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Imaging Elevated Brain Arachidonic Acid Signaling in Unanesthetized Serotonin Transporter (5-HTT)-Deficient Mice

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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. As postsynaptic 5-HT$_{2A/2C}$ receptors are coupled to cytosolic phospholipase A$_2$ (cPLA$_2$), 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 were also 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 cPLA$_2$ activity was elevated in 5-HTT-deficient mice; cyclooxygenase activity and prostaglandin E$_2$ and F$_{2\alpha}$ and thromboxane B$_2$ concentrations were reduced. Head-twitch responses to DOI, although 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 cPLA$_2$ stimulation through 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.
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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, 2008a; Mathews *et al*, 2004; Murphy *et al*, 2008; Murphy and Lesch, 2008). They also show 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

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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 three- and sixfold 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 A$_2$ (cPLA$_2$), and thereby tonically activate cPLA$_2$ (Berg *et al*, 1998a; Clark *et al*, 1995; Felder *et al*, 1990). cPLA$_2$ 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 (eg, 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 study to see whether this cascade is upregulated in 5-HTT-deficient mice.

Brain AA signaling involving cPLA$_2$-coupled neuroreceptors can be imaged in unanesthetized rodents by infusing radiolabeled AA i.v. 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, as AA cannot 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 study, 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 cPLA$_2$ 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, as COX-1 and -2 have been reported to be functionally coupled to cPLA$_2$ 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 whether 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 study 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 nine-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. DOI (Sigma-Aldrich, St Louis MO). [1-^{14}C]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 into 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 O_2 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 3–4 h in a warming environment maintained at 25°C. Starting 20 min after s.c. DOI or saline injection, 45 μl [1-^{14}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-^{14}C]AA infusions 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 earlier, more 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),

$$k^* = \frac{c^*_{\text{brain}^{(20\text{ m})}}}{\int_0^{20} c^*_{\text{plasma}}{}^{\text{dt}}} \quad (1)$$

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 the onset of [1-^{14}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,

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

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

Brain cPLA$_2$ 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 vol 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 cPLA$_2$ activity, using a cPLA$_2$ assay kit and secretory PLA$_2$ and Ca^{2+}-independent PLA$_2$ 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 PGE_2 formation (pg PGE_2/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 1 M HCl. PGE_2 was extracted with ethyl acetate and quantified using a PGE_2 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 PGE_2, $PGF_{2\alpha}$, and TXB_2 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 PGE_2, $PGF_{2\alpha}$ and TXB_2 kits (Oxford Biochemical Research, Oxford, MI).

Head-Twitch Responses

Mice were administered PCPA (300 mg/kg i.p.) or vehicle twice daily for 3 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, $cPLA_2$ 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 five 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, and 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 mean ± SD, with statistical significance taken as $p \leqslant 0.05$.

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 [(nCi/ml × s) ± SD, $n = 4$–6] are 5-HTT$^{+/+}$ plus saline, 135 476 ± 21 938; 5-HTT$^{+/+}$ plus DOI, 116 030 ± 19 123; 5-HTT$^{+/-}$ plus saline, 130 028 ± 10 446; 5-HTT$^{+/-}$ plus DOI, 106 044 ± 11 708; 5-HTT$^{-/-}$ plus saline, 113 179 ± 18 727, and 5-HTT$^{-/-}$ plus DOI, 105 875 ± 11 947.

Plasma Concentrations of Unlabeled 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 with 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 acid, 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-injected mice, but reduced in 5-HTT$^{+/-}$ and 5-HTT$^{-/-}$ mice.

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 × 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. Cerebral cortex, olfactory tubercle, hippocampus, nucleus accumbens, caudate-putamen, geniculate nucleus, thalamus, mammillary nucleus, mesencephalon, and rhombencephalon were affected in both genotypes. In the two regions with statistically insignificant genotype × 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 nonblood–barrier regions (2 of 3, average 42%). In the

Table 1 Unesterified plasma fatty-acid concentrations in 5-HTT$^{+/+}$, 5-HTT$^{+/-}$, and 5-HTT$^{-/-}$ mice after s.c. saline or DOI administration

	5-HTT$^{+/+}$		5-HTT$^{+/-}$		5-HTT$^{-/-}$		5-HTT × DOI interaction	5-HTT effect	DOI effect
Fatty acid	**Saline ($n=5$)**	**DOI ($n=6$)**	**Saline ($n=5$)**	**DOI ($n=5$)**	**Saline ($n=5$)**	**DOI ($n=5$)**	***p*-value**	***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 are not reported for statistically significant 5-HTT × DOI interaction. In cases of statistically insignificant 5-HTT × DOI interaction, Bonferroni's post-test was performed. *$p<0.05$; **$p<0.01$; 5-HTT$^{+/+}$+DOI *vs* 5-HTT$^{+/+}$ saline; 5-HTT$^{+/-}$ saline *vs* 5-HTT$^{+/+}$ saline, 5-HTT$^{+/-}$+DOI *vs* 5-HTT$^{+/-}$ saline, 5-HTT$^{-/-}$ saline *vs* 5-HTT$^{+/+}$ saline, 5-HTT$^{-/-}$+DOI vs 5-HTT$^{-/-}$ saline.

two regions with insignificant genotype × 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 × 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 two regions with statistically insignificant genotype × 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 unlabeled unesterified AA from plasma into brain phospholipids, J_{in}, were calculated by multiplying individual regional values of k^* by the plasma concentration of unlabeled unesterified AA (Eq. 2; data not shown). Each of the 92 regions showed a statistically significant 5-HTT genotype × 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 deletions 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 cPLA$_2$ Activity

An *in vitro* assay with calcium chelators showed that brain cPLA$_2$ 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 Ca^{2+} 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 μM DOI did not significantly affect COX activity (102.9 ± 9.8 *vs* 112.4 ± 13.9 pg/min/mg protein), indicating that DOI did not inhibit COX enzymes. On the other hand, 100 μM Celebrex, a selective COX-2 inhibitor used as a positive control, inhibited COX activity by 68% (35.5 ± 4.2 *vs* 112.4 ± 13.9 pg/min/mg protein, $n=5$, $p<0.001$).

Brain PGE$_2$, PGF$_{2\alpha}$, and TXB$_2$ Concentrations

As illustrated in Table 3, the basal brain PGE$_2$ concentration was decreased significantly by 74 and 90% in 5-HTT$^{+/-}$ and 5-HTT$^{-/-}$ mice, respectively, compared with 5-HTT$^{+/+}$

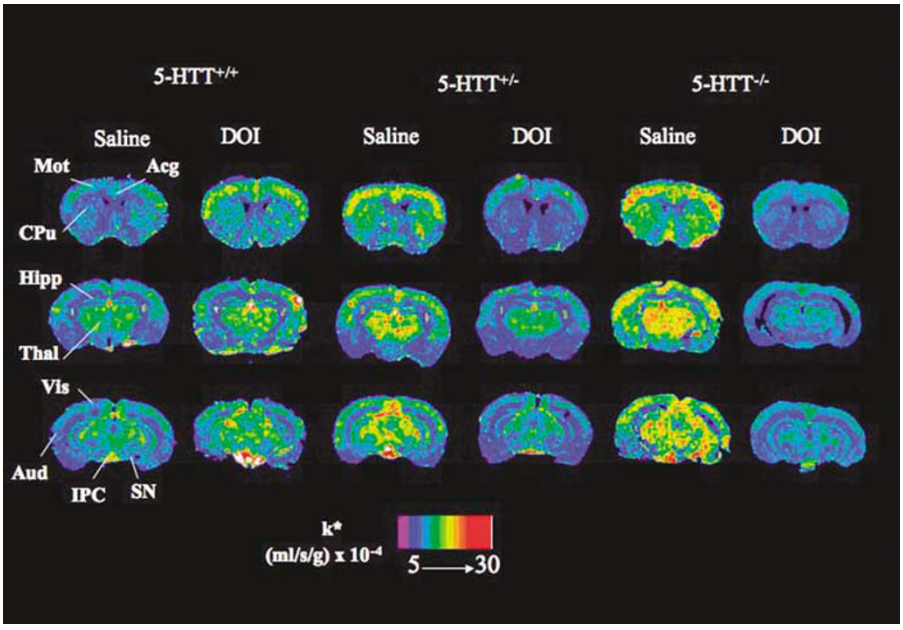

Figure 1 Coronal autoradiographs of brain showing effects of DOI and 5-HTT genotype on regional AA incorporation coefficients k^* in mice. Values of k^* (ml/s/g brain $\times 10^{-4}$) are given on a color scale from 5 (blue) to 30 (red). Acg, anterior cingulate cortex; Aud, auditory cortex; CPu, caudate-putamen; Hipp, hippocampus; IPC, interpeduncular nucleus; Mot, motor cortex; SN, substantia nigra; Thal, thalamus; Vis, visual cortex.

mice. Brain $PGF_{2\alpha}$ was decreased significantly by 23 and 35% in 5-HTT$^{+/-}$ and 5-HTT$^{-/-}$ mice, respectively, and brain TXB_2 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 an earlier 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 ($F(1,57) = 3.22$, $p = 0.078$), and 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, 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 $\sim$48% fewer HTR than did 5-HTT$^{+/+}$ 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 of 92 regions by 21–71%, and in 5-HTT$^{+/-}$ mice in 45 regions by 20–67%, compared to 5-HTT$^{+/+}$ mice. Comparable increases were found for J_{in} as well. The increases were accompanied by elevated brain $cPLA_2$ 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, PGE_2 (−74 and -90%, respectively), $PGF_{2\alpha}$ (−24 and −35%, respectively), and TXB_2 (−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 PLA_2-coupled postsynaptic 5-$HT_{2A/2C}$ receptors, likely tonically activate $cPLA_2$ and increase AA release from membrane phospholipid, thereby increasing baseline values of k^* and J_{in} for AA. Tonic activation of $cPLA_2$-coupled neuroreceptors also has been reported to increase $cPLA_2$ 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 through 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

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

	5-HTT$^{+/+}$		5-HTT$^{+/-}$		5-HTT$^{-/-}$		Genotype × drug interaction	Genotype effect	Drug effect
Brain region	**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**
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)	0014 (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)
Pyriform 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)
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)
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)
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)
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)
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)
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)

Table 2 Continued

Brain region	5-HTT$^{+/+}$ Saline ($n=5$)	5-HTT$^{+/+}$ DOI ($n=6$)	5-HTT$^{+/-}$ Saline ($n=5$)	5-HTT$^{+/-}$ DOI ($n=5$)	5-HTT$^{-/-}$ Saline ($n=5$)	5-HTT$^{-/-}$ DOI ($n=5$)	Genotype × drug interaction *p* and F-values	Genotype effect *p*- and F-values	Drug effect *p*- and F-values
Preoptic area (LPO/MPO)	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)
Suprachiasmatic nu	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)
Globus pallidus	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)
Bed nu stria terminalis	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)
Olfactory tubercle	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)
Diagonal band dorsal	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)
Ventral	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)
Amygdala	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)
Hippocampus									
CA1	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)
CA2	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)
CA3	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)
Dentate gyrus	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)
SLM	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)
Accumbens nu	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)
Caudate putamen									
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)
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)

Table 2 Continued

Brain region	5-HTT$^{+/+}$ Saline ($n=5$)	5-HTT$^{+/+}$ DOI ($n=6$)	5-HTT$^{+/-}$ Saline ($n=5$)	5-HTT$^{+/-}$ DOI ($n=5$)	5-HTT$^{-/-}$ Saline ($n=5$)	5-HTT$^{-/-}$ DOI ($n=5$)	Genotype × drug interaction *p* and F-values	Genotype effect *p*- and F-values	Drug effect *p*- and F-values
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)
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)
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)
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)
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)
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)
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)

Table 2 Continued

Brain region	5-HTT$^{+/+}$		5-HTT$^{+/-}$		5-HTT$^{-/-}$		Genotype × drug interaction	Genotype effect	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
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)
Nonblood–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)

nu, nucleus; SLM, stratum lacunosum-moleculae of hippocampus $k^* = (ml/s/g) \times 10^{-4}$, DOI: 1.5 mg/kg s.c. (each value is a mean ± SD).
In cases of statistically significant genotype 5-HTT × drug interaction, Bonferroni's post-tests were realized. *$p<0.05$; **$p<0.01$; ***$p<0.001$; 5-HTT$^{+/+}$+DOI *vs* 5-HTT$^{+/+}$ saline, 5-HTT$^{+/-}$ saline *vs* 5-HTT$^{+/+}$ saline, 5-HTT$^{+/-}$+DOI *vs* 5-HTT$^{+/-}$ saline, 5-HTT$^{-/-}$ saline *vs* 5-HTT$^{+/+}$ saline, and 5-HTT$^{-/-}$+DOI *vs* 5-HTT$^{-/-}$ saline.

transcriptionally upregulate $cPLA_2$ 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 PGE_2, $PGF_{2\alpha}$, and TXB_2 concentrations indicate that AA loss was not through 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). Although there is a limitation 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 $cPLA_2$ activity, but such 'uncoupling' of the enzymes also was noted in COX-2 knockout mice (Bosetti *et al*, 2004; Zhang *et al*, 2002). $cPLA_2$ and COX-2 normally are functionally coupled and colocalized 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 $5\text{-}HT_{2A/2C}$ receptor density or their availability due to internalization, as binding studies indicate that $5\text{-}HT_{2A}$ receptor density is reduced only in the striatum but is increased in the hypothalamus and septum of the mice, whereas $5\text{-}HT_{2C}$ 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 $5\text{-}HT_{2A/2C}$ 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 $cPLA_2$-coupled $5\text{-}HT_{2A/2C}$ 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), as 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 whether it also

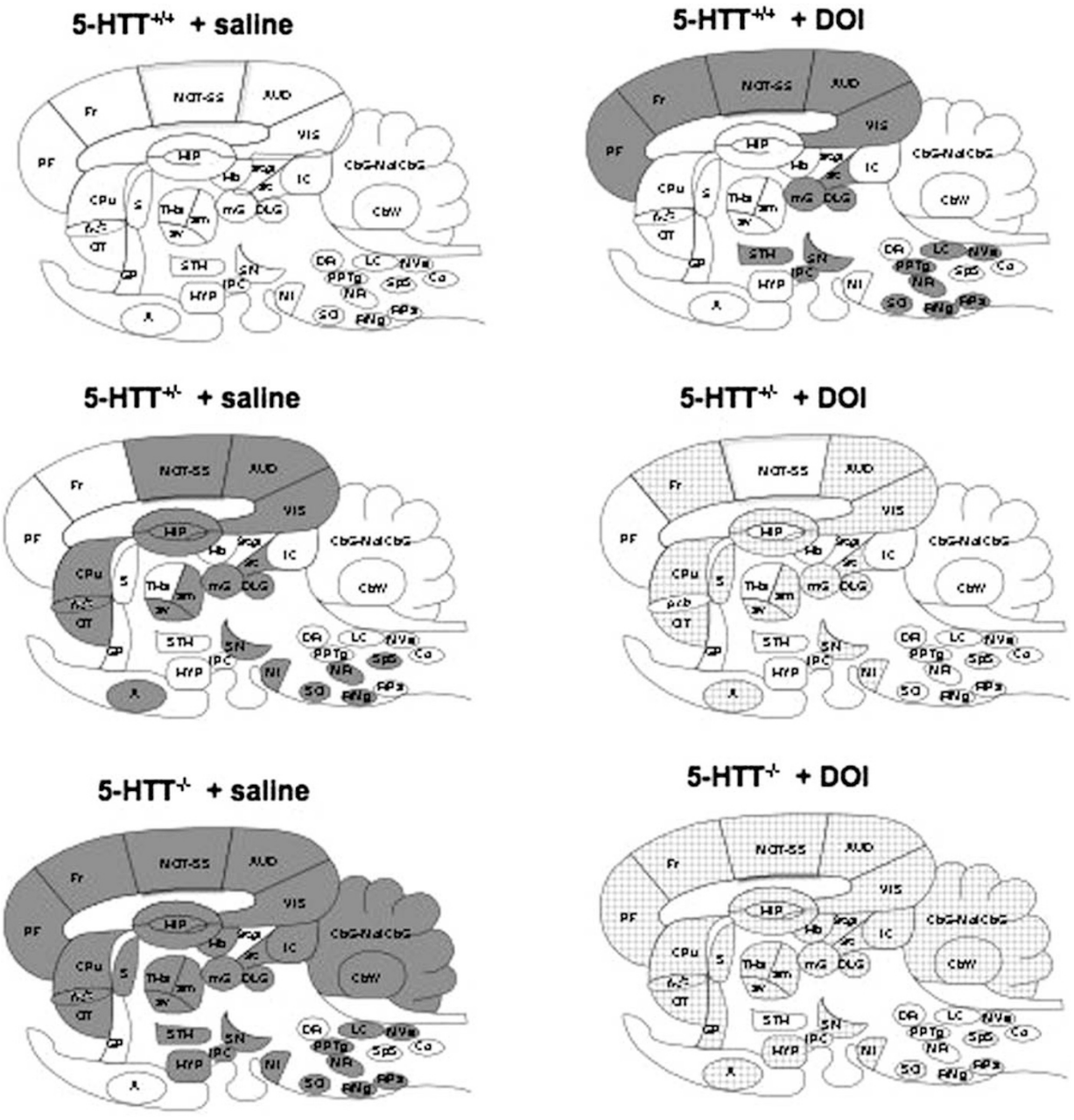

Figure 2 Difference patterns of k^* responses to DOI in sagittal representation of 5-HTT mouse brain. Regions in which k^* was increased significantly ($p<0.05$) are solid gray, regions in which k^* was decreased significantly are hatched. The 5-HTT$^{+/-}$ + DOI image is compared with the 5-HTT$^{+/+}$ + 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.

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 this study, 5-HTP induced fewer HTR in 5-HTT$^{-/-}$ *vs* 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.

Table 3 Effect of 5-HTT genotype on global brain $cPLA_2$ 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***[a]
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*** [b]
$PGF_{2\alpha}$ (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*** [b]

Values are mean ± SD. Bonferroni's multiple comparison tests were performed: ***$p<0.001$; 5-HTT$^{-/-}$ and 5-HTT$^{+/-}$ *vs* 5-HTT$^{+/+}$.
[a]$p<0.001$ 5-HTT$^{-/-}$ *vs* 5-HTT$^{+/-}$.
[b]$p<0.01$.

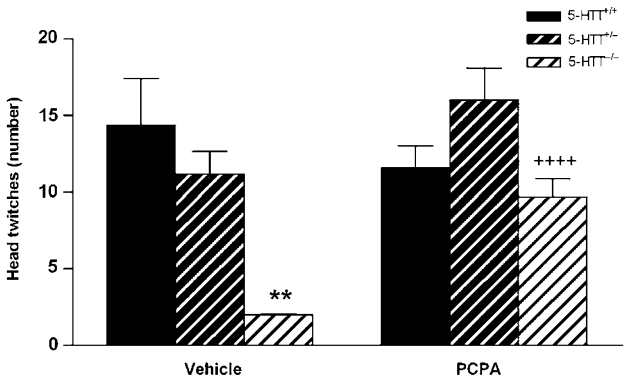

Figure 3 Effects of brain 5-HT depletion by PCPA pretreatment on DOI-induced head twitches in 5-HTT mice. At baseline (vehicle pretreatment), DOI induced fewer head twitches in 5-HTT$^{-/-}$ mice compared to 5-HTT$^{+/+}$ mice, whereas DOI-induced head twitches were similar between 5-HTT$^{+/+}$ and 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 mean ± 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.

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 $cPLA_2$ 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.

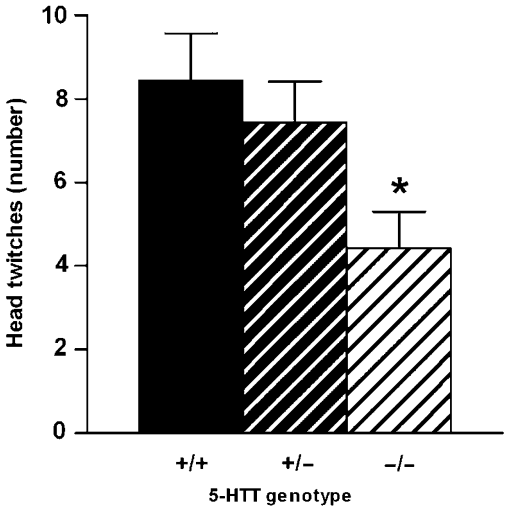

Figure 4 5-HTP-induced head twitches in 5-HTT mice. The 5-HT precursor 5-HTP, which increases 5-HT levels, induced fewer head twitches in 5-HTT$^{-/-}$ mice compared to 5-HTT$^{+/+}$ mice, with no difference between 5-HTT$^{+/-}$ and 5-HTT$^{+/+}$ mice. Data represent the mean ± SEM; $n=9$–16 per group.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

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 adrenocorticotropic 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 study reported that baseline values did not differ significantly between 5-HTT$^{-/-}$ and 5-HTT$^{+/+}$ mice, unlike the current findings. However, the earlier study is likely erroneous. Frozen brain sections in the earlier study were exposed to X-ray film for 6–10 weeks, rather than for 4 weeks as was carried out in this study, 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 the 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, 2007b; Rao *et al*, 2008; Rapoport and Bosetti, 2002).

The new data in this study 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 i.v. injection of [1-^{11}C]AA. In such PET studies, the coefficient of variation of k^* for AA ranges from 12 to 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 shown in eight 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 showed that reduced or absent 5-HTT function in mice results in the 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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DISCLOSURE/CONFLICT OF INTEREST

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REFERENCES

Alexandre C, Popa D, Fabre V, Bouali S, Venault P, Lesch KP *et al* (2006). Early life blockade of 5-hydroxytryptamine 1A receptors normalizes sleep and depression-like behavior in adult knockout mice lacking the serotonin transporter. *J Neurosci* **26**: 5554–5564.

Anton RF, Wallis C, Randall CL (1983). *In vivo* regional levels of PGE and thromboxane in mouse brain: effect of decapitation, focused microwave fixation, and indomethacin. *Prostaglandins* **26**: 421–429.

Baca-Garcia E, Vaquero-Lorenzo C, Diaz-Hernandez M, Rodriguez-Salgado B, Dolengevich-Segal H, Arrojo-Romero M *et al* (2007). Association between obsessive-compulsive disorder and a variable number of tandem repeats polymorphism in intron 2 of the serotonin transporter gene. *Prog Neuropsychopharmacol Biol Psychiatry* **31**: 416–420.

Basselin M, Chang L, Bell JM, Rapoport SI (2006a). Chronic lithium chloride administration attenuates brain NMDA receptor-initiated signaling via arachidonic acid in unanesthetized rats. *Neuropsychopharmacology* **31**: 1659–1674.

Basselin M, Murphy DL, Rapoport SI (2007a). Increased brain arachidonic acid signaling can be imaged *in vivo* in serotonin transporter deficient mice: Basis for future clinical PET studies (abstract). *Annual Meeting American College Neuropsychopharmacology,* Boca Ratan, FL. 9–13 December 2007 Poster Session III Poster 123.

Basselin M, Villacreses NE, Chen M, Bell JM, Rapoport SI (2007b). Chronic carbamazepine administration reduces NMDA receptor-initiated signaling via arachidonic acid in rat brain. *Biol Psychiatry* **62**: 934–943.

Basselin M, Villacreses NE, Langenbach R, Ma K, Bell JM, Rapoport SI (2006b). Resting and arecoline-stimulated brain metabolism and signaling involving arachidonic acid are altered in the cyclooxygenase-2 knockout mouse. *J Neurochem* **96**: 669–679.

Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A *et al* (1998). Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ('Ecstasy') in serotonin transporter-deficient mice. *Mol Pharmacol* **53**: 649–655.

Berg KA, Maayani S, Goldfarb J, Clarke WP (1998a). Pleiotropic behavior of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor agonists. *Ann N Y Acad Sci* **861**: 104–110.

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP (1998b). Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol Pharmacol* **54**: 94–104.

Bosetti F (2007). Arachidonic acid metabolism in brain physiology and pathology: lessons from genetically altered mouse models. *J Neurochem* **102**: 577–586.

Bosetti F, Langenbach R, Weerasinghe GR (2004). Prostaglandin E_2 and microsomal prostaglandin E synthase-2 expression are decreased in the cyclooxygenase-2-deficient mouse brain despite compensatory induction of cyclooxygenase-1 and Ca^{2+}-dependent phospholipase A_2. *J Neurochem* **91**: 1389–1397.

Bosetti F, Weerasinghe GR (2003). The expression of brain cyclooxygenase-2 is down-regulated in the cytosolic phospholipase A_2 knockout mouse. *J Neurochem* **87**: 1471–1477.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H *et al* (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**: 386–389.

Cesana R, Ceci A, Ciprandi C, Borsini F (1993). Mesulergine antagonism towards the fluoxetine anti-immobility effect in the forced swimming test in mice. *J Pharm Pharmacol* **45**: 473–475.

Chang MC, Arai T, Freed LM, Wakabayashi S, Channing MA, Dunn BB *et al* (1997). Brain incorporation of [1-^{11}C]-arachidonate in normocapnic and hypercapnic monkeys, measured with positron emission tomography. *Brain Res* **755**: 74–83.

Clark JD, Schievella AR, Nalefski EA, Lin LL (1995). Cytosolic phospholipase A_2. *J Lipid Mediat Cell Signal* **12**: 83–117.

Demar Jr JC, Lee HJ, Ma K, Chang L, Bell JM, Rapoport SI *et al* (2006). Brain elongation of linoleic acid is a negligible source of the arachidonate in brain phospholipids of adult rats. *Biochim Biophys Acta* **1761**: 1050–1059.

Demar Jr JC, Ma K, Chang L, Bell JM, Rapoport SI (2005). alpha-Linolenic acid does not contribute appreciably to docosahexaenoic acid within brain phospholipids of adult rats fed a diet enriched in docosahexaenoic acid. *J Neurochem* **94**: 1063–1076.

Esaki T, Cook M, Shimoji K, Murphy DL, Sokoloff L, Holmes A (2005). Developmental disruption of serotonin transporter function impairs cerebral responses to whisker stimulation in mice. *Proc Natl Acad Sci USA* **102**: 5582–5587.

Esposito G, Giovacchini G, Liow J-S, Bhattacharjee AK, Greenstein D, Schapiro M *et al* (2008). Imaging neuroinflammation in Alzheimer disease with radiolabeled arachidonic acid and PET. *J Nucl Med* **49**: 1414–1421.

Felder CC, Kanterman RY, Ma AL, Axelrod J (1990). Serotonin stimulates phospholipase A_2 and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proc Natl Acad Sci USA* **87**: 2187–2191.

Fitzpatrick F, Soberman R (2001). Regulated formation of eicosanoids. *J Clin Invest* **107**: 1347–1351.

Folch J, Lees M, Sloane Stanley GH (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**: 497–509.

Fox MA, Andrews AM, Wendland JR, Lesch K-P, Holmes A, Murphy DL (2007a). A pharmacological analysis of mice with a targeted disruption of the serotonin transporter. *Psychopharmacology (Berl)* **195**: 147–166.

Fox MA, Jensen CL, French HT, Stein AR, Huang SJ, Tolliver TJ *et al* (2008a). Neurochemical, behavioral, and physiological effects of pharmacologically enhanced serotonin levels in serotonin transporter (SERT)-deficient mice. *Psychopharmacology* **201**: 203–218.

Fox MA, Jensen CL, Gallagher PS, Murphy DL (2007b). Receptor mediation of exaggerated responses to serotonin-enhancing drugs in serotonin transporter (SERT)-deficient mice. *Neuropharmacology* **53**: 643–656.

Fox MA, Stein AS, French HT, Murphy DL (2008b). Head twitches in serotonin transporter (SERT)-deficient mice: 5-HT_{1A} and 5-HT_{2A} receptor interactions. *Fundam Clin Pharmacol* **22**: S91.

Franklin KBJ, Paxinos G (1997). *The Mouse Brain in Stereotaxic Coodinates*. Academic Press, Inc.: San Diego.

Giovacchini G, Lerner A, Toczek MT, Fraser C, Ma K, DeMar JC *et al* (2004). Brain incorporation of [^{11}C]arachidonic acid, blood volume, and blood flow in healthy aging: a study with partial-volume correction. *J Nucl Med* **45**: 1471–1479.

Gottschalk LA, Stone WM, Gleser GC, Iacono JM (1969). Anxiety and plasma free fatty acids (FFA). *Life Sci* **8**: 61–68.

Holman RT (1986). Control of polyunsaturated acids in tissue lipids. *J Am Coll Nutr* **5**: 183–211.

Homberg JR, Olivier JD, Smits BM, Mul JD, Mudde J, Verheul M *et al* (2007). Characterization of the serotonin transporter knockout rat: A selective change in the functioning of the serotonergic system. *Neuroscience* **146**: 1662–1676.

Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD *et al* (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* **78**: 815–826.

Jennings KA, Sheward WJ, Harmar AJ, Sharp T (2008). Evidence that genetic variation in 5-HT transporter expression is linked to changes in 5-HT_{2A} receptor function. *Neuropharmacology* **54**: 776–783.

John TM, Viswanathan M, Etches RJ, Pilo B, George JC (1987). Influence of corticosterone infusion on plasma levels of catecholamines, thyroid hormones, and certain metabolites in laying hens. *Poult Sci* **66**: 1059–1063.

Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P (1996). COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proc Natl Acad Sci USA* **93**: 2317–2321.

Lee HJ, Rao JS, Chang L, Rapoport SI, Bazinet RP (2008). Chronic N-methyl-D-aspartate administration increases the turnover of arachidonic acid within brain phospholipids of the unanesthetized rat. *J Lipid Res* **49**: 162–168.

Lee HJ, Rao JS, Ertley RN, Chang L, Rapoport SI, Bazinet RP (2007). Chronic fluoxetine increases cytosolic phospholipase A_2 activity and arachidonic acid turnover in brain phospholipids of the unanesthetized rat. *Psychopharmacology (Berl)* **190**: 103–115.

Li D, He L (2007). Meta-analysis supports association between serotonin transporter (5-HTT) and suicidal behavior. *Mol Psychiatry* **12**: 47–54.

Li Q, Wichems CH, Ma L, Van de Kar LD, Garcia F, Murphy DL (2003). Brain region-specific alterations of 5-HT2A and 5-HT2C receptors in serotonin transporter knockout mice. *J Neurochem* **84**: 1256–1265.

Marek GJ, Aghajanian GK (1996). LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT_{2A} receptors on interneurons in rat piriform cortex. *J Pharmacol Exp Ther* **278**: 1373–1382.

Marques FZ, Hutz MH, Bau CH (2006). Influence of the serotonin transporter gene on comorbid disorders among alcohol-dependent individuals. *Psychiatr Genet* **16**: 125–131.

Masoliver E, Menoyo A, Perez V, Volpini V, Rio ED, Perez J *et al* (2006). Serotonin transporter linked promoter (polymorphism) in the serotonin transporter gene may be associated with antidepressant-induced mania in bipolar disorder. *Psychiatr Genet* **16**: 25–29.

Mathews TA, Fedele DE, Coppelli FM, Avila AM, Murphy DL, Andrews AM (2004). Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. *J Neurosci Methods* **140**: 169–181.

Motulsky H (2003). *Prism 4 Statistics Guide-Statistical Analyses for Laboratory and Clinical Researchers*. GraphPad Software Inc.: San Diego, pp 82–84.

Murphy DL, Fox MA, Timpano KR, Moya P, Ren-Patterson R, Andrews AM *et al* (2008). How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. *Neuropharmacology* **55**: 932–960.

Murphy DL, Lerner A, Rudnick G, Lesch KP (2004). Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol Interv* **4**: 109–123.

Murphy DL, Lesch KP (2008). Targeting the murine serotonin transporter: insights into human neurobiology. *Nat Rev Neurosci* **9**: 85–96.

Ong WY, Sandhya TL, Horrocks LA, Farooqui AA (1999). Distribution of cytoplasmic phospholipase A_2 in the normal rat brain. *J Hirnforsch* **39**: 391–400.

Pardue S, Rapoport SI, Bosetti F (2003). Co-localization of cytosolic phospholipase A_2 and cyclooxygenase-2 in Rhesus monkey cerebellum. *Brain Res Mol Brain Res* **116**: 106–114.

Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, Boovariwala A, Willeit M *et al* (2007). Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [^{11}C] DASB positron emission tomography study. *Biol Psychiatry* **62**: 327–331.

Qu Y, Chang L, Klaff J, Seemann R, Greenstein D, Rapoport SI (2006). Chronic fluoxetine upregulates arachidonic acid incorporation into the brain of unanesthetized rats. *Eur Neuropsychopharmacol* **16**: 561–571.

Qu Y, Villacreses N, Murphy DL, Rapoport SI (2005). 5-$HT_{2A/2C}$ receptor signaling via phospholipase A_2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. *Psychopharmacology (Berl)* **180**: 12–20.

Rao JS, Ertley RN, Rapoport SI, Bazinet RP, Lee H-J (2007). Chronic NMDA administration to rats up-regulates frontal cortex cytosolic phospholipase A_2 and its transcription factor, activator protein-2. *J Neurochem* **102**: 1918–1927.

Rao JS, Lee HJ, Rapoport SI, Bazinet RP (2008). Mode of action of mood stabilizers: is the arachidonic acid cascade a common target? *Mol Psychiatry* **13**: 585–596.

Rapoport SI (2001). *In vivo* fatty acid incorporation into brain phospholipids in relation to plasma availability, signal transduction and membrane remodeling. *J Mol Neurosci* **16**: 243–261.

Rapoport SI, Bosetti F (2002). Do lithium and anticonvulsants target the brain arachidonic acid cascade in bipolar disorder? *Arch Gen Psychiatry* **59**: 592–596.

Rapoport SI, Chang MCJ, Spector AA (2001). Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. *J Lipid Res* **42**: 678–685.

Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI (1992). A quantitative method for measuring regional *in vivo* fatty-acid incorporation into and turnover within brain phospholipids: Review and critical analysis. *Brain Res Brain Res Rev* **17**: 187–214.

Rubino T, Realini N, Castiglioni C, Guidali C, Vigano D, Marras E *et al* (2008). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cereb Cortex* **18**: 1292–1301.

Rutkowska M, Jamontt J, Gliniak H (2006). Effects of cannabinoids on the anxiety-like response in mice. *Pharmacol Rep* **58**: 200–206.

Sapirstein A, Saito H, Texel SJ, Samad TA, O'Leary E, Bonventre JV (2005). Cytosolic phospholipase $A_2\alpha$ regulates induction of brain cyclooxygenase-2 in a mouse model of inflammation. *Am J Physiol Regul Integr Comp Physiol* **288**: R1774–R1782.

Serretti A, Benedetti F, Zanardi R, Smeraldi E (2005). The influence of Serotonin Transporter Promoter Polymorphism (SERTPR) and other polymorphisms of the serotonin pathway on the efficacy of antidepressant treatments. *Prog Neuropsychopharmacol Biol Psychiatry* **29**: 1074–1084.

Shimizu T, Wolfe LS (1990). Arachidonic acid cascade and signal transduction. *J Neurochem* **55**: 1–15.

Sokoloff L (1999). Energetics of functional activation in neural tissues. *Neurochem Res* **24**: 321–329.

Stenfors C, Ross SB (2002). Evidence for involvement of 5-hydroxytryptamine$_{1B}$ autoreceptors in the enhancement of serotonin turnover in the mouse brain following repeated treatment with fluoxetine. *Life Sci* **71**: 2867–2880.

Tabachnick BG, Fidell LS (2001). *Computer-assisted research design and analysis*. Allyn and Bacon: Boston (novol), pp 184–188.

Toborek M, Malecki A, Garrido R, Mattson MP, Hennig B, Young B (1999). Arachidonic acid-induced oxidative injury to cultured spinal cord neurons. *J Neurochem* **73**: 684–692.

Uher R, McGuffin P (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. *Mol Psychiatry* **13**: 131–146.

Wendland JR, Moya PR, Kruse MR, Ren-Patterson RF, Jensen CL, Timpano KR *et al* (2008). A novel, putative gain-of-function haplotype at Slc6a4 associates with obsessive-compulsive disorder. *Hum Mol Genet* **17**: 717–723.

Willins DL, Meltzer HY (1997). Direct injection of 5-HT$_{2A}$ receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharmacol Exp Ther* **282**: 699–706.

Xu J, Weng YI, Simonyi A, Krugh BW, Liao Z, Weisman GA *et al* (2002). Role of PKC and MAPK in cytosolic PLA_2 phosphorylation and arachadonic acid release in primary murine astrocytes. *J Neurochem* **83**: 259–270.

Xu L, Han C, Lim K, Wu T (2008). Activation of cytosolic phospholipase $A_2\alpha$ through nitric oxide-induced S-nitrosylation. Involvement of inducible nitric-oxide synthase and cyclooxygenase-2. *J Biol Chem* **283**: 3077–3087.

Zhang J, Goorha S, Raghow R, Ballou LR (2002). The tissue-specific, compensatory expression of cyclooxygenase-1 and -2 in transgenic mice. *Prostaglandins Other Lipid Mediat* **67**: 121–135.