CHAPTER 2

Effects of n-3 PUFA enriched and n-3 PUFA deficient diets in naïve and Aβ-treated female rats

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Abstract

Depression is one of the most common psychiatric diseases and the prevalence of depressive symptoms in women is almost twice compared to men, although the reasons of this gender difference are not fully understood yet. Recently, soluble Aβ_{1-42} peptide has been receiving great importance in the development of depression, also since depression is highly comorbid with Alzheimer’s disease and other neurodegenerative illnesses. Accordingly, we have previously shown that central Aβ injection is able to elicit depressive-like phenotype in male rats. In the present study, we reproduced for the first time the Aβ-induced depressive-like model in female rats, evaluating behavioural and neurochemical outcomes. Moreover, we studied the effect of lifelong exposure to either n-3 PUFA enriched or n-3 PUFA deficient diet, in female rats, both intact and after central Aβ administration. Our results confirmed the Aβ-induced depressive-like profile also in female rats. Moreover, chronic exposure to n-3 PUFA deficient diet led to highly negative alterations in behavioural and neurochemical parameters, while lifelong exposure to n-3 PUFA enriched diet was able to restore the Aβ-induced depressive-like profile in female rats. In conclusion, the Aβ-induced depressive-like profile was reversed by n-3 PUFA supplementation, indicating a possible therapeutic role of n-3 PUFA in the treatment of the burden of depressive disorders.
2.1 Introduction

Depression is one of the most common psychiatric diseases and the prevalence of depressive symptoms has reached epidemic proportions during the last few decades (Gorman, 2006). In this regard, several studies reported that depression is more prevalent in women compared to men (Gorman, 2006; Kokras et al., 2015; Marcus et al., 2005). Although the reasons of this gender difference are not fully understood yet, women show different response to sex hormones, that might ultimately influence behaviour and brain functions (Marrocco & McEwen, 2016).

In particular, estrogens modulate several neural and behavioural functions, including mood, cognitive function, blood pressure regulation, motor coordination, pain, and opioid sensitivity (McEwen & Milner, 2017). In addition, it has been shown that estrogens also affect neurotrophic functions and monoamine neurotransmission in several brain areas, thus they might ultimately be involved in the pathogenesis of depressive-like disorders (Borrow & Cameron, 2014). These evidence suggest that the antidepressant therapy should be personalized, taking into account also gender differences (Sloan & Kornstein, 2003; Thiels, Linden, Grieger, & Leonard, 2005). In addition, a series of studies indicated that estrogens modulate the metabolic production of different endogenous and exogenous molecules (M. Barton et al., 2017; Laredo, Villalon Landeros, & Trainor, 2014; Migliaccio, Davis, Gibson, Gray, & Korach, 1992). Among these molecules, it has been reported that estrogens stimulate the conversion of essential fatty acids into their longer chain metabolites, such as α-linolenic acid conversion into docosahexanoic acid (DHA) (Burdge & Wootton, 2002; Giltay, Gooren, Toorians, Katan, & Zock, 2004). DHA is a key n-3 polyunsaturated fatty acid (PUFA) involved in the Central Nervous System (CNS) development (Colangelo et al., 2017) and, thus, fundamental during pregnancy and early stage of childhood (Echeverria, Valenzuela, Catalina Hernandez-Rodas, & Valenzuela, 2017). DHA and arachidonic acid (AA, 20:4n-6) are biologically important PUFAs, and can be supplied either directly from diet or by metabolic conversion of their essential precursors α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), respectively (Morgese, Tucci, et al., 2017). DHA, AA and their mediators modulate several processes, such as signal pathways, membrane fluidity, neurotransmission, neuroinflammation and cell survival (Echeverria et al., 2017). During embryonic life and lactation, PUFAs intake exclusively depends on maternal diet, as the metabolic conversion of essential precursors cannot be accomplished (Lafourcade et al., 2011). Indeed, in utero exposure to unbalanced diet can be an important risk factor for mental disorders in later adulthood. Modern western diets are
characterized by low fish consumption and more junk food, resulting in n-3 PUFA deficiency and abnormal n-6 PUFA increase, respectively (Simopoulos, 2011). This unbalanced n-6/n-3 ratio is considered to be detrimental for the CNS functioning. Indeed, recent research suggests an etiological role for n-3 PUFAs deficiency in mood disorders, such as Major Depressive Disorder (MDD) (Grosso et al., 2016; McNamara & Welge, 2016). Accordingly, different epidemiological studies reported an inverse correlation between n-3 PUFA intake and depressive symptoms among United States women (Beydoun et al., 2013; Beydoun et al., 2015). In this regard, we have previously shown that lifelong deficiency of n-3 PUFA leads to a depressive-like phenotype associated with reduced serotonin (5-HT) levels and increased soluble amyloid beta (Aβ)_{1-42} concentrations (Morgese, Tucci, et al., 2017) in male rats. The Aβ_{1-42} peptide, produced through proteolytic cleavage of the amyloid precursor protein (APP), has been demonstrated to have powerful neurotoxic effects (Pomara & Sidtis, 2007). Recently, soluble Aβ_{1-42} peptide has been received great importance in the development of depression, also since depression is highly comorbid with Alzheimer’s disease and other neurodegenerative illnesses (Schiavone, Tucci, et al., 2017; Sun et al., 2008). In our previous studies, we injected soluble Aβ_{1-42} in the ventricular area of male rats, provoking a depressive-like phenotype (Colaianna et al., 2010), accompanied by reduced cortical 5-HT and neurotrophins, such as Nerve Grow Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF).

Although the majority of animal studies on depression use males in order to avoid the variability that hormonal cycle could induce (Altemus, 2006), the US National Institute of Health is strongly encouraging preclinical research on females (Kokras et al., 2015). For this reason, considering also the higher incidence of depressive disorders in women, the development of preclinical models of depressive-like profile in females is becoming necessary (D’Souza & Sadananda, 2017).

In the present study, we reproduced for the first time the Aβ-induced depressive-like model in female rats, evaluating behavioural and neurochemical outcomes. Moreover, we studied the effect of lifelong exposure to either n-3 PUFA enriched or n-3 PUFA deficient diet, in female rats, both intact and after Aβ central administration.
2.2 Materials and Methods

Animals

Adult (250-300g) Wistar rats (Harlan, S. Pietro al Natisone, Udine) were used in this study. They were housed at constant room temperature (22±1°C) and relative humidity (55±5%) under a 12 h light/dark cycle (lights on at the 7 A.M.) with ad libitum access to food and water. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of the Italian Ministry of Health (D.L. 26/2014), the Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2004), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures involving animals were conducted in accordance to ARRIVE guidelines. Animal welfare was daily monitored through the entire period of experimental procedures. No signs of distress were evidenced, anyway all efforts were made to minimize the number of animals used and their suffering.

Diets

One male and two female rats were housed together for mating. Animals were exposed to specific diets mimicking lifelong n-3 PUFA deficiency or supplementation, as previously described (Aid et al., 2003; Lafourcade et al., 2011; Morgese et al., 2016). In particular, after mating dams were randomly assigned to the group fed with either a diet containing 6% total fat in the form of only rapeseed oil (n-3 enriched, rich in linolenic acid 18:3n-3) or peanut oil (n-3 deficient, rich in linoleic acid 18:2n-6) throughout gestation and lactation. As control group, dams were fed with a diet containing 6% total fat in the form of 3% of peanut oil plus 3% of rapeseed oil, called control diet. After weaning, offspring continued to be subjected to the same diet throughout life. All experiments were carried out in female eight-week-old rats.

Effects on experiments carried out may be influenced by the time of their estrous cycle (Jans, Lieben, & Blokland, 2007). Pro-estrous/estrus events tend to be dictated by lighting times, but under normal lighting schedules (as in the present study) tend to occur during the late afternoon to early hours of the morning (Witcher & Freeman, 1985). Hence, all animal procedures were performed in the morning (usually 09.00–12.00 h) to reduce estrous cycle effects. Serum estradiol concentrations were measured to take account of possible differences in the estrous cycle.
**Aβ administration**

The Aβ<sub>1-42</sub> peptide was purchased from Tocris (Bristol, UK) and was dissolved in sterile double-distilled water (vehicle) at a concentration of 4 μM as previously describe (Colaianna et al., 2010). All solutions were freshly prepared. 7-weeks-old rats were anesthetized with 3.6 ml/kg Equithesin intraperitoneally (i.p.; composition: 1.2 g sodium pentobarbital; 5.3 g chloral hydrate; 2.7 g MgSO<sub>4</sub>; 49.5 ml propylene glycol; 12.5 ml ethanol and 58 ml distilled water) and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The skin was shaved, disinfected and cut with a sterile scalpel to expose the skull and a hole was drilled to insert the infusion needle (30-gauge stainless steel tubing; Cooper’s Needles, Birmingham, UK). Coordinates for icv infusions were based on the atlas of Paxinos and Watson (1998): AP = -0.5, ML = +1.2 and DV = -3.2 from bregma, with the incisor bar set at -3.3 mm. Soluble Aβ (5 μl) was delivered through a 25 μl Hamilton microsyringe at 2 μl/min infusion rate over a period of 2.5 min, with an additional 5 min allowed to elapse prior to removal of the infusion needle. Control rats were infused with vehicle only, because reverse Aβ<sub>42-1</sub>, used in preliminary experiments, had no effect on the measured neurochemical parameters and was indistinguishable from vehicle alone (unpublished observations). The injection placement of needle track was verified at the time of dissection. All experimental procedures were performed 7 days after icv administration (SHAM or Aβ-treated groups).

**Forced swimming test**

The forced swimming test (FST) is a reliable task for discriminating depressive state in animals and is widely used for predicting antidepressant properties of drugs (Porsolt, Bertin, & Jalfre, 1977). On the first of the two test days, animals were placed individually in inescapable Perspex cylinders (diameter 23 cm; height 70 cm) filled with water at constant temperature of 25±1°C at 30 cm of height (Cryan, Valentino, & Lucki, 2005).

During the preconditioning period, animals were videotaped for 15 min. Then, rats were removed and dried before to be returned to their home cages. Twenty-four h later, each rat was positioned in the water-filled cylinder for 5 min. This session was video-recorded and subsequently scored by an observer blind to the treatment groups. During the test sessions, the frequency that rats spent performing the following behaviors were measured: struggling (time spent in tentative of escaping), swimming (time spent moving around the cylinder) and immobility (time spent
remaining afloat making only the necessary movements to keep its head above the water). Data were expressed as frequency on 5 sec counts.

**Post-mortem tissue analysis**

Rats were euthanized and brains were immediately removed and cooled on ice for dissection of target region, namely PFC, according to the atlas of Paxinos and Watson (1998). Tissues were frozen and stored at -80°C until analysis was performed.

**HPLC quantifications**

5-HT, 5-hydroxyindolacetic acid (5-HIAA) and dopamine (DA) concentrations were determined by HPLC coupled with an electrochemical detector (Ultimate ECD, Dionex Scientific, Milan, Italy). Separation was performed by a LC18 reverse phase column (Kinetex, 150 mm×4.2 mm, ODS 5 μm; Phenomenex, Castel Maggiore- Bologna, Italy). The detection was accomplished by a thin-layer amperometric cell (Dionex, ThermoScientifics, Milan, Italy) with a 5 mm diameter glassy carbon electrode at a working potential of 0.400 V vs. Pd. The mobile phase used was 75 mM NaH2PO4, 1.7 mM octane sulfonic acid, 0.3 mM EDTA, acetonitrile 10%, in distilled water, buffered at pH 3.0. The flow rate was maintained by an isocratic pump (Shimadzu LC-10 AD, Kyoto, Japan) at 0.7 ml/min. Data were acquired and integrated using Chromeleon software (version 6.80, Thermo Scientific Dionex, San Donato Milanese, Italy).

**ELISA quantifications**

PFC samples were analyzed for NGF quantifications by using ELISA kits provided by Cloud-Clone Corporation (Houston, Texas, USA). Assays were performed according to the manufacturer’s instructions. Briefly, tissues were diluted (10% tissue weight/total volume) with ice-cold medium containing phosphate-buffered saline (PBS) and protease inhibitor cocktail (Sigma-Aldrich, Milan, Italy). Samples were homogenized and centrifuged at 10.000 x g at 4°C for 20 min. The supernatant was collected and assays were performed according to the manufacturer’s instructions. To normalize data and negate differences due to sample collection, protein concentration was determined by using the BCA assay kit. Each sample analysis was carried out in duplicate to avoid intra-assay variations.

Plasma samples were analyzed for soluble Aβ1-42 by using an ELISA kit provided by Cloud-Clone Corporation (Houston, Texas, USA). Assays were performed according to the manufacturer’s instructions. Each sample analysis was carried out in duplicate to avoid intra-assay variations.

**Statistical analyses**
Results were expressed as mean ± S.E.M. Behavioural and neurobiological data were analyzed by using one or two-way analysis of variance (ANOVA) followed by Bonferroni post hoc analyses, as required. P value was set at 0.05.

2.3 Results

Effects of n-3 PUFA deficient diet on depressive-like behaviour using FST

To investigate the influence of lifelong exposure to n-3 PUFA deficient and n-3 PUFA enriched diet on depressive-like behaviour, we performed the forced swimming test (FST). Our results show that n-3 PUFA deficient diet significantly increased the immobility frequency compared to control diet (Figure 1A, One-way ANOVA followed by Bonferroni’s multiple comparison test, $F=4.351$, $P<0.05$ n-3 deficient versus CTRL). Moreover, there were no significant differences in struggling frequency (Figure 1B, One-way ANOVA followed by Bonferroni’s multiple comparison test, n.s.), while swimming was significantly decreased in n-3 PUFA deficient diet-exposed animals (Figure 1C, One-way ANOVA followed by Bonferroni’s multiple comparison test, $F=4.929$, $P<0.05$ n-3 deficient versus CTRL).
Figure 1: Effects of control, n-3 PUFA enriched and n-3 PUFA deficient diets on FST. Frequency measure of immobility (A), struggling (B), and swimming (C) behaviours in female rats fed from conception until 5 weeks post-weaning with control diet (white bar), n-3 PUFA enriched diet (grey bar), and n-3 PUFA deficient diet (black bar). Data are expressed as mean ± SEM (n=12-13 per group). One-way ANOVA followed by Bonferroni’s multiple comparison test, #P < 0.05 vs. CTRL.

Effects of n-3 PUFA deficient diet on plasmatic Aβ levels
We quantified plasmatic soluble Aβ_{1-42} peptide in offspring of rats fed with n-3 PUFA enriched and n-3 PUFA deficient diets. We found that animals exposed throughout their life to n-3 PUFA deficient diet had a significant increase in plasmatic Aβ levels compared to controls (Figure 2, one-way ANOVA followed by Bonferroni’s multiple comparison test, F=9.164, P<0.01 n-3 deficient versus CTRL).
Figure 2 Effects of control diet (white bar), n-3 PUFA enriched diet (grey bar), and n-3 PUFA deficient diet (black bar) on plasmatic soluble Aβ levels. Data are expressed as mean ± SEM (n=6-7 per group). One-way ANOVA followed by Bonferroni’s multiple comparison test **P < 0.01 vs. CTRL.

Effects of n-3 PUFA enriched diet on Aβ-induced depressive-like behaviour using FST

Our group has previously demonstrated that Aβ soluble peptide is able to evoke a depressive-like state (Colaianna et al.), thus we tested whether lifelong exposure to n-3 PUFA enriched diet would prevent such Aβ-induced alterations in female offspring rats. As shown in Figure 3A and 3C, n-3 PUFA enriched diet prevented the depressive effect of Aβ. Indeed, immobility frequency was significantly increased and swimming frequency was significantly reduced in Aβ treated rats compared to SHAM operated only in control animals (Figure 3A, Two-way ANOVA followed by Bonferroni’s multiple comparison test; F(1,32)=6.258, P<0.01, Aβ versus SHAM rats; Figure 3C, Two-way ANOVA followed by Bonferroni’s multiple comparison test; F(1,32)=13.57, P<0.01, Aβ versus SHAM rats), while no differences were evidenced in struggling frequency among groups (Figure 3B, Two-way ANOVA followed by Bonferroni’s multiple comparison test; n. s.).
Figure 3 Effects of control and n-3 PUFA enriched diet on Aβ-induced depressive-like behaviour. Frequency measure of immobility (A), struggling (B), and swimming (C) behaviours in female rats SHAM-operated (white bar) and Aβ-operated (black bar). Data are expressed as mean±SEM (n=9-12 per group). Two-way ANOVA followed by Bonferroni’s multiple comparison test P<0.01, vs. SHAM rats.

Effects of n-3 PUFA deficient and n-3 PUFA enriched diets on serotonin levels and turnover in PFC

In order to better investigate behavioural results, we performed also neurochemical analyses. In particular, we quantified serotonin (5-HT) content and 5-HT turnover (5-HIAA/5-HT ratio) in PFC. We found that cortical 5-HT concentrations were significantly lower in animals pre- and post-natal fed with n-3 PUFA deficient diet compared to controls (Figure 4A, one-way ANOVA followed by Bonferroni’s multiple comparison test, F=3.546, P<0.05 n-3 deficient versus CTRL). Moreover, 5-HT turnover was significantly increased in n-3 PUFA deficient rats compared to controls animals (Figure 4B, one-way ANOVA followed by Bonferroni’s multiple comparison test, F=6.086, P<0.05 n-3 PUFA versus n-6/n-3 CTRL). We also quantified 5-HT content in PFC of female rats exposed during their entire life to n-3 PUFA enriched or control diet 7 days after Aβ icv injection. In
particular, Aβ injection significantly reduced 5-HT content in control rats (Figure 4C, two-way ANOVA followed by Bonferroni’s multiple comparison test, $F_{(1,17)}=3.431$ $P<0.05$ Aβ-treated vs SHAM operated rats), while in n-3 PUFA fed animals no differences were retrieved between groups, indicating a protective effect of this diet towards Aβ-induced impairment (Figure 4C, two-way ANOVA followed by Bonferroni’s multiple comparison test, n.s., Aβ-treated vs SHAM operated rats). In regard to 5-HT turnover, no differences were found among experimental groups (Figure 4D, two-way ANOVA followed by Bonferroni’s multiple comparison test, n.s.).

**Figure 4** Effects of control (white bar), n-3 PUFA enriched (grey bar) and n-3 PUFA deficient (dark bar) diets on cortical 5-HT levels (A) and 5-HIAA/5-HT ratio (B) in naive animal. Data are expressed as mean ± SEM. One-way ANOVA followed by Bonferroni’s multiple comparison test, *P<0.05 vs. CTRL. Effects of control and n-3 PUFA enriched diet on cortical 5-HT levels (C) and 5-HIAA/5-HT ratio (D) in SHAM- (white bar) and Aβ-operated (dark bar) females. Data are expressed as mean ± SEM (n=5-7 per group). Two-way ANOVA followed by Bonferroni’s multiple comparison test, *P<0.05 vs. SHAM-operated.
Effects of n-3 PUFA deficient and n-3 PUFA enriched diets on dopamine levels in PFC

We quantified cortical dopamine in female offspring fed with n-3 PUFA enriched; n-3 PUFA deficient or control diets and no significant differences were found (Figure 5A, One-way ANOVA followed by Bonferroni’s multiple comparison test, n.s.). We also analyzed dopamine content in PFC of female rats exposed during their entire life to n-3 PUFA enriched or control diets 7 days after Aβ icv injection; we found a significant increase in dopamine content of Aβ-treated animals compared to SHAM operated only in n-3 PUFA fed animals, suggesting a specific interaction with dopaminergic system only in presence of Aβ (Figure 5B, two-way ANOVA followed by Bonferroni’s multiple comparison test, F\(_{1,20}\)=5.873, P<0.05, Aβ-treated vs SHAM operated rats).

Figure 5 Effects of control (white bar), n-3 PUFA enriched (grey bar) and n-3 PUFA deficient (dark bar) diets on cortical DA levels in naive (A), SHAM- (white bar) and Aβ-operated (dark bar) females (B). Data are expressed as mean ± SEM (n=6 per group). One- and Two-way ANOVA followed by Bonferroni’s multiple comparison test *P<0.05 vs. SHAM-operated.

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Effects of n-3 PUFA deficient and n-3 PUFA enriched diets on cortical NGF protein content

To endorse our results on behavioral analyses, we measured NGF protein levels in PFC of our experimental groups. We found that NGF was significantly reduced in n-3 PUFA deficient animals compared to animal exposed to n-3 PUFA enriched and control diets (Figure 6A, One-way ANOVA followed by Bonferroni’s multiple comparison test, F=7.514, P<0.001 n-3 deficient versus n-3 enriched, P<0.05 n-3 deficient vs CTRL diet).

Interestingly, cortical NGF concentrations significantly increased after Aβ administration in n-3 PUFA fed animals compared to controls, still confirming a protective role of this diet towards Aβ-induced impairments (Figure 6B, Two-way ANOVA followed by Bonferroni’s multiple comparison test, F(1,16)=4.835 ,P<0.05 n-3 enriched vs CTRL diet).

Figure 6 Effects of control (white bar), n-3 PUFA enriched (grey bar) and n-3 PUFA deficient (dark bar) diets on cortical NGF levels in naïve (A), SHAM- (white bar) and Aβ-operated (dark bar) females (B). Data are expressed as mean ± SEM
(n=5-6 per group). One- and Two-way ANOVA followed by Bonferroni’s multiple comparison test #P<0.05 vs. CTRL, **P<0.01 vs. n-3 enriched, #P<0.05 vs. Aβ-operated CTRL diet.

2.4 Discussion
In the present study, we showed that chronic exposure to n-3 PUFA deficient diet leads to highly negative alterations in behavioural and neurochemical parameters, while lifelong exposure to n-3 PUFA enriched diet is able to restore the Aβ-induced depressive-like profile in female rats. From a behavioural point of view, our results showed an increase in immobility frequency and a decrease in swimming frequency in FST in female adult offspring fed during their entire life with n-3 PUFA deficient diet. FST is a reliable test widely used to assess depressive-like state and screen antidepressants activity in rodents (Li et al., 2017). This test is based on learned helplessness that results in depressive-like symptoms, such as immobility increase and swimming and struggling decrease. These results are in line with our previous study, in which we reported a significant increase in immobility and decrease in swimming and struggling frequency in male rats fed with a diet poor in n-3 PUFA, confirming the positive effect of n-3 PUFA supplementation (Morgese, Tucci, et al., 2017). In order to rule out whether the increased immobility frequency and the decreased swimming frequency could be due to locomotor impairments, we performed OF test, whose results indicated no differences in vertical or horizontal activity in all experimental groups. Thus, the impairment retrieved in the FST could not be attributed to alteration in locomotion, but it might be related instead to neurobiological alterations induced by low n-3 PUFA consumption.
In addition, we quantified plasmatic concentrations of Aβ in female animals receiving a diet either rich or poor in n-3 PUFA. Our results showed that plasmatic Aβ levels were significantly increased in female rats fed with poor n-3 PUFA diet compared to controls. In good agreement, our previous study in male rats showed that a diet poor in n-3 PUFA increased plasmatic Aβ levels compared to controls, while high n-3 PUFA diet significantly decreased such levels (Morgese et al., 2016). Recently, the Aβ peptide, particularly in its soluble forms, is gaining more and more attention in the study of depressive disorders (Colaianna et al., 2010; Pomara & Sidiatis, 2007; Schiavone, Tucci, et al., 2017; Sun et al., 2008). In this regard, our group has previously demonstrated that central Aβ administration can evoke a depressive like-phenotype in rats characterized by increased immobility frequency in the FST and by reduced cortical 5-HT and neurotrophin levels (Colaianna et al., 2010). Regarding Aβ and n-3 PUFA interaction, recent studies suggest a crucial role played
by n-3 PUFA in the production/clearance of the Aβ peptide (Hopperton, Trepanier, Giuliano, & Bazinet, 2016; Lim et al., 2005). Indeed, it has been shown that n-3 PUFA, by increasing membrane fluidity, promote the Aβ interaction with membrane lipid bilayers, influencing the peptide aggregation process (Emendato et al., 2016). Thus, we can speculate that in our model the decrease availability of n-3 PUFA in plasmalemma, secondary to deficiency in n-3-PUFA consumption, may lead to less interaction of Aβ species to the membrane, ultimately resulting in higher soluble Aβ levels.

To better understand the link between Aβ and PUFA and to investigate possible gender differences, we administered the soluble Aβ peptide in female offspring fed with n-3 PUFA enriched or control diet. 7 days after Aβ icv, we performed the FST and we found that in control animals immobility frequency was significantly increased and swimming frequency was significantly decreased in Aβ-treated females compared to SHAM operated animals, confirming the efficacy of the Aβ-depressive-like model also in females. Conversely, in n-3 PUFA fed animals, there were no differences between Aβ injected and SHAM operated animals, indicating a protective role of n-3 PUFA diet on the depressive-like phenotype induced by soluble Aβ injection.

From a neurochemical point of view, we focused on 5-HT, 5-HT metabolism and DA in PFC. In this regard, we found that cortical 5-HT was significantly decreased in n-3 PUFA deficient females and 5-HIAA/5-HT ratio was significantly increased, confirming the deleterious effects of a diet poor in n-3 PUFA. Furthermore, cortical 5-HT was significantly reduced in Aβ-treated animals compared to SHAM operated animals, both fed with control diet, consolidating the Aβ-induced depressive-like profile. As widely known, 5-HT and its metabolism impairment are strongly involved in the pathogenesis of depression and Selective Serotonin Reuptake Inhibitors (SSRI) are the most used pharmacological treatment for major depressive disorder (Salaminios et al., 2017). Moreover, in an interesting clinical study, Barton and Colleagues found an elevated brain 5-HT turnover in unmedicated patients with depression (D. A. Barton et al., 2008) and several studies also reported a decrease in brain 5-HT turnover after classical or natural antidepressant treatments (Ahmed & Azmat, 2017; S. H. Lin et al., 2015). Interestingly, we found that n-3 PUFA enriched diet was able to restore 5-HT levels in Aβ treated animals. Among the several mechanisms that have been proposed to explain the influence of n-3 PUFA on the 5-HT synthesis, release and function in the brain, one of the most important might be the DHA modulation of 5-HT receptors accessibility (Patrick & Ames, 2015). In particular, DHA increases cell membrane fluidity in postsynaptic
neurons, thus, in low DHA conditions, the membrane becomes less fluid and the binding of serotonin to its receptor decreases significantly, due to the lower accessibility of serotonin receptors (Jones, Arai, & Rapoport, 1997; Paila, Ganguly, & Chattopadhyay, 2010). This effect is not limited to the serotonin receptors, but also affects the dopamine receptors and other neurotransmitter receptors (Paila et al., 2010). Furthermore, n-3 PUFA might influence serotonin neurotransmission acting through the inflammatory pathways. Interestingly, McNamara and colleagues showed that n-3 PUFA deficiency was positively correlated with pro-inflammatory cytokine production, leading to an increase in central 5-HT turnover, while n-3 PUFA supplementation prevented this negative effect (McNamara, Able, Rider, Tso, & Jandacek, 2010).

As regard other monoaminergic neurotransmissions, several evidence pointed out to an important role of dopaminergic system in the pathogenesis of depression (Finan & Smith, 2013; Hori & Kunugi, 2013; Tye et al., 2013). In our model, we found no differences in cortical DA in naïve animals fed with n-3 PUFA enriched or n-3 PUFA deficient diet, but after Aβ injection, DA was significantly increased in animals exposed to n-3 PUFA enriched diet compared to SHAM operated animals.

Recent preclinical studies have indicated the involvement of dopaminergic receptors, either D1, D2 or D3, in the antidepressant effects (Pytka et al., 2016). In addition, it was shown that lesion of dopamine neurons in ventral tegmental area lead to dopamine depletion in the nucleus accumbens, producing depressive-like phenotype in the animals (Furlanetti, Coenen, & Dobrossy, 2016).

In this regard, very little is known about relationships between PUFA status and dopaminergic functioning in major depression. In a clinical study, DHA was inversely correlated with homovanillic acid, the main DA metabolite, in cerebrospinal fluid, indicating a possible link between n-3 PUFA status and dopaminergic tone in the brain (Sublette et al., 2014). Moreover, Zimmer and Colleagues demonstrated that in n-3 PUFA deficient rats, dopamine vesicles are specifically decreased in frontal cortex, inducing modification in dopamine metabolism (Zimmer et al., 2002). The mechanism leading to this modification might involve different pathways, such as vesicle turnover and membrane fluidity. Hence, even if drugs acting on dopaminergic system play a marginal role in the treatment of depression, this still remains a field open for future investigations.
In the searching for biological substrate involved in depressive state, neurotrophins also play a significant role. In this regard, we found a decrease of cortical NGF in female rats exposed to n-3 PUFA deficient diet, further confirming the putative pro-depressive effect of a diet poor in n-3 PUFA. Moreover, in Aβ treated animals, NGF was significantly increased in n-3 PUFA fed animals compared to controls, supporting the hypothesis of a possible therapeutic effect of n-3 PUFA in pathological conditions. Accordingly, it has been demonstrated that neurotrophic factors are relevant in neurodegenerative diseases, such as Parkinson’s and Alzheimer’s disease (Iulita et al., 2017; Triaca & Calissano, 2016). Since neurodegenerative symptoms also occur in depression, a neurotrophin hypothesis of depression has been raised. In particular, it has been shown that NGF decrease contributes to the etiology of depression (Song, Zhang, & Manku, 2009). Interestingly, it has been reported that dopamine agonists, such as bromocriptine, bergolide, cabergolide, may promote the synthesis and secretion of NGF (Ohta et al., 2010; Ohta et al., 2003). These data are in line with our results, in which Aβ treated females exposed to n-3 PUFA enriched diet showed an increase in cortical dopamine and also in cortical NGF. These results suggest a beneficial effect of n-3 PUFA supplementation in Aβ- induced depressive-like symptoms, acting also through dopamine neurotransmission. Our hypothesis is supported by previous clinical studies in randomized placebo-controlled trials in which the antidepressant efficacy of n-3 PUFA supplementation is equivalent to and also additive to the effects of classical antidepressants (Gertsik, Poland, Bresee, & Rapaport, 2012; Jazayeri et al., 2008; J. J. Liu et al., 2013).

Although our group is a pioneer on the depressive-like model induced by soluble Aβ icv injection (Colaianna et al., 2010; Morgese et al., 2015; Morgese, Schiavone, et al., 2017; Morgese et al., 2014; Morgese, Tucci, et al., 2017; Schiavone, Tucci, et al., 2017; Trabace, 2014; Trabace et al., 2007; Tucci et al., 2014), this is the first study using female rats. In particular, female trends confirm male results regarding Aβ-induced depressive-like profile. Literature about estrogens involvement in the pathogenesis of depression is controversial. In this regard, there are several studies supporting the influence of hormonal fluctuations in the development of mental illnesses, in particular pointing toward the importance of sex hormones as neuro-endocrine modulators (Fink, Sumner, McQueen, Wilson, & Rosie, 1998; Kudielka & Kirschbaum, 2005; Soares, Castro, Reis-Henriques, Monteiro, & Santos, 2012). Nevertheless different studies reported the influence of social factors, such as marital and employment status, as a trigger in the development of depression in women (Bulloch, Williams, Lavorato, & Patten, 2009; Diaz, Guendelman, &
From our point of view, females did not show differences compared to males, revealing the same Aβ-induced phenotype.

Furthermore, n-3 PUFA supplementation showed differences in naïve females compared to Aβ treated animals, indicating a positive effect only in presence of Aβ-induced dysfunctions. These results endorse the hypothesis of a possible therapeutic use of n-3 PUFA supplementation, acting in synergy with antidepressants or even alone. In support to our findings, several studies supported the benefits of n-3 PUFA supplementation in the treatment of post partum depressive symptoms (Chong et al., 2015; Sparling, Henschke, Nesbitt, & Gabrysch, 2017), while a recent study reported no efficacy of daily n-3 PUFA supplementation in the prevention of maternal depressive symptoms (Vaz, Farias, Adegboy, Nardi, & Kac, 2017).

Conversely, the deleterious effects of n-3 PUFA deficiency are widely shown and the inverse correlation between low n-3 PUFA intake and increase of depressive-like symptoms is extensively reported (Beydoun et al., 2013; Beydoun et al., 2015; Grosso et al., 2016). Although the underlying mechanisms of action are still unclear and different hypotheses have been raised, mainly based on up or down regulation of physiological n-3 PUFA pathways. Among these pathways, we focused on the modulation of n-3 PUFA supplementation or deficiency on monoamine neurotransmission, especially 5-HT, DA and their metabolism. In particular, the highly unsaturated nature of EPA and DHA results in the influence of membrane fluidity and signal transduction. Thus, n-3 PUFA supplementation provokes changes that may affect different neurotransmitter systems, particularly altering the regulation of dopaminergic and serotonergic neurotransmission, which are dysfunctional in depressed patients (Grosso, Galvano, et al., 2014).

Furthermore, DHA is important in brain development and plays a critical role in neuronal signaling pathways regulated by neurotrophins. In particular, high cortical accretion of DHA upregulates mRNA expressions of key neurotrophins, such as NGF. Consistent with the health benefits of n-3 PUFA intake, NGF increase has been shown to ameliorate the symptoms of neurodegenerative disorders, such as Alzheimer’s depressive disorder (Wiener et al., 2015).

To the best of our knowledge, this is the first study that evaluates the effect of Aβ icv injection in female rats, reporting depressive-like behaviour and neurochemical impairments. In conclusion, the Aβ-induced depressive-like profile was reversed by n-3 PUFA supplementation, indicating a possible therapeutic role of n-3 PUFA in the treatment of the burden of depressive disorders.