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Imaging elevated brain arachidonic acid signaling in unanesthetized serotonin transporter (5-HTT)-deficient mice

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Abstract

Certain polymorphisms reduce serotonin (5-HT) reuptake transporter (5-HTT) function and increase susceptibility to psychiatric disorders. Heterozygous (5-HTT^{+/-}) deficient mice, models for humans with these polymorphisms, have elevated brain 5-HT concentrations and behavioral abnormalities. Since postsynaptic 5-HT_{2A/2C} receptors are coupled to cytosolic phospholipase A₂ (cPLA₂), which releases arachidonic acid (AA) from membrane phospholipid, 5-HTT deficient mice may have altered brain AA signaling and metabolism. To test this hypothesis, signaling was imaged as an AA incorporation coefficient *k** in unanesthetized homozygous knockout (5-HTT^{-/-}), 5-HTT^{+/-} and wild-type (5-HTT^{+/+}) mice, following saline (baseline) or 1.5 mg/kg s.c. DOI, a partial 5-HT_{2A/2C} receptor agonist. Enzyme activities, metabolite concentrations, and head-twitch responses to DOI also were measured. Baseline *k** was widely elevated by 20-70% in brains of 5-HTT^{+/-} and 5-HTT^{-/-} compared to 5-HTT^{+/+} mice. DOI increased *k** in 5-HTT^{+/+} mice, but decreased *k** in 5-HTT-deficient mice. Brain cPLA₂ activity was elevated in 5-HTT-deficient mice; cyclooxygenase activity and prostaglandin E₂ and F_{2α} and thromboxane B₂ concentrations were reduced. Head-twitch responses to DOI, while robust in 5-HTT^{+/+} and 5-HTT^{+/-} mice, were markedly fewer in 5-HTT^{-/-} mice. Pretreatment with *para*-chlorophenylalanine, a 5-HT synthesis inhibitor, restored head twitches in 5-HTT^{-/-} mice to levels in 5-HTT^{+/+} mice. We propose that increased baseline values of *k** in 5-HTT deficient mice reflect tonic cPLA₂ stimulation *via* 5-HT_{2A/2C} receptors occupied by excess 5-HT, and that reduced *k** and head twitch responses to DOI reflected displacement of receptor-bound 5-HT by DOI with a lower affinity. Increased baseline AA signaling in humans having polymorphisms with reduced 5-HTT function might be identified using positron emission tomography.

Keywords

serotonin, arachidonic acid; serotonin transporter; deficient mice; phospholipase A₂; eicosanoids

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Disclosure/Conflict of Interest

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Introduction

Extracellular serotonin (5-hydroxytryptamine, 5-HT) in brain is regulated in part by the presynaptic serotonin reuptake transporter (5-HTT, SLC6A4). Mice with a partial (5-HTT^{+/-}) or complete (5-HTT^{-/-}) 5-HTT deletion, compared with wild-type (5-HTT^{+/+}) mice, differ with regard to brain anatomy; brain concentrations, reuptake, synthesis, and release of 5-HT; 5-HT and GABA receptor densities; programmed cell death, and brain glucose metabolism (Bengel *et al*, 1998; Esaki *et al*, 2005; Fox *et al*, 2007a; Fox *et al*, 2008a; Mathews *et al*, 2004; Murphy *et al*, 2008; Murphy and Lesch 2008). They also demonstrate increased anxiety-and depression-like behaviors and reduced aggressiveness on various tests (Fox *et al*, 2007a; Murphy *et al*, 2008; Murphy and Lesch 2008).

Reduced serotonergic function in 5-HTT^{+/-} and 5-HTT^{-/-} mice is thought to be comparable to reduced serotonergic function in humans who carry one or two short (S) compared with long (L) alleles of the promoter-region polymorphism of 5-HTT (5-HTTLPR), or who express rs25531 or rs25532 variants of the 5-HTT allele (Murphy *et al*, 2008; Murphy and Lesch 2008). Thus, studying 5-HTT deficient mice could elucidate dysfunctional serotonergic neurotransmission in humans with these polymorphisms, and suggest new methods for identifying and quantifying this dysfunction.

For example, humans carrying one “S” 5-HTTLPR allele have 50% reductions in 5-HTT expression and function in lymphocytes, platelets and brain, compared with those with the LL genotype (Hu *et al*, 2006; Murphy *et al*, 2008; Murphy and Lesch 2008; Praschak-Rieder *et al*, 2007; Wendland *et al*, 2008). They also have comparatively elevated anxiety, depression, and aggression-related personality traits and increased susceptibility to depression associated with major negative life events (Caspi *et al*, 2003; Uher and McGuffin 2008). They respond poorly to selective serotonin reuptake inhibitors (SSRIs) (Hu *et al*, 2006; Murphy *et al*, 2004; Serretti *et al*, 2005), and are at increased risk for bipolar disorder, comorbid disorders accompanying alcoholism, and suicide (Baca-Garcia *et al*, 2007; Li and He 2007; Marques *et al*, 2006; Masoliver *et al*, 2006). Extracellular striatal 5-HT concentrations are 3- and 6-fold higher, respectively, in 5-HTT^{+/-} and 5-HTT^{-/-} than 5-HTT^{+/+} mice (Mathews *et al*, 2004). 5-HT_{2A} receptor density is reduced in the striatum but increased in the hypothalamus and septum of 5-HTT^{-/-} compared with 5-HTT^{+/+} mice, whereas 5-HT_{2C} receptor density is elevated in the amygdala and choroid plexus (Li *et al*, 2003).

Elevated extracellular 5-HT concentrations would be expected to increase 5-HT occupancy of the postsynaptic 5-HT_{2A/2C} receptors that are coupled to cytosolic phospholipase A₂ (cPLA₂), and thereby tonically activate cPLA₂ (Berg *et al*, 1998a; Clark *et al*, 1995; Felder *et al*, 1990). cPLA₂ when activated selectively releases arachidonic acid (AA, 20:4n-6) from membrane phospholipid, to initiate the AA signaling cascade (Fitzpatrick and Soberman 2001; Shimizu and Wolfe 1990). AA and its metabolites (e.g. prostaglandins and endocannabinoids) can modify sleep, neural firing, neurotransmitter release, nociception, cerebral blood flow and gene transcription (Bosetti 2007). We therefore thought it of interest in this paper to see if this cascade is upregulated in 5-HTT deficient mice.

Brain AA signaling involving cPLA₂-coupled neuroreceptors can be imaged in unanesthetized rodents by infusing radiolabeled AA intravenously and measuring tracer uptake into brain with quantitative autoradiography (Rapoport 2001; Robinson *et al*, 1992). k^* for AA at baseline or following drug is independent of changes in cerebral blood flow, thus only reflecting brain AA metabolism (Chang *et al*, 1997). The flux J_{in} of AA, which represents the rate of regional brain AA consumption since AA can't be synthesized *de novo* in vertebrate tissue or converted from its circulating precursor, linoleic acid (18:2n-6) in

brain (DeMar *et al*, 2006; Holman 1986; Rapoport *et al*, 2001), can be calculated as the product of k^* and the unesterified plasma AA concentration.

In this paper, we used *in vivo* brain AA imaging to test whether the reported high levels of brain extracellular 5-HT in 5-HTT deficient mice would tonically stimulate 5-HT_{2A/2C} receptors to augment cPLA₂ activity, and thereby elevate baseline values of k^* and J_{in} , and of AA-derived eicosanoid concentrations. We also examined whether these changes would be accompanied by elevated cyclooxygenase (COX) activity and concentrations of COX-derived eicosanoids, since COX-1 and -2 have been reported to be functionally coupled to cPLA₂ in brain (Bosetti and Weerasinghe 2003; Fitzpatrick and Soberman 2001; Kaufmann *et al*, 1996; Ong *et al*, 1999; Pardue *et al*, 2003; Sapirstein *et al*, 2005; Xu *et al*, 2008). Additionally, we checked if k^* responses to (+/-)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), a partial 5-HT_{2A/2C} agonist (Marek and Aghajanian 1996), would be reduced in 5-HTT deficient mice by displacing already bound 5-HT. The 1.5 mg/kg s.c. DOI dose that we chose has been reported to increase k^* for AA significantly in mouse brain regions rich in 5-HT_{2A/2C} receptors (Qu *et al*, 2005). Finally, we quantified head-twitch responses (HTRs) to DOI as a behavioral test of 5-HT_{2A} receptor function (Willins and Meltzer 1997), before and after pharmacological alteration of extracellular 5-HT (Cesana *et al*, 1993; Fox *et al*, 2007b). Parts of this work have been presented in abstract form (Basselin *et al*, 2007a).

Materials and Methods

Animals

Experiments were conducted following the "Guide for the Care and Use of Laboratory Animals" (National Institute of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Five- to 9-month-old male 5-HTT^{+/-} and 5-HTT^{-/-} mice and their littermate 5-HTT^{+/+} controls, derived from a C57BL/6J genetic background (Bengel *et al*, 1998), were maintained in an animal facility in which temperature, humidity and light cycle were regulated, with free access to water and a fixed diet (Rodent NIH-31 auto 18-4, Zeigler Bros, Gardners, PA). The diet contained (as percent of total fatty acids) 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α -linolenic, 0.02% AA, 2.0% eicosapentaenoic, and 2.3% docosahexaenoic acid.

Drugs

Unanesthetized mice received 0.9% NaCl (saline) or 1.5 mg/kg s.c. (\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI, Sigma-Aldrich, St. Louis MO). [1 -¹⁴C]AA in ethanol (53 mCi/mmol; 99.4% pure, Moravek Biomedicals, Brea, CA) was evaporated and resuspended in 5 mM HEPES buffer, pH 7.4, which contained 50 mg/ml of bovine serum albumin essentially fatty acid free (Sigma-Aldrich). Tracer purity was ascertained to exceed 99% by gas chromatography, after converting AA to its methyl ester with 1% sulfuric acid in anhydrous methanol. The 5-HT synthesis inhibitor *para*-chlorophenylalanine (PCPA, 30 mg/ml prepared in distilled deionized water) and the 5-HT precursor 5-hydroxy-L-tryptophan (5-HTP, 5 mg/ml prepared in 5% Tween 80 in distilled water) were obtained from Sigma-Aldrich.

Surgical procedures and tracer infusion

A mouse was anesthetized with 2-3% halothane in O₂, and PE 10 polyethylene catheters were inserted into its right femoral artery and vein as reported (Basselin *et al*, 2006b; Qu *et al*, 2005). The wound site was closed with 454 Instant Adhesive (Loctite Corp. Hartford, CT) and the mouse was wrapped loosely, with its upper body remaining free, in a fast-

setting plaster cast taped to a wooden block. It was allowed to recover from anesthesia for 3 to 4 h in a warming environment maintained at 25°C. Starting 20 min after s.c. DOI or saline injection, 45 µl [$1\text{-}^{14}\text{C}$]AA (300 µCi/kg) was infused for 3 min through the femoral vein at a rate of 15 µl/min, using a Hamilton syringe and an infusion pump (Harvard Apparatus Model 22, Holliston, MA). Ten 15-20 µl arterial blood samples were collected at 0, 0.25, 1.0, 1.5, 2.0, 2.8, 3.2, 5.0, 10 and 19 min to determine radioactivity of unesterified AA in the plasma. At 20 min the mouse was killed by an overdose of Nembutal® (50 mg/kg, i.v.). The brain was removed quickly within < 30 s, frozen in 2-methylbutane in dry ice at -40°C, and stored at -80°C until sectioned.

Chemical analysis

The blood samples collected before, during and after [$1\text{-}^{14}\text{C}$]AA infusion were centrifuged immediately (30 s at 18,000 g) to obtain plasma, which was stored at -80°C. Total lipids were extracted from 5 µl of thawed plasma with 1 ml chloroform:methanol (2:1, by vol.) and 0.5 ml 0.1 M KCl, using a modified method of Folch (Folch *et al*, 1957). Radioactivity was determined in 100 µl of the lower organic phase by liquid scintillation counting. As reported previously, greater than 95-98% of total plasma and brain radioactivity at 5 min was radiolabeled AA (Lee *et al*, 2007). Concentrations of unlabeled unesterified fatty acids were determined in 100-150 µl of frozen arterial plasma collected by heart puncture. Total lipids were extracted by the modified Folch method, and were separated by thin layer chromatography on silica gel 60 plates using the solvent system: heptane/diethyl ether/acetic acid (60:40:3, by vol). Unesterified fatty acids were scraped from the plate and methylated with 1% sulfuric acid (by vol) in anhydrous methanol for 3 h at 70°C, then separated and quantified by gas chromatography using heptadecanoic acid (17:0) as an internal standard.

Quantitative autoradiography and calculations

Frozen brains were cut in serial 20-µm thick coronal sections on a cryostat at -20°C, then placed for 4 weeks together with calibrated [^{14}C]methylmethacrylate standards (Amersham, Arlington Heights, IL) on Ektascan C/RA film (Eastman Kodak Company, Rochester, NY). Radioactivity (nCi/g of brain) in 92 anatomically identified regions (Franklin and Paxinos 1997) was measured bilaterally six times by quantitative densitometry, using the public domain NIH Image program 1.62 (<http://rsb.info.nih.gov/nih-image/>). Regional AA incorporation coefficients k^* (ml/s/g brain) of AA were calculated as (Robinson *et al*, 1992),

$$k^* = \frac{c_{brain}^* (20 \text{ min})}{\int_0^{20} c_{plasma}^* dt} \quad (\text{Eq. 1})$$

where c_{brain}^* (nCi/g brain) is brain radioactivity at 20 min after the onset of infusion as determined by densitometry, c_{plasma}^* (nCi/ml plasma) is the arterial plasma concentration of labeled unesterified AA as determined by scintillation counting, and t (min) is time after onset of [$1\text{-}^{14}\text{C}$]AA infusion. Integrals of plasma radioactivity (input function in denominator) were determined in each experiment by trapezoidal integration, and divided into c_{brain}^* to calculate k^* for each experiment.

Regional rate of incorporation of unesterified AA from plasma into brain phospholipids, J_{in} (fmol/s/g), was calculated as,

$$J_{in} = k^* c_{plasma} \quad (\text{Eq. 2})$$

where c_{plasma} (nmol/ml) is the plasma concentration of unlabelled unesterified AA.

Brain cPLA₂ activity

In separate experiments, mice were anesthetized with Nembutal® (50 mg/kg, i.p.) and decapitated. The brain was rapidly excised, frozen in 2-methylbutane maintained at -40°C with dry ice, and stored at -80°C . Brain hemispheres were homogenized using a Teflon-glass homogenizer in 2 volumes of ice-cold buffer containing 10 mM HEPES, pH 7.5, 1 mM EDTA, 0.34 M sucrose and protease inhibitor cocktail tablet (Complete, Roche, Mannheim, Germany). Homogenates were centrifuged at 14,000 g for 20 min, then at 100,000 g for 1 h at 4°C . Supernatants corresponding to the cytosolic fraction were assayed for cPLA₂ activity, using a cPLA₂ assay kit and secretory PLA₂ and Ca²⁺-independent PLA₂ inhibitors (Cayman, Ann Arbor MI).

Brain COX activity

Brain hemispheres (see above) were homogenized using a Teflon-glass homogenizer in 1 ml of ice-cold lysate buffer containing 10 mM Tris-HCl, pH 7.8, 1% Igepal CA-630, 0.15 M NaCl, and 1 mM EDTA. Homogenates were centrifuged at 14,000 g for 20 min at 4°C . Brain COX activity was measured as the rate of PGE₂ formation (pg PGE₂/min/mg cytosolic protein) in homogenate cytosolic fractions diluted 1:5 with lysate buffer in the presence of 10 mM phenol, 18.2 mM (-)-epinephrine, 4.6 mM L-glutathione reduced, and 10 μM porcine hematin. The reaction was started by adding AA (Oxford Biochemical Research, Oxford, MI) to a final concentration of 0.1 mM, and the mixture was incubated at 37°C for 15 min. The reaction was terminated by adding 250 μl of 1M HCl. PGE₂ was extracted with ethyl acetate and quantified using a PGE₂ immunoassay kit (Oxford Biochemical Research, Oxford, MI). A sample not containing AA was assayed and used for the blank determination.

In the same study, test drugs, Celebrex® (400 mg. Pfizer Inc, New York, NY, obtained from NIH Division of Veterinary Medicine, Bethesda, MD), a specific COX-2 inhibitor, and DOI were dissolved in dimethylsulfoxide at a concentration of 0.1% and in saline, respectively. These drugs were added to the mixture 10 min before adding AA (see above).

Brain PGE₂, PGF_{2 α} and TXB₂ concentrations

In separate experiments, mice were anesthetized with Nembutal® (50 mg/kg, i.p.) and subjected to head-focused microwave irradiation (5.5 kW, 0.9 s; Cober Electronics, Stamford, CT) to stop postmortem changes (Anton *et al*, 1983). Frozen half-brains were weighed, homogenized with 18 vol of hexane:isopropanol (3:2 by vol) using a glass Tenbroeck homogenizer and the homogenate was centrifuged for 5 min at 800 g. Tissue residues then were rinsed with 3×2 vol of the same solvent. The resultant lipid extract was concentrated to dryness under nitrogen and resuspended in enzyme immunoassay buffer provided with the polyclonal PGE₂, PGF_{2 α} and TXB₂ kits (Oxford Biochemical Research, Oxford, MI).

Head-twitch responses

Mice were administered PCPA (300 mg/kg i.p.) or vehicle twice daily for three days (Cesana *et al*, 1993). On the fourth day, 18 h after the final dose of PCPA, mice were placed in a Plexiglas container. Following 15 min of habituation, DOI (2.5 mg/kg i.p.) was administered. HTR were counted for five 1-min periods starting 5 min after drug administration, and were summed over these five periods. In a separate experiment, HTR following administration of 5-HTP (80 mg/kg i.p.) were assessed in a similar manner (Fox *et al*, 2007b).

Statistical analysis

A one-way analysis of variance (ANOVA) with a Bonferroni's post-test was used to compare mean body weights, cPLA₂ and COX activities and eicosanoid concentrations using GraphPad Prism version 4.0b for Macintosh (GraphPad Software, San Diego CA, www.graphpad.com). A two-way ANOVA was employed to examine the effects of two factors, genotype (5-HTT^{-/-} or 5-HTT^{+/-} vs. 5-HTT^{+/+}) and drug (DOI vs. saline) using SPSS 16.0 (SPSS Inc., Chicago, IL, http://www.spss.com) on the arterial input function, plasma unesterified fatty acid concentrations, k^* and J_{in} . We report all main effect statistics (p and F values), although main effects in the context of a significant interaction may be difficult to interpret (Motulsky 2003; Tabachnick and Fidell 2001). A one-way ANOVA with Bonferroni's post-test with correction for 5 comparisons (5-HTT^{+/+} plus DOI vs. 5-HTT^{+/+} saline; 5-HTT^{+/-} saline vs. 5-HTT^{+/+} saline, 5-HTT^{+/-} plus DOI vs. 5-HTT^{+/-} saline, 5-HTT^{-/-} saline vs. 5-HTT^{+/+} saline, 5-HTT^{-/-} plus DOI vs. 5-HTT^{-/-} saline) was performed. For k^* and J_{in} , corrections for multiple comparisons across regions were not made because the purpose of this exploratory study was to identify regions that were involved in individual drug effect.

One-way (genotype) or two-way (genotype × drug condition) ANOVAs followed by Bonferroni's post-tests were used to assess differences in 5-HTP- and DOI-induced HTR, respectively. Data are reported as means ± SD, with statistical significance taken as $p \leq 0.05$.

Results

Body weight and arterial plasma input function

Mean body weight was significantly higher in 5-HTT^{-/-} ($p < 0.01$) than 5-HTT^{+/-} mice (38.9 ± 5.7 g ($n = 10$) vs. 29.5 ± 5.4 g ($n = 11$)). Body weight equaled 34.2 ± 2.8 g ($n = 10$) in 5-HTT^{+/-} mice.

A two-way ANOVA revealed a significant effect of DOI ($p = 0.008$) on integrated arterial plasma radioactivity (denominator of Eq. 1; plasma input function). Input functions [(nC/ml × s) ± SD, $n = 4-6$] are: 5-HTT^{+/+} plus saline, $135,476 \pm 21,938$; 5-HTT^{+/+} plus DOI, $116,030 \pm 19,123$; 5-HTT^{+/-} plus saline, $130,028 \pm 10,446$; 5-HTT^{+/-} plus DOI, $106,044 \pm 11,708$; 5-HTT^{-/-} plus saline, $113,179 \pm 18,727$ and 5-HTT^{-/-} plus DOI, $105,875 \pm 11,947$.

Plasma concentrations of unlabelled unesterified fatty acids

A two-way ANOVA showed significant interactions between 5-HTT genotype and DOI for plasma concentrations of unesterified palmitoleic, stearic, oleic, and arachidonic acids (Table 1). Subsequent one-way ANOVAs with Bonferroni's post-tests showed that palmitoleic and oleic acid concentrations were higher in 5-HTT^{-/-} and 5-HTT^{+/-} mice than in 5-HTT^{+/+} mice by 57% and 34%, respectively, and that DOI compared to saline decreased palmitoleic acid in 5-HTT^{+/-} and 5-HTT^{-/-} mice by 52% and 38%, respectively. The mean unesterified plasma AA concentration did not differ significantly between groups. Where 5-HTT × DOI interactions were insignificant, the 5-HTT genotype had a main effect for palmitic, linoleic and α -linolenic acids, and DOI had a main effect for linoleic and α -linolenic acids.

Regional brain AA incorporation coefficients k^*

Figure 1 illustrates color-coded coronal autoradiographs of k^* for AA from brains of 5-HTT^{+/+}, 5-HTT^{+/-} and 5-HTT^{-/-} mice injected with either saline (baseline) or DOI. 5-HTT^{+/-} mice and to a greater extent 5-HTT^{-/-} mice had higher baseline values of k^* (Eq. 1) than the 5-HTT^{+/+} mice. Values of k^* were elevated in 5-HTT^{+/+} mice injected with DOI

compared with saline, but reduced in 5-HTT^{+/-} and 5-HTT^{-/-} mice injected with DOI compared with saline.

Mean AA incorporation coefficients k^* in each of 92 brain regions were compared among the different experimental groups and conditions using a two-way ANOVA. As illustrated in Table 2, 90 brain regions (but not the bed nucleus of the stria terminalis and the dorsal raphe nucleus, highlighted) had statistically significant genotype \times drug interactions.

Effect of 5-HTT genotype on baseline values of k^* —A one-way ANOVA with a Bonferroni's post-test showed that partial and total 5-HTT deletion significantly increased mean baseline values of k^* for AA in 45 (by 20-67%) and 72 (by 21-71%) regions, respectively. Affected in both genotypes were cerebral cortex, olfactory tubercle, hippocampus, nucleus accumbens, caudate-putamen, geniculate nucleus, thalamus, mammillary nucleus, mesencephalon and rhombencephalon. In the 2 regions with statistically insignificant genotype \times drug interactions, 5-HTT genotype did not have any main effect.

Effect of DOI in 5-HTT^{+/+} mice—DOI compared with saline significantly increased k^* for AA (by 17-65%) in 42 of 92 regions of the 5-HTT^{+/+} mice (Table 2). Positively affected regions included cerebral cortex (21 of 25 regions, average 33%), suprachiasmatic nucleus (39%), hippocampus CA1 (17%), caudate-putamen ventral (23%), geniculate nucleus (29%), subthalamic nucleus (26%), mesencephalon (6 of 9 regions, average 38%), rhombencephalon (6 of 10 regions, average 51%), white matter (1 of 4 regions, 19%) and non-blood barrier regions (2 of 3, average 42%). In the 2 regions with insignificant genotype \times drug interactions, DOI did not have any significant main effect.

Effect of DOI in 5-HTT^{+/-} and 5-HTT^{-/-} mice—DOI compared with saline did not significantly increase k^* in any of the 42 regions where 5-HTT genotype \times drug interactions were statistically significant (Table 2), but significantly reduced k^* in 31 (-12 to -31%) and 83 (-16 to -63%) regions in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively. In the 2 regions with statistically insignificant genotype \times drug interactions, DOI did not have any main effect.

Patterns of significant differences in k^* —Figure 2 presents difference patterns of k^* responses to DOI in sagittal representations of the mouse brain. The 5-HTT^{+/-} + DOI image compared with the 5-HTT^{+/+} + saline image illustrates the positive regional effects of DOI in the wild-type mice, whereas the 5-HTT^{+/-} + saline image and the 5-HTT^{-/-} + saline image compared with the 5-HTT^{+/+} + saline image illustrates the positive effects of a partial and complete deletion of 5-HTT, respectively, on baseline values of k^* . The 5-HTT^{+/-} + DOI and the 5-HTT^{-/-} + DOI images compared with the 5-HTT^{+/-} + saline and the 5-HTT^{-/-} + DOI saline images show the negative effects of acute DOI on k^* for AA, in mice with a partial and complete deletion of 5-HTT.

Regional incorporation rates of unlabeled unesterified AA from plasma into brain

Baseline and DOI-induced rates of incorporation of unlabelled unesterified AA from plasma into brain phospholipids, J_{in} , were calculated by multiplying individual regional values of k^* by the plasma concentration of unlabelled unesterified AA (Eq. 2) (data not shown). Each of the 92 regions showed a statistically significant 5-HTT genotype \times drug interaction with regard to J_{in} . In 5-HTT^{+/+} mice, baseline J_{in} ranged from 4.19 fmol/s/g in the internal capsule (white matter) to 23.4 fmol/s/g in the choroid plexus. The partial and total 5-HTT deletion significantly increased J_{in} in 15 and 68 brain regions, respectively. DOI elevated J_{in}

significantly in 71 out of 92 regions in the 5-HTT^{+/+} mice, whereas the drug significantly decreased J_{in} in 68 and 83 out of 92 regions in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively.

Brain cPLA₂ activity

An *in vitro* assay with calcium chelators showed that brain cPLA₂ activity was increased by 29% ($p < 0.001$) and 34.5% ($p < 0.001$) in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively, compared with 5-HTT^{+/+} mice (Table 3). Activity did not differ significantly between the 5-HTT^{+/-} and 5-HTT^{-/-} mice. We did not analyze brains following DOI because we could not reproduce the intracellular Ca²⁺ concentrations associated with DOI *in vivo*.

Brain COX activity

Brain COX activity was decreased by 49.2% ($p < 0.001$) and 74.2% ($p < 0.001$) in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively, compared to 5-HTT^{+/+} mice (Table 3). COX activity was 49% less in 5-HTT^{-/-} than in 5-HTT^{+/-} mice. Preincubation of 5-HTT^{+/+} homogenate with 100 μ M DOI did not significantly affect COX activity (102.9 \pm 9.8 versus 112.4 \pm 13.9 pg/min/mg protein), indicating that DOI did not inhibit COX enzymes. On the other hand, 100 μ M Celebrex[®], a selective COX-2 inhibitor used as a positive control, inhibited COX activity by 68% (35.5 \pm 4.2 versus 112.4 \pm 13.9 pg/min/mg protein, $n = 5$, $p < 0.001$).

Brain PGE₂ PGF_{2 α} and TXB₂ concentrations

As illustrated in Table 3, the basal brain PGE₂ concentration was decreased significantly by 74% and 90% in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively, compared with 5-HTT^{+/+} mice. Brain PGF_{2 α} was decreased significantly by 23% and 35% in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively, and brain TXB₂ was decreased significantly by 34% and 72% in 5-HTT^{+/-} and 5-HTT^{-/-} mice, respectively.

Head-twitch responses

After vehicle pretreatment, 5-HTT^{-/-} mice had 86% fewer DOI-induced HTR than 5-HTT^{+/+} mice ($p = 0.006$), similar to a previous report (Qu *et al*, 2005); the number of responses did not differ significantly between 5-HTT^{+/-} and 5-HTT^{+/+} mice, although 5-HTT^{+/-} mice had an intermediate response (decreased 22% compared to 5-HTT^{+/+} mice) (Figure 3). PCPA pretreatment increased the number of HTR in 5-HTT^{-/-} mice by 386% ($p < 0.0001$), with a trend toward an increase in 5-HTT^{+/-} mice ($p = 0.08$), but had no significant effect in 5-HTT^{+/+} mice. After PCPA pretreatment, DOI-induced HTR did not differ significantly among the three genotypes. These findings suggest that 5-HT depletion “normalized” DOI-induced HTR in 5-HTT^{-/-} mice (main effect of genotype [$F(2,57) = 6.33$, $p = 0.003$], main effect of pretreatment drug [$F(1,57) = 3.22$, $p = 0.078$], genotype \times pretreatment drug interaction [$F(2,57) = 3.13$, $p = 0.05$]).

Administration of the 5-HT precursor 5-HTP is reported to increase 5-HT syndrome behaviors (Fox *et al*, 2008a; Fox *et al*, 2007b) and brain 5-HT concentrations 2-5 fold in 5-HTT^{+/+} and 5-HTT^{+/-} mice and 4.5-12 fold in 5-HTT^{-/-} mice (Fox *et al*, 2008a). 5-HTT^{-/-} mice given 5-HTP displayed ~48% fewer HTR than did 5-HTT^{+/+} mice ($p = 0.037$), whereas there was no difference between 5-HTT^{+/+} and 5-HTT^{+/-} mice given 5-HTP (Figure 4) (main effect of genotype [$F(2,36) = 4.24$, $p = 0.022$]).

Discussion

Baseline AA incorporation coefficients k^* were increased significantly in 5-HTT^{-/-} mice in 72 regions of 92 regions by 21-71%, and in 5-HTT^{+/-} mice in 45 regions by 20-67%, compared with 5-HTT^{+/+} mice. Comparable increases were found for J_{in} as well. The increases were accompanied by elevated brain cPLA₂ activity (29% and 35% in 5-HTT^{+/-}

and 5-HTT^{-/-} mice, respectively), decreased COX activity (-49% and -74%, respectively) and decreased concentrations of the COX-derived AA metabolites, PGE₂ (-74% and -90%, respectively), PGF_{2α} (-24% and -35%, respectively) and TXB₂ (-34% and -72%, respectively). The partial 5-HT_{2A/2C} agonist DOI increased k* in 42 regions in wild-type mice, but decreased k* in 31 and 83 regions, respectively, in the 5-HTT^{+/-} and 5-HTT^{-/-} mice. DOI-induced HTR were reduced in 5-HTT^{-/-} mice, but this decreased response was “normalized to 5-HTT^{+/+} levels after 5-HT depletion by pretreatment with PCPA.

Together, these studies suggest that elevated extracellular 5-HT levels in 5-HTT deficient mice, by increasing 5-HT occupancy of PLA₂-coupled postsynaptic 5-HT_{2A/2C} receptors, likely tonically activate cPLA₂ and increase AA release from membrane phospholipid, thereby increasing baseline values of k* and J_{in} for AA. Tonic activation of cPLA₂-coupled neuroreceptors also has been reported to increase cPLA₂ expression (mRNA and activity) in rats treated chronically with a subconvulsive dose of N-methyl-D-aspartic acid (NMDA) to stimulate NMDA receptors (Lee *et al*, 2008; Rao *et al*, 2007), or with fluoxetine, a 5-HTT inhibitor, to stimulate 5-HT_{2A/2C} receptors *via* elevated extracellular 5-HT (Lee *et al*, 2007; Qu *et al*, 2006; Stenfors and Ross 2002). In these and the present case, excess neuroreceptor-induced AA release may have activated protein kinase C and nuclear transcription factor-κB, to transcriptionally upregulate cPLA₂ expression by a feedback mechanism (Toborek *et al*, 1999; Xu *et al*, 2002).

Although the increased J_{in} for AA in the deficient mice represented increased AA loss by brain metabolism (Demar *et al*, 2005; Holman 1986; Rapoport *et al*, 2001), the reduced brain COX activity and PGE₂, PGF_{2α} and TXB₂ concentrations indicate that AA loss was not *via* COX-mediated pathways, but by other pathways such as β-oxidation, formation of endocannabinoids, or oxidation by cytochrome P450 epoxygenase or lipoxygenase (Fitzpatrick and Soberman 2001; Shimizu and Wolfe 1990). While a limitation of this study is that we do not know the exact pathways of increased loss, these might be determined in the future by measuring brain COX-2, COX-1, 5-lipoxygenase and cytochrome P450 epoxygenase activities and their metabolic products in the 5-HTT-deficient mice, in relation to altered behavior (Fox *et al*, 2007a; Murphy and Lesch 2008). In this regard, endocannabinoids derivatives such as anandamide can induce anxiety-like behaviors in rodents (Rubino *et al*, 2008; Rutkowska *et al*, 2006). We do not have a ready explanation for the reductions in COX activity and in eicosanoid concentrations, in the face of increased cPLA₂ activity, but such “uncoupling” of the enzymes also was noted in COX-2 knockout mice (Bosetti *et al*, 2004; Zhang *et al*, 2002). cPLA₂ and COX-2 normally are functionally coupled and co-localized on postsynaptic membranes in rodent brain (Bosetti and Weerasinghe 2003; Fitzpatrick and Soberman 2001; Kaufmann *et al*, 1996; Ong *et al*, 1999; Pardue *et al*, 2003; Sapirstein *et al*, 2005; Xu *et al*, 2008).

The decreased k* and J_{in} responses to DOI in the 5-HTT deficient mice are not due to reductions in 5-HT_{2A/2C} receptor density or their availability due to internalization, as binding studies indicate that 5-HT_{2A} receptor density is reduced only in the striatum but is increased in the hypothalamus and septum of the mice, whereas 5-HT_{2C} receptor density is elevated in the amygdala and choroid plexus (Li *et al*, 2003). The hypothalamus, septum and amygdala belong to the limbic system, which is involved in emotional regulation. Altered 5-HT_{2A/2C} receptors and their signaling may contribute to some of the behavioral changes observed in these mice, such as increased anxiety-like behaviors and reduced aggressiveness on various tests (Fox *et al*, 2007a; Murphy *et al*, 2008; Murphy and Lesch 2008).

One possibility for the decreased k* and J_{in} responses to DOI is that, as a partial agonist, DOI displaced bound 5-HT from cPLA₂-coupled 5-HT_{2A/2C} receptors, and produced less activation compared with 5-HT (Marek and Aghajanian 1996). Such displacement also can

explain the decreased DOI-induced HTR in 5-HTT^{-/-} mice, replicating an earlier report (Qu *et al*, 2005)), since PCPA pretreatment, sufficient to deplete extracellular 5-HT by 67-94% in wild-type or 5-HTT^{-/-} mice (Cesana *et al*, 1993; Fox *et al*, 2008b), returned the DOI-induced HTR in 5-HTT^{-/-} mice to wild-type levels. Consistent with this interpretation, 5-HTT overexpressing mice have lower levels of extracellular 5-HT and higher DOI-induced HTR than do wild-type mice (Jennings *et al*, 2008). Postnatal PCPA administration is reported to prevent some aspects of the adult 5-HTT^{-/-} behavioral phenotype (Alexandre *et al*, 2006) and it would be worthwhile to see if it also prevented some of the differences in the AA signal (Fox *et al*, 2007a).

The serotonin precursor 5-HTP, which increases 5-HT levels in 5-HTT-deficient mice (Fox *et al*, 2008a), induces HTR in mice. In the current study, 5-HTP induced fewer HTR in 5-HTT^{-/-} versus 5-HTT^{+/+} mice, indicating that excessive baseline levels of synaptic 5-HT increases HTR. Brain extracellular concentrations of dopamine, glutamate and acetylcholine are unchanged in 5-HTT-deficient mice and rats (Homberg *et al*, 2007; Mathews *et al*, 2004), and likely did not contribute to the elevations in k* and J_{in} for AA in the 5-HTT deficient mice.

In the 5-HTT^{+/+} mice, statistically significant elevations in k* for AA in response to DOI occurred in the neocortex, mesencephalon and rhombencephalon, which have high 5-HT_{2A} receptor densities. The olfactory tubercle, hypothalamus, amygdala, hippocampus and choroid plexus, which contain mainly 5-HT_{2C} receptors (Li *et al*, 2003), were not activated significantly, suggesting that the k* responses to DOI were mediated mainly by 5-HT_{2A} receptors. However, stimulation of 5-HT_{2A} or 5-HT_{2C} receptors by different agonists can activate cPLA₂ to release AA (Berg *et al*, 1998b). Selective 5-HT_{2A} and 5-HT_{2C} antagonists could be used to distinguish the roles of the two receptor subtypes.

Dorsal raphe neurons of 5-HTT^{-/-} and 5-HTT^{+/-} mice exhibit decreased firing rates (Murphy and Lesch 2008), and 5-HTT^{-/-} mice have widespread reductions in brain glucose metabolism, a measure of energy consumption (Esaki *et al*, 2005; Sokoloff 1999), despite their elevated values of k* and J_{in}. The obesity of the 5-HTT^{-/-} mice is consistent with published data and is associated with increased plasma levels of insulin, leptin, cholesterol and triglycerides (Murphy and Lesch 2008). Their high plasma unesterified fatty acid concentrations may be related to adrenocorticotrophic hormone and corticosterone elevations and to anxiety-related behaviors (Gottschalk *et al*, 1969; John *et al*, 1987; Murphy and Lesch 2008).

The baseline values of k* for AA in the 5-HTT^{+/+} mice agree with published values (Basselin *et al*, 2006b; Qu *et al*, 2005). An earlier paper reported that baseline values did not differ significantly between 5-HTT^{-/-} and 5-HTT^{+/+} mice, unlike the current findings. However, the previous report is likely erroneous. Frozen brain sections in the previous work were exposed to X-ray film for 6-10 weeks, rather than for 4 weeks as was done in the present paper, which resulted in saturation at high optical densities, with loss of linearity and discrimination (Basselin M, unpublished observations).

The 5-HTT^{+/-} mouse is considered a model for humans who carry the “S” compared with “L” allele of the 5-HTTLPR, or who express rs25531 or rs25532 variants of the 5-HTT allele with regard to levels of 5-HTT expression and function (Murphy *et al*, 2008; Murphy and Lesch 2008). Individuals with these lesser-expressing 5-HTT polymorphisms are at risk for multiple psychiatric disorders, including bipolar disorder (Masoliver *et al*, 2006; Murphy *et al*, 2008; Murphy and Lesch 2008). In this regard, elevated brain AA metabolism has been suggested to contribute to and be a risk factor for bipolar disorder (Basselin *et al*, 2006a; Basselin *et al*, 2007b; Rao *et al*, 2008; Rapoport and Bosetti 2002).

The new data in this paper suggest that baseline k^* and J_{in} for AA would be elevated in individuals with the “S” 5-HTTLPR allele. This prediction might be tested with the use of positron emission tomography (PET) in human subjects in the resting state, following the intravenous injection of [^{11}C]AA. In such PET studies, the coefficient of variation of k^* for AA ranges from 12-16% (Esposito *et al*, 2008; Giovacchini *et al*, 2004). A power analysis (<http://statpages.org/#Power>) shows that statistically significant ($\alpha = 5$, statistical power = 0.8) resting-state differences of 20% (the lower range of significant elevations in the 5-HTT deficient mice) thus could be demonstrated with 8 subjects each belonging to long and short 5-HTTLPR groups. To date, PET studies assessing acute drug responses of the AA signal have not been performed in humans, but in any case, if DOI were given it would be expected to produce untoward hallucinogenic effects (Marek and Aghajanian 1996).

The present findings extend our knowledge of altered neurotransmission involving 5-HT, which is thought to contribute to depression and anxiety disorders. The data demonstrated that reduced or absent 5-HTT function in mice results in an upregulation of baseline AA signaling involving 5-HT. Given the parallels between phenotypic abnormalities in 5-HTT-deficient mice and in human mood and anxiety disorders, these data provide a model for humans with 5-HTT polymorphisms and mutations that affect 5-HTT expression and function.

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Abbreviations

AA	arachidonic acid
COX	cyclooxygenase
PLA ₂	phospholipase A ₂
cPLA ₂	cytosolic PLA ₂
PET	positron emission tomography
PGE ₂	prostaglandin E ₂
PGF _{2α}	prostaglandin F _{2α}
5-HT	serotonin
5-HTP	5-hydroxy-L-tryptophan
5-HTT	serotonin reuptake transporter
HTR	head-twitch responses
PCPA	<i>para</i> -chlorophenylalanine
PET	positron emission tomography
TXB ₂	thromboxane B ₂
DOI	(\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride
SSRI	selective serotonin reuptake inhibitor

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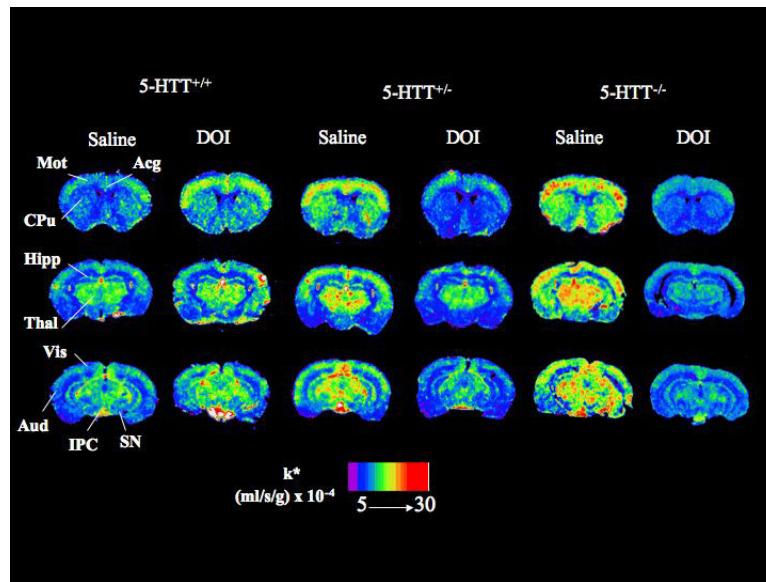


Figure 1.

Coronal autoradiographs of brain showing effects of DOI and 5-HTT genotype on regional AA incorporation coefficients k^* in mice. Values of k^* ($\text{ml/s/g brain} \times 10^{-4}$) are given on a color scale from 5 (blue) to 30 (red). Abbreviations: Acg, anterior cingulate cortex; Aud, auditory cortex; CPu, caudate-putamen; Hipp, hippocampus; IPC, interpeduncular nucleus; Mot, motor cortex; SN, substantia nigra; Thal, thalamus; Vis, visual cortex.

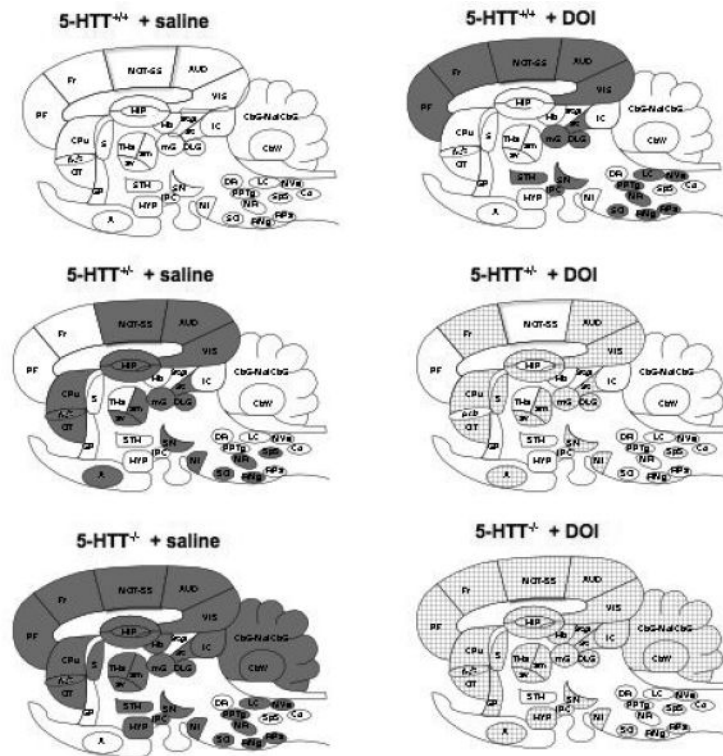


Figure 2.

Difference patterns of k^* responses to DOI in sagittal representation of 5-HTT mouse brain. Regions in which k^* was increased significantly ($p < 0.05$) are solid grey, regions in which k^* was decreased significantly are hatched. The 5-HTT^{+/-} + DOI image is compared with the 5-HTT^{+/+} + saline image. The 5-HTT^{+/-} + saline and the 5-HTT^{-/-} + saline images are compared with the 5-HTT^{+/+} + saline image. The 5-HTT^{+/-} + DOI and the 5-HTT^{-/-} + DOI images are compared with the 5-HTT^{+/-} + saline and the 5-HTT^{-/-} + saline, respectively. List of regions: A, amygdala; Acb, nucleus accumbens; AUD, auditory cortex; am, anteromedial thalamic nucleus; av, anteroventral thalamic nucleus; CbG, cerebellar gray matter; CbW, cerebellar white matter; Co, cochlear nucleus; CPu, caudate putamen; DLG, dorsal lateral geniculate nucleus; DR, dorsal raphe; Fr, frontal cortex; GP, globus pallidus; Hb, habenular complex; HIP, hippocampus; HYP, hypothalamus; IC, inferior colliculus; IPC, interpeduncular nucleus; LC, locus coeruleus; MI, mammillary nucleus; mG, medial geniculate nucleus; MolCbG, molecular layer of cerebellar gray matter; MOT, motor cortex; MR, median raphe; MVe, medial vestibular nucleus; OT, olfactory tubercle; PF, prefrontal cortex; PPTg, pedunculopontine tegmental nucleus; SN, substantia nigra; S, septum; SO, superior olive; Sp5, spinal trigeminal nucleus; SS, somatosensory cortex; SC, superior colliculus; SCgl, gray layer of superior colliculus; STH, subthalamic nucleus; ThA, thalamus; VIS, visual cortex.

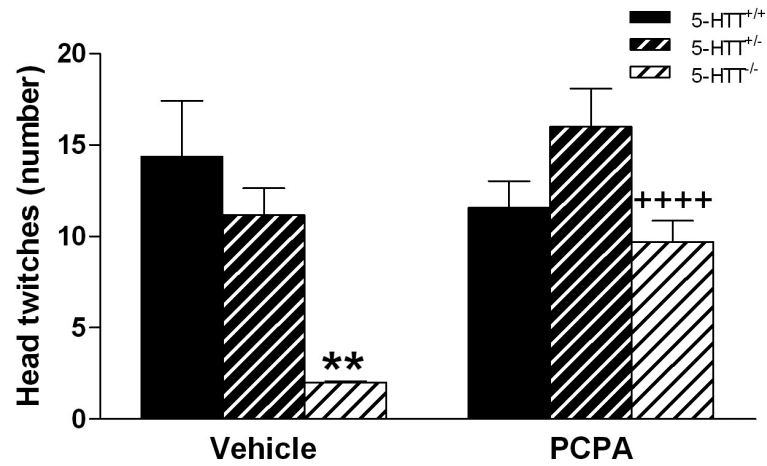


Figure 3.

Effects of brain 5-HT depletion by PCPA pretreatment on DOI-induced head twitches in 5-HTT mice. At baseline (vehicle pretreatment), DOI induced fewer head twitches in 5-HTT^{-/-} mice compared to 5-HTT^{+/+} mice, whereas DOI-induced head twitches were similar between 5-HTT^{+/+} and 5-HTT^{+/-} mice. Pretreatment with PCPA, which depletes 5-HT levels, increased DOI-induced head twitches in 5-HTT^{-/-} mice, with a trend toward a significant increase in 5-HTT^{+/-} mice ($p = 0.08$), compared to their vehicle-pretreated counterparts. Data represent the means \pm SEM, $n = 7-13$ per group. ** $p < 0.01$ compared to 5-HTT^{+/+} mice in the same pretreatment condition; ++++ $p < 0.0001$ compared to vehicle-pretreated mice of the same 5-HTT genotype.

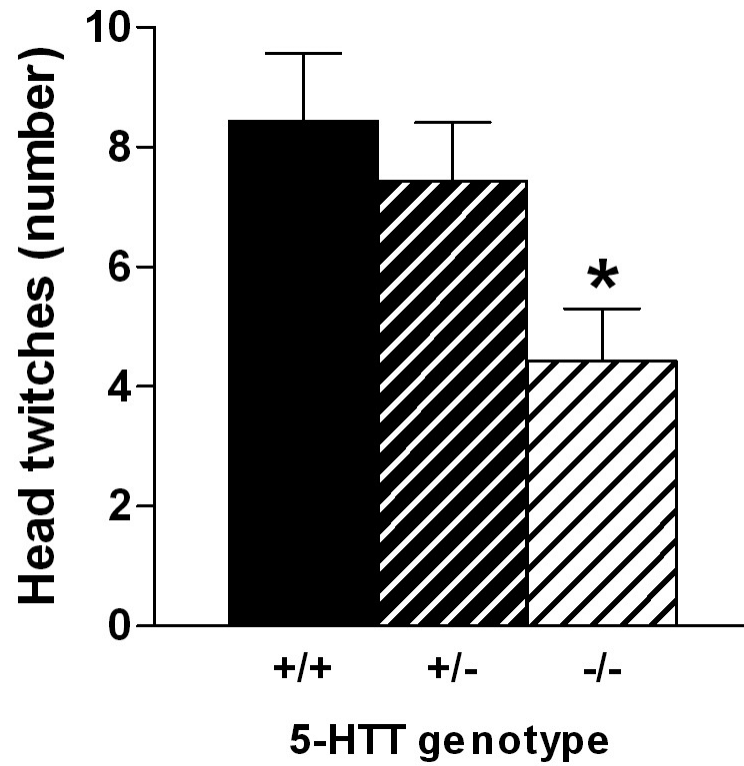


Figure 4. 5-HTP-induced head twitches in 5-HTT mice. The 5-HT precursor 5-HTP, which increases 5-HT levels, induced fewer head twitches in 5-HTT^{-/-} mice compared to 5-HTT^{+/+} mice, with no difference between 5-HTT^{+/-} and 5-HTT^{+/+} mice. Data represent the means \pm SEM; n = 9-16 per group. * p < 0.05 compared to 5-HTT^{+/+} mice.

Table 1

Unesterified plasma fatty acid concentrations in 5-HTT^{+/+}, 5-HTT^{+/-} and 5-HTT^{-/-} mice after s.c. saline or DOI administration

Fatty Acid	5-HTT ^{+/+}		5-HTT ^{+/-}		5-HTT ^{-/-}		5-HTT × DOI interaction		5-HTT effect		DOI effect
	Saline (n = 5)	DOI (n = 6)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	p-value	DOI	p-value	p-value	
Palmitate (16:0)	94.0 ± 26.5	118.0 ± 37.7	104.6 ± 26.9	77.3 ± 23.4	128.4 ± 21.5	128.7 ± 19.6	0.118		0.016		0.923
Palmitoleate (16:1n-7)	24.8 ± 3.1	22.9 ± 3.8	32.9 ± 12.2	15.8 ± 7.1 ^{***}	39.0 ± 6.3 [*]	24.3 ± 3.2 ^{**}	0.031				
Stearate (18:0)	37.0 ± 11.9	45.5 ± 15.1	27.3 ± 5.5	33.5 ± 10.1	40.8 ± 5.0	28.2 ± 5.6	0.047				
Oleate (18:1n-9)	108.6 ± 15.0	119.3 ± 16.7	106.0 ± 29.4	76.5 ± 11.9	145.8 ± 12.9 ^{**}	127.2 ± 7.7	0.032				
Linoleate (18:2n-6)	124.1 ± 14.7	118.3 ± 15.9	111.4 ± 20.1	94.5 ± 28.1	165.0 ± 12.9	123.9 ± 15.9	0.106			<0.001	0.004
α-Linolenate (18:3n-3)	8.79 ± 1.34	7.27 ± 0.89	8.32 ± 2.43	6.08 ± 1.56	11.6 ± 1.2	10.4 ± 1.6	0.732			<0.001	0.007
Arachidonate (20:4n-6)	8.23 ± 2.15	10.2 ± 0.3	7.09 ± 1.04	5.94 ± 1.55	7.80 ± 1.54	7.15 ± 0.90	0.028				
Docosahexaenoate (22:6n-3)	18.1 ± 5.9	18.2 ± 4.8	18.8 ± 6.6	18.6 ± 6.2	21.9 ± 7.0	22.1 ± 23.4	0.997			1.000	0.280

Concentrations are nmol/ml plasma. Values are mean ± SD.

Main effects not reported if statistically significant 5-HTT × DOI interaction. In cases of statistically insignificant 5-HTT × DOI interaction, Bonferroni's post-test was performed.

5-HTT^{+/+} plus DOI versus 5-HTT^{+/+} saline; 5-HTT^{+/-} plus DOI versus 5-HTT^{+/-} saline, 5-HTT^{+/-} plus DOI versus 5-HTT^{+/+} saline, 5-HTT^{-/-} plus DOI versus 5-HTT^{-/-} saline, 5-HTT^{-/-} plus DOI versus 5-HTT^{+/+} saline

* p < 0.05

** p < 0.01

TABLE 2
 Arachidonic acid incorporation coefficients (k*) in 5-HTT^{+/+}, 5-HTT^{+/-} and 5-HTT^{-/-} mice at baseline and in response to DOI

Brain region	5-HTT ^{+/+}			5-HTT ^{+/-}			5-HTT ^{-/-}			Genotype × Drug Interaction		
	Saline (n = 5)	DOI (n = 6)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	p and (F) values	Genotype effect p and (F) values	Drug effect p and (F) values			
Prefrontal cortex layer I	6.47 ± 0.44	8.84 ± 1.59*	7.90 ± 1.36	6.56 ± 1.14	9.41 ± 1.44**	4.89 ± 0.77**	< 0.001 (21.2)	0.598 (0.53)	0.014 (7.05)			
Prefrontal cortex layer IV	8.29 ± 0.91	10.4 ± 1.5	10.4 ± 1.0	8.70 ± 1.40	10.6 ± 1.9*	6.32 ± 0.71**	< 0.001 (16.2)	0.162 (1.96)	0.009 (8.06)			
Primary olfactory cortex	7.34 ± 0.76	9.19 ± 1.28*	9.25 ± 0.78*	7.93 ± 0.59	8.92 ± 1.19	5.50 ± 0.62***	< 0.001 (21.4)	0.007 (6.05)	0.008 (8.30)			
Frontal cortex (10)												
Layer I	7.27 ± 1.12	8.14 ± 0.51	8.44 ± 0.69	7.87 ± 1.06	9.80 ± 1.12*	7.01 ± 1.19*	0.034 (3.89)	0.565 (0.58)	0.137 (2.36)			
Layer IV	9.16 ± 1.30	10.4 ± 1.2	12.1 ± 0.5*	9.00 ± 0.83*	11.8 ± 1.8*	8.10 ± 1.09*	0.001 (9.17)	0.476 (0.76)	0.002 (12.17)			
Frontal cortex (8)												
Layer I	7.37 ± 1.00	10.3 ± 1.9**	9.48 ± 1.07	8.49 ± 0.42	10.3 ± 1.5**	6.17 ± 0.95***	< 0.001 (20.62)	0.388 (0.98)	0.111 (2.73)			
Layer IV	9.67 ± 1.08	13.0 ± 1.9**	11.8 ± 0.8	9.10 ± 0.58*	13.7 ± 1.9**	6.48 ± 0.78***	< 0.001 (23.44)	0.073 (2.91)	0.004 (10.34)			
Pyramidal cortex	6.11 ± 0.61	8.12 ± 1.34*	7.87 ± 0.86	6.91 ± 1.48	8.49 ± 1.22**	3.98 ± 0.44***	< 0.001 (23.95)	0.061 (3.14)	0.006 (8.85)			
Anterior cingulate cortex	9.74 ± 1.00	12.3 ± 1.4**	11.9 ± 0.9*	10.1 ± 1.2	12.7 ± 1.4**	7.37 ± 1.22***	< 0.001 (29.10)	0.124 (2.27)	0.002 (12.57)			
Motor cortex												
Layer I	6.42 ± 1.10	8.09 ± 0.52	8.82 ± 1.57*	7.76 ± 0.68	9.92 ± 2.69**	7.06 ± 0.91*	0.006 (6.28)	0.133 (2.20)	0.169 (2.00)			
Layer II - III	6.80 ± 0.97	9.07 ± 0.71*	9.67 ± 1.31**	8.76 ± 1.00	10.4 ± 2.0***	6.94 ± 0.65***	< 0.001 (15.77)	0.059 (3.17)	0.107 (2.79)			
Layer IV	8.96 ± 1.22	12.1 ± 1.3***	11.8 ± 0.8**	10.4 ± 1.3	13.0 ± 1.3***	7.89 ± 0.73***	< 0.001 (33.37)	0.356 (1.078)	0.013 (7.09)			
Layer V	7.04 ± 0.49	9.94 ± 1.17***	10.2 ± 1.5***	8.80 ± 0.99	9.82 ± 1.35**	6.34 ± 0.75***	< 0.001 (23.35)	0.023 (4.42)	0.113 (2.70)			
Layer VI	6.58 ± 0.53	9.59 ± 1.08***	9.62 ± 1.14***	8.94 ± 1.07	9.80 ± 1.08***	6.19 ± 1.10***	< 0.001 (32.08)	0.008 (5.86)	0.219 (1.59)			
Somatosensory cortex												
Layer I	6.99 ± 0.65	10.0 ± 0.8***	9.38 ± 0.74**	8.53 ± 0.97	10.4 ± 1.2***	8.70 ± 1.21*	< 0.001 (18.88)	0.064 (3.08)	0.606 (0.274)			
Layer II - III	8.13 ± 0.85	11.2 ± 0.7***	11.1 ± 1.1***	8.81 ± 0.80**	10.9 ± 1.3***	8.07 ± 1.01***	< 0.001 (28.65)	0.567 (0.58)	0.065 (3.71)			
Layer IV	9.66 ± 0.89	13.1 ± 1.9**	12.2 ± 1.1*	10.9 ± 0.8	12.9 ± 1.8**	9.86 ± 0.97**	< 0.001 (16.24)	0.937 (0.07)	0.549 (0.37)			
Layer V	9.01 ± 1.09	12.1 ± 1.7**	12.0 ± 1.2**	10.6 ± 1.3	11.7 ± 1.7*	7.92 ± 1.00***	< 0.001 (16.90)	0.082 (2.77)	0.151 (2.19)			
Layer VI	9.19 ± 1.08	11.4 ± 0.9*	11.4 ± 1.0*	9.92 ± 0.89	10.6 ± 1.7	8.65 ± 1.02	< 0.001 (10.56)	0.138 (2.14)	0.303 (1.11)			

Brain region	5-HTT ^{+/+}			5-HTT ^{-/-}			Genotype × Drug Interaction			Drug effect	
	Saline (n = 5)	DOI (n = 6)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	p and (F) values	p and (F) values	p and (F) values	p and (F) values	
Auditory cortex											
Layer I	7.62 ± 1.43	9.84 ± 1.11*	11.3 ± 0.8***	8.56 ± 1.15**	11.2 ± 1.0***	6.84 ± 1.01***	< 0.001 (25.55)	0.052 (3.34)	< 0.001 (17.21)	< 0.001 (17.21)	
Layer IV	9.23 ± 1.43	12.1 ± 1.3**	12.1 ± 0.9**	9.76 ± 0.67*	11.9 ± 1.6**	7.40 ± 0.34***	< 0.001 (30.22)	0.046 (3.48)	0.003 (10.43)	0.003 (10.43)	
Layer VI	8.09 ± 1.21	10.0 ± 0.8**	10.9 ± 0.4***	9.07 ± 0.68**	9.98 ± 1.1**	6.38 ± 0.25***	< 0.001 (33.00)	< 0.001 (12.95)	< 0.001 (16.79)	< 0.001 (16.79)	
Visual cortex											
Layer I	7.17 ± 1.22	9.42 ± 1.04*	9.99 ± 1.71**	8.26 ± 1.06	10.0 ± 0.7**	6.47 ± 0.31***	< 0.001 (19.21)	0.148 (2.07)	0.017 (6.51)	0.017 (6.51)	
Layer IV	8.64 ± 0.65	12.3 ± 2.4***	11.5 ± 1.1**	9.04 ± 0.72*	10.2 ± 1.1	7.40 ± 0.34*	< 0.001 (20.78)	0.015 (5.01)	0.275 (1.25)	0.275 (1.25)	
Layer VI	8.31 ± 0.86	10.9 ± 1.9*	11.6 ± 0.9**	8.31 ± 0.51**	10.7 ± 1.8*	7.03 ± 0.30***	< 0.001 (20.01)	0.174 (1.88)	0.005 (9.69)	0.005 (9.69)	
Preoptic area (LPO/MPO)											
Suprachiasmatic nu	6.46 ± 0.50	7.60 ± 2.07	7.89 ± 0.46	6.44 ± 0.57	6.95 ± 0.71	4.58 ± 0.57**	0.002 (7.87)	0.011 (5.45)	0.027 (5.55)	0.027 (5.55)	
Globus pallidus	6.42 ± 0.81	8.93 ± 1.85*	7.44 ± 1.09	6.20 ± 1.02	7.56 ± 1.45	4.12 ± 0.54***	< 0.001 (15.59)	0.009 (5.77)	0.117 (2.63)	0.117 (2.63)	
Bed nu stria terminalis											
Olfactory tubercle	5.83 ± 0.65	6.33 ± 0.95	7.08 ± 0.59	6.27 ± 0.54	6.77 ± 0.87	4.13 ± 0.51***	< 0.001 (12.80)	0.003 (7.42)	0.001 (14.68)	0.001 (14.68)	
Diagonal band Dorsal	6.63 ± 0.86	7.22 ± 1.21	7.09 ± 0.70	6.49 ± 1.05	7.58 ± 0.45	6.50 ± 0.42	0.088 (2.68)	0.801 (0.22)	0.252 (1.38)	0.252 (1.38)	
Ventral	8.67 ± 1.06	9.91 ± 1.11	10.7 ± 0.8*	8.29 ± 0.94**	11.1 ± 1.4**	6.54 ± 0.43***	< 0.001 (22.5)	0.352 (1.09)	< 0.001 (28.29)	< 0.001 (28.29)	
Amygdala	7.97 ± 0.37	9.00 ± 2.3	8.81 ± 0.57	7.07 ± 1.09	10.9 ± 1.3**	5.01 ± 0.58***	< 0.001 (19.32)	0.551 (0.61)	< 0.001 (22.93)	< 0.001 (22.93)	
Hippocampus	8.09 ± 0.93	8.49 ± 1.83	8.59 ± 1.06	6.38 ± 1.30*	11.5 ± 0.7**	4.23 ± 0.68***	< 0.001 (22.97)	0.320 (1.19)	< 0.001 (50.10)	< 0.001 (50.10)	
CA1	6.78 ± 0.28	7.57 ± 0.79	8.70 ± 0.96*	6.68 ± 1.28*	8.32 ± 1.1	5.38 ± 0.89***	< 0.001 (11.27)	0.152 (2.03)	< 0.001 (16.86)	< 0.001 (16.86)	
CA2	5.91 ± 0.44	6.89 ± 0.62*	8.23 ± 1.10**	6.31 ± 0.37**	7.17 ± 0.97	5.49 ± 0.27*	0.001 (9.41)	0.036 (3.82)	0.009 (8.06)	0.009 (8.06)	
CA3	6.25 ± 0.39	7.27 ± 0.94	8.14 ± 0.42***	6.83 ± 0.38*	7.72 ± 0.43*	5.39 ± 1.06***	< 0.001 (16.75)	0.013 (5.18)	0.002 (12.70)	0.002 (12.70)	
Dentate gyrus	6.26 ± 0.70	7.57 ± 0.56	8.15 ± 0.53***	7.00 ± 0.70*	8.29 ± 0.91**	4.66 ± 0.50***	< 0.001 (36.45)	0.004 (7.00)	< 0.001 (23.38)	< 0.001 (23.38)	
SLM	7.01 ± 0.70	7.83 ± 0.85	8.84 ± 0.65**	7.65 ± 0.64	8.71 ± 0.83**	5.56 ± 0.62***	< 0.001 (19.47)	0.006 (6.29)	< 0.001 (20.09)	< 0.001 (20.09)	
Accumbens nu	9.27 ± 1.12	11.0 ± 1.0	11.9 ± 1.2*	9.74 ± 0.47	11.3 ± 1.1	7.96 ± 1.46**	0.001 (9.97)	0.171 (1.90)	0.021 (6.12)	0.021 (6.12)	
Caudate putamen	6.95 ± 0.69	7.72 ± 1.09	9.18 ± 0.62**	7.89 ± 0.92	8.73 ± 0.79*	5.84 ± 0.66***	< 0.001 (13.07)	0.003 (7.53)	0.001 (14.57)	0.001 (14.57)	
Dorsal	7.45 ± 0.91	8.66 ± 0.95	9.32 ± 1.49*	7.55 ± 0.30*	10.2 ± 0.8**	5.07 ± 0.58***	< 0.001 (31.28)	0.161 (1.96)	< 0.001 (33.00)	< 0.001 (33.00)	

Brain region	5-HTT ^{+/+}			5-HTT ^{-/-}			Genotype × Drug Interaction			Drug effect	
	Saline (n = 5)	DOI (n = 6)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	p and (F) values	p and (F) values	p and (F) values	p and (F) values	
Ventral	7.65 ± 0.32	9.39 ± 0.91 ^{**}	9.21 ± 0.74 [*]	7.53 ± 0.36 [*]	11.4 ± 1.4 ^{***}	5.55 ± 0.64 ^{***}	<0.001 (32.41)	0.951 (0.05)	<0.001 (24.79)	<0.001 (24.79)	
Lateral	7.34 ± 0.46	8.66 ± 0.75	9.67 ± 1.27 [*]	8.12 ± 0.73	11.8 ± 1.9 ^{***}	5.76 ± 0.64 ^{***}	<0.001 (25.09)	0.192 (1.76)	<0.001 (23.54)	<0.001 (23.54)	
Medial	7.28 ± 0.45	7.98 ± 0.94	9.26 ± 0.88 [*]	7.90 ± 0.52	10.9 ± 1.9 ^{***}	5.24 ± 0.61 ^{***}	<0.001 (26.61)	0.124 (2.28)	<0.001 (33.34)	<0.001 (33.34)	
Septal nucleus lateral	6.46 ± 0.74	6.30 ± 0.77	7.58 ± 0.74	6.19 ± 0.42 [*]	8.48 ± 0.67 ^{***}	4.69 ± 0.61 ^{***}	<0.001 (19.33)	0.241 (1.51)	<0.001 (54.26)	<0.001 (54.26)	
Septal nucleus medial	8.33 ± 1.01	9.22 ± 1.86	8.68 ± 0.62	7.26 ± 0.72	10.9 ± 1.1 ^{**}	4.86 ± 0.65 ^{***}	<0.001 (25.32)	0.146 (2.08)	<0.001 (29.12)	<0.001 (29.12)	
Diencephalon											
Habenular nu lateral	14.0 ± 1.6	16.5 ± 2.3	15.3 ± 1.7	13.8 ± 0.9	17.7 ± 2.0 ^{**}	9.03 ± 0.91 ^{***}	<0.001 (29.60)	0.050 (3.38)	<0.001 (18.81)	<0.001 (18.81)	
Habenular nu medial	13.2 ± 2.3	16.8 ± 2.1	18.1 ± 4.8	13.7 ± 0.7	17.3 ± 1.7 ^{**}	8.41 ± 0.37 ^{***}	<0.001 (17.49)	0.032 (3.98)	0.001 (13.54)	0.001 (13.54)	
Lateral geniculate nu	10.7 ± 1.1	13.5 ± 1.0 ^{***}	13.4 ± 1.1 ^{**}	10.5 ± 0.8 ^{***}	14.6 ± 1.3 ^{***}	8.13 ± 0.63 ^{***}	<0.001 (55.61)	0.247 (1.48)	<0.001 (35.7)	<0.001 (35.7)	
Medial geniculate nu.	11.0 ± 1.3	14.4 ± 0.9 ^{***}	14.4 ± 1.1 ^{***}	11.5 ± 0.7 ^{**}	15.3 ± 0.9 ^{***}	10.5 ± 1.8 ^{***}	<0.001 (31.85)	0.333 (1.15)	0.017 (6.55)	0.017 (6.55)	
Thalamus											
Ventroposterior lateral nu	10.9 ± 0.9	12.7 ± 1.9	12.2 ± 1.00	9.32 ± 1.16 [*]	13.3 ± 1.0 [*]	7.31 ± 1.04 ^{***}	<0.001 (24.22)	0.034 (3.89)	<0.001 (27.53)	<0.001 (27.53)	
Ventroposterior medial nu	10.1 ± 1.5	12.6 ± 1.9	12.9 ± 1.5	9.62 ± 0.96 [*]	13.1 ± 1.4 [*]	7.79 ± 1.18 ^{***}	<0.001 (20.57)	0.303 (1.25)	0.001 (14.76)	0.001 (14.76)	
Paratenial nu	8.70 ± 1.38	10.5 ± 1.1	11.5 ± 1.3	9.21 ± 0.97	12.2 ± 1.9	6.73 ± 0.76 ^{**}	<0.001 (21.40)	0.257 (1.44)	<0.001 (19.40)	<0.001 (19.40)	
Anteroventral nu	12.8 ± 1.3	14.4 ± 1.5	16.8 ± 2.5 [*]	12.4 ± 0.7 [*]	17.7 ± 2.1 [*]	10.4 ± 0.5 ^{***}	0.002 (8.28)	0.786 (0.24)	0.001 (14.30)	0.001 (14.30)	
Anteromedial nu	9.51 ± 1.68	10.9 ± 1.1	13.2 ± 1.5 ^{**}	10.1 ± 1.1 [*]	13.4 ± 2.5 ^{**}	7.23 ± 1.08 ^{***}	<0.001 (15.27)	0.072 (2.93)	<0.001 (21.79)	<0.001 (21.79)	
Reticular nu	9.98 ± 1.23	11.6 ± 1.9	10.7 ± 1.7	10.1 ± 0.7	12.7 ± 2.0 [*]	7.35 ± 0.89 ^{***}	<0.001 (14.55)	0.521 (0.67)	0.014 (6.94)	0.014 (6.94)	
Paraventricular nu	9.25 ± 1.39	10.6 ± 1.0	11.5 ± 1.3 [*]	9.62 ± 0.78	12.2 ± 1.9 ^{**}	6.73 ± 0.76 ^{***}	<0.001 (19.97)	0.167 (1.93)	<0.001 (20.51)	<0.001 (20.51)	
Parafascicular nu	9.46 ± 1.33	11.3 ± 1.3	10.8 ± 0.8	10.1 ± 1.5	12.5 ± 2.7 ^{**}	6.17 ± 0.94 ^{***}	<0.001 (19.25)	0.194 (1.75)	0.005 (9.66)	0.005 (9.66)	
Subthalamic nu	10.4 ± 1.1	13.1 ± 1.0 [*]	12.6 ± 1.1	12.2 ± 1.2	14.5 ± 2.3 ^{***}	12.4 ± 0.8	0.004 (7.06)	0.044 (3.56)	0.856 (0.034)	0.856 (0.034)	
Hypothalamus											
Supraoptic nu	7.04 ± 0.67	7.24 ± 0.87	8.89 ± 0.44 ^{**}	7.37 ± 0.46 [*]	7.75 ± 0.95	4.23 ± 0.68 ^{***}	<0.001 (17.76)	<0.001 (22.63)	<0.001 (39.58)	<0.001 (39.58)	
Lateral	6.34 ± 0.21	7.23 ± 1.05	7.95 ± 0.99	6.37 ± 0.35	8.19 ± 1.88 [*]	4.30 ± 0.57 ^{***}	<0.001 (14.64)	0.145 (2.09)	<0.001 (17.64)	<0.001 (17.64)	
Anterior	6.42 ± 0.39	7.44 ± 1.27	7.96 ± 0.87	6.29 ± 0.49	9.21 ± 2.28 ^{**}	4.83 ± 0.56 ^{***}	<0.001 (13.73)	0.930 (0.072)	0.001 (15.62)	0.001 (15.62)	
Periventricular	5.20 ± 0.36	6.48 ± 1.20	7.01 ± 0.65	5.05 ± 0.64 [*]	7.58 ± 1.90 ^{**}	4.01 ± 0.47 ^{***}	<0.001 (15.25)	0.863 (0.148)	0.001 (14.75)	0.001 (14.75)	

Brain region	5-HTT ^{+/+}			5-HTT ^{-/-}			Genotype × Drug Interaction		
	Saline (n = 5)	DOI (n = 6)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	p and (F) values	Genotype effect p and (F) values	Drug effect p and (F) values
Arcuate	6.41 ± 0.90	7.77 ± 1.55	4.34 ± 1.03	6.15 ± 0.29	8.01 ± 1.34	4.88 ± 0.74 ^{***}	<0.001 (11.41)	0.406 (0.94)	0.017 (6.48)
Ventromedial	6.64 ± 0.82	8.04 ± 1.45	7.84 ± 1.15	6.35 ± 0.28	7.98 ± 1.50	4.57 ± 0.69 ^{***}	<0.001 (12.94)	0.086 (2.71)	0.006 (8.89)
Posterior	8.91 ± 1.88	11.7 ± 1.8	9.38 ± 0.56	8.07 ± 0.68	17.7 ± 3.3 ^{***}	6.74 ± 1.02 ^{***}	<0.001 (39.83)	0.001 (9.66)	<0.001 (24.29)
Mammillary nu	6.45 ± 0.82	8.41 ± 1.61	9.25 ± 0.78 ^{**}	8.15 ± 0.55 ^{**}	9.96 ± 1.71 ^{***}	7.39 ± 1.09 ^{**}	<0.001 (14.37)	0.063 (3.09)	0.008 (8.22)
Mesencephalon									
Interpeduncular nu	14.4 ± 2.6	23.1 ± 3.2 ^{***}	19.5 ± 3.6	15.7 ± 1.1	23.6 ± 4.2 ^{***}	13.7 ± 2.2 ^{***}	<0.001 (25.93)	0.658 (0.43)	0.137 (2.37)
Substantia nigra	7.01 ± 0.98	9.75 ± 1.20 ^{**}	10.8 ± 1.0 ^{***}	8.14 ± 1.06 ^{**}	9.68 ± 0.62 ^{**}	7.42 ± 1.47 [*]	<0.001 (20.06)	0.074 (2.90)	0.077 (3.39)
Pretectal area	9.49 ± 1.14	12.7 ± 2.5 [*]	12.1 ± 1.5	10.2 ± 1.4	13.0 ± 1.9 [*]	9.81 ± 1.36 [*]	0.001 (10.01)	0.918 (0.09)	0.344 (0.93)
Grey layer Sup colliculus	9.00 ± 1.56	10.5 ± 1.2	11.1 ± 0.7	9.52 ± 0.76	11.1 ± 1.8	7.37 ± 1.17 ^{***}	<0.001 (11.10)	0.181 (1.83)	0.010 (7.72)
Superior colliculus	9.70 ± 1.16	13.0 ± 2.2 [*]	14.0 ± 2.5 ^{**}	9.68 ± 0.75 ^{**}	13.4 ± 1.9 [*]	8.09 ± 1.23 ^{***}	<0.001 (19.16)	0.380 (1.06)	0.003 (11.12)
Inferior colliculus	14.7 ± 1.3	18.0 ± 0.9	18.5 ± 2.2	15.3 ± 1.7	20.5 ± 3.7 ^{**}	15.6 ± 2.1 [*]	0.001 (9.53)	0.228 (1.57)	0.063 (3.78)
Median raphe nu	7.70 ± 1.12	10.3 ± 0.9 [*]	11.3 ± 0.9 ^{***}	11.0 ± 1.5	11.3 ± 0.9 ^{***}	11.4 ± 1.5	0.016 (4.89)	<0.001 (13.97)	0.064 (3.25)
Dorsal raphe nu	8.56 ± 1.18	11.2 ± 1.5	10.9 ± 0.8	11.1 ± 1.1	11.4 ± 1.5	10.6 ± 0.9	0.173 (1.89)	0.585 (0.55)	0.556 (0.36)
Pedunculopontine tegmental nu	7.88 ± 0.87	9.94 ± 1.48 [*]	9.56 ± 0.64	8.62 ± 0.84	9.56 ± 0.64 ^{**}	8.89 ± 0.362	0.002 (8.49)	0.737 (0.31)	0.655 (0.21)
Rhombencephalon									
Flocculus	9.46 ± 1.02	10.8 ± 0.8	10.4 ± 1.1	10.3 ± 0.6	12.2 ± 1.3 ^{**}	8.98 ± 1.43 ^{***}	<0.001 (12.24)	0.585 (0.55)	0.099 (2.94)
Cerebellar gray matter	7.93 ± 0.58	10.1 ± 1.2	11.4 ± 1.2 ^{**}	8.79 ± 0.94 [*]	12.5 ± 1.5 ^{***}	7.95 ± 1.74 ^{***}	<0.001 (19.67)	0.062 (3.11)	0.001 (13.82)
Molecular layer cerebellar gray	11.2 ± 1.2	15.9 ± 1.7 ^{**}	14.2 ± 1.8	13.0 ± 1.2	16.8 ± 2.4 ^{***}	12.3 ± 2.4 ^{**}	<0.001 (16.33)	0.407 (0.93)	0.602 (0.28)
Raphe magnus nu	8.12 ± 0.84	11.3 ± 1.3 [*]	11.3 ± 2.1 [*]	10.5 ± 1.0	14.1 ± 2.8 ^{***}	11.2 ± 2.0	0.003 (7.66)	0.004 (6.97)	0.794 (0.07)
Raphe pallidus nu	8.08 ± 1.7	11.5 ± 1.6 [*]	10.4 ± 1.0	8.96 ± 1.23	12.3 ± 3.7 [*]	8.1 ± 1.0 [*]	0.001 (10.26)	0.813 (0.21)	0.297 (1.13)
Locus coeruleus	9.09 ± 0.60	12.5 ± 1.9 [*]	11.7 ± 1.3	12.6 ± 1.9	14.6 ± 2.3 ^{***}	13.8 ± 1.8	0.032 (3.98)	0.001 (9.67)	0.077 (3.39)
Cochlear nu	9.92 ± 1.52	11.9 ± 1.8	11.8 ± 0.5	11.7 ± 1.8	14.9 ± 1.0	12.9 ± 1.3	0.012 (5.26)	<0.001 (12.28)	0.945 (0.01)
Spinal trigeminal nu, interpolar	9.68 ± 1.22	12.6 ± 1.5	13.8 ± 2.1 ^{**}	9.53 ± 0.62 ^{**}	12.8 ± 1.8	9.45 ± 2.32 [*]	<0.001 (13.20)	0.782 (0.25)	0.011 (7.63)
Superior olive	7.12 ± 0.52	11.6 ± 3.1 [*]	11.9 ± 0.9 [*]	9.77 ± 1.31	11.3 ± 2.8 [*]	8.86 ± 2.9 ^{***}	<0.001 (12.71)	<0.001 (18.41)	<0.001 (17.13)
Medial vestibular nu	9.84 ± 0.85	16.2 ± 3.2 ^{***}	13.1 ± 2.1	10.5 ± 0.9	14.8 ± 2.6 ^{**}	7.60 ± 2.30 ^{***}	<0.001 (25.62)	0.165 (1.94)	0.171 (1.98)

Brain region	5-HTT ^{+/+}			5-HTT ^{+/-}			5-HTT ^{-/-}			Genotype × Drug Interaction		Drug effect		
	Saline (n = 5)	DOI (n = 6)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	Saline (n = 5)	DOI (n = 5)	p and (F) values	p and (F) values	p and (F) values	p and (F) values		
White matter														
Corpus callosum	5.34 ± 0.60	6.06 ± 0.58	6.28 ± 0.77	5.25 ± 0.40	5.39 ± 0.26	3.85 ± 0.43 ^{***}	< 0.001 (13.01)	< 0.001 (14.52)	0.004 (10.22)					
Zona incerta	7.55 ± 0.63	9.04 ± 1.91	8.36 ± 1.07	7.84 ± 0.35	11.0 ± 1.9 ^{**}	7.95 ± 0.75 ^{***}	< 0.001 (23.01)	0.829 (0.190)	0.001 (12.97)					
Internal capsule	5.09 ± 0.36	6.05 ± 1.08 [*]	5.83 ± 0.57	5.32 ± 0.48	6.11 ± 0.89 [*]	3.72 ± 0.88 ^{***}	< 0.001 (12.27)	0.110 (2.42)	0.028 (5.45)					
Cerebellar white matter	5.66 ± 0.50	5.75 ± 0.64	5.95 ± 0.39	5.91 ± 0.33	7.45 ± 1.35 ^{**}	3.87 ± 0.65 ^{***}	< 0.001 (21.17)	0.662 (0.42)	< 0.001 (20.41)					
Non-blood-brain barrier regions														
Subfornical organ	7.41 ± 1.16	10.7 ± 1.7 [*]	8.93 ± 0.81	8.59 ± 0.55	12.7 ± 3.5 ^{***}	7.92 ± 0.89 ^{**}	< 0.001 (14.10)	0.131 (2.21)	0.352 (0.90)					
Median eminence	5.84 ± 0.78	8.17 ± 1.19 [*]	7.08 ± 0.87	5.09 ± 0.90 [*]	9.11 ± 1.47 ^{***}	4.41 ± 0.70 ^{***}	< 0.001 (31.53)	0.127 (2.25)	0.001 (15.60)					
Choroid plexus (third ventricle)	28.5 ± 3.2	31.0 ± 3.7	28.4 ± 6.4	29.3 ± 1.7	35.5 ± 9.5	24.1 ± 3.1 ^{**}	0.011 (5.47)	0.893 (0.11)	0.174 (1.96)					

Abbreviations: nu, nucleus; SLM, stratum lacunosum-moleculae of hippocampus

k* = (ml/s/g) × 10⁻⁴, DOI: 1.5 mg/kg s.c. Each value is a mean ± S.D.

In cases of statistically significant genotype 5-HTT × drug interaction, Bonferroni¹ post-test were realized.

5-HTT^{+/+} plus DOI vs 5-HTT^{+/+} saline; 5-HTT^{+/+} saline vs 5-HTT^{+/+} plus DOI vs 5-HTT^{+/+} saline, 5-HTT^{+/+} saline vs 5-HTT^{+/+} plus DOI vs 5-HTT^{+/+} saline, 5-HTT^{+/+} plus DOI vs 5-HTT^{+/+} saline, 5-HTT^{+/+} plus DOI vs 5-HTT^{+/+} saline

* p < 0.05

** p < 0.01

*** p < 0.001

Table 3Effect of 5-HTT genotype on global brain cPLA₂ and COX activities and eicosanoid concentrations

	5-HTT ^{+/+}	5-HTT ^{+/-}	5-HTT ^{-/-}
cPLA ₂ activity (nmol/min/g protein)			
	(n = 6)	(n = 7)	(n = 5)
	896.3 ± 83.7	1155 ± 95 ^{***}	1207 ± 83 ^{***}
COX activity (pg PGE ₂ /min/mg protein)			
	(n = 6)	(n = 7)	(n = 5)
	112.4 ± 13.9	57.1 ± 9.6 ^{***}	29.0 ± 3.6 ^{***B}
Eicosanoid concentration			
	(n = 4)	(n = 4)	(n = 4)
PGE ₂ (ng/g brain)	23.6 ± 1.8	6.12 ± 1.18 ^{***}	2.44 ± 0.75 ^{***A}
PGF _{2α} (pg/g brain)	63.5 ± 4.3	48.5 ± 3.6 ^{***}	41.3 ± 3.1 ^{***}
TXB ₂ (pg/g brain)	102.4 ± 24.3	67.4 ± 4.3 ^{***}	28.4 ± 2.4 ^{***A}

Values are means ± SD. Bonferroni's multiple comparison tests were performed:

5-HTT^{-/-} and 5-HTT^{+/-} versus 5-HTT^{+/+}***
p < 0.001^A
p < 0.01^B
p < 0.001 5-HTT^{-/-} versus 5-HTT^{+/-}