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## 5-HT<sub>2A/2C</sub> receptor signaling via phospholipase A<sub>2</sub> and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter

Received: 10 December 2004 / Accepted: 15 December 2004 / Published online: 15 April 2005  
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**Abstract** *Subjects:* The serotonin reuptake transporter (SERT) helps to regulate brain serotonergic transmission and is the target of some antidepressants. To further understand SERT function, we measured a marker of regional brain phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activation in SERT knockout mice (SERT<sup>-/-</sup>) and their littermate controls (SERT<sup>+/+</sup>). *Methods:* Following administration of 1.5 mg/kg s.c. (±)-2, 5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), a 5-HT<sub>2A/2C</sub> receptor agonist, to unanesthetized mice injected intravenously with radiolabeled arachidonic acid (AA), PLA<sub>2</sub> activation, represented as the regional incorporation coefficient *k*<sup>\*</sup> of AA, was determined with quantitative autoradiography in each of 71 brain regions. *Results:* In SERT<sup>+/+</sup> mice, DOI significantly increased *k*<sup>\*</sup> in 27 regions known to have 5-HT<sub>2A/2C</sub> receptors, including the frontal, motor, somatosensory, pyriform and cingulate cortex, white matter, nucleus accumbens, caudate putamen, septum, CA1 of hippocampus, thalamus, and hypothalamus. In contrast, DOI did not increase *k*<sup>\*</sup> significantly in any brain region of SERT<sup>-/-</sup> mice. Head twitches following DOI, which also were measured, were robust in SERT<sup>+/+</sup> mice but were markedly attenuated in SERT<sup>-/-</sup> mice. *Conclusions:* These

results show that a lifelong elevation of the synaptic 5-HT concentration in SERT<sup>-/-</sup> mice leads to downregulation of 5-HT<sub>2A/2C</sub> receptor-mediated PLA<sub>2</sub> signaling via AA and of head twitches, in response to DOI.

**Keywords** Serotonin · Phospholipase A<sub>2</sub> · Arachidonic acid · Signal transduction · Imaging · Brain · SERT · Knockout · DOI

**Abbreviations** AA: arachidonic acid · DOI: (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane · 5-HT: 5-hydroxytryptamine, serotonin · PLA<sub>2</sub>: phospholipase A<sub>2</sub> · SERT: serotonin reuptake transporter · SSRI: selective serotonin reuptake inhibitor · sn: stereospecifically numbered

### Introduction

Disturbed neurotransmission involving serotonin (5-hydroxytryptamine, 5-HT) is thought to contribute to depression and anxiety disorders. Thus, in vivo neuroimaging and postmortem studies have demonstrated altered expression of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor subtypes in brains of patients with these disorders (Biver et al. 1997; Drevets et al. 1999). In addition, the risk of depression is increased in unaffected subjects having the short allele of the promoter-region polymorphism (5HTTLPR) of the serotonin reuptake transporter (*SERT*) gene, causing a comparatively low rate of 5-HT reuptake (Smits et al. 2004). Finally, the efficacy of selective serotonin reuptake inhibitors (SSRIs) in treating depression and anxiety disorders supports a role for serotonergic dysfunction in these disorders (Fuller 1995; Murphy et al. 2004).

Identifying mechanisms underlying this dysfunction and understanding how effective drugs ameliorate it might help to understand the etiology of depression and anxiety disorders and to develop better therapies for them. One possibility is that the dysfunction affects signaling pathways that are coupled via G proteins to 5-HT receptors. These pathways can involve (1) cyclic adenylyl cyclase and cAMP,

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(2) phospholipase C and the phosphatidylinositol cycle (Nishizuka 1986; Berridge 1986; Mann et al. 1995), and (4) phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and the arachidonic acid (AA, 20:4n-6) cascade (Rapoport and Bosetti 2002). With regard to the AA cascade, it is known that the binding of 5-HT to postsynaptic 5-HT<sub>2A/2C</sub> receptors can activate PLA<sub>2</sub> to release AA from the stereospecifically numbered (*sn*)-2 position of membrane phospholipids (Berg et al. 1998; Felder et al. 1990).

We have developed a method to image brain PLA<sub>2</sub> activation in unanesthetized rodents given drugs acting at receptors coupled to PLA<sub>2</sub> (Basselin et al. 2003; DeGeorge et al. 1991; Qu et al. 2003a,b; Rapoport 2003; Robinson et al. 1992). In light of the suggested role for SERT changes in human depression and anxiety disorders (see above), we decided to use this method to see if 5-HT<sub>2A/2C</sub>-mediated signaling involving PLA<sub>2</sub> is disturbed in SERT knockout mice (SERT<sup>-/-</sup> mice) (Bengel et al. 1998). Compared with control SERT<sup>+/+</sup> mice, SERT<sup>-/-</sup> mice show, among other effects, a sixfold elevation in brain extracellular 5-HT concentration and a 60–80% reduction in intracellular 5-HT concentration (Bengel et al. 1998; Mathews et al. 2004). They also demonstrate regionally diverse changes in brain 5-HT<sub>2A/2C</sub> and 5-HT<sub>1A</sub> receptor densities (Li et al. 2000, 2003).

Our fatty acid method provides values for incorporation coefficients  $k^*$  of AA from plasma into phospholipids of individual brain regions (DeGeorge et al. 1991; Rapoport 2003; Robinson et al. 1992). Increments in  $k^*$  in response to a drug acting at receptors coupled to PLA<sub>2</sub> have been shown to represent PLA<sub>2</sub> activation and AA release from the *sn*-2 position of membrane phospholipid. This is because a fraction of the AA that is released from the phospholipid will be lost by conversion to eicosanoids,  $\beta$ -oxidation, or other pathways, whereas the remainder will be reincorporated into the phospholipids (Chang et al. 1997; Rapoport 2003). The quantity lost is rapidly replaced by diet-derived unesterified AA in plasma, as AA cannot be synthesized de novo nor significantly converted from its precursor linoleic acid (18:2n-6) in mammalian brain (Carlson 1999; DeMar et al. 2004). The extent of replacement, proportional to PLA<sub>2</sub> activation, can be estimated by injecting radiolabeled AA intravenously and measuring regional values of  $k^*$  (brain radioactivity/integrated plasma radioactivity) with quantitative autoradiography.  $k^*$  following drug is independent of changes in brain blood flow, thus marking only PLA<sub>2</sub> activation, and represents tracer largely incorporated in synaptic membrane phospholipids (Chang et al. 1997; DeGeorge et al. 1991; Jones et al. 1996).

We reported that acute administration of the 5-HT<sub>2A/2C</sub> receptor agonist, DOI [(±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane], to unanesthetized rats increased  $k^*$  for AA in brain areas containing 5-HT<sub>2A/2C</sub> receptors, as did acute administration of the SSRI, fluoxetine (Qu et al. 2003a,b). The DOI-induced increments could be blocked by pretreatment with the 5-HT<sub>2A/2C</sub> receptor antagonist, mianserin, and comparable increments following other drugs have been shown to be blunted by preinjected manoalide, a PLA<sub>2</sub> inhibitor (Rapoport 2003). Acute DOI in rats also

increases brain protein and mRNA levels of cyclooxygenase-2 (Mackowiak et al. 2002), an enzyme in the AA cascade that converts unesterified AA to prostaglandin E<sub>2</sub> and other eicosanoids. Together, the observations indicate that both acute DOI and acute fluoxetine activate PLA<sub>2</sub> via 5-HT<sub>2A/2C</sub> receptors—DOI by binding to the receptors, fluoxetine by increasing endogenous 5-HT binding to them—and thereby provoke AA hydrolysis from phospholipid (Shimizu and Wolfe 1990).

In this study, we determined baseline values of  $k^*$  for AA and  $k^*$  responses to acute DOI in unanesthetized SERT<sup>-/-</sup> and SERT<sup>+/+</sup> mice. We also measured DOI-provoked head twitches (Goodwin et al. 1984; Wettstein et al. 1999). We administered 1.5 mg/kg s.c. DOI, a dose within the range (1–2.5 mg/kg) reported to cause head twitches and to increase  $k^*$  for AA in rodents (Basselin et al. 2005; Qu et al. 2003a; Wettstein et al. 1999).

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## Materials and methods

### Materials

Radiolabeled [1-<sup>14</sup>C]arachidonic acid ([1-<sup>14</sup>C]AA) (specific activity = 200 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). Its radiochemical purity exceeded 96% on thin-layer chromatography. DOI was purchased from Sigma Research Biochemicals International (Natick, MA). Pentobarbital sodium was purchased from Richmond Veterinary Supply Co. (Richmond, VA).

### Animals

The experimental protocol was approved by the Animal Care and Use Committee of the National Institute of Child Health and Human Development and conformed to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication 86-23). Male SERT<sup>-/-</sup> mice on a C57BL/6J background were created by homologous recombination (Bengel et al. 1998). They were from the F9 generation and 12 weeks old, weighing 30 to 35 g. They and their SERT<sup>+/+</sup> littermate controls were housed in groups of four to five per cage in a room with a 12-h light–12-h dark cycle and with controlled humidity and temperature. They were provided with ad lib food and water.

### Drug administration and tracer infusion

The mice were divided into four experimental groups of nine animals each: (1) wild-type controls (SERT<sup>+/+</sup>) given s.c. saline; (2) wild-type controls given 1.5 mg/kg s.c. DOI; (3) SERT<sup>-/-</sup> mice given s.c. saline; (4) SERT<sup>-/-</sup> mice given 1.5 mg/kg s.c. DOI. Mice in each group were subjected to the *in vivo* fatty acid method to image brain PLA<sub>2</sub> signaling in terms of the incorporation coefficient  $k^*$  (see



**Fig. 1** Comparison of DOI-induced head twitches between SERT<sup>+/+</sup> and SERT<sup>-/-</sup> mice. Each column shows mean $\pm$ SE head twitches between 30 and 35 min following DOI or saline administration ( $n=5-6$ ). \*\*\*Differs from SERT<sup>+/+</sup> mean ( $p<0.005$ ) by unpaired  $t$  test

Eq. 1), as described elsewhere (DeGeorge et al. 1991; Qu et al. 2003a,b; Rapoport 2003; Robinson et al. 1992).

Briefly, after a mouse was anesthetized with halothane (1–3% v/v in O<sub>2</sub>), polyethylene PE 10 catheters (Becton Dickinson, Sparks, MD) containing heparinized (100 IU/ml) saline were surgically implanted into a femoral artery and vein. The incision site was infiltrated with 1% lidocaine and closed with Duro Superglue (Manco, Avon, OH). The mouse was wrapped loosely, its upper body free, in a fast-setting plaster cast secured to a wooden block, and allowed to recover from anesthesia in a temperature-controlled and sound-dampened box for 4 h. Body temperature was kept at 36–37°C using a rectal thermometer and a feedback heating device (YSI indicating temperature controller, Yellow Springs Instruments, Yellow Springs, OH). Arterial

blood gases and pH (blood gas analyzer Model 238, Ciba-Corning, Medfield, MA) also were monitored.

After recovering, the mouse was given s.c. either 100  $\mu$ l saline or DOI (1.5 mg/kg in 100  $\mu$ l saline). Twenty minutes later, 0.3 mCi/kg [1-<sup>14</sup>C]AA in 45  $\mu$ l of 5 mM HEPES buffer (pH 7.4) containing 50 mg/ml fatty-acid-free bovine serum albumin (Sigma), was infused into the femoral vein at a rate of 15  $\mu$ l/min for 3 min, using an infusion pump (Harvard Instruments, Holliston, MA). Ten 15- $\mu$ l arterial blood samples were collected at fixed times (0, 0.25, 0.50, 1.0, 1.5, 2.0, 2.8, 3.2, 5.30, 6.0 10, and 19 min) from the beginning of infusion until the animal was killed at 20 min with 5 mg i.v. sodium pentobarbital, after which its brain was removed and frozen in 2-methylbutane at –50°C. Lipids were extracted from arterial plasma and radioactivity in the extract was measured by liquid scintillation counting.

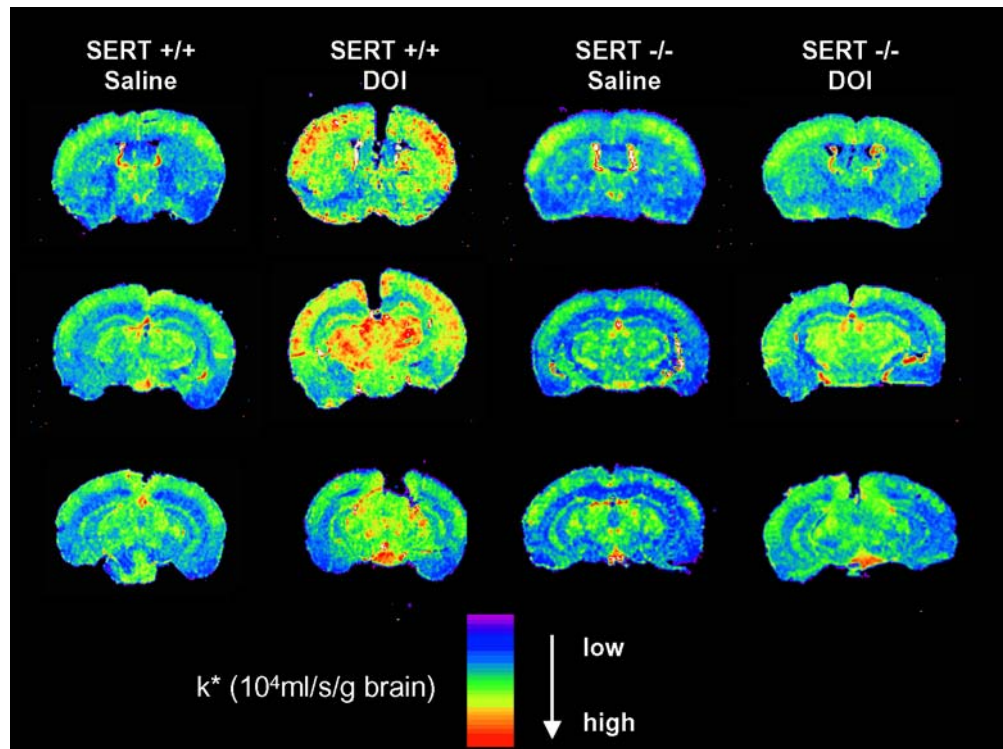
#### Head twitches

Head twitches, rapid movements of the head with little or no involvement of the trunk (Goodwin et al. 1984), were counted visually with the use of a stopwatch between 30 and 35 min after the s.c. administration of DOI or saline.

#### Autoradiography and calculations

Brains were cut in coronal sections on a cryostat (Hacker Instruments, Fairfield, NJ) at –20°C. Sets of three adjacent 20- $\mu$ m sections were collected at 140- $\mu$ m intervals on glass coverslips and dried. The sections were exposed to autoradiographic film (EMC, Eastman Kodak Company,

**Fig. 2** Coronal autoradiographs demonstrating incorporation coefficients  $k^*$  for arachidonic acid, from brain of SERT<sup>+/+</sup> mouse given saline s.c.; SERT<sup>+/+</sup> mouse given DOI (1.5 mg/kg s.c.); SERT<sup>-/-</sup> mouse given saline; SERT<sup>+/+</sup> mouse given DOI.  $k^*$  is color coded



**Table 1** Comparison of regional arachidonic acid incorporation coefficients  $k^*$  in mouse brain

Brain region	Abbreviation <sup>a</sup>	SERT+/+		SERT-/-	
		Saline	DOI	Saline	DOI
Cerebral cortex					
Frontal association cortex	FrA	7.18±1.20	12.50±0.40***	9.15±0.60	7.62±0.56
Primary motor cortex	M1	7.49±1.1	11.53±0.77***	9.21±0.55	8.21±0.43
Secondary motor cortex	M2	7.05±1.1	11.00±0.98**	8.89±0.51	7.85±0.49
Primary somatosensory cortex layer II–III	S1 II–III	6.72±0.96	10.94±1.11***	8.36±0.49	7.98±0.40
Primary somatosensory cortex layer IV	S1 IV	7.80±1.21	11.70±1.12*	9.79±0.97	9.69±0.59
Primary somatosensory cortex layer V–VI	S1 V–VI	7.53±0.99	11.33±1.17**	9.41±0.57	8.78±0.53
Cingulate cortex	Cg	7.52±1.29	12.14±1.31**	9.53±0.50	9.01±0.54
Primary visual cortex layer II–III	V1 II–III	9.45±1.10	10.70±2.33	7.42±1.15	7.59±0.72
Primary visual cortex layer IV	V1 IV	9.67±1.21	11.59±2.49	8.07±1.38	8.88±0.73
Primary visual cortex layer V–VI	V1 V–VI	9.37±1.19	10.80±2.3	8.21±1.25	8.21±0.66
Primary auditory cortex II–III	AuI II–III	7.82±1.14	9.71±2.01	7.05±1.08	6.96±0.74
Primary auditory cortex IV	AuI IV	8.25±1.21	10.43±2.12	7.61±1.37	8.02±0.78
Primary auditory cortex V–VI	AuI V–VI	7.97±1.14	9.85±2.06	7.08±1.08	7.45±0.73
White matter					
Corpus callosum	cc	4.10±0.76	7.80±0.77***	5.62±0.49	6.46±0.64
Internal capsule	ic	4.57±0.67	7.51±0.88**	6.31±0.70	4.97±0.59
Olfactory regions					
Piriform cortex	Pir	8.15±1.34	13.50±1.15**	10.25±0.76	10.01±0.78
Basal ganglia and related areas					
Accumbens nucleus	Acb	5.92±1.1	9.97±0.79**	7.03±0.53	6.01±0.76
Caudate putamen	CPU	5.76±0.84	10.24±0.84***	7.42±0.55	6.51±0.47
Substantia nigra, reticular part	SNR	9.63±1.25	11.40±2.44	8.88±1.20	8.38±0.68
Substantia nigra, compact part	SNC	9.12±1.25	12.57±2.22	8.31±1.20	7.70±0.69
Lateral globus pallidus	LGP	5.90±0.83	9.37±1.12**	7.95±0.68	7.04±0.45
Subthalamic nucleus	Sth	10.99±1.36	13.71±2.17	11.51±1.47	11.29±1.24
Basolateral amygdaloid nucleus	BL	5.66±0.74	8.96±1.45*	7.65±0.99	6.17±0.57
Septum					
Lateral septal nucleus, intermediate part	LSI	5.08±0.85	7.97±0.80**	6.25±0.51	5.75±0.66
Medial septal nucleus	MS	6.75±1.08	10.04±1.08*	7.73±0.54	7.75±0.54
Nucleus vertical limb, diagonal band	VDB	6.78±1.15	10.05±1.10*	7.73±0.55	7.58±0.60
Nucleus horizontal limb, diagonal band	HDB	5.52±1.01	9.39±0.95**	7.48±0.53	6.90±0.64
Hippocampal formation					
CA1 field of the hippocampus	CA1	8.29±1.03	12.96±1.91*	10.46±1.07	9.20±0.60
CA2 field of the hippocampus	CA2	8.61±1.01	12.38±1.81*	10.33±1.27	8.36±0.64
CA3 field of the hippocampus	CA3	9.99±1.05	13.50±1.83	11.76±1.10	10.02±0.73
Dentate gyrus	DG	10.04±1.00	15.46±2.85*	11.45±1.13	10.41±1.04
Thalamus					
Paraventricular thalamic nuclei, anterior	PVA	8.82±0.91	12.80±1.36**	10.102±0.84	8.91±0.56
Parataenial thalamic nuclei	PT	8.86±0.91	11.96±1.34*	10.61±0.69	8.89±0.61
Anteroventral thalamic nucleus	AV	10.55±0.72	15.12±1.97*	13.46±1.37	13.11±1.04
Anteromedial thalamic nuclei	AM	9.22±0.91	12.03±1.24	11.59±1.24	9.20±0.52
Reticular thalamic nuclei	Rt	8.45±0.84	11.74±1.23*	10.20±0.79	8.84±0.53
Ventral posterolateral thalamic nuclei	VPL	9.62±1.26	14.86±2.03*	10.68±1.51	9.74±0.72
Ventral posteromedial thalamic nuclei	VPM	9.39±1.19	14.24±1.89*	10.46±1.55	9.72±0.72
Parafascicular thalamic nuclei	PF	9.68±1.00	14.17±2.02*	10.67±1.67	10.12±0.76
Medial thalamic nuclei	MHb	12.80±1.70	17.00±2.21	12.98±1.03	14.29±1.40
Lateral thalamic nuclei	LHb	9.79±0.98	14.94±2.02**	11.69±1.20	11.28±0.87
Dorsal lateral geniculate nuclei	DLG	9.88±1.09	14.85±1.94*	10.92±1.67	10.53±0.82
Medial geniculate nuclei, dorsal/medial geniculate nuclei, medial	MGD/MGV	10.93±1.34	12.33±2.71	9.58±1.49	9.70±0.58
Hypothalamus					
Subfornical organ	SFO	15.16±0.88	21.73±4.31	19.06±2.05	17.31±3.4
Lateral hypothalamic area	LH	5.88±0.88	9.18±1.02**	7.69±0.69	6.19±0.58

**Table 1** (continued)

Brain region	Abbreviation <sup>a</sup>	SERT+/+		SERT-/-	
		Saline	DOI	Saline	DOI
Medial forebrain bundle	mfb	5.77±0.85	9.08±0.91**	7.30±0.71	6.42±0.71
Arcuate hypothalamic nuclei, lateroposterior/medioposterior	ArcLP/MP	6.60±0.97	10.65±1.61*	7.82±1.32	9.58±0.92
Periventricular hypothalamic nuclei	Pe	7.27±1.05	10.81±1.21*	8.38±0.69	7.82±0.67
Arcuate hypothalamic nuclei, dorsal	ArcD	7.55±1.03	10.92±1.76*	8.89±0.97	7.82±0.67
Ventromedial hypothalamic nuclei	VMH	7.21±0.89	10.36±1.53*	9.41±0.98	7.41±0.89
Posterior hypothalamic area	PH	7.90±0.88	11.91±1.70*	9.43±0.96	8.69±0.69
Medial mammillary nuclei, medial	MM	16.02±1.49	15.24±3.41	12.90±3.28	14.48±1.39
Medial mammillary nuclei, lateral/lateral mammillary nuclei	ML/LM	7.30±0.99	9.34±1.41	8.51±1.94	9.18±1.69
Median eminence	ME	7.30±0.99	9.34±1.41	8.51±1.94	9.18±1.69
Brainstem and spinal cord					
Raphe magnus nuclei	RMg	10.08±2.01	7.60±1.38	8.70±1.48	11.22±1.87
Raphe pallidus nuclei	Rpa	9.56±1.53	8.09±1.32	7.66±1.50	8.85±1.04
Median raphe nuclei	MnR	9.48±1.70	9.20±1.77	7.92±1.16	8.71±0.66
Dorsal raphe nuclei	DR	10.63±1.61	12.81±1.95	9.16±1.41	10.57±0.85
Locus coeruleus	LC	11.76±1.49	9.28±2.09	8.81±1.33	10.81±1.08
Superior colliculus	SuG	11.44±1.42	10.18±1.56	9.45±1.27	9.61±0.59
Interpeduncular nucleus	IPC	13.72±1.93	13.42±2.3	11.17±2.04	11.62±0.90
Pedunculopontine tegmental nuclei	PPTg	9.02±1.39	9.63±2.07	7.77±1.13	8.69±0.90
Central nuclei inferior colliculus	CIC	14.58±1.60	12.25±2.30	11.00±1.53	14.75±1.28
Medial vestibular nuclei (parvicel)	MVePC	12.29±1.52	10.53±1.75	10.53±1.63	13.18±2.11
Dorsal cochlear nuclei	DC	7.21±1.48	7.61±2.00	6.97±1.71	6.64±1.26
Spinal trigeminal nuclei, interpolar	Sp51	9.99±1.82	8.40±1.44	8.90±1.45	8.85±0.82
Superior colliculus	SC	9.97±1.29	12.60±2.80	11.61±3.40	8.89±0.89
Cerebellum					
Cerebellar lobules 2	Cb2	11.16±2.15	9.61±1.67	9.49±1.51	10.88±0.69
Cerebellar lobules simple	CbSim	10.44±1.97	7.95±1.20	11.91±2.51	9.54±0.71
Flocculus	Fl	11.09±1.50	9.90±1.53	10.90±1.98	13.33±1.27
Choroid plexus	ChP	30.22±8.54	26.87±4.30	23.20±2.15	25.86±3.22

Values for  $k^*$  are mean±SEM (ml/s/g brain × 10<sup>4</sup>) ( $n=6-9$ )

<sup>a</sup>Abbreviations from Franklin and Paxinos (1997). One-way ANOVA and Bonferroni multiple comparison test were used to compare  $k^*$  values between DOI and saline in SERT+/+ mice, between DOI and saline in SERT-/- mice, and between saline injections in SERT+/+ and SERT-/- mice. There is no significant difference in any brain region in the second and third comparisons

\* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , DOI vs saline in SERT+/+ mice

Rochester, NY) for 6–10 weeks, together with calibrated [<sup>14</sup>C]methylmethacrylate standards (Amersham, Arlington Heights, IL). A fourth adjacent section was collected and stained with cresyl violet to identify brain regions from a mouse-brain atlas (Franklin and Paxinos 1997).

Regional brain radioactivity was measured in sextuplicate by quantitative densitometry, using the public domain image analysis program NIH Image (version 1.55) created by Wayne Rasband (National Institutes of Health, Bethesda, MD) that was installed on a Macintosh computer (Apple Computer, Cupertino, CA). Regional AA incorporation coefficients  $k^*$  were calculated as:

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

where  $k^*$  is in units of milliliters per second per gram (because specific gravity of brain approximates 1),  $c^*_{\text{brain}}$  is brain radioactivity at 20 min after the onset of infusion, in

units of nanocuries per gram,  $c^*_{\text{plasma}}$  is plasma fatty acid radioactivity in units of nanocuries per milliliter, and  $t$  is time after onset of [<sup>14</sup>C]AA infusion.

Data were analyzed using Prism software for the Macintosh (Abacus Concepts, Berkeley, CA) and are reported as means±SEM. A one-way ANOVA and a Bonferroni multiple comparison test were used to compare  $k^*$  values between DOI and saline injections in SERT+/+ mice, between DOI and saline injections in SERT-/- mice, and between saline injections in SERT+/+ and SERT-/- mice. Statistical significance was taken as  $p<0.05$ .

## Results

### Behavioral effects of DOI

Head twitches were not noted in either SERT+/+ or SERT-/- mice given s.c. saline. Twitches were provoked by s.c. DOI in SERT+/+. In SERT-/- mice, DOI-induced

twitches were reduced by 86% compared with twitches in the SERT<sup>+/+</sup> mice (Fig. 1).

#### DOI's effects on incorporation coefficients ( $k^*$ )

Figure 2 presents representative autoradiographs of coronal brain sections from a wild-type control mouse (SERT<sup>+/+</sup>) given saline s.c., a SERT<sup>+/+</sup> mouse given DOI s.c., a SERT<sup>-/-</sup> mouse given saline, and a SERT<sup>-/-</sup> mouse given DOI. The figure illustrates increments in  $k^*$  for AA in the brain of the SERT<sup>+/+</sup> mouse given DOI compared with the mouse given saline, but not in the SERT<sup>-/-</sup> mouse given DOI compared with one given saline.

Table 1 presents mean regional incorporation coefficients ( $k^*$ ) for AA in the four experimental groups in each of 71 brain regions identified by quantitative autoradiography. The first data column provides values for  $k^*$  that are comparable to values reported in unanesthetized rats (Basselin et al. 2003; DeGeorge et al. 1991; Qu et al. 2003b). The first two data columns in the table show that in SERT<sup>+/+</sup> mice, 1.5 mg/kg DOI compared with saline produced statistically significant increments in  $k^*$  in white matter (2 of 2 regions, average 75%), olfactory regions (66%) and cerebral cortex (7 of 13 regions, average 58%), septum (3 of 4 regions, average 59%), thalamus (5 of 12 brain regions, average 48%), hypothalamus (5 of 11 regions, 55%), the CA1 field of the hippocampus (56%), nucleus accumbens (68%), caudate putamen (78%), and globus pallidus (59%). In SERT<sup>-/-</sup> mice, in contrast, DOI compared with saline did not change  $k^*$  significantly in any of the 71 regions examined. Additionally, there was no significant difference between any mean  $k^*$  in SERT<sup>-/-</sup> mice compared with SERT<sup>+/+</sup> mice given saline (comparison of data columns 3 and 1 in Table 1).

## Discussion

Regional brain PLA<sub>2</sub> activation, represented as an increment in the regional brain incorporation coefficient  $k^*$  for AA, was profoundly reduced in SERT<sup>-/-</sup> mice compared with SERT<sup>+/+</sup> mice given 1.5 mg/kg s.c. DOI, a 5-HT<sub>2A/2C</sub> receptor agonist. This observation suggests that 5-HT<sub>2A/2C</sub>-mediated signaling via AA is markedly downregulated in the SERT<sup>-/-</sup> mouse. There are several possible causes for this.

5-HT<sub>2A</sub> receptors in the rodent brain are much more widespread than are 5-HT<sub>2C</sub> receptors. Studies indicate that 5-HT<sub>2A</sub> receptor density is decreased by 30–40% in the claustrum, cerebral cortex, and striatum of SERT<sup>-/-</sup> mice, but increased in the hypothalamus and septum, whereas 5-HT<sub>2C</sub> receptor density is elevated in the amygdala and choroid plexus (Li et al. 2003; Rioux et al. 1999). In addition to the changes in 5-HT<sub>2A/2C</sub> receptor densities, other possible causes for a reduced  $k^*$  response to DOI in SERT<sup>-/-</sup> mice may be replacement of high-affinity by low-affinity postsynaptic 5-HT<sub>2A/2C</sub> receptors (Egan et al. 2000; Glennon 1994), reduced 5-HT<sub>2A</sub> receptor availability due

to internalization (Berry et al. 1996; Roth et al. 1998), reduced coupling of 5-HT<sub>2A/2C</sub> receptors to PLA<sub>2</sub> enzymes via G proteins, or changes in the coupled PLA<sub>2</sub> enzymes themselves (Dennis 1994). However, G<sub>q</sub> and G<sub>11</sub> protein levels appear to be unchanged (Li et al. 2003).

Other differences in the brain of the SERT<sup>-/-</sup> mouse include the absence of SERT binding sites and of 5-HT uptake capacity (Bengel et al. 1998). There is a sixfold increase in the extracellular 5-HT concentration, a marked reduction in the rate of clearance of released 5-HT, and a 60–80% reduction in the intracellular 5-HT concentration (Bengel et al. 1998; Mathews et al. 2004; Montanez et al. 2003). In addition, electrophysiological and neurochemical measurements show that presynaptic and postsynaptic 5-HT<sub>1A</sub> receptors are desensitized and decreased in number (Gobbi et al. 1997; Li et al. 2001). With regard to behavior, SERT<sup>-/-</sup> mice are more anxious on tests of anxiety than are SERT<sup>+/+</sup> mice and show reduced aggression in the resident–intruder test (Ansorge et al. 2004; Holmes et al. 2003).

In rats, DOI provokes head twitches, skin jerks, and forepaw tapping, behaviors that are considered part of a “5-HT syndrome” (Wettstein et al. 1999). The responses usually appear at a dose of 1.0 mg/kg and peak at 2.5 mg/kg. The marked head twitch responses to 1.5 mg/kg DOI in our SERT<sup>+/+</sup> mice (Fig. 1) are consistent with this dose–response relation. The twitches are thought to be mediated mainly by 5-HT<sub>2A</sub> receptors (Schreiber et al. 1995; Willins and Meltzer 1997), and have been correlated with 5-HT<sub>2A</sub> receptor density in the frontal cortex and hippocampus in some but not all reports (Yamada et al. 1995). Fewer head twitches following DOI in SERT<sup>-/-</sup> than SERT<sup>+/+</sup> mice may be related to altered 5-HT<sub>2A</sub>-mediated signaling by any of a number of signaling cascades, including phospholipase C (Gray and Roth 2001; Raymond et al. 2001) and, in view of the data in this paper, a reduced AA cascade (Berg et al. 1998; Felder et al. 1990; Qu et al. 2003a,b; Shimizu and Wolfe 1990). Whereas the contributions of the two first signaling pathways remain to be evaluated, a role for AA or its eicosanoid products is suggested by evidence that the synthetic cannabinoid, anandamide, a potential source of exogenous AA, inhibits DOI-provoked head twitches in mice, independently of its binding to cannabinoid receptors, and that anandamide's effect on twitches can be prevented by cyclooxygenase inhibitors (Egashira et al. 2004).

$k^*$  for AA was not increased significantly following DOI in any of the 71 brain regions examined in the SERT<sup>-/-</sup> mice, whereas it was widely and significantly elevated in the SERT<sup>+/+</sup> mice (Table 1). At baseline in the SERT<sup>+/+</sup> mice, furthermore, values for  $k^*$  were comparable to values reported in unanesthetized rats (Qu et al. 2003b). DOI-induced increments in the SERT<sup>+/+</sup> mice were evident in the cerebral cortex and in white matter, olfactory regions, septum, thalamus, hypothalamus, hippocampus, nucleus accumbens, caudate putamen, and globus pallidus, regions with high densities mainly of 5-HT<sub>2A</sub> and to a lesser extent 5-HT<sub>2C</sub> receptors, whereas the choroid plexus, which contains mainly 5-HT<sub>2C</sub> receptors, was not activated (Li et al.

2003; McKenna and Peroutka 1989; Pazos and Palacios 1985). The choroid plexus can be activated, however, in rats by DOI at a dose of 2.5 mg/kg i.p. (Qu et al. 2003a).

Secondary activation of PLA<sub>2</sub> is always a possibility in drug studies, via any of a number of different downstream receptor types coupled to PLA<sub>2</sub> (Bayon et al. 1997; Felder et al. 1990; Vial and Piomelli 1995; Weichel et al. 1999). This may explain why some regions having low levels of 5-HT<sub>2A/2C</sub> receptors (Li et al. 2003), e.g., septal nuclei, had significant elevations in *k*\* for AA (Table 1). However, the fact that DOI stimulation of *k*\* can be entirely blocked by mianserin (Qu et al. 2003a) indicates that increases in *k*\* arise from primary activation of 5-HT<sub>2A/2C</sub> receptors. We chose a lower DOI dose (1.5 mg/kg) than we previously used in rats (2.5 mg/kg) (Qu et al. 2003a) to reduce the extent of secondary activation, and the pattern of *k*\* responses to 1.5 mg/kg DOI in SERT<sup>+/+</sup> mice was comparable to the pattern following 1.0–2.5 mg/kg DOI in rats (Basselin et al. 2005; Qu et al. 2003a).

We reported that acute fluoxetine, like acute DOI, increased *k*\* for AA in rat brain regions having 5-HT<sub>2A/2C</sub> receptors (Qu et al. 2003b) and ascribed the increases to fluoxetine's ability to increase endogenous 5-HT in the synaptic cleft to activate postsynaptic 5-HT<sub>2A/2C</sub> receptors coupled to PLA<sub>2</sub> (Fuller 1995). Chronic fluoxetine also has been shown to increase 5-HT extracellular concentrations and turnover in rodent brain (Guan and McBride 1988; Stenfors and Ross 2002), and we later reported that 3 weeks of repeated fluoxetine administration to rats, followed by 3 days of washout, increased baseline values of *k*\* for AA but did not alter *k*\* responses to DOI (Rapoport et al. 2003). Together, the data in this paper (Fig. 1 and Table 1) and those following chronic fluoxetine (Rapoport et al. 2003) suggest that a lifelong (Mathews et al. 2004) but a not short period (3 weeks) of elevated extracellular 5-HT profoundly downregulates PLA<sub>2</sub>-mediated AA signaling coupled to 5-HT<sub>2A/2C</sub> receptors. Behavioral measurements in adult mice given fluoxetine between postnatal days 4 and 21 are consistent with a developmental sensitivity to elevated extracellular 5-HT (Ansorge et al. 2004).

Causes for the profound downregulation in SERT<sup>-/-</sup> mice of PLA<sub>2</sub> signaling involving AA, in response to DOI, remain to be elucidated, and several possibilities have been discussed above. Using specific 5-HT<sub>2A</sub> and HT<sub>2C</sub> antagonists (Hayashi et al. 2004; Komiyama et al. 2004) to distinguish the roles of the two receptor subtypes would help in this regard, as might a dose–response study with DOI. With regard to head twitches in response to DOI, signaling cascades other than the AA cascade remain to be evaluated (see above). In addition, studying heterozygous SERT<sup>+/-</sup> mice, which have an abnormally elevated brain 5-HT concentration but to a lesser extent than do SERT<sup>-/-</sup> mice (Gobbi et al. 2001; Mathews et al. 2004; Shen et al. 2004), may be more relevant for understanding the role of 5-HT in anxiety and depression in humans who have the short 5HTTLPR SERT allele (Murphy et al. 2004; Smits et al. 2004). Eventually, brain 5-HT<sub>2A/2C</sub>-mediated signaling coupled to PLA<sub>2</sub> might be imaged in such subjects with

positron emission tomography (Esposito et al. 2003; Giovacchini et al. 2002).

**Acknowledgements** This work was supported in part by the National Alliance for Research on Schizophrenia and Depression (NARSAD) under a Distinguished Investigator Award to S.I. Rapoport.

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