European Neuropsychopharmacology (2006) 16, 561—571

www.elsevier.com/locate/euroneuro

Chronic fluoxetine upregulates arachidonic acid incorporation into the brain of unanesthetized rats

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Received 7 August 2005; received in revised form 29 October 2005; accepted 17 January 2006

KEYWORDS
Serotonin;
Phospholipase A_2;
Arachidonic;
Fluoxetine;
Rat;
5-$HT_{2A/2C}$;
Receptor;
Depression

Abstract Serotonergic 5-$HT_{2A/2C}$ receptors can be coupled to phospholipase A_2 (PLA_2) activation to release the second messenger, arachidonic acid (AA), from membrane phospholipids. We wished to see if this signaling process in rat brain would be altered by chronic administration followed by 3 days of washout of the selective serotonin reuptake inhibitor, fluoxetine. We injected [^{3}H]AA intravenously in unanesthetized rats and used quantitative autoradiography to determine the incorporation coefficient k^* for AA (regional brain radioactivity/integrated plasma radioactivity), a marker of PLA_2 activation, in each of 86 brain regions. k^* was measured following acute i.p. saline or (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI, 1.0 mg/kg i.p.), a 5-$HT_{2A/2C}$ receptor agonist, in rats injected for 21 days with 10 mg/kg i.p. fluoxetine or saline daily, followed by 3 days without injection. Acute DOI produced statistically significant increments in k^* in brain regions with high densities of 5-$HT_{2A/2C}$ receptors, but the increments did not differ significantly between the chronic fluoxetine- and saline-treated rats. Additionally, chronic fluoxetine compared with saline widely and significantly increased baseline values of k^*. These results suggest that 5-$HT_{2A/2C}$ receptor-initiated AA signaling is unaffected by chronic fluoxetine plus 3 days of washout in the rat, but that baseline AA signaling is nevertheless upregulated. This upregulation likely occurs independently of significant active drug in brain, considering the short brain half-lives of it and its norfluoxetine metabolite. Such upregulation may contribute to fluoxetine's efficacy against human depression.
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Abbreviations: AA, arachidonic acid; DOI, (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane; 5-HT, 5-hydroxytryptamine (serotonin); $rCMR_{glc}$, regional cerebral metabolic rate for glucose; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic PLA_2; $sPLA_2$, secretory PLA_2; PLC, phospholipase C; SERT, serotonin reuptake transporter.

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0924-977X/$ - see front matter. Published by Elsevier B.V. and ECNP.
doi:10.1016/j.euroneuro.2006.01.008

1. Introduction

Fluoxetine (Prozac) is used widely to treat depression, bipolar disorder, obsessive—compulsive disorder, and anxiety disorder (Amsterdam et al., 1998; Boerlin et al., 1998; Fuller, 1995). It is considered a selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor that elevates 5-HT in the serotonergic synaptic cleft (Fuller and Wong, 1977; Wong et al., 1995), but it can increase extracellular norepinephrine and dopamine as well (Bymaster et al., 2002). Its therapeutic action when chronically administered has been ascribed to desensitization of pre-synaptic 5-HT_{1A} and 5-HT_{1B} autoreceptors, further augmenting extracellular 5-HT (Blier and de Montigny, 1994; Newman et al., 2004; Rutter et al., 1994).

In rodents, chronic fluoxetine followed by 3 days of washout was reported to elevate brain extracellular 5-HT and to increase 5-HT synthesis and turnover rates (Stenfors and Ross, 2002a). These changes likely represent neuroplastic changes, as brain half-lives of fluoxetine and its active metabolite, norfluoxetine, 5 and 15 h, respectively, suggest that little of any of these drugs remains in brain after 3 days of washout (Caccia et al., 1990).

5-$HT_{2A/2C}$ receptors can be coupled to phospholipase A_2 (PLA_2) activation via G proteins, to release the second messenger arachidonic acid (AA 20:4 $n-6$) from membrane phospholipid (Axelrod, 1990; Berg et al., 1998; Garcia and Kim, 1997; Kurrasch-Orbaugh et al., 2003; Qu et al., 2003b). A method exists to image this coupling in the brain of rats administered drugs that modify 5-HT binding to 5-$HT_{2A/2C}$ receptors (Basselin et al., 2005; DeGeorge et al., 1991; Qu et al., 2003a). The method involves injecting radiolabeled AA intravenously, determining at 15—20 min brain radioactivity with quantitative autoradiography, and calculating regional brain incorporation coefficients k^* for AA (brain radioactivity/integrated plasma radioactivity). k^* is proportional to PLA_2 activation and is independent of changes in cerebral blood flow (Chang et al., 1997; DeGeorge et al., 1991; DeMar et al., 2004; Jones et al., 1996; Rapoport, 2001, 2003; Robinson et al., 1992).

Using this method in unanesthetized rats, we reported that 2.5 mg/kg i.p. DOI significantly increased k^* for AA in brain regions with high densities of 5-$HT_{2A/2C}$ receptors, and that the increases could be blocked by pretreatment with the 5-$HT_{2A/2C}$ antagonist, mianserin (Qu et al., 2003a). Acute fluoxetine (10 mg/kg i.p.) increased k^* for AA in comparable regions (Qu et al., 2003b), consistent with fluoxetine increasing extracellular 5-HT (Fuller and Wong, 1977; Wong et al., 1995). However, behavioral and k^* responses to 1.5 mg/kg s.c. DOI were absent in mice in which the selective serotonin reuptake transporter (SERT) was knocked out ($SERT^{(-/-)}$ mice), compared with wild type controls (Qu et al., 2005). As extracellular 5-HT is elevated in $SERT^{(-/-)}$ mice (Bengel et al., 1998), this suggested that a life-long exposure to elevated 5-HT depresses 5-$HT_{2A/2C}$ receptor-mediated activation of PLA_2.

Chronic fluoxetine's therapeutic mechanism of action is not completely understood, and it is possible that AA signaling contributes to it (Kucia et al., 2003; Qu et al., 2003b; Sublette et al., 2004). We therefore decided to use our fatty acid method to examine the effect of chronic fluoxetine with washout on 5-$HT_{2A/2C}$-initiated PLA_2 activation in unanesthetized rats. We compared k^* for AA in adult rats given i.p. saline or fluoxetine for 3 weeks (10 mg/kg i.p. daily), followed by 3 days without injection. In both groups, we measured k^* following acute administration of DOI (1.0 mg/kg i.p.) or of i.p. saline (baseline). An abstract of part of this work has been published (Rapoport et al., 2003).

2. Experimental procedures

2.1. Materials

[5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid ([3H]AA) at a specific activity of 200 Ci/mmol was purchased from Moravek Biochemicals (Brea, CA). Its radiochemical purity on thin layer chromatography exceeded 97%. Fluoxetine and (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) were purchased from Sigma Research Biochemicals International (Natick, MA). Sodium pentobarbital was purchased from Richmond Veterinary Supply (Richmond, VA).

2.2. Animals

The 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 Fischer-344 rats (Charles River Laboratories, Wilmington, MA), weighing 290—320 g and 12-weeks old, were housed under standard laboratory conditions with a 12 h light—12 h dark cycle, and with free access to laboratory chow and water. They were divided into a control group of 16 animals that were injected daily for 21 days with saline (2.5 ml/kg i.p.) and a treatment group of 16 animals that received daily for 21 days fluoxetine (10 mg/kg i.p.). At the end of the 21 days, the saline and fluoxetine injections were discontinued for 3 days.

2.3. Arterial and venous catheterization

The in vivo fatty acid method to image brain PLA2 signaling is described in detail elsewhere (DeGeorge et al., 1991; Qu et al., 2003a; Rapoport, 2001; Robinson et al., 1992). Briefly, a rat was anesthetized with halothane (1—3% v/v in O_2), and PE 50 polyethylene catheters (Clay Adams, Lincolnshire, IL) filled with heparinized saline (100 IU/ml) were surgically implanted into a femoral artery and vein. The incision was infiltrated with a local anesthetic (1% lidocaine) and closed with wound clips. The hindquarters of the rat then were wrapped loosely in a fast-setting plaster cast secured to a wooden block, and the rat was allowed to recover from anesthesia in a temperature-controlled sound-dampened box for 4 h. Body temperature was maintained at 36—37 °C using a rectal thermometer and a feedback heating device.

2.4. Drug administration and tracer infusion

After the rat had recovered from anesthesia, 125 μl of arterial blood was withdrawn to measure pH, pO_2 and pCO_2

(Blood Gas Analyzer Model 238 pH, Ciba Corning, Medfield, MA). Then, 0.3 ml saline or DOI (1.0 mg/kg in 0.3 ml saline) was injected i.p. Twenty min later, the rat was infused for 5 min with 1.75 m Ci/kg [^{3}H]AA in 2 ml of 5 mM HEPES buffer, pH 7.4, containing 50 mg/ml fatty acid-free bovine serum albumin. The fluid was infused at a rate of 400 μl/min into the indwelling venous catheter using an infusion pump (Harvard Instruments, Holliston, MA). Timed 125 μl arterial blood samples were collected from the start of infusion to 20 min, when the rat was killed with 65 mg i.v. sodium pentobarbital. The brain was removed, rapidly frozen in 2-methylbutane at −50 °C, and stored at −80 °C for quantitative autoradiography. Plasma was separated from arterial blood by centrifugation, and plasma lipids were extracted (Folch et al., 1957). Radioactivity in the organic fraction due to [^{3}H]AA was measured by liquid scintillation counting.

2.5. Autoradiography and calculations

Frozen brains were cut coronally on a cryostat (Hacker Instruments, Fairfield, NJ) at −20 °C. Sets of three adjacent 20 μm sections were collected at 140 μm intervals, mounted on glass coverslips and dried on a hot plate. The sections were exposed for 7–14 days, together with [^{3}H]methyl-methacrylate autoradiographic standards (Amersham, Arlington Heights, IL), to [^{3}H]phosphor imaging plates (Fuji Medical Systems, Stamford, CT). The plates were scanned by a scanner system (BAS 5000, West Lafayette, IN) following the manufacturer's instructions. Adjacent sections were stained with cresyl violet to identify each of 86 brain regions from a rat-brain atlas (Paxinos and Watson, 1987).

Regional brain radioactivity on the autoradiographs was measured in sextuplicate by quantitative densitometry, using phosphor-imaging software (Image Gauge V3.45, Fuji). Regional brain incorporation coefficients k^* were calculated as,

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

k^* is in units of milliliter per second per gram (equal to s^{-1} as brain specific gravity approximates 1); c^*_{brain} (20 min) is brain radioactivity at 20 min after the onset of infusion, in units of nCi per gram; c^*_{plasma} is plasma AA radioactivity in units of nCi per milliliter; t is time after onset of [^{3}H]AA infusion.

Data for each of 86 regions were analyzed with a 2 × 2 ANOVA to determine the statistical significance of Main effects (chronic fluoxetine vs. chronic saline, acute DOI vs. acute saline), as well as of their Interactions. Significance was taken as $p \leq 0.05$.

3. Results

3.1. Physiological parameters

As shown in Table 1, chronic fluoxetine followed by 3 days of washout had no effect on a number of measured physiological parameters. Acute DOI (1.0 mg/kg i.p.) significantly increased mean arterial systolic blood pressure and decreased mean heart rate in both chronic saline- and fluoxetine-treated rats.

Table 1 Physiological parameters in rats administered fluoxetine chronically, followed by 3 days off drug; and acute DOI or saline

	Chronic saline-injected rats			Chronic fluoxetine-injected rats		
	Baseline (i.p. saline)	DOI (1.0 mg/kg i.p.)		Baseline (i.p. saline)	DOI (1.0 mg/kg i.p.)	
		Before	After		Before	After
Body temperature (°C)	36.1 ± 0.3	36.3 ± 0.3	36.3 ± 0.3	36.8 ± 0.3	36.7 ± 0.1	36.2 ± 0.2[a]
Arterial blood pressure (mm Hg) (systolic/diastolic)	131.2 ± 4.6/75.6 ± 3.9	131.5 ± 4.5/81.5 ± 2.8	151.8 ± 6.1[a]/97.5 ± 4.2	118.2 ± 4.6/72.2 ± 4.2	127.4 ± 3.3/75.4 ± 2.2	142.0 ± 3.8[a]/85.0 ± 4.3
Heart rate (bpm)	432 ± 12	428 ± 13	383 ± 11[a]	435 ± 15	411 ± 9	377 ± 11[a]
Arterial pH	7.5 ± 0.02		7.4 ± 0.01	7.4 ± 0.01		7.4 ± 0.01
Arterial pCO_2 (mm Hg)	35.3 ± 2.6		40.9 ± 1.4	40.3 ± 1.4		40.0 ± 1.3
Arterial pO_2 (mmHg)	107.8 ± 7.1		88.8 ± 1.3	94.3 ± 3.2		102 ± 5.8

†Mean ± SEM.
Animal number = 6–9.
[a] $p<0.05$, paired t-tests were used to means in same animal before and after DOI injection.

3.2. Effects of chronic fluoxetine with or without acute DOI

Fig. 1 presents representative autoradiographs of coronal brain sections from: (A) a "control," chronic saline-treated rat (given acute i.p. saline); (B) a control rat that received acute DOI 1.0 mg/kg i.p; (C) a chronic fluoxetine-treated rat injected acutely with i.p. saline; (D) a chronic fluoxetine-treated rat that received acute DOI 1.0 mg/kg i.p. The autoradiographs illustrate increased values of k^* for AA in B and C compared with A, and in D compared with A, B and C.

Fig. 2 illustrates data that were subjected to a 2×2 ANOVA in two typical brain regions, frontal cortex IV and motor cortex II—III. In both regions, the Main effects of fluoxetine and of DOI were statistically significant, whereas their Interactions were not. Fluoxetine's significant Main effect shows that it increased k^* for AA in rats given both acute saline and acute DOI, whereas the significant Main effect of DOI indicates that acute DOI compared with acute saline increased k^* for AA in rats treated chronically with fluoxetine and saline. The insignificant Interaction means that chronic fluoxetine did not change the DOI response.

Data such as these are summarized in Table 2 for each of the 86 brain regions examined. The first data column in the table gives values of k^* in response to acute saline, in control rats that had been given i.p. saline for 21 days followed by 3 days without injection; the second, k^* in control rats injected acutely with DOI; the third, values of k^* following acute saline in rats treated with fluoxetine (10 mg/kg i.p. daily for 21 days followed by 3 days off drug); the fourth, k^* in response to acute DOI in chronic fluoxetine-treated animals.

In none of the 86 regions was there a statistically significant Interaction with regard to k^* for AA between chronic fluoxetine and acute DOI. This means that chronic fluoxetine did not affect DOI-initiated, 5-$HT_{2A/2C}$-mediated signaling via PLA_2 in any brain region. Indeed, k^* responses following DOI were equally robust in the chronic saline and fluoxetine-injected animals, as the Main effect of acute DOI was statistically significant in 61 (71%) of 86 regions. DOI elevated k^* for AA by 15—25% (average 22%) in these 61 regions.

Table 2 also shows that 77 (90%) of the 86 regions had a statistically significant Main effect of fluoxetine in the 2×2 ANOVA. In these regions, chronic fluoxetine with washout compared with chronic saline significantly elevated k^* for AA, to the same extent in acute saline- and DOI-injected animals (insignificant Interaction).

4. Discussion

Ten mg/kg i.p. fluoxetine compared with i.p. saline, administered to unanesthetized male rats daily for 21 days

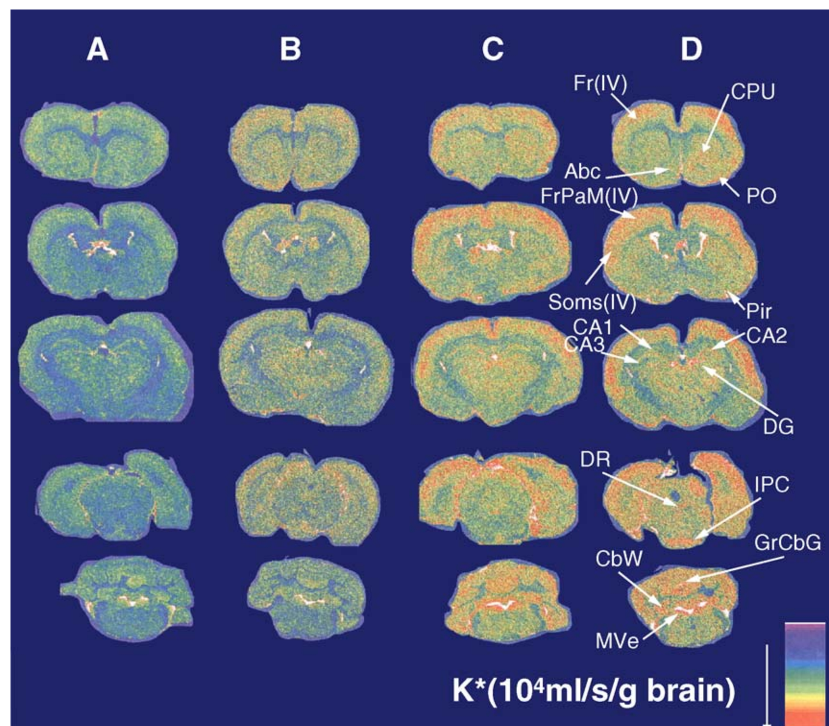

Figure 1 Coronal autoradiographs demonstrating arachidonic acid incorporation coefficients k^*. Brain of (A) control rat given acute saline 3 days after receiving i.p. saline for 21 days; (B) control rat given acute DOI (1.0 mg/kg i.p.), 3 days after receiving i.p. saline for 21 days; (C) rat given fluoxetine (10 mg/kg i.p. daily) for 21 days, followed by 3 day washout, then i.p. saline on day 24; (D) rat given fluoxetine (10 mg/kg i.p. daily) for 21 days, followed by 3 day washout, then acute DOI (1.0 mg/kg i.p.). k^* is color-coded. Abbreviations: Fr(IV), frontal cortex, layer IV; FrPaM (IV), frontal motor (layer IV); Soms, somatosensory cortex; IPC, interpeduncular nucleus; CPU, caudate putamen; CA1, CA2, CA3, DG, regions of the hippocampus; Pir, pyriform cortex; PO, olfactory cortex; GrCbG, granular layer, cerebellar gray; CbW, cerebellar white; DR, dorsal raphe; MVe, medial vestibular nucleus; Abc, nucleus accumbens.

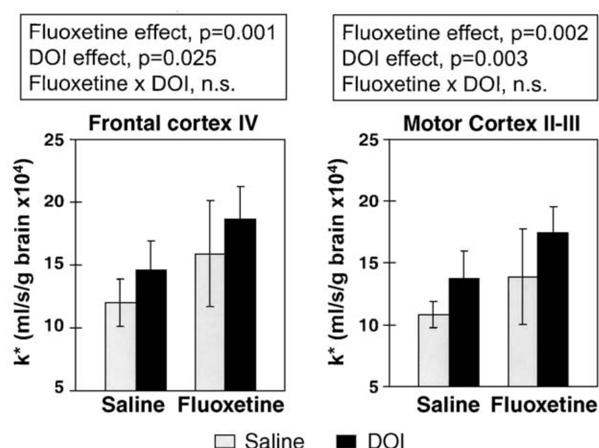

Figure 2 k^* for arachidonic acid (Eq. (1)) in each of two brain regions, in rats treated chronically with fluoxetine (10 mg/kg i.p) or saline for 21 days, and injected with DOI (1.0 mg/kg i.p.) or saline on day 24. Means ± SEM. Statistics calculated by 2 × 2 ANOVA. $n = 6$–9.

followed in both cases by 3 days without injection, did not significantly alter DOI-induced elevations in k^* for AA in any of 86 brain regions (statistically insignificant Interactions). To the extent that DOI-initiated elevations represent 5-$HT_{2A/2C}$-mediated activation of PLA_2 to release AA (see Introduction) (Basselin et al., 2005; Berg et al., 1998; Kurrasch-Orbaugh et al., 2003; Qu et al., 2003a), and that 5-$HT_{2A/2C}$ receptors are largely post-synaptic (Aghajanian and Marek, 1999; Cooper et al., 2003; Staley et al., 1998), these results indicate that chronic fluoxetine with washout did not alter post-synaptic 5-$HT_{2A/2C}$ signaling via PLA_2. On the other hand, chronic fluoxetine plus washout significantly elevated values of k^* in acute saline and DOI-injected rats (significant fluoxetine Main effect). This elevation likely was due to neuroplastic upregulation of baseline AA signaling involving 5-HT (see below). It corresponds to increased AA incorporation into brain phosphatidylcholine (Bazinet, unpublished results), as was found in another drug activation study (DeGeorge et al., 1991; Rapoport, 2001).

5-$HT_{2A/2C}$ receptors can be coupled via $G_{q/11}$ proteins to PLA_2 activation (Kurrasch-Orbaugh et al., 2003), independently of PLC activation (Berg et al., 1998; Garcia and Kim, 1997; Kurrasch-Orbaugh et al., 2003). Consistent with PLA_2 coupling, we reported that acute 2.5 mg/kg i.p. DOI in rats significantly increased k^* for AA by an average of 60% (Qu et al., 2003a) in regions containing 5-$HT_{2A/2C}$ receptors (Li et al., 2003; McKenna et al., 1989; Pazos and Palacios, 1985), and that the increments could be blocked by pretreatment with mianserin, a 5-$HT_{2A/2C}$ receptor antagonist. In the present study, 1.0 mg/kg i.p. DOI increased k^* in comparable regions by 22% on average (Table 2), consistent with a dose–response relation. Affected were regions in neocortex, thalamus, olfactory cortex, nucleus accumbens, globus pallidus and amygdala, all of which are reported to have high densities of 5-HT_{2A} receptors; 5-HT_{2C} receptors also are reported in some of them (Li et al., 2003; McKenna et al., 1989; Pazos and Palacios, 1985; Xu and Pandey, 2000).

The statistically insignificant fluoxetine × DOI Interactions with respect to k^* (Table 2 and Fig. 2) indicate that post-synaptic 5-$HT_{2A/2C}$ receptor signaling via PLA_2, was unaffected by chronic fluoxetine. In this regard, chronic fluoxetine has been reported to increase, decrease or not change 5-$HT_{2A/2C}$ receptor density in different brain regions (Cadogan et al., 1993; Hrdina and Vu, 1993; Tilakaratne et al., 1995), and to reduce binding of 5-HT2A receptors to G-proteins after 1 but not 7 days off drug (Gobbi et al., 1997; Hensler, 2002; Li et al., 1996). The lack of a significant effect of 3 weeks of fluoxetine plus washout on DOI-induced increments in k^* contrasts with reduced k^* increments to DOI in SERT knockout compared with wild-type mice (Qu et al., 2005). The difference suggests that a life-long (Mathews et al., 2004) but not a 3-week elevation of synaptic 5-HT will downregulate 5-$HT_{2A/2C}$ receptor signaling involving PLA_2.

We have ascribed increases in k^* for AA in rats given acute fluoxetine to an acute elevation of endogenous 5-HT to activate PLA_2 via 5-$HT_{2A/2C}$ receptors (Qu et al., 2003b). However, chronic fluoxetine is considered to increase extracellular 5-HT to a greater extent than acute fluoxetine, by desensitizing the pre-synaptic 5-HT_{1A} and 5-HT_{1B} autoreceptors that normally suppress 5-HT release from pre-synaptic elements (Blier and de Montigny, 1994; Newman et al., 2004; Rutter et al., 1994). Consistent with this mechanism, 5-HT_{1B} receptors remained desensitized for as long as 4 days after chronic fluoxetine (el Mansari et al., 1995; Stenfors and Ross, 2002b), whereas 5-HT_{1A} receptors were desensitized 2 days after stopping fluoxetine or gepirone (5-H_{1A} agonist) (Blier and de Montigny, 1987; Invernizzi et al., 1996; Le Poul et al., 2000). Chronic fluoxetine downregulated G-protein alpha subunits involved in 5-HT_{1A} activation, but these effects were absent after 7 days off drug (Gobbi et al., 1997). After a 1 day but not a 7 day washout, SERT was upregulated in rat brain (Gobbi et al., 1997; Hrdina and Vu, 1993).

The significantly elevated baseline values of k^* at 3 days after chronic fluoxetine (significant Main effect of fluoxetine) likely represented a neuroplastic effect involving 5-HT transmission, as half-lives in rat brain of fluoxetine and its active metabolite norfluoxetine, 5 and 15 h respectively, indicate that these compounds were largely absent from brain after 3 days (Caccia et al., 1990). This neuroplastic effect did not involve altered post-synaptic signaling via 5-$HT_{2A/2C}$ receptors, in view of the unchanged k^* responses to DOI after washout (see above). Upregulated overall signaling, however, is consistent with observations in mice that brain 5-HT synthesis (measured as 5-hydroxy-L-tryptophan accumulation) and 5-HT turnover (measured as the 5-HIAA / 5-HT ratio), and well as brain 5-HT, were elevated 3 days after chronic fluoxetine (Stenfors and Ross, 2002b). In rats or guinea pigs, extracellular 5-HT was elevated at 1 day but not 4 days after fluoxetine withdrawal (Invernizzi et al., 1996; Schmidt et al., 1988; Wong et al., 1995). One or 2 days following chronic fluoxetine in rats, c-fos formation was increased in response to acute DOI, and PLC-mediated hydrolysis of phosphatidylinositol was increased in response to acute 5-HT or DOI (Cadogan et al., 1993; Damjanoska et al., 2003; Hrdina and Vu, 1993; Tilakaratne et al., 1995).

Table 2 Regional AA incorporation coefficients k^* (ml/s/g brain $\times 10^4$) in rats treated chronically with fluoxetine (10 mg/kg daily, 21 days, washout for 3 days) or saline, then given acute DOI (1.0 mg/kg i.p.) or saline

Brain region	Chronic saline		Chronic fluoxetine		Fluoxetine effect	DOI effect	Fluoxetine × DOI effect
Number animals	Saline	DOI	Saline	DOI	*P* value	*P* value	*P* value
	6	8	9	7			
Cerebral cortex							
Prefrontal cortex IV	11.27 ± 1.45	13.16 ± 2.38	13.95 ± 3.61	16.78 ± 2.46	0.004	0.028	0.647
Frontal cortex layer II–III	11.17 ± 1.23	14.04 ± 2.40	14.08 ± 3.32	16.95 ± 2.17	0.004	0.005	0.997
Frontal cortex layer IV	12.00 ± 1.88	14.60 ± 2.37	15.93 ± 4.20	18.67 ± 2.61	0.001	0.025	0.952
Motor cortex layer II–III	10.81 ± 1.07	13.72 ± 2.28	13.91 ± 3.82	17.47 ± 2.07	0.002	0.003	0.743
Motor cortex layer IV	12.18 ± 1.60	15.30 ± 2.13	16.38 ± 5.26	19.99 ± 2.62	0.002	0.013	0.851
Motor cortex layer V–VI	10.66 ± 1.32	12.96 ± 1.80	13.90 ± 3.95	16.38 ± 1.81	0.002	0.02	0.925
Somatosensory cortex layer II–III	11.35 ± 1.57	13.84 ± 2.21	14.83 ± 3.89	17.94 ± 2.13	0.001	0.01	0.761
Somatosensory cortex layer IV	12.01 ± 1.36	14.67 ± 2.26	16.21 ± 4.46	19.42 ± 2.71	0.001	0.016	0.81
Somatosensory cortex layer V–VI	10.63 ± 1.49	12.61 ± 2.18	13.64 ± 3.98	16.39 ± 2.16	0.003	0.028	0.709
Anterior cingulate cortex	11.34 ± 1.20	13.48 ± 2.20	14.11 ± 3.60	16.71 ± 2.31	0.004	0.021	0.811
Auditory cortex layer II–III	10.60 ± 1.30	13.04 ± 1.85	13.80 ± 3.5	17.10 ± 2.64	0.001	0.006	0.655
Auditory cortex layer IV	11.80 ± 1.75	14.47 ± 2.09	15.96 ± 4.21	20.07 ± 3.40	0.001	0.007	0.541
Auditory cortex layer V–VI	10.96 ± 1.87	12.76 ± 2.07	13.48 ± 3.57	16.48 ± 2.08	0.003	0.019	0.537
Visual cortex layer II–III	10.74 ± 1.13	13.02 ± 2.24	13.04 ± 3.14	16.44 ± 1.77	0.002	0.003	0.518
Visual cortex layer IV	12.18 ± 2.04	14.00 ± 2.25	14.14 ± 3.73	17.96 ± 2.98	0.011	0.014	0.361
Visual cortex layer V–VI	10.86 ± 1.54	12.17 ± 1.95	12.56 ± 3.28	15.67 ± 2.05	0.007	0.019	0.317
White matter							
Corpus callosum	5.81 ± 0.64	7.42 ± 2.25	7.01 ± 1.79	8.04 ± 1.39	0.158	0.045	0.649
Internal capsule	5.49 ± 0.45	6.34 ± 1.23	6.54 ± 1.44	7.84 ± 1.51	0.011	0.031	0.637
Anterior commissure	6.56 ± 0.93	7.45 ± 1.29	8.16 ± 1.93	9.71 ± 1.54	0.002	0.038	0.558
Olfactory regions							
Olfactory Cortex	13.43 ± 0.91	16.09 ± 2.50	16.36 ± 4.03	19.60 ± 2.46	0.005	0.01	0.784
Pyriform cortex	11.80 ± 1.16	14.11 ± 266	14.59 ± 3.41	18.37 ± 3.53	0.003	0.009	0.502
Basal ganglia and related areas							
Nucleus accumbens	10.08 ± 1.10	11.04 ± 1.96	12.94 ± 3.19	14.80 ± 1.90	0.001	0.106	0.6
Caudate putamen dorsal	9.77 ± 1.02	11.40 ± 2.04	12.35 ± 3.48	15.28 ± 1.94	0.001	0.017	0.476
Caudate putamen ventral	10.34 ± 1.10	11.76 ± 2.18	12.55 ± 2.85	15.06 ± 2.15	0.003	0.026	0.516

Caudate putamen lateral	9.97 ± 1.38	11.88 ± 2.05	12.25 ± 3.14	14.86 ± 2.04	0.005	0.015	0.685
Caudate putamen medial	10.23 ± 0.99	11.20 ± 2.40	12.35 ± 3.63	14.89 ± 2.32	0.006	0.085	0.432
Bed nucleus stria preoptic nucleus	8.93 ± 0.83	10.33 ± 1.81	10.82 ± 2.84	12.89 ± 2.19	0.009	0.038	0.679
Bed nucleus stria suprachiasmatic nucleus	11.03 ± 2.78	14.23 ± 1.86	9.59 ± 1.22	11.15 ± 2.27	0.01	0.007	0.323
Bed nucleus stria terminals	8.61 ± 1.4	9.65 ± 1.28	10.11 ± 2.75	11.82 ± 1.86	0.019	0.072	0.653
Entopeduncular nucleus	7.33 ± 3.17	9.29 ± 1.73	9.86 ± 3.22	11.59 ± 1.87	0.018	0.065	0.909
Globus pallidus	8.93 ± 1.51	9.42 ± 1.65	10.06 ± 3.05	12.00 ± 2.14	0.035	0.158	0.394
Amygdala basolateral/basomedial	8.06 ± 0.80	9.82 ± 1.66	9.68 ± 2.50	11.84 ± 1.47	0.012	0.007	0.769
Amygdala subthalamic nucleus	9.37 ± 1.31	11.44 ± 2.19	11.17 ± 3.18	13.91 ± 1.12	0.016	0.007	0.687
Substantia nigra							
Pars reticulata	9.59 ± 1.65	10.74 ± 2.01	11.24 ± 2.81	13.4 ± 1.90	0.014	0.052	0.541
Pars compacta	9.72 ± 1.83	10.66 ± 2.06	10.70 v 2.44	13.19 ± 1.76	0.031	0.035	0.322
Septum							
Lateral septal nucleus	8.82 ± 1.34	8.98 ± 1.76	9.94 ± 2.59	11.12 ± 1.58	0.032	0.361	0.488
Medial septal nucleus	9.28 ± 1.16	11.60 v 2.80	11.03 ± 2.52	12.91 ± 2.29	0.09	0.023	0.802
Dorsal diagonal band	9.41 ± 1.28	11.26 ± 2.34	11.43 ± 2.39	14.03 ± 2.02	0.005	0.008	0.645
Ventral diagonal band	9.36 ± 1.20	10.68 ± 2.09	11.10 ± 2.68	14.05 ± 2.34	0.004	0.015	0.328
Hippocampal formation							
Ammon's horn CA1	10.95 ± 1.53	13.26 ± 2.30	13.76 ± 4.31	17.05 ± 3.11	0.008	0.023	0.674
Ammon's horn CA2	11.16 ± 2.50	12.60 ± 2.73	13.45 ± 4.41	16.18 ± 1.61	0.017	0.083	0.581
Ammon's horn CA3	11.09 ± 2.13	12.43 ± 1.90	13.04 ± 3.79	15.53 ± 1.77	0.015	0.061	0.559
Dentate gyrus	11.60 ± 3.10	12.05 ± 2.07	14.58 ± 4.51	17.98 ± 3.91	0.002	0.157	0.273
Dorsal lateral geniculate nucleus	10.31 ± 1.53	12.47 ± 2.30	12.64 ± 3.52	14.89 ± 2.30	0.017	0.026	0.964
Parafascicular nucleus	11.30 ± 4.52	11.96 ± 2.10	12.06 ± 3.80	13.83 ± 1.71	0.274	0.314	0.641
Thalamus							
Paratenial nuclei	9.85 ± 1.01	11.30 ± 1.75	12.20 ± 2.98	14.34 ± 2.05	0.002	0.034	0.672
Anteroventral nuclei	12.46 ± 1.47	15.04 ± 2.46	15.80 ± 4.22	18.17 ± 2.31	0.006	0.032	0.922
Anteromedial nuclei	9.98 ± 1.21	11.72 ± 1.99	12.46 ± 3.46	14.85 ± 1.40	0.003	0.025	0.714
Reticular nuclei	10.03 ± 1.29	12.64 ± 2.17	13.10 ± 3.92	15.04 ± 2.03	0.011	0.03	0.738
Paraventricular nuclei	9.02 ± 1.16	10.83 ± 1.59	11.57 ± 3.09	13.29 ± 2.37	0.006	0.045	0.957
Ventroposterior medial thalamus	9.63 ± 1.53	11.89 ± 2.12	11.84 ± 3.38	13.85 ± 1.87	0.029	0.026	0.89
Ventroposterior lateral thalamus	9.64 ± 1.23	11.73 ± 2.39	11.90 ± 3.23	14.07 ± 1.82	0.015	0.024	0.962
Lateral habenular nucleus	10.95 ± 1.67	13.22 ± 2.02	13.27 ± 3.99	16.00 ± 1.50	0.015	0.017	0.815
Medial habenular nucleus	14.18 ± 2.65	11.87 ± 1.49	14.37 ± 4.17	18.24 ± 2.17	0.006	0.009	0.482
Medial geniculate nucleus	11.58 ± 2.84	12.09 ± 1.90	12.58 ± 2.93	15.45 ± 1.90	0.023	0.073	0.202

(continued on next page)

Table 2 (*continued*)

Brain region	Chronic saline		Chronic fluoxetine		Fluoxetine effect	DOI effect	Fluoxetine × DOI effect
Number animals	Saline	DOI	Saline	DOI	*P* value	*P* value	*P* value
	6	8	9	7			
Hypothalamus							
Supraoptic nuclei	11.85 ± 1.47	14.57 ± 3.66	19.52 ± 9.43	22.46 ± 12.80	0.018	0.366	0.971
Subfornical organ nuclei	19.01 ± 6.44	18.69 ± 7.35	20.01 ± 8.44	21.64 ± 7.58	0.488	0.817	0.73
Lateral nuclei	8.08 ± 1.10	9.71 ± 1.87	9.85 ± 2.37	11.43 ± 1.8	0.017	0.026	0.972
Anterior nuclei	8.21 ± 0.93	9.59 ± 1.82	10.37 ± 2.55	11.90 ± 1.61	0.004	0.05	0.918
Periventricular nuclei	9.96 ± 2.52	11.36 ± 1.86	10.48 ± 2.97	14.59 ± 2.06	0.045	0.005	0.141
Arcuate nuclei	8.47 ± 0.67	9.97 ± 2.03	9.82 ± 2.21	11.69 ± 1.50	0.029	0.017	0.788
Ventromedial nuclei	8.29 ± 10.2	9.60 ± 1.64	9.85 ± 2.80	11.74 ± 1.34	0.016	0.035	0.689
Posterior nuclei	9.56 ± 1.36	11.40 ± 1.99	11.98 ± 3.11	15.11 ± 1.72	0.001	0.006	0.45
Mammillary nucleus	8.55 ± 10.9	8.61 ± 3.34	10.07 ± 2.55	11.58 ± 1.49	0.018	0.384	0.42
Medial forebrain bundle	8.72 ± 1.35	10.07 ± 2.09	10.45 ± 2.86	12.19 ± 2.09	0.028	0.075	0.818
Median eminence	10.06 ± 2.68	17.76 ± 8.98	12.50 ± 6.25	19.24 ± 11.69	0.52	0.024	0.875
Brainstem and spinal cord							
Raphe magnus nuclei	8.54 ± 2.76	10.18 ± 2.54	9.27 ± 1.96	12.17 ± 2.48	0.138	0.017	0.485
Raphe pallidus nuclei	9.16 ± 2.24	10.17 ± 2.27	9.15 ± 2.84	10.76 ± 2.89	0.76	0.184	0.755
Raphe median nuclei	10.23 ± 2.39	10.39 ± 1.73	10.88 ± 3.14	12.71 ± 3.03	0.138	0.327	0.395
Raphe dorsal nuclei	10.66 ± 2.47	12.41 ± 2.18	11.2 ± 3.59	14.35 ± 2.19	0.232	0.023	0.499
Locus coeruleus	10.20 ± 2.21	11.98 ± 2.28	12.17 ± 3.78	14.29 ± 2.11	0.048	0.069	0.868
Cochlear nucleus	14.25 ± 3.46	16.71 ± 5.58	17.09 ± 7.58	18.05 ± 3.62	0.322	0.416	0.721
Vestibular nucleus (medial)	13.69 ± 3.69	16.53 ± 3.58	16.23 ± 3.94	19.31 ± 4.28	0.075	0.049	0.933
Inferior colliculus	13.81 ± 2.68	15.95 ± 2.72	16.69 ± 4.84	20.20 ± 3.03	0.012	0.041	0.607
Superior colliculus	11.10 ± 1.90	12.81 ± 2.50	13.52 ± 4.42	17.43 ± 2.32	0.005	0.021	0.347
Pretectal area	11.49 ± 2.74	11.82 ± 4.34	15.20 ± 4.29	17.46 ± 1.53	0.001	0.334	0.471
Pedunculopontine nucleus	8.41 ± 1.96	9.84 ± 1.85	9.25 ± 2.10	11.59 ± 1.96	0.087	0.016	0.535
Deep layers of superior colliculus	12.27 ± 2.25	16.09 ± 2.86	18.13 ± 6.13	28.40 ± 11.58	0.001	0.009	0.207
Interpeduncular nucleus	11.46 ± 1.57	13.71 ± 3.51	12.77 ± 1.94	14.11 ± 3.50	0.413	0.093	0.659
Spinal tract V nucleus	9.82 ± 2.88	10.66 ± 1.96	10.73 ± 2.39	13.20 ± 1.62	0.046	0.056	0.333
Cerebellum							
Cerebellum gray matter	10.9 ± 1.95	12.28 ± 2.54	12.76 ± 3.57	14.74 ± 3.41	0.063	0.142	0.79
Molecular layer, gray matter	11.42 ± 2.34	12.42 ± 2.05	13.74 ± 15.35	15.35 ± 3.46	0.046	0.307	0.808
Granular layer, gray matter	15.30 ± 3.15	16.68 ± 1.84	18.17 ± 5.30	19.07 ± 3.88	0.077	0.43	0.866
Flocculus	12.36 ± 3.09	14.27 ± 2.47	14.50 ± 3.73	17.93 ± 2.21	0.014	0.023	0.499
Cerebellum white matter	5.89 ± 1.24	6.53 ± 1.69	5.98 ± 2.05	6.85 ± 2.46	0.778	0.302	0.873
Choroid plexus	52.32 ± 8.21	58.17 ± 18.99	62.83 ± 17.14	70.63 ± 29.33	0.132	0.363	0.896

Data in each region were subjected to a 2 × 2 ANOVA.
Table gives means × SEM for k^* (ml/s/g brain $\times 10^4$).

In addition to reflecting altered baseline 5-HT neurotransmission, the significant baseline elevations in k^* for AA following chronic fluoxetine plus washout could have been related to elevated extracellular dopamine and/or norepinephrine levels(Bymaster et al., 2002), since dopaminergic D_2 and adrenergic receptors also can be coupled to PLA_2 activation (Bhattacharjee et al., 2005; Kanterman et al., 1991; Muthalif et al., 1996; Pavoine et al., 1999; Vial and Piomelli, 1995). Additional neuroreceptor interactions may have played a role, as 5-$HT_{1A/1B}$ heteroreceptors can regulate dopamine release, whereas adrenergic receptors can modulate 5-HT release (Ng et al., 1999; Szabo and Blier, 2001).

Chronic fluoxetine has been reported to increase baseline PLA_2 activity in rat brain (Kucia et al., 2003), and such a change may have contributed to the upregulated baseline values of k^* that we found in this study. For example, in a rat model of neuroinflammation caused by 6days of intracerebroventricular infusion of lipopolysaccharide, an elevated baseline k^* for AA was accompanied by increased brain activities of AA-selective cytosolic $cPLA_2$ and of secretory $sPLA_2$ (Dennis, 1994; Lee et al., 2004; Rosenberger et al., 2004), and by increased AA turnover and incorporation in brain phospholipids.

It has been suggested that the manic symptoms of bipolar disorder are associated with elevated brain AA metabolism (Rapoport and Bosetti, 2002; Sublette et al., 2004), which is consistent with evidence that chronic administration to rats of the mood stabilizers, lithium and carbamazepine, reduced brain $cPLA_2$ activity and AA incorporation in brain phospholipids (Bazinet et al., 2005; Chang et al., 1996; Ghelardoni et al., 2004; Rintala et al., 1999). Thus, the upregulation of baseline k^* for AA by chronic fluoxetine as a marker of reduced brain AA metabolism, if confirmed, may be related to fluoxetine's efficacy against clinical depression. In this regard, fluoxetine has been noted to frequently "switch" patients with bipolar depression or obsessive—compulsive disorder to a manic state (Calabrese et al., 1999; Go et al., 1998).

In unanesthetized rats, acute or chronic fluoxetine, as well as acute DOI, are reported to reduce regional cerebral metabolic rates for glucose, $rCMR_{glc}$ (Freo et al., 2000; Freo et al., 1991; Sokoloff, 1999). These decreases contrast with the increases produced by the drugs on k^* for AA (Table 2) (Qu et al., 2003a; Qu et al., 2003b). The discrepancy may arise because AA incorporation largely represents postsynaptic (Aghajanian and Marek, 1999; Cooper et al., 2003; Staley et al., 1998) 5-$HT_{2A/2C}$ initiated PLA_2-mediated release and reincorporation of AA at the neuronal cell body or dendrites, whereas rCMRglc responses represent mainly ATP consumption by downstream pre-synaptic axon terminals, whose activity is reduced by DOI or fluoxetine (Ashby et al., 1990; Czachura and Rasmussen, 2000; Rapoport, 2001; Sokoloff, 1999).

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, and by the NIH intramural research program.

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