Fluoxetine Potentiation of Omega-3 Fatty Acid Antidepressant Effect: Evaluating Pharmacokinetic and Brain Fatty Acid-Related Aspects in Rodents

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ABSTRACT: We previously reported that combined fluoxetine administration at antidepressant doses renders additive antidepressant effects, whereas non-antidepressant doses potentiate the omega-3 fatty acid antidepressant effect. In the present study, we aimed to evaluate putative pharmacokinetic and brain omega-3 fatty acid-related aspects for fluoxetine potentiation of omega-3 fatty acid antidepressant effect in rats. Coadministration of omega-3 fatty acids with a non-antidepressant dose of fluoxetine (1 mg/kg day) failed to affect both brain fluoxetine concentration and norfluoxetine plasma concentration profile. Fluoxetine plasma concentrations remained below the sensitivity limit of the detection method. Either antidepressant (10 mg/kg day) or non-antidepressant (1 mg/kg day) doses of fluoxetine in combination with omega-3 fatty acids increased hippocampal docosapentaenoic acid (DPA, 22:5 omega-3) levels. Although individual treatments had no effects on DPA concentration, DPA increase was higher when omega-3 were combined with the non-antidepressant dose of fluoxetine. Chronic DPA administration exerted antidepressant-like effects in the forced swimming test while increasing hippocampal docosahexaenoic (22:6 omega-3) and DPA levels. Our results suggest no pharmacokinetic interaction and reveal specific hippocampal DPA changes after fluoxetine and omega-3 combined treatments in our experimental conditions. The DPA role in the synergistic effect of fluoxetine and omega-3 combined treatments will be for sure the focus of future studies. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3316–3325, 2014

Keywords: omega-3 fatty acids; antidepressants; fluoxetine; forced swimming test; depression; drug effects; drug-like properties; pharmacodynamic; preclinical pharmacokinetic; physiological model

INTRODUCTION

Polyunsaturated fatty acids omega-6 and omega-3, as linoleic acid (LA, 18:2) and alpha-linolenic acid (ALA; l8:3), respectively, are essential nutrients for optimal health that must be obtained from the diet as they cannot be synthesized *de novo*.[1] These fatty acids are precursors of omega-6 and omega-3 long-chain polyunsaturated fatty acid families. Among them, arachidonic acid (AA; 20:4 omega-6) and docosahexaenoic acid (DHA; 22:6 omega-3) are highly abundant in the brain[2,3]; whereas others, such as the omega-3 eicosapentaenoic acid (EPA; 20:5), are present at very low levels.[4] In recent years, there has been a growing interest in certain omega-3 fatty acids, such as DHA and EPA in mood disorders. Epidemiological data show a negative correlation between omega-3 fatty acid enriched diet consumption and rates of depression[5–8] as well as omega-3 fatty acid serum levels and the severity of this disease.[9–12] Preclinical evidence has demonstrated the antidepressant effect of omega-3 fatty acids (DHA plus EPA) administered alone[13–17] or in combination with antidepressant drugs such as fluoxetine or mirtazapine.[17] Also, clinical studies have shown the efficacy of omega-3 fatty acid monotherapy[18–21] or combined treatments with antidepressants for major depressive disorder, childhood depression, resistant depression, and bipolar depression.[22,23]

Recent studies in our laboratory have shown that the combined administration of antidepressant doses of fluoxetine and omega-3 fatty acids in rats renders additive antidepressant effects. Moreover, non-antidepressant doses of fluoxetine potentiate the antidepressant effect of omega-3 fatty acids.[17] However, the mechanisms underlying omega-3 fatty acids and fluoxetine synergistic effects in combined treatments are not yet well understood. The aim of the present study was to examine putative pharmacokinetic and brain omega-3 fatty acid-related aspects for fluoxetine potentiation of omega- 3 fatty acid antidepressant effect. To this aim, we evaluated fluoxetine/norfluoxetine plasma and brain levels as well as omega-6 and omega-3 fatty acid levels in the hippocampus and cerebral cortex under omega-3 fatty acids and fluoxetine single or combined chronic treatments.

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EXPERIMENTAL PROCEDURES

Animals and Drugs

Adult male Wistar rats weighing 200–390 g at the beginning of the experiment were used. Three or four animals were housed in polyethylene cages (55 × 38 × 30 cm^3) in a temperature-controlled room (20 ± 1°C) on a 12:12-hour light-dark cycle (8 AM lights on) with free access to food and water except during testing. Animals were used only once in each test. All studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA. The experimental protocols were approved by the Ethics Committee for the Care and Use of Laboratory Animals of the School of Pharmacy and Biochemistry at the University of Buenos Aires. Special care was taken to minimize the number of animals used and their suffering.

All chemical substances were of analytical grade. Fluoxetine–HCl (Gador Laboratory, Buenos Aires, Argentina), norfluoxetine (Sigma–Aldrich, Inc., St. Louis, Missouri), and docosapentaenoic acid (DPA; 22: 5 omega-3; Nu-Chek Prep, Inc., Elysian, Minnesota) were used.

Experimental Design

In the first set of experiments, rats were randomly assigned into six groups: control + saline (c-sal), control + fluoxetine 10 mg/kg (c-flx 10), control + fluoxetine 1 mg/kg (c-flx 1), omega-3 + saline (omega-3-sal), omega-3 + fluoxetine 10 mg/kg (omega-3-flx 10), and omega-3 + fluoxetine 1 mg/kg (omega-3-flx 1). Control animals (c-sal and c-flx) were fed with the standard diet (casein 20%). Omega-3 sal and omega-3 flx rats were fed with standard diet supplemented with fish oil (Tables 1 and 2).

Prior to each experiment, fluoxetine–HCl was dissolved in distilled water and administered by intraperitoneal (i.p.) injection in a volume equivalent to 1 cc/kg. Control groups received daily i.p. injections of saline solution (0.9% NaCl). The antidepressant dose of fluoxetine (10 mg/kg day) was chosen according to previous studies that demonstrated a robust effect in the forced swimming test (FST) employing similar conditions.[17,24,25] The fluoxetine dose lacking antidepressant effect (1 mg/kg day) was also chosen according to previous studies.[17,26] Rats were injected with fluoxetine or saline solution once a day for 16 days and test sessions took place 24 h later (day 17) in accordance with previous studies.[17,27]

To evaluate the possible antidepressant effect of DPA (22:5 omega-3), at the beginning of the study, another set of animals were randomly divided into two groups: rats that orally received DPA (150 mg/kg day) or olive oil (control) for 16 days. The dose of DPA used in the present study was based on a previous report.[28] The weight of the animals was recorded every day for 17 days.

Table 1. Diet Composition

	Control	Omega-3 Fatty Acid Supplemented Diet
	g/kg Diet	
Calcium caseinate	200	200
Corn oil	50	50
Choline chlorhydrate	1.5	1.5
Vitamin mixture[a]	10	10
Mineral mixture[b]	35	35
Maltodextrin	696.9	696.9
Salmon oil	–	11.93

[a]Composition of vitamin supplement triturated in sucrose (g/kg of diet): D-calcium panthotenate, 1.60; nicotinic acid, 3.00; D-biotin, 0.02; menadione, 0.029; thiamine HCl, 0.60; riboflavin, 0.60; folic acid, 0,20; DL-alpha-tocopherol acetate (500 μ/g), 15.00; retinyl palmitate, (400 UI/g), 0.228; pyridoxine HCl, 0.70; cyanocobalamin 0,1% (triturated in mannitol 1:1000), 2.50; cholecalciferol, (250,000 U/g), 0.40; sucrose, 975.123.

[b]Composition (g/kg of diet) as follows: K_2HPO_4, 322.5; $CaCO_3$, 357; NaCl, 74; MgO, 0.8; $MgSO_4.7H_2O$, 146.9; $ZnSO_4.5H_2O$, 0.63; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.008; KI, 0.0078; $Na_2SeO3.5H_2O$, 0.1025; iron and ammonium citrate, 6.06; ZnCl, 1.79; sucrose 91.

Table 2. Fatty Acid Composition of Control and Omega-3-Supplemented Diets[a]

	Experimental Diet	
Fatty Acids	Control	Omega-3 Diet
14:0 Myristic	0.11	7.25
16:1 Palmitoleic	0.16	9.63
16:0 Palmitic	6.75	18.44
18:0 Stearic	3.04	3.51
18:1 Oleic	29.2	14.43
18:2 *n-6* Linoleic	58.8	3.06
18:3 *n-3* Alpha-linolenic	0.11	0.69
20:4 *n-6* Arachidonic	0.24	1.55
20:5 *n-3* Eicosapentaenoic	NC	17.39
22:5 *n-3* Docosapentaenoic	NC	2.68
22:6 *n-3* Docosahexaenoic	NC	12.13

[a]Values are expressed as g/100 g total fatty acids, determined by gas chromatography according to previous studies.[17]

Diet Composition

Omega-3 fatty acids (720 mg/kg day) were administered as a diet supplement in food enriched with salmon oil for 16 days according to previous studies.[17] Each 1000 mg of highly concentrated salmon oil contained approximately 30% omega-3 fatty acids (17% EPA and 13% DHA), rendering 40 mg of salmon oil per gram of the enriched food. Omega-3 fatty acids were added every morning. The amount of diet eaten per day in the different groups was quantified and the average value was employed to calculate the omega-3 dose administered during the 16-day treatment. Diets were equivalent in overall fat, protein, carbohydrate, and caloric content (Table 1).

Total lipid content of the diet was 50 g/100g of wet weight and the three main unsaturated fatty acids present in the control diet were oleic acid (29.2%), LA (58.8%), and ALA (0.11%); whereas EPA, DPA, and DHA were not detected (Table 2).

Forced Swimming Test

Experimental Procedure

In this study, we used the FST, a well-accepted model to test the antidepressant-like action of agents and to identify in rats treatments with antidepressant efficacy in humans.[29] Stress is a well-known risk factor in the development of depression. The FST employs forced swimming stimuli as stressor to generate a behavior characterized by increased immobility time. In our protocol, two sessions were conducted: an initial 15-min pretest on day 1 followed by a 5-min test on day 17. Chronic omega-3 fatty acids and/or fluoxetine treatment began on day 1 after the pretest session.[17] Swimming sessions were conducted by placing rats in individual Plexiglas cylinders (46 cm tall × 20 cm

in diameter) that had been previously filled with water (23°C–25°C) up to 30 cm from bottom. All swimming sessions were carried out between 1200 and 1800 h.

At the end of both swimming sessions, rats were removed from the cylinders, dried with towels, placed in heated cages for 15 min, allowed to rest and recover, and then returned to their home cages. The cylinders were emptied and cleaned between rats. Each animal was assigned randomly to a treatment, and was only employed for one pretest/test session.

Behavioral Scoring

For behavioral sampling, rats were rated at 5 s intervals throughout the duration of the test session. At each 5 s interval, the predominant behavior was assigned to one of three categories: (1) immobility: floating in the water without struggling, and making only those movements necessary to keep the head above the water; (2) swimming: making active swimming motions, more than necessary to merely keep the head above water (i.e., moving around in the cylinder); and (3) climbing: making active movements with forepaws in and out of the water, usually directed against the walls. Scores for each behavior were expressed as total behavioral counts per 5 min session.[17,30,31]

Open Field Test

To ensure that the alterations in the duration of immobility in the FST are not the result of changes in motor activity,[32] a set of animals were subjected to the open field test. All rats underwent the forced swimming pretest on day 1 and a group of animals were subjected to a 5-min open-field session on day 17 without being retested in the FST.

The apparatus used consisted of a black, square open field (60 cm by 60 cm) with the floor divided in squares (15 × 15 cm^2) by means of white lines. The open field was placed in a quiet room only illuminated with a 75 W electric bulb hung 75 cm above it. Testing was performed between 1400 and 1700 h. Each animal was carefully placed in the center of the field and the number of times the rat entered different squares were counted blindly by an observer for 5 min. After each animal was removed, the open field was carefully cleaned with a damp cloth.

Fatty Acid Analysis

At the end of the FST, rats were sacrificed in a CO_2 chamber followed by decapitation. Brains were rapidly removed and hippocampi and cerebral cortexes dissected on ice and stored at −80°C until analysis. Total lipids were extracted from aliquots of tissue homogenate according to the method of Folch et al.[33] Then, they were converted to their methyl esters and analyzed by Chrompack CP-900S GC using a flame ionization detector (Chrompack International, Middelburg, Holland) and a fused silica capillary column (88 m × 0.25 mm × 0.1 μm, Model CP-SIL) with nitrogen as a carrier gas. The oven temperature program was set at 70°C for 4 min, then increased 13°C per minute to 170°C, then 1°C per minute to 270°C, and finally held at 200°C for 15 min. Fatty acid peaks were identified by comparison of the retention times with standards of known composition (Nu-Chek Prep, Inc., Elysian, Minnesota). Fatty acid concentration was expressed as percentage of total fatty acids.

Measurement of Fluoxetine and Norfluoxetine Concentrations

Plasma and Brain Sample Collection

Following chronic treatment (16 days) with fluoxetine (1 mg/kg) alone or in combination with omega-3 fatty acids (720 mg/kg), 250 μL blood samples were collected into heparinized tubes on day 17 over a time course of 0.17, 0.33, 0.50, 1, 2, 4, 8, and 24 h via venipuncture of the lateral tail vein. Plasma samples were obtained by centrifugation at 2,000*g* at 4°C for 15 min. Supernatants were stored at −20°C until use. For brain samples, cerebral cortexes and hippocampi stored at −80°C were homogenized in 10 vol (w/v) of 0.1 N perchloric acid, centrifuged at 15,000*g* at 4°C for 20 min and the resultant supernatants were kept at −20°C until use.

HPLC

Levels of fluoxetine and norfluoxetine in brain and blood samples were measured by liquid chromatography with fluorescence detection according to Vlase et al.[34] with modifications employing a phenomenex Luna ODS column 5 mm, C18, 150 × 4.6 mm^2 (Waters Spherisorb, Wexford, Ireland) and a fluorescence detector (FL-3000; Thermo Finnigan, Les Ulis, France). The excitation and emission wavelengths used were 230 and 312 nm, respectively. The optimal composition of the mobile phase was achieved by a mixture of acetonitrile and 40 mM potassium dihydrogen phosphate buffer (31:69). The assay for fluoxetine and norfluoxetine was linear over the range of 50–1000 ng/mL. Intraday and interday coefficient of variation of the chromatographic method was less than 10%. Quantification of fluoxetine and norfluoxetine was made using phenytoin as internal standard. Fluoxetine and norfluoxetine were extracted by liquid procedure from the plasma sample for quantification by liquid chromatography. Briefly, an aliquot of internal standard (phenytoin 2 μg/mL), 100 μL sodium hydroxide 0.1 N and 1 mL of ethyl ether were added to 50 μL of plasma sample. The mixture was vortexed for 2 min and centrifuged at 1,000*g* for 10 min. The organic layer was transferred into a conical tube and evaporated under nitrogen gas. The dry extract was reconstituted with 100 μL of mobile phase and injected into the chromatographic system.

Statistical Analysis

For each experiment, two-way analysis of variance (ANOVA) with treatment and diet as factors were performed. Subsequent post-hoc analysis was carried out using Tukey's comparison test. $p < 0.05$ was considered as statistically significant. Statistical significance between two samples was evaluated by Student's *t*-test.

RESULTS

Antidepressant-Like Effects of Fluoxetine and/or Omega-3 Fatty Acid Treatments

In accordance with our previous study,[17] a 16-day treatment with either antidepressant fluoxetine (10 mg/kg) or diet supplemented with omega-3 fatty acids (720 mg/kg) significantly decreased immobility, concomitant with an increase in swimming without modifying the climbing behavior of adult male rats (Fig. 1a). The same doses of fluoxetine and omega-3 fatty acids administered together reduced immobility and increased swimming behavior. These effects were significantly higher than the

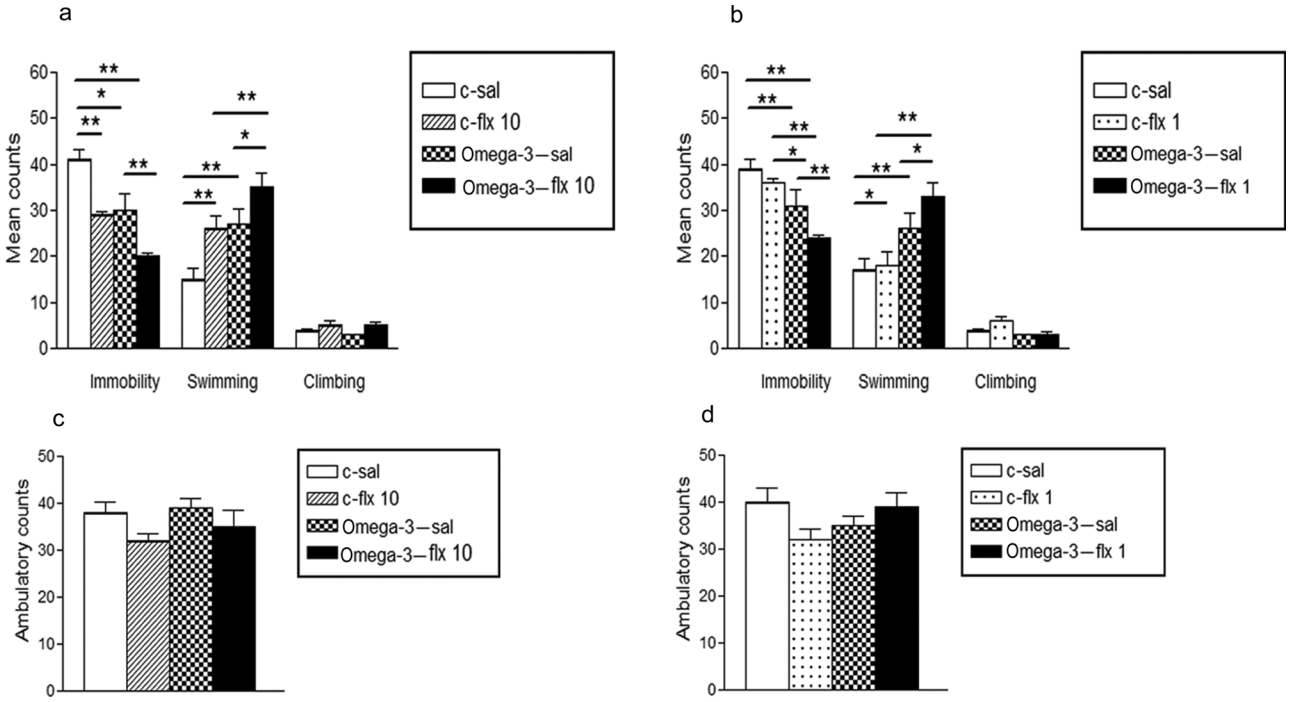

Figure 1. Effect of fluoxetine, omega-3 fatty acids and their combined treatments on behaviors in the forced swimming test (FST) and open field test (OFT). (a) Fluoxetine (10 mg/kg) and/or omega-3 fatty acids (720 mg/kg) in the FST. (b) Fluoxetine (1 mg/kg) and/or omega-3 fatty acids (720 mg/kg) in the FST. (c) Fluoxetine (10 mg/kg) and/or omega-3 fatty acids (720 mg/kg) in the OFT. (d) Fluoxetine (1 mg/kg) and/or omega-3 fatty acids (720 mg/kg) in the OFT. Bars represent the mean number of counts over the 5-min period of the test (± SEM). $^{**}p < 0.01$, $n = 6$–12 rats per group. Data were analyzed by two-way ANOVA followed by Tukey's test for multiple comparisons. c-sal, control diet- saline; c-flx 1, control diet-fluoxetine 1 mg/kg; c-flx 10, control diet-fluoxetine 10 mg/kg; omega-3-sal, omega-3 diet-saline; omega-3-flx 1, omega-3 diet-fluoxetine 1 mg/kg; omega-3-flx 10, omega-3 diet-fluoxetine 10 mg/kg.

individual effects obtained for fluoxetine or omega-3 fatty acid single administration and resulted to be additive (Fig. 1a). Administration of a subeffective dose of fluoxetine (1 mg/kg) had no significant effect on FST (Fig. 1b). However, the combined treatment of omega-3 fatty acids and fluoxetine (1 mg/kg) decreased immobility and increased swimming behavior in a magnitude that exceeded omega-3 individual effects. A significant interaction among diet and treatment was found, indicating the potentiation of omega-3 fatty acid antidepressant-like effect by fluoxetine.

Then, the effect of fluoxetine and/or dietary supplementation with omega-3 fatty acids on spontaneous locomotor activity was evaluated. Animals treated with either fluoxetine or omega-3 fatty acids alone or in combination exhibited equal locomotion behavior (Figs. 1c and 1d), confirming the specificity of the FST results.

Coadministration of Omega-3 Fatty Acids with Fluoxetine Fail to Alter Both Plasma Norfluoxetine Concentration and Brain Levels of Fluoxetine

A possible explanation for the potentiation effect of subeffective doses of fluoxetine on omega-3 fatty acid antidepressant-like effect could involve higher bioavailability and/or changes in drug metabolism exerted by omega-3 fatty acids.

To test this hypothesis, we measured plasma concentrations of fluoxetine and its main metabolite norfluoxetine after a 16-day treatment with fluoxetine (1 mg/kg) alone or in combination with omega-3 fatty acids (720 mg/kg). Norfluoxetine plasma concentration–time curve after last fluoxetine injection on day 17 is shown in Figure 2a. Both treatments generated similar norfluoxetine plasma concentration profiles (AUC ± SEM in ng/mL h: c-flx 1 = 3370 ± 258 vs. omega-3 flx 1 = 3180 ± 312; $p > 0.05$). As expected, fluoxetine plasma levels were low and resulted below the detection limit. Then, to rule out a possible interaction at the blood brain barrier level, we determined brain fluoxetine concentrations. As shown in Figure 2b, coadministration of omega-3 fatty acids with fluoxetine did not modify fluoxetine concentration either in cerebral cortex or in the hippocampus.

Fluoxetine and Omega-3 Fatty Acids Increase Hippocampal DPA Levels in Combined but Not in Individual Treatments

Other possible mechanism for the synergistic effect of fluoxetine on the omega-3 fatty acid antidepressant-like effect could involve changes in brain fatty acid composition. To evaluate this hypothesis, omega-6 and omega-3 fatty acid levels in the hippocampus were measured in all animal groups. Omega-6 fatty acids dihomo-gamma-linolenic acid (DGLA; 20:3), AA (20:4), and docosatetraenoic acid (DTA; 22:4) levels were not modified by either omega-3 fatty acid supplementation or fluoxetine treatments (1 and 10 mg/kg) administered alone or in combination (Fig. 3a). On the contrary, regarding omega-3 fatty acid levels in the hippocampus, omega-3 fatty acid supplementation induced an increase in DHA without modifying EPA and DPA (22:5) levels, whereas fluoxetine alone (1 or 10 mg/kg) had no significant effect on the concentration of any of these omega-3

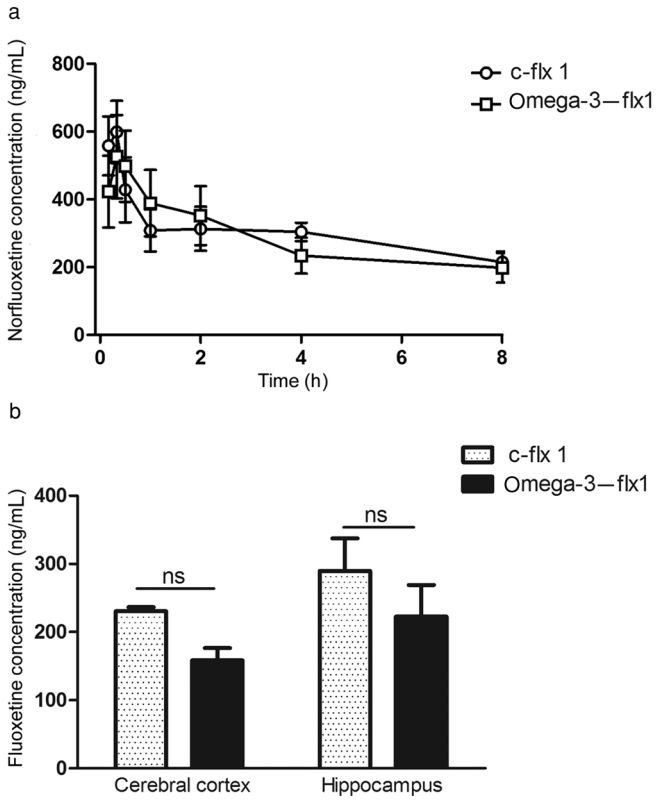

Figure 2. Norfluoxetine plasma levels and brain fluoxetine concentration after single or combined treatments of fluoxetine with omega-3 fatty acids. (a) Time course for norfluoxetine plasma levels and (b) fluoxetine concentrations in the cerebral cortex and the hippocampus after 16-day treatment with fluoxetine (1 mg/kg) alone or in combination with omega-3 fatty acids (720 mg/kg). Results are expressed as mean values (±SEM, $n = 6$–9 or $n = 4$ animals per group for plasma and brain samples, respectively). ns, nonsignificant between bars, two-way ANOVA.

fatty acids in this brain area (Fig. 3b). Surprisingly, coadministration of fluoxetine (both 1 and 10 mg/kg) and omega-3 fatty acids significantly increased DPA levels (Fig. 3b). It is worth noting that the combination of the lower dose of fluoxetine (1 mg/kg) with omega-3 fatty acids increased DPA concentrations to a greater extent than the higher dose of fluoxetine (10 mg/kg) with omega-3 fatty acids.

Oral Administration of DPA Exerts Antidepressant-Like Effects in the FST

To determine whether DPA could have contributed to the synergistic effect of fluoxetine on the omega-3 antidepressant-like effect, DPA was chronically administered and its effect evaluated in the FST. DPA-treated rats exhibited significantly lower immobility time with a concomitant increase in swimming without modifying climbing behavior (Fig. 4a). On the contrary, DPA failed to modify locomotor activity as evaluated by the OFT (Fig. 4b). As swimming behavior could be influenced by body weight,[35] animals were weighted before the beginning of DPA administration and throughout the 16-day treatment. Chronic DPA administration did not modify body weight gain when compared with controls (Fig. 4c).

Chronic DPA Administration Renders Increased Hippocampal DHA and DPA Levels with No Changes in EPA or Omega-6 Fatty Acids

Then, the effect of DPA chronic treatment on omega-6 and omega-3 fatty acids brain levels were evaluated both in the cerebral cortex and the hippocampus. The content of either omega-6 (DGLA, AA, and DTA) or omega-3 (EPA, DPA, and DHA) fatty acids was not affected by DPA treatment in the cerebral cortex (Figs. 5a and 5b).

In the hippocampus, chronic DPA administration did not modify omega-6 (DGLA, AA, or DTA) levels when compared with the control group (Fig. 5c). Interestingly, DPA induced a significant increase in DPA and DHA levels without modifying EPA levels in this brain structure (Fig. 5d).

DISCUSSION

We have previously reported that combined treatments of omega-3 fatty acids with antidepressant drugs may provide therapeutic benefits in terms of final antidepressant action and side effect profile.[17] Omega-3 fatty acid supplementation is able to specifically reduce the immobility time with a concomitant increase in swimming behavior, a behavioral profile consistent with an antidepressant-like action in the FST.[17,36] Also, omega-3 fatty acid supplementation in combination with chronic antidepressant doses of fluoxetine has significantly higher antidepressant-like effects than individual treatments. Indeed, the combined treatment renders an additive antidepressant-like effect at therapeutic doses of antidepressants.[14,17] Surprisingly, supplementation with omega-3 fatty acids in combination with chronic low non-antidepressant doses of fluoxetine exerts a significantly higher antidepressant-like effect than omega-3 fatty acids alone, suggesting that the omega-3 fatty acid antidepressant-like effect can be potentiated with inactive doses of fluoxetine.[17] This is consistent with the idea that lowering the antidepressant dose may help reduce the magnitude of their side effects. However, the mechanisms underlying additive and synergistic effects of omega-3 fatty acids in combination with fluoxetine remain largely unknown.

We aimed this study to explore putative mechanisms involved in additive/synergistic effects of fluoxetine and omega-3 combined treatments. It is known that fluoxetine is extensively metabolized by the hepatic cytochrome P450 enzyme system to form an active N-demethylated metabolite norfluoxetine,[37] which has a similar potency to that of the parent drug.[38,39] It has been described that a significant part of the clinical efficacy of fluoxetine is attributable to norfluoxetine.[40,41] Studies carried out on rats show that norfluoxetine concentration in plasma and brain reaches considerably higher levels than those of fluoxetine during the period of drug treatment. Although fluoxetine accumulates more markedly in rat brain than norfluoxetine, it disappears completely from plasma and brain after treatment stops, whereas norfluoxetine persists up to 7 days after treatment withdrawal.[41] Herein, we studied whether coadministration of omega-3 fatty acids could affect norfluoxetine plasma concentration. We show that coadministration of omega-3 fatty acids fails to modify norfluoxetine concentration profile in plasma after chronic treatment with a low dose of fluoxetine. In accordance with the literature,[42–44] fluoxetine plasma concentrations were below the sensitivity limit of our detection method. Thus, to rule out a possible modulation at

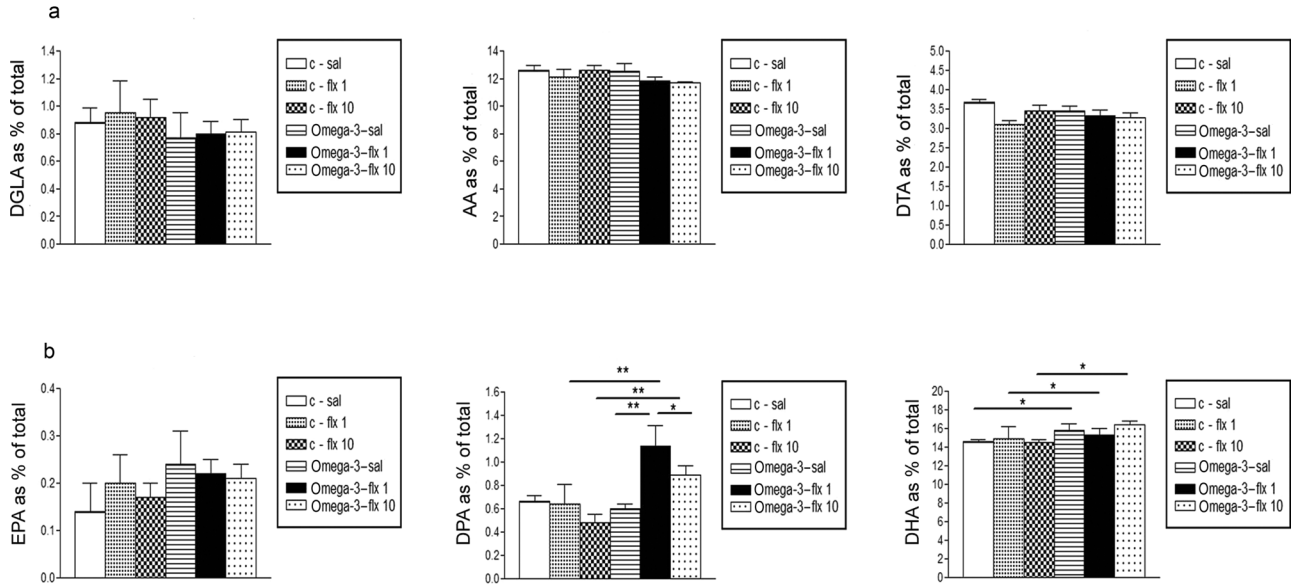

Figure 3. Omega-6 and omega-3 hippocampal levels after single and combined treatments of fluoxetine- and omega-3-supplemented diet. Effects of fluoxetine (1 and 10 mg/kg), omega-3 fatty acids (720 mg/kg) and their combined treatments on (a) omega-6 (DGLA, AA, and DTA) and (b) omega-3 (EPA, DPA, and DHA) fatty acid levels in the hippocampus. Results are expressed as percentage of total fatty acids (mean ± SEM, $n = 6$–9 animals). *$p < 0.05$; **$p < 0.01$, one-way ANOVA followed by Tukey`s test for multiple comparisons. DGLA, dihomo-gamma-linolenic acid (20:3, omega-6); AA, arachidonic acid (20:4, omega-6); DTA, docosatetraenoic acid (22:4, omega-6); EPA, eicosapentaenoic acid (20:5, omega-3); DPA, docosapentaenoic acid (22:5, omega-3); DHA, docosahexaenoic acid (22:6, omega-3).

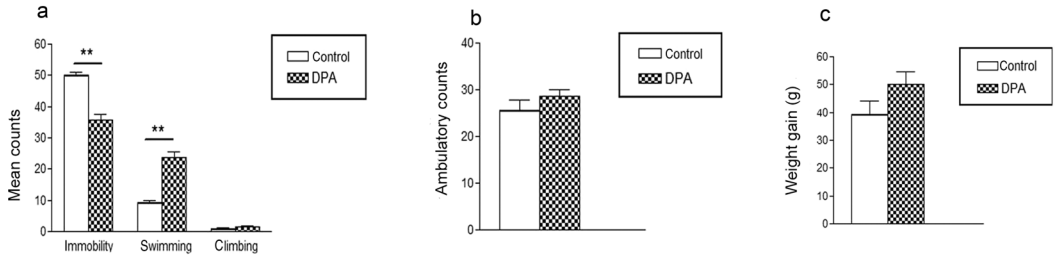

Figure 4. Effect of chronic DPA treatment. After a 16-day oral administration of DPA, its effects in the (a) forced swimming test (FST), (b) open field test (OFT), and (c) on animal weight gain were evaluated. Results are expressed as mean values (±SEM, $n = 7$–10 animals per group). Control, olive oil, DPA, docosapentaenoic acid (150 mg/kg day). **$p < 0.01$, Student's t-test.

the blood brain barrier level, we investigated whether coadministration of omega-3 fatty acids could affect brain fluoxetine concentrations. We show that coadministration of omega-3 fatty acids fails to modify fluoxetine concentrations either in the cerebral cortex or in the hippocampus after chronic treatment with a low dose of fluoxetine. It should be noted that a change in brain norfluoxetine concentration is improbable as it distributes similarly to its parent drug. Therefore, any contribution of a pharmacokinetic interaction to the synergistic/additive antidepressant-like effects observed in the FST in our conditions seems to be unlikely.

The brain is a tissue with high content of omega-6 and omega-3 fatty acids, particularly AA and DHA. These two fatty acids are the major constituents of neural cell membrane phospholipids,[45] whereas DHA helps maintain the integrity and fluidity of these membranes, and can also modify the function of membrane proteins including enzymes, receptors, membrane transporters, ion channels, and the expression of different genes in the brain.[46] In recent years, a large number of studies have been conducted to investigate the influence of diet supplemented with various fatty acids on the lipid composition of the membrane. In many studies, it has been asserted that chronic fatty acid supplementation slowly modifies the cellular membrane phospholipid concentrations that finally lead to changes in neurotransmission.[47,48] However, previous studies have reported that the omega-3 fatty acid supplementation exerts beneficial pharmacological effect regardless of changes in brain membrane phospholipid composition.[17,49]

The two major bioactive components of omega-3 fatty acids, DHA and EPA, are synthesized in the human body from ALA or acquired directly from the diet containing fish or fish products.[50] Epidemiologic and clinical studies suggest that consumption of fish oil (EPA and DHA) is associated with benefits in mood disorders, particularly depression,[51] bipolar disorder,[52–54] and childhood depression.[22] The results of the meta-analysis appear to indicate that EPA and not DHA may be the responsible fatty acid for the antidepressant effects.[55,56] Concerning preclinical studies, most reports on the omega-3 fatty acid antidepressant-like effect have been performed using fish oil (EPA plus DHA) employing the FST.[13–15,18] In contrast,

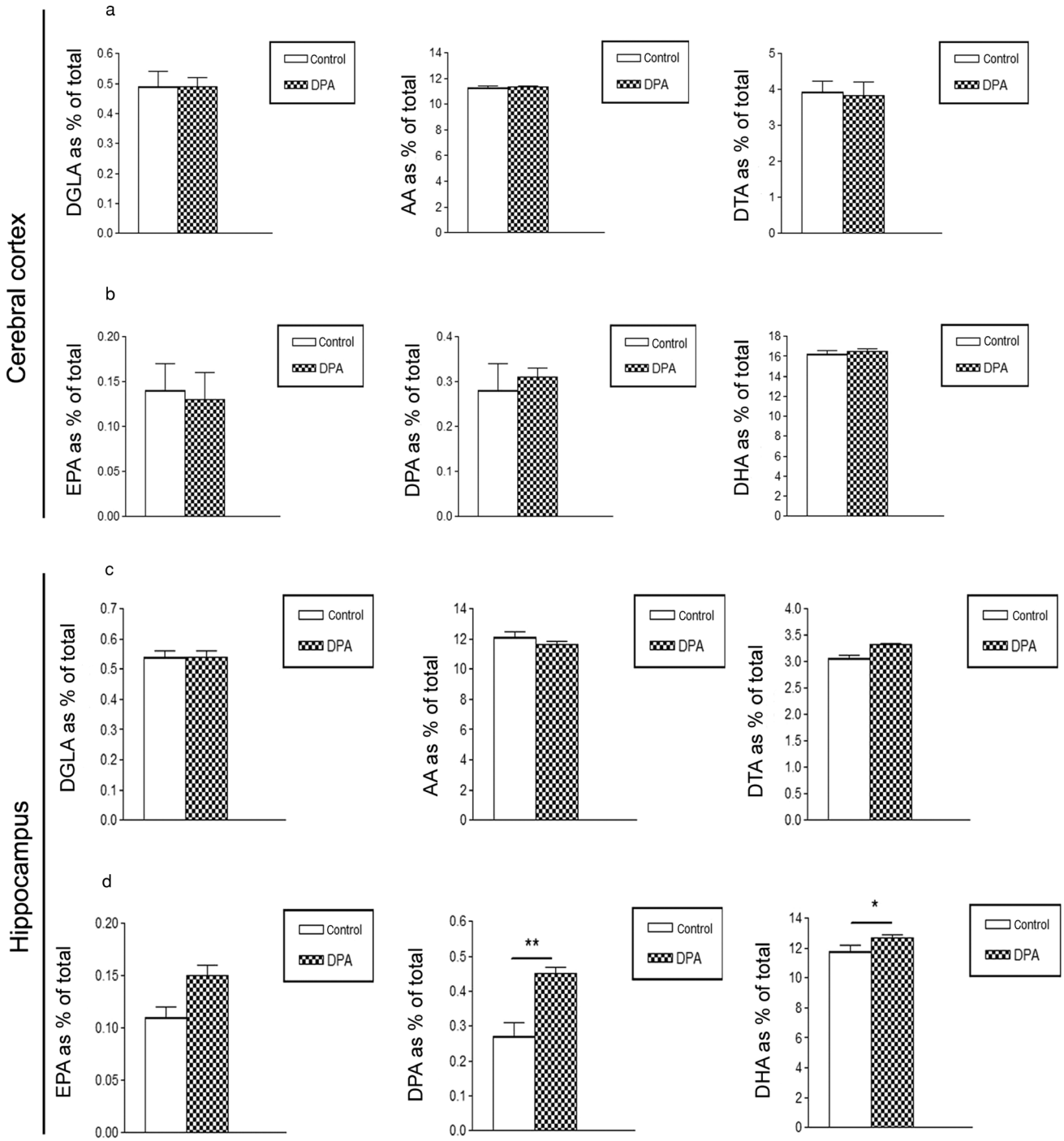

Figure 5. Fatty acid composition in the cerebral cortex and the hippocampus after chronic DPA administration. (a) Omega-6 and (b) omega-3 fatty acid composition in cerebral cortex and (c) omega-6 and (d) omega-3 fatty acid composition in the hippocampus of rats treated with DPA (150 mg/kg day) or control diet for 16 days. Results are expressed as mean values (±SEM, $n = 6–9$) of the total fatty acid content. *$p < 0.05$; **$p < 0.01$, Student's t-test.

a few studies have specifically investigated the effects of EPA alone and have shown that both acute and chronic treatment with EPA fail to induce antidepressant-like effects in the FST in mice.[57] Although it seems possible that these results are because of ineffective doses and/or length of treatment with EPA, the evidence points out DHA as an omega-3 fatty acid with antidepressant action.

Our study shows that chronic omega-3 fatty acids and/or fluoxetine (1 and 10 mg/kg) treatment did not significantly alter brain EPA concentration. In contrast, diet supplementation with omega-3 fatty acids leads to a significant increase in DHA concentration in the hippocampus, which has been associated with antidepressant effects.[15,58–60] Furthermore, our study demonstrates that a shorter omega-3 fatty acid treatment

(16 days) is enough to increase the concentration of DHA in the brain.

Another very significant finding of the present study is that omega-3 fatty acid supplementation in combination with chronic fluoxetine treatment leads to a significant increase in DPA concentrations in the hippocampus. Moreover, we show that DPA exerts antidepressant-like effects in rats. To our knowledge, this is the first report showing the antidepressant-like effect of DPA. Interestingly, supplementation with omega-3 fatty acids in combination with a low non-antidepressant dose of fluoxetine (1 mg/kg) increases DPA levels in a greater extent than the combined treatment with omega-3 fatty acids and fluoxetine at antidepressant doses (10 mg/kg). In this regard, previous studies have found that chronic fluoxetine treatment positively regulates the expression of enzyme-encoding genes, such as delta-5 desaturase and delta-6 desaturase.[61,62] The delta-5 and delta-6 desaturases, encoded by fatty acid desaturase 1 (*FADS1*) and 2 (*FADS2*) genes, respectively, are rate-limiting enzymes in the metabolism of omega-3 and omega-6 fatty acids.[63] Particularly, fluoxetine effect on delta-5 and delta-6 desaturase expression is weaker than that of other antidepressants and fits an U dose–response curve.[62] Taking this in mind, it could be thought that synergistic/additive effects of combined treatment with omega-3 fatty acids and fluoxetine might involve an increase in the hippocampal levels of DPA through the fluoxetine regulation of omega-3 fatty acid biosynthesis. As fluoxetine single treatment at either dose did not increase hippocampal levels of DPA in our experimental conditions, it can be speculated that the weak fluoxetine effect on desaturase expression could render increased fatty acid levels only under excess of substrates. This speculation might explain the specific DPA level increase seen in our study after combined treatments with omega-3 fatty acids. In addition, the fluoxetine U dose–response curve on desaturase expression might as well account for the higher increase in DPA levels produced by the lower dose of this antidepressant. Further studies are guaranteed to shed light on this matter.

In agreement with a previous report,[28] we show no increase in brain EPA levels after DPA treatment. Furthermore, the concentration of EPA following treatment with control diet results relatively low in cerebral cortex and hippocampal phospholipids and comparable with the EPA concentration reported in human brain.[64] Very importantly, herein we show that DPA treatment significantly increases DPA and DHA concentrations in the hippocampus, but not in cerebral cortex. Moreover, the magnitude of the hippocampal DPA increase induced by chronic oral DPA administration is similar to that obtained after fluoxetine and omega-3 combined treatments. Considering that DHA in the brain may be directly obtained from the diet, as preformed DHA, or synthesized *in vivo* from other common dietary omega-3 fatty acids such as ALA, EPA, or DPA,[65–68] a possible explanation for the increased DHA level in the hippocampus after DPA treatment might be that DPA is directly converted to DHA in the brain. Unlike a previous study,[28] we show that oral treatment with DPA increases both DPA and DHA levels in the hippocampus. This discrepancy with the only increment in DPA levels showed by Kaur et al.[28] could be because of the higher dose and the longer treatment employed in our study.

In summary, our findings demonstrate that chronic treatment with omega-3 fatty acid do not increase norfluoxetine plasma or brain fluoxetine concentrations when administered in combined treatment with fluoxetine and therefore suggest the absence of a pharmacokinetic interaction in the synergistic effect seen in combined treatments in our experimental conditions. Besides the expected DHA increase induced by chronic omega-3 administration, our findings reveal other changes in brain omega-3 fatty acid composition after fluoxetine and omega-3 combined treatments. Moreover, these changes are specific for the hippocampal region and involve an increase in the omega-3 fatty acid DPA. Future studies are needed to further explore the DPA role in the synergistic effect of combined treatments, which could shed light on the DPA efficacy for the treatment of depressive disorders.

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C.H.L. and A.R. developed the study concept and design. C.H.L. conducted the animal study. C.H.L. and A.R. analyzed, interpreted the data, and drafted the manuscript. M.F.P. contributed to obtain brain samples. N.S. participated in diet design. P.G. carried out the fatty acid analysis. C.H. worked on fluoxetine/norfluoxetine measurements. C.H.L., A.R., and N.S. made critical revisions of the manuscript. All authors read and approved the final manuscript. The authors have no conflict of interest.

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