Published in final edited form as:

Pharmacol Biochem Behav. 2013 December ; 0: . doi:10.1016/j.pbb.2013.09.010.

Omega-3 Fatty Acid Deficiency Does Not Alter the Effects of Chronic Fluoxetine Treatment on Central Serotonin Turnover or Behavior in the Forced Swim Test in Female Rats

Robert K. McNamara^{1,*}, Jessica A. Able¹, Yanhong Liu¹, Ronald Jandacek², Therese Rider², Patrick Tso², and Jack W. Lipton^{1,#}

¹Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 45267

²Department of Pathology, University of Cincinnati, Cincinnati OH 45237

Abstract

While translational evidence suggests that long-chain omega-3 fatty acid status is positively associated with the efficacy of selective serotonin reuptake inhibitor drugs, the neurochemical mechanisms mediating this interaction are not known. Here we investigated the effects of dietary omega-3 (*n*-3) fatty acid insufficiency on the neurochemical and behavioral effects of chronic fluoxetine (FLX) treatment. Female rats were fed diets with (CON, *n*=56) or without (DEF, *n*=40) the *n*-3 fatty acids during peri-adolescent development (P21-P90), and one half of each group were administered FLX (10 mg/kg/d) for 30 d (P60-P90) prior to testing. In adulthood (P90), regional brain serotonin (5-HT) and 5-hydroxyindoleacetic (5-HIAA) concentrations, presynaptic markers of 5-HT neurotransmission, behavioral responses in the forced swim test (FST), and plasma FLX and norfluoxetine (NFLX) concentrations were investigated. Peri-adolescent *n*-3 insufficiency led to significant reductions in cortical docosahexaenoic acid (DHA, 22:6*n*-3) composition in DEF (−25%, *p* 0.0001) and DEF+FLX (−28%, *p* 0.0001) rats. Untreated DEF rats exhibited significantly lower regional 5-HIAA/5-HT ratios compared with untreated CON rats, but exhibited similar behavioral responses in the FST. In both CON and DEF rats, chronic FLX treatment similarly and significantly decreased 5-HIAA concentrations and the 5-HIAA/5-HT ratio in the hypothalamus, hippocampus, and nucleus accumbens, brainstem tryptophan hydroxylase-2 mRNA expression, and immobility in the FST. While the FLX-induced reduction in 5-HIAA concentrations in the prefrontal cortex was significantly blunted in DEF rats, the reduction in the 5-HIAA/5-HT ratio was similar to CON rats. Although plasma FLX and NFLX levels were not significantly different in DEF and CON rats, the NFLX/FLX ratio was significantly lower in DEF +FLX rats. These preclinical data demonstrate that *n*-3 fatty acid deficiency does not significantly reduce the effects of chronic FLX treatment on central 5-HT turnover or behavior in the FST in female rats.

© 2013 Elsevier Inc. All rights reserved.

*Corresponding author: Robert K. McNamara, Ph.D. Department of Psychiatry and Behavioral Neuroscience University of Cincinnati College of Medicine 260 Stetson Street Cincinnati, OH 45219-0516 PH: 513-558-5601 FAX: 513-558-4805 robert.mcnamara@uc.edu.

#Current address: Department of Translational Science & Molecular Medicine, Michigan State University, College of Human Medicine, 333 Bostwick Ave NE, Grand Rapids, MI 49503.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Omega-3 fatty acid; Docosahexaenoic acid; Fluoxetine; Serotonin; Female; Forced swim test

1. Introduction

Patients with major depressive disorder (MDD) exhibit significant deficits in long-chain omega-3 (LCn-3) fatty acids, including eicosapentaenoic acid and docosahexaenoic acid (DHA), compared with healthy subjects [1-5]. The prevalence rate of MDD is approximately 2-fold greater in females following puberty [6], and low estrogen levels are associated with lower erythrocyte or plasma phospholipid DHA levels [7-10]. Moreover, cross-sectional epidemiological surveys suggest that low dietary LCn-3 fatty acid intake is associated with increased risk of depression in females but not in males [11,12]. We previously reported that DHA, the principal LCn-3 fatty acid in brain, is significantly reduced in the postmortem prefrontal cortex (PFC) of female, but not male, patients with MDD [13]. While these findings suggest that the link between LCn-3 fatty acid deficiency and vulnerability to depressive symptoms is more robust in females, there is currently a dearth of evidence regarding the neurobiological basis of this association and whether LCn-3 fatty acid status influences antidepressant treatment response.

Multiple lines of clinical evidence suggest that a dysregulation in serotonin (5-hydroxytryptamine, 5-HT) neurotransmission is central to the pathophysiology and treatment of MDD [14,15]. Emerging evidence from preclinical studies suggest that dietary-induced elevations in LCn-3 fatty acid status increase 5-HT concentrations in rat frontal cortex [16], attenuate reductions in frontal cortex 5-HT content in response to chronic stress [17], and decrease depression-like behavior in the forced swimming test (FST)[18]. Furthermore, the combination of LCn-3 fatty acids and the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX) is significantly more effective than FLX alone for reducing depression-like behavior in the FST [19,20]. Importantly, adjunctive LCn-3 fatty acid supplementation has also been found to significantly augment the antidepressant effects of SSRIs in patients with MDD [21-23]. In contrast, dietary *n*-3 fatty acid insufficiency during perinatal development is associated with elevated 5-HT₂ receptor density in the frontal cortex [24] and impaired fenfluramine-induced elevations in extracellular 5-HT concentrations in the hippocampus [25]. While these translational findings suggest that LCn-3 fatty acid status augments SSRI efficacy through common effects on 5-HT neurotransmission, this mechanism has not been systematically investigated.

The primary objective of the present study was to use a rat model to investigate the impact of LCn-3 fatty acid deficiency on the effects of chronic FLX treatment on central 5-HT neurotransmission and behavioral responses in the FST. Similar to other rodent models of depression including the olfactory bulbectomized rats [26] and Flinders sensitive line rats [27], we previously reported that peri-adolescent (P21-P90), but not perinatal or post-pubescent, *n*-3 fatty acid insufficiency is associated with decreased 5-HT turnover in the rat PFC [28]. The peri-adolescent model, unlike the perinatal and post-pubescent models, also leads to reductions in adult PFC DHA levels that are similar in magnitude to those observed in the postmortem PFC of adult female MDD patients [13]. Based on the evidence reviewed above, our specific hypothesis was that LCn-3 fatty acid deficiency would significantly blunt the effects of chronic FLX treatment on limbic forebrain 5-HT turnover and behavior (i.e., reduced immobility) in the FST.

2. Materials and Methods

2.1. Animals and diets

Adult (P60) male and nulliparous female Long-Evans hooded rats (Harlan Farms, Indianapolis, IN) were paired, and males removed 2 weeks later. Dams and litters were maintained on standard Teklad rodent chow throughout gestation and nursing. Immediately following weaning (P21), female rats from different litters were randomly assigned to either -linolenic acid (ALA)-fortified diet (control, CON, TD.04285, Harlan-TEKLAD, Madison, WI)(n=56) or ALA-free diet (deficient, DEF, TD.04286)(n=40). Both ALA-fortified and ALA-free diets were matched for all non-fat nutrients (**Table 1**). Independent fatty acid composition analyses found that the both diets were closely matched with the exception of ALA (18:3n-3), which was absent from the deficient diet and represented 4.6% of total fatty acid composition in the control diet (**Table 1**). Rats were housed 2 per cage with food and water available *ad libitum*, and were maintained under standard vivarium conditions on a 12:12 h light:dark cycle. All experimental procedures were approved by the University of Institutional Animal Care and Use Committee (Protocol #06-03-01-01), and adhere to the guidelines set by the National Institutes of Health.

2.2. Tissue collection

Adult (P90) rats were sacrificed by decapitation during the light portion of the cycle, brains extracted and immediately immersed in ice-cold 0.9% NaCl for 2 min. The brain was then dissected to isolate the PFC (the olfactory tubercle and residual striatal tissue were removed from the PFC), nucleus accumbens, hippocampus, hypothalamus, and brainstem. Brain tissues were immediately placed in a cryotubes, flash frozen in liquid nitrogen, and stored at -80°C. Trunk blood was collected into EDTA-coated tubes, and plasma isolated by centrifugation and stored at -80°C. At the time of sacrifice, vaginal smears were collected onto glass slides, dried, and stained (Dipp-Kwik Stain kit, American Master/Tech Scientific Inc., Lodi, CA) to determine position in the ovarian cycle (metestrus [diestrus D1], diestrus, proestrus, estrus) based on cell morphology [29].

2.3. Chronic fluoxetine administration

One half of CON (n=28) and DEF (n=20) rats were randomly assigned to receive chronic FLX through their drinking water for 30 d (P60-P90). Administration through drinking water mimics oral administration in human patient populations, obviates daily injection stress, and unlike mini-pumps allows for the maintenance of drug dose in accordance with age-related increases in body weight. For three days prior to drug delivery, 24 h water consumption was determined for each cage using bottle weights (1 g water = 1 ml water), and ml water intake/mean kg body weight calculated. Based on daily ml/kg water consumption, FLX solution (4 mg/ml) (Mallinckrodt Inc., St. Louis MO) was added to drinking water at a concentration necessary to deliver a mean daily dose of 10 mg/kg/d. In pilot studies we found that oral administration of 10 mg/kg/d of FLX produced FLX levels in rat plasma (~100 ng/ml) that are commonly observed in plasma from human MDD patients treated with therapeutic doses of FLX (~100 ng/ml)[30]. Moreover, we selected the 10 mg/kg/d dose based on prior dose-ranging studies finding that it produced robust alterations in rat brain 5-HT turnover [31] and behavior in the FST [32]. Red opaque drinking bottles were used to protect FLX from light degradation. Fresh solutions were prepared and FLX concentration adjusted to mean body weight every 3 days. Plasma FLX and norfluoxetine (NFLX) concentrations (ng/ml) were determined by liquid chromatography tandem mass spectrometry (Medtox Laboratories, Inc., St. Paul Minnesota) by a technician blinded to treatment.

2.4. HPLC-ECD

Regional brain tissue 5-HT content and 5-HIAA concentrations (ng per mg protein) were determined by high performance liquid chromatography with electrochemical detection (HPLC-ECD), as described in detail previously [28]. Briefly, an antioxidant solution (0.4 N perchlorate, 1.34 mM ethylenediaminetetraacetic acid (EDTA) and 0.53 mM sodium metabisulfite) was added to the samples followed by homogenization using an ultrasonic tissue homogenizer (Biologics, Gainesville, VA). A fraction of the tissue homogenate was dissolved in 2% sodium dodecyl sulfate (SDS) (w/v) for protein determination (Pierce BCA Protein Reagent Kit, Rockford, IL). The remaining suspension was spun at $14,000 \times g$ for 20 min at 4°C. The supernatant was reserved for HPLC. Samples were separated on a Microsorb MV C-18 column (5 μ m, 4.6 $\times$ 250 mm, Varian, Walnut Creek, CA) and simultaneously examined for 5-HT and 5-HIAA. Compounds were detected using a 12-channel coulometric array detector (CoulArray 5200, ESA, Chelmsford, MA) attached to a Waters 2695 Solvent Delivery System (Waters, Milford, MA). Samples were processed by a technician blinded to treatment. Our primary measure of interest was the 5-HIAA/5-HT ratio, and prior studies have found that this product/precursor ratio is highly sensitive to both chronic FLX treatment [31] and *n*-3 fatty acid deficiency [28].

2.5. Brainstem mRNA expression

Frozen brainstem tissue was homogenized (BioLogics Model 300 V/T ultrasonic homogenizer, Manassas, VA) in Tri Reagent (MRC Inc., Cincinnati, OH), and total RNA isolated and purified using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total RNA was treated to remove potential DNA contamination using RNase-free DNase (Qiagen, Valencia, CA), and RNA quantified using a Nanodrop instrument (Nanodrop Instruments, Wilmington, DE). The real-time reverse transcriptase polymerase chain reaction (RT-PCR) procedure has been described in detail previously [28]. Primers and fluorogenic probes (Midland Certified Reagent Company, Midland, TX) were designed using Primer Express v.2.0 software (Applied Biosystems, Foster City, CA) based on the rat mRNA sequences for GAPDH (GenBank accession number: NM_017008), serotonin transporter (SERT, NM_013034), tryptophan hydroxylase-2 (TPH-2, NM_173839), and presynaptic 5-HT_{1A} receptors (NM_012585). Primer and probe sequences are provided in **Table 2**. Each probe was conjugated to a FAM reporter at the 5' end and a TAMRA quencher at the 3' end. The reverse primer spanned an exon-exon junction to obviate genomic DNA contamination. Reverse transcription was performed using the 9600 GeneAmp thermocycler (Perkin-Elmer, Norwalk, CT). mRNA quantities were normalized to GAPDH mRNA values obtained from the same tissue. All samples were processed by a technician blinded to treatment.

2.6. Forced swim test

Because previous studies have found that exposure to the FST leads to significant alterations in central neurochemistry and brainstem mRNA expression [33,34], FST experiments were conducted in a separate cohort of CON (n=17), CON+FLX (n=16), DEF (n=10), and DEF +FLX (n=10) rats. The modified FST procedure follows that described in Cyran et al [35]. Adult (P90) rats were placed into a clear cylindrical tank (46 cm high, 20 cm diameter) filled with water (23-25°C) to a 30-cm depth for a 15-min pretrial followed 24 h later with the 5-min test trial. Behavior was recorded with an overhead video camera, and analyzed using the time-sampling technique by a trained technician that was blind to treatment. Cumulative behavioral scores were calculated for the 15-min pretrial and the 5-min test trial. Climbing was defined as upward-directed movements of the forepaws against the side of the swim chamber. Swimming was defined as movement throughout the chamber not directed at the side of the swim chamber. Immobility was defined as no activity other than that required too

maintain the head above the water (floating). Latency to first immobility maintained for 2 sec was also scored based on a previous study finding that LCn-3 supplementation increased the latency to first immobility using this criterion [18]. After each trial, rats were placed under a heating lamp for 5 min and then returned to their home cage. Between each trial FST cylinders were emptied, rinsed, and refilled with fresh water.

2.7. Gas chromatography

The gas chromatography procedure used to determine PFC fatty acid composition has been described in detail previously [10]. Briefly, total fatty acid composition was determined with a Shimadzu GC-2010 equipped with an auto-injector (Shimadzu Scientific Instruments Inc., Columbia MD). The column was a DB-23 (123-2332): 30 m (length), I.D. 0.32 mm wide bore, film thickness of 0.25 μ M (J&W Scientific, Folsom CA). Analysis of fatty acid methyl esters is based on areas calculated with Shimadzu Class VP 4.3 software. Fatty acid composition data is expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). All samples were processed by a technician blinded to treatment.

2.8. Statistical analyses

Primary outcome measures were analyzed with a two-way ANOVA, with Diet (CON, DEF), and Treatment (FLX, Vehicle) as the main factors. Pairwise comparisons were made with Fishers LSD protected *t*-tests. Plasma FLX and NFLX concentrations in CON and DEF rats were analyzed with two-tailed *t*-tests ($\alpha=0.05$). Estrous phase was analyzed by assigning the position in the estrous phase with an arbitrary number (1=metestrus, 2=diestrus, 3=proestrus, 4=estrus), and group differences determined using the Kruskal-Wallis nonparametric test. Statistical analyses were performed using GB-STAT (V.10, Dynamic Microsystems, Inc., Silver Springs MD).

3. Results

3.1. Food intake, body weight, and estrous phase

For fluid intake (ml/kg/d), the main effects of Diet, Treatment, and the Diet x Treatment interaction were not significant. For food intake (g/kg/d), the main effect of Treatment was significant $F(1,49)=14.8$, $p=0.01$, and the main effect of Diet, $F(1,49)=1.92$, $p=0.2$, and the Diet x Treatment interaction, $F(1,49)=0.002$, $p=0.9$ were not significant. Both CON+FLX ($p=0.03$) and DEF+FLX ($p=0.01$) rats consumed significantly less food compared with CON and DEF rats, respectively, and food consumption of CON+FLX and DEF+FLX rats were not different ($p=0.6$). Mean body weights at baseline (P60) for CON (210 ± 4.2 g), CON+FLX (207 ± 3.7 g), DEF (204 ± 5.3 g), and DEF+FLX (209 ± 2.4 g) groups were not significantly different ($p=0.8$). For endpoint body weight, the main effect of Treatment was significant, $F(1,49)=38.1$, $p=0.0001$, and the main effect of Diet and the Diet x Treatment interaction were not significant. Mean endpoint body weights of CON+FLX (226 ± 4.2 g) and DEF+FLX (231 ± 2.9 g) rats were significantly lower than CON (270 ± 4.9 g, $p=0.0001$) and DEF (264 ± 8.3 g, $p=0.009$) rats, respectively, and the mean body weights of CON+FLX and DEF+FLX rats were not different ($p=0.4$). At the time of sacrifice the majority of rats in the CON ($n=24/28$), CON+FLX ($n=22/28$), DEF ($n=16/20$), and DEF+FLX ($n=17/20$) groups were in metestrus or diestrus, and remaining rats were in proestrus or estrus. The frequency of rats in each phase of the estrous cycle did not differ between each of the four treatment groups ($p=0.7$).

3.2. Plasma FLX and NFLX concentrations

Mean FLX-fluid intake by CON+FLX rats (47 ± 2.3 ml/kg/d) did not differ from DEF+FLX rats (44 ± 3.1 ml/kg/d, $p=0.4$). Plasma FLX and NFLX concentrations are presented in **Table**

3. There were no significant differences in plasma FLX ($p=0.21$), NFLX ($p=0.32$), or FLX +NFLX ($p=0.64$) concentrations between CON+FLX and DEF+FLX rats. The NFLX/FLX ratio was significantly lower in DEF+FLX rats compared with CON+FLX rats ($p=0.04$). FLX and NFLX were not detected in plasma from CON and DEF rats not treated with FLX (<10 ng/ml).

3.3. PFC fatty acid composition

For PFC DHA composition, the main effect Diet, $F(1,43)=475$, $p=0.0001$, and Treatment, $F(1,43)=10.1$, $p=0.003$, were significant, and the Diet x Treatment interaction, $F(1,43)=0.8$, $p=0.4$, was not significant. PFC DHA composition was significantly lower in DEF (-25% , $p=0.0001$) and DEF+FLX (-28% , $p=0.0001$) rats relative to CON and CON+FLX rats, respectively. Lower DHA levels were observed in DEF+FLX versus DEF rats ($p=0.03$), and there was a trend for lower DHA levels in CON+FLX versus CON rats ($p=0.08$) (**Fig. 1A**). For PFC AA composition, the main effect Diet, $F(1,43)=1.3$, $p=0.3$, and Treatment, $F(1,43)=0.02$, $p=0.9$, and the Diet x Treatment interaction, $F(1,43)=1.9$, $p=0.62$, were not significant (**Fig. 1B**). For the AA:DHA ratio, the main effect Diet, $F(1,43)=309$, $p=0.0001$, and Treatment, $F(1,43)=6.9$, $p=0.01$, were significant, and the Diet x Treatment interaction, $F(1,43)=0.34$, $p=0.6$, was not significant. The AA:DHA ratio was significantly higher in DEF ($+27\%$, $p=0.0001$) and DEF+FLX ($+27\%$, $p=0.0001$) rats relative to CON and CON+FLX rats, respectively (**Fig. 1C**). A higher AA/DHA ratio was observed in CON+FLX versus CON rats ($p=0.03$), and there was a trend for a higher AA/DHA ratio in DEF+FLX versus DEF rats ($p=0.1$).

3.4. Regional 5-HT turnover

For PFC 5-HT concentrations, the main effect of Diet was significant, $F(1,39)=10.1$, $p=0.003$, and the main effect of Treatment and the Diet x Treatment interaction were not significant (**Fig. 2A**). For PFC 5-HIAA concentrations, the main effects of Diet, $F(1,39)=4.3$, $p=0.04$, and Treatment, $F(1,39)=40.3$, $p=0.0001$, were significant, and the Diet x Treatment interaction was not significant. For the PFC 5-HIAA/5-HT ratio, the main effects of Diet, $F(1,39)=4.4$, $p=0.04$, and Treatment, $F(1,39)=53.7$, $p=0.0001$, were significant, and the Diet x Treatment interaction approached significance, $F(1,39)=3.4$, $p=0.07$. For hippocampus 5-HT concentrations, the main effects of Diet and Treatment, and the Diet x Treatment interaction were not significant (**Fig. 2B**). For hippocampus 5-HIAA concentrations, the main effect of Treatment was significant, $F(1,39)=45.5$, $p=0.0001$, and the main effect of Diet and the Diet x Treatment interaction were not significant. For the hippocampus 5-HIAA/5-HT ratio, the main effect of Treatment was significant, $F(1,39)=61.6$, $p=0.0001$, and the main effect of Diet and the Diet x Treatment interaction were not significant. For nucleus accumbens 5-HT concentrations, the main effect of Diet was significant, $F(1,39)=5.1$, $p=0.03$, and the main effect of Treatment and the Diet x Treatment interaction were not significant (**Fig. 2C**). For nucleus accumbens 5-HIAA concentrations, the main effect of Treatment was significant, $F(1,39)=93.1$, $p=0.0001$, and the main effect of Diet and the Diet x Treatment interaction were not significant. For the nucleus accumbens 5-HIAA/5-HT ratio, the main effects of Diet, $F(1,39)=10.6$, $p=0.002$, and Treatment, $F(1,39)=123.4$, $p=0.0001$, were significant, and the Diet x Treatment interaction was not significant. For hypothalamus 5-HT concentrations, the main effect of Diet was significant, $F(1,39)=9.5$, $p=0.004$, and the main effect of Treatment and the Diet x Treatment interaction were not significant (**Fig. 2D**). For hypothalamus 5-HIAA concentrations, the main effects of Diet, $F(1,39)=4.9$, $p=0.04$, and Treatment $F(1,39)=33.9$, $p=0.0001$, were significant, and the Diet x Treatment interaction was not significant. For the hypothalamus 5-HIAA/5-HT ratio, the main effect of Treatment was significant, $F(1,39)=33.7$, $p=0.0001$, the main effect of Diet approached significance, $F(1,39)=3.4$, $p=0.08$, and the Diet x Treatment interaction was not significant.

3.5. Brainstem mRNA expression

For GAPDH mRNA expression, the main effects of Diet and Treatment, and the Diet $\times$ Treatment interaction were not significant. For SERT mRNA expression, the main effects of Diet and Treatment, and the Diet $\times$ Treatment interaction, were not significant (**Fig. 3A**). For TPH-2 mRNA expression, the main effect of Treatment was significant, $F(1,39)=7.7$, $p=0.008$, and the main effect of Diet and the Diet $\times$ Treatment interaction were not significant (**Fig. 3B**). For 5-HT_{1A} mRNA expression, the main effects of Diet and Treatment, and the Diet $\times$ Treatment interaction, were not significant (**Fig. 3C**).

3.6. Forced swim test

During the 5-min test trial, the main effect of Treatment was significant for latency to first immobility, $F(1,49)=6.8$, $p=0.01$ (**Fig. 4A**), immobility scores, $F(1,49)=5.2$, $p=0.02$ (**Fig. 4B**), and swimming scores, $F(1,49)=4.1$, $p=0.04$ (**Fig. 4C**), and the main effects of Diet and the Diet $\times$ Treatment interaction were not significant. For climbing (**Fig. 4D**), the main effect of Diet was significant, $F(1,49)=4.4$, $p=0.04$, and the main effect of Treatment and the Diet $\times$ Treatment interaction were not significant.

4. Discussion

This study investigated the hypothesis that LC *n*-3 fatty acid deficiency would significantly blunt the neurochemical and behavioral effects of chronic FLX treatment. In support of this hypothesis, we found that the reduction in PFC 5-HIAA concentrations following chronic FLX treatment was significantly blunted in DEF rats compared with CON rats. However, the reduction in the PFC 5-HIAA/5-HT ratio was not blunted, and chronic FLX treatment had similar effects on 5-HIAA and the 5-HIAA/5-HT ratio in other brain regions. Furthermore, chronic FLX treatment similarly reduced brainstem TPH-2 mRNA expression and depressive-like behaviors in the FST in CON and DEF rats. Consistent with prior rat FLX treatment studies [36,37], food intake and body weight were similarly reduced in both CON and DEF rats following chronic FLX treatment. We also found that DEF+FLX rats exhibited a significantly lower NFLX/FLX ratio compared with CON+FLX rats, suggesting that *n*-3 fatty acid deficiency may impair FLX metabolism. Lastly, we found that untreated DEF rats exhibited constitutive reductions in 5-HT turnover in all brain regions examined compared with untreated CON rats, but did not exhibit altered behavioral responses in the FST. Taken collectively, these findings demonstrate that peri-adolescent *n*-3 fatty acid deficiency leading to PFC DHA deficits and regional reductions in 5-HT turnover does not significantly alter the effects of chronic FLX treatment on 5-HT turnover or behavior in the FST in female rats.

This study has three notable limitations. First, we did not include a separate measure of motor activity to rule out the potential contribution of motor hyperactivity to the observed behavioral responses in the FST. However, a previous study found that FLX (10 mg/kg) treatment significantly reduced (vs. increased) locomotor activity in open field activity chambers [32]. Therefore, the observed reduction in immobility, and increases in latency to the first immobility and swimming, observed in FLX-treated rats are likely not attributable to a general increase in locomotor activity. Nevertheless, additional studies will be required to rule this out. Second, the small number of rats in each phase of the estrous cycle precludes determination of the potential influence of position in the estrous on primary outcome measures. However, the majority of rats in each treatment group were in metestrus or diestrus at the time of sacrifice, and the frequency of rats in each phase did not differ significantly between treatment groups. Moreover, we and others have found that chronically ovariectomized rats do not exhibit significant alterations in regional brain 5-HT or 5-HIAA concentrations [28,38,39], and behavioral studies have found that reductions in

immobility following FLX treatment were similar in rats in different estrous phases and in ovariectomized rats [40,41]. Nevertheless, in view of evidence that position in the estrous cycle can interact with dietary manipulations to impact behavior in the FST [41], larger studies are warranted to address potential interactions with *n*-3 fatty acid deficiency and/or chronic FLX treatment. Third, we did not evaluate the fatty acid composition of brain regions other than the PFC to evaluate relationships with 5-HIAA and 5-HT concentrations. However, a previous study found that the reductions in PFC DHA following dietary ALA deficiency are very similar to other brain regions including the hippocampus, hypothalamus, and striatum [42], and we observed similar reductions in the 5-HIAA/5-HT ratio in all brain regions of DEF rats.

Chronic FLX treatment robustly reduced 5-HIAA concentrations and the 5-HIAA/5-HT ratio in different limbic forebrain regions of female rats, consistent with a prior study using male rats [31]. The present study additionally demonstrates that the effect of chronic FLX treatment on central 5-HT turnover was not significantly altered by LC *n*-3 fatty acid deficiency. Importantly, FLX-induced reductions in 5-HT turnover could not be attributed to group differences in the estrous cycle, and we and others previously reported that ovariectomized rats with or without estrogen replacement do not exhibit significant alterations in forebrain 5-HT turnover [28,43]. In all FLX-treated rats the reduction in 5-HT turnover was primarily attributable to reductions in 5-HIAA, a finding that is consistent with the clinical observation that chronic FLX treatment significantly reduce 5-HIAA concentrations in cerebrospinal fluid and internal jugular venoarterial plasma of MDD patients [44,45]. Consistent with a prior study using male rats [46], we also found that chronic FLX treatment decreased brainstem expression of tryptophan hydroxylase-2 (TPH-2), the rate-limiting enzyme in the biosynthesis of 5-HT, in both CON and DEF rats. In contrast to some previous studies [33,46,47], neither SERT nor 5-HT_{1A} mRNA expression were significantly altered in FLX-treated CON or DEF rats. Together, these findings suggest that chronic FLX treatment leads to robust reductions in central 5-HT turnover and biosynthesis which is not significantly altered by *n*-3 fatty acid deficiency.

The present study also found that untreated DEF rats exhibited constitutive decreases in 5-HT turnover in the PFC as well as other limbic forebrain structures which is consistent with our previous findings [28]. This reduction in 5-HT turnover was primarily due to elevations in 5-HT, as 5-HIAA concentrations were similar with the exception of the hypothalamus where they were significantly elevated. It is noteworthy that constitutive reductions in forebrain 5-HT turnover have also been observed in Flinders sensitive line rat model of depression [27], which is associated with similar elevations in the AA/DHA ratio secondary to elevations in AA composition [48], and in the olfactory bulbectomized rat model of depression [26]. However, unlike Flinders sensitive line rats [49] and olfactory bulbectomized rats [50], peri-adolescent *n*-3 fatty acid deficiency did not significantly increase immobility in the FST. The latter finding suggests that *n*-3 fatty acid deficiency alone is not sufficient to increase behavioral indices of depression in the FST. In view of emerging evidence that suggests that the combination of postnatal stress and *n*-3 fatty acid deficiency has synergistic effects on different behavioral indices of emotion in adulthood [51,52], additional studies are warranted to investigate whether this interaction is required to impact behavior in the FST.

Both CON and DEF rats exhibited similar FLX intake resulting in plasma FLX concentrations that are within the human female plasma range [30]. However, rat plasma NFLX concentrations were approximately three-fold greater and the NFLX/FLX ratio (an index of FLX metabolism) approximately 7-fold greater than is observed in female MDD patients [30]. Importantly, we observed trends for higher plasma FLX and lower plasma NFLX concentrations in FLX-treated DEF rats, and the NFLX/FLX ratio was significantly

lower compared with CON rats. These findings suggest that *n*-3 fatty acid deficiency may impair the conversion of FLX to NFLX which is mediated by the liver cytochrome 450 system. Interestingly, a previous study found that increasing LC*n*-3 fatty acid status elevated rat liver cytochrome P-450 activity [53]. Although rat plasma FLX and NFLX concentrations are highly correlated with brain FLX and NFLX concentrations [31], and both FLX and NFLX similarly reduce 5-HIAA concentrations in rat brain [54], we found that both CON and DEF rats exhibited similar neurochemical and behavioral responses following chronic FLX treatment. This suggests that this apparent deficit in FLX metabolism was not sufficient to significantly alter the pharmacological effects of FLX. Nevertheless, additional dose-ranging studies are warranted to investigate whether LC*n*-3 fatty acid effects on liver cytochrome P-450 activity and FLX metabolism can account for the augmenting effects of LC*n*-3 fatty acids on FLX-induced changes in behavior [19,20] and clinical efficacy [21-23].

In conclusion, the present study demonstrates that peri-adolescent *n*-3 fatty acid deficiency leading to PFC DHA deficits and regional alterations in 5-HT neurochemistry does not significantly alter the effects of chronic FLX treatment on 5-HT turnover or behavior in the FST in female rats. Although we obtained preliminary evidence for an apparent deficit in FLX metabolism in DEF rats, it was not associated with differential neurochemical or behavioral responses to FLX. Therefore, the present data do not support our hypothesis that LC*n*-3 fatty acid deficiency would blunt the neurochemical and behavioral effects of chronic FLX treatment. Nevertheless, additional studies are warranted to investigate alternative mechanisms mediating the augmenting effect of LC*n*-3 fatty acids on SSRI treatment response.

Acknowledgments

This work was supported in part by National Institute of Mental Health grants MH073704 and MH074858 (R.K.M.), DK59630 (P.T.), and core facilities provided by NS058830 (J.W.L.).

REFERENCES

- Assies J, Pouwer F, Lok A, Mocking RJ, Bockting CL, Visser I, Abeling NG, Duran M, Schene AH. Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case-control study. *PLoS One*. 2010; 5(5):e10635. [PubMed: 20498721]
- Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *J Affect Disord*. 1998; 48:149–155. [PubMed: 9543204]
- Lin PY, Huang SY, Su KP. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biol Psychiatry*. 2010; 68:140–147. [PubMed: 20452573]
- McNamara RK, Jandacek R, Rider T, Tso P, Dwivedi Y, Pandey GN. Selective deficits in erythrocyte docosahexaenoic acid composition in adult patients with bipolar disorder and major depressive disorder. *J Affect Disord*. 2010; 126:303–311. [PubMed: 20413162]
- Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatry*. 1998; 43:315–319. [PubMed: 9513745]
- Kessler RC. Epidemiology of women and depression. *J Affect Disord*. 2003; 74:5–13. [PubMed: 12646294]
- Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr*. 2002; 88:411–420. [PubMed: 12323090]
- Giltay EJ, Gooren LJ, Toorians AW, Katan MB, Zock PL. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr*. 2004; 80:1167–1174. [PubMed: 15531662]

9. Lohner S, Fekete K, Marosvölgyi T, Decsi T. Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publications. *Ann Nutr Metab.* 2013; 62:98–112. [PubMed: 23327902]
10. McNamara RK, Able J, Jandacek R, Rider T, Tso P. Gender differences in rat erythrocyte and brain docosahexaenoic acid composition: role of ovarian hormones and dietary omega-3 fatty acid composition. *Psychoneuroendocrinology.* 2009; 34:532–539. [PubMed: 19046819]
11. Tanskanen A, Hibbeln JR, Tuomilehto J, Uutela A, Haukkala A, Viinamäki H, Lehtonen J, Vartiainen E. Fish consumption and depressive symptoms in the general population in Finland. *Psychiatr Serv.* 2001; 52:529–531. [PubMed: 11274502]
12. Timonen M, Horrobin D, Jokelainen J, Laitinen J, Herva A, Räsänen P. Fish consumption and depression: the Northern Finland 1966 birth cohort study. *J Affect Disord.* 2004; 82:447–452. [PubMed: 15555697]
13. McNamara RK, Hahn CG, Jandacek R, Rider T, Tso P, Stanford KE, Richtand NM. Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Biol Psychiatry.* 2007; 62:17–24. [PubMed: 17188654]
14. Arango V, Underwood MD, Mann JJ. Serotonin brain circuits involved in major depression and suicide. *Prog Brain Res.* 2002; 136:443–453. [PubMed: 12143401]
15. Vaswani M, Linda FK, Ramesh S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. *Prog Neuropsychopharmacol Biol Psychiatry.* 2003; 27:85–102. [PubMed: 12551730]
16. Chalon S, Delion-Vancassel S, Belzung C, Guilloteau D, Leguisquet AM, Besnard JC, Durand G. Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. *J. Nutr.* 1998; 128:2512–2519. [PubMed: 9868201]
17. Vancassel S, Leman S, Hanonick L, Denis S, Roger J, Nollet M, Bodard S, Kousignian I, Belzung C, Chalon S. n-3 polyunsaturated fatty acid supplementation reverses stress- induced modifications on brain monoamine levels in mice. *J Lipid Res.* 2008; 49:340–348. [PubMed: 17991757]
18. Carlezon WA Jr, Mague SD, Parow AM, Stoll AL, Cohen BM, Renshaw PF. Antidepressant-like effects of uridine and omega-3 fatty acids are potentiated by combined treatment in rats. *Biol Psychiatry.* 2005; 57:343–350. [PubMed: 15705349]
19. Laino CH, Fonseca C, Sterin-Speziale N, Slobodianik N, Reinés A. Potentiation of omega-3 fatty acid antidepressant-like effects with low non-antidepressant doses of fluoxetine and mirtazapine. *Eur J Pharmacol.* 2010; 648:117–126. [PubMed: 20826148]
20. Lakhwani L, Tongia SK, Pal VS, Agrawal RP, Nyati P, Phadnis P. Omega-3 fatty acids have antidepressant activity in forced swimming test in Wistar rats. *Acta Pol Pharm.* 2007; 64:271–276. [PubMed: 17695151]
21. Jazayeri S, Tehrani-Doost M, Keshavarz SA, Hosseini M, Djazayeri A, Amini H, Jalali M, Peet M. Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. *Aust N Z J Psychiatry.* 2008; 42:192–198. [PubMed: 18247193]
22. Gertsik L, Poland RE, Bresee C, Rapaport MH. Omega-3 fatty acid augmentation of citalopram treatment for patients with major depressive disorder. *J Clin Psychopharmacol.* 2012; 32:61–64. [PubMed: 22198441]
23. Peet M, Horrobin DF. A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch Gen Psychiatry.* 2002; 59:913–919. [PubMed: 12365878]
24. Delion S, Chalon S, Hérault J, Guilloteau D, Besnard JC, Durand G. Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotonergic neurotransmission in rats. *J Nutr.* 1994; 124:2466–2476. [PubMed: 16856329]
25. Kodas E, Galineau L, Bodard S, Vancassel S, Guilloteau D, Besnard JC, Chalon S. Serotonergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat. *J Neurochem.* 2004; 89:695–702. [PubMed: 15086526]

26. Hellweg R, Zueger M, Fink K, Hörtnagl H, Gass P. Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. *Neurobiol Dis.* 2007; 25:1–7. [PubMed: 16990008]
27. Zangen A, Overstreet DH, Yadid G. High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. *J Neurochem.* 1997; 69:2477–2483. [PubMed: 9375680]
28. McNamara RK, Able J, Liu Y, Jandacek R, Rider T, Tso P, Lipton JW. Omega-3 fatty acid deficiency during perinatal development increases serotonin turnover in the prefrontal cortex and decreases midbrain tryptophan hydroxylase-2 expression in adult female rats: dissociation from estrogenic effects. *J Psychiatr Res.* 2009; 43:656–663. [PubMed: 18986658]
29. Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, Young E. Strategies and methods for research on sex differences in brain and behavior. *Endocrinology.* 2005; 146:1650–1673. [PubMed: 15618360]
30. Amsterdam JD, Fawcett J, Quitkin FM, Reimherr FW, Rosenbaum JF, Michelson D, Hornig-Rohan M, Beasley CM. Fluoxetine and norfluoxetine plasma concentrations in major depression: a multicenter study. *Am J Psychiatry.* 1997; 154:963–969. [PubMed: 9210747]
31. Unceta N, Barrondo S, de Azúa IR, Gómez-Caballero A, Goicolea MA, Sallés J, Barrio RJ. Determination of fluoxetine, norfluoxetine and their enantiomers in rat plasma and brain samples by liquid chromatography with fluorescence detection. *J Chromatogr B Analyt Technol Biomed. Life Sci.* 2007; 852:519–528.
32. Hemby SE, Lucki I, Gatto G, Singh A, Thornley C, Matasi J, Kong N, Smith JE, Davies HM, Dworkin SI. Potential antidepressant effects of novel tropane compounds, selective for serotonin or dopamine transporters. *J Pharmacol Exp Ther.* 1997; 282:727–733. [PubMed: 9262336]
33. Drossopoulou G, Antoniou K, Kitraki E, Papathanasiou G, Papalexi E, Dalla C, Papadopoulou-Daifoti Z. Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. *Neuroscience.* 2004; 126:849–857. [PubMed: 15207320]
34. Shishkina GT, Kalinina TS, Dygalo NN. Effects of swim stress and fluoxetine on 5-HT_{1A} receptor gene expression and monoamine metabolism in the rat brain regions. *Cell Mol Neurobiol.* 2012; 32:787–794. [PubMed: 22453856]
35. Cryan JF, Page ME, Lucki I. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology (Berl).* 2005; 182:335–344. [PubMed: 16001105]
36. Lauzurica N, García-García L, Fuentes JA, Delgado M. Hypophagia and induction of serotonin transporter gene expression in raphe nuclei of male and female rats after short-term fluoxetine treatment. *J Physiol Biochem.* 2013; 69:69–74. [PubMed: 22730080]
37. Lightowler S, Wood M, Brown T, Glen A, Blackburn T, Tulloch I, Kennett G. An investigation of the mechanism responsible for fluoxetine-induced hypophagia in rats. *Eur J Pharmacol.* 1996; 296:137–143. [PubMed: 8838449]
38. Bitar MS, Ota M, Linnoila M, Shapiro BH. Modification of gonadectomy-induced increases in brain monoamine metabolism by steroid hormones in male and female rats. *Psychoneuroendocrinology.* 1991; 16:547–557. [PubMed: 1725825]
39. Lu H, Yuri K, Ito T, Yoshimoto K, Kawata M. The effects of oestrogen and progesterone on serotonin and its metabolite in the lateral septum, medial preoptic area and ventromedial hypothalamic nucleus of female rats. *J Neuroendocrinol.* 1998; 10:919–926. [PubMed: 9870749]
40. Estrada-Camarena E, López-Rubalcava C, Hernández-Aragón A, Mejía-Mauries S, Picazo O. Long-term ovariectomy modulates the antidepressant-like action of estrogens, but not of antidepressants. *J Psychopharmacol.* 2011; 25:1365–1377. [PubMed: 21890587]
41. Allen PJ, D'Anci KE, Kanarek RB, Renshaw PF. Sex-specific antidepressant effects of dietary creatine with and without sub-acute fluoxetine in rats. *Pharmacol Biochem Behav.* 2012; 101:588–601. [PubMed: 22429992]
42. Xiao Y, Huang Y, Chen ZY. Distribution, depletion and recovery of docosahexaenoic acid are region-specific in rat brain. *Br J Nutr.* 2005; 94:544–550. [PubMed: 16197579]

43. Lu H, Yuri K, Ito T, Yoshimoto K, Kawata M. The effects of oestrogen and progesterone on serotonin and its metabolite in the lateral septum, medial preoptic area and ventromedial hypothalamic nucleus of female rats. *J Neuroendocrinol*. 1998; 10:919–926. [PubMed: 9870749]
44. Barton DA, Esler MD, Dawood T, Lambert EA, Haikerwal D, Brenchley C, Socratous F, Hastings J, Guo L, Wiesner G, Kaye DM, Bayles R, Schlaich MP, Lambert GW. Elevated brain serotonin turnover in patients with depression: effect of genotype and therapy. *Arch Gen Psychiatry*. 2008; 65:38–46. [PubMed: 18180427]
45. De Bellis MD, Geraciotti TD Jr, Altemus M, Kling MA. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. *Biol Psychiatry*. 1993; 33:636–641. [PubMed: 7687151]
46. Dygalo NN, Shishkina GT, Kalinina TS, Yudina AM, Ovchinnikova ES. Effect of repeated treatment with fluoxetine on tryptophan hydroxylase-2 gene expression in the rat brainstem. *Pharmacol Biochem Behav*. 2006; 85:220–227. [PubMed: 16982082]
47. Le Poul E, Boni C, Hanoun N, Laporte AM, Laaris N, Chauveau J, Hamon M, Lanfumey L. Differential adaptation of brain 5-HT1A and 5-HT1B receptors and 5-HT transporter in rats treated chronically with fluoxetine. *Neuropharmacology*. 2000; 39:110–122. [PubMed: 10665824]
48. Green P, Gispan-Herman I, Yadid G. Increased arachidonic acid concentration in the brain of Flinders Sensitive Line rats, an animal model of depression. *J Lipid Res*. 2005; 46:1093–1096. [PubMed: 15805551]
49. Overstreet DH, Friedman E, Mathé AA, Yadid G. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neurosci Biobehav Rev*. 2005; 29:739–759. [PubMed: 15925699]
50. Wang YQ, Tu ZC, Xu XY, Li R, Qu WM, Urade Y, Huang ZL. Acute administration of fluoxetine normalizes rapid eye movement sleep abnormality, but not depressive behaviors in olfactory bulbectomized rats. *J Neurochem*. 2012; 120:314–324. [PubMed: 22035172]
51. Mathieu G, Denis S, Lavialle M, Vancassel S. Synergistic effects of stress and omega-3 fatty acid deprivation on emotional response and brain lipid composition in adult rats. *Prostaglandins Leukot Essent Fatty Acids*. 2008; 78:391–401. [PubMed: 18579362]
52. Mathieu G, Oualian C, Denis I, Lavialle M, Gisquet-Verrier P, Vancassel S. Dietary n-3 polyunsaturated fatty acid deprivation together with early maternal separation increases anxiety and vulnerability to stress in adult rats. *Prostaglandins Leukot Essent Fatty Acids*. 2011; 85:129–136. [PubMed: 21784625]
53. Valdes E, Vega P, Avalos N, Orellana M. Dietary fish oil and cytochrome P-450 monooxygenase activity in rat liver and kidney. *Lipids*. 1995; 30:955–958. [PubMed: 8538384]
54. Bourdeaux R, Desor D, Lehr PR, Younos C, Capolaghi B. Effects of fluoxetine and norfluoxetine on 5-hydroxytryptamine metabolism in blood platelets and brain after administration to rats. *J Pharm Pharmacol*. 1998; 50:1387–1392. [PubMed: 10052854]

Highlights

- Long-chain omega-3 fatty acid status is positively associated with selective serotonin reuptake inhibitor (SSRI) antidepressant efficacy. Here we tested the hypothesis that omega-3 fatty deficiency would reduce the neurochemical and behavioral effects of chronic fluoxetine treatment.
- In control and omega-3 fatty acid deficient rats, chronic fluoxetine treatment had similar effects on 5-HT turnover in limbic forebrain regions and brainstem markers of presynaptic 5-HT neurotransmission.
- In control and omega-3 fatty acid deficient rats, chronic fluoxetine treatment had similar effects on behavior in the forced swim test.
- Untreated omega-3 fatty acid deficient rats exhibited constitutive reductions in 5-HT turnover in limbic forebrain structures, but did not exhibit abnormal behavioral responses in the FST.

Figure 1.

DHA (22:6n-3) composition (**A**), arachidonic acid (AA, 20:4n-6) composition (**B**), and the AA:DHA ratio (**C**) in PFC of CON, CON+FLX, DEF, and DEF+FLX rats (n=10-12/group). Values (wt % total fatty acids) are group mean $\pm$ S.E.M. *** P 0.001 vs. CON or CON+FLX rats, # P 0.05 vs. DEF rats in (**A**) and vs. CON rats in (**C**).

Figure 2.

Mean concentrations of 5-HT and 5-HIAA, and the 5-HIAA/5-HT ratio, in the prefrontal cortex (A), hippocampus (B), nucleus accumbens (C), and hypothalamus (D) of CON, CON+FLX, DEF, and DEF+FLX rats (n=10-12/group). Values are group mean $\pm$ S.E.M.

* P 0.05 vs. CON, *** P 0.001 vs. CON and DEF rats, # P 0.05 vs. CON+FLX.

Figure 3.

Mean serotonin transporter (SERT)(A), tryptophan hydroxylase-2 (TPH-2)(B), and 5-HT_{1A} receptor (C) mRNA expression in the brainstem of CON, CON+FLX, DEF, and DEF+FLX rats (n=10-12/group). Values (mRNA/GAPDH mRNA) are group mean $\pm$ S.E.M. **P* 0.05, ***P* 0.01 vs. CON or DEF rats.

Figure 4. Latency to first immobility (**A**) and immobility (**B**), swimming (**C**), and climbing (**D**) scores during the 5-min test trial in CON, CON+FLX, DEF, and DEF+FLX rats (n=10-17/group). Values are group mean $\pm$ S.E.M. * P 0.05, ** P 0.01 vs. CON or DEF rats.

Table 1**Diet Compositions**

Ingredient¹	ALA+	ALA-
Casein, vitamin free	20	20
Carbohydrate		
Cornstarch	20	20
Sucrose	27	27
Dextrose	9.9	9.9
Maltose-dextrin	6	6
Cellulose	5	5
Mineral mix	3.5	3.5
Vitamin mix	1	1
L-Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
TBHQ	0.002	0.002
Fat		
Hydrogenated coconut oil	4.5	5.1
Safflower	1.9	1.9
Flaxseed	0.6	0
Fatty acid composition ²		
C8:0	4.3	5.0
C10:0	3.8	4.2
C12:0	29	32.8
C14:0	11	12.5
C16:0	8.3	8.7
C18:0	9.4	10.2
18:1n-9	6.7	4.7
18:2n-6	22.7	21.9
20:4n-6	nd	nd
18:3n-3	4.6	nd
22:6n-3	nd	nd

nd = not detected

¹ g/100 g diet² wt % of total fatty acids

Table 2

Primer and Probe Sequences

Gene	Primer & Probe Sequence	Accession Number
<i>SERT</i>	F: 5'-AACTCCTGGAACACTGGCAACT-3'	<i>NM_013034</i>
	R: 5'-GCAGGACATGGCGCAAGTA-3'	
	P: 5'-CAACTACTTCGCCCAGGACAACATCACC-3'	
<i>TPH-2</i>	F: 5'-TACCTGAGCCCAAGAGACTTCCT-3'	<i>NM_173839</i>
	R: 5'-GCTCATGGCATGTGTCTGGTT-3'	
	P: 5'-CTCCGACCCCTCTACACCCCG-3'	
<i>5-HT_{1A}</i>	F: 5'-TGTTGCTCATGCTGGTTCTCTAC-3'	<i>NM_012585</i>
	R: 5'-CTGACAGTCTTGCGGATTTCG-3'	
	P: 5'-CGCATCTTCAGAGCCGCACGC-3'	

F = Forward primer

R = Reverse Primer

P = Probe

Table 3

Plasma Fluoxetine and Norfluoxetine Concentrations

	CON+FLX	DEF+FLX	<i>p</i> -value ²
Fluoxetine (FLX) ¹	87.5 ± 12.1	114.8 ± 15.9	0.21
Norfluoxetine (NFLX)	551.5 ± 38.8	488.1 ± 49.7	0.32
FLX+NFLX	639.0 ± 48.9	602.8 ± 60.2	0.64
NFLX/FLX	7.7 ± 1.1	4.5 ± 0.5	0.04

¹Values are mean concentration (ng/ml) ± S.E.M.

²Unpaired *t*-test (two-tailed)