Prostaglandins, Leukotrienes and Essential Fatty Acids 109 (2016) 29–38

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Dairy fat blend improves brain DHA and neuroplasticity and regulates corticosterone in mice

A.L. Dinel [a,b,*], C. Rey [a,b,c], C. Bonhomme [d], P. Le Ruyet [e], C. Joffre [a,b], S. Layé [a,b]

[a] Nutrition et Neurobiologie Intégrée, INRA UMR 1286, 33076 Bordeaux, France
[b] University of Bordeaux, Bordeaux, France
[c] ITERG, Institut des Corps Gras, 33600 Pessac, France
[d] Lactalis Nutrition Europe, Torce F-35370, France
[e] Lactalis, R&D, Retiers F-35240, France

ARTICLE INFO

Article history:
Received 20 January 2016
Received in revised form
22 March 2016
Accepted 24 March 2016

Keywords:
Dairy lipid
DHA
Microglia
Neurogenesis
HPA axis
PUFA

ABSTRACT

Mimicking the breast milk lipid composition appears to be necessary for infant formula to cover the brain's needs in n-3 PUFA. In this study, we evaluated the impact of partial replacement of vegetable oil (VL) in infant formula by dairy fat (DL) on docosahexaenoic acid (DHA) brain level, neuroplasticity and corticosterone in mice. Mice were fed with balanced VL or balanced DL diets enriched or not in DHA and arachidonic acid (ARA) from the first day of gestation. Brain DHA level, microglia number, neurogenesis, corticosterone and glucocorticoid receptor expression were measured in the offsprings. DL diet increased DHA and neuroplasticity in the brain of mice at postnatal day (PND) 14 and at adulthood compared to VL. At PND14, ARA and DHA supplementation increased DHA in VL but not in DL mice brain. Importantly, DHA and ARA supplementation further improved neurogenesis and decreased corticosterone level in DL mice at adulthood. In conclusion, dairy lipids improve brain DHA level and neuroplasticity.

1. Introduction

Breastfeeding is highly recommended by the World Health Organization (WHO), at least for the first six months of life, as it provides young infant the nutrients they need for a healthy growth. Importantly, for brain development, the needs in essential fatty acids from the n-3 and n-6 families are crucial. Because the organism cannot produce these fatty acids, alpha-linolenic acid (ALA) and linoleic acid (LA) have to be provided by the maternal diet. Even though infant formulas follow the recommended guidelines in terms of fat composition for most fatty acids and especially essential fatty acids, they are far from breast milk composition as they are usually prepared using only vegetable oils. Recently, infant formulas prepared with dairy fat have been re-introduced in the panel of commercial infant formulas {Delplanque, 2015 #1305}. Furthermore in an attempt to most adequately mimic the composition of mother's milk, long chain fatty acids (LCPUFA) can be added. Infant formulas containing dairy fats [1] or evaporated milk [2] result in an intermediate LCPUFA status compared to individuals fed with vegetable fat formulas or breast milk. However, the use of lipid formulation does not necessarily take into account brain's need in PUFAs.

Docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) are essential PUFAs and key structural components of neuronal membrane phospholipids [3,4]. DHA and ARA are metabolized from precursors ALA and LA [5] that cannot be synthetized *de novo* by mammals and have to be provided through the diet. The demand for complex lipids such as DHA is important to form vital cell membrane structures during perinatal period [6] and it accumulates in the brain during the later stages of gestation and lactation via placenta and maternal milk or infant formula [6–9]. Previous studies have shown that dairy fat-based formula with LA/ALA ratio leads to optimal DHA levels in brain pups [10,11]. Moreover, dairy products have been shown to have beneficial impact on the bioconversion of ALA in humans or animals [12–14].

DHA and ARA are necessary for normal brain development and function [15–19]. DHA promotes neuroplasticity, neurogenesis, synaptogenesis and neuroimmune interactions [16,20–23]. Kang et al. [24] recently discovered that the Fat-1 transgenic mouse, which has enriched levels of DHA in the brain because it can convert n-6 to n-3 fatty acids, exhibits increased hippocampal neurogenesis. Neurogenesis is also increased in the hippocampus of aged rats supplemented with DHA [25]. In opposite, Bertrand

* Corresponding author at: Nutrition et Neurobiologie Intégrée, INRA UMR 1286, 33076 Bordeaux, France.

http://dx.doi.org/10.1016/j.plefa.2016.03.013
0952-3278/

et al. demonstrated that neurogenesis is decreased in the DHA-deficient embryonic rat brain [26].

PUFA are also powerful immunomodulators and n-3 PUFAs such as DHA and their derivatives (resolvins, neuroprotectins, maresins) exert anti-inflammatory effects and inhibit the production of proinflammatory cytokines [20]. Microglia are the main cellular component of the brain innate immune system and are key players in the regulation and maintenance of central nervous system (CNS) homeostasis [27–29]. It has been recently demonstrated that microglia play a key role during brain development, especially in neural circuit formation [30,31], by engulfing and eliminating synapses during development, leading to maturation of the CNS network [30–32]. Modulation of n-3/n-6 PUFA ratio impacts microglia activity with functional effect during perinatal and adult periods [22,23].

Numerous studies have shown that, in animal models of nutritional n-3 PUFA deprivation, brain DHA decrease leads to anxiety and depressive-like behaviors [33,34]. Chronic n-3 PUFA supplementation has beneficial effects on anxiety, cognitive and depressive-like behaviors in animals submitted to stress [35]. Supplementation with n-3 PUFAs has been shown to prevent the disruption of stress axis functionality associated with the development of neuropsychiatric disorders. N-3 PUFA deficiency induces hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis, reflected by plasma corticosterone elevation whereas dietary n-3 PUFA supplementation induces resilience to the effects of chronic social stress on emotional behaviors, by preventing HPA axis hyperactivity [36].

Providing the adequate amount of n-3 PUFA for optimal DHA accretion in early life is of high importance to promote neuroplasticity. The aim of this study was to evaluate the short and long term impact of partial replacement of vegetable oil in infant formula by dairy fat supplemented or not in DHA and ARA on brain DHA level and neuroplasticity (microglial cells number, neurogenesis) and corticosterone as well as glucocorticoid receptor phosphorylation.

2. Material and methods

2.1. Animals and experimental design

All experiments were conducted according to the INRA Quality Reference System, and to relevant French (Directive 87/148, Ministère de l'Agriculture et de la Pêche) and European (Directive 86/609, November 24th 1986, European Community) legislations. They followed ethic protocols approved by the Région Aquitaine Veterinary Services (Direction Départementale de la Protection des Animaux, approval ID: A33-063-920) and by ethics committee (No. 50120112-A). Every effort was made to minimize suffering and the number of animals used. Adult male and female swiss mice (CD-1 mice) were obtained from Janvier (St Berthevin, France) before mating. They were housed in same sex pairs in polypropylene cages and maintained in a temperature and humidity controlled pathogen-free facility on a 12 h light-dark cycle with ad libitum access to food and water.

Mice were fed with diets containing 5% of total lipids from different sources: balanced vegetable lipids (VL), VL supplemented in DHA (0.2%) and ARA (0.4%) (VL+DHA+ARA), balanced dairy lipids (DL) and DL supplemented in DHA (0.2%) and ARA (0.4%) (DL+DHA+ARA). The composition of the diets is described in Tables 1 and 2. Diets were given to mice since the first day of gestation and to the offsprings until adulthood (post-natal day, PND90). Litters were harmonized at 12 pups/litter with as many males as possible and a minimum of 3 females [37,38]. All studies were performed in males at PND14 and PND90.

Table 1
Composition of the diets (g/kg diet).

Ingredient	Amount
Casein	180
Cornstarch	460
Sucrose	230
Cellulose	20
Fat	50
Mineral mix[a]	50
Vitamin mix[b]	10

[a] Composition (g/kg): sucrose, 110.7; $CaCO_3$, 240; K_2HPO_4, 215; $CaHPO_4$, 215; $MgSO_4,7H_2O$, 100; NaCl, 60; MgO, 40; $FeSO_4,7H_2O$, 8; $ZnSO_4,7H_2O$, 7; $MnSO_4,H_2O$, 2; $CuSO_4,5H_2O$, 1; $Na_2SiO_7,3H_2O$, 0.5; $AlK(SO_4)_2,12H_2O$, 0.2; K_2CrO_4, 0.15; NaF, 0.1; $NiSO_4,6H_2O$, 0.1; H_2BO_3, 0.1; $CoSO_4,7H_2O$, 0.05; KIO_3, 0.04; $(NH_4)_6Mo_7O_{24},4H_2O$, 0.02; LiCl, 0.015; Na_2SeO_3, 0.015; NH_4VO_3, 0.01.

[b] Composition (g/kg): sucrose, 549.45; retinyl acetate, 1; cholecalciferol, 0.25; DL-α-tocopheryl acetate, 20; phylloquinone, 0.1; thiamin HCl, 1; riboflavin, 1; nicotinic acid, 5; calcium pantothenate, 2.5; pyridoxine HCl, 1; biotin, 1; folic acid, 0.2; cyanobalamin, 2.5; choline HCl, 200; DL-methionine, 200; p-aminobenzoic acid, 5; inositol, 10.

Table 2
Composition of fatty acid in the diets.

Fatty acids	VL	VL+DHA+ARA	DL	DL + DHA + ARA
8:0	–	–	0.7	0.7
10:0	–	–	1.6	1.6
12:0	0.2	0.2	1.9	1.9
14:0	0.8	0.9	6.2	6.1
15:0	0.1	0.1	0.6	0.6
16:0	28.8	28.8	18.4	18.5
17:0	0.1	0.1	0.3	0.3
18:0	3.9	4.0	7.0	7.1
20:0	0.4	0.4	0.3	0.3
22:0	0.3	0.3	0.4	0.4
24:0	0.2	0.2	0.2	0.2
Saturated	**34.7**	**34.8**	**37.5**	**37.6**
16:1n-9	–	–	0.1	–
16:1n-7	0.2	0.2	0.9	0.9
17:1	–	–	0.1	0.1
18:1 trans	0.1	0.1	1.3	1.2
18:1n-9	47.1	46.2	45.4	44.8
18:1n-7	1.1	1.1	1.3	1.3
20:1n-9	0.3	0.3	0.4	0.4
Monounsaturated	**48.8**	**48.2**	**49.4**	**48.7**
18:2n-6	15.1	15.0	10.8	10.8
20:4n-6	–	0.4	–	0.4
n-6	**15.1**	**15.4**	**10.8**	**11.2**
18:3n-3	1.3	1.3	2.2	2.2
22:6n-3	–	0.2	–	0.2
n-3	**1.3**	**1.5**	**2.2**	**2.4**
Polyunsaturated	**16.5**	**17.0**	**13.1**	**13.7**
n-6/n-3	**11.3**	**10.0**	**4.8**	**4.6**

A first cohort (n=30/dietary group, N=120) was used to evaluate the impact of the different diets on microglial number, neurogenesis as well as corticosterone and GR expression and phosphorylation in offspring's brain at PND14. A second cohort (n=15/dietary group, N=60) was used to evaluate long term effect of diets on neurogenesis and HPA axis activation at PND90. Plasma, total brain or brain structures (cortex and hippocampus) were collected and frozen in isopentane for further analysis.

2.2. Biochemical/histological measurements

2.2.1. Analysis of fatty acids in the cortex

Total lipids were analyzed in cortex of PND14 and PND90 mice as previously described [17]. Briefly, lipids from the cortex were extracted according to the method of Folch [39] and fatty acids were transmethylated according to the method of Morrison and Smith [40].

Table 3
Fatty acid composition of cortex at PND14.

	VL	VL+DHA+ARA	DL	DL+DHA+ARA	Statistical effects		
					Lipid quality effect	Supplemen-tation effect	Interaction
14:0	0.76+0.04BCE	0.74+0.032c	0.81+0.02b	0.92+0.005a	<0.01	NS	<0.05
15:0	0.09+0.003c	0.07+0.003a	0.09+0.005c	0.07+0.003b	<0.001	NS	<0.001
16:0	26.1+0.15	25.8+0.15	26.6+0.18	26.2+0.18	<0.05	0.06	NS
17:0	0.11+0.06c	0.10+0.03a	0.11+0.005c	0.13+0.003BCE	<0.05	NS	<0.01
18:0	19.2+0.21	19.4+0.03	19.2+0.16	19.6+0.06	NS	<0.05	NS
20:0	0.1+0.008	0.1+0.006	0.1+0.006	0.1+0.01	0.05	NS	NS
22:0	0.1+0.006	0.1+0.003	0.1+0.007	0.05+0.02	0.05	NS	NS
24:0	0.1+0.005	0.1+0.006	0.1+0.003	0.1+0.003	NS	NS	NS
Saturated	**46.5+0.20**	**46.4+0.14**	**47.1+0.13**	**47.2+0.16**	**<0.01**	**NS**	**NS**
16:1n-9	1.1+0.04	1.1+0.02	1.0+0.005	1.0+0.04	<0.01	NS	NS
16:1n-7	1.03+0.002ab	1.0+0.002a	0.98+0.025b	1.09+0.034a	NS	NS	<0.05
18:1t	0.1+0.006	0.1+0.003	0.1+0.01	0.1+0.004	NS	NS	NS
18:1n-9	10.4+0.03	10.2+0.05	10.5+0.14	10.1+0.10	NS	<0.05	NS
18:1n-7	2.9+0.03	2.8+0.01	2.9+0.05	2.8+0.02	NS	<0.01	NS
20:1n-9	0.3+0.006	0.3+0.006	0.3+0.011	0.3+0.010	NS	NS	NS
20:1n-7	0.1+0.004	0.1+0.00	0.1+0.004	0.1+0.006	NS	<0.05	NS
24:1n-9	0.1+0.007	0.1+0.01	0.1+0.005	0.1+0.009	NS	NS	NS
Monounsaturated	**16.1+0.06**	**15.6+0.01**	**15.9+0.18**	**15.6+0.06**	**NS**	**<0.01**	**NS**
18:2n-6	0.56+0.013ab	0.61+0.009a	0.54+0.006b	0.52+0.009b	<0.001	NS	<0.01
20:2n-6	0.14+0.006c	0.16+0.00ac	0.15+0.004c	0.13+0.003b	<0.01	NS	<0.01
20:3n-6	0.37+0.02	0.37+0.01	0.41+0.003	0.39+0.007	0.05	NS	NS
20:4n-6	13.2+0.19	13.2+0.02	12.4+0.07	12.7+0.11	<0.001	NS	NS
22:4n-6	3.0+0.07	2.9+0.02	2.5+0.09	2.6+0.02	<0.0001	NS	NS
22:5n-6	2.6+0.04a	1.7+0.01b	1.4+0.02c	1.0+0.03d	<0.0001	<0.0001	<0.0001
n-6	**19.9+0.17a**	**18.9+0.03c**	**17.4+0.10d**	**17.4+0.11bd**	**<0.0001**	**<0.01**	**<0.01**
22:5n-3	0.16+0.01BCE	0.14+0.01c	0.18+0.01b	0.21+0.01a	<0.0001	NS	<0.01
22:6n-3	11.0+0.30d	12.7+0.13c	13.0+0.13b	13.6+0.22ab	<0.0001	<0.001	<0.05
n-3	**11.1+0.29d**	**12.9+0.13c**	**13.2+0.14b**	**13.8+0.22ab**	**<0.0001**	**<0.001**	**<0.05**
20:3n-9	0.3+0.04	0.2+0.01	0.4+0.01	0.3+0.02	<0.05	<0.05	NS
Polyunsaturated	**31.3+0.15**	**32.0+0.10**	**31.0+0.19**	**31.5+0.11**	**<0.05**	**<0.01**	**NS**
DMA16:0	2.7+0.05	2.6+0.01	2.7+0.01	2.6+0.02	0.06	<0.05	NS
DMA 18:0	2.0+0.06	2.0+0.02	2.0+0.02	1.9+0.03	<0.05	NS	NS
DMA18:1n-9	0.6+0.01	0.5+0.01	0.6+0.02	0.5+0.01	NS	NS	NS
DMA18:1n-7	0.50+0.006c	0.46+0.000a	0.47+0.008b	0.47+0.008ab	NS	<0.01	<0.01
DMA	**5.8+0.10**	**5.7+0.03**	**5.7+0.04**	**5.4+0.03**	**<0.05**	**<0.05**	**NS**
n-6/n-3	**1.8+0.06a**	**1.5+0.02b**	**1.3+0.01c**	**1.2+0.03c**	**<0.0001**	**<0.001**	**<0.01**
AA/DHA	**1.21+0.047a**	**1.03+0.012b**	**0.95+0.005c**	**0.93+0.023c**	**<0.0001**	**<0.01**	**<0.05**
Δ9 desaturase	**0.5+0.01**	**0.5+0.003**	**0.5+0.01**	**0.5+0.01**	**NS**	**<0.05**	**NS**
Δ6 desaturase	**0.7+0.03**	**0.6+0.01**	**0.75+0.01**	**0.7+0.01**	**<0.0001**	**NS**	**NS**
Δ5 desaturase	**35.8+1.96**	**35.4+1.08**	**30.4+0.32**	**32.5+0.79**	**<0.01**	**NS**	**NS**

Values are means ± SEM. Statistical significance between groups was analyzed by two-way ANOVA (lipid quality × supplementation) followed by Bonferroni post hoc test when appropriate. VL: vegetable lipids; DL: dairy lipids; AA: arachidonic acid; DHA: docosahexaenoic acid; DMA: dimethylacetals; Δ9 desaturase: 18:1n-9/18:0; Δ6 desaturase: 20:3n-6/18:2n-6; Δ5 desaturase: 20:4n-6/:20:3n-6.

2.2.2. Microglia purification

At PND14 of age, microglia (approximately 3×10^5 cells/brain, 85% of microglia) were isolated from whole brain homogenates as previously described [41]. Microglial cells were re-suspended in Phosphate Buffer Saline solution (PBS)/0.1% Bovine to perform flow cytometry analysis.

2.2.3. Flow cytometry

Microglial preparations were incubated with anti-CD16/CD32 antibody (eBiosciences, Paris, France) to block Fc receptors for 10 min on ice. Cells were washed and then incubated for 45 min with the appropriate conjugated antibodies: anti-CD11b-APC, anti-CD45-PerCP Cy5.5 (Biolegend, Saint Quentin Yvelines, France). Cells were washed and resuspended in PBS/BSA 0.1%. Non-specific binding was assessed using non-specific, isotype-matched antibodies. Antigen expression was determined using a Becton–Dickinson LSRFortessa™ multicolor cytometer. Ten thousand events were recorded for each sample and isotype matched-conjugate. Data were analyzed using FlowJo software and gating for each antibody was determined based on non-specific binding of appropriate negative isotype stained controls [41].

2.2.4. Immunohistochemical detection of Doublecortin (DCX)-positive cells

After transcardiac perfusion with PBS (pH 7.4), followed by 4% paraformaldehyde (PFA), brains were removed, post-fixed for 4 h in PFA, cryoprotected in 30% sucrose for 24 h, snap frozen in liquid nitrogen, and stored at −80 °C before sectioning. Free-floating (30 μm) coronal sections containing the hippocampus (from −0.9 mm to −3.1 mm relative to bregma) were collected on a cryostat for immunohistochemistry. Neurogenesis in the hippocampus was evaluated as previously described [37]. The number of DCX-positive cells was measured on three sections representing the same three levels of the dentate gyrus for all animals [42]. Cells were discriminated between mature and immature forms since immature cells present small neuronal processes.

2.2.5. Measurement of neurotrophins, neurotrophin receptor and glucocorticoid receptors by western-blot

Western blot analyses of the neurotrophic factor BDNF and its receptor TrkB and glucocorticoid receptors GR and its phosphorylated form (P-GR) were performed according to the method described in Dinel et al. [37].

Table 4
Fatty acid composition of cortex at PND90.

	VL	VL+DHA+ARA	DL	DL+DHA+ARA	Statistical effects		
					Lipid quality effect	Supplemen-tation effect	Interaction
14:0	0.10+0.005b	0.09+0.000c	0.11+0.002b	0.14+0.003a	<0.0001	<0.05	<0.0001
15:0	0.04+0.005	0.03+0.005	0.05+0.000	0.05+0.003	<0.001	NS	NS
16:0	22.3+0.54	22.6+0.13	22.4+0.21	22.2+0.24	NS	NS	NS
17:0	0.12+0.004	0.12+0.003	0.13+0.006	0.15+0.003	<0.01	NS	NS
18:0	20.9+0.16	21.3+0.13	21.5+0.12	21.5+0.06	<0.05	NS	NS
20:0	0.20+0.0011	0.21+0.003	0.21+0.003	0.25+0.012	<0.05	<0.01	NS
22:0	0.15+0.017c	0.16+0.004b	0.14+0.006c	0.21+0.007a	NS	<0.01	<0.05
24:0	0.16+0.013b	0.16+0.003b	0.15+0.016b	0.24+0.015a	<0.05	<0.01	<0.01
Saturated	**44.0+0.65**	**44.7+0.22**	**44.7+0.25**	**44.8+0.26**	**NS**	**NS**	**NS**
16:1n-9	0.20+0.010	0.14+0.023	0.20+0.005	0.18+0.007	NS	<0.05	NS
16:1n-7	0.5+0.003	0.5+0.006	0.5+0.017	0.6+0.022	NS	NS	NS
18:1t	0.04+0.002	0.04+0.003	0.04+0.00	0.04+0.003	NS	NS	NS
18:1n-9	11.9+0.27	12.2+0.07	12.0+0.13	12.1+0.05	NS	NS	NS
18:1n-7	3.3+0.01	3.2+0.02	3.3+0.04	3.3+0.05	NS	NS	NS
20:1n-9	0.69+0.09	0.71+0.02	0.66+0.04	0.72+0.02	NS	NS	NS
20:1n-7	0.20+0.016	0.20+0.008	0.20+0.004	0.21+0.003	NS	NS	NS
22:1n-9	0.1+0.009	0.1+0.005	0.1+0.009	0.1+0.003	NS	NS	NS
24:1n-9	0.6+0.09	0.6+0.03	0.5+0.07	0.8+0.06	NS	NS	NS
Monounsaturated	**17.6+0.42**	**17.7+0.11**	**17.6+0.20**	**18.1+0.05**	**NS**	**NS**	**NS**
18:2n-6	0.31+0.009	0.29+0.012	0.32+0.012	0.31+0.009	NS	NS	NS
20:2n-6	0.08+0.004	0.08+0.003	0.08+0.005	0.07+0.015	NS	NS	NS
20:3n-6	0.33+0.009	0.32+0.004	0.40+0.014	0.39+0.017	<0.001	NS	NS
20:4n-6	9.6+0.02	9.4+0.8	9.3+0.2	8.9+0.08	<0.01	<0.05	NS
22:4n-6	2.6+0.15	2.5+0.05	2.2+0.03	2.1+0.06	<0.01	NS	NS
22:5n-6	1.5+0.14a	1.0+0.09b	0.7+0.03c	0.6+0.03c	<0.0001	<0.05	<0.05
n-6	**14.5+0.13**	**13.6+0.06**	**13.1+0.18**	**12.5+0.15**	**<0.0001**	**<0.0001**	**NS**
22:5n-3	0.13+0.033	0.09+0.030	0.19+0.009	0.17+0.003	<0.05	NS	NS
22:6n-3	15.5+0.14	16.0+0.07	16.4+0.10	16.7+0.19	<0.0001	<0.05	NS
n-3	**15.6+0.12**	**16.1+0.05**	**16.6+0.09**	**16.8+0.19**	**<0.0001**	**<0.05**	**NS**
20:3n-9	0.2+0.004	0.1+0.004	0.2+0.01	0.2+0.02	<0.0001	<0.01	NS
Polyunsaturated	**30.3+0.14**	**29.8+0.05**	**29.9+0.10**	**29.5+0.10**	**<0.01**	**<0.01**	**NS**
DMA16:0	2.1+0.07	2.0+0.03	2.1+0.05	1.9+0.04	NS	<0.05	NS
DMA18:0	3.8+0.09	3.7+0.02	3.7+0.03	3.5+0.09	<0.05	<0.05	NS
DMA18:1n-9	1.0+0.07	1.0+0.03	0.9+0.06	1.0+0.03	NS	NS	NS
DMA18:1n-7	1.2+0.08	1.1+0.04	1.1+0.06	1.2+0.03	NS	NS	NS
DMA	**8.1+0.29**	**7.8+0.08**	**7.8+0.11**	**7.6+0.13**	**NS**	**NS**	**NS**
n-6/n-3	**0.93+0.012**	**0.85+0.006**	**0.78+0.014**	**0.74+0.015**	**<0.0001**	**<0.001**	**NS**
AA/DHA	**0.6+0.009**	**0.6+0.004**	**0.6+0.014**	**0.5+0.009**	**<0.001**	**<0.05**	**NS**
Δ9 desaturase	**0.6+0.016**	**0.6+0.005**	**0.6+0.007**	**0.6+0.00**	**NS**	**NS**	**NS**
Δ6 desaturase	**1.1+0.05**	**1.1+0.05**	**1.2+0.09**	**1.2+0.02**	**<0.05**	**NS**	**NS**
Δ5 desaturase	**28.8+0.83**	**29.6+0.33**	**23.4+1.13**	**23.2+0.92**	**<0.0001**	**NS**	**NS**

Values are means ± SEM. Statistical significance between groups was analyzed by two-way ANOVA (lipid quality × supplementation) followed by Bonferroni post hoc test when appropriate. VL: vegetable lipids; DL: dairy lipids; AA: arachidonic acid; DHA: docosahexaenoic acid; DMA: dimethylacetals; Δ9 desaturase: 18:1 n-9/18:0; Δ6 desaturase: 20:3n-6/18:2n-6; Δ5 desaturase: 20:4n-6/:20:3n-6.

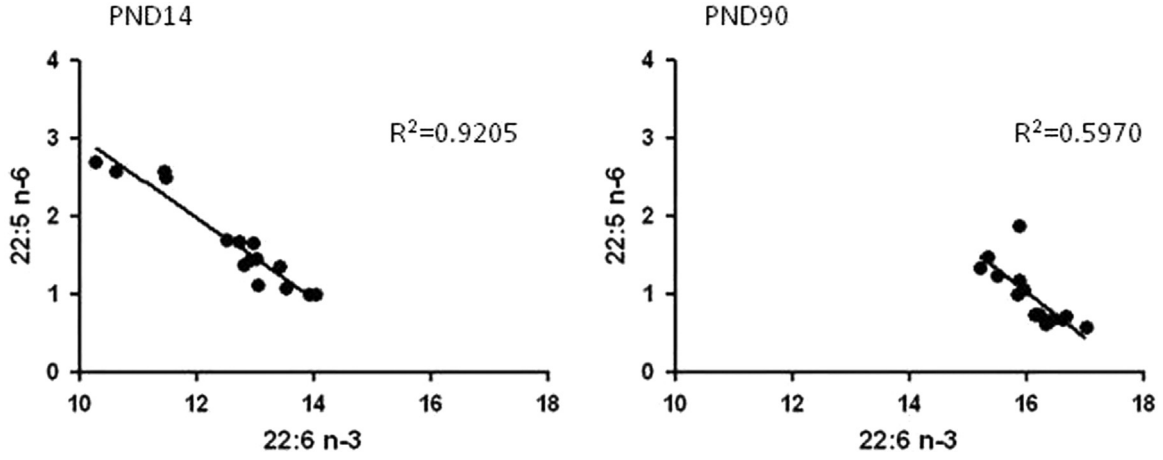

Fig. 1. Correlation between level of DPA n-6 and DHA in cortex of mice at PND 14 and PND 90. ***p < 0.001 means significant correlation.

2.2.6. Assessment of corticosterone level by enzyme immunoassay

A commercially prepared Enzyme Immunoassay kit was used to measure corticosterone concentrations in plasma (Corticosterone enzyme immunoassay kit, Arbor Assay, USA) according to the manufacturer's instructions. The sensitivity of the assay was 18.6 pg/mL [43].

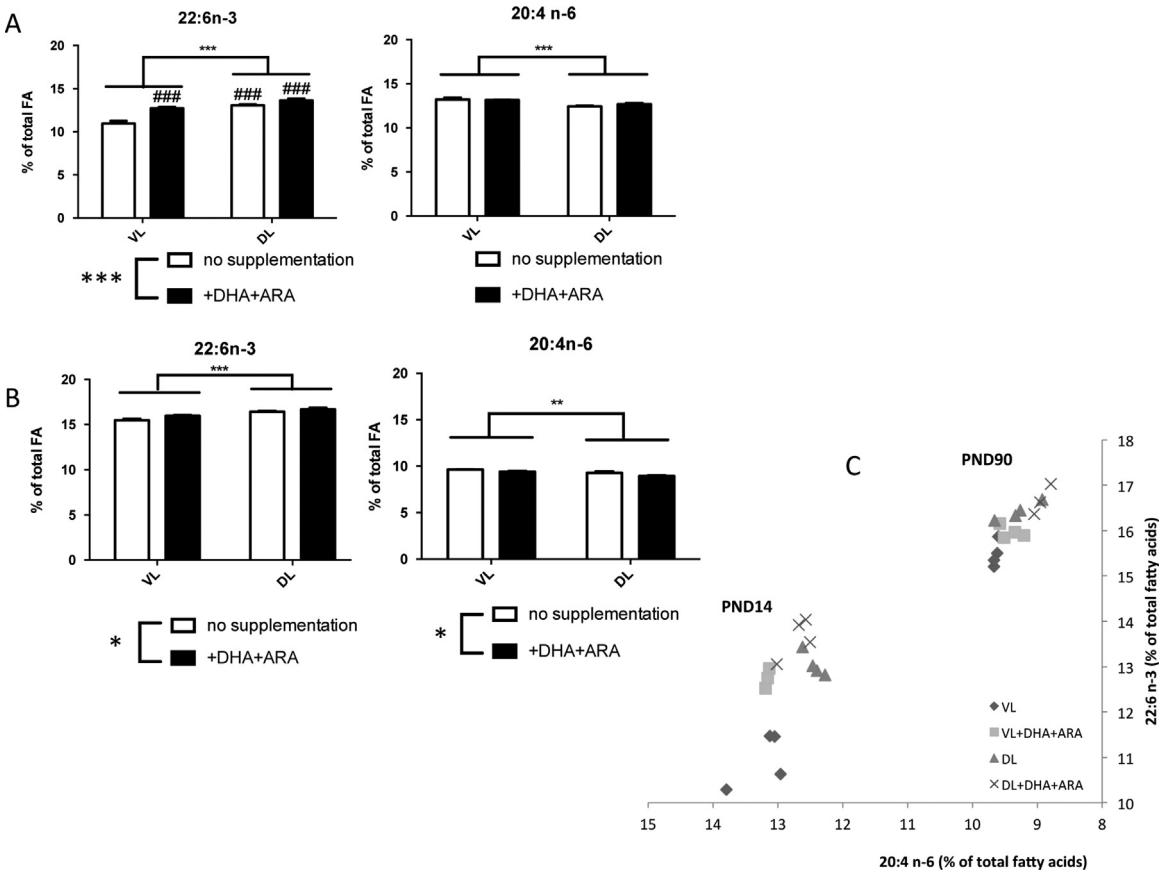

Fig. 2. Fatty acid compositions in PFC. Percentage of DHA and ARA were measured by HPLC on prefrontal cortex at postnatal day (PND) 14 (A) and 90 (B). Data are expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, for lipid quality or supplementation factors effect. ###, p < 0.001 for comparison to VL group. (c) Comparison between DHA and ARA accretion in the different groups.

3. Statistical analysis

All data were expressed as the mean ± SEM. A p-value < 0.05 was considered as significant. Data were statistically analyzed using a two-way ANOVA comparison factor 1: lipid quality (VL, DL), factor 2: supplementation (ARA+DHA) followed by a Bonferroni post-hoc when interaction was significant.

4. Results

4.1. Effect of lipid quality and DHA+ARA supplementation on fatty acid level in mice cortex at PND14 and PND90

At PND14, the analysis of n-3 PUFA revealed a lipid quality effect ($F(1,11)=49.39$, $p<0.001$), a DHA+ARA supplementation effect ($F(1,11)=29.20$, $p<0.001$) and an interaction lipid quality × supplementation effect ($F(1,11)=6.949$, $p<0.05$). N-6 PUFA changes were the opposite of n-3 PUFA modification and statistical analysis demonstrated a lipid quality effect ($F(1,11)=265.4$, $p<0.001$), a DHA+ARA supplementation effect ($F(1,11)=17.70$, $p<0.01$) and an interaction lipid quality × supplementation effect ($F(1,11)=13.87$, $p<0.01$). At PND90, n-3 PUFAs were increased in DL diet compared to VL ($F(1,11)=63.52$, $p<0.001$) and DHA+ARA supplementation increased n-3 PUFA level ($F(1,11)=9.478$, $p<0.05$). In opposite, n-6 PUFAs were decreased by DL diet compared to VL ($F(1,11)=85.12$, $p<0.001$) and DHA+ARA supplementation induced a decrease in n-6 PUFA ($F(1,11)=26.07$, $p<0.001$). Data are presented in Table 3 (PND14) and Table 4 (PND90).

Interestingly, as expected, DHA level was significantly correlated with docosapentaenoic acid (DPA) n-6 level at PND14 ($p<0.0001$, $R^2=0.9205$) and at PND90 ($p<0.001$, $R^2=0.5970$) (Fig. 1).

At PND14, lipid quality ($F(1,11)=45.79$, $p<0.001$), DHA+ARA supplementation ($F(1,11)=28.74$, $p<0.001$) and their interactions ($F(1,11)=7.278$, $p<0.5$) significantly increased DHA (22:6n-3) level (Fig. 2A). Post-hoc analysis revealed a significant increase of DHA levels in VL+DHA+ARA ($p<0.001$), DL ($p<0.001$) and DL+DHA+ARA ($p<0.001$), compared to VL. ARA level was decreased in DL mice as compared to VL mice (lipid quality effect, $F(1,11)=24.33$, $p<0.001$). At PND90, there was a significant effect of lipid quality ($F(1,11)=43.39$, $p<0.001$) and of supplementation ($F(1,11)=8.877$, $p<0.5$) on DHA levels. ARA level was significantly lower in DL compared to VL (lipid quality effect, $F(1,11)=16.84$, $p<0.01$). A significant effect of DHA+ARA supplementation was revealed ($F(1,11)=8.967$, $p<0.5$) (Fig. 2B). Fig. 2C summarizes the effect of diets on DHA and ARA in the cortex of mice at PND14 and PND90 and emphasizes that DL diet efficiently increase DHA independently of age and supplementation.

4.2. Effect of lipid quality and DHA+ARA supplementation on microglia number in mice brain at PND14

Microglia number in the developing brain is dependent on DHA [41]. Using CD11b and CD45 as markers, brain microglia and macrophages were analyzed by flow cytometry (Fig. 3). The representative bivariate dot plots show that the majority of viable cells displayed low levels of CD11+/CD45 in all dietary groups (Fig. 3A). Statistical analysis revealed a significant effect of lipid

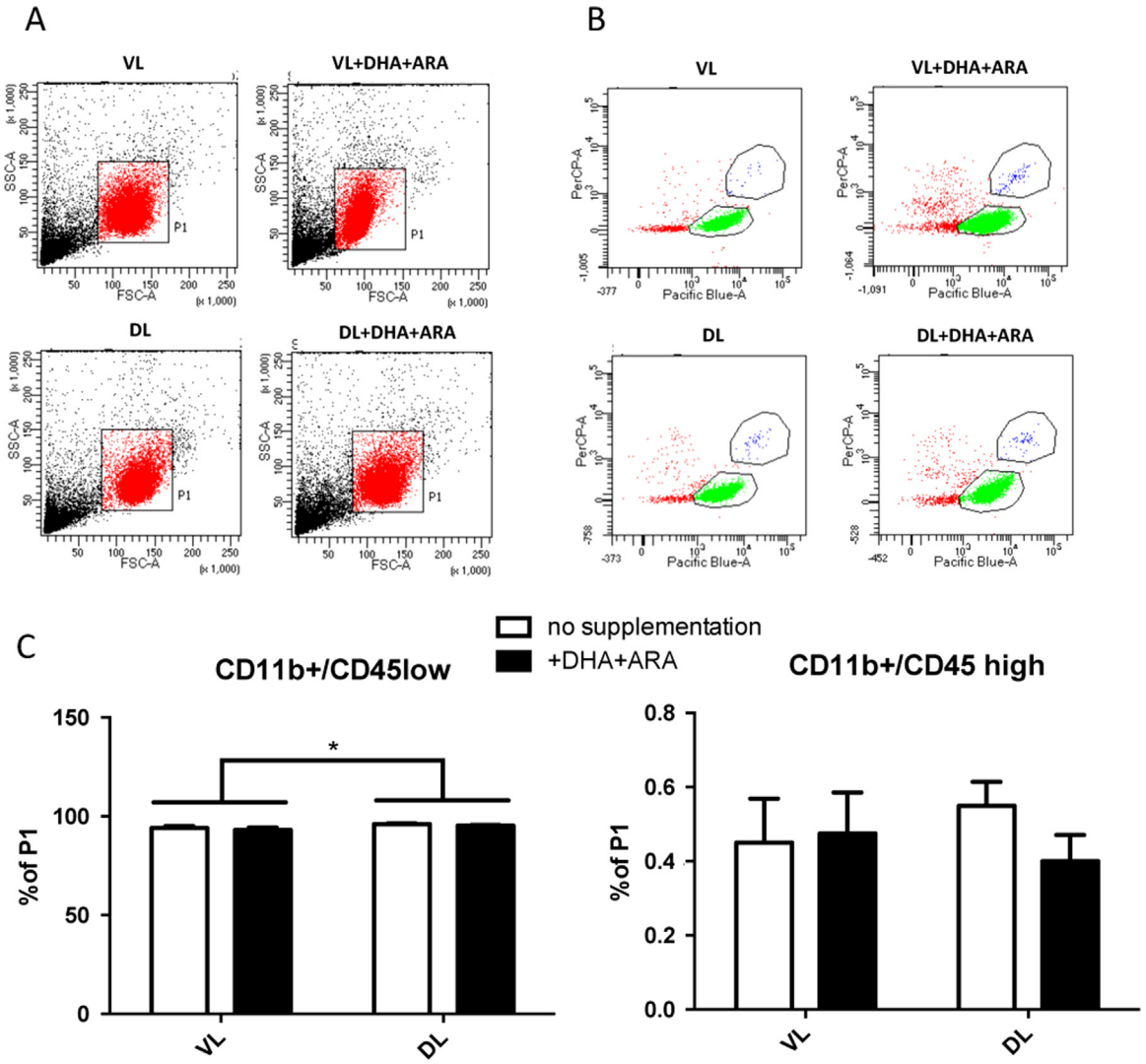

Fig. 3. Flow cytometric analysis of microglial phenotype. (A) Flow cytometric analysis of the cell suspensions. A first population (P1) was selected based on granulocity and size of the cells (SSC for side scatter:granularity; FSC for forward scatter: size) (B) Representative bivariate dot plots of Percoll isolated cells (P1 population) gated on CD11b+/CD45low expression for microglia in saline or LPS conditions. All analyses were completed using the CD11b+/CD45low microglia. (C) Average percentage of events that were CD11b+/CD45low and CD11b+/CD45high. Data are expressed as mean ± SEM, *$p < 0.05$, for lipid quality factors effect.

quality on Cd11b+/CD45 low cell number ($F(1,13)=7.852$, $p < 0.05$) with no effect on CD11b+/Cd45 high cells (Fig. 3B and C). These results suggest that DL increases microglia number in the brain of PND14 mice.

4.3. Effect of lipid quality and DHA+ARA supplementation on neurogenesis in mice hippocampus at PND14 and PND90

DCX labeling of proliferating neurons [42] was used to study the effect of diets on neurogenesis [37] at PND 14 (Fig. 4A) and PND90 (Fig. 5A). Intensity of DCX-labeling was first measured in the dentate gyrus of PND14 mice, as an index of neuronal proliferation [44]. The intensity of DCX labeling was significantly increased in the dentate gyrus of DL mice compared to VL mice ($F(1,37)=6.083$, $p < 0.05$) (Fig. 3A). No significant effect of supplementation was revealed. At PND90, the total number of DCX positive cells was not affected by the diet and/or supplementation. However, the number of immature DCX positive cell was significantly reduced in the DL group ($F(1,16)=8.980$, $p < 0.01$) with an additional effect when ARA+DHA was present in DL mice (lipid quality × supplementation, $F(1,16)=5.470$, $p < 0.05$, $p < 0.05$) (Fig. 5A).

4.4. Effect of lipid quality and DHA+ARA supplementation on neurotrophic factors in mice hippocampus at PND14 and PND90

BDNF has been implicated in regulating adult neurogenesis in the subgranular zone of the dentate gyrus [45]. Since neurogenesis was modulated by diets, we then assessed the expression of BDNF and its receptor (TrkB high, TrkB low) by western-blot in the hippocampus of PND14 and PND90 mice [46] (Figs. 4 and 5). At PND14 (Fig. 4B), BDNF expression was significantly increased in the hippocampus of mice fed with DL compared to those fed with VL (diet effect, $F(1,11)=8.584$, $p < 0.05$). The expression of the TrkB receptor subunit high (145 kDa) was significantly decreased by the supplementation ($F(1,12)=6.216$, $p < 0.05$). No significant effect of the diets was detected on TrkB low subunit. At PND90 (Fig. 5B), expression of BDNF ($F(1,18)=5.044$, $p < 0.05$) and TrkB low ($F(1,18)=7.115$, $p < 0.05$) were significantly decreased in the hippocampus of DL mice compared to VL. In addition, the expression of high TrkB subunit was significantly decreased in the hippocampus of mice fed with DL+DHA+ARA compared to those fed with VL+DHA+ARA (interaction lipid quality × supplementation, $F(1,18)=7.899$, $p < 0.05$; $p < 0.05$).

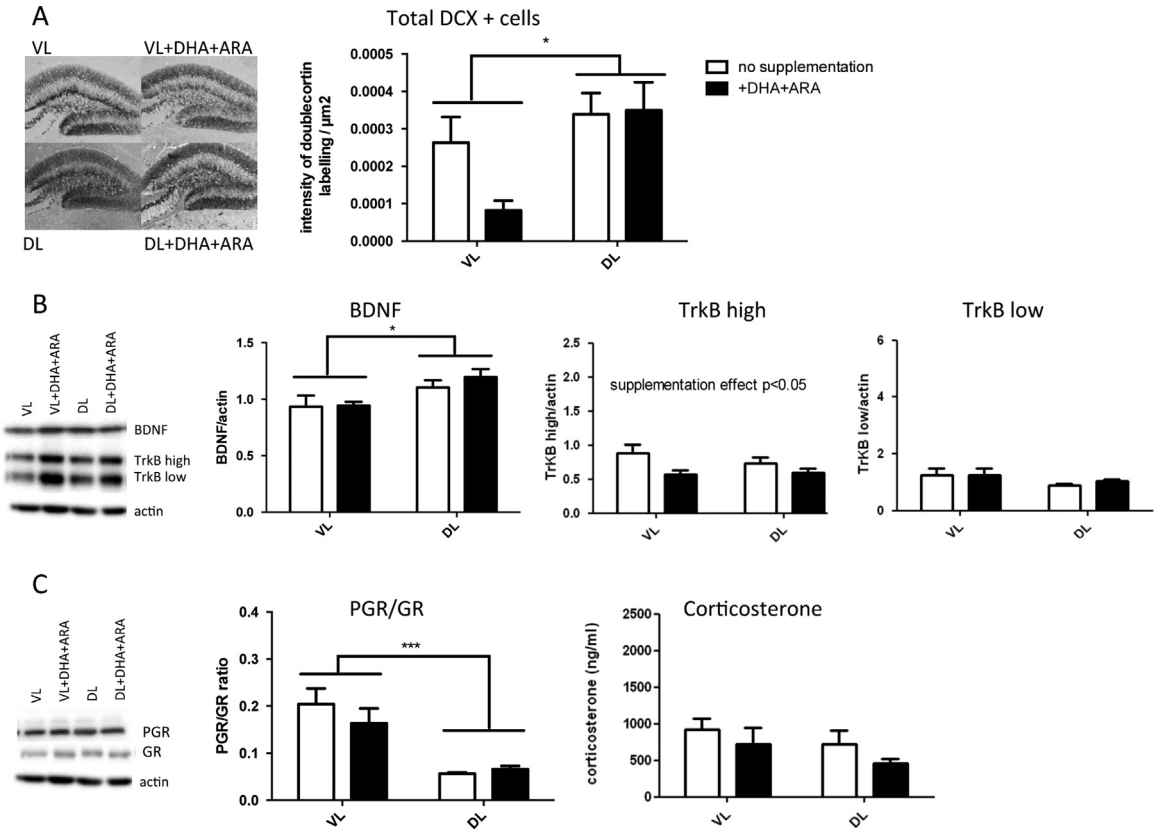

Fig. 4. Evaluation at postnatal day 14 (PND14) of (A): doublecortin (DCX)-positive cells in the dentate gyrus of the hippocampus. Lipid quality effect, $p < 0.05$ (B): BDNF protein level and its TrkB receptor in the hippocampus. BDNF: lipid quality effect, $p < 0.05$; TrkB high: supplementation effect, $p < 0.05$ (C): phosphorylated glucocorticoid receptor/glucocorticoid receptor ratio of the hippocampus and corticosterone level in plasma. PGR/GR: lipid quality effect, $p < 0.001$. Data are expressed as mean ± SEM.2 way ANOVA analyses were realized. *$p < 0.05$, ***$p < 0.001$, for lipid quality or supplementation factors effect.

4.5. Effect of lipid quality and DHA+ARA supplementation on corticosterone and glucocorticoid receptor phosphorylation in mice hippocampus at PND14 and PND90

BDNF is regulated by the activity of the HPA axis [47] and DHA is a strong regulator of corticosterone and GR phosphorylation in the brain [36,48]. Therefore, total and phosphorylated GR (PGR) expressions in the hippocampus and blood corticosterone - both used as a marker of HPA axis activity - were measured at PND14 and PND90 [49]. No significant effect of lipid quality or supplementation was revealed for GR expression in the hippocampus of juvenile and adult mice (data not shown). Importantly, PGR/GR ratio was significantly decreased in the hippocampus of PND14 mice fed with DL compared to those fed with VL ($F(1,12)=27.56$, $p < 0.001$) (Fig. 4C). No significant difference was revealed for corticosterone level (Fig. 4C). At PND90, DL significantly increased PGR/GR ($F(1,18)=4.754$, $p < 0.05$) while only supplementation in DL mice had a significant effect on corticosterone (supplementation effect $F(1,19)=10.07$, $p < 0.01$) (Fig. 5C). In addition, interaction between lipid quality and supplementation was revealed (PGR/GR $F(1,18)=5.097$, $p < 0.05$; corticosterone $F(1,19)=5.429$, $p < 0.05$, $p < 0.01$F,p). As a result, DL+DHA+ARA significantly reduced corticosterone level as compared to DL ($p < 0.01$) (Fig. 5C).

5. Discussion

The present study demonstrates the importance of dairy lipids for DHA accretion in the developing and adult brain and further demonstrates that the quality of lipids in the diet impacts brain neuroplasticity on both short (PND14) and long (PND90) term.

Here, we first show that dairy lipids in the maternal diet induce an increase in DHA level in the prefrontal cortex of offsprings at PND14 and PND90. These results are in accordance with previous results showing that dairy lipids consumption for 6 weeks at weaning significantly increases brain DHA [10]. Beneficial effect of dairy lipids on brain DHA accretion could be due to short- and medium-chain fatty acids [10]. These fatty acids are highly oxidized after absorption [50,51]. They may thereby spare ALA, one of the best b-oxidation substrates, from oxidation, and favor ALA partitioning towards the desaturation and elongation pathways and its conversion into very LCPUFA [10]. Indeed, short chain fatty acids were increased in the offspring brain at PND14 and PND90 (Table 1). However, their role in brain DHA accretion of offsprings fed with dairy lipids remains to be demonstrated. DHA and n-6 DPA levels were negatively correlated in brain of PND14 and PND90 mice (Fig. 1), suggesting that DHA increase is at the expense of n-6 DPA as previously reported [52]. Importantly, DHA increase as compared to ARA decrease is higher in dairy lipid diet group, even when supplementation with ARA+DHA was given to the mice (Fig. 2(C)). In our work, DHA was only measured in the cortex since the same animals were used for assessing the impact of diets on neuroplasticity. Because previous work reported that diets enriched in n-3 PUFAs similarly increased DHA level in all brain structures [15,53,54], one could assume that the increase in DHA observed in the cortex of dairy fed mice also occurs in other brain structures. Altogether, these data emphasize the importance of diary diet for brain DHA accretion.

We previously showed that brain DHA influences microglia activity at birth and at PND21 [29] as well as at adulthood [16,18,22]. Here, we found that the number of microglia is increased in the brain of PND14 offspring fed with a dairy diet.

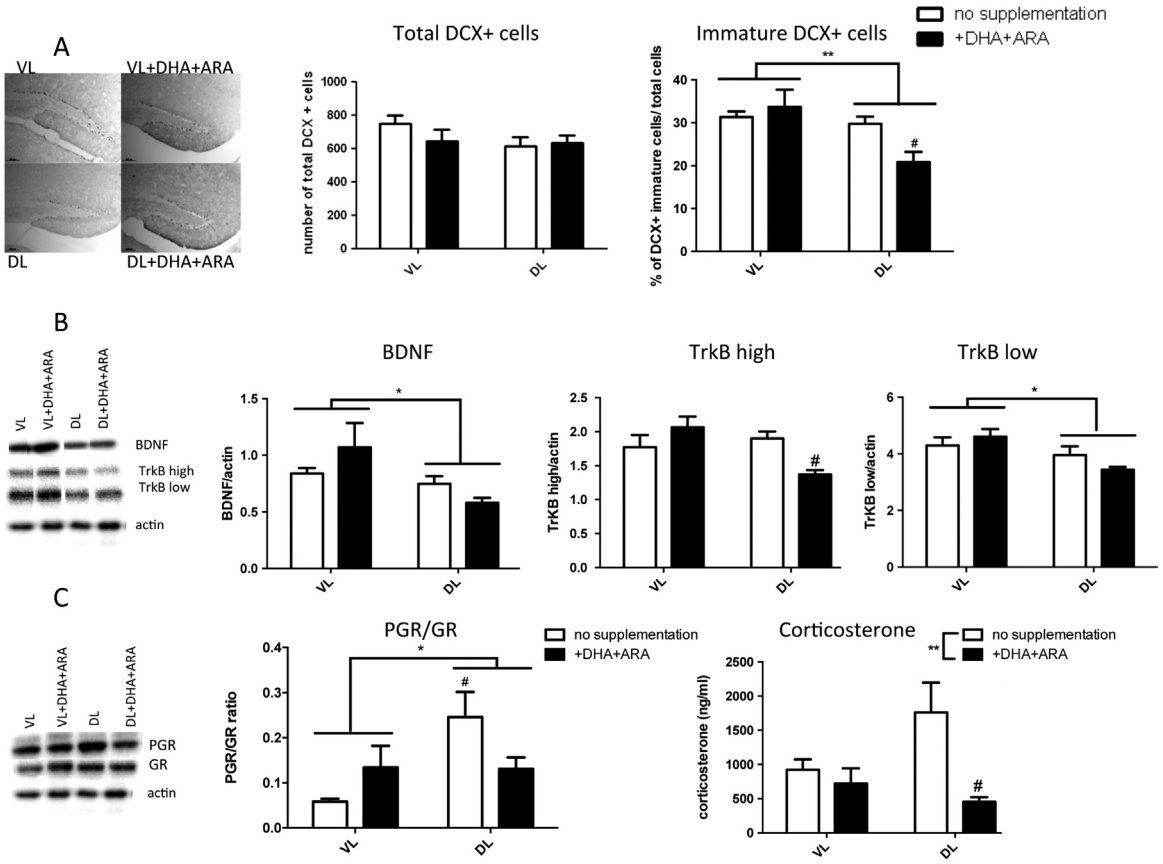

Fig. 5. Evaluation at postnatal day 90 (PND90) of (A): doublecortin (DCX)-positive cells in the dentate gyrus of the hippocampus. % of immature cells: diet effect, $p < 0.01$. (B): BDNF protein level and its TrkB receptor in the hippocampus. BDNF: diet effect, $p < 0.05$; TrkB high: diet effect, $p < 0.05$ (C): phosphorylated glucocorticoid receptor/glucocorticoid receptor ratio of the hippocampus and corticosterone level in plasma. PGR/GR: diet effect, $p < 0.05$; corticosterone: supplementation effect, $p < 0.01$. Data are expressed as mean ± SEM.2 way ANOVA analyses were realized. $^{*}p < 0.05$, $^{**}p < 0.01$, for lipid quality or supplementation factors effect. # $p < 0.05$ for post-hoc analysis.

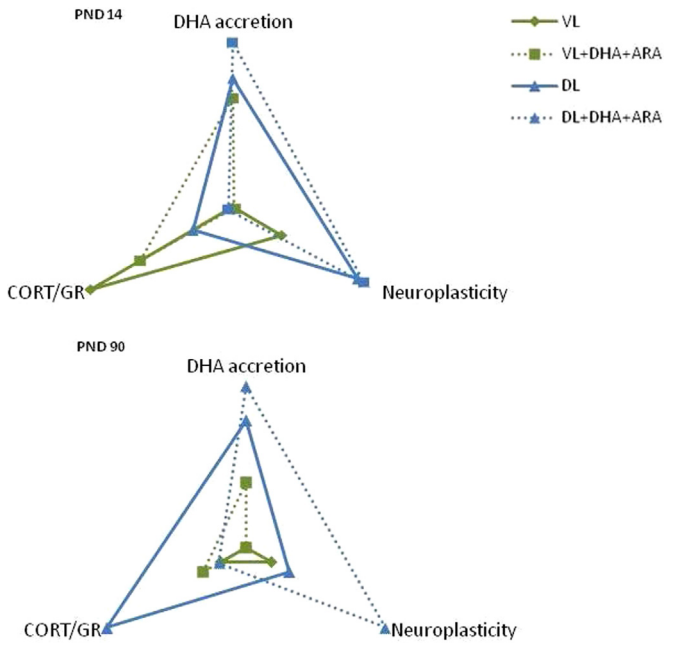

Fig. 6. Graphic representation of impact of lipid quality in diet at postnatal day 14 and 90. Results are normalized by fraction between maximum result and minimum result and represented through 3 axis. At PND14: axe 1: DHA accretion, axe 2, neuroplasticity (number of microglia, BDNF protein expression, neurogenesis), axe 3: CORT/GR (corticosterone level, PGR/GR protein expression). At PND90: axe 1: DHA accretion, axe 2: neuroplasticity (BDNF protein expression, neurogenesis), axe 3: CORT/GR (corticosterone level, PGR/GR protein expression).

During development, microglia engulfs dead neurons and synaptic material, therefore playing a major role in brain maturation [30]. Impairment of synaptic pruning results in an excess of immature synapses and is associated with a persistence of immature brain circuitry [30]. One could assume that the increase in microglia number observed in the brain of dairy fed pups could be beneficial, since synaptic pruning is particularly efficient at PND14 [55] and pruning abnormalities are observed in neurodevelopmental disorders such as autism [56,57]. However, further studies need to be performed to evaluate the biological relevance of this increase.

Dairy lipids efficiently promote neurogenesis in both juvenile and adult mice as revealed by the increased number of DCX positive cells. This effect could be linked to increased DHA, since dietary supplementation with DHA has previously been shown to promote neurogenesis, neuritogenesis and neurite growth in hippocampal neurons in the newborn [58] as well as in the adult [25,59–61]. Importantly, dairy lipids decrease the number of immature DCX positive neurons (identified as cells with small processes), in accordance with previous work with n-3 PUFA-rich or deprived diets [62–64]. In addition, dairy lipid significantly increased BDNF expression in the hippocampus of PND14 mice. This is in accordance with results showing that DHA decrease in the prefrontal cortex is accompanied by a decrease of BDNF expression [47], while n-3 PUFA dietary supplementation associated with vitamin B12 increases the level of DHA and BDNF in the hippocampus of rats [65]. Since the neurotrophin BDNF is a critical mediator of neuronal survival, differentiation, and plasticity [66], this neurotrophic factor could be involved in the improvement of neurogenesis observed in juveniles fed with dairy lipid. However, this effect could be specific to the developmental period since at

adulthood, BDNF and its receptor were both decreased by DL diet. Recent findings suggested that the ontogenetic stage during which neurons are generated is crucial for their function. Neurons born during early postnatal life and neurons born in adulthood are activated under different behavioral testing conditions [67].

We further found that GR phosphorylation was decreased in the hippocampus of dairy lipids mice at PND14 with no change in corticosterone level. On the contrary, GR phosphorylation and corticosterone levels were higher in DL adult mice. DHA is a potent modulator of the activity of the HPA axis, both at the level of corticosterone and GR phosphorylation [48]. Opposite regulation of HPA axis at PND14 and at adulthood could be linked to age as HPA axis maturates at PND14 [68]. At PND14, the stress system shifts from a hyporesponsive state to an adult state, but the development of brain stress marker continue [69]. Indeed, high fat diet exacerbates the neuroendocrine response to stress and causes prolonged secretion of glucocorticoids in adults, while it inhibits HPA response in juvenile, suggesting that the type of fatty acid (saturated vs. unsaturated) can display quite different effects on the HPA axis [70]. When supplemented with DHA+ARA, DL effect on HPA axis activity is reversed with decreased glucocorticoid phosphorylation and the corticosterone level at adulthood but not at PND14. As DHA accretion in the brain of DL+ARA+DHA mice (as compared to DL) is higher at PND14 than at adulthood, one could assume an early life impact of supplementation on HPA axis. However, this remains to be demonstrated.

In conclusion, our data suggest that dairy lipids potently influence DHA accretion, microglia number, neurogenesis and HPA axis activity as graphically represented in Fig. 6. Perinatal exposure to DL defines an early and specific profile with the promotion of brain DHA and neuroplasticity distinct to the one found with VL. DHA and ARA supplementation does not allow reaching DL profile. At adulthood, DL mice profile maintains a high level of brain DHA and neuroplasticity as compared to VL. DHA and ARA supplementation influence HPA axis activity. Our results bring new data to understand the effect of dairy lipids in brain development and function and could help to evaluate their use in infant formulas.

Funding

This work was partially funded by Lactalis.

References

[1] T.A. Sanders, D.J. Naismith, A comparison of the influence of breast-feeding and bottle-feeding on the fatty acid composition of the erythrocytes, Br. J. Nutr. 41 (1979) 619–623.
[2] M.L. Courage, U.R. McCloy, G.R. Herzberg, W.L. Andrews, B.S. Simmons, A. C. McDonald, C.N. Mercer, J.K. Friel, Visual acuity development and fatty acid composition of erythrocytes in full-term infants fed breast milk, commercial formula, or evaporated milk, J. Dev. Behav. Pediatr. 19 (1998) 9–17.
[3] I. Carrie, P. Guesnet, J.M. Bourre, H. Frances, Diets containing long-chain n-3 polyunsaturated fatty acids affect behaviour differently during development than ageing in mice, Br. J. Nutr. 83 (2000) 439–447.
[4] T. Moriguchi, N. Salem Jr., Recovery of brain docosahexaenoate leads to recovery of spatial task performance, J. Neurochem. 87 (2003) 297–309.
[5] A.J. Sinclair, Long-chain polyunsaturated fatty acids in the mammalian brain, Proc. Nutr. Soc. 34 (1975) 287–291.
[6] M.T. Clandinin, J.E. Chappell, S. Leong, T. Heim, P.R. Swyer, G.W. Chance, Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements, Early Hum. Dev. 4 (1980) 121–129.
[7] M.T. Clandinin, J.E. Chappell, S. Leong, T. Heim, P.R. Swyer, G.W. Chance, Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements, Early Hum. Dev. 4 (1980) 131–138.
[8] P.J. Morgane, R. Austin-LaFrance, J. Bronzino, J. Tonkiss, S. Diaz-Cintra, L. Cintra, T. Kemper, J.R. Galler, Prenatal malnutrition and development of the brain, Neurosci. Biobehav. Rev. 17 (1993) 91–128.
[9] H.M. Su, Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance, J. Nutr. Biochem. 21 (2010) 364–373.
[10] B. Delplanque, Q. Du, G. Agnani, P. Le Ruyet, J.C. Martin, A dairy fat matrix providing alpha-linolenic acid (ALA) is better than a vegetable fat mixture to increase brain DHA accretion in young rats, Prostaglandins Leukot. Essent. Fat. Acids 88 (2013) 115–120.
[11] Q. Du, J.C. Martin, G. Agnani, N. Pages, P. Leruyet, P. Carayon, B. Delplanque, Dairy fat blends high in alpha-linolenic acid are superior to n-3 fatty-acid-enriched palm oil blends for increasing DHA levels in the brains of young rats, J. Nutr. Biochem. 23 (2012) 1573–1582.
[12] J.M. Naughton, A.J. Sinclair, K. O'Dea, M.S. Steel, Effects of dietary butter enrichment on the fatty acid distribution of phospholipid fractions isolated from rat platelets and aortae, Biochim. Biophys. Acta 962 (1988) 166–172.
[13] H. Dabadie, E. Peuchant, M. Bernard, P. LeRuyet, F. Mendy, Moderate intake of myristic acid in sn-2 position has beneficial lipidic effects and enhances DHA of cholesteryl esters in an interventional study, J. Nutr. Biochem. 16 (2005) 375–382.
[14] V. Rioux, D. Catheline, M. Bouriel, P. Legrand, Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5 n-3 and C20:3 n-6 in the rat, Reprod. Nutr. Dev. 45 (2005) 599–612.
[15] Y. Xiao, Y. Huang, Z.Y. Chen, Distribution, depletion and recovery of docosahexaenoic acid are region-specific in rat brain, Br. J. Nutr. 94 (2005) 544–550.
[16] V.F. Labrousse, A. Nadjar, C. Joffre, L. Costes, A. Aubert, S. Gregoire, L. Bretillon, S. Laye, Short-term long chain omega3 diet protects from neuroinflammatory processes and memory impairment in aged mice, Plos One 7 (2012) e36861.
[17] T. Larrieu, C. Madore, C. Joffre, S. Laye, Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice, J. Physiol. Biochem. 68 (2012) 671–681.
[18] A. Moranis, J.C. Delpech, V. De Smedt-Peyrusse, A. Aubert, P. Guesnet, M. Lavialle, C. Joffre, S. Laye, Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice, Brain Behav. Immun. 26 (2012) 721–731.
[19] D.W. Luchtman, C. Song, Cognitive enhancement by omega-3 fatty acids from child-hood to old age: findings from animal and clinical studies, Neuropharmacology 64 (2013) 550–565.
[20] P.C. Calder, Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br. J. Clin. Pharmacol. 75 (2013) 645–662.
[21] V. De Smedt-Peyrusse, F. Sargueil, A. Moranis, H. Harizi, S. Mongrand, S. Laye, Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization, J. Neurochem. 105 (2008) 296–307.
[22] J.C. Delpech, C. Madore, C. Joffre, A. Aubert, J.X. Kang, A. Nadjar, S. Laye, Transgenic increase in n-3/n-6 fatty acid ratio protects against cognitive deficits induced by an immune challenge through decrease of neuroinflammation, Neuropsychopharmacology 40 (2015) 525–536.
[23] C. Madore, A. Nadjar, J.C. Delpech, A. Sere, A. Aubert, C. Portal, C. Joffre, S. Laye, Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes, Brain Behav. Immun. (2014).
[24] J.X. Kang, E.D. Gleason, Omega-3 Fatty acids and hippocampal neurogenesis in depression, CNS Neurol. Disord. Drug Targets 12 (2013) 460–465.
[25] E. Kawakita, M. Hashimoto, O. Shido, Docosahexaenoic acid promotes neurogenesis in vitro and in vivo, Neuroscience 139 (2006) 991–997.
[26] P. Coti Bertrand, J.R. O'Kusky, S.M. Innis, Maternal dietary (n-3) fatty acid deficiency alters neurogenesis in the embryonic rat brain, J. Nutr. 136 (2006) 1570–1575.
[27] U.K. Hanisch, Functional diversity of microglia - how heterogeneous are they to begin with? Front. Cell. Neurosci. 7 (2013) 65.
[28] M. Prinz, J. Priller, S.S. Sisodia, R.M. Ransohoff, Heterogeneity of CNS myeloid cells and their roles in neurodegeneration, Nat. Neurosci. 14 (2011) 1227–1235.
[29] C. Madore, A. Nadjar, J.C. Delpech, A. Sere, A. Aubert, C. Portal, C. Joffre, S. Laye, Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes, Brain Behav. Immun. 41 (2014) 22–31.
[30] R.C. Paolicelli, G. Bolasco, F. Pagani, L. Maggi, M. Scianni, P. Panzanelli, M. Giustetto, T.A. Ferreira, E. Guiducci, L. Dumas, D. Ragozzino, C.T. Gross, Synaptic pruning by microglia is necessary for normal brain development, Science 333 (2011) 1456–1458.
[31] D.P. Schafer, E.K. Lehrman, B. Stevens, The "quad-partite" synapse: microglia-synapse interactions in the developing and mature CNS, Glia 61 (2013) 24–36.
[32] M.E. Tremblay, R.L. Lowery, A.K. Majewska, Microglial interactions with synapses are modulated by visual experience, Plos Biol. 8 (2010) e1000527.
[33] J.C. DeMar Jr., K. Ma, J.M. Bell, M. Igarashi, D. Greenstein, S.I. Rapoport, One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores in rats, J. Lipid Res. 47 (2006) 172–180.
[34] M. Lafourcade, T. Larrieu, S. Mato, A. Duffaud, M. Sepers, I. Matias, V. De Smedt-Peyrusse, V.F. Labrousse, L. Bretillon, C. Matute, R. Rodriguez-Puertas, S. Laye, O.J. Manzoni, Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions, Nat. Neurosci. 14 (2011) 345–350.

[35] A.C. Ferraz, A.M. Delattre, R.G. Almendra, M. Sonagli, C. Borges, P. Araujo, M. L. Andersen, S. Tufik, M.M. Lima, Chronic omega-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol, Behav. Brain Res. 219 (2011) 116–122.
[36] T. Larrieu, L.M. Hilal, C. Fourrier, V. De Smedt-Peyrusse, N. Sans, L. Capuron, S. Laye, Nutritional omega-3 modulates neuronal morphology in the prefrontal cortex along with depression-related behaviour through corticosterone secretion, Transl. Psychiatry 4 (2014) e437.
[37] A.-L. Dinel, C. Joffre, P. Trifilieff, A. Aubert, A. Foury, P. Le Ruyet, S. Laye, Inflammation early in life is a vulnerability factor for emotional behavior at adolescence and for lipopolysaccharide-induced spatial memory and neurogenesis alteration at adulthood, J. Neuroinflamm. 11 (2014).
[38] A.L. Lepinay, T. Larrieu, C. Joffre, N. Acar, I. Garate, N. Castanon, G. Ferreira, B. Langelier, P. Guesnet, L. Bretillon, P. Parnet, S. Laye, M. Darnaudery, Perinatal high-fat diet increases hippocampal vulnerability to the adverse effects of subsequent high-fat feeding, Psychoneuroendocrinology 53 (2015) 82–93.
[39] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[40] W.R. Morrison, L.M. Smith, Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol, J. Lipid Res. 5 (1964) 600–608.
[41] C. Madore, C. Joffre, J.C. Delpech, V. De Smedt-Peyrusse, A. Aubert, L. Coste, S. Laye, A. Nadjar, Early morphofunctional plasticity of microglia in response to acute lipopolysaccharide, Brain Behav. Immun. 34 (2013) 151–158.
[42] C. Boitard, N. Etchamendy, J. Sauvant, A. Aubert, S. Tronel, A. Marighetto, S. Laye, G. Ferreira, Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice, Hippocampus 22 (2012) 2095–2100.
[43] D. Bonhomme, V. Pallet, G. Dominguez, L. Servant, N. Henkous, P. Lafenetre, P. Higueret, D. Beracochea, K. Touyarot, Retinoic acid modulates intrahippocampal levels of corticosterone in middle-aged mice: consequences on hippocampal plasticity and contextual memory, Front. Aging Neurosci. 6 (2014) 6.
[44] A. Kunze, A. Achilles, S. Keiner, O.W. Witte, C. Redecker, Two distinct populations of doublecortin-positive cells in the perilesional zone of cortical infarcts, BMC Neurosci. 16 (2015) 20.
[45] E.G. Waterhouse, J.J. An, L.L. Orefice, M. Baydyuk, G.Y. Liao, K. Zheng, B. Lu, B. Xu, BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission, J. Neurosci. 32 (2012) 14318–14330.
[46] F. Calabrese, A.C. Rossetti, G. Racagni, P. Gass, M.A. Riva, R. Molteni, Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity, Front. Cell. Neurosci. 8 (2014) 430.
[47] J.S. Rao, R.N. Ertley, H.J. Lee, J.C. DeMar Jr., J.T. Arnold, S.I. Rapoport, R. P. Bazinet, n-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism, Mol. Psychiatry 12 (2007) 36–46.
[48] T. Larrieu, M.L. Hilal, V. De Smedt-Peyrusse, N. Sans, S. Layé, Nutritional Omega-3 Deficiency Alters Glucocorticoid Receptor-Signaling Pathway and Neuronal Morphology in Regionally Distinct Brain Structures Associated with Emotional Deficits, Neural Plasticity.
[49] A.-L. Dinel, C. Andre, A. Aubert, G. Ferreira, S. Laye, N. Castanon, Lipopolysaccharide-induced brain activation of the indoleamine 2,3-dioxygenase and depressive-like behavior are impaired in a mouse model of metabolic syndrome, Psychoneuroendocrinology 40 (2014) 48–59.
[50] V. Rolland, S. Roseau, G. Fromentin, S. Nicolaidis, D. Tome, P.C. Even, Body weight, body composition, and energy metabolism in lean and obese Zucker rats fed soybean oil or butter, Am. J. Clin. Nutr. 75 (2002) 21–30.
[51] H. Bendixen, A. Flint, A. Raben, C.E. Hoy, H. Mu, X. Xu, E.M. Bartels, A. Astrup, Effect of 3 modified fats and a conventional fat on appetite, energy intake, energy expenditure, and substrate oxidation in healthy men, Am. J. Clin. Nutr. 75 (2002) 47–56.
[52] F. Destaillats, C. Joffre, N. Acar, F. Joffre, J.B. Bezelgues, B. Pasquis, C. Cruz-Hernandez, S. Rezzi, I. Montoliu, F. Dionisi, L. Bretillon, Differential effect of maternal diet supplementation with alpha-Linolenic adcid or n-3 long-chain polyunsaturated fatty acids on glial cell phosphatidylethanolamine and phosphatidylserine fatty acid profile in neonate rat brains, Nutr. Metab. 7 (2010) 2.
[53] I. Carrie, M. Clement, D. de Javel, H. Frances, J.M. Bourre, Specific phospholipid fatty acid composition of brain regions in mice. Effects of n-3 polyunsaturated fatty acid deficiency and phospholipid supplementation, J. Lipid Res. 41 (2000) 465–472.
[54] I. Marteinsdottir, D.F. Horrobin, C. Stenfors, E. Theodorsson, A.A. Mathe, Changes in dietary fatty acids alter phospholipid fatty acid composition in selected regions of rat brain, Prog. Neuropsychopharmacol. Biol. Psychiatry 22 (1998) 1007–1021.
[55] R.C. Paolicelli, C.T. Gross, Microglia in development: linking brain wiring to brain environment, Neuron Glia Biol. 7 (2011) 77–83.
[56] M.K. Belmonte, G. Allen, A. Beckel-Mitchener, L.M. Boulanger, R.A. Carper, S. J. Webb, Autism and abnormal development of brain connectivity, J. Neurosci. 24 (2004) 9228–9231.
[57] K. Teffer, K. Semendeferi, Human prefrontal cortex: evolution, development, and pathology, Prog. Brain Res. 195 (2012) 191–218.
[58] F. Calderon, H.Y. Kim, Docosahexaenoic acid promotes neurite growth in hippocampal neurons, J. Neurochem. 90 (2004) 979–988.
[59] N.B. Boneva, T. Yamashima, New insights into "GPR40-CREB interaction in adult neurogenesis" specific for primates, Hippocampus 22 (2012) 896–905.
[60] C. He, X. Qu, L. Cui, J. Wang, J.X. Kang, Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid, Proc. Natl. Acad. Sci. USA 106 (2009) 11370–11375.
[61] L.G. Robson, S. Dyall, D. Sidloff, A.T. Michael-Titus, Omega-3 polyunsaturated fatty acids increase the neurite outgrowth of rat sensory neurones throughout development and in aged animals, Neurobiol. Aging 31 (2010) 678–687.
[62] K. Gharami, M. Das, S. Das, Essential role of docosahexaenoic acid towards development of a smarter brain, Neurochem. Int. 89 (2015) 51–62.
[63] S.C. Dyall, G.J. Michael, A.T. Michael-Titus, Omega-3 fatty acids reverse age-related decreases in nuclear receptors and increase neurogenesis in old rats, J. Neurosci. Res. 88 (2010) 2091–2102.
[64] G. Bertrand, C. Martageix, V. Jallu, F. Vitry, C. Kaplan, Predictive value of sequential maternal anti-HPA-1a antibody concentrations for the severity of fetal alloimmune thrombocytopenia, J. Thromb. Haemost. 4 (2006) 628–637.
[65] R.S. Rathod, A.A. Khaire, A.A. Kale, S.R. Joshi, Beneficial effects of omega-3 fatty acids and vitamin B12 supplementation on brain docosahexaenoic acid, brain derived neurotrophic factor, and cognitive performance in the second-generation Wistar rats, Biofactors 41 (2015) 261–272.
[66] M.V. Chao, Neurotrophins and their receptors: a convergence point for many signalling pathways, Nat. Rev. Neurosci. 4 (2003) 299–309.
[67] S. Tronel, V. Lemaire, V. Charrier, M.F. Montaron, D.N. Abrous, Influence of ontogenetic age on the role of dentate granule neurons, Brain Struct. Funct. 220 (2015) 645–661.
[68] M.V. Schmidt, L. Enthoven, M. van der Mark, S. Levine, E.R. de Kloet, M.S. Oitzl, The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse, Int. J. Dev. Neurosci. 21 (2003) 125–132.
[69] M.V. Schmidt, S. Levine, M.S. Oitzl, M. van der Mark, M.B. Muller, F. Holsboer, E.R. de Kloet, Glucocorticoid receptor blockade disinhibits pituitary-adrenal activity during the stress hyporesponsive period of the mouse, Endocrinology 146 (2005) 1458–1464.
[70] C.D. Walker, Nutritional aspects modulating brain development and the responses to stress in early neonatal life, Prog. Neuropsychopharmacol. Biol. Psychiatry 29 (2005) 1249–1263.