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3,4-Methylenedioxymethamphetamine Induces Differential Regulation of Tryptophan Hydroxylase 2 Protein and mRNA Levels in the Rat Dorsal Raphe Nucleus

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Abstract

Previous investigations with 3,4-methylenedioxymethamphetamine (MDMA) have suggested that administration of this drug results in a degeneration of 5-HT nerve terminals and subsequent alterations in 5-HT neurotransmission. However, only limited investigations have examined the effects of MDMA on the dorsal raphe nucleus. The present study was designed to assess the effect of MDMA on the rate-limiting enzyme in 5-HT biosynthesis, tryptophan hydroxylase (TPH), by measuring TPH2 protein and mRNA levels in rat dorsal raphe (DR) nucleus. Rats were administered MDMA (20 mg/kg, s.c.) or saline twice daily for 4 days and killed 14 days later. Tissue sections of the DR were processed for quantitative immunoautoradiography and *in situ* hybridization histochemistry for measurements of the levels of TPH2-immunoreactivity (IR) and TPH2 mRNA. To assess 5-HT axon terminal integrity after MDMA treatment, the density of 5-HT transporter (SERT) binding sites was measured by quantitative autoradiography using [¹²⁵I]RTI-55 ((-)-2beta-carbomethoxy-3 beta-(4-iodophenyl) tropane) (¹²⁵I-RTI-55) as a ligand. TPH2-IR levels were significantly decreased by 45% in the mid DR and by 40% in the caudal DR in the MDMA-treated rats compared with saline-injected rats. In contrast, TPH2 mRNA levels were significantly increased by 24% in the mid DR and by 12% in the caudal DR. MDMA treatment significantly decreased ¹²⁵I-RTI-55 labeled SERT binding sites in the striatum, nucleus accumbens and cingulate cortex demonstrating a loss of 5-HT terminals. The increase in TPH2 mRNA levels in both the mid DR and caudal DR of MDMA-treated rats may reflect a compensatory mechanism in the injured 5-HT neurons to increase TPH2 protein synthesis. Taken together, our results suggest that a serious defect occurs in the biosynthesis of TPH2 in the DR following MDMA administration.

Keywords

amphetamine; 5-HT; 5-HT transporter; immunoautoradiography; *in situ* hybridization; drug abuse

3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”) is an amphetamine derivative, which is commonly used as a recreational drug because of its euphoric and empathic properties, and thus has become a popular drug of abuse among the adolescent and young adult population. Unfortunately, many individuals perceive MDMA as a “safe” drug. However, evidence from

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both human and animal studies reveals that MDMA exerts long-term neurotoxic effects on 5-HT neurons (Battaglia et al., 1991; Fischer et al., 1995; Lew et al., 1996; Hatzidimitriou et al., 1999). Specifically, immunocytochemical studies have reported that MDMA preferentially destroys the fine, small fusiform varicose 5-HT axon terminals originating from the dorsal raphe (DR) nucleus causing the degeneration of 5-HT terminals in various cortical and subcortical regions. In addition, evidence from biochemical studies has repeatedly demonstrated that the MDMA-induced loss of 5-HT terminals includes a marked reduction of 5-HT transporter (SERT) binding sites throughout these regions (Battaglia et al., 1991; Aguirre et al., 1995; Fischer et al., 1995; Lew et al., 1996).

While many studies have characterized the MDMA-induced loss of 5-HT terminals, very few studies have investigated the effects of MDMA on the midbrain neurons that contain the biosynthetic machinery for synthesizing 5-HT. Aguirre and colleagues (1997) reported a decrease in 5-HT_{1A} autoreceptor binding density and mRNA levels in rat brainstem 7 days after MDMA treatment. This study represents the first report of an alteration in a key regulator of 5-HT neurons in the brainstem raphe nuclei following MDMA-induced 5-HT axon degeneration, and suggests that the decrease in 5-HT_{1A} autoreceptor synthesis reflects a compensatory mechanism of 5-HT neurons to adapt to the loss of 5-HT axons after repeated MDMA administration (Aguirre et al., 1995, 1997). In contrast, in a recent PET study of the effects of chronic MDMA, Cumming and colleagues (2007) failed to detect alterations in 5-HT_{1A} autoreceptor binding sites in several brain regions of Göttingen minipigs, including the mesencephalon and pons. Given this inconsistency, it is critical to determine whether alterations occur in other 5-HT markers in raphe neurons following MDMA treatment.

One important key regulator in the 5-HT neurotransmission is tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of 5-HT (Jequier et al., 1967) and also a phenotypic marker for serotonergic neurons. TPH exists in two different isoforms encoded by two different genes: the original TPH, referred now as TPH1 and the newly discovered isoform, identified as TPH2 (Walther et al., 2003). Both TPH isoforms are highly homologous and share 71% of their amino acid sequence identities. Despite this similarity, the two enzymes show different biochemical properties (McKinney et al., 2005). They also differ by their anatomical distribution with TPH2 being exclusively expressed in the brain, whereas TPH1 is most abundant in the pineal gland and periphery (Patel et al., 2004; Salli et al., 2004; Zill et al., 2007). In the brain, TPH2 mRNA is mainly synthesized in the raphe nuclei (Zill et al., 2007) and presumably transports to cortical and subcortical brain regions through axons.

Considering the amount of research that has accumulated over the past decade on the serotonergic effects of MDMA, there have been relatively few biochemical studies conducted to examine the biosynthetic integrity of TPH following MDMA administration. Three earlier studies examined the acute effects of MDMA on the activity of TPH in several terminal fields and found that MDMA produced a rapid and sustained decrease in TPH activity in the striatum, frontal cortex and hippocampus (Stone et al., 1986, 1987; Schmidt et al., 1987). In addition, a reduction in the density of tryptophan hydroxylase-immunoreactive (TPH-IR) fibers was recently found in serotonergic nerve terminal axons in several brain regions after MDMA treatment (Ádori et al., 2006; Xie et al., 2006). It is surprising that no studies have examined TPH2 protein or mRNA expression in the DR nucleus following MDMA administration, particularly since several previous reports found significant reductions in TPH enzyme activity and increases in TPH mRNA in the DR after 5-HT nerve terminal depletion or destruction by selective TPH inhibitors such as p-chlorophenylalanine (PCPA, a specific and irreversible inhibitor of TPH) and fenfluramine (FEN, a 5-HT releasing agent and reuptake inhibitor) or the established 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) (Park et al., 1994; Raison et al., 1996; Semple-Rowland et al., 1996; Ljubic-Thibal et al., 1999). Given the long-term decreases in TPH activity previously reported in various terminal fields after MDMA

treatment, we predict that MDMA would produce alterations in TPH2 protein and mRNA levels in DR following a neurotoxic dosage regimen.

The aim of the present study was to examine the effects of repeated administration of MDMA on TPH2 protein and mRNA levels in the rat DR nucleus using immunoautoradiography and *in situ* hybridization histochemistry.

Experimental Procedures

Animal treatments

All procedures for the treatment of animals were performed in strict compliance with the *NIH Guide for the Care and Use of Laboratory Animals* and approved by the IACUC at the University of Mississippi Medical Center. Animal experiments were all conducted in a fashion that minimized the number of rodents used and their suffering. Male Sprague–Dawley rats weighing between 225 and 250 g were used. Animals were housed individually and provided with food and water *ad libitum*. The room was maintained on a 12-h light/dark cycle, with lights on 7 am to 7 pm. All experiments were performed during the light phase. All rats were gently handled on a daily basis for 1 week preceding the experiments. Rats were randomly assigned to the following treatment groups: saline (1 ml/kg, s.c. twice daily for 4 days, $N=7$); MDMA (20 mg/kg, s.c. twice daily for 4 days, $N=7$). Fourteen days after the last dose of MDMA or saline rats were immediately killed by decapitation.

Histological assessment

Upon completion of the treatment, animals were killed by decapitation and the brains rapidly removed, blocked and frozen on powdered dry ice. Prior to sectioning, the cryostat knife was cleaned with diethylpyrocarbonate (DEPC) -treated sterile water to remove any RNAase that may have been present. Serial frozen 12 μ m thick coronal sections encompassing the entire rostral–caudal extent of the DR, prefrontal cortex, hippocampus and striatum were cut on a cryostat (-20°C). Frozen tissue sections were thaw-mounted onto gelatin-coated slides and stored at -80°C until processed. Every 10th tissue section was reserved for Nissl staining and used as reference slides.

Immunoautoradiography

Details of the immunoautoradiography procedure have been previously described Bonkale et al. (2004). Slide-mounted frozen tissue sections containing the DR region were removed from the -80°C freezer, warmed to -20°C and placed into 4% paraformaldehyde for 14–17 h. The sections were then rinsed in phosphate-buffered saline (PBS) and then incubated for 60 min in PBS with 4% of goat serum and 0.1% Triton X-100 to reduce background. The tissue sections were incubated at 4°C for 24 h with primary antiserum to TPH (mouse anti-TPH, 1:10,000, Sigma), diluted in PBS containing 0.3% Triton X-100, 2.0% goat serum and 0.05% bovine serum albumin. The sections were then washed 3 $\times$ for 5 min in 0.1% Triton X-100 in PBS and then incubated with ^{125}I -labeled secondary antibody (donkey anti-mouse IgG) (TPH) using a dilution of 1:200 (0.5 $\mu\text{Ci/ml}$) in 0.1% Triton X-100 in PBS for 120 min at room temperature. Following this incubation, the sections were washed in 0.1% Triton X-100 in PBS followed by PBS. The slides were then dipped into 70% ethanol, dried and co-exposed with iodinated plastic standards to Biomax MR film (Eastman Kodak Corporation, Rochester, NY, USA) for 24–48 h. The exposure times were selected so that the resultant autoradiographic signal is within the optimum range of film sensitivity.

The densitometric quantitation of the film autoradiograms was performed using a computer-based image analysis system (MCID Elite) from Imaging Research Inc. (St. Catherines, Ontario) as previously described (Bonkale et al., 2004). The quantification of the

autoradiographic images was accomplished using a standard curve consisting of optical density values converted to radioactivity units/mg protein (nCi/mg) using the iodinated standards. The mid and caudal DR levels were defined according to Abrams et al. (2004). To determine the sampling boundaries of the specific level of the DR on the film autoradiograms, the corresponding Nissl-stained image was digitized and the cytoarchitectonic boundaries of each specific level outlined. This template was then superimposed on the autoradiographic image and the radioactivity values measured within the brain region. TPH-IR values (nCi/mg) for each brain region were derived by interpolation from the standard curve. Non-specific binding of the secondary antibody to tissue sections was measured for each case, and this average background value was subtracted from each data point.

***In situ* hybridization histochemistry**

The details of the *in situ* hybridization procedure have been previously published (Austin et al., 1994). Sterile, RNAase-free conditions were used throughout this procedure. Rodent specific cDNAs for TPH2 mRNA transcripts were generated using forward and reverse sequence specific primers, total RNA isolated from rat brain and RT-PCR according to the method of Kerner et al. (1998). After amplification of cDNAs, nested primers with SP6 and T7 RNA polymerase sequences attached were used to amplify the cDNA, thus providing cDNA templates for RNA probe synthesis. Riboprobes were labeled with ^{35}S -CTP and synthesized using the *in vitro* transcription procedure according to the manufacturer's recommendations (Maxiscript kit, Ambion, TX, USA). Labeled riboprobes were separated from unincorporated nucleotides using Nuc-trap push columns (Stratagene). Slide-mounted tissue sections were fixed, acetylated and then dehydrated and delipidated in a series of ethanol and chloroform washes. Hybridization buffer containing 10^6 d.p.m. of ^{35}S -dATP-labeled cDNA or cRNA probe is pipetted on to each tissue section. Tissue sections are incubated at 37 °C (oligos) or 56 °C (riboprobes) for 20–24 h and then washed in a series of formamide/NaCl/sodium citrate (SSC) buffers to remove nonspecific binding of the probe. Following the washes, the hybridized sections were exposed to B-max Hyperfilm (Amersham, Arlington Heights, IL, USA). Radioactive ^{14}C plastic standards, (American Radiolabeled Chemicals (ARC) Inc., St. Louis, MO, USA) that were cross-calibrated to brain paste standards containing known amounts of ^{35}S , were co-exposed to the film with the hybridized slides.

The quantification of film autoradiograms was accomplished using the MCID Elite imaging system as described previously (Austin and O'Donnell, 1999; Chang et al., 2000). Dpm/area values for each brain region were derived by interpolation from the standard curve.

Receptor autoradiography

These procedures have been previously described by Stockmeier and colleagues (1996). Briefly, radioligand binding to the SERT was determined autoradiographically using [^{125}I] RTI-55 ((-)-2beta-carbomethoxy-3 beta-(4-iodophenyl) tropane) (^{125}I -RTI-55) as a ligand (McGregor et al., 2003). Frozen tissue sections were removed from the -80 °C freezer and dried in a desiccator. After a 15 min preincubation in phosphate buffer (10 mM NaH_2PO_4 , 0.1 M sucrose, pH 7.4) at room temperature, sections were incubated for 1 h at room temperature with ^{125}I -RTI-55 (50 nM, Perkin-Elmer) in phosphate buffer in the presence of 1 μM mazindol (block DAT binding). Non-specific binding was measured in duplicate serial sections co-incubated with 10 μM fluoxetine-HCl (Lilly, IN, USA). After incubation, the sections were washed twice for 20 min each in ice-cold buffer.

After the incubations described above, all sections were dipped in ice-cold water, air-dried and stored for 24–48 h at 4 °C in sealed slide boxes with Drierite capsules. The sections and tritiated plastic standards (ARC) were placed into a Fuji Phosphor Imaging System or exposed to Biomax MR film (Kodak) in film cassettes for 6–10 h ^{125}I -RTI-55. Films were developed with

Kodak D-19 developer and fixed with Kodak Rapid Fix. The exposure times were selected so that the resultant autoradiographic signal is within the optimum range of film sensitivity.

The autoradiographic images of radioligand binding in the forebrain sections were quantified using the M5 imaging system. The gray level transmittance per pixel was converted to relative optical density by MCID software and expressed as fmol of radioligand bound per mg protein by calibrating to images from co-exposed tritiated plastic standards or iodinated plastic standards. The plastic standards were previously calibrated by co-exposure with sections of brain paste containing known amounts of tritium and protein. Using Nissl-stained sections digital templates are drawn to correspond to the striatum, hippocampal subfields and prefrontal cortical regions, and these templates were superimposed on the autoradiographic images. Images of total and nonspecific ^{125}I -RTI-55 binding of are digitized and superimposed, and non-specific binding is digitally subtracted from total binding to yield specific binding.

Statistical analyses

Data were expressed as mean $\pm$ S.E.M. Differences between the control and MDMA treated group were analyzed by Student *t*-test. All statistical assumptions were met and a value of $P<0.05$ was considered significant.

Results

Effects of MDMA on TPH2 protein and mRNA levels in the mid and caudal DR

TPH2 protein and mRNA levels in the mid and caudal DR of control and MDMA-treated rats were measured by using immunautoradiography and *in situ* hybridization histochemistry. The analysis of the film autoradiograms revealed that the distribution of TPH2 protein in the mid and caudal DR (Fig. 1A and 1C) closely overlaps that of TPH2 mRNA (Fig. 2A and 2C). The densitometric quantitation of the film autoradiograms revealed that TPH2 protein levels were significantly decreased by 45% in the mid DR ($t=3.06$, $P=0.018$, Fig. 1A') and by 40% in the caudal DR ($t=3.16$, $P=0.016$, Fig. 1B') in the MDMA-treated rats compared with saline-injected rats. Autoradiographic images illustrating the regional differences in TPH2 protein expression in the mid and caudal DR of an MDMA-treated rat and saline-treated rat are shown in Fig. 1A–D. Also, the area of the mid and caudal DR, delineated by the autoradiographic signal, was decreased by 12% and 18%, respectively, in the MDMA group compared with the saline group although these changes did not reach statistical significance.

In contrast to the TPH2 protein data, densitometric quantitation of *in situ* autoradiograms revealed that TPH2 mRNA levels were significantly increased by 24% in the mid DR ($t=3.81$, $P=0.007$, Fig. 2A') and by 12% in the caudal DR ($t=3.36$, $P=0.01$, Fig. 2B') in the MDMA-treated rats compared with saline-injected rats. Autoradiographic images illustrating regional differences in TPH2 mRNA expression in the middle and caudal DR of an MDMA-treated rat and saline-treated rat are shown in Fig. 2A–D. The area of the middle and caudal DR defined by TPH2 mRNA did not differ between treatment groups.

Effects of MDMA on SERT binding sites in forebrain regions

Tissue sections of the forebrain from the same rats described above were processed for receptor autoradiography using ^{125}I -RTI-55 as a ligand to measure the density of SERT binding sites in the striatum, nucleus accumbens and cingulate cortex. MDMA treatment significantly decreased SERT binding sites in the striatum ($t=5.81$, $P=0.0004$), nucleus accumbens ($t=3.67$, $P=0.008$), and cingulate cortex ($t=8.15$, $P=0.0012$, Fig. 3).

Discussion

Our results revealed that repeated MDMA administration significantly decreased TPH2 protein levels, but significantly elevated TPH2 mRNA concentrations in the mid and caudal regions of the DR. These data are the first to demonstrate alterations in the biosynthesis of the rate-limiting enzyme, TPH2, in the DR nucleus following MDMA administration. The MDMA-induced reduction in TPH2 protein in the DR is consistent with earlier biochemical investigations that reported a decrease in TPH activity in several serotonergic nerve terminal regions of the rat brain following administration of MDMA. A reduction in TPH activity was found in the neostriatum, hippocampus and cortex of rat 18 h after 10 mg/kg MDMA treatment (Stone et al., 1986). Furthermore, a decrease in rat cortical TPH activity was also observed 12 h following 20 mg/kg MDMA administration (Schmidt and Taylor, 1987). These authors did not attribute these findings to a direct action of MDMA on TPH, but rather to both an inhibition of 5-HT synthesis and increase in release.

Our findings extend previous results that reported reductions in TPH enzyme activity and protein levels in the DR after treatment with amphetamines substituted. For example, a 10% decrease in TPH activity as a result of PCPA administration was found in the DR and caudal brainstem (Park et al., 1994). In contrast, several studies have demonstrated that 5,7-DHT, a substance that destroys serotonergic containing neurons, had an opposite effect on TPH enzyme activity in rat midbrain. Stachowiak and colleagues (1986) found an increase in TPH enzyme activity in the midbrain 3 days after intraventricular injection of 5,7-DHT. They suggested that this increase occurred in residual serotonergic neurons in the midbrain to compensate neuronal loss caused by 5,7-DHT. This interpretation also is in agreement with the observation of Clewans and Azmitia (1984) who reported that TPH enzyme activity gradually increased in the rat midbrain, reaching a maximum by 28 days after administration of 5,7-DHT. It is tempting to speculate that an increase in TPH enzyme activity in the midbrain after 5,7-DHT treatment appears to represent a compensatory response to neuronal loss while its decrease following treatment with substituted amphetamines, including MDMA may be attributed to functional changes that occur in serotonergic neurons.

The MDMA-induced increase in TPH2 mRNA in the rat mid and caudal DR regions is in agreement with a recent study that reported that TPH mRNA levels measured by RT-PCR were selectively increased in the rat frontal cortex 24h after MDMA injection (García-Osta et al., 2004). However, it is difficult to interpret the functional significance of elevated TPH mRNA in presumably 5-HT nerve terminals in the frontal cortex following MDMA in the context of our results of increased TPH2 mRNA in the cell body regions of the DR after MDMA. Little is known regarding the functional significance of mRNA transcripts localized to nerve terminals. However, perhaps the MDMA-induced increase in TPH mRNA levels in the frontal cortex reflects a demand for local TPH synthesis resulting in transport of TPH mRNA from the cell body to the nerve terminal and increased TPH mRNA synthesis in the DR. The increase of TPH mRNA levels in the DR following MDMA administration is similar to that produced by selective TPH inhibitors, including PCPA. In particular, an increase in the steady state levels of TPH mRNA has been demonstrated in the DR and median raphe nuclei after treatment with PCPA (Cortès et al., 1993; Park et al., 1994). A similar increase also was observed in residual serotonergic cell bodies of the DR following 5,7-DHT administration (Bendotti et al., 1990; Park et al., 1993). Finally, Ljubic-Thibal and colleagues (1999) demonstrated that a selective destruction of serotonergic nerve terminals in the rat dorsolateral hypothalamus caused by 5,7-DHT resulted in an increase in TPH mRNA in subpopulation DR serotonergic neurons. The observations reported in the present study agree with the suggestion that the increase in TPH2 mRNA levels in the DR may reflect a compensatory response to the deficits in 5-HT terminals and axons as a result of damage caused by MDMA.

Although the mechanisms by which MDMA increases TPH2 mRNA expression remain unknown, alterations in the level of gene expression are determined by several factors, including rate of gene transcription initiation, mRNA stability, mRNA translation efficiency, and posttranslational regulation such as protein modifications. With regard to TPH mRNA, two potential mechanisms have been postulated to explain its upregulation by TPH selective inhibitors in serotonergic cell bodies and terminal areas (Bendotti et al., 1993; Cortès et al., 1993; Park et al., 1994; Ljubic-Thibal et al., 1999; García-Osta et al., 2004). First, it has been proposed that an increase in TPH mRNA may be a result of an upregulation of gene transcription, which is a consequence of depletion of 5-HT concentrations in the cell bodies and nerve terminal regions (Bendotti et al., 1993; Cortès et al., 1993; Park et al., 1994). TPH promoter contains cAMP response-element (CRE) binding sites that are involved in gene transcription. It has been shown that the recovery of 5-HT levels correlated with an induction of CRE-binding activity and increase of TPH mRNA expression in rat frontal cortex following MDMA administration (García-Osta et al., 2004; Martínez-Turrillas et al., 2006). These authors suggested that MDMA may upregulate TPH gene expression via a cAMP response element-binding protein (CREB) –dependent mechanism. Alternatively, it has been proposed that the stability of TPH mRNA might contribute to an increase of TPH mRNA levels.

The present data demonstrate that changes in TPH2 mRNA abundance do not necessarily reflect changes in TPH2 protein levels. Discrepancy between TPH mRNA and TPH activity has also been previously reported following treatment with TPH inhibitors (Bendotti et al., 1993). One interpretation of this phenomenon is that MDMA produces a reduction in the rate of translation of TPH mRNA to TPH protein. This explanation is supported by an earlier study showing that *in vivo* administration of amphetamine decreased protein synthesis in rat brain through a dissociation of polyribosomes (Widelitz et al., 1975). However, when Stone and co-workers (1987) measured TPH activity in the striatum, frontal cortex and hippocampus after repeated MDMA treatment (5 doses, every 6 h), they found that the decrease in TPH activity persisted for at least 110 days. This observation is inconsistent with a simple inhibition of protein synthesis.

In addition to translational regulation, posttranslational modifications have been implicated in the regulation of TPH protein and activity. For example, TPH is phosphorylated by a variety of protein kinases, including cAMP-dependent protein kinase (PKA) as well as calcium calmodulin-dependent protein kinase (Ehret et al., 1989; Johansen et al., 1995). Recently, it was shown that MDMA causes a decline in PKA activity in rat prefrontal cortex and hippocampus (Crawford et al., 2006), and thus may contribute to a decrease in TPH protein and activity.

The neurotoxic effects of MDMA on serotonergic axon and terminals can be quantified by measuring 5-HT uptake sites or SERT protein levels (Battaglia et al., 1987; De Silva et al., 2005; Xie et al., 2006). Thus, reduction in 5-HT uptake sites or SERT protein levels would reflect loss of serotonergic axon and terminals. We found a reduction in ¹²⁵I-RTI-55 binding to SERT in the striatum, nucleus accumbens and cingulate cortex. Our results are comparable to previously published reports of MDMA-induced reductions in SERT binding sites in various forebrain regions (Battaglia et al., 1987; Lew et al., 1996), and confirm that the repeated dosing regimen of MDMA used in the present study destroyed 5-HT nerve terminals in several forebrain regions. Taken together, these data suggest that 5-HT containing cell bodies and 5-HT axons terminal are differentially vulnerable to the neurotoxic effects of MDMA.

There is growing body of evidence that repeated MDMA abuse can produce physiological changes in the human brain and can lead to the development of serious cognitive and neuropsychological problems. Several recent PET imaging studies reported serotonergic alterations in the brain of MDMA abusers. McCann et al. (1998) reported significant reductions

in SERT binding sites in several cortical regions, including frontal, cingulate, occipital and parietal cortices as well as in subcortical regions such as putamen, hypothalamus, and midbrain in the MDMA users compared with controls. These *in vivo* imaging studies provide convincing evidence that repeated MDMA abuse can result in persistent functional, structural and serotonergic alterations in the human brain that may lead to an increased risk of behavioral problems or psychiatric illness.

Conclusion

In summary, the present study is the first to demonstrate that chronic MDMA administration decreases TPH2 protein, whereas it increases TPH2 mRNA levels in the cell bodies of serotonergic neurons in the rat DR nucleus. Upregulation of TPH2 mRNA may reflect a compensatory mechanism in the injured 5-HT neurons to increase TPH protein synthesis and restore 5-HT levels. Further studies will be required to understand the mechanisms by which MDMA induces alterations in the biosynthesis of the transcriptional and translational products of the TPH2 gene in the rat DR.

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Abbreviations

CRE	cAMP response-element
DR	dorsal raphe
MDMA	3,4-methylenedioxymethamphetamine
PBS	phosphate-buffered saline
PCPA	p-chlorophenylalanine
PKA	cAMP-dependent protein kinase
SERT	5-HT transporter
TPH	tryptophan hydroxylase
TPH-IR	tryptophan hydroxylase-immunoreactive
5,7-DHT	5,7-dihydroxytryptamine
¹²⁵I-RTI-55	[125I]RTI-55 ((-)-2beta-carbomethoxy-3 beta-(4-iodophenyl) tropane)

Fig. 1.

Effect of chronic administration of MDMA on TPH2 immunoreactivity in the mid (A') and caudal (B') anatomical levels of the DR. Rats were injected twice daily with saline ($n=5$) or MDMA ($n=5$, 20 mg/kg) for 4 days and killed by decapitation 14 days after the last dose of MDMA or saline, as described in Experimental Procedures. Data are expressed as mean $\pm$ S.E.M. for each treatment group. * Significant difference ($P<0.05$, Student's t -test) from control group. (A, B) TPH2 immunoreactivity in the mid DR of saline- (A) and MDMA- (B) injected rats. (C, D) TPH2 immunoreactivity in the caudal DR of saline- (C) and MDMA- (D) injected rats. Compared with controls, note marked decrease in MDMA-treated rats.

Fig. 2. Effect of chronic administration of MDMA on TPH2 mRNA in the mid (A') and caudal (B') anatomical levels of the DR. Data are expressed as mean $\pm$ S.E.M. for each treatment group. * Significant difference ($P < 0.05$, Student's *t*-test) from control group. (A, B) TPH2 mRNA in the mid DR of saline- (A) and MDMA- (B) injected rats. (C, D) TPH2 mRNA in the caudal DR of saline- (C) and MDMA- (D) injected rats. Compared with controls, note marked increase in MDMA-treated rats.

Fig. 3. Effect of chronic administration of MDMA on SERT binding in the striatum, nucleus accumbens and cingulate cortex. Data are expressed as mean $\pm$ S.E.M. for each treatment group.
* Significant difference ($P<0.05$, Student's t -test) from control group.