

Altered Brain Serotonin Homeostasis and Locomotor Insensitivity to 3,4-Methylenedioxymethamphetamine (“Ecstasy”) in Serotonin Transporter-Deficient Mice

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ABSTRACT

The sodium-dependent, high affinity serotonin [5-hydroxytryptamine (5-HT)] transporter (5-HTT) provides the primary mechanism for inactivation of 5-HT after its release into the synaptic cleft. To further evaluate the function of the 5-HTT, the murine gene was disrupted by homologous recombination. Despite evidence that excess extracellular 5-HT during embryonic development, including that produced by drugs that inhibit the 5-HTT, may lead to severe craniofacial and cardiac malformations, no obvious developmental phenotype was observed in the 5-HTT^{-/-} mice. High affinity [³H]5-HT uptake was completely absent in 5-HTT^{-/-} mice, confirming a physiologically effective knockout of the 5-HTT gene. 5-HTT binding sites labeled with [¹²⁵I]3β-(4'-iodophenyl)tropan-2β-carboxylic acid

methyl ester were reduced in a gene dose-dependent manner, with no demonstrable binding in 5-HTT^{-/-} mutants. In adult 5-HTT^{-/-} mice, marked reductions (60–80%) in 5-HT concentrations were measured in several brain regions. While (+)-amphetamine-induced hyperactivity did not differ across genotypes, the locomotor enhancing effects of (+)-3,4-methylenedioxymethamphetamine, a substituted amphetamine that releases 5-HT via a transporter-dependent mechanism, was completely absent in 5-HTT^{-/-} mutants. Together, these data suggest that the presence of a functional 5-HTT is essential for brain 5-HT homeostasis and for 3,4-methylenedioxymethamphetamine-induced hyperactivity.

The 5-HT system is an important modulator of many developmental, behavioral, and physiological processes. The 5-HTT plays a key role in the regulation of serotonergic neurotransmission and has been implicated in depression, anxiety, and substance abuse (Lesch *et al.*, 1993a; Vanhoutte *et al.*, 1993). 5-HTT is the primary target for widely used 5-HT reuptake-inhibiting antidepressant drugs such as fluoxetine, as well as drugs of abuse like MDMA (“ecstasy”) (Rudnick and Wall, 1992; Levi and Raiteri, 1993; Blakely *et al.*, 1994). As part of continued efforts directed at elucidating the role of 5-HTT in normal behavior and in disease states (Lesch *et al.*, 1993b; Lesch *et al.*, 1994; Lesch *et al.*, 1996), an animal model with a targeted disruption of the 5-HTT gene was generated. A genomic segment containing exon 2 of the murine 5-HTT gene was replaced with a PGK-*neo* gene cas-

sette by homologous recombination in ES cells. As reported in an article describing the organization of the murine 5-HTT gene (Bengel *et al.*, 1997), this segment of the 5-HTT gene contains the start codon, a conserved residue of transmembrane domain 1 that participates in substrate transport (Barker *et al.*, 1997), and several post-translational modification sites. Therefore, it was anticipated that a 5-HTT gene lacking exon 2 would result in an inactive or highly dysfunctional gene product without altering the expression of neighboring genes (Olson *et al.*, 1996).

Materials and Methods

Targeting construct and Southern blot analysis. A mouse c129 genomic P1 library (Genomic Systems, St. Louis, MO) was screened by a polymerase chain reaction targeting exon 2 (Kp1, 5'-TGAGATTACCAAGGGGACG; Kp2, 3'-CCTCCACCATCTGGTAGCAT). Two clones, P1(20) and P1(242), were purified and further characterized by restriction mapping and Southern blot analysis. 3' and 5' DNA fragments encompassing exon 2 with an overall length of 7.5 kb were inserted into the pPNT-*neo* replacement targeting

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); 5-HTT, 5-hydroxytryptamine transporter; MDMA, 3,4-methylenedioxymethamphetamine; ES, embryonic stem; kb, kilobase pair; RTI-55, 3β-(4'-iodophenyl)tropan-2β-carboxylic acid methyl ester.

vector, containing a *neo* and TK cassette under the control of the PGK promoter (Fig. 1A) (Tybulewicz et al., 1991). A 1.1 kb *Bam*HI/*Hind*III fragment containing 5-HTT exon 2 was replaced by a 1.8 kb PGK neomycin-polyA expression cassette. Before electroporation, the targeting construct was linearized at the single *Not*I restriction site of pPNT-*neo*. 129 R1 ES cells were cultured, transfected, and subjected to double selection. DNA was digested with *Asp*718 and hybridized with a 3' probe that recognized a 5-HTT sequence external to the construct (Fig. 1B). In addition, recombinant ES cell clones were identified by Southern blot analysis, with the use of a 5' *Hind*III/*Bam*HI probe to confirm accurate gene targeting (data not shown). After confirmation of two targeted ES cell clones (ES 49, ES 53; targeting frequency = 2/61), both clones were microinjected in C57BL/6J blastocysts to obtain chimeric progeny. Chimeric males were mated to CD-1 and C57BL/6J female mice; pups were genotyped by Southern blot analysis of tail biopsies (Fig. 1C). After confirmation of germline transmission, 5-HTT^{+/-} mice were mated to produce 5-HTT^{-/-} mutants.

Animals. CD-1 mice (25 g) were purchased from Charles River Laboratories (Wilmington, MA) and C57BL/6J mice (25 g) from Jackson Laboratories (Bar Harbor, ME). Animals were housed in groups of 3–5 per cage with food and water *ad libitum* in a facility approved by the American Association for Accreditation of Laboratory Animal Care (12-hr light-dark cycle). Experimental protocols adhered to National Institutes of Health guidelines and were approved by the National Institute of Mental Health Animal Care and Use Committee. The following experiments were performed with 5-HTT^{-/-}, 5-HTT^{+/-} mice on the CD-1 background and control littermates.

Tissue preparation and brain neurochemistry. Mice were killed by cervical dislocation and their brains were rapidly removed and dissected over ice. After removal of frontal cortex, the brain was bisected sagittally and the brain stem, hippocampus, and striatum were dissected from right hemisphere samples (Sidman et al., 1971). The left hemispheres were frozen in isopentane on dry ice for autoradiography. Samples for 5-HT analysis were stored at -70° before high performance liquid chromatography using electrochemical detection, as described previously (Andrews and Murphy, 1993).

5-HTT autoradiography. [¹²⁵I]RTI-55 binding to the 5-HTT was quantified from [¹²⁵I] microscale standards using National Institutes of Health image software and is expressed as nanocuries per milligram of tissue (mean ± standard error). The density of regions of

interest was measured in each of three adjacent sections from four animals per genotype. Left hemispheres were sectioned sagittally (20 μm) at -20° and thaw-mounted on gelatin-coated slides. [¹²⁵I]RTI-55 binding was performed as described previously (Silverthorn et al., 1995) using LR 1111 [1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)homopiperazine] (1 mM) to inhibit binding of [¹²⁵I]RTI-55 to dopamine uptake sites. Nonspecific binding was determined in the presence of 1 μM paroxetine and represented <10% of the total binding.

[³H]5-HT uptake studies. Brain stem and cortex samples from three mice of each genotype used in four experiments for [³H]5-HT uptake were homogenized in 15 volumes of 0.32 M sucrose using a motor driven Teflon pestle and a glass mortar. The homogenates were centrifuged for 10 min at 1,000 × *g*. The supernatants were then centrifuged for 10 min at 17,000 × *g*. The pellets were resuspended in sucrose at a final concentration of 1–2 mg/ml. 5-HT uptake was measured using six concentrations of [³H]5-HT (10–100 nM) as described previously (Maarten and O'Reilly, 1990) with some minor modifications. Tubes containing 120 mM NaCl, 20 mM Tris-HCl, 5 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 10 mM glucose, 1 mM ascorbic acid, and 0.1 mM pargyline plus [³H]5-HT were preincubated for 5 min at 37°. Brain synaptosomes were then added to each tube and uptake was allowed to occur for 5 min at 37°. The process was terminated by immersing the tubes in ice water followed by rapid filtration through Whatman GF/B filters. Radioactivity was measured using liquid scintillation counting. Specific uptake was defined as that occurring at 37° minus nonspecific uptake determined in tubes containing 0.1 mM fluoxetine incubated at 0°. Sodium dependence of [³H]5-HT uptake was evaluated by substituting LiCl (120 mM) for NaCl in one set of experiments.

Locomotor activity. Testing was conducted during the light phase (10:00–15:00 hr). Initially, a pilot study was performed to determine the dose of (+)-MDMA required to produce locomotor stimulation in CD-1 mice. Within the range of 1.0 to 10 mg/kg, a dose of 5.0 mg/kg (+)-MDMA produced maximal locomotor stimulation; therefore, this dose was used in subsequent experiments. Mice [male mice 8–23 weeks of age for the MDMA study; female mice 8–14 weeks of age for the (+)-amphetamine study] of the three genotypes (5 to 9 mice per group) were brought from the animal colony to the testing room and placed in individual cages at least 1 hr before testing. Locomotor activity was assessed in either Plexiglas test

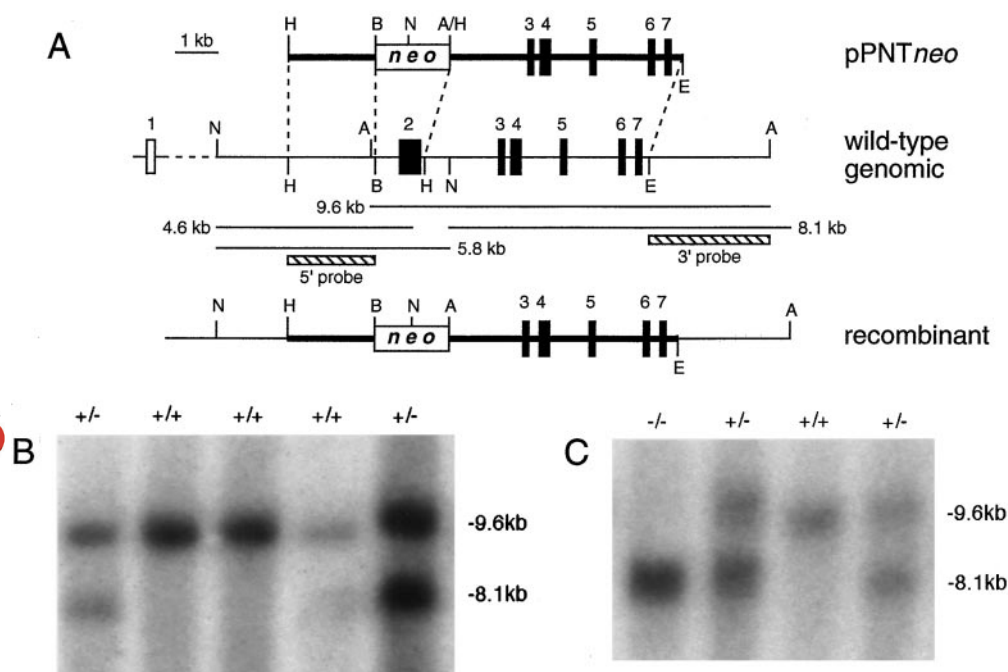


Fig. 1. Targeted disruption of the gene encoding the murine 5-HTT. A, Restriction map and exon/intron organization of the wild-type 5-HTT gene, the targeting vector pPNT-*neo*, and the recombinant 5-HTT allele. Black and open boxes represent coding and untranslated exons, respectively. The map of the targeting vector depicts the replacement of exon 2 and flanking genomic sequences by the PGK neomycin-polyA expression cassette. Solid lines indicate the *Asp*718 and *Nco*I restriction fragment sizes for wild-type and recombinant DNA. Hatched boxes, 5' and 3' probes used for Southern blot analysis. Letters indicate restriction sites: A, *Asp*718; B, *Bam*HI; E, *Eco*RI; H, *Hind*III; N, *Nco*I; S, *Sph*I; X, *Xba*I. B, Southern blot analysis from ES cell transfectants. C, Tail biopsies obtained from littermates produced by heterozygous cross-breeding. Wild-type and mutant alleles correspond to 9.6- and 8.1-kb fragments, respectively.

chambers (29.5 × 25.5 × 29 cm) using electronic counters that detected interruptions of eight independent photocell beams located 15 mm above the floor (Coulbourn, Allentown, PA) or in a two-chambered Digiscan (Accuscan, Columbus, OH). Animals were allowed to become habituated to the test chamber before injection with either drug [(+)-MDMA or (+)-amphetamine, 5 mg/kg intraperitoneally] or saline. Cumulative beam disruptions were recorded at 10, 30, and 60 min.

Drugs and chemicals. [¹²⁵I]RTI-55 (2200 Ci/mmol) and [³H]5-HT creatinine sulfate (24–27 Ci/mmol) were purchased from Dupont NEN (Boston, MA). Fluoxetine HCl was kindly provided by Eli Lilly Laboratories (Indianapolis, IN). Paroxetine HCl was a gift from SmithKline Beecham (Essex, UK). LR 1111 was a gift from R. B. Rothman (National Institute on Drug Abuse, Rockville, MD). MDMA HCl and (+)-amphetamine HCl were a gift from the Research Technology Branch of the National Institute on Drug Abuse. All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO) or a comparable source and were of analytical grade.

Statistics. Data were analyzed initially by one- or two-way analysis of variance using the Statistical Analysis System (SAS Institute, Cary, NC). Significant differences between groups were evaluated using *post hoc* Student-Newman-Keuls multiple comparisons tests or *t* tests. Welch *t* tests were used in some comparisons when unequal variances across the results in the different genotypes were observed. All values are expressed as mean ± standard error, with differences of *p* < 0.05 considered statistically significant.

Results

Microinjection of two recombinant ES cell clones with the predicted mutant allele (Fig. 1B) into C57BL/6J mouse blastocysts 3.5 days old produced chimeric mice. Southern blot analysis after tail biopsy using both 3' and 5' probes confirmed accurate targeting and excluded the possibility of additional integrations (Fig. 1C). After germline transmission was established, homozygous 5-HTT^{-/-} mutants were generated from fertile heterozygote 5-HTT^{+/-} progeny with an expected 1:2:1 ratio of 5-HTT^{+/+} to 5-HTT^{+/-} and 5-HTT^{-/-} genotypes. 5-HTT^{-/-} mice exhibited normal weight gain and showed no tendency toward increased lethality compared with their 5-HTT^{+/+} littermates. In addition, they were fertile and produced normal litter sizes when crossed with each other. Obvious developmental defects or behavioral abnormalities were not seen in mutant 5-HTT^{+/-} or 5-HTT^{-/-} mice observed into adulthood.

Quantitative autoradiography of the brain 5-HTT using

[¹²⁵I]RTI-55 was performed to examine the effects of the disruption of the 5-HTT gene. A ~50% reduction in uptake site density was measured in 5-HTT^{+/-} mice whereas an absence of binding was observed in all brain regions in the 5-HTT^{-/-} mutants (Fig. 2 and 3A). Direct studies of 5-HTT function were conducted by measuring [³H]5-HT uptake over a 10-fold concentration range in synaptosomes prepared from brain stem and cortex. The resulting saturation isotherms of [³H]5-HT uptake were similar for the 5-HTT^{+/+} and 5-HTT^{+/-} mice (Fig. 4), and nonlinear regression analysis did not reveal differences in the *V*_{max} (21 ± 5 pmoles/mg protein/5 min) and *K*_m (45 ± 7 nM) in brain stem or the *V*_{max} (15 ± 4) and *K*_m (30 ± 4) in cortex of 5-HTT^{+/-} mutants compared with control mice. [³H]5-HT uptake was absent in 5-HTT^{-/-} mutants (Fig. 4). The residual [³H]5-HT accumulation was sodium independent and represented less than 3% of that found in 5-HTT^{+/+} mice. *V*_{max} and *K*_m were indeterminate in both brain regions in 5-HTT^{-/-} mutants.

To evaluate the importance of functional 5-HTT in regulating 5-HT and other neurotransmitters, monoamine concentrations in various brain regions were measured using high performance liquid chromatography. In contrast to essentially normal levels of 5-HT in 5-HTT^{+/+} and 5-HTT^{+/-} mice, 5-HTT^{-/-} mutants showed 60–80% reductions in 5-HT concentrations in brain stem, frontal cortex, hippocampus, and striatum (Fig. 3B). Smaller reductions in 5-hydroxyindoleacetic acid in these brain regions were also statistically significant, whereas concentrations of norepinephrine, dopamine, and their metabolites were unchanged in the 5-HTT^{-/-} mutants (data not shown).

Consequences of the disruption of the 5-HTT gene on behavior were evaluated in studies of locomotor activity using (+)-MDMA and (+)-amphetamine. Initial studies demonstrated similar levels of baseline (saline) locomotor activity across the three genotypes (Fig. 5). Administration of (+)-MDMA to 5-HTT^{+/+} mice resulted in a 3-fold increase in locomotor activity relative to saline-treated littermate controls that persisted over the 60 min study period (Fig. 5A). Cumulative (+)-MDMA-induced hyperactivity in the 5-HTT^{+/-} mice was attenuated by ~50% at all time points relative to 5-HTT^{+/+} mice treated with (+)-MDMA. The 5-HTT^{-/-} mice, on the other hand, displayed no increase in locomotor activity after (+)-MDMA administration. Interest-

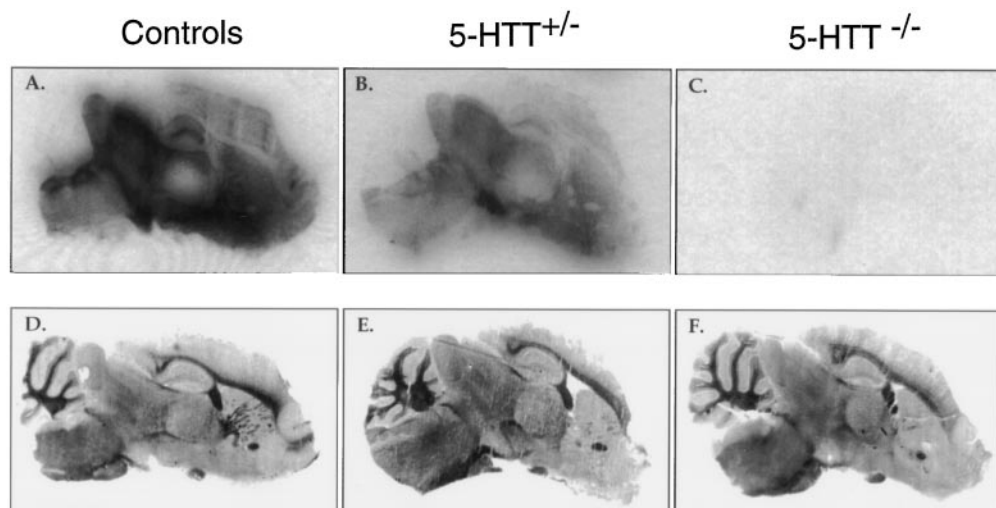


Fig. 2. Autoradiograms of [¹²⁵I]RTI-55 binding to the 5-HTT. *Top row*, changes in 5-HTT density in sagittal brain sections at the level of the substantia nigra; *lower row*, same sections stained with alcian blue. Results depicted are representative of mice from the following groups: A and D, 5-HTT^{+/+}; B and E, 5-HTT^{+/-}; C and F, 5-HTT^{-/-}.

ingly, at the 10- and 30-min time points, these mice exhibited a significant paradoxical reduction in locomotor activity relative to the saline-treated controls. Administration of (+)-amphetamine to 5-HTT^{+/+} mice resulted in a 5-fold increase in locomotor activity relative to saline-treated littermate controls that persisted over the 60 min study period (Fig. 5B). Cumulative (+)-amphetamine-induced hyperactivity did not differ across genotypes.

Discussion

5-HTT becomes transcriptionally active at embryonic day 10 when it regulates cranial neural crest migration (Shuey et al., 1992; Moiseiwitsch and Lauder, 1995). Despite many 5-HTT-related changes in brain 5-HT homeostasis in adult mice with a null mutation of the 5-HTT, apparent anatomical alterations were not observed in mice examined postnatally and observed into adulthood. The lack of major anatomical anomalies is unexpected in the face of a series of reports indicating that inhibition of the 5-HTT by fluoxetine, sertraline, and amitriptyline or by excess 5-HT in cultured mouse embryos perturbs craniofacial morphogenesis (Shuey et al., 1992; Yavarone et al., 1993; Moiseiwitsch and Lauder, 1995).

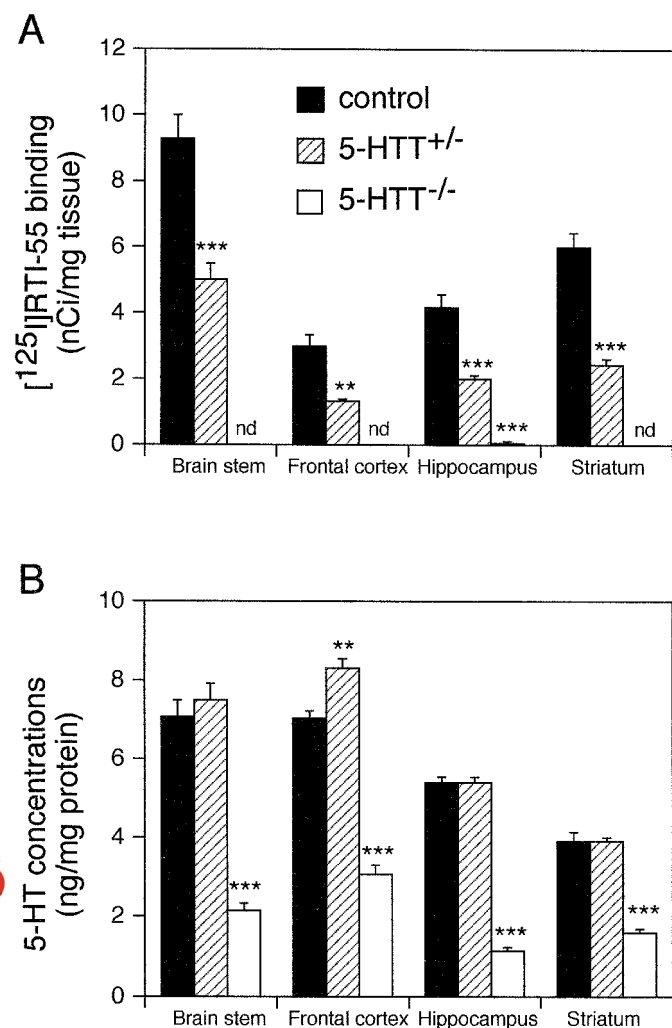


Fig. 3. A, Quantitative differences in [¹²⁵I]RTI-55-labeled 5-HTT in four brain regions across the three genotypes (four mice per group). B, Regional 5-HT concentrations (eight mice per group). Significance of differences compared with 5-HTT^{+/+} mice: **, $p < 0.01$ and *** $p < 0.001$.

Our findings conflict with a report that rats treated with high doses of fluoxetine during pregnancy have smaller pups with poorer weight gain (Vorhees et al., 1994). Detailed morphological analyses of cortical and subcortical structures where 5-HT has been suggested to play a trophic role during development are required to clarify the impact of 5-HTT inactivation on the formation and plasticity of brain structures (Cases et al., 1996; Lebrand et al., 1996).

The observation of congenital malformations in rodent models indicates that these developmental changes might result from excess 5-HT and specifically from treatment with selective 5-HT reuptake-inhibiting antidepressants, has raised considerable concern because of the very wide use of these drugs and the inevitable consequence that some women became pregnant during treatment with these agents. However, several preliminary reports and one very recent follow-up study of women who became pregnant and continued treatment with fluoxetine revealed no evidence of neurodevelopmental changes in preschool children (Nulman et al., 1997). Another recent study also found no increase in major or minor structural anomalies or gestational problems in mothers receiving fluoxetine during the first two trimesters; minor anomalies and gestational difficulties were more fre-

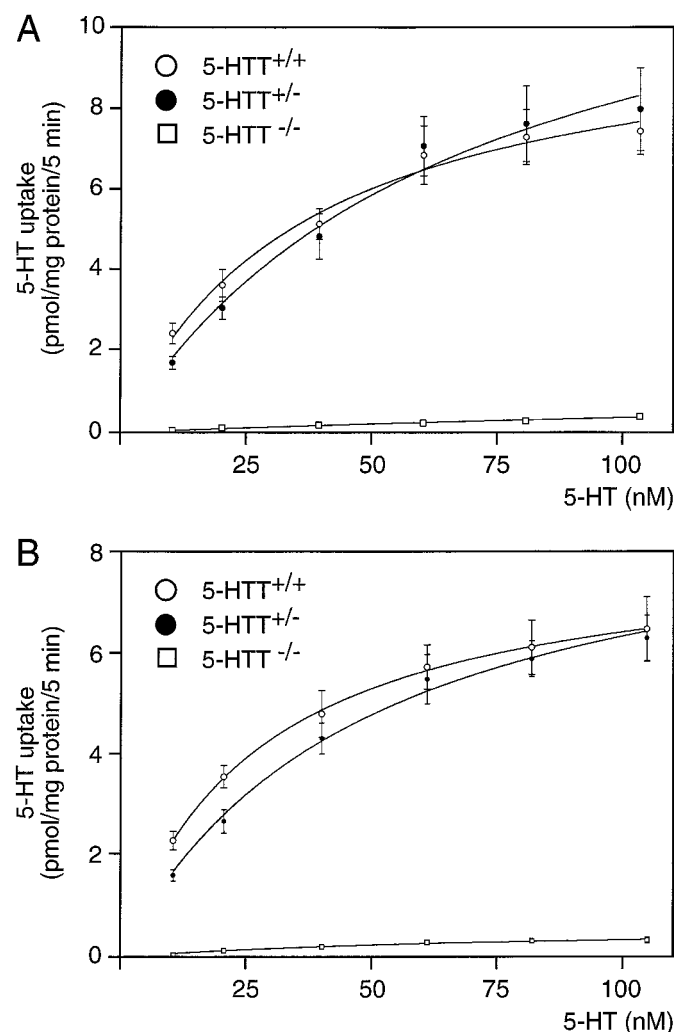


Fig. 4. [³H]5-HT uptake in 5-HTT^{+/+} and 5-HTT^{-/-} mutant mice compared with littermate controls in brain stem (A) and cortex (B).

quent, however, in mothers whose exposure to fluoxetine began in the third trimester (Chambers *et al.*, 1996), although an editorial accompanying this paper raises some methodological issues regarding these findings (Robert, 1996).

Although developmental processes in mice and humans cannot be readily equated, the lack of any apparent developmental abnormalities in our mice suggests that major compensatory mechanisms and neuroadaptive changes occur in 5-HTT^{-/-} mice during embryonic and subsequent neurodevelopment. Transporter function was clearly ablated in 5-HTT^{-/-} mice, but 5-HT uptake was nearly unaltered in 5-HTT^{+/-} mice. The data on uptake function contrast with those from [¹²⁵I]RTI-55 binding, which showed a gene dose-dependent reduction in uptake sites. The lack of parallel changes in uptake versus binding site density confirms the existence and physiological relevance of additional modes of post-translational regulation for the cell membrane 5-HT transporting mechanism (Blakely *et al.*, 1994; Qian *et al.*,

1997). Adaptive changes in 5-HT synthesis, turnover, or metabolism were, in fact, evidenced in our study by the substantial depletion of neuronal 5-HT and 5-hydroxyindoleacetic acid in the four brain regions evaluated. These reductions in 5-HT may reflect negative feedback mechanisms affecting the synthesis of 5-HT. In this regard, modest reductions of brain 5-HT have been reported in rodents treated chronically with antidepressants like fluoxetine and clomipramine (Ratnay, 1991; Feenstra *et al.*, 1996; Cabrera-Vera *et al.*, 1997). The possibility that subtle changes in the dynamics of 5-HT transport in 5-HTT^{+/-} mice exert acute or long-term effect on neurodevelopment and adult brain plasticity requires further detailed assessment at the cellular and molecular level.

The substituted amphetamine, MDMA, is an indirect sympathomimetic with effects on the serotonergic system (Ratnay, 1991; Steele *et al.*, 1994; Green *et al.*, 1995; White *et al.*, 1996). It has been suggested that the primary mechanism of action of MDMA involves the release of endogenous 5-HT from presynaptic nerve terminals, presumably via reversal of the plasma membrane 5-HT transport (Rudnick and Wall, 1992; Wichems *et al.*, 1995; Gudelsky and Nash, 1996). The net effect of this agent is an increase in 5-HT in the synapse and a prolonged effect of 5-HT. In rodents, administration of MDMA is associated with several behavioral effects, one of which is enhanced locomotor activity (Callaway *et al.*, 1990; Ratnay, 1991; Steele *et al.*, 1994; Green *et al.*, 1995; White *et al.*, 1996). This increase in locomotion is mediated by the indirect agonist effect of MDMA and not via a direct effect of MDMA on receptors. Although activation of the serotonergic system has previously been thought to be inhibitory on motor output, recent studies have indicated that the release of presynaptic 5-HT produces hyperactivity.

The importance of presynaptic release of 5-HT by MDMA in locomotor enhancement has previously been demonstrated in studies with rats (Callaway *et al.*, 1990) and more recently in our preliminary dose-ranging studies with CD-1 mice (data not shown) where pretreatment with selective serotonin reuptake-inhibiting antidepressants attenuated the (+)-MDMA-induced hyperactivity. In the current study, we employed (+)-MDMA as a pharmacologic challenge in mice lacking the 5-HTT to probe the effects of the 5-HTT disruption on the serotonergic system. We demonstrated a gene dose-dependent decrease in the hyperactivity induced by (+)-MDMA, suggesting a requirement for a functional 5-HTT in MDMA-induced locomotor activity in mice. An alternative theory to this finding may be that the 5-HTT^{-/-} mice respond in this fashion because they have depleted 5-HT levels, and therefore are unable to release sufficient 5-HT. However, the ~50% decrease of locomotion induced by (+)-MDMA in the 5-HTT^{+/-} mice who have 5-HT levels comparable with the 5-HTT^{+/+} mice argues against this possibility. The decrease in (+)-MDMA-induced hyperactivity in the 5-HTT^{+/-} mice and the complete absence in the 5-HTT^{-/-} mice suggests that the 5-HTT is not only the molecular target for MDMA, but that the 5-HTT also mediates its behavioral and molecular effects on the serotonergic system.

Increased locomotor activity in rodents is also produced by (+)-amphetamine and cocaine, but these effects have been attributed to a primary action of these agents on dopaminergic neurotransmission. Selective lesions of mesolimbic dopamine neurons by different methods prevent amphetamine- or cocaine-induced increases in locomotor activity (Kelly and

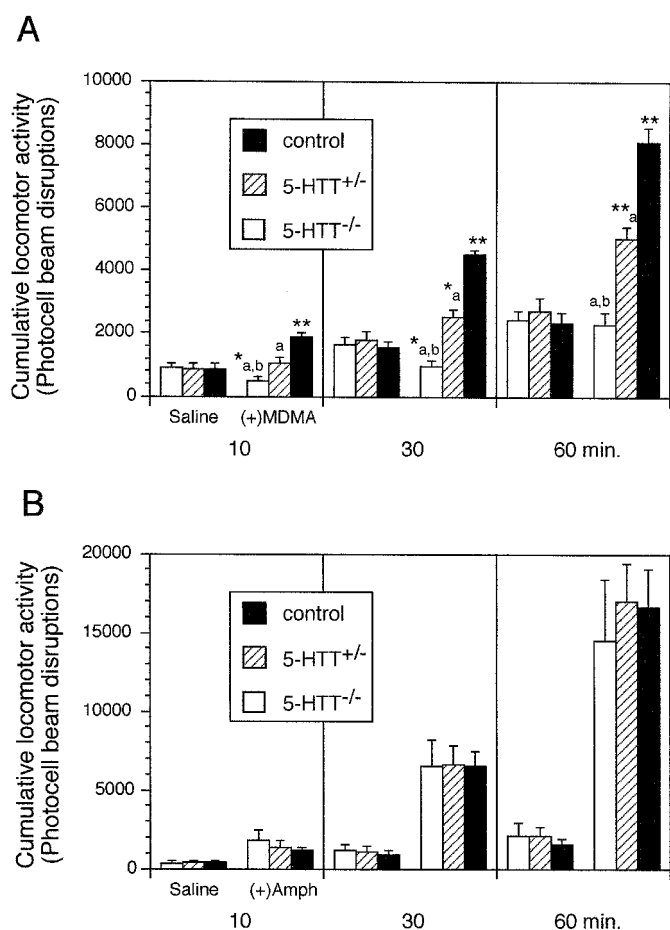


Fig. 5. A, Effect of (+)-MDMA on cumulative locomotor activity. Male mice were injected intraperitoneally with (+)-MDMA (5 mg/kg) or saline, and cumulative photocell beam disruptions were recorded at 10, 30, and 60 min (eight mice per group). *, $p < 0.05$ and **, $p < 0.01$, Significance of differences from respective saline-treated control groups; a, $p < 0.05$, significance of difference from (+)-MDMA-treated 5-HTT^{+/+} group; b, $p < 0.05$, significance of difference from (+)-MDMA-treated 5-HTT^{+/-} group. Error bars, standard error, shown when larger than the dimensions of the symbols. B, Effect of (+)-amphetamine [(+)-Amph] on cumulative locomotor activity. Female mice were injected intraperitoneally with (+)-amphetamine (5 mg/kg) or saline, and cumulative photocell beam disruptions were recorded at 10, 30, and 60 min (five to nine mice per group). Error bars, standard error.

Iversen, 1976; Koob *et al.*, 1981). Mice lacking the dopamine transporter are also insensitive to the locomotor stimulant effects of both these drugs of abuse (Giros *et al.*, 1996). In the present study, no differences across 5-HTT genotypes in the marked locomotor stimulant effects of (+)-amphetamine used at the same dose as MDMA were observed. In ongoing investigations of the effects of cocaine on locomotor activity and other behaviors in these mice, no genotype-related differences in the locomotor effects were observed (Wichems *et al.*, manuscript in preparation). Thus, mice lacking the 5-HTT differ only in their locomotor responses to the one substituted amphetamine that targets the 5-HTT, (+)-MDMA, but not to (+)-amphetamine or cocaine.

Intense interest has been focused on understanding the role of 5-HT in functions as widely varied as sleep, appetite, temperature regulation, pain perception, and motor activity (Vanhoutte *et al.*, 1993). Of equal interest is evidence suggesting that imbalances in brain 5-HT neurotransmission may contribute to conditions such as depression, alcoholism, and drug abuse, as well as obsessive-compulsive disorder and other anxiety disorders (Lesch *et al.*, 1993a, 1993b; Owens and Nemeroff, 1994; Melo *et al.*, 1996; Murphy *et al.*, 1996). Recently, the short, low-activity variant of a polymorphism in the human 5-HTT gene's 5' regulatory region was found to be associated with anxiety-related personality traits (Lesch *et al.*, 1996), adding further data implicating 5-HT in the regulation of anxiety and mood states in primates as well as analogous characteristics in other vertebrate and even invertebrate species (Walters *et al.*, 1981; Griebel, 1995; Yeh *et al.*, 1996). Despite the existence of 14 or more different 5-HT receptor subtypes, it is the single cell membrane 5-HTT that is believed to be the primary modulator of both the 5-HT signaling and the tone of the serotonergic system. However, the precise physiological role of the 5-HTT in the brain has not been evaluated adequately. We anticipate that 5-HTT-deficient mice will prove to be a useful model for the continued study of the mechanism of action of drugs used in the treatment of disorders involving serotonergic dysfunction. Furthermore, these mice represent a powerful tool for the investigation of some drugs of abuse, including (+)-MDMA, which, in addition to having psychostimulant effects, may also produce 5-HT-related neurotoxicity similar to that found after administration of other serotonergic agents (Schmidt *et al.*, 1987; Andrews and Murphy, 1993; Steele *et al.*, 1994).

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