMA treatment.[26] It is also possible that MDMA treatment alters the activity of 5-HT$_{1B}$ receptors without changing 5-HT$_{1B}$ mRNA or binding levels in most brain regions. Our detection of a large change in striatal 5-HT$_{1B}$ binding and a similar trend in globus pallidus suggests that MDMA may have complex posttranscriptional effects on 5-HT$_{1B}$ levels that are an important focus of further inquiry. These posttranscriptional effects might include an increased translational efficiency or increased receptor stability. It seems likely, though, that altered 5-HT$_{1B}$ receptor function is an acute rather than a chronic consequence of MDMA abuse, since all indices returned to control levels at 14 days.

This study has several limitations. *In situ* hybridization detects messenger RNA, which probably correlates with the capacity of a neuron to synthesize the mature protein over time, but it only reflects the steady state mRNA concentration at the time of measurement. We performed no functional assessment of 5-HT$_{1B}$ activity to complement the *in situ* hybridization and radioligand binding data. Binding site analysis does not indicate the functional status of the receptor. However, this study suggests that MDMA has acute effects on the posttranscriptional regulation of some 5-HT$_{1B}$ receptors that may contribute to the behavioral effects of MDMA. These are important topics for future study. Nevertheless, the combined utilization of these techniques offers a greater degree of molecular and anatomical precision than many other techniques.

The serotonergic system has been increasingly implicated in the physiology of drug addiction and abuse. In particular, 5-HT$_{1B}$ receptors are involved in increasing alcohol and cocaine self-administration.[13,14,27] Although different experimental designs have led to divergent conclusions (perhaps because 5-HT$_{1B}$ receptors in different brain regions have opposing effects on drug reinforcement), it now appears that these receptors both mediate the acute behavioral effects of drugs including MDMA as well as impact reinforcement mechanisms leading to addictive self administration. However, the anatomical loci of the 5-HT$_{1B}$ receptors involved in these actions remain uncertain.

In conclusion, we found that MDMA treatment in a variety of dosing schedules did not change 5-HT$_{1B}$ mRNA levels in several brain regions but may have complex posttranscriptional effects on 5-HT$_{1B}$ receptor regulation in striatum and related subcortical regions.

Acknowledgements

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References

1 McKenna DJ, Peroutka SJ. Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). *J Neurochem* 1990; **54**: 14–22.
2 Mamounas LA, Mullen CA, O'Hearn E, Molliver ME. Dual serotoninergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. *J Comp Neurol* 1991; **314**: 558–586.
3 Gartside SE, McQuade R, Sharp T. Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on 5-HT cell firing and release: comparison between dorsal and median raphe 5-HT systems. *Neuropharmacology* 1997; **36**: 1697–1703.
4 Rudnick G, Wall SC. The molecular mechanism of 'ecstasy' [3,4-methylenedioxymethamphetamine (MDMA)]: serotonin transporters are targets from MDMA-induced serotonin release. *Proc Natl Acad Sci USA* 1992; **89**: 1817–1821.
5 Ricaurte GA, Markowska AL, Wenk GL, Hatzidimitriou G, Wlos J, Olton DS. 3,4-methylenedioxymethamphetamine, serotonin and memory. *J Pharmacol Exp Ther* 1993; **266**: 1097–1105.
6 Ali SF, Newport GD, Scallet AC, Binienda Z, Ferguson SA, Bailey JR *et al*. Oral administration of 3,4-methylenedioxymethamphetamine (MDMA) produces selective serotonergic depletion in the nonhuman primate. *Neurotoxicol Teratol* 1993; **15**: 91–96.
7 Battaglia G, Sharkey J, Kuhar MJ, de Souza EB. Neuroanatomic specificity and time course of alterations in rat brain serotonergic pathways induced by MDMA (3,4-methylenedioxymethamphetamine): assessment using quantitative autoradiography. *Synapse* 1991; **8**: 249–260.
8 Scanzello CR, Hatzidimitriou G, Martello AL, Katz JL, Ricaurte GA. Serotonergic recovery after (±)3,4-(methylenedioxy) methamphetamine injury: observations in rats. *J Pharmacol Exp Ther* 1993; **264**: 1484–1491.
9 Insel TR, Battaglia G, Johannessen JN, Marra S, De S-EB. 3,4-Methylenedioxymethamphetamine ('ecstasy') selectively destroys brain serotonin terminals in rhesus monkeys. *J Pharmacol Exp Ther* 1989; **249**: 713–720.
10 Ricaurte GA, Martello AL, Katz JL, Martello MB. Lasting effects of (±)-3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in nonhuman primates: neurochemical observations. *J Pharmacol Exp Ther* 1992; **261**: 616–622.
11 Rempel NL, Callaway CW, Geyer MA. Serotonin$_{1B}$ receptor activation mimics behavioral effects of presynaptic serotonin release. *Neuropsychopharmacology* 1993; **8**: 201–211.
12 Callaway CW, Geyer MA. Tolerance and cross-tolerance to the activating effects of 3,4-methylenedioxymethamphetamine and a 5-hydroxytryptamine$_{1B}$ agonist. *J Pharmacol Exp Ther* 1992; **263**: 318–326.
13 Crabbe JC, Phillips TJ, Feller DJ, Hen R, Wenger CD, Lessov CN *et al*. Elevated alcohol consumption in null mutant mice lacking 5-HT$_{1B}$ serotonin receptors. *Nature Genet* 1996; **13**: 98–101.
14 Rocha BA, Scearce L-K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC *et al*. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature* 1998; **393**: 175–178.
15 Boschert M, Amara DA, Segu L, Hen R. The mouse 5-hydroxytryptamine$_{1B}$ receptor is localized predominantly on axon terminals. *Neuroscience* 1994; **58**: 167–182.
16 Hoyer D, Middlemiss DN. Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol Sci* 1989; **10**: 130–132.

17 Hartig PR, Hoyer D, Humphrey PPA, Martin GR. Alignment of receptor nomenclature with the human genome: classification of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes. *Trends Pharmacol Sci* 1996; **17**: 103–105.
18 McCann UD, Ricaurte GA. Lasting neuropsychiatric sequelae of (±)methylenedioxymethamphetamine ('ecstasy') in recreational users. *J Clin Psychopharmacol* 1991; **11**: 302–305.
19 McGuire PK, Cope H, Fahy TA. Diversity of psychopathology associated with use of 3,4-methylenedioxymethamphetamine ('Ecstasy'). *Br J Psychiatry* 1994; **165**: 391–395.
20 Neumaier JF, Root DC, Hamblin MW. Chronic fluoxetine reduces serotonin transporter mRNA and 5-HT1b mRNA in a sequential manner in the rat dorsal raphe nucleus. *Neuropsychopharmacology* 1996; **15**: 515–522.
21 Neumaier JF. Quantitative *in situ* hybridization detection of 5-HT receptor mRNA in rat brain using storage phosphor imaging. *Am Biotech Lab* 1998; **16**: 61–62.
22 Neumaier JF, Szot P, Peskind E, Dorsa DM, Hamblin MW. Serotonergic lesioning differentially effects presynaptic and postsynaptic 5-HT$_{1B}$ receptor mRNA levels in rat brain. *Brain Res* 1996; **722**: 50–58.
23 Pranzatelli MR, Razi P. Drug-induced regulation of [^{125}I]iodocyanopindolol-labeled 5-hydroxytryptamine$_{1B}$ receptor binding sites in the central nervous system. *Neuropsychopharmacology* 1994; **10**: 259–264.
24 Callaway CW, Rempel N, Peng RY, Geyer MA. Serotonin 5-HT$_1$-like receptors mediate hyperactivity in rats induced by 3,4-methylenedioxymethamphetamine. *Neuropsychopharmacology* 1992; **7**: 113–127.
25 Offord SJ, Ordway GA, Frazer A. Application of [^{125}I]iodocyanopindolol to measure 5-hydroxytryptamine$_{1B}$ receptors in the brain of the rat. *J Pharm Exp Ther* 1988; **244**: 144–153.
26 Geyer MA. Serotonergic functions in arousal and motor activity. *Behav Brain Res* 1995; **73**: 1–2.
27 Parsons LH, Weiss F, Koob GF. Serotonin$_{1B}$ receptor stimulation enhances cocaine reinforcement. *J Neurosci* 1998; **18**: 10078–10089.