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Dissociation between Serotonin Neurotoxicity and Brain-Derived Neurotrophic Factor Induction following Neonatal MDMA Exposure in Rats

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Key Words
3,4-Methylenedioxymethamphetamine · Brain-derived neurotrophic factor · Ecstasy · Rats · Serotonin transporter

Abstract
Early developmental treatment of rats with 3,4-methylenedioxymethamphetamine (MDMA) was previously found to cause an abnormal pattern of forebrain serotonergic axon density in adulthood consisting of a cortical hypoinnervation and a striatal hyperinnervation. The present study tested the hypothesis that this reorganization was due to regional differences in brain-derived neurotrophic factor (BDNF) expression. Rats received MDMA (10 mg/kg, s.c., b.i.d.) on postnatal days (PD) 1–4, after which brain tissues were collected on PD 11, 30, and 67 for analysis. BDNF protein levels were found to be elevated in the occipital cortex but not in the hippocampus or striatum following MDMA administration. Serotonin transporter binding (an index of serotonergic fiber integrity) was significantly reduced in the hippocampus at PD 11 but returned to normal by PD 30, whereas the cortex exhibited a delayed reduction that was not manifested until PD 30. These results do not support the view that a region-specific enhancement in BDNF expression mediates the abnormal serotonergic reinnervation observed following neonatal MDMA exposure.

B.J.P. and J.D.F. contributed equally to this work.

Introduction

The chemical 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') is a popular recreational drug, particularly in the United States, western Europe, and Australia [Degenhardt et al., 2004; Johnson et al., 2007; UNODCC, 2007]. Acutely, MDMA induces neuronal release of serotonin (5-HT), dopamine, and norepinephrine, and also inhibits the reuptake of these monoamines [Green et al., 2003]. In the long-term, MDMA damages serotonergic axons in adult rats and possibly also humans [Boot et al., 2000; Callahan et al., 2001; Ricaurte et al., 2000]. As many ecstasy users are women of reproductive age [Ho et al., 2002], it is important to fully appreciate the consequences of early developmental MDMA exposure. We recently demonstrated an increase in the number of cells immunoreactive for cleaved caspase-3, an indicator of apoptosis, as well as significant reductions in serotonin transporter (SERT) binding in various brain areas after neonatal MDMA exposure in rats [Meyer et al., 2004]. Nine months after the drug treatments, SERT-immunoreactive fiber density was found to be decreased in the visual and somatosensory cortices, but increased in the dorsal caudate-putamen and nucleus accumbens. Normal fiber density was observed in the hippocampus and lateral hypothalamus. In consideration with other research [Hatzidimitriou et al., 1999; Kelly et al. 2002; Winslow and Insel, 1990], these findings suggest that MDMA may cause a reorganization of the ascending serotonergic pathways, reflecting differential regrowth of serotonergic

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axons in different terminal areas after initial drug-induced damage.

The molecular mechanisms underlying the regrowth and reorganization of damaged serotonergic fibers are not well understood. However, one candidate molecule that has been implicated in such regrowth is brain-derived neurotrophic factor (BDNF). The serotonergic neurons of the raphe nuclei of rats express mRNA for trkB, the high-affinity tyrosine kinase receptor for BDNF [Madhav et al., 2001]. The presence of trkB indicates that these cells are potential targets of BDNF or other neurotrophins such as neurotrophin-3 and neurotrophin-4/5, which can also activate trkB. Several studies have suggested a possible role for BDNF in serotonergic fiber sprouting following injury. For example, manipulations that increase local BDNF concentrations were found to stimulate the sprouting of descending serotonergic axons following axotomy by spinal transection [Bregman et al., 1997; Jin et al., 2000]. More recently, Ramer et al. [2007] showed that interference with trkB function blocked spinal serotonergic fiber sprouting after dorsal root injury. Most importantly, in rats treated with the 5-HT neurotoxin *p*-chloroamphetamine, sprouting of cortical serotonergic fibers following drug-induced denervation was promoted either by local infusion of BDNF protein [Mamounas et al., 2000] or by BDNF overexpression using an adenoviral vector [Grider et al., 2005].

Considering the above-cited evidence for BDNF-mediated serotonergic fiber growth, the present study tested the hypothesis that regional differences in BDNF expression are responsible for the differential pattern of forebrain serotonergic reinnervation previously observed after neonatal MDMA [Meyer et al., 2004]. We predicted that BDNF would be up-regulated in all forebrain regions following MDMA, but that the magnitude of this upregulation would vary in accordance with the pattern of SERT-immunoreactive fiber density found in adulthood [i.e. caudate-putamen (striatum) > hippocampus > visual (occipital) cortex].

Materials and Methods

Animals

Sprague-Dawley rats (CD strain; Charles River, Kingston, N.Y., USA) were maintained and bred in a temperature- and humidity-controlled colony room (temperature = 21 ± 2°C). Adult females were paired with stud males until the appearance of a sperm plug, which was used to determine the onset of pregnancy. Pregnant females were housed in standard plastic tubs with woodchip bedding. The day after parturition, litters were culled to 8 pups, usually with an equal number of females and males. A between-litters design was used in which 11 litters were treated with MDMA and 11 litters received saline vehicle. (+/–)MDMA HCl (Sigma, St. Louis, Mo., USA or Research Triangle Institute, Research Triangle Park, N.C., USA) was administered twice daily at a dose of 10 mg/kg (1 mg/ml) per injection. The first injection was given between 08:00 and 10:00, and the interdose interval was 4 h. Treatments were administered on postnatal days (PD) 1–4, a developmental period in rats that roughly corresponds to early in the third trimester of gestation in humans [Dobbing and Sands, 1979; Romijn et al., 1991]. Because sex does not appear to appreciably modify the serotonergic neurotoxicity of perinatally administered MDMA [Meyer et al., 2004; Piper, 2007], the female offspring were used in the present study while the males participated in a separate experiment [Piper and Meyer, 2006]. One animal from each litter was killed for tissue collection at PD 11, PD 30, and PD 65–70, corresponding to neonatal, juvenile, and young adult stages of development. Pups kept for the later time points were weaned on PD 21, and housed with same-sex littermates in a separate colony room. The 5-day sample collection period for postpubertal rats, henceforth designated PD 67 for simplicity, occurred so that tissue collection could be performed during diestrus to control for the influence of the estrous cycle on BDNF expression [Gibbs, 1998]. Stage of estrous cycle was determined by means of vaginal swabs, and diestrus was defined by the presence of cornified cells in the vaginal epithelium.

Brain Sample Collection

Animals were lightly anesthetized by CO_2 inhalation and then decapitated. The hippocampus, striatum, and occipital cortex were rapidly removed over ice and stored at –70°C for later study. In those cases in which the same animal contributed to both BDNF and SERT measurements, tissue samples from each side of the brain were stored separately for the respective analyses.

BDNF Analysis

All litters were used for determination of regional BDNF concentrations at all 3 time points (PD 11, 30, and 67; n = 11 per group at each age). Tissue samples were homogenized by means of a motor-driven Teflon pestle in either 10 vol or 300 μl (whichever was greater) of ice-cold lysis buffer consisting of 20 mM Tris-HCl, 5 mM EDTA, 0.1% Triton X-100, 0.5% bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin (pH 8.0). Homogenates were centrifuged at 4°C at 23,000 *g* for 15 min and the supernatant was analyzed in duplicate for its BDNF content by means of the Promega E_{max}® ImmunoAssay System (Promega, Madison, Wisc., USA) according to the manufacturer's directions. Absorbances were read at 450 nm using a Bio-Rad microplate reader, and the resulting BDNF values were expressed per unit wet weight of tissue.

SERT Binding

Six of the 11 litters from each treatment condition were also used to determine regional SERT binding as an index of MDMA-induced serotonergic neurotoxicity. SERT assays were performed only at the 2 early time points, since we already had information on longer-term changes in SERT following the same neonatal MDMA treatment regimen as used in the present study [Meyer et al., 2004]. Tissue samples were homogenized using a Kinematica Polytron (Brinkmann Instruments, Westbury, N.Y., USA) in ei-

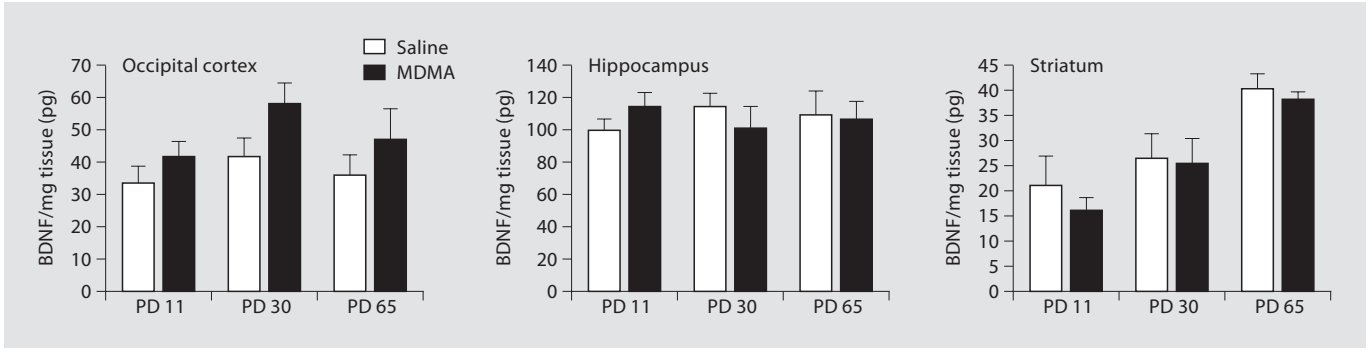

Fig. 1. The influence of neonatal MDMA treatment on regional BDNF concentrations at different ages (n = 11 per group). There was a significant overall increase in BDNF in the MDMA-treated compared to the control animals in the occipital cortex ($p < 0.025$).

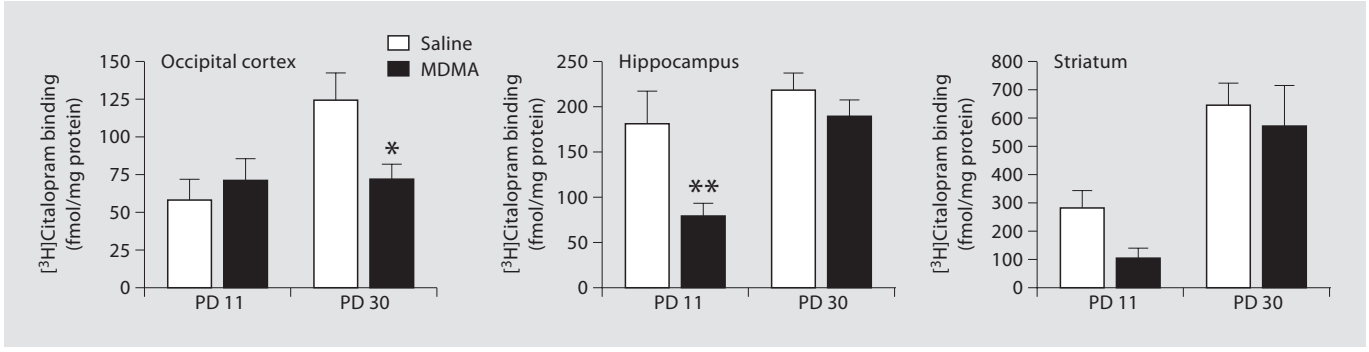

Fig. 2. The influence of neonatal MDMA treatment on regional SERT binding at different ages (n = 6 per group). * p = 0.017, ** p = 0.005 compared to the respective control group at that age.

ther 40 (hippocampus and cortex) or 80 (striatum) vol of ice-cold buffer containing 10 mM sodium phosphate, 5 mM potassium chloride, and 120 mM sodium chloride (pH 7.4). Homogenates were centrifuged at 4°C at 20,000 *g* for 20 min. The homogenization and centrifugation steps were repeated twice to yield a washed membrane fraction. Samples were then assayed in triplicate for SERT binding as described in Piper et al. [2005], using 1.0 nM [^{3}H]citalopram with or without 10 μM fluoxetine to define nonspecific binding. Protein was measured by means of the Bio-Rad DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, Calif., USA) with bovine γ-globulin as the standard.

Data Analysis

Statistical analyses were performed using SYSTAT 10.2 (Systat Software, San Jose, Calif., USA). BDNF values for each brain area were first analyzed by means of 2-way analyses of variance (ANOVA) with treatment and age as between-subjects variables. This enabled us to ascertain possible treatment-related changes in regional BDNF concentrations over time. With respect to the SERT binding data, however, we were principally interested in comparing the MDMA-treated animals to the controls at each age. Therefore, for this analysis we performed a simple 1-way ANOVA with a generalized linear model for each brain area, in which we compared the 4 treatment/age conditions against each other followed by the appropriate contrasts (PD 11 and 30: saline vs. MDMA).

Results

BDNF levels in each brain area and at each age are shown in figure 1. ANOVA of BDNF in the occipital cortex revealed a main effect of drug treatment [$F(1,58) = 5.56$, $p < 0.025$], but no main effect of age or treatment × age interaction. Mean BDNF concentrations were higher in the MDMA-treated animals compared to the controls at each age, with the greatest increase (39%) occurring at PD 30. Levels of BDNF in the hippocampus were unaltered either by treatment or by age. Striatal BDNF significantly increased with age [$F(2,54) = 13.11$, $p < 0.001$], but was unaffected by treatment.

The [^{3}H]citalopram binding data are presented in figure 2. One-way ANOVA of the results from the occipital cortex was highly significant [$F(3,20) = 4.23$, $p = 0.018$], and the post hoc contrasts showed that the MDMA-treated and control animals differed significantly at PD 30 [$F(1,20) = 6.71$, $p = 0.017$], but not at PD 11. The hippocampus likewise showed a strong overall effect of condition [$F(3,20) = 6.95$, $p = 0.002$], but in this case the treatment groups differed significantly at PD 11 [$F(1,20) = 9.92$, $p = 0.005$] and not at PD 30. Finally, the striatum also exhibited an overall effect of condition [$F(3,20) = 7.84$, $p = 0.001$], but the greater variability in results from this brain area precluded obtaining a significant group difference either at PD 11 or 30.

Discussion

The first key finding of this study is that the serotonergic neurotoxic effects of neonatal MDMA treatment were manifested in a regionally and temporally selective manner. Specifically, the drug-exposed animals showed reduced [^{3}H]citalopram binding in the hippocampus 1 week after drug treatments, but the binding values recovered by PD 30. In contrast, SERT binding in the occipital cortex was unaffected at PD 11, but was significantly reduced at the later time point. This outcome is congruent with our earlier report of a delayed reduction in [^{3}H]paroxetine binding to SERT in the parietal cortex following MDMA [Meyer et al., 2004]. These results also extend upon prior studies demonstrating an age-dependent modulation of the dynamic response of the serotonergic system to early developmental MDMA treatment [reviewed in Piper, 2007].

Second, the present results do not support our hypothesis that regional differences in BDNF upregulation are responsible for the long-term abnormalities in forebrain 5-HT innervation following neonatal MDMA exposure. Given the previously discussed evidence for BDNF-mediated sprouting of injured serotonergic axons, we hypothesized that MDMA administration would provoke regionally specific increases in BDNF expression that would parallel the degree of recovery in the 3 selected brain areas. Instead, we found a clear dissociation between BDNF and altered SERT levels (used here as an index of serotonergic fiber/terminal density). The occipital cortex was the only area in which BDNF was up-regulated in the MDMA-treated animals, yet this area showed a delayed neurotoxic effect of the drug treatment despite such upregulation. In contrast, the hippocampus exhibited no change in BDNF expression, even though this area exhibited signs of early serotonergic neurotoxicity followed by apparent recovery.

Several previous studies have reported altered BDNF levels after MDMA or other serotonergic neurotoxins. For example, Koprich et al. [2003] found that rats given daily MDMA administration at PD 11–20 showed increased concentrations of BDNF in the hippocampus, frontal cortex, striatum, and brainstem when measured the day after the final drug treatment. Except for the brainstem, all of these areas also displayed MDMA-related reductions in 5-HT and 5-HIAA content. More recently, Martínez-Turrillas et al. [2006] found that young adult rats given a single 10 mg/kg dose of MDMA showed a transient depletion of 5-HT in the frontal cortex that was paralleled by a transient increase in BDNF mRNA expression in this area. In contrast, the hippocampus displayed a more dramatic and persistent (at least 7 days) reduction in 5-HT, yet there was a time-dependent downregulation of BDNF gene expression that was manifested in all hippocampal subfields. Finally, 2 additional studies involving other serotonergic neurotoxins given to adult rats or mice similarly found complex region- and time-dependent changes in BDNF protein or gene expression [Luellen et al., 2006; Zetterström et al., 1999]. Several conclusions can be drawn from these results along with those of the present study. First, it seems evident that administration of a serotonergic neurotoxin does not reliably lead to increased BDNF expression either in adult or developing animals. Second, even under conditions where upregulation is observed, there are often regional differences that are not yet fully understood. Finally, it is important to keep in mind that the relationship between BDNF and 5-HT is reciprocal. Not only is BDNF capable of stimulating the sprouting of serotonergic fibers, but 5-HT stimulates BDNF expression [Mattson et al., 2004]. Since substituted amphetamines such as MDMA and *p*-chloroamphetamine acutely release 5-HT, we suggest that short-term increases in BDNF following administration of such compounds are likely due, at least in part, to this releasing action rather than to their neurotoxic effects. According to this view, if 5-HT neurotoxins did cause upregulation of BDNF as a trophic response to stimulate serotonergic fiber regrowth, the effect might be characterized by a delayed onset but a prolonged time course.

In conclusion, we report that neonatal MDMA administration decreased SERT binding in a temporally and regionally specific manner. The predicted increase in BDNF expression only occurred in the occipital cortex, despite the fact that this area was shown in a previous study to dis-

play a prolonged serotonergic denervation after the same treatment paradigm. These results do not support the hypothesis that differential BDNF induction mediates regional differences in serotonergic recovery following neonatal MDMA exposure. However, it remains possible that MDMA administration leads to enhanced BDNF signaling by mechanisms not investigated here, such as increased trkB expression and/or enhanced activity of downstream effectors such as phosphatidylinositol 3-kinase and Akt.

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References

Boot BP, McGregor IS, Hall W (2000): MDMA (ecstasy) neurotoxicity: assessing and communicating the risks. Lancet 355:1818–1821.

Bregman BS, McAtee M, Dai HN, Kuhn PL (1997): Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. Exp Neurol 148: 475–494.

Callahan BT, Cord BJ, Ricaurte GA (2001): Long-term impairment of anterograde axonal transport along fiber projections originating in the rostral raphe nuclei after treatment with fenfluramine or methylenedioxymethamphetamine. Synapse 40:113–121.

Degenhardt L, Barker B, Topp L (2004): Patterns of ecstasy use in Australia: findings from a national household survey. Addiction 99: 187–195.

Dobbing J, Sands J (1979): Comparative aspects of the brain growth spurt. Early Hum Dev 3: 79–83.

Gibbs RB (1998): Levels of trkA and BDNF mRNA, but not NGF mRNA, fluctuate across the estrous cycle and increase in response to acute hormone replacement. Brain Res 787:259–268.

Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003): The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). Pharmacol Rev 55:463–508.

Grider MH, Mamounas LA, Le W, Shine HD (2005): In situ expression of brain-derived neurotrophic factor of neurotrophin-3 promotes sprouting of cortical serotonergic axons following a neurotoxic lesion. J Neurosci Res 84:404–412.

Hatzidimitriou G, McCann UD, Ricaurte GA (1999): Altered serotonin innervation patterns in the forebrain of monkeys treated with (+/–)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. J Neurosci 19: 5096–5107.

Ho EL, Karimi-Tabesh L, Koren G (2002): Characteristics of pregnant women who use ecstasy (3,4-methylenedioxymethamphetamine). Neurotoxicol Teratol 23:561–567.

Jin Y, Tessler A, Fischer I, Houle JD (2000): Fibroblasts genetically modified to produce BDNF support regrowth of chronically injured serotonergic axons. Neurorehabil Neural Repair 14:311–317.

Johnson LD, O'Malley PM, Bachman JG, Schulenberg JE (2007): Monitoring the future national survey results on drug use, 1975–2006. Volume I: secondary school students (NIH publication No. 07-6205). Bethesda, National Institute on Drug Abuse.

Kelly PA, Ritchie IM, Quate L, McBean DE, Olverman HJ (2002): Functional consequences of perinatal exposure to 3,4-methylenedioxymethamphetamine in rat brain. Brit J Pharmacol 137:963–970.

Koprich JB, Campbell NG, Lipton JW (2003): Neonatal 3,4-methylenedioxymethamphetamine (ecstasy) alters dopamine and serotonin neurochemistry and increases brain-derived neurotrophic factor in the forebrain and brainstem of the rat. Dev Brain Res 147: 177–182.

Luellen BA, Szapacs ME, Materese CK, Andrews AM (2006): The neurotoxin 2′-NH_2-MPTP degenerates serotonin axons and evokes increases in hippocampal BDNF. Neuropharmacology 50:297–308.

Madhav TR, Pei Q, Zetterström TS (2001): Serotonergic cells of the rat raphe nuclei express mRNA of tyrosine kinase B (trkB), the high-affinity receptor for brain derived neurotrophic factor (BDNF). Mol Brain Res 93: 56–63.

Mamounas LA, Altar CA, Blue ME, Kaplan DR, Tessarollo L, Lyons WE (2000): BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain. J Neurosci 20:771–782.

Martínez-Turrillas R, Moyano S, Del Río J, Frechilla D (2006): Differential effects of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on BDNF mRNA expression in rat frontal cortex and hippocampus. Neurosci Lett 402:126–130.

Mattson MP, Maudsley S, Martin B (2004): BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci 27:589–594.

Meyer JS, Grande M, Johnson K, Ali SF (2004): Neurotoxic effects of MDMA ('ecstasy') administration to neonatal rats. Int J Dev Neurosci 22:261–271.

Piper BJ (2007): A developmental comparison of the neurobehavioral effects of ecstasy (MDMA). Neurotoxicol Teratol 29:288–300.

Piper BJ, Meyer JS (2006): Increased responsiveness to MDMA in adult rats treated neonatally with MDMA. Neurotoxicol Teratol 28: 95–102.

Piper BJ, Fraiman JB, Meyer JS (2005): Repeated MDMA ('ecstasy') exposure in adolescent male rats alters temperature regulation, spontaneous motor activity, attention, and serotonin transporter levels. Dev Psychobiol 47:145–157.

Ramer LM, McPhail LT, Borisoff JF, Soril LJJ, Kaan TKY, Lee JHT, Saunders JWT, Hwi LPR, Ramer MS (2007): Endogenous trkB ligands suppress functional mechanosensory plasticity in the deafferented spinal cord. J Neurosci 27:5812–5822.

Ricaurte GA, McCann UD, Szabo Z, Scheffel U (2000): Toxicodynamics and long-term toxicity of the recreational drug, 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). Toxicol Lett 112:143–146.

Romijn HJ, Hofman MA, Gramsbergen A (1991): At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? Early Hum Dev 26: 61–67.

United Nations Office of Drug Control and Crime (2007): 2007 World Drug Report (ISBN: 978–92–1–148222–5). New York, United Nations.

Winslow JT, Insel TR (1990): Serotonergic modulation of rat pup ultrasonic vocal development: studies with 3,4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 254:212–220.

Zetterström TSC, Pei Q, Madhav TR, Coppell AL, Lewis L, Grahame-Smith DG (1999): Manipulations of brain 5-HT levels affect gene expression for BDNF in rat brain. Neuropharmacology 38:1063–1073.