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(±)3,4-Methylenedioxymethamphetamine (“Ecstasy”) Treatment Modulates Expression of Neurotrophins and Their Receptors in Multiple Regions of Adult Rat Brain

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Abstract

(±)3,4-Methylenedioxymethamphetamine (MDMA), a widely used drug of abuse, rapidly reduces serotonin levels in the brain when ingested or administered in sufficient quantities, resulting in deficits in complex route-based learning, spatial learning, and reference memory. Neurotrophins are important for survival and preservation of neurons in the adult brain, including serotonergic neurons. In this study, we examined the effects of MDMA on the expression of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) and their respective high-affinity receptors, tropomyosin receptor kinase (trk)B and trkC, in multiple regions of the rat brain. A serotonergic-depleting dose of MDMA (10 mg/kg × 4 at 2-hour intervals on a single day) was administered to adult Sprague-Dawley rats, and brains were examined 1, 7, or 24 hours after the last dose. Messenger RNA levels of BDNF, NT-3, trkB, and trkC were analyzed by using in situ hybridization with cRNA probes. The prefrontal cortex was particularly vulnerable to MDMA-induced alterations in that BDNF, NT-3, trkB, and trkC mRNAs were all upregulated at multiple time points. MDMA-treated animals had increased BDNF expression in the frontal, parietal, piriform, and entorhinal cortices, increased NT-3 expression in the anterior cingulate cortex, and elevated trkC in the entorhinal cortex. In the nigrostriatal system, BDNF expression was upregulated in the substantia nigra pars compacta, and trkB was elevated in the striatum in MDMA-treated animals. Both neurotrophins and trkB were differentially regulated in several regions of the hippocampal formation. These findings suggest a possible role for neurotrophin signaling in the learning and memory deficits seen following MDMA treatment.

INDEXING TERMS

BDNF; NT-3; trkB; trkC; MDMA; corticosterone

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The use of the popular illicit drug ($\pm$)3,4-methylene-dioxymethamphetamine (MDMA; Ecstasy) is linked to cognitive deficits in humans, including learning and memory impairments (Parrott, 2000). Recreational use of MDMA in humans affects various aspects of memory performance that can continue even after abstinence from the drug (Morgan, 1999; Gouzoulis-Mayfrank et al., 2000; Verkes et al., 2001) and occur concurrently with serotonergic (5-hydroxytryptamine [5-HT]) system dysfunction (Verkes et al., 2001). Adult animals treated with MDMA exhibit cognitive impairments including deficiencies in Cincinnati water maze learning (a test of spatial and egocentric learning) and in selective aspects of Morris water maze navigation and reference memory (spatial learning and memory) (Sprague et al., 2003; Able et al., 2006; Skelton et al., 2008). Acutely, MDMA induces hyperthermia, locomotor hyperactivity, and head weaving (part of the 5-HT syndrome) in rats (McNamara et al., 1995; Green et al., 2003) as well as reductions in brain 5-HT (O'Callaghan and Miller, 1994) and similar physiological and neurochemical changes in humans (Parrott, 2002).

Many of the behavioral effects of MDMA in animals are associated with neurochemical changes in the 5-HT system (Callaway et al., 1990; White et al., 1996; Green et al., 2003). In addition, we and others have shown that MDMA causes dopamine reductions in the striatum when administered multiple times on a single day (McGregor et al., 2003; Cohen et al., 2005; Able et al., 2006; Skelton et al., 2008). However, the consequences of MDMA exposure for other systems, such as neurotrophic factors, have received little attention. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) not only influence proliferation, neurotransmitter phenotype, migration, and neurite outgrowth during development, but also promote the survival and maintenance of adult neurons. A variety of factors can influence the levels of BDNF and NT-3, including brain injury and physiological activity, as well as stress (Gall and Lauterborn 1992; Smith et al., 1995a; Thoenen, 1995, 2000; Hicks et al., 1997, 1999; Poo, 2001). For example, in the hippocampus, BDNF levels are decreased whereas NT-3 levels are increased after exposure to chronic stress (Smith et al., 1995a; Song et al., 2006). The expression of BDNF is also decreased in the hippocampus after corticosterone administration (Schaaf et al., 1998), and MDMA exposure can increase levels of corticosterone (Nash et al., 1988), suggesting that MDMA may alter neurotrophin expression. Because 5-HT and BDNF have complementary roles in neural plasticity and modulate one another (Mattson et al., 2004), MDMA may interact with both to induce imbalance.

There are data demonstrating that drugs of abuse including MDMA can alter BDNF expression, but less is known about the changes in NT-3 expression. For example, drugs that affect the dopaminergic system, such as amphetamine and cocaine, upregulate BDNF or modulate protein expression in various brain regions including the prefrontal cortex (PFC), piriform cortex, amygdala, striatum, and hypothalamus (Meredith et al., 2002; Fumagalli et al., 2007). Chronic morphine treatment and withdrawal differentially regulate BDNF and NT-3 in the locus coeruleus (LC) (Numan et al., 1998) and nucleus paragigantocellularis (Hatami et al., 2007). Administration of MDMA to adult rats increases BDNF mRNA in the PFC up to 48 hours after injection (Martínez-Turrillas et al., 2006). In contrast, BDNF mRNA levels were decreased in all hippocampal subfields 48 hours to 7 days after MDMA treatment (Martínez-Turrillas et al., 2006). In neonatal rats exposed to MDMA from postnatal days (P) 11–20, BDNF protein levels were increased in the frontal cortex, striatum, hippocampus, and brainstem when examined on P21 (Koprach et al., 2003). However, the short-term effects of MDMA on BDNF and NT-3 after a 5-HT-depleting regimen of the drug in adulthood are not known.

In the present study, we examined the effects of an MDMA binge-dosing regimen on expression of neurotrophins in the brain. Specifically, the mRNA levels of BDNF and NT-3

and their respective high-affinity receptors tropomyosin receptor kinase (trk)B and trkC were examined after MDMA treatment. Because dopaminergic changes have been demonstrated following MDMA administration, we also evaluated tyrosine hydroxylase (TH) mRNA expression levels. Given the wide distribution of neurotrophin expression in the brain, we focused on areas involved in learning and memory and motor control. In addition, plasma levels of corticosterone were determined. We hypothesized that a serotonergic-depleting regimen of MDMA would alter neurotrophin expression in a regionally specific fashion.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley CD IGS rats (250–275 g) were acquired from Charles River Laboratories (Raleigh, NC). Three days after arrival, rats were implanted under isoflurane anesthesia with subcutaneous temperature transponders (IPTT-200, Biomedic Data Systems, Seaford, DE). Animals were housed two per cage under normal conditions (14-hour light/10-hour dark cycle with lights on at 6:00 AM), and all procedures were in compliance with the Cincinnati Children's Research Foundation and University of Cincinnati Institutional Animal Care and Use Committees.

MDMA administration

Rats were randomly assigned to MDMA (10 mg/kg, expressed as the freebase [$>95\%$ pure]; National Institute on Drug Abuse, Bethesda, MD) or saline (SAL) treatment groups. The drug was administered subcutaneously in the dorsum. Beginning at 7:00 AM at an ambient room temperature of $24 \pm 1^\circ\text{C}$, animals were given a total of four doses at 2-hour intervals over a 6-hour period. During this dosing period, body temperatures were monitored every 30 minutes, until 2 hours after the last injection. If an animal's temperature reached 40.2°C , the animal was placed in shallow water until the temperature fell below 40.2°C . This procedure was used to prevent animals from dying due to MDMA-induced hyperthermia, but it does not alter the neurochemical or behavioral effects of the drug (Able et al., 2006; Skelton et al., 2008). Rats were decapitated, and blood and brain tissue were collected at 1, 7, or 24 hours after the last injection ($n = 3\text{--}5/\text{time point/group}$). The brains were removed, mounted onto tissue-freezing medium (Ted Pella, Redding, CA), and frozen in dry ice.

Corticosterone assay

Blood was collected in ice-chilled polypropylene tubes containing 2% EDTA (0.05 ml). Blood was centrifuged at 4°C for 15 minutes. Plasma was aliquoted and stored at -80°C until assayed. Plasma was diluted 3:1 in assay buffer and assayed in duplicate for corticosterone with an enzyme immunoassay kit (Immunodiagnostic Systems, Fountain Hills, AZ).

In situ hybridization

Frozen brains were serially sectioned throughout the rostrocaudal aspects of the PFC, striatum, hippocampus, ventral midbrain, and LC at a thickness of $10\text{ }\mu\text{m}$ in a cryostat and thaw-mounted onto Superfrost Plus microslides (VWR, Batavia, IL). Sections were stored at -20°C until hybridization. Adjacent sections from each area and time point were subsequently hybridized with ^{35}S -labeled cRNA probes for detection of BDNF, trkB, NT-3, and trkC mRNAs, as well as for TH mRNA in the ventral midbrain and LC.

For pretreatment and hybridization of tissue sections, the slides were processed as described previously (Seroogy and Herman, 1997; Numan and Seroogy, 1997; Numan et al., 2005; Braun et al., 2011). Briefly, for pretreatment, the slides were brought to room temperature

and fixed in 4% paraformaldehyde (pH 7.4) for 10 minutes. The slides were subsequently placed in a series of washes made with diethyl pyrocarbonate (DEPC)-treated water consisting of 0.1 M phosphate-buffered saline (PBS; pH 7.4), followed by 0.1 M PBS/0.2% glycine, and again 0.1 M PBS. The sections were then washed in triethanolamine (pH 8.0) containing 0.25% acetic anhydride for 10 minutes. Lastly, the sections were dehydrated in a sequence of ethanol washes of increasing concentration, delipidated in chloroform, and air-dried.

The probes used for hybridization were prepared from linearized cDNA plasmids by using the proper RNA polymerase and labeled with ³⁵S-UTP (PerkinElmer, Boston, MA). The BDNF and NT-3 cDNA plasmids (gifts from Christine Gall and Julie Lauterborn, University of California at Irvine) produced RNA transcripts of 540 and 550 bases, respectively. The BDNF cRNA included 384 bases complementary to the rat BDNF mRNA coding region (nucleotides 388–771; Isackson et al., 1991; Gall et al., 1992; Scarisbrick et al., 1999; GenBank accession number: NM_012513.3), whereas the NT-3 cRNA was complementary to 392 bases of the rat NT-3 mRNA coding region (nucleotides 481–873; Ernfors et al., 1990; Maisonpierre et al., 1990; Gall et al., 1992; Scarisbrick et al., 1999; GenBank accession number: NM_031073.2). The trkB and trkC plasmids resulted in antisense RNA transcripts of 200 and 577 bases, respectively (Lamballe et al., 1991; Jelsma et al., 1993; Dixon and McKinnon, 1994). The trkB riboprobe detects the kinase-specific, full-length form of the rat trkB receptor (nucleotides 1358–1558; Middlemas et al., 1991; Goodness et al., 1997; GenBank accession number: NM_012731.1), whereas the trkC riboprobe recognizes both the full-length and truncated forms of the rat trkC receptor (nucleotides 272–829; Valenzuela et al., 1993; Dixon and McKinnon, 1994; GenBank accession number: NM_019248.1). The TH cDNA construct for rat TH (kindly provided by James Herman, University of Cincinnati) resulted in an RNA transcript of 372 bases (nucleotides 62–433; Zhang et al., 2010; GenBank accession number: NM_012740).

The ³⁵S-labeled probes were generated as previously described (Seroogy and Herman, 1997) by using the RNA polymerase specific for each vector (T3 for BDNF and NT-3; T7 for TH, trkB, and trkC). The hybridization solution for each probe contained the following: deionized formamide, 50% dextran sulfate, 10 mg/ml denatured salmon sperm DNA, 15 mg/ml tRNA, 5 M dithiothreitol, DEPC H₂O, and the appropriate ³⁵S-labeled cRNA probe. The tissue was hybridized with 50 µl of the solution and coverslipped. The resulting concentration of the hybridization solution was 1×10^6 cpm/slide. The slides were then placed in a sealed, humidified chamber and incubated at 60°C for 18–24 hours. Following posthybridization treatment, the slides were exposed to BioMax MR film (Kodak, Rochester, NY) for the appropriate amount of time for each probe (TH, 1 day; BDNF, 10–15 days; trkB, 8–17 days; NT-3, 18–26 days; trkC, 5–9 days) for detection and localization of the signal. The films were subsequently developed with GPX developer and fixer (both Kodak).

Analysis

The film autoradiograms were analyzed by using Scion Image (NIH) software. The optical density (OD) of the hybridization signal for each probe was obtained at each time point to compare densities of hybridization between MDMA- and SAL-treated animals. Anatomical delineations were determined according to the rat brain atlas of Paxinos and Watson (2007). The regions of interest measured are shown schematically in Figure 1. Measurements were taken from four to six sections per region analyzed per animal for each cRNA probe. Care was taken to ensure that film images were not saturated, as illumination parameters with Scion Image were set to avoid the possibility of saturation (mean gray levels for all densitometric measurements were well under 256). A background control value was taken by measuring an unlabeled region on each section (white matter, e.g., corpus callosum), and

the value was subtracted from the OD of the region examined. The resulting value is the mean corrected gray level.

The MDMA data are shown as a percentage of the control values. The hybridization data were analyzed by using t-tests at each time point. A regression analysis was performed to determine possible correlations of hippocampal BDNF mRNA levels with systemic corticosterone levels. Temperature and corticosterone data (treatment $\times$ time point) were analyzed by using factorial analysis of variance (ANOVA; time was a within-subject factor for temperature and a between-subject factor for corticosterone). Differences were considered significant at $P < 0.05$; trends were noted at $P < 0.10$. Photomicrograph images were prepared in PowerPoint and modified to the correct size in Adobe Photoshop (Adobe Systems, San Jose, CA). No adjustments to contrast or brightness were made.

RESULTS

Temperature and corticosterone levels

The body temperature of the rats was monitored during and 2 hours after treatment. Prior to administration of MDMA or SAL, there were no significant differences between groups. The main effect of treatment approached significance, $F(1,15) = 4.20$, $P < 0.06$, and the treatment $\times$ time interaction was significant, $F(16,240) = 16.55$, $P < 0.0001$. Following the first dose at 30 minutes, MDMA-treated animals had lower body temperatures than SAL-treated rats (Fig. 2A). However, the temperature of MDMA-treated rats steadily increased and from 240 to 480 minutes (with the exception of 270 minutes), the temperatures of MDMA-treated animals were significantly higher than those of SAL animals.

Plasma was assessed for corticosterone at 1, 7, and 24 hours after the last dose. Corticosterone levels were increased in MDMA-treated animals overall (Fig. 2B; $F(1,23) = 7.54$, $P < 0.02$). The interaction of treatment $\times$ time was not significant, although time was significant ($P < 0.001$). Animals showed elevated corticosterone levels at the 1- and 7-hour time points compared with the 24-hour time point.

Alterations in gene expression

BDNF mRNA—Changes in the expression of BDNF were the most prevalent of the five mRNAs examined. In the PFC (Fig. 3A, B, E), BDNF expression was increased in MDMA-treated animals 1 hour after the last dose ($t(4) = 3.71$, $P < 0.05$), and remained elevated at 7 hours ($t(8) = 5.60$, $P < 0.001$) compared with SAL-treated animals. No differences were noted at 24 hours. The increase in BDNF expression was seen throughout the entire PFC.

In both frontal and parietal cortices, a similar pattern of alterations in BDNF mRNA expression was observed. In the frontal cortex (Fig. 3C, D, F), BDNF hybridization signal was elevated in MDMA-treated rats at the 1-hour ($t(4) = 3.81$, $P < 0.05$) and 7-hour ($t(8) = 3.82$, $P < 0.01$) time points compared with SAL-treated rats. There were no significant changes at 24 hours. In the parietal cortex (Fig. 3C, D, G), the cRNA labeling was increased significantly only at 7 hours in the MDMA-treated animals compared with the controls ($t(8) = 9.86$, $P < 0.05$). A trend toward increased BDNF mRNA levels was also seen at 1 hour ($P = 0.06$).

BDNF cRNA hybridization in the hippocampal CA1 region (Fig. 4A–F, G) remained unchanged at 1 and 7 hours following MDMA, but was significantly elevated at 24 hours ($t(7) = 3.94$, $P < 0.01$) compared with SAL-treated animals. In contrast to the increases in labeling observed in the cortical and CA1 regions, MDMA-treated rats exhibited a decrease in BDNF hybridization signal in the CA3 region (Fig. 4A–F, H), at 1 hour ($t(7) = 2.35$, $P < 0.05$) and 7 hours ($t(8) = 5.09$, $P < 0.01$) after the last dose compared with controls. A

similar pattern was detected in the stratum granulosum of the dentate gyrus, where expression of BDNF mRNA was decreased at both 1 hour ($t(7) = 5.06$, $P < 0.01$) and 7 hours ($t(8) = 9.53$, $P < 0.001$) after MDMA treatment compared with controls (Fig. 4A–F, I). By 24 hours, expression levels in the both the CA3 and dentate gyrus regions were not significantly different from those of controls.

Significant increases in BDNF mRNA hybridization were observed in both the piriform and entorhinal cortices. In the piriform cortex (Fig. 5), BDNF levels were elevated in the MDMA-treated rats at 1 hour ($t(4) = 4.74$, $P < 0.01$) and 7 hours ($t(8) = 4.258$, $P < 0.01$), and although the increase diminished by 24 hours post-MDMA, it remained significant ($t(7) = 2.62$, $P < 0.05$) relative to controls. In the entorhinal cortex (Fig. 6), elevated BDNF hybridization signal was relatively constant throughout the times examined, with increases at 1 hour ($t(8) = 3.42$, $P < 0.05$), 7 hours ($t(8) = 2.94$, $P < 0.05$), and 24 hours ($t(7) = 2.98$, $P < 0.05$) after MDMA treatment compared with SAL controls.

In the substantia nigra pars compacta (SNpc), a significant elevation in BDNF mRNA levels was seen only at 24 hours in the MDMA-treated rats compared with controls ($t(8) = 2.56$, $P < 0.05$) (Fig. 7). Additional areas analyzed for changes in BDNF expression included the anterior cingulate cortex (AC Ctx), ventral tegmental area (VTA), and LC. No significant differences in hybridization were observed in these regions (data not shown).

trkB mRNA—Expression of trkB, the high-affinity receptor for BDNF, was examined in the same regions as BDNF. Although hybridization for trkB mRNA was not as plastic as that of BDNF, receptor expression was altered in several areas. In the striatum (Fig. 8A, B, G), trkB mRNA hybridization was elevated in MDMA-treated rats compared with controls at 1 hour ($t(4) = 4.66$, $P < 0.01$) and 7 hours ($t(8) = 8.63$, $P < 0.001$), but not 24 hours. Whereas no changes in trkB mRNA expression were observed in the PFC at the 1- and 7-hour time points, trkB mRNA labeling was significantly increased in the MDMA-treated rats at the 24-hour time point ($t(7) = 2.81$, $P < 0.05$) compared with SAL controls (Fig. 8C, D, H). Similar to BDNF, trkB mRNA levels were increased in the hippocampal CA1 region in MDMA-treated rats at 24 hours ($t(7) = 4.08$, $P < 0.01$) compared with SAL-treated rats (Fig. 8E, F, I). No significant changes were observed in the other regions of the hippocampal formation, in other cortical regions, or in the SNpc, VTA, or LC (data not shown).

NT-3 mRNA—In the PFC (Fig. 9A, B, G), NT-3 mRNA hybridization was increased in medial aspects (infralimbic and prelimbic regions) in the MDMA-treated rats compared with controls at 1 hour after the last dose ($t(4) = 11.25$, $P < 0.001$), but returned to control levels by 7 hours post-treatment and remained so at 24 hours. In the AC Ctx (Fig. 9C, D, H), NT-3 expression was elevated in MDMA animals compared with controls at both 1 hour ($t(4) = 4.78$, $P < 0.01$) and 7 hours ($t(7) = 2.95$, $P < 0.05$), but expression was similar by 24 hours. A slight, but significant, increase in NT-3 mRNA hybridization was detected in the dentate gyrus of MDMA-treated rats at the 1-hour time point ($t(6) = 2.94$, $P < 0.05$) compared with SAL-treated rats (Fig. 9E, F, I). However, by 7 hours after the last dose, a slight decrease in NT-3 mRNA expression was evident in the dentate gyrus of MDMA-treated rats ($t(8) = 2.92$, $P < 0.05$) (Fig. 9I), with similar expression at 24 hours compared with SAL controls. No alterations in NT-3 mRNA levels were found in the frontal, parietal, piriform, or entorhinal cortices, the CA2 region of the hippocampus, the SNpc, the VTA, or the LC at any of the time points after the MDMA regimen (data not shown).

trkC mRNA—Due to a freezer malfunction, the tissue from the 1-hour time point of the PFC and striatum was lost before it could be processed for trkC hybridization. In the entorhinal cortex, trkC mRNA levels were increased only at 1 hour post-treatment ($t(8) = 2.34$, $P < 0.05$) in MDMA-treated rats compared with SAL-treated controls (Fig. 10A, B,

G). In contrast, in the LC, trkC mRNA levels were decreased 7 hours post-MDMA ($t(6) = 4.67$, $P < 0.01$) compared with SAL-treated animals (Fig. 10C, D, H). Although no changes in trkC mRNA levels were detected in the PFC at 7 hours after the last dose, hybridization was significantly increased at 24 hours ($t(6) = 2.79$, $P < 0.05$) in animals treated with MDMA compared with SAL controls, and this was restricted to the inner cortical layers (Fig. 10 E, F, I). No alterations in trkC hybridization signal were found in the striatum, frontal, parietal, anterior cingulate or piriform cortices, hippocampal formation, SNpc, or VTA (data not shown).

TH mRNA—Levels of TH mRNA were examined in the SNpc, VTA, and LC following MDMA administration, and no significant changes in expression were noted at any time point (data not shown).

Hippocampal BDNF mRNA and corticosterone correlations

Because previous studies have shown that corticosterone regulates BDNF expression in the hippocampus (Schaaf et al., 1997, 1998), correlations of BDNF mRNA in the various subregions of the hippocampal formation with corticosterone levels were examined. For the CA1 region and dentate gyrus, there were negative correlations of corticosterone and BDNF mRNA at the 1-hour time point ($r = -0.72$ and -0.82 , for both, $P < 0.05$, respectively). No significant correlations were observed for the CA3 region and BDNF mRNA at the 1-hour time point. For the 7- and 24-hour time points, no significant correlations were observed in any region.

DISCUSSION

The present results demonstrate that MDMA treatment differentially regulates gene expression of neurotrophins and their receptors in multiple regions of the rat brain (see Table 1 for summary). In the forebrain, levels of BDNF mRNA were upregulated in several areas at different intervals after MDMA treatment, whereas increases in NT-3 expression were restricted to the prefrontal and anterior cingulate cortices, as well as the dentate gyrus. Both trkB and trkC receptor mRNAs were elevated by MDMA in the PFC only at 24 hours. Increased expression of trkC was found in the entorhinal cortex, whereas trkB mRNA levels were upregulated in the hippocampal CA1 region. The MDMA-induced modulation of neurotrophins and their high-affinity receptors was not limited to cortical and limbic regions. Alterations were also observed in the nigrostriatal system, with expression of BDNF upregulated in the SNpc and trkB in the striatum. Moreover, in addition to the above-noted increases in neurotrophin expression following MDMA administration, several brain regions exhibited decreased hybridization, including the hippocampal CA3 region and dentate gyrus for both BDNF and NT-3, and the locus coeruleus for trkC. Axons projecting from the dorsal raphe nuclei to the forebrain, cortex, striatum, and hippocampus exhibit MDMA-associated morphological changes (O'Hearn et al., 1988), so it was not entirely unexpected that MDMA would have effects on neurotrophin expression in these regions, but the exact pattern was unknown. Overall, the present data indicate that MDMA-induced modulation of BDNF and trkB mRNA levels are more widespread than previously appreciated (Martínez-Turrillas et al., 2006). This is also the first study to demonstrate alterations in NT-3 and trkC expression following MDMA exposure.

Martínez-Turrillas et al. (2006) also examined the effects of MDMA on BDNF in the hippocampus and PFC of adult rats, and some different patterns were observed in that study compared with ours, although only the 24-hour time point overlapped between experiments. For example, increases in BDNF mRNA were noted in the frontal cortex at 24 and 48 hours after a single dose of MDMA (10 mg/kg), with decreases in hippocampal regions CA1,

CA3, and the dentate gyrus at 48 hours and 7 days after MDMA (Martínez-Turrillas et al. 2006). In the current experiment, no decrease in BDNF mRNA in the CA3 region or dentate gyrus at 24 hours was noted (though there were decreases at the 1- and 7-hour time points), and we found that BDNF mRNA in the CA1 region increased at 24 hours following 4 doses of MDMA. No significant increases similar to those observed by Martínez-Turrillas et al. (2006) in BDNF in the PFC at 24 hours were found.

The differences in BDNF expression between the two studies may be attributable to dosing regimens. Martínez-Turrillas et al. (2006) administered a single dose of MDMA (10 mg/kg), whereas in the present study MDMA (10 mg/kg) was administered four times with a 2-hour interval between each dose. The current dosing regimen was chosen because it and very similar regimens are widely reported in the literature to induce long-term 5-HT reductions (Shankaran and Gudelsky, 1999; Morley et al., 2001; Shankaran et al., 2001; Clemens et al., 2004; Bauman et al., 2007, 2008; Bhide et al., 2009) and, therefore, models similar changes reported in human chronic MDMA users. Nonetheless, both experiments demonstrate that MDMA has significant regionally specific effects on neurotrophins.

Although we did not examine these rats specifically for neurotransmitter loss, we have previously found that this dosing regimen results in serotonin depletion in the hippocampus, striatum, and prefrontal cortex, as well as dopamine loss in the striatum (Able et al., 2006; Skelton et al. 2008). These neurotransmitter alterations may play a role in the changes of neurotrophin expression. Neurotrophins, particularly BDNF, play an important role in synaptic plasticity. For example, an increase in BDNF levels can cause dendritic growth and can result in enhanced synaptic efficacy (Thoenen, 1995). Plasticity is important for cognition and plays a role in perception, learning, and memory (Black, 1999). This implies that alterations in neurotrophins within regions important for cognition, learning, and memory, particularly the hippocampus and various cortical regions, may have behavioral consequences (Rattiner et al., 2005).

We and others have shown that MDMA exposure produces lasting effects on learning and memory following exposure in adolescent or adult animals (Sprague et al., 2003; Piper and Meyer, 2004; Able et al., 2006; Skelton et al., 2008). Subregions of the hippocampus play roles in learning and memory processes, including spatial learning and novelty detection (Kesner et al., 2004). The loss of neurotrophic factors, as observed in this study, may increase the susceptibility of hippocampal neurons to maladaptive plasticity, which over a period of time could contribute to learning and memory changes. The decrease in BDNF may be tied to the increase in corticosterone that was seen in MDMA-treated animals throughout the dosing period. Elevation of corticosterone has been linked to a decrease of BDNF expression in the hippocampus in several animal models (Smith et al., 1995a; Schaaf et al., 1997, 1998; Song et al., 2006). It is unclear whether the increases in BDNF, trkB, and NT-3 mRNAs observed in the hippocampal subfields result in increased protein levels that are sufficient to protect these neurons from injury or whether the loss of BDNF expression in the earlier time points results in an environment permissive to the harmful effects of MDMA exposure and the associated increased corticosterone levels.

Why the hippocampus is vulnerable to decreases in BDNF mRNA expression compared with other regions after MDMA exposure is not fully understood. The time points examined were within 24 hours after MDMA exposure, so it is unlikely that the reduction in BDNF expression in the hippocampus is the result of neuronal loss. It seems more likely that increased corticosterone in combination with reduced 5-HT altered BDNF expression acutely. In this regard, activation of 5-HT receptors can result in activation of cyclic adenosine monophosphate response element binding (CREB) activation and stimulation of transcription of the BDNF gene (Mattson et al., 2004). It is possible that the acute release of

5-HT after MDMA exposure activated 5-HT receptors and caused an upregulation of BDNF mRNA levels as noted in the CA1 region. How this might result in a decrease in BDNF in the CA3 region and dentate gyrus is unknown. Serotonin is depleted in the hippocampus after MDMA exposure (García-Osta et al., 2004), and reductions may occur more quickly and/or to a greater extent in some subregions relative to others. Regardless of the mechanism, the regionally specific decreases in BDNF expression might explain why some cognitive skills are affected by MDMA, but not others.

Both the PFC and the AC Ctx are important areas in the regulation of stress, memory, and mood, so it is not surprising that MDMA affected both neurotrophins and their receptors in these regions. Serotonin content in forebrain is reduced by MDMA (Green et al., 2003), and these reductions are long-lasting (Mayerhofer et al., 2001) but not permanent. In human users, there is evidence of specific effects on executive functions after MDMA use, and these functions are associated with the PFC (Reay et al., 2006). Both NT-3 and BDNF were increased in the PFC shortly after treatment, whereas their receptors were not upregulated until 24 hours later. We found no significant changes in BDNF mRNA in the AC Ctx after MDMA, although a loss of BDNF in this region after exposure to stress has been reported (Smith et al., 1995c). Instead, we observed elevations of NT-3 mRNA at the early post-MDMA time points that were not maintained at 24 hours.

Following MDMA exposure, BDNF expression was elevated at 7 hours in the parietal cortex. Loss of 5-HT cell bodies has been described in this region following MDMA exposure (Schmued, 2003). In addition, the parietal cortex sends projections to the entorhinal cortex, which has an important function in egocentric learning (Parron and Save, 2004). We have previously reported egocentric deficits in the Cincinnati water maze after treatment with MDMA (Able et al., 2006; Skelton et al., 2008). However, these deficits are seen at longer intervals after MDMA treatment and cannot be directly attributed to the BDNF expression changes seen here. The entorhinal cortex also plays an important role in transmitting sensory information to the hippocampus, as well as receiving output from hippocampal subfields (Kerr et al., 2007). BDNF mRNA levels were upregulated at all three time points, and trkB receptor mRNA was elevated at 1 hour in the entorhinal cortex. Both BDNF and NT-3 can be retrogradely transported by neurons in both the hippocampus and the entorhinal cortex (DiStefano et al., 1992).

Levels of BDNF mRNA were increased at all three time points in the piriform cortex. This region is part of the olfactory system, and extends projections to the medial PFC. Olfactory perception is dependent on learning and memory, and the piriform cortex plays an important part in object recognition and discrimination of odors (Wilson and Stevenson, 2003; Keller et al., 2005). Expression of BDNF mRNA increases in the piriform cortex after exposure to stressors (Nibuya et al., 1995; Katoh-Semba et al., 1999), and it has been suggested that such increases serve to protect neurons (Nibuya et al., 1995), but may not always be fully effective (Schmidt-Kastner et al., 1996). Upregulation of BDNF mRNA is also observed in the piriform and entorhinal cortices during the formation of recognition memory (Broad et al., 2002), and there are reports that MDMA interferes with novel object recognition learning (Morley et al., 2001; Piper and Meyer, 2004; but see Able et al., 2006).

Neurotrophin expression levels were also altered in regions outside cortical and limbic regions. We observed MDMA-induced changes within the striatum, with trkB mRNA expression exhibiting a substantial increase at the 1- and 7-hour time points. This striatal trkB upregulation paralleled the similarly large increase in BDNF expression in the cerebral cortex (PFC, frontal and parietal cortices; see Fig. 3) at the same early post-MDMA time points. Because BDNF produced in the cortex is anterogradely transported to the striatum (Altar et al., 1997), such temporal correspondence may suggest MDMA-induced enhanced

BDNF/trkB trophic communication in corticostriatal systems, at least early on following drug administration. In the SNpc, levels of BDNF mRNA were increased by 24 hours post-MDMA. BDNF and NT-3, as well as their receptors trkB and trkC, are synthesized by dopaminergic neurons in the SNpc (Seroogy et al., 1994; Numan and Seroogy, 1999). Nigral BDNF is also anterogradely transported to the striatum where it activates trkB receptors (Altar et al., 1994, 1997). The delayed elevation of nigral BDNF mRNA (at the 24-hour time point) relative to the early striatal trkB mRNA increase (at the 1- and 7-hour time points) is less consistent with MDMA-induced augmented nigrostriatal neurotrophin signaling because the striatal trkB levels have already returned to control levels by 24 hours post-treatment. Finally, we observed no changes in TH mRNA within the SNpc at any post-MDMA time point. It should be noted that upregulation of trkB mRNA in the striatum has also been seen following administration of the catecholamine-specific neurotoxin 6-hydroxydopamine (Numan and Seroogy, 1997), probably in response to the loss of dopaminergic afferent innervation. The present results raise the possibility that loss of 5-HT afferent innervation similarly induces a compensatory elevation of trkB expression in the striatum.

In the LC, a region that contains noradrenergic neurons, chronic stress causes an upregulation in NT-3 and TH mRNA expression (Smith et al., 1995b), whereas BDNF mRNA is decreased (Hemmerle et al., 2007). The LC was the only other area besides the hippocampus to demonstrate a decrease in mRNA expression after MDMA exposure, in this case in trkC mRNA at 7 hours. However, there were no changes in either NT-3 or BDNF mRNAs.

In conclusion, MDMA has selective regional effects on BDNF and NT-3 mRNAs. Now that we have established that there are changes in expression after MDMA treatment in regions associated with cognition, memory, and egocentric learning, future studies assessing longer intervals will be necessary to elucidate the relationship between the behavioral changes and neurotrophin expression.

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Figure 1.

A–E: Schematic representations (modified from the atlas of Paxinos and Watson, 2007) of specific brain regions measured. SNpc, substantia nigra pars compacta; VTA, ventral tegmental area; DG, dentate gyrus; CA1, hippocampal region CA1; CA3, hippocampal region CA3.

Figure 2.

Temperature and corticosterone measurements. **A:** Body temperature recordings of saline (SAL)- and MDMA-dosed rats during and for 2 hours after treatment. Arrows show when each dose was given. As expected by the end of dosing, there was a hyperthermic response to MDMA. **B:** Corticosterone levels of SAL- and MDMA-treated rats at 1, 7, and 24 hours after the last dose. There was a treatment main effect of MDMA on corticosterone levels. *, $P < 0.05$.

Figure 3.

A, B Film autoradiographs showing enhanced expression of BDNF mRNA in prefrontal cortex at 7 hours after MDMA treatment. **C, D**: Film autoradiographs showing increased BDNF expression in frontal (Fr Ctx) and parietal (Par Ctx) cortices at 7 hours post-MDMA administration. **E–G**: Quantification of the hybridization signal revealed increased expression of BDNF mRNA at both 1 and 7 hours post-administration of MDMA in the prefrontal cortex (E) and frontal cortex (F). In the parietal cortex (G) there was only a significant increase of mRNA expression at 7 hours, with a trend toward an increase noted at 1 hour post-MDMA. Open bar, saline-treated; black bar, MDMA-treated. Data are expressed as mean $\pm$ SEM. #, $P = 0.06$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with respective control values. Scale bar = 1,000 μ m in A (applies to A, B) and C (applies to C, D).

Figure 4.

A–F: Film autoradiograms showing BDNF cRNA hybridization in rostral hippocampal formation of saline-treated (Control; A, C, E) and MDMA-treated (B, D, F) rats at 1, 7, and 24 hours after last exposure. **G–I:** Densitometric analysis of BDNF mRNA expression in the hippocampal formation of MDMA- and SAL-treated rats. **G:** Expression of BDNF mRNA was increased in region CA1 of the hippocampus at 24 hours after MDMA treatment. **H:** In region CA3 of the hippocampus a decrease in mRNA expression was detected at 1 and 7 hours. **I:** BDNF mRNA levels in the stratum granulosum of the dentate gyrus (DG) were decreased at both 1 and 7 hours. Open bar, saline-treated; black bar, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with respective control values. Scale bar = 1,000 μ m in A (applies to A–F).

Figure 5.

A, B Representative autoradiograms of BDNF expression in the piriform cortex (Pir Ctx) at 7 hours post-MDMA treatment. **C:** Expression of BDNF mRNA in the Pir Ctx after MDMA exposure. Quantification of in situ hybridization signal revealed a significant increase in BDNF at 1, 7, and 24 hours post-MDMA administration. Open bar, saline-treated; black bar, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$ compared with respective control values. Scale bar = 500 μ m in A (applies to A, B).

Figure 6.

A, B Representative autoradiograms of BDNF expression in the entorhinal cortex (Ent Ctx) at 7 hours after last MDMA exposure. **C**: Levels of BDNF mRNA expression are increased in the Ent Ctx at 1, 7, and 24 hours post-MDMA administration. Open bar, saline-treated; black bar, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$ compared with respective control values. Scale bar = 500 μ m in A (applies to A, B).

Figure 7.

A, B BDNF expression in the substantia nigra pars compacta (SNpc) at 24 hours post-MDMA treatment. **C:** Quantification indicated that BDNF mRNA levels are increased significantly in the SNpc 24 hours after MDMA administration. Open bars, saline-treated; black bars, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$. Scale bar = 500 μ m in A (applies to A, B).

Figure 8.

A–F: Film autoradiograms showing elevated *trkB* mRNA expression in MDMA- versus saline-treated (Control) animals in the striatum (A, B), prefrontal cortex (PFC) (C, D), and region CA1 of the hippocampus (E, F) at various time points post-MDMA treatment. **G–I:** Densitometric analysis of *trkB* mRNA expression in the striatum, PFC, and hippocampus. **G:** Elevated levels of *trkB* mRNA were detected in the striatum at 1 and 7 hours post-MDMA. **H:** An increase of *trkB* mRNA was seen in the PFC at 24 hours after MDMA treatment. **I:** Hybridization signal for *trkB* mRNA was increased in the CA1 region of the hippocampus only at 24 hours post-MDMA. Open bar, saline-treated; black bar, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with respective control values. Scale bar = 1,000 μ m in A (applies to A, B), C (applies to C, D), and E (applies to E, F).

Figure 9.

A–F: Film autoradiograms showing modulation of NT-3 mRNA expression in the prefrontal cortex (PFC; A, B), anterior cingulate cortex (AC Ctx; C, D), and hippocampus (E, F) at various time points following MDMA administration. **G–I:** Densitometric analysis of NT-3 mRNA expression in limbic cortical regions and hippocampal dentate gyrus. **G:** Expression of NT-3 mRNA is increased in the medial PFC 1 hour after MDMA administration, but returns to control levels by 7 hours after exposure. **H:** In the AC Ctx, NT-3 mRNA levels are increased at 1 and 7 hours post-administration of MDMA. **I:** Levels of NT-3 mRNA in the dentate gyrus (DG) initially are increased at the 1-hour time point and then decreased at the 7-hour time point before returning to control levels by 24 hours after MDMA. Open bars, saline-treated; black bars, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with respective control values. Scale bar = 500 μ m in A (applies to A, B), C (applies to C, D), and E (applies to E, F).

Figure 10.

A–F: Film autoradiograms showing modulation of trkC mRNA expression in the entorhinal cortex (Ent Ctx; A, B), locus coeruleus (LC; C, D), and prefrontal cortex (PFC; E, F) at various time points following MDMA administration. **G–I:** Densitometric analysis of trkC mRNA expression in cortical regions and LC. **G:** In the Ent Ctx, expression of trkC is increased at 1 hour post-MDMA administration and returns to control levels by 7 hours. **H:** Quantification of trkC expression in the LC reveals a decrease at 7 hours with no changes detected at other time points. **I:** Expression of trkC mRNA in the PFC is increased at 24 hours after MDMA administration. Open bar, saline-treated; black bar, MDMA-treated. Data are expressed as mean $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$ compared with respective control values. Scale bar = 1,000 μ m in A (applies to A, B); 500 μ m in C (applies to C, D) and E (applies to E, F).

TABLE 1

Summary of MDMA-Induced Neurotrophin Alterations by Brain Region

Brain region	Neurotrophin/receptor											
	BDNF			trkB			NT-3			trkC		
	1 h	7 h	24 h	1 h	7 h	24 h	1 h	7 h	24 h	1 h	7 h	24 h
Prefrontal cortex	↑	↑	↔	↔	↔	↑	↑	↔	↔	nd	↔	↑
Anterior cingulate cortex	↔	↔	↔	↔	↔	↔	↑	↑	↔	nd	↔	↔
Frontal cortex	↑	↑	↔	↔	↔	↔	↔	↔	↔	nd	↔	↔
Parietal cortex	↔	↑	↔	↔	↔	↔	↔	↔	↔	nd	↔	↔
Piriform cortex	↑	↑	↑	↔	↔	↔	↔	↔	↔	nd	↔	↔
Entorhinal cortex	↑	↑	↑	↔	↔	↔	↔	↔	↔	↑	↔	↔
Striatum	na	na	na	↑	↔	↔	na	na	na	nd	↔	↔
Hippocampal CA1 region	↔	↔	↑	↔	↔	↑	na	na	na	↔	↔	↔
Hippocampal CA3 region	↓	↓	↔	↔	↔	↔	na	na	na	↔	↔	↔
Dentate gyrus	↓	↓	↔	↔	↔	↔	↑	↓	↔	↔	↔	↔
Ventral tegmental area	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
Substantia nigra pars compacta	↔	↔	↑	↔	↔	↔	↔	↔	↔	↔	↔	↔
Locus coeruleus	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔

Abbreviations: na, not applicable, i.e., no measurable expression is found in these regions; nd, not determined; BDNF, brain-derived neurotrophic factor; MDMA, (±)3,4-methylenedioxymethamphetamine; NT-3, neurotrophin-3; trk, tropomyosin receptor kinase; ↑, increased expression; ↓, decreased expression; ↔, no change in expression.