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Transient postnatal fluoxetine leads to decreased brain arachidonic acid metabolism and cytochrome P450 4A in adult mice

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Abstract

Fetal and perinatal exposure to selective serotonin (5-HT) reuptake inhibitors (SSRIs) has been reported to alter childhood behavior, while transient early exposure in rodents is reported to alter their behavior and decrease brain extracellular 5-HT in adulthood. Since 5-HT_{2A/2C} receptor-mediated neurotransmission can involve G-protein coupled activation of cytosolic phospholipase A₂ (cPLA₂), releasing arachidonic acid (ARA) from synaptic membrane phospholipid, we hypothesized that transient postnatal exposure to fluoxetine would decrease brain ARA metabolism in adult mice. Brain ARA incorporation coefficients k^* and rates J_{in} were quantitatively imaged following intravenous [1-¹⁴C]ARA infusion of unanesthetized adult mice that had been injected daily with fluoxetine (10 mg/kg i.p.) or saline during postnatal days P4–P21. Expression of brain ARA metabolic enzymes and other relevant markers also was measured. On neuroimaging, k^* and J_{in} was decreased widely in early fluoxetine- compared to saline-treated adult mice. Of the enzymes measured, cPLA₂ activity was unchanged, while Ca²⁺-independent iPLA₂ activity was increased. There was a significant 74% reduced protein level of cytochrome P450 (CYP) 4A, which can convert ARA to 20-HETE. Reduced brain ARA metabolism in adult mice transiently exposed to postnatal fluoxetine, and a 74% reduction in CYP4A protein, suggest long-term effects independent of drug presence in brain ARA metabolism, and in CYP4A metabolites. Comparable changes in humans might contribute to reported altered behavior following early SSRI.

Keywords

fluoxetine; arachidonic acid; mouse; neurodevelopment; postnatal; neuroimaging; serotonin; SSRI; Cytochrome P450 4A; metabolism; brain

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Introduction

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) such as fluoxetine are approved for treating depression, anxiety and personality disorders in women during pregnancy and lactation, and in children and adolescents. Their use during pregnancy has been associated with premature birth, neonatal cardiovascular abnormalities, pulmonary hypertension, and disturbed behavior, and increased risk for autism spectrum disorder in offspring [1, 2]. In addition, infants may be at risk because of exposure to SSRIs *via* breast milk [3]. SSRIs also were reported to increase suicidality in pediatric patients [4], but a recent review called this into question [5]. In contrast, several studies suggest that antidepressant use during pregnancy has no major long-term effects on neurodevelopment and behavior in the offspring [6, 7], thus the issue remains controversial.

Nevertheless, adult rodents that were exposed transiently (P1 to P21) to an SSRI show decreased brain extracellular 5-HT [8], increased density of the presynaptic 5-HT reuptake transporter (5-HTT) [9, 10], and structurally abnormal serotonergic neurons [11] and dendritic spines [12]. The early-exposed adult rodents also demonstrate depressive-like behaviors [13–16] and, less consistently, anxiety-like behaviors [13, 17]. The P1 to P21 postnatal period in rodents coincides with a brain growth spurt, rapid dendritic and axonal outgrowth, synaptogenesis and myelination, and peak establishment of neural connections and susceptibility to xenobiotics [18–20]. It corresponds to the period of maturation of the human brain monoaminergic system, which begins during the third trimester of pregnancy and continues through the first 2–3 years of life [13, 21].

Changes in behavior and brain integrity in adult rodents following transient SSRI exposure may be related to disturbed arachidonic acid (ARA, 20:4 n-6) neurotransmission and metabolism, since ARA is released from synaptic membrane phospholipid during neurotransmission involving 5-HT_{2A/2C} receptors [22–25]. As a second messenger, ARA can modify multiple aspects of brain function and structure, and it is a precursor of a large number of bioactive eicosanoid products within the brain ARA metabolic cascade [26, 27].

We have developed a method to measure brain ARA signaling in unanesthetized rodents, which involves infusing radiolabeled ARA intravenously and using quantitative autoradiography to quantify regional brain radioactivity, representing tracer ARA incorporation into synaptic membrane phospholipid [25, 28]. A mathematical model is used to calculate ARA incorporation coefficients and rates, k^* and J_{in} , respectively [26, 29]. Since the brain ARA lost by metabolism cannot be synthesized *de novo* from 2-carbon fragments, or elongated significantly (< 1%) from its shorter chain polyunsaturated precursor within brain, linoleic acid (18:2n-6) [30], J_{in} stoichiometrically equals ARA metabolic loss [31].

In the present study, we used our *in vivo* infusion method to determine whether transient postnatal exposure of rats to fluoxetine would alter brain k^* and J_{in} for ARA during adulthood. We thought changes in these parameters might occur because of the changes noted above in serotonin synaptic markers in the adult brain of perinatally exposed rodents. We also measured expression of other ARA metabolic markers, including phospholipase A₂ (PLA₂) enzymes involved in neurotransmission, downstream ARA oxidizing enzymes, and 5-HTT. Part of this work has been published in abstract form [32].

Methods

Animals

Experiments were conducted following the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication 86–23), under a protocol approved by the Animal Care and Use Committee of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Untimed pregnant C57BL/6 mice (Charles River Laboratories International, Wilmington, MA, USA) were housed until they gave birth. Postnatal drug or saline was administered as reported [13]. Briefly, on postnatal day 4 (P4), male pups were assigned randomly to a saline (bacteriostatic 0.9% NaCl injection, USP, Hospira, Lake Forest, IL, USA; i.p.) group, to a fluoxetine hydrochloride (10.0 mg/kg, i.p., dissolved in saline; Sigma-Aldrich, St. Louis, MO, USA) treatment group, given at a therapeutically equivalent human dose [13]. Mice were injected once per day, using a sterile insulin syringe ($29\text{G} \times \frac{1}{2}$, 0.3 ml), from P4 to P21. This regimen produces therapeutically relevant plasma concentrations of fluoxetine (360 ± 123 ng/ml) and its metabolite norfluoxetine (708 ± 168 ng/ml) at P22 [13].

Lactating dams and pups had free access to water and food (Rodent NIH-07, Ziegler Bros, Gardens, PA, USA). This diet contains soybean, alfalfa, and fishmeal, and has 5% crude fat by weight. By gas-liquid chromatography, the chow contains (as % of total fatty acids) 30.6% saturated, 33.5% monounsaturated, 47.1% linoleic, 4.9% α -linolenic, 0.27% ARA, 1.68% eicosapentaenoic, and 2.2% docosahexaenoic acid (DHA, 22:6n-3) [33]. At weaning (P28), dams were removed, and the pups were sexed and housed in separate cages (3 to 5 mice per cage) until adulthood (P90–P120).

Surgical Procedures and Tracer Infusion

Between P90 and P120, male mice that had been treated postnatally with saline or fluoxetine were anesthetized with isoflurane (2–3% v/v in O_2), and polyethylene PE 10 catheters were implanted into the right femoral artery and vein [33]. A mouse was allowed to recover from anesthesia for 3 h with its hindquarters loosely wrapped and taped to a wooden block in a sound-dampened, temperature-controlled chamber. During recovery, body temperature was maintained at 37°C with a rectal probe and a heating element (TACT-2DF Temperature controller, Physitemp Instruments, Clifton, NJ, USA). Then [$1\text{-}^{14}\text{C}$]ARA (45 μl , 300 $\mu\text{Ci/ml}$ 53 mCi/mmol; 99.4% pure, Moravsek Biochemicals, Brea, CA) in 5 mM HEPES buffer (pH 7.4), containing 50 mg/ml fatty acid-free bovine serum albumin, was infused through the femoral vein (3 min, 15 $\mu\text{l/min}$) using a Hamilton syringe and an infusion pump (Harvard Apparatus Model 22, Holliston, MA, USA). Ten arterial blood samples (15–20 μl) were collected at fixed times during infusion (0, 0.25, 1.0, 1.5, 2.0, 2.8, 3.2, 5.0, 10, and 19 min). At 20 min, the mouse was euthanized by an overdose of Nembutal (50 mg/kg, i.v.). Cardiac puncture was performed to rapidly collect 0.5 ml blood, and the mouse was decapitated. The brain was removed, quickly frozen in 2-methylbutane at -40°C , then stored at -80°C for later sectioning. Radiotracer purity exceeded 99% by thin layer chromatography and gas-liquid chromatography, and was measured after converting labeled ARA to its methyl ester with 1% sulfuric acid in anhydrous methanol.

Chemical Analysis

Arterial blood samples (20 μl) were collected before, during and after [$1\text{-}^{14}\text{C}$]ARA infusion and were centrifuged (18,000 g for 30 s). Total lipids were extracted from 5 μl plasma with chloroform:methanol (1 ml, 2:1, v/v) and KCl (0.5 ml, 0.1M), using a modified Folch procedure [34]. The lower organic phase (100 μl) of the extract was used to measure the labeled unesterified ARA concentration by scintillation spectrometry (Model 2200CA,

Packard Instruments, Downers Grove, IL, USA). As reported, 95–98% of total plasma and brain radioactivity at 5 min was radiolabeled ARA [35].

Concentrations of unesterified unlabeled fatty acids were determined in the arterial plasma (100–150 μ l) collected by cardiac puncture. Total lipids were extracted [34] and separated by thin layer chromatography on 60 silica gel plates (Whatman, Clifton, NJ, USA), using the solvent system heptane: diethylether: glacial acetic acid (60:40:3, v/v/v). Unesterified fatty acids were scraped from the plate and methylated with 1% H_2SO_4 in anhydrous methanol (3 h at 70°C), then separated by gas-liquid chromatography and quantified using an internal standard, heptadecanoic acid (17:0).

Quantitative Autoradiography and Calculations

Twenty-mm thick sections were cut from the left hemisphere and then placed with [^{14}C]methylmethacrylate standards (Amersham, Arlington Heights, IL, USA) on Ektascan C/RA film (Eastman Kodak, Rochester, NY, USA) for 4 weeks. Radioactivity (nCi/g brain wet weight) in 92 identified regions was measured bilaterally six times by quantitative densitometry (NIH Image program 1.62). Regional incorporation coefficients of unesterified plasma ARA into brain phospholipids, k^* (ml/s/g brain), were calculated as [29]:

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

$c_{brain}^*(20)$ (nCi/g brain wet wt.) is brain radioactivity 20 min after beginning infusion, c_{plasma}^* (nCi/ml plasma) is labeled plasma unesterified ARA, and t (min) is time after beginning infusion. Integrated plasma radioactivity (input function) was determined by trapezoidal integration and used to calculate k^* for each experiment. Regional rates of incorporation of unesterified ARA from plasma into brain phospholipid, J_{in} (nmol/s/g), were calculated as:

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

where c_{plasma} is the plasma concentration (nmol/ml) of unlabeled unesterified ARA. These rates stoichiometrically equal regions rates of ARA metabolic loss [26, 36].

Preparation of cytosolic and membrane fractions from whole brains

In separate experiments, non-infused mice were anesthetized with Nembutal (50 mg/kg, i.p.) and decapitated. Brains ($n = 10$ saline, $n = 12$ fluoxetine) were removed quickly and frozen in 2-methylbutane at -40°C on dry ice, then stored at -80°C . Half-brains were homogenized using a Tenbroeck tissue grinder on ice (Kontes Glass, Vineland, NJ, USA) in 2 vol of 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 (Beckman L8-M Ultracentrifuge, Fullerton, CA USA). The supernatant corresponded to the cytosolic fraction. The pellet then was resuspended in 0.2 M Tris, pH 7.4 containing 0.2% Triton X-100 and protease inhibitors (Sigma-Aldrich), incubated on ice for 1 h with occasional stirring, and finally centrifuged at 100,000 g for 1 h at 4°C . The soluble supernatant was used as the membrane fraction. Protein concentrations of both fractions were determined by the Bradford method [37].

Whole brain PLA₂ activities

Cytosolic fractions were assayed for Ca^{2+} -dependent ARA selective cytosolic cPLA₂ and Ca^{2+} -independent docosahexaenoic acid (DHA) selective iPLA₂ activities, as described

[38]. Activity of secretory sPLA₂ was measured using a sPLA₂ assay kit (Cayman, Ann Arbor, MI, USA).

Western blotting

Proteins (25 µg) from membrane and cytosolic fractions were separated using 4–20% sodium dodecyl sulfate polyacrylamide gel (Bio-Rad, Hercules, CA, USA) and transferred to a nitrocellulose membrane. After blocking (Blocking buffer, Sigma-Aldrich), blots were incubated overnight at 4°C with primary antibody diluted in blocking buffer [anti-iPLA₂-VIA (1:500), anti-cyclooxygenase (COX)-2 (1:200), anti-5-lipoxygenase (LOX) (1:500), anti-12-LOX (1:500), anti-cytochrome P 450 epoxygenase (CYP)2C9 (1:1000) and anti-5-HTT antibodies (1:500) (Abcam, Cambridge, MA, USA), anti-COX-1 (1:200), anti-15-LOX (1:200), anti-CYP4A (1:200) (Santa-Cruz, CA, USA) and anti-membrane prostaglandin E₂ synthase (mPGES) (1:500) (Cayman, Ann Arbor, MI, USA)]. After washing, blots were incubated (90 min, room temperature) with the appropriate horseradish peroxidase-conjugated secondary antibodies (Bio-Rad) and peroxidase activity was determined by chemiluminescence using the SuperSignal West Pico reagents (Thermo Fisher Scientific, Rockford, IL, USA). The relative protein expression was determined after normalization with the β-actin protein (Sigma-Aldrich) using the ImageJ software, and expressed as per cent of the saline-treated group (control). Due to limited success with survival, n = 9 for the membrane fraction from saline mice, and n = 8 for the saline group in iPLA₂, COX-2 and cytosolic 5-HTT expression assays.

Statistical analysis

Data are reported as mean ± SD or SEM. An unpaired two-tailed *t*-test was used to compare means between fluoxetine- and saline-treated mice, with statistical significance tested at *p* *0.05, **0.02 or ***0.01. For *k** and *J_{in}*, corrections for multiple comparisons across regions were not made because this study is exploratory and its goal was to screen for patterns of regional changes in brain ARA incorporation [39, 40]. Adjustments for multiple comparisons with large bodies of observation, as found in neuroimaging of multiple regions, leads to a too ready rejection of the null hypothesis [41].

Results

Body weight and arterial plasma input function

Fluoxetine injection from P4 to P21 significantly reduced body weight. At P12, fluoxetine-treated mice weighed 11% less than saline-treated mice (5.90 ± 0.50 g, *n* = 11 vs. 6.60 ± 0.50 g, *n* = 7; *p* = 0.012), and the decrease was maintained until P21 (*p* = 0.04). Body weight also was reduced at P90–P120 (−13%; *p* = 0.003).

Fluoxetine treatment did not affect steady-state plasma radioactivity during the 3-min [$1\text{-}^{14}\text{C}$]ARA infusion in the adult mice. Integrated plasma radioactivity did not differ between fluoxetine- and saline-treated mice ($153,587 \pm 20,044$ (*n* = 11) vs. $144,630 \pm 15,953$ (*n* = 7) nCi × s/ml ± SD).

Plasma concentrations of unlabeled unesterified fatty acids

There was no statistically significant difference in the plasma concentration of any of eight measured unlabeled unesterified fatty acids between fluoxetine- and saline-treated mice (data not shown), including ARA (4.82 ± 1.67 (*n* = 11 fluoxetine) vs. 5.77 ± 2.07 (*n* = 7 saline) nmol/ml plasma).

Regional brain ARA incorporation coefficients, k^*

Figure 1 presents color-coded autoradiographs of coronal brain slices from saline- and fluoxetine-treated mice, illustrating regional incorporation coefficients k^* for ARA, obtained using Eq. 1. Compared to saline, fluoxetine-treated mice showed widespread decreases in k^* . Statistically significant 17% to 29% (average $21 \pm 3\%$) reductions were found in 24 of 92 regions (33%), 7 regions at $p < 0.01$, 8 regions at $p < 0.02$, and 9 regions at $p < 0.05$ (Table 1). Affected were gray matter regions of the neocortex, diencephalon, mesencephalon and rhombencephalon (Table 1). No significant change was noted in white matter, while k^* in the choroid plexus was significantly reduced by 23%. Chronic fluoxetine did not elevate k^* significantly in any brain region.

Regional ARA incorporation rates J_{in}

Baseline incorporation rates J_{in} of unlabeled unesterified ARA from plasma into brain were calculated by multiplying individual regional values of k^* by the unlabeled unesterified plasma ARA concentration in each experiment (Eq. 2) (Supplementary Table 1). Seventy-eight of the 92 brain regions (85%) showed a statistically significant decreased J_{in} following fluoxetine compared with saline treatment ($p < 0.05$), thus much of the brain (Supplementary Figure 1). In saline-treated mice, J_{in} ranged from 37.7×10^{-4} nmol/s/g in the internal capsule to 161.7×10^{-4} nmol/s/g in the choroid plexus. In fluoxetine-treated mice, J_{in} ranged from 29.6×10^{-4} nmol/s/g in the internal capsule to 94.5×10^{-4} nmol/s/g in the choroid plexus.

Whole brain enzyme activities and protein levels

Whole brain cytosolic activities of cPLA₂ and sPLA₂, each of which can release ARA from phospholipid [42], did not differ significantly between fluoxetine- and saline-treated mice (Table 2). DHA-preferring Ca²⁺-independent iPLA₂ [43, 44] activity was significantly increased by 32% ($p = 0.004$) in fluoxetine-treated mice, but mean iPLA₂-VIA protein was unaltered (Table 2). There was no significant change in cytosolic protein levels of COX-1, COX-2, 5-LOX, 12-LOX, 15-LOX, and CYP2C9 or mPGES between treatment groups (Table 3). However, CYP4 A1, A2 and A3 protein levels were reduced by 74% after early fluoxetine treatment ($p = 0.004$) (Table 2). We quantified these three isoforms together as their molecular weights were too close to be distinguished.

Whole brain 5-HTT protein level

By varying exposure time in Western blotting, three isoforms of 5-HTT were detected in the brain membrane fraction, with molecular weights of 37 kDa, 56 kDa and 68 kDa (Figure 2A), but only the 37 kDa and 56 kDa isoforms were observed in the cytosol (Figure 2B). This expression profile is consistent with a study reporting cleavages of the native 56 kDa 5-HTT protein in rat brain [45]. Postnatal fluoxetine had no significant effect on 5-HTT isoform protein levels in membrane or cytosolic fractions of whole brain.

Discussion

Transient postnatal fluoxetine, administered daily from P4 to P21, significantly decreased baseline brain ARA incorporation coefficients k^* in 24 of 92 measured regions in adult male mice (P90 to P120), as well as ARA incorporation rates, J_{in} , in 78 regions. Postnatal fluoxetine also significantly increased brain iPLA₂ activity and decreased CYP4A protein, but did not change protein levels of other measured markers (iPLA₂-VIA, COX-2, 5-LOX, 12-LOX, CYP2C9, COX-1, 15-LOX, or mPGES) or of 5-HTT. Body weight was reduced 13% by postnatal fluoxetine, similar to prior reports [11, 17, 46].

Significantly reduced ARA incorporation coefficients and rates following transient postnatal fluoxetine contrast with increased ARA incorporation in adult 5-HTT knockout mice, and in adult rats treated chronically with fluoxetine, in each case of which brain cPLA₂ activity and extracellular 5-HT concentration are increased [33, 35, 47–50]. However, our finding reduced ARA incorporation coefficients and rates in postnatally fluoxetine exposed adult rats corresponds to evidence that extracellular 5-HT is reduced and that cortical 5-HTT is increased in adult mice exposed postnatally to an SSRI [8–10], although we did not find a change in whole brain 5-HTT in the present study. Thus, in each of the three conditions, fluoxetine treatment of adult rats, 5-HTT knockout adult mice, and transient postnatal fluoxetine exposure of rats measured during adulthood (present study), the direction of changes in baseline ARA incorporation corresponds to the direction of change of extracellular 5-HT, suggesting that the baseline ARA signal in each of these three conditions reflects extracellular 5-HT.

Depressed serotonergic transmission appears to be a risk factor for clinical depression. Thus, lowering brain 5-HT level by tryptophan depletion can induce depressive symptoms, and major depression can be ameliorated with SSRIs [51]. In agreement, transient postnatal fluoxetine in rats, which reduces ARA incorporation (this study) and extracellular 5-HT in adulthood, also increases depressive-like behaviors in adulthood [52–54]. The 5-HTT knockout mouse, having elevated extracellular 5-HT [50] and increased ARA metabolism [33] shows mainly anxiety-like behaviors [55], while tests of depression have yielded less consistent results [56, 57].

Decreased baseline brain ARA incorporation coefficients and rates following postnatal fluoxetine in the present study were not associated with reduced activity cPLA₂ or of sPLA₂, both of which hydrolyze ARA from membrane phospholipid [42]. Activity of iPLA₂, which preferentially hydrolyzes DHA from membrane phospholipid [43, 44, 58], was elevated, but iPLA₂ VIA protein was not significantly different from control, suggesting post-translation upregulation. Following dietary PUFA manipulation, the change of iPLA₂ expression is opposite in direction to changes of cPLA₂ and sPLA₂ expression [59, 60].

As noted in the Introduction, J_{in} for ARA stoichiometrically equals the rate of ARA metabolic loss from brain, since ARA cannot be synthesized *de novo* in vertebrates nor elongated significantly in brain from circulating precursors derived from plasma [31, 36]. On the other hand, k^* represents the “avidity” of brain for unesterified plasma ARA, and depends on diffusional and enzymatic processes within the brain deacylation-reacylation cycle [29, 61–63]. Therefore, the significantly decreased values of k^* and J_{in} for ARA of 33% and 85% of the 92 regions studied, respectively, suggest downregulation of brain affinity for ARA and of ARA metabolic loss [64, 65]. Our data also suggest that one pathway that is downregulated involves ARA metabolism by CYP4A, whose protein level was reduced by 74%. CYP4A oxidizes ARA to 20-hydroxyeicosatrienoic acid (20-HETE) [66], which influences cerebrovascular function and can be produced by stimulation of 5-HT_{1B} receptors [67–70]. Future studies might use lipidomic methods [71] to test whether 20-HETE or other ARA metabolites are reduced following postnatal fluoxetine. Additionally, regional expression of enzymes might show differences not evidenced by global analysis.

Long-term effects of transient exposure to fluoxetine during rodent neurodevelopment suggest epigenetic or other neuroplastic mechanisms, and epigenetic changes associated with SSRI administration to both neonatal and adult rats have been reported [72, 73][74]. These potential mechanisms remain to be studied in more detail. Effects are not due to remaining fluoxetine or its metabolites, which have elimination half-lives of less than 15 h [75].

In summary, transient postnatal fluoxetine caused a downregulation of global baseline brain ARA metabolism in adult mice, suggesting long-term quasi-permanent consequences of early SSRI exposure. The reduced ARA metabolism may be related to the reported reduction in extracellular 5-HT, since ARA hydrolysis is coupled to 5-HT_{2A/2C} receptors, and to reduced conversion of unesterified ARA to 20-HETE by CYP4A, whose expression was 74% reduced.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Autoradiographs showing ARA incorporation coefficients k^* in coronal brain sections of saline- and fluoxetine-treated mice. Values of k^* are color-coded. Abbreviations: ACg, anterior cingulate cortex; Aud, auditory cortex; CPu, caudate putamen; Hipp, hippocampus; IPC, interpeduncular nucleus; Mot, motor cortex; SN, substantia nigra; Thal, thalamus; Vis, visual cortex.

Fig. 2.

5-HTT protein isoforms in brain membranes and cytosol of fluoxetine and saline treated rats. Proteins were measured in brain membrane (A) or cytosolic (B) fractions by Western blotting (representative immunoblots are displayed, with adjusted exposure time for each isoform). 37 kDa, 56 kDa and 68 kDa isoforms were detected in the membrane fraction and 37 kDa and 56 kDa isoforms were detected in the cytosol. After normalization to β -actin protein, results are expressed as % of the control values (means $\pm$ SEM, n = 8–9 for saline and n = 12 for fluoxetine).

Table 1

Postnatal fluoxetine exposure decreases baseline arachidonic acid incorporation coefficients k^* in adult mice.

Brain Region	Saline (n = 7)	Fluoxetine (n = 12)
	k*, (ml/s/g) × 10 ^{−4}	
<i>Telencephalon</i>		
Prefrontal cortex layer I	7.85 ± 1.13	7.44 ± 2.05
Prefrontal cortex layer IV	8.72 ± 1.12	7.84 ± 2.17
Primary olfactory cortex	8.18 ± 0.99	7.21 ± 2.21
Frontal cortex (10)		
Layer I	8.40 ± 1.37	7.30 ± 2.13
Layer IV	9.27 ± 1.50	8.08 ± 2.51
Frontal cortex (8)		
Layer I	9.03 ± 1.23	7.50 ± 2.22
Layer IV	9.52 ± 1.37	8.12 ± 2.32
Pyriform cortex	7.93 ± 1.05	6.41 ± 1.67*
Anterior cingulate cortex	11.48 ± 1.33	9.30 ± 1.87**
Motor cortex		
Layer I	8.22 ± 1.36	7.17 ± 1.56
Layer II – III	9.38 ± 1.10	7.84 ± 1.97
Layer IV	11.07 ± 0.70	9.10 ± 2.45
Layer V	10.93 ± 1.04	8.80 ± 2.19
Layer VI	10.22 ± 1.14	8.11 ± 1.79**
Somatosensory cortex		
Layer I	8.80 ± 1.49	7.30 ± 1.85
Layer II–III	9.96 ± 1.32	8.13 ± 1.97
Layer IV	11.55 ± 0.95	9.72 ± 2.35
Layer V	11.15 ± 1.06	8.88 ± 1.91**
Layer VI	10.29 ± 1.07	8.30 ± 1.70**
Auditory cortex		
Layer I	8.14 ± 1.34	7.02 ± 2.13
Layer IV	9.37 ± 1.67	8.01 ± 2.41
Layer VI	8.88 ± 1.64	7.03 ± 1.90
Visual cortex		
Layer I	8.29 ± 1.60	6.87 ± 1.94
Layer IV	9.84 ± 1.28	7.75 ± 2.19
Layer VI	8.93 ± 1.19	7.08 ± 2.13
Preoptic area (LPO/MPO)	8.52 ± 1.04	6.74 ± 1.49**
Suprachiasmatic nu	9.37 ± 2.08	6.65 ± 1.69***
Globus pallidus	8.89 ± 0.84	7.96 ± 2.09
Bed nu stria terminalis	8.28 ± 1.04	6.65 ± 1.56***
Olfactory tubercle	7.79 ± 1.45	6.99 ± 1.90
Diagonal band Dorsal	9.70 ± 1.32	7.37 ± 1.85***

Brain Region	Saline (n = 7)	Fluoxetine (n = 12)
Ventral	9.24 ± 0.85	7.01 ± 1.88***
Amygdala basolat/med	6.78 ± 1.69	6.16 ± 1.71
Hippocampus CA1	6.70 ± 1.73	6.19 ± 2.09
CA2	7.30 ± 1.75	6.59 ± 2.57
CA3	7.67 ± 1.63	7.01 ± 2.35
Dentate gyrus	8.55 ± 1.81	7.56 ± 2.54
SLM	11.17 ± 2.63	9.61 ± 3.21
Accumbens nucleus	8.35 ± 1.08	7.26 ± 2.08
Caudate putamen		
Dorsal	9.23 ± 0.77	7.27 ± 1.86**
Ventral	9.21 ± 0.99	7.46 ± 1.96*
Lateral	9.36 ± 0.80	7.66 ± 1.99*
Medial	9.21 ± 0.80	7.18 ± 1.76**
Septal nucleus lateral	7.96 ± 0.65	6.40 ± 1.57*
medial	9.26 ± 0.95	7.54 ± 1.90*
Diencephalon		
Habenular nu lateral	14.35 ± 2.94	12.66 ± 4.18
Habenular nu medial	13.54 ± 2.97	12.19 ± 4.45
Lateral geniculate nu dorsal	12.08 ± 1.78	9.38 ± 3.31
Medial geniculate nu	13.21 ± 2.05	9.97 ± 2.66
Thalamus		
Ventroposterior lateral nu	12.21 ± 2.16	9.40 ± 3.44
Ventroposterior medial nu	11.84 ± 2.13	9.08 ± 2.96*
Paratenial nu	11.94 ± 1.05	9.92 ± 2.65
Anteroventral nu	15.26 ± 1.19	14.06 ± 3.96
Anteromedial nu	13.13 ± 1.12	10.57 ± 3.02
Reticular nu	12.91 ± 1.00	10.65 ± 2.89
Paraventricular nu	11.37 ± 1.59	9.39 ± 2.52
Parafascicular nu	11.58 ± 2.15	8.82 ± 3.39
Subthalamic nucleus	11.19 ± 2.00	9.42 ± 2.96
Hypothalamus		
Supraoptic nu	8.54 ± 0.99	7.51 ± 1.35
Lateral	7.99 ± 1.38	7.10 ± 1.53
Anterior	7.85 ± 1.25	6.99 ± 1.54
Periventricular	7.85 ± 1.29	7.15 ± 1.40
Arcuate	8.23 ± 1.75	7.22 ± 1.39
Ventromedial	8.26 ± 1.86	7.36 ± 2.08
Posterior	8.38 ± 2.02	6.79 ± 1.64
Mammillary nucleus	7.54 ± 1.85	6.79 ± 1.64
Mesencephalon		
Interpeduncular nucleus	16.45 ± 2.64	13.44 ± 3.59
Substantia nigra	9.45 ± 0.97	7.63 ± 2.24

Brain Region	Saline (n = 7)	Fluoxetine (n = 12)
Pretectal area	11.51 ± 1.79	8.97 ± 2.20*
Superior colliculus	11.23 ± 2.57	9.07 ± 1.86
Deep layers	10.47 ± 1.37	8.81 ± 1.79
Inferior colliculus	19.52 ± 3.54	14.52 ± 2.80***
Median Raphe nu	10.94 ± 3.91	9.31 ± 1.44
Dorsal Raphe nu	10.06 ± 2.47	9.24 ± 1.67
Pedunculopontine tegmental nu	10.64 ± 2.07	8.04 ± 1.45***
<i>Rhombencephalon</i>		
Flocculus	12.89 ± 1.19	12.48 ± 2.70
Cerebellar gray matter	11.73 ± 2.13	10.76 ± 2.24
Molecular layer cerebellar gray matter	12.49 ± 1.99	12.17 ± 1.90
Raphe magnus nu	11.26 ± 3.15	8.77 ± 1.37*
Raphe pallidus nu	11.26 ± 2.52	8.74 ± 1.55**
Locus coeruleus	12.93 ± 2.84	10.96 ± 2.41
Cochlear nu	13.20 ± 3.97	12.05 ± 2.15
Spinal trigeminal nu	10.31 ± 2.34	10.67 ± 1.63
Superior olive	11.27 ± 2.71	9.41 ± 1.57
Medial vestibular nu	19.29 ± 2.14	15.98 ± 2.98*
<i>White matter</i>		
Corpus callosum	6.89 ± 1.13	5.48 ± 1.59
Internal Capsule	5.88 ± 1.44	6.15 ± 1.48
Cerebellar white matter	7.35 ± 0.85	6.92 ± 1.34
<i>Non-blood-brain barrier regions</i>		
Subfornical organ	8.58 ± 0.86	7.53 ± 1.53
Median eminence	7.34 ± 1.84	7.28 ± 2.07
Choroid plexus	25.48 ± 3.08	19.62 ± 4.34***

Values are mean ± S.D.

Abbreviations: nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-molecule of hippocampus.

Unpaired t-test with P < 0.05*, 0.02**, 0.01***

Table 2

Effects of postnatal fluoxetine exposure on whole-brain enzyme activities and protein levels during adulthood

	Saline (n = 10)	Fluoxetine (n = 12)
<i>Activity</i>		
cPLA ₂ (pmol/min/mg protein)	5.42 ± 0.41	5.70 ± 0.28
sPLA ₂ (nmol/min/mg protein)	104.4 ± 7.9 (n = 9)	123.8 ± 11.6
iPLA ₂ (pmol/min/mg protein)	47.1 ± 4.5 (n = 9)	62.0 ± 2.1 ^{**} (+31%)
<i>Protein (% fluoxetine/saline)</i>		
iPLA ₂ -VIA	100.0 ± 15.6 (n = 8)	108.3 ± 14.4
COX-1 (69 kDa)	100.0 ± 12.7	105.6 ± 17.6
COX-2 (69 kDa)	100.0 ± 15.9 (n = 8)	102.5 ± 12.3
5-LOX (78 kDa)	100.0 ± 13.5	104.5 ± 9.2
12-LOX (75 kDa)	100.0 ± 12.3	101.9 ± 6.9
15-LOX (79 kDa)	100.0 ± 8.6	93.7 ± 6.9
mPGES (33 kDa)	100.0 ± 10.7 (n = 9)	105.45 ± 6.9
CYP2C9 (59 kDa)	100.0 ± 8.1	79.9 ± 7.1
CYP4A (50-52-54 kDa)	100.0 ± 20.0	25.8 ± 13.3 ^{**} (-74%)

Values are mean ± SEM.

^{**}
P < 0.01, unpaired t-test