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Review

Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life

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Abstract – Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are the major polyunsaturated fatty acids in the membranes of brain and retinal cells. Animals specifically deficient in dietary n-3 fatty acids have low DHA content in their membranes, reduced visual acuity and impaired learning ability. Studies on bottle-fed human infants have shown that adding DHA and AA to milk replacer-formulas can bring their concentrations in the infant blood lipids to values as high as those produced by breast-feeding and significantly improves mental development and maturation of visual function. In older subjects, diverse neuropsychiatric and neurodegenerative diseases have been associated to decreased blood levels of n-3 PUFA. Low intakes of fish or of n-3 PUFA in populations have been associated with increased risks of depression and Alzheimer disease, and n-3 PUFA, especially eicosapentaenoic acid (EPA, 20:5n-3), have shown efficacy as adjunctive treatment – and in some cases as the only treatment – in several psychiatric disorders. The mechanisms by which polyunsaturated fatty acids have an impact on neuronal functions will be reviewed: the modulation of membrane biophysical properties, regulation of neurotransmitter release, synthesis of biologically active oxygenated derivatives, and nuclear receptor-mediated transcription of genes responsive to fatty acids or to their derivatives.

docosahexaenoic acid / arachidonic acid / retina / brain / milk feeding / eicosanoids / synaptic terminals / phototransduction / neuroprotection / nuclear receptors / neuropsychiatric diseases / neurodegenerative diseases

Abbreviations

AA: arachidonic acid (20:4n-6), ABC: ATP-binding cassette transporters, ACS2: acyl-CoA synthase 2, ADHD: attention-deficit hyperactivity disorders, AFSSA: Agence Française de Sécurité Sanitaire des Aliments, AOX: peroxi-

somal acylCoA-oxidase, aP2: adipocyte lipid-binding protein 2, ApoE: apolipoprotein E, B-FABP: brain fatty acid-binding protein, CAR β : constitutive androstane receptor β , CD36: type B scavenger receptor, CNS: central nervous system, COUP-TF1: chicken ovalbumin upstream promoter-transcription factor 1, COX1 and COX2:

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cyclo-oxygenases 1 and 2, CYP2B: cytochrome P450-2B, DHA: docosahexaenoic acid (22:6n-3), DGLA: dihomo- γ -linolenic acid (20:3n-6), DHAm_{ax}: plateau value of DHA content in membrane phospholipids, DHA50: dietary dose of DHA required to reach half the DHAm_{ax}, DPA: docosapentaenoic (22:5n-6 or 22:5n-3), EPA: eicosapentaenoic acid (20:5n-3), ERG: electroretinogram, 8s-HETE: 8s-hydroxyeicosatetraenoic acid, HMG-CoAS: mitochondrial hydroxy-methyl-glutarylCoA synthase, HNF-4: hepatocyte nuclear factor-4, 3-KAT2: 3-keto acylCoA thiolase 2, LA: linoleic acid (18:2n-6), α -LNA: alpha-linolenic acid (18:3n-3), 5- and 15-LOX: 5- and 15-lipoxygenases, L-PBE: L-peroxisomal bifunctional enzyme, LTB₄: leukotriene B₄, LXR: liver X receptor, NF κ B: nuclear factor κ B, NPD1: "neuroprotectin D1", PBREM: phenobarbital responsive enhancer module, PE: phosphatidylethanolamines, PEPCK: phosphoenolpyruvate carboxykinase, 15d-PGJ₂: 15-deoxy prostaglandin J₂, PLA₂: phospholipase A₂, PP: peroxisome proliferators, PPAR: peroxisome proliferators-activated receptors, PPRE: peroxisome proliferator-responsive element, PTE-1b: peroxisomal thioesterase 1b, PUFA: polyunsaturated fatty acids, 9c-RA: 9cis retinoic acid, RAR: retinoic acid receptor, RXR: 9cis-retinoid X receptor, SREBP-1c: sterol regulatory element binding protein 1c, SCD: stearoyl-CoA desaturase, UCP: uncoupling proteins.

1. ESSENTIAL POLYUNSATURATED FATTY ACIDS (PUFA) IN THE FOOD CHAIN: ANIMALS NEED 20 AND 22 CARBON ATOMS, PLANTS SUPPLY ONLY 18

The incorporation of polyunsaturated fatty acids (PUFA) in the nerve cell membranes of the brain and retina is one of the processes of perinatal development that contributes to the functional maturation of the central nervous system (CNS). In vertebrates, two major PUFA contribute to the framework of the nerve cell membranes, arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3). The preferential incorporation of DHA in the brain and retina, as compared to other tissues, is a remarkable constant throughout the evolution of species. AA is the main long-chain derivative of the precursor of the n-6 series

of essential fatty acids, linoleic acid (LA, 18:2n-6), whereas DHA is formed from the precursor of the n-3 series, alpha-linolenic acid (α -LNA, 18:3n-3) (see review in [1]) (Fig. 1). Both precursors, which are themselves very minor constituents of the nerve cell membranes, are synthesised in variable proportions by plants, algae, bacteria and fungi, but not by vertebrate animals. In most animals, and some algae, bacteria and fungi, but not in plants, LA and α -LNA are transformed into long chain PUFA, including which AA and DHA, respectively (Fig. 1). Thus, the concentration of AA and DHA in animal tissues and animal fats depends, in part, on the intake of precursors contained in foods. Man and omnivorous and carnivorous animals can directly ingest AA and DHA from animal preys or foods, whereas herbivorous animals exclusively depend on the supply of precursors, LA and α -LNA.

Starting in the 1970s, the development of animal models specifically deficient in n-3 fatty acids, using vegetable oils very rich in LA and very poor in α -LNA [2], has allowed the discovery of the essential role of DHA in the maturation of visual [3] and cerebral functions [4]. In n-3 PUFA deficient animals, alterations of retinal and brain functions are accompanied by a decrease in retinal and cerebral concentrations of DHA and by its replacement in tissue lipids by an n-6 long-chain PUFA, docosapentaenoic acid (n-6 DPA, 22:5n-6) (Fig. 1). This structural replacement is possibly related to the functional alterations observed in n-3 deficient animals, with one of the most thoroughly investigated being that which occurs during the development of visual acuity.

2. DHA, AN ESSENTIAL FACTOR IN THE MATURATION OF VISUAL ACUITY

In rodents and in the rhesus monkey, a chronic dietary deficiency in n-3 fatty acids, i.e. starting from conception and continuing after birth and weaning, induces a sharp

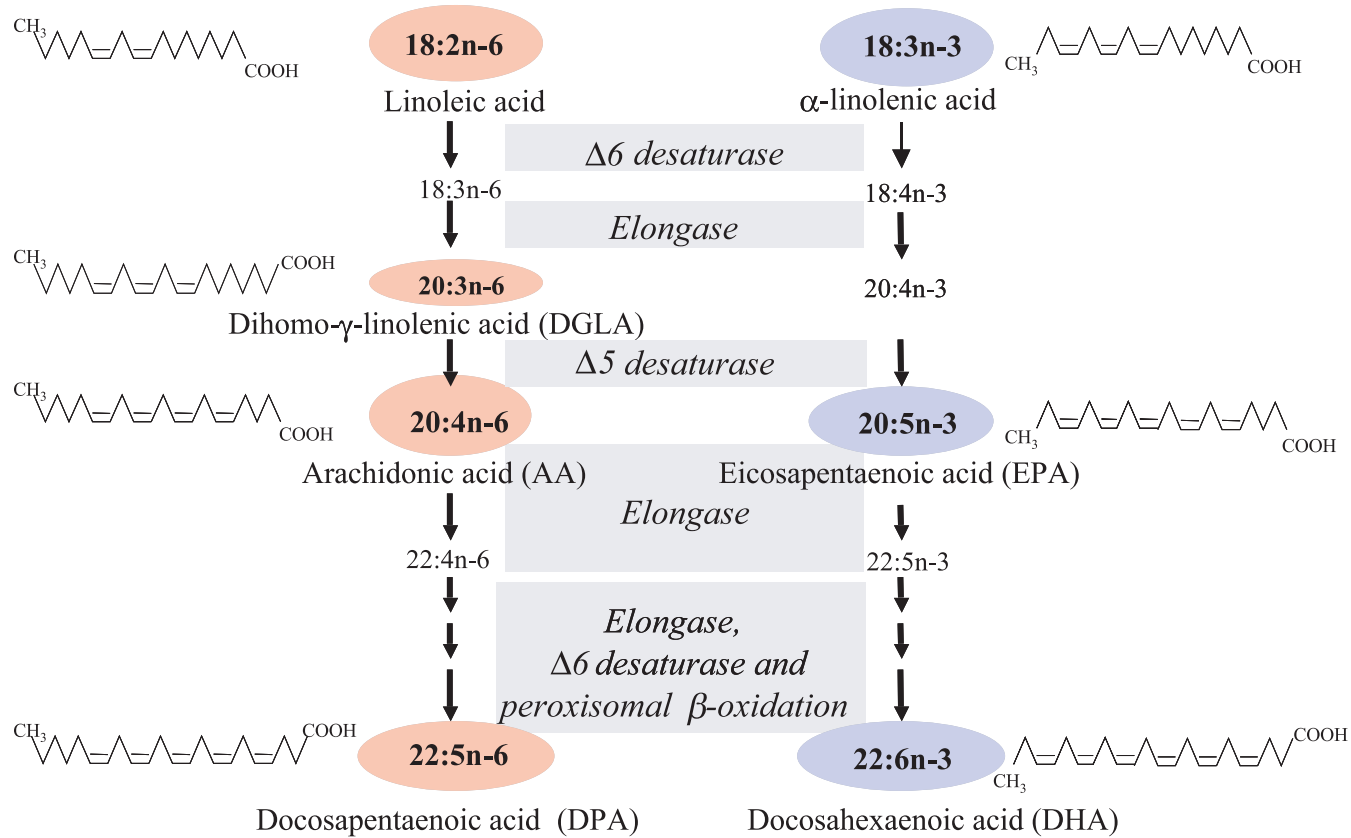


Figure 1. Fatty acid metabolism of essential fatty acids of the n-6 and n-3 series via the elongation-desaturation pathway (based on data reviewed in [1]).

decrease (from 50 to 80%) of the DHA concentration in nerve cells and results in impairments of cerebral and visual functions [4–6]. Cerebral performances have been evaluated in rodents by their capacity to elaborate and memorise a stress-escape procedure (electrical shock [4]), or to perform in an elevated plus-maze or in a water maze [7]. Their visual performances have been determined by electroretinography (ERG) [3, 4]. The analysis of components of ERG allow to characterise the response of photoreceptor cells (hyperpolarised a-wave) and those of bipolar cells and Müller cells (depolarised b-wave). Visual acuity in primates can also be evaluated by considering the visual reflex (preferential looking acuity) [5, 6] or by measuring the visual-evoked potential at the surface of the occipital cortex. These studies have shown that a diet containing vegetable oils with a very high LA/ α -LNA ratio (greater than 200), such as sunflower, groundnut or safflower seed oils, induces a significant decrease in the electrophysiological responses, as compared to a diet with a LA/ α -LNA ratio between 5 and 10, obtained by adding rapeseed or soybean oil. In n-3 PUFA deficient monkeys, these alterations consisted in an increase in the delay of the ERG peak of cone and rod cells, a decrease in the a-wave amplitude [5] and an increase in the recovery time in darkness [6, 8]. These n-3 PUFA deficiency-induced alterations have long-lasting effects on the visual acuity of young primates, even after a repletion by supplementation of the diet with fish oil for several months [9]. These studies suggest that the DHA status reached during the period of perinatal development is critical for the maturation of visual function [9]. More recently, the ERG measured in newborn baboons, not deficient at birth in n-3 PUFA and fed with a formula supplemented or not in DHA for 4 weeks, has shown that the parameters of the a-wave (initial slope change, amplitude and implicit time) are all positively correlated with the retinal concentration in DHA [10].

The data obtained in deficient animals have raised the question of the neurosensory consequences of n-3 fatty acid intakes in newborn humans fed with milk replacer formulas, containing corn or sunflower oils poor in – or almost devoid of – α -LNA.

3. BREAST VS. BOTTLE FEEDING: EFFECTS ON PUFA STATUS AND NEURAL DEVELOPMENT OF INFANTS

3.1. Breast milk and milk replacers have a different impact on the development of mental and visual functions

At the beginning of the 1990s, it was shown that feeding low birth-weight premature infants with milk replacer formulas containing corn oil as the only source of lipids decreases the sensitivity of the photoreceptor cells measured by ERG [11, 12], reduces the visual-evoked potential and impairs the forced-choice preferential-looking [13]. These authors showed for the first time that feeding such a milk replacer also induces a decrease in visual acuity of 4 month old children born at term, as compared to breast-fed infants [13]. Since then, numerous studies have been conducted in infants born at term or prematurely, breast-fed or bottle-fed with formulas supplemented or not with n-3 long-chain fatty acids (reviewed in [14]). One can conclude that supplementation with DHA (or with DHA and EPA) either improves the visual acuity of the young child in a persistent or a transient way, or has no effect. It is noteworthy that no study has detected any deleterious effect of long-chain PUFA supplementation on the visual acuity of the child. The differences associated with the type of feeding are less marked in the child than those shown in animal models, and in some cases are even inexistent. In normal conditions of development, newborn humans are not born with n-3 deficiency at birth, and the amplitudes

of the structural modifications generated by bottle-feeding as compared to breast-feeding are in no way comparable to the collapse of the DHA status caused by chronic deficiencies in n-3 PUFA.

Post mortem analyses of the cortex of infants has shown that the DHA cerebral status is, at the age of 12 months, decreased by 20% with bottle feeding as compared to breast feeding [15], which represents a 3 to 4 times less important decrease than in cases of chronic deficiencies. In addition, it is impossible to standardise the intake in DHA of maternal milk since this depends on the dietary habits of the mothers; this makes breast-feeding as a nutritional and functional reference very aleatory, although it is generally considered as being the "golden standard". The determination of the functional capacities of children should also consider confounding factors, including the post-conception age, which are even more difficult to control since the distribution of children into the different feeding groups cannot always be done in a random way. A satisfying approach consists in comparing, in infants born at term, the effects of bottle feeding with formulas containing LA and α -LNA in equilibrated proportions, and randomly supplemented or not with AA and DHA. Recent studies realised in these conditions show that the score of mental development determined at the age of 18 months is better in children receiving supplements in AA and DHA, at concentrations of 0.72% and 0.36% of total milk fatty acids, and that their visual acuity at the age of 1 year is 5/20ths higher as compared to children never receiving a supplement (but receiving the two precursors in an equilibrated proportion) [16, 17]. It is to be noted that before being placed in one of the two groups (with a supplement or not), all the infants were breast-fed during their first 4 to 6 months of life, and they all had thus received during this pre-experimental period a DHA intake representing on average 0.4% of the total fatty acids in maternal milk. The authors of this study established a linear relationship between the concentration in DHA in the

erythrocytes, which is itself a reflection of the quantities ingested by the child, and the visual acuity determined by the visual evoked potential method.

3.2. PUFA supplementation of milk replacers: long-chain or precursors?

Since the DHA status of infants fed with corn oil formulas is markedly lower than that of breast-fed infants, the readjustment of the balance between LA and α -LNA in milk replacer formulas has appeared necessary [18]. However, raising α -LNA in milk formula is not sufficient to reproduce the DHA blood levels of breast-fed infants. The human newborn possesses the enzymatic capacity to synthesise DHA from α -LNA, but to a limited extent [19], and it was found to decrease with gestational age [20]. The fraction of dietary α -LNA which is actually converted to DHA in infants is still to be determined, but it is suspected to be low. In adults, it has been estimated at less than 0.1% [21]. Finally, only milk replacer formulas supplemented with preformed DHA can ensure the same DHA status as that of breast-fed infants [22, 23].

The equilibrium between the n-6 and n-3 series in formulas can be obtained by co-supplementing with AA, so that the AA/DHA ratio mimics that of human milk (generally comprised between 1.3 and 2.0) [24, 25]. It is well known that the PUFA levels in peripheral tissues such as the heart, intestine and liver, and particularly the levels of AA and DHA, are much more sensitive to dietary influences than the PUFA levels of nerve tissue [26, 27]. Thus, in formula-fed newborn pigs, we have shown that supplementing the formula with DHA and EPA, but not with AA, decreased the concentration of AA by 65% in cardiac phosphatidylethanolamines (PE) as compared to sow milk-fed piglets, but only by 10% in brain cortex PE [27]. This lowering effect on the AA content was avoided by using a formula containing egg phospholipids, with 0.6% long chain n-6 PUFA and 0.5% long chain n-3 PUFA, allowing to reach the maximal

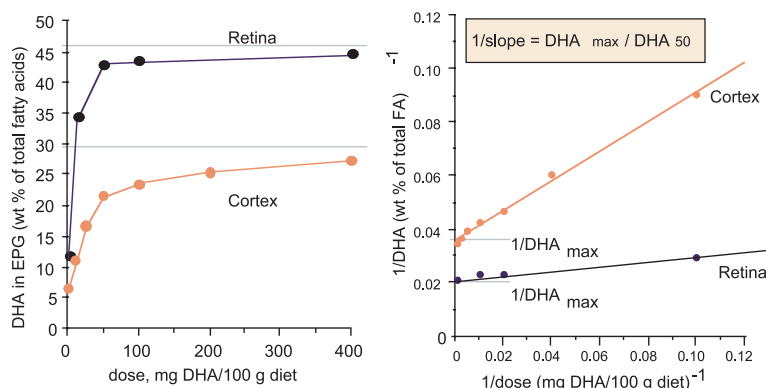


Figure 2. The plateau-value of DHA incorporation (expressed in % of total fatty acids) in the rat brain and retina phosphatidylethanolamine (PE) fractions was computed from reciprocal plotting: the reciprocal of the DHA content (1/DHA) in PE was plotted relative to the reciprocal of the dose (1/dose), the dose being the concentration of dietary DHA expressed in mg per 100 g of diet (from [29]). The double reciprocal plot results in the value of 1/dose tending toward zero as the external DHA tends toward infinite amounts, which defines the theoretical status of DHAm_{ax}. The ordinate at the origin is thus the reciprocal of the DHAm_{ax}, and the dose giving rise to twice the value of the ordinate at the origin, i.e., one half the DHAm_{ax}, gives the DHA₅₀. The straight line drawn through the double reciprocal data is thus described by the general equation: (1/DHA) = (1/DHAm_{ax}) + a (1/dose), where a = slope. The reciprocal of the slope (1/a = DHAm_{ax}/DHA₅₀) is a reflection of the specific avidity of the tissue phospholipid for DHA (the lower the slope, the higher the avidity for DHA).

incorporation of DHA in the brain and retina without altering the AA status of the other tissues [27, 28]. Most of the milk replacer formulas commercialised in occidental countries, and almost all in France, are not supplemented in long-chain PUFA (AA or DHA) but only in precursors (LA and α -LNA), which ironically makes the bottle-fed human infant the only mammal that does not receive 20 and 22 carbon PUFA during the first months of his existence.

4. USING AN ANIMAL MODEL OF DHA DEFICIENCY AND DIETARY REPLENISHMENT TO ESTIMATE THE INFANT'S BRAIN REQUIREMENTS IN DHA

The estimation of DHA needs in infants raises the question of the correspondence between the PUFA levels in circulating lipids

(the only that are accessible to analysis) and those in nerve tissues. We have proposed a method based on the dose-response curve linking brain DHA status and dietary DHA in rats [29], and on the dose-response curve linking erythrocyte DHA level and DHA concentration of maternal milk in breast-fed infants [24].

In the deficient rat model, the level of DHA in nerve tissue phospholipids from young rats born to mothers chronically deprived of n-3 fatty acids defines the lowest physiological level of DHA incorporation. When graded, increasing amounts of dietary DHA are fed throughout pre- and postnatal life to rats born to n-3 PUFA deficient mothers, the brain and retina DHA levels measured after weaning are related to DHA concentrations in the diet according to a hyperbolic dose-response curve, reaching a plateau (Fig. 2, left). The plateau values (DHAm_{ax}) were extrapolated from linear

regression after double-reciprocal plotting (Fig. 2, right). Each phospholipid class of brain areas and retina can be characterised by its DHAm_{ax}, and by the dietary dose (DHA50) required to reach half the DHAm_{ax}. The tissue avidity for DHA is indicated by the ratio of DHAm_{ax} to DHA50: the higher the ratio, the higher the efficiency for taking up DHA from the blood to the tissue and matching its DHAm_{ax}. We found that, among rat nerve tissues, the retina had the highest DHAm_{ax} (46% of total fatty acids in PE) but the lowest DHA50 (4 mg DHA·100 g⁻¹ diet), and thus the highest DHAm_{ax}/DHA50 ratio (11.5). In the PE fractions from different brain areas, the DHAm_{ax}/DHA50 ratios were comprised between 1.6 and 1.9, indicating that tissue avidity for dietary DHA is 6 to 7 times lower in the brain than in the retina [29]. The method we propose to estimate the DHA requirement of the human infant is based on the assumptions that (1) the requirement is met when the DHA levels in nerve tissue reach 90% of the DHAm_{ax}, and that (2) the dose-response curves and the DHAm_{ax}/DHA50 ratios are roughly similar in young rats and in infants. According to the rat model, about 10 times the DHA50 calculated for brain PE, i.e. 180 mg DHA·100 g⁻¹ diet, is needed to reach 90% of the DHAm_{ax} in the brain and 97% in the retina. This dietary supply corresponds to a DHA concentration in milk of 0.8% of total fatty acids. The validity of this estimation based on animal model values is supported by a dose-response study by Gibson et al. in infants, who found that the DHA content in erythrocyte phospholipids from infants fed maternal milk containing 0.8% DHA reaches 91% of its plateau-value (calculated from [24]). Therefore, we can estimate that the DHA status in infants fed a milk containing 0.8% DHA (corresponding to 0.4% of energy and to around 200 mg·day⁻¹) will match 90 to 97% of its maximum value in erythrocytes, brain and retina. In France, the mean DHA content in breast milk ranges from 0.3 to 0.4% of total fatty acids [25], leading theoretically to 85% of the infant erythrocyte DHAm_{ax} (calculated from [24]),

and by extrapolation from the rat model, to 80 and 95% of the brain and retina DHAm_{ax} values [29].

5. RECOMMENDED DIETARY INTAKES FOR N-3 FATTY ACIDS AND THEIR CONSUMPTION IN WESTERN COUNTRIES

5.1. Recommendations

Numerous official committees of nutrition such as the Agence Française de Sécurité Sanitaire des Aliments AFSSA [30, 31] and scientific societies such as the International Society for the Study of Fatty Acids and Lipids ISSFAL [32, 33] have proposed recommendations for n-3 PUFA to cover human requirements throughout life (Fig. 3). The recommended values are generally expressed both as g·day⁻¹ and as % of total energy intake. The following daily intakes are recommended for α -LNA: 0.8–1% of energy (corresponding for adult females and males to 1.6 and 2.2 g·day⁻¹), and for DHA: 0.05–0.1% of energy, i.e. 100–200 and 120–240 mg·day⁻¹ for females and males, respectively. The recommended daily intakes reach 2.2 g α -LNA and 300 mg DHA for lactating women. For the 6-month-old infant, the α -LNA recommended intakes range from 0.45–0.6% of energy [31, 33] to 1.5% (corresponding to an intake of 1 g·day⁻¹ for a 650 Kcal diet) [31]. In breast milk and infant formulas, α -LNA amounts to 1 to 3% of total fatty acids. The DHA recommended intakes for the newborn are mainly based on the breast milk contents, which are submitted to great variations among populations [34, 35]. The ISSFAL estimates as adequate a DHA intake of 0.15% of energy, i.e. around 80 mg·day⁻¹ (equivalent to 80 mg·L⁻¹ of milk and to 0.35% of total fatty acids). As stated above, we estimated as optimal for DHA incorporation in the developing brain and retina, a DHA intake of 0.8% of total fatty acids [29], corresponding to around 180 mg of DHA ingested daily (Fig. 3). The value of

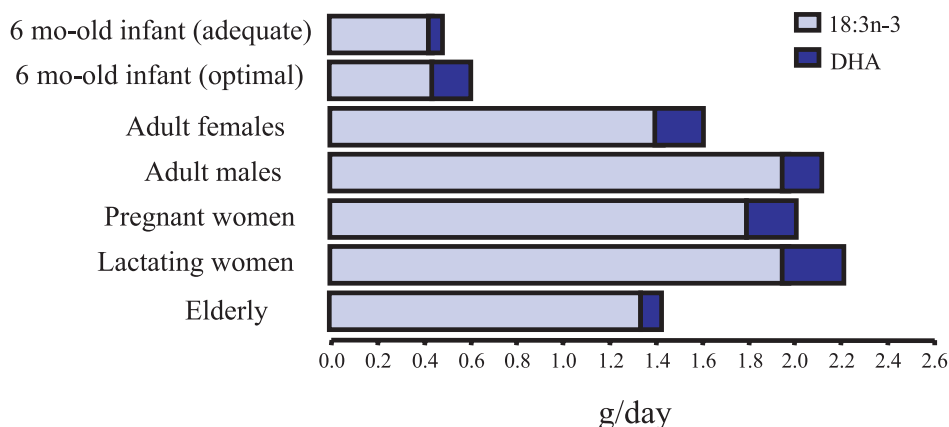


Figure 3. Recommended dietary intakes for n-3 fatty acids ($\text{g}\cdot\text{day}^{-1}$) throughout life.

0.8% of total fatty acids is in a range similar to the DHA concentrations found in the breast milk of women having fish and seafood consumption habits [34, 36].

5.2. Intakes

The estimated daily intake of n-3 PUFA in Western countries varies largely, but is often under the recommended intakes. The mean intake of α -LNA rarely reaches the recommended intakes [37–42]. The mean DHA intakes mainly depend on fish consumption, which can differ greatly between countries [43]. In some countries, it can be below the recommended intake (90–125 vs. 200–300 $\text{mg}\cdot\text{day}^{-1}$) [39], but it may be well over in countries with fish-eating habits [41]. In France, the mean intake of α -LNA is one of the lowest in Europe (0.5–0.7 $\text{g}\cdot\text{day}^{-1}$ in women and 0.6–0.9 $\text{g}\cdot\text{day}^{-1}$ in men, or 0.3–0.4% of energy) [37, 42, 44], less than half of the recommended intake. These low α -LNA intakes, while those in LA are much higher, have to be related to the high mean value (close to 20) of the LA to α -LNA ratio that we have found in the milk of French mothers [25, 35]. However, the mean intakes of long-chain n-3 PUFA (400 $\text{mg}\cdot\text{day}^{-1}$ in women and 500 $\text{mg}\cdot\text{day}^{-1}$ in men), and especially that of DHA

(225 $\text{mg}\cdot\text{day}^{-1}$ in women and 270 $\text{mg}\cdot\text{day}^{-1}$ in men), appear to meet the recommendations, although with very large individual variations [42]. Therefore, it would be useful to increase the intake of α -LNA by consuming α -LNA-rich vegetable oils, such as rapeseed or soybean oils, and to encourage the consumption of fatty fish in persons who never eat fish, or who eat fish only rarely.

6. N-3 PUFA AND NEUROPSYCHIATRIC DISEASES

6.1. Clinical data

Essential PUFA can affect brain functions beyond the critical period of perinatal development. Alterations of n-6 and n-3 status have been associated with psychiatric pathologies in children and adults. Decreases in the blood levels of n-3 and/or n-6 PUFA have been observed in patients with depression [45–50], bipolar disorder [51, 52], schizophrenia [53–57], child hyperactivity [58] and autism [59]. Whether these alterations of the essential fatty acid contents of blood lipids are implicated in the etiology of the diseases, and whether they are causes or only side consequences of the

pathologies are still matters of debate. In the case of schizophrenia, decreased levels of both n-3 PUFA (EPA, n-3 DPA, DHA) and AA in blood lipids have been repeatedly observed [53–57]. These alterations are correlated to the intensity of psychotic symptoms of nontreated patients [57] and tend to be normalised by a neuroleptic treatment [56, 57], which suggests that the decrease of blood PUFA levels is tightly involved in the pathophysiology of the disease. There is to-date little argument, however, that a lower PUFA status due to dietary deficiencies could be by itself an independent cause or even a risk factor for this disease with a strong genetic background. At variance, patients with depression showed decreased levels of one or several n-3 PUFA (α -LNA, EPA, n-3 DPA, DHA) in blood lipids (as compared to non-depressed controls), but not of n-6 PUFA [45–49]. Some studies have shown a negative correlation between the n-3 PUFA levels or the EPA/AA ratio in erythrocytes and the depression score of the patients [45, 48]. In one study [48], the levels of α -LNA, EPA, DPA and DHA in erythrocytes were correlated positively with their respective dietary intakes, which suggests that their lower levels found in depressed patients could be a consequence of a dietary deficiency. A study on post-partum depression found that women who have developed postpartum depression had less DHA in plasma lipids at delivery than women who did not, which suggests a causal relation between a lower n-3 PUFAs status at delivery and the ulterior onset of depression [50].

6.2. Epidemiological studies

The possible relations between fish or n-3 PUFA intakes or PUFA status and depression or other mood disorders have been further investigated in epidemiological population studies. Several international comparison studies have searched the existence of an association between mean fish consumption and the prevalence of affective disorders

in different countries around the world. A strong decreasing relation was found between the prevalence of major depression [60], postpartum depression [61] and bipolar spectrum disorders [62] and the apparent mean fish and seafood consumption calculated from FAO statistics. In the case of postpartum depression, both a greater fish and seafood consumption and a higher DHA content of mother's milk predicted a lower postpartum depression prevalence [61]. No such relation was observed with the prevalence of schizophrenia, suggesting that it would be specific to affective disorders [62]. In these ecological studies, however, the relations found can be in part due to many possible confounding factors (genetic, cultural, environmental), especially since the countries with the lowest rates of mood disorders are mainly Asian countries (Taiwan, Korea, Japan, Singapore, Hong-Kong, Malaysia), but there are also other countries such as Iceland or Chile.

Other types of epidemiological studies (cross-sectional, case-control, nested case-control and cohorts) dealing with fish or n-3 PUFA intake and depression or depression-related endpoints are summarised in Table I. One large cohort study in Japan found a negative association between fish consumption at the baseline and suicide rate during follow-up [63]. A case-control study in China found a strong association between suicide attempt and low EPA and DHA levels in red blood cells [64]. Five cross-sectional studies in Finland, New Zealand and Japan found a significant negative association of depression or depressive symptoms with fish consumption [65–68], or with the intake of α -LNA [69]. When men and women were analysed separately, this association was only seen in women [65, 68]. Two small cross-sectional studies in population samples from Crete and a nested case-control study in the Netherlands found lower levels of α -LNA or of DHA in the blood or adipose tissue of depressed subjects than of non-depressed subjects [70–72]. Finally, only one large cohort of men smokers in Finland did not report any inverse association of fish

Table I. Fish and polyunsaturated fatty acid intake and depression: epidemiologic observational studies.

Country, period	Study type	Sample size: cases/controls or cases (cohort) and age (yr)	Fish or n-3 PUFA intake (contrast for OR or RR), or blood or tissue biomarker	Method/endpoints	Odds ratio (OR) or relative risk (RR) (95% confidence interval) or main result, and <i>P</i> for trend or difference	Reference
Japan, 1965–1982 (follow-up period)	Cohort	870 (265 118) men and women ≥ 40 at inclusion	daily vs. non-daily fish intake	death register/suicide	0.81 (0.72–0.91) <i>P</i> not determined	[63]
China, 2002	Case-control	100/100 men and women (all ages)	EPA and DHA % in red cell PL 4th vs. 1st quartile	hospital admitted patients: suicide attempt cases vs. accident trauma controls	EPA: 0.12 (0.04–0.36) <i>P</i> = 0.0001 DHA: 0.21 (0.07–0.60) <i>P</i> = 0.002	[64]
Finland, 1992	Cross-sectional	896 (3 403) men and women 25–64	≥ 1 fish meal/week ⁻¹ vs. less often	self-reported depressive symptoms (BDI)	0.76 (0.64–0.91); 0.71 (0.56–0.90) in women (<i>P</i> < 0.01), not significant in men	[65]
Finland, 1999	Cross-sectional	not reported (1 767) men and women, 25–64	≥ 2 fish meals/week ⁻¹ vs. less often	self-reported depressive symptoms (BDI)	0.63 (0.43–0.94) <i>P</i> = 0.02	[66]
New Zealand, 1996–1997	Cross-sectional	(4 644) men and women, ≥ 15	any fish intake vs. no fish intake	self-reported mental health (SF–36)	higher mental health score in fish consumers <i>P</i> < 0.01	[67]
Crete, 1999	Cross-sectional	22 (143) men and women, mean 39	% fatty acids in adipose tissue	self-reported depressive symptoms (Zung)	less DHA in mildly depressed subjects, <i>P</i> < 0.01	[70]
Crete, 2000	Cross-sectional	24 (67) men, mean 85	% fatty acids in adipose tissue	depressive symptoms (GDS–15)	less α -LNA in depressed subjects <i>P</i> < 0.02	[71]
Netherlands 1997–1999	Nested case-control	264/461 among 3884 men and women, ≥ 60	% fatty acids in plasma phospholipids	psychiatric interview	less DHA and more AA in depressed subjects <i>P</i> = 0.05	[72]
Japan	Cross-sectional	436 (771) men and women lung cancer patients, mean 64	α -LNA, EPA and DHA intakes (4th vs. 1st quartile)	depression (HADS-D)	α -LNA: 0.50 (0.31–0.71); <i>P</i> = 0.004 no association with EPA or DHA	[69]
Finland, 1985–1994 (follow-up period)	Cohort (ATBC study)	8,612 (27 111) male smokers 50–69	fish, fish and vegetable n-3 PUFA intakes (3rd vs. 1st tertile)	self-reported depressed mood during follow-up	fish intake: 1.06 (1.00–1.12); <i>P</i> = 0.04; no association with α -LNA, EPA or DHA	[73]
Finland, 1997	Cross-sectional (in a cohort)	107 to 483 (8 463) men and women, mean 31	≥ 1 fish meal/week vs. less often	self-reported depression (HSCL–25) and doctor-diagnosed lifetime depression	in women: lifetime depression 0.83 (0.62–1.11); current depression 0.71 (0.55–0.91); both 0.42 (0.24–0.71), no association in men	[68]

or n-3 PUFA intakes with depression [73]. On the whole, most studies have found a negative association of depression or depressive symptoms with fish or n-3 PUFA intake, or with n-3 PUFA levels in blood or adipose tissue. The relation was seen among women, not (or not significantly) among men. Thus, there is a strong suspicion from epidemiological studies that a low fish or n-3 PUFA intake can increase depression or suicide risk, especially in women, but studies with a prospective design are still needed to prove it.

6.3. Intervention studies: specific effects for EPA and DHA?

Randomised, double-blind, placebo-controlled intervention studies have been undertaken to test the effect of n-3 PUFA (fish oil, EPA or DHA) in psychiatric patients with diverse diagnoses. Those of these studies which deal with mood disorders are summarised in Table II. EPA at the dose of 1 or 2 g·day⁻¹ has shown efficacy in reducing symptoms of treated depressive patients [74, 75], but not, curiously, at a higher dose [75]. A high dose of EPA (6 g·day⁻¹) did not improve bipolar patients [76]. However fish oil providing 4–6 g EPA and 2–3 g DHA·day⁻¹ appeared to improve both depressive and bipolar patients [77, 78], including bipolar patients receiving no other treatment [77]. In the latter trial on bipolar patients, the improvement due to fish oil treatment consisted in a lower rate of depressive relapses [77]. EPA at 1 g·day⁻¹ was efficient in reducing both aggression and depression symptoms of women with untreated borderline personality disorder [79]. In contrast, DHA showed no effect on depression in the two studies where it was tested as the only treatment [80, 81]. In particular, DHA at a dose sufficient to prevent its depletion during pregnancy and lactation (200 mg·day⁻¹) has no effect on postpartum depression rate when given to breast-feeding women after delivery [81], which suggests that either higher doses are required, or that supplementing during preg-

nancy is necessary, or that DHA by itself is not active in preventing postpartum depression.

EPA or DHA as the only or adjunctive treatment have been tested in psychiatric disorders other than mood disorders. EPA as adjunctive treatment helps to improve schizophrenic patients when given at the dose of 2–3 g·day⁻¹ [82–84], but not, again, at higher doses [83], whereas DHA showed no effect [82]. One trial on children with learning problems and attention-deficit hyperactivity disorder (ADHD) symptoms has shown a significant improvement in subjects receiving a mixture of n-6 and n-3 PUFA as the only treatment [85]. In a series of open cases, young girls with anorexia nervosa improved or recovered when treated with EPA (1 g·day⁻¹) in addition to a standard treatment [86].

On the whole, EPA at doses varying from 1 to 3 g·day⁻¹, or fish oil at higher doses appear to improve psychiatric patients with astonishingly diverse diagnoses. DHA showed no effect in the few trials where it was tested. In schizophrenic patients, improvement due to EPA treatment was surprisingly correlated with the rise of red blood cell AA level [83, 87]. The fact that high doses (≥ 4 g·day⁻¹) of pure EPA are not efficient (or less than lower doses) in schizophrenic, depressive and bipolar patients, might be related to a decrease (or a lack of restoration) of the membrane levels of AA [84]. Other trials are warranted to determine the efficacy of n-3 PUFA in psychiatric patients. Owing to the high prevalence of depression in Western countries, an increasing interest is borne to the potential preventive and therapeutic role of n-3 PUFA in depression, in particular: (1) in cases where a standard antidepressant treatment is not indicated or not desired (depression of low or moderate intensity, depression during pregnancy); (2) as a maintenance or preventive treatment in at-risk subjects (patients with previous depressive episodes, pregnant women, dysthymic subjects, etc).

Table II. n-3 polyunsaturated fatty acids and mood or mood-related disorders: randomised, placebo-controlled interventional studies. Borderline personality disorder (BPD) is generally not classified in mood disorders; we include here one trial on BPD, since it involves the evaluation of depressive symptoms.

Country, sample size and duration	Diagnosis	Intervention	Endpoints	Results	Reference
Israel 3 men and 17 women 4 weeks	Past episodes of depression and current major depression, treated but resistant	Adjunctive ethyl-EPA 2 g·day ⁻¹ , or placebo	Depression score (Hamilton Depression Rating Scale) at baseline and after 1–4 weeks	Depression score lower in EPA group than in placebo after 2 weeks; 50% decrease after 4 weeks on EPA, whereas no decrease in placebo group	[74]
UK 11 men and 59 women 12 weeks	Persistent major depression despite ongoing treatment	Adjunctive ethyl-EPA 1, 2 or 4 g·day ⁻¹ , or placebo	Depression scores (HDRS, MADRS, BDI) at baseline and after 4, 8 and 12 weeks	Depression scores lower in the 1 g EPA·day ⁻¹ group than in the placebo group at all time points; no or marginal effect of EPA at the doses of 2·day ⁻¹ and 4g·day ⁻¹	[75]
Taiwan 4 men and 18 women 8 weeks	Major depression, ongoing treatment	Adjunctive fish oil, 4.4 g EPA + 2.2 g DHA·day ⁻¹ , or placebo	Depression score (HDRS) at baseline and after 8 weeks	Depression score lower in the group receiving fish oil than in the placebo group after 8 weeks	[78]
USA 35 men and women 6 weeks	Major depression, no ongoing treatment	DHA 2g·day ⁻¹ , or placebo	Depression scores (HDRS, MADRS)	No effect of DHA on depression scores	[80]
USA 89 women at delivery 4 months	Women at delivery, planning to breast-feed for at least 4 months	DHA (as algae oil) 200 mg·day ⁻¹ , or placebo	Self-rated depression score (BDI) at baseline, 3 weeks, 2 and 4 months ; postpartum depression score (EPDS) at 18 months	No effect of DHA on depression scores or depression rate at any time point	[81]
USA 10 men and 20 women 4 months	Bipolar disorder I or II, ongoing mood-stabilizing treatment (22) or no treatment (8)	Fish oil, 6.2 g EPA + 3.4 g DHA·day ⁻¹ , or placebo, adjunctive or alone	Duration of time to exit the trial because of a depressive relapse requiring a new treatment	Greater mean duration of time remaining in the study and less depressive relapses in the fish oil-treated group, including among the patients receiving no other treatment	[77]
USA 121 men and women 4 months	Bipolar disorder: current acute depression (59) or rapid cycling (62)	Adjunctive EPA 6 g·day ⁻¹ , or placebo	Depression score (?)	No clinical response of EPA	[76]
USA 20 women 8 weeks	Borderline personality disorder, not treated	Ethyl-EPA 1 g·day ⁻¹ , or placebo	Aggression score (MOAS) and depression score (MADRS) at baseline and after 2–8 weeks	Past episodes of depression and current major depression, treated but resistant Better improvement of patients receiving EPA than those receiving placebo on both aggression and depression scores	[79]

7. PUFA STATUS AND NEURODEGENERATIVE DISEASES

7.1. PUFA status in Alzheimer disease

Modifications of the PUFA status have also been associated with neurodegenerative diseases, in particular Alzheimer disease. Alzheimer disease is the most frequent neurodegenerative dementia occurring in elderly people. Contrary to vascular demen-tias, which are secondary to vascular events, the etiology of Alzheimer disease appears to be idiopathic, and only concerns brain cells. Case-control studies have shown that patients affected with Alzheimer disease present 30 to 50% lower concentrations of EPA and DHA in their erythrocyte or serum lipids than normal subjects of the same age [88, 89]. In both a cross-sectional study and a prospective study in population samples of mean-old age (45–75 years), a higher intake of marine n-3 PUFA or a higher percentage of EPA and DHA in erythrocytes were associated with a lower risk of cognitive decline [90, 91]. Several prospective studies have examined if a relationship exists between baseline consumption of sea products rich in n-3 PUFA and the incidence of Alzheimer disease. People eating fish or seafood once a week or more have a risk of developing Alzheimer disease in the following years 30 to 70% lower than people eating seafood only rarely or never [92–94]. In one of these studies, the detailed analysis of the n-3 PUFA intakes showed that a decreased risk was strongly associated with the intake of DHA, but not with the intakes of EPA or α -LNA [93]. A 70% decreased risk was found in persons with a daily intake of 100 mg DHA (highest quintile), as compared to those with a daily intake of 30 mg DHA (lowest quintile) [93]. This result could suggest that, on the contrary to depression, dietary DHA, rather than EPA, is involved in neuroprotective processes which prevent or delay the development of Alzheimer disease, but more evidence, in particular from intervention studies, is needed to support this hypothesis. In fact,

in observational studies, it is generally difficult to separately analyse the effects of dietary EPA and DHA, since both of them are always strongly correlated with fish and seafood intake. In addition to the association observed with Alzheimer disease, isolated, small uncontrolled open clinical trials suggest a possible efficacy of n-3 PUFA in other neurological diseases: multiple sclerosis [95], epilepsy [96], and Huntington disease [97].

7.2. Trial of DHA treatment in rat studies

Since the protecting effect of DHA seems to be associated with well-installed food habits, it is possible that the mechanisms implicated concern the concentration of this fatty acid in brain tissue membranes. This hypothesis seems to be supported by the results of a Japanese study using an animal model for the infusion of the amyloid A β peptide by intracerebral microdialysis, reproducing the neurofibrillar degeneration of Alzheimer disease [98]. Infused rats presented lipoperoxidation products and neuronal apoptosis signs, and their learning capacities strongly decreased. These effects were cancelled if the rats had ingested 100 mg DHA daily during 12 weeks before the infusion of the A β peptide, which increased the concentration of DHA in the brain by 30% as compared to rats not receiving any supplement. In the third session of the test implicating the memory function, the performances of the rats pretreated with DHA and infused with the amyloid peptide were higher than those of placebo rats and closer to those of rats pretreated with DHA and not being infused. The authors suggested that DHA has an antioxidant neuroprotecting effect on the apoptotic process induced by free radicals and lipoperoxidation products.

8. MECHANISMS OF ACTION OF N-3 PUFA IN THE BRAIN AND RETINA: FOUR HYPOTHESES

The different hypotheses emitted to explain the mechanisms of action of PUFA

illustrates the wide diversity of the regulated functions by these components. Although the different pathways may be strongly interconnected at all the molecular, cellular and physiological levels, four hypotheses will be successively and separately presented within the framework of this paper.

8.1. PUFA are precursors of active mediators

8.1.1. *Synthesis of eicosanoids*

This pathway of PUFA action on nerve cells starts with their release from the internal sn-2 position of membrane phospholipids under the action of a hormone- or a calcium-dependent phospholipase A2 (PLA2). PUFA with 20 carbon atoms (AA, EPA, and dihomo- γ -linolenic acid or DGLA, 20:3n-6) are then converted in oxygenated products, eicosanoids (Fig. 4). The reactions are catalysed by 15-lipoxygenase (15-LOX), 5-lipoxygenase (5-LOX) or cyclo-oxygenases (COX1 and COX2). The 15-LOX and 5-LOX pathways lead to the formation of lipoxins and leukotrienes involved in immune and inflammatory reactions, whereas COX pathways produce endoperoxides, which are notably active in the constriction of blood vessels and in platelet aggregation. Prostaglandins, prostacyclins and thromboxanes of the 1, 2, and 3 series are produced by the COX pathway from respectively DGLA, AA and EPA. The 5-LOX pathway leads to leucotrienes of the series 4 from AA and of the series 5 from EPA. Since the fatty acid substrates are competing for the same enzymes, the biosynthesis of endoperoxides of the series 1 and 3 counteracts that of the series 2, and the biosynthesis of leucotrienes of the series 5 counteracts that of the series 4. The dietary equilibrium between the n-6 and n-3 fatty acids thus determines the balance in the synthesis of active mediators whose properties are generally opposed. AA generates proagregant and vasoconstrictor (series 2 endoperoxides) and proinflammatory (series 4 leucotrienes) mediators, whereas the mediators

issued from EPA have antiagregating and vasodilating properties (endoperoxides of the series 3), and non-inflammatory properties (series 5 leucotrienes).

8.1.2. *Eicosanoids and the regulator role of astrocytes*

The AA-derived eicosanoids probably have an action in the brain. By binding on astrocyte membrane receptors, the series 2 prostaglandins, produced in response to an influx of calcium ions, may be involved in the regulation of the glutamate release in the synaptic cleft (for a review see [99]). The released glutamate modulates neuronal excitability and synaptic transmission at the pre-synaptic level. On the contrary, glutamate uptake by astrocytes (Fig. 5), avoids its potentially neurotoxic accumulation in the synaptic cleft. A defect in the astrocytic uptake of glutamate may be the cause of neuronal damages acquired during traumatisms (ischemia, lesions) and could be associated to several neurodegenerative pathologies. Moreover, the excessive release of AA could alter glutamate transporters and contribute to the extracellular accumulation of glutamate. The effects of oxidative stress on glutamate transport is possibly mediated by a product of PUFA peroxidation, 4-hydroxynonenal, which may interact with the GLT-1 astrocyte transporter (EAAT2) and induce its inactivation [100]. Under the action of different stimuli, especially in response to proinflammatory cytokines [101], astrocytes in the synaptic cleft also release a sPLA2 which releases AA from the sn-2 position of membrane phospholipids. Compared to AA, EPA and DHA are less good substrates for LOX and COX, and they modulate the nature and amounts of the oxygenated derivatives [102–105]. Increasing the dietary intake of n-3 PUFA could thus slow down the inflammatory cycle maintained by the action of cytokines on astrocytes, i.e. the secretion of sPLA2, a large release of AA and production of PGE2 in the extracellular space, inducing in return the synthesis of inflammatory interleukins by astrocytes [99].

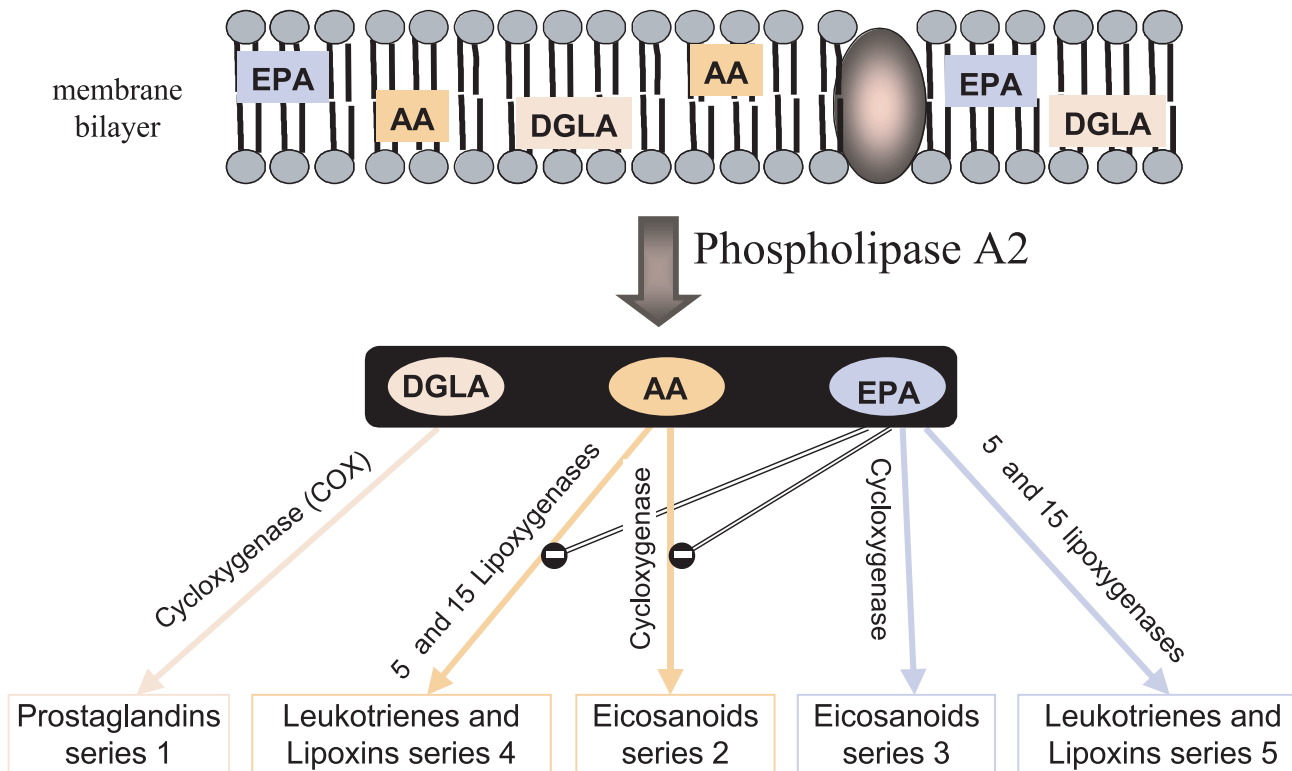


Figure 4. PUFA with 20 carbon atoms (AA, EPA, and DGLA) are released from membrane phospholipids and oxygenated to give eicosanoids. The oxygenation is catalysed by the 15-lipoxygenase (15-LOX), 5-lipoxygenase (5-LOX) or the cyclo-oxygenases (COX1 and COX2). The 15-LOX and 5-LOX pathways produce lipoxins and leukotrienes implicated in the immune and inflammatory reactions, and the COX pathway produces endoperoxides implicated in the constriction of blood vessels and in platelet aggregation. The prostaglandins, prostacyclins and thromboxanes of the 1, 2, and 3 series are produced by the COX pathway from respectively DGLA, AA and EPA. 5-LOX leads to leukotrienes of the series 4 from AA and of the series 5 from EPA.

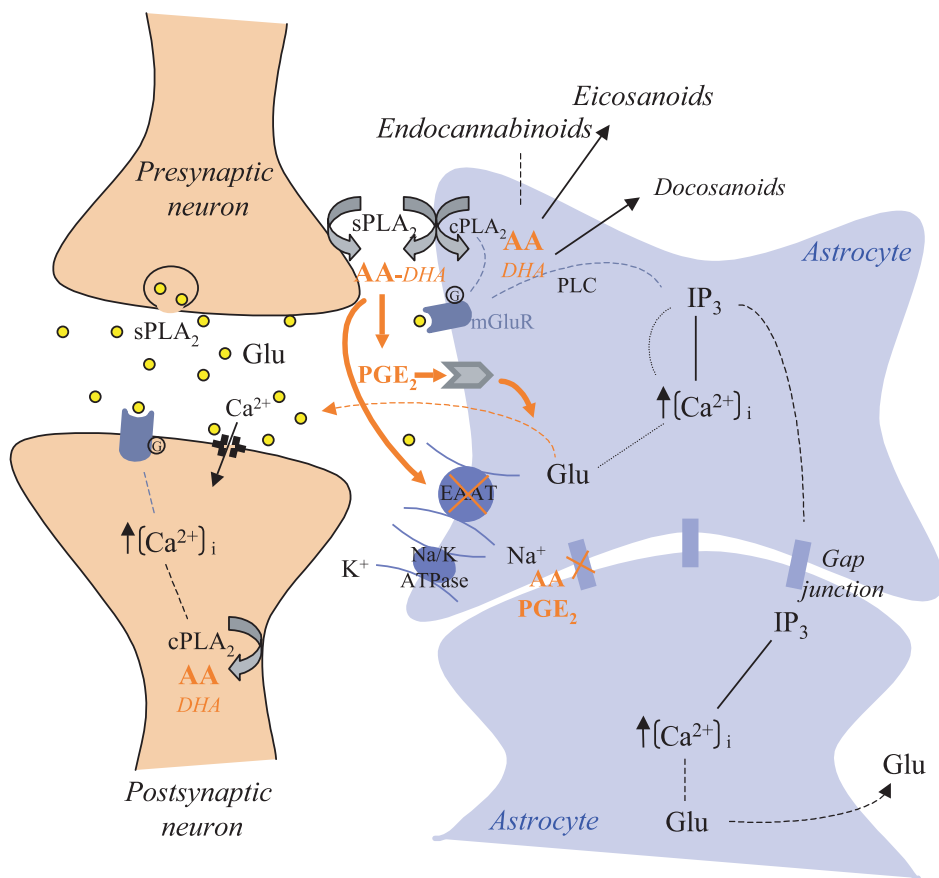


Figure 5. Implication of PUFA in the synaptic regulation by astrocytes: the release of glutamate (Glu) during neuronal activation induces a calcium influx in the postsynaptic neurons and an activation of neuronal and astrocytic glutamate receptors. The activation of metabotropic receptors (mGluR) results in an increase of intracellular calcium concentration through the IP_3 pathway [phospholipase C (PLC) activation, IP_3 increase, calcium release of intracellular pools]. Calcium-mediated activation of the cytoplasmic phospholipase A_2 (cPLA $_2$) produces the release of AA and DHA from membrane phospholipids. The cPLA $_2$ may be directly activated through G-protein-linked mGluR. A secretory PLA $_2$ (sPLA $_2$), may be released from the presynaptic terminal. Lipid signalling can modulate neuronal function and survival in several ways : (1) Free AA regulates the extracellular concentration of glutamate by inhibiting the glutamate uptake by the astrocyte transporter (EAAT), and by activating its release in a Ca^{2+} -dependent mechanism via the stimulation of the astrocyte receptors for PGE $_2$ (prostaglandins E $_2$). (2) Free AA and DHA may enter the enzymatic oxygenation pathways, producing the respective eicosanoids and docosanoids which can also regulate the synaptic transmission. (3) AA and PGE $_2$ can regulate through the IP_3 -mediated propagation of Ca^{2+} waves throughout the glial cells through inhibition of gap junctions.

We recently showed that the concentrations in DHA and AA in the phospholipid membranes of cultured astrocytes vary as a function of the concentration of the fatty

acids in this medium, and notably that the concentration of AA is inversely related to the concentration of DHA [106]. In addition, we observed that supplemental DHA

has an effect on the morphology of astrocytes from primary culture, which exhibit a stellar shape different from that induced by AA (Denis et al., unpublished results). In the brain, the reversible process of astrocytic stellation allows the emission and the retraction of the astrocytic processes around the synapses, thus regulating the neuronal energy flux and the ion and neurotransmitter homeostasis in the synaptic cleft (review in [107]). The hypothesis can be made that the respective proportions of DHA and AA in membranes may have an impact on the morphological plasticity and on the different astrocyte functions involved in the regulation of synaptic transmission.

8.1.3. New mediators, the docosanoids

The nerve membranes contain much more AA than EPA, which makes the local synthesis of eicosanoids from cerebral EPA quite questionable. However, these membranes contain almost as much DHA as AA, or even more in the case of the retina, and bioactive docosanoids could be produced from DHA via the same enzymatic pathways as those of PUFA made of 20 carbons. The biosynthesis in the brain of a new mediator formed from enzymatic oxygenation of DHA was recently evidenced in a model of cerebral ischemia and reperfusion in the mouse [108]. The ischemic stroke results in the release of lipoperoxides and cytokines, the increase of COX2 expression and the infiltration of leukocytes. Among the liberated mediators, the authors isolated a new di-hydroxylated compound issued from the oxygenation of DHA by 15-LOX, the 10,17S-docosatriene, which once isolated and perfused in another animal can strongly inhibit the inflammatory responses induced by the ischemic stroke. According to the authors, this pathway of enzymatic oxygenation of endogenous DHA may contribute to protect the brain against injury produced by oxidising stress. Very recently, they have shown that the 10,17S-docosatriene molecule, so called “neuroprotectin D1” (NPD1), is synthesised by human retinal pigment epithelial cells treated with a cal-

cium ionophore or with DHA [109]. NPD1 added to retinal pigment epithelial cells potently counteracted the apoptotic DNA damage triggered by treatments with oxidative agents. NPD1 also up-regulated the expression of antiapoptotic proteins (Bcl-2 and Bcl-xL), while it decreased those of proapoptotic proteins (Bax, Bad) and COX2 [109]. Overall, NPD1 protected cells from oxidative stress-induced apoptosis. The NPD1 pathway will very probably lead to new perspectives in research to understand the protective effect of DHA-derived mediators in hypoxic-ischemic situations.

8.2. PUFA are components of membrane domains

8.2.1. Membrane lipids do not mix uniformly

Since the fluid mosaic model of Singer and Nicholson, the membrane concept has evolved. The protein-protein interactions, within and around the membrane clearly depend on the quality of their lipid micro-environment, and membrane domains (“lipid rafts”) have received increasing attention as potential platforms for proteins in signalling and trafficking. The theory of lipid rafts (reviews in [110, 111]) conceptualises that separation of discrete liquid-ordered and liquid-disordered phase domains occurs in membranes containing sufficient amounts of sphingolipids, sterols and saturated phosphatidylcholines. These clusters of ordered lipids are characterised by their insolubility, at low temperature, in nonionic detergents. Then, hydrophobic proteins can be locally packaged with these lipid clusters and be organised as membrane rafts. The organisation, and thus the possibilities for interactions and activations of these lipid raft-interacting proteins, are very different from those of proteins preferentially embedded in the fluid phase, the latter being composed of PUFA-rich phospholipids having a low degree of order of their acyl chains and allowing a much larger freedom of movement. Studies on artificial membranes have focussed on

the possible link between DHA, the more unsaturated among structural fatty acids (characterised by a low degree of order within the membrane phospholipids) and the capacity of rhodopsin to receive photons and to transduce them into a biochemical signal.

8.2.2. Photo-activation of rhodopsin in artificial membranes

The rhodopsin model perfectly illustrates a category of proteins, whose activity requires rapid and reversible conformational changes, and thus a high flexibility of their membrane environment. Among all membrane proteins of the organism, rhodopsin, located in the membrane disks of the external segment of the cone and rod cells of the retina, is probably the molecule whose immediate lipid environment has the highest DHA concentration (estimated at least one fatty acid out of two). The photo-induced activation of rhodopsin is coupled with the isomerisation of its chromophore, 11-*cis*-retinal, to an all-*trans* configuration. Through the cascade activation of a G protein and of phosphodiesterase, rhodopsin translates the light signal into an electric signal at the origin of visual perception. The photo-activation of one molecule of rhodopsin results in the activation of 1000 to 2000 molecules of phosphodiesterase per second, which provokes the hydrolysis of 100 000 molecules of cyclic CMP and the closing off of the ion channels thus blocking within 100 ms the influx of around one million Na⁺ ions (for a review see [112]). Spectrophotometry methods allow to measure the equilibrium constant (*K_{eq}*) that rules over the photo-induced conformational changes of rhodopsin. By using artificial membranes of phosphatidylcholine made up of different associations of fatty acids, Litman and Mitchell showed that the *K_{eq}* value characteristic of native membranes of the retina is only reached in the artificial membrane when it is entirely made up of DHA [113]. The same authors have shown that the incorporation of cholesterol in the phosphatidylcholine membranes (at 30 mol%) also

has the effect of reducing the *K_{eq}* value, and that this inhibitory effect is less pronounced in those membranes that are entirely made up of DHA [114]. In membranes made up of myristic acid (14:0) and of 30 mol% cholesterol, a particularly rigid conformation that mimicks lipid rafts, the amount of photoinduced transformation of rhodopsin almost reaches zero [114]. These data prove that DHA is required for the first step of phototransduction and suggest that its abundance in the photoreceptor membranes can be efficiently opposed to the rigidifying effect of cholesterol [115]. The next step of the phototransduction process is binding the photoactivated rhodopsin (metarhodopsin MII) on the Gt subunit of transducin. In artificial membranes, this interaction also depends on the respective proportions of DHA and cholesterol: DHA allows the quasi-instantaneous formation of the MII-Gt complex, whereas cholesterol increases the latency time preceding its formation [116]. Altogether, these data prove that the role of DHA in visual perception begins at the initiation step of the phototransduction molecular process. Due to the 6 double bonds of DHA, the ring of membrane phospholipids that encircle rhodopsin can be deformed very rapidly and reversibly, making it thus easier for rhodopsin to change its conformation and to recover its initial state. Finally, the authors used the n-3 deficiency model in the rat to demonstrate that the disk membranes of the n-3 deficient retina, where 80% of DHA are replaced by 22:5n-6 (n-6 DPA), exhibit a higher degree of order of the phospholipid acyl chains relative to non-deficient rats [117]. The structural change results in reduced rhodopsin activation, rhodopsin-transducin (Gt) coupling, cGMP phosphodiesterase activity, and slower formation of metarhodopsin II (MII) and the MII-Gt complex [117]. These very recent data provide a solid molecular basis for connecting the changes in retinal membrane composition to the reduced amplitude and delayed response of the electroretinogram a-wave observed in n-3 chronic deficiency in rodents and nonhuman primates.

8.3. Impact of n-3 PUFA deficiency on vesicular neurotransmission

The synaptic release of neurotransmitters constitutes another membrane process directly implicated in the functioning of the CNS. The process initially concerns the maturation of vesicles carrying neurotransmitters and their storage in the active zone of the presynaptic terminals. The arrival of the action potential causes an influx of calcium that induces the fusion of vesicular and plasma membranes, releasing via exocytosis the neurotransmitters in the synaptic cleft. An opposite process allows to recruit membrane proteins and to regenerate by endocytosis small empty vesicles for a new cycle of maturation and presynaptic storage. The neurotransmitters activate the post-synaptic receptors that translate the entry of calcium ions, permitting the propagation of the action potential within the neuronal network. All functions of the nervous system, from sensorial perception to learning and memory, are governed by the synaptic coupling between exocytosis and endocytosis. The understanding of the complex protein machinery implicated in the vesicular processes has considerably evolved over the last 10 years (review in [118]). The specific role of membrane PUFA in the vesicular processes has been closely studied over the last few years. The use of a chronically n-3 fatty acid deficient rat model has showed the role of n-3 PUFA in the storage and presynaptic releasing processes of several neurotransmitters. The monoaminergic and cholinergic systems have been more specifically studied, due to their implication both in the regulation of important physiological functions and in the control of cognitive processes including attention, motivation and memory. These studies have shown in n-3 deficient rats, an increase in spontaneous release of dopamine in the nucleus accumbens [119, 120], acetylcholine [121] or serotonin [122] in the hippocampus (Fig. 6). In contrast, the drug-induced release of these neurotransmitters is significantly reduced [120, 122, 123]. This phe-

nomenon could result from an increased basal leakage of the neurotransmitter in the synaptic cleft that would reduce its storage in the vesicles (Fig. 7). During a nervous stimulation, generated for example in a learning situation, the amplitude of neurotransmitter release would then be reduced leading to a lower efficiency of the nervous influx. Concerning the study of the dopaminergic system, it was shown that the n-3 deficiency decreases the number of dopaminergic vesicles in the frontal cortex. These results suggest that n-3 deficiency leads to a hypo-functioning of the mesocortical dopaminergic system, and a model for the general dysfunctioning of the dopaminergic mesocortico-limbic loop has been proposed to explain the behavioural perturbations observed in deficient animals [124]. The membrane deficit in DHA can be one possible explanation of the effects of n-3 deficiency on neurotransmitter storage and release. Besides, modifications caused by n-3 PUFA deficiency in the neurotransmission process could also partly result from alterations in brain energy metabolism, such as glucose utilisation and oxidative phosphorylation [125]. It is therefore assumed that multiple interactions exist among membrane PUFA and vesicular neurotransmission processes, with significant incidences on learning capacity and behaviour.

8.4. PUFA and their metabolites regulate gene transcription

8.4.1. *Fatty acids are natural ligands of nuclear receptors*

In 1990, Issemann and Green discovered that molecules used for the treatment of hyperlipidemia act by binding on transcription factors (PPARs, peroxisome proliferators-activated receptors) that belong to the superfamily of steroid hormone receptors [126]. Since it has been shown that fatty acids are natural endogenous activators of PPARs [127], the concept of regulation of biological functions by PUFA and their

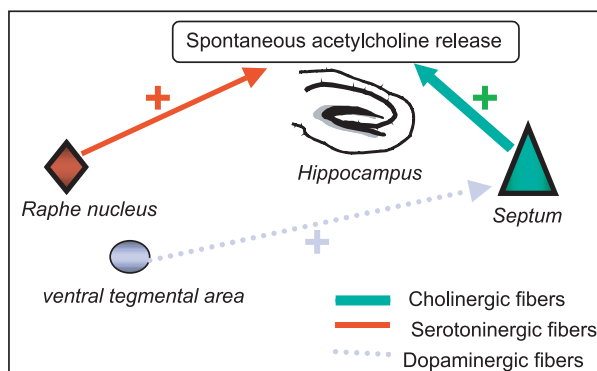


Figure 6. A schematic representation of functional interactions between dopaminergic, serotonergic and cholinergic neurotransmission systems in the control of Ach release in the hippocampus. In n-3 deficiency, the increase of dopamine synthesis in the ventral tegmental area and the alterations of serotonin release in the hippocampus could participate in the higher spontaneous Ach release (from [120–122]).

oxygenated derivatives has progressed considerably. Binding to PPARs allows fatty acids to partly control their metabolic fate by directly inducing the transcription of genes coding for proteins involved in mitochondrial and peroxisomal β -oxidation [128]. PPARs interact with responsive elements of the genome made up of two AGGTCA hexamers separated by one base (DR1 repeat) [129]. The genes that possess this type of sequence upstream of their promoter, or a similar sequence, are susceptible to be activated by the transcriptional heterodimer formed by the association of a PPARs with RXR (retinoid receptor) [130]. The PPARs are themselves coded by 3 different genes, differently expressed according to cell type, and translated into 3 isoforms: PPAR α , PPAR γ and PPAR β/δ . RXR also decomposes into 3 isotypes (α , β and γ). PPAR-RXR heterodimers can also repress the transcription of genes normally activated by other transcription factors by occupying in an inoperative way the target sequence corresponding to their promoters.

A large number of combinations is possible between the different isotypes of PPARs and RXR (including their respective ligands) that may induce varying effects on the target gene transcription level, whether they

are directly or indirectly involved in lipid metabolism. The preferential distribution of the PPARs and RXR isotypes in the different tissues and cell types contributes to functional specificities: catabolism of lipids associated with a predominance of PPAR α in the liver, brown adipose tissue and skeletal muscles; differentiation and lipogenesis in the adipose tissue, and uptake of oxidised LDL in macrophages, both preferentially expressing PPAR γ . The tissue distribution of PPAR δ is ubiquitous but this isotype is generally more abundant in most of the different cerebral regions [131], the digestive tract, kidneys, heart, diaphragm and oesophagus [132]. In the mouse skeletal muscle, PPAR δ has been shown to control fatty acid oxidation by regulating genes involved in fatty acid transport, β -oxidation, and mitochondrial respiration [133, 134].

8.4.2. PUFA as possible regulators of gene transcription in the CNS

It is known that transcription of the genes encoding the first two enzymes of the peroxisomal-oxidation pathway, acylCoA-oxidase (AOX) and L-peroxisomal bifunctional enzyme (L-PBE), is mediated through the RXR-PPAR heterodimer which binds

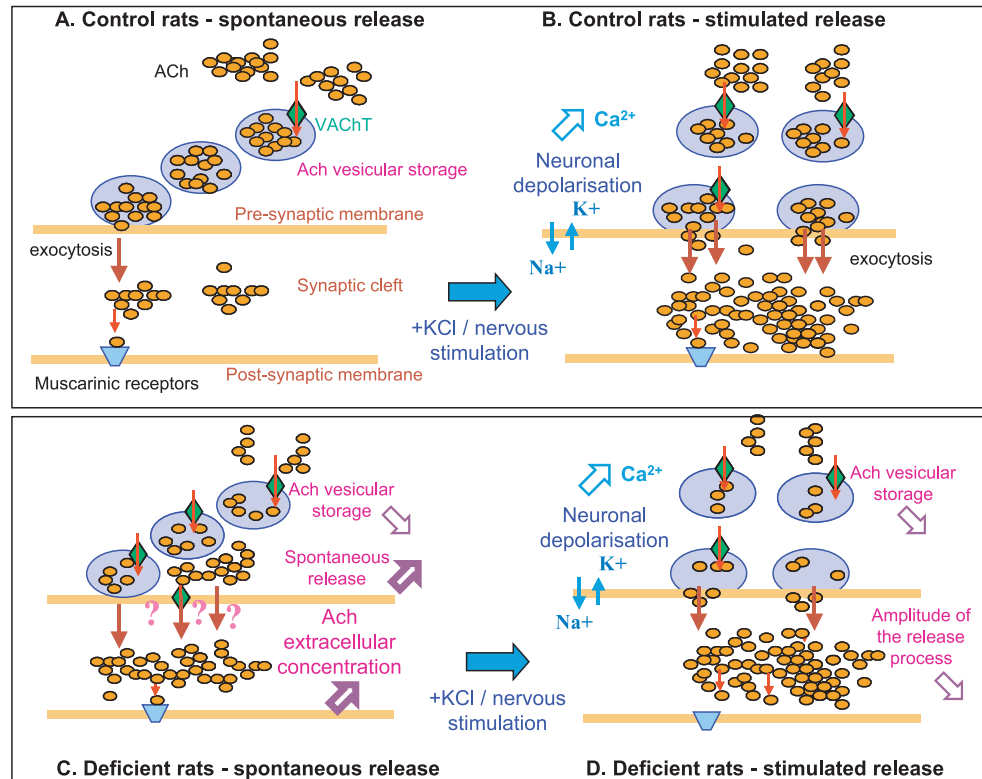


Figure 7. A schematic representation of acetylcholine (ACh) release in the synaptic cleft in resting (spontaneous release) and neuronal activation (stimulated release) conditions, in control (A – B) and n-3 PUFA deficient rats (C – D). In the resting state, a small amount of ACh is released in the extracellular space in control rats (A), whereas this spontaneous release is enhanced in n-3 PUFA deficient rats (C). In stimulated conditions (KCl infusion), cytoplasmic ACh is stored in vesicles through the vesicular ACh transporter (VAVhT). Full vesicles can be recruited for exocytosis and high levels of ACh are released (B). In deficient rats (D), the ACh leakage that occurs in resting conditions leads to storage depletion and then induces reduced ACh release under stimulation (D).

to the corresponding peroxisome proliferator-responsive elements (PPRE) [130] (AOX initiates the peroxysomal oxidation of the very long-chain fatty acids, allowing the shortening of neurotoxic compounds and the synthesis of DHA from its upstream precursors). However, the specific role of PPARs in the different regions of the CNS, in a possible relation with the regulation of the fatty acid metabolism, is an unresolved question. We have recently made the hypothesis that in retinoblastoma cells, the expression of AOX and the terminal step of DHA synthesis could be regulated by the upstream precursors of DHA through the modulation of PPAR δ -mRNA abundance (PPAR δ is the major isotype in these cells) [135]. In cultured oligodendrocytes, the chemically-induced activation of PPAR δ favoured the cell morphological differentiation and induced the synthesis of the myelin basic protein, suggesting that PPAR δ could play a role in nervous conduction by regulating the formation and the maintenance of myelin [136]. Although PUFAs, or some of their metabolites, are likely to exert the role of PPARs ligands in the CNS, the endogenous ligands of the brain PPARs, especially of the δ -isotype, have not been clearly identified.

8.4.3. The pivotal role of RXR

Actually, it appears that RXR, the obligatory transcriptional partner of PPARs [130], constitutes a very plausible potential target for DHA. This ubiquitous factor regulates the transcription of a large number of genes implicated in the pathways of proliferation, differentiation, and apoptosis in several types of neural cells [137]. RXR α also regulates the transcription of most of the genes implicated in lipid metabolism through its interaction with other transcription factors, such as the constitutive androstane receptor, CAR β [138], the retinoic acid receptor, RAR α [139] and the liver X receptors, LXR (also expressed in the rodent brain) [140]. The multiple roles of RXR α , a transcription factor positioned at the cross-road of genes encoding for miscellaneous receptors, trans-

porters and enzymes of the lipid metabolism, are schematically illustrated in Figure 8.

Finally, the binding of DHA on the ligand and binding domain of RXR α has been evidenced in the mouse brain [141], and two recent studies using transfected cells demonstrated that DHA potentially activates the transcription of a reporter gene mediated by RXR α [142, 143]. These novel data support the concept that DHA, and possibly other PUFA, could activate one or several RXR signalling pathways in the CNS. Therefore, the putative effects of DHA on gene transcription in the brain, possibly recruiting different RXR heterodimers (Fig. 8), and their possible influence on neural function, opens up a large field of investigations.

8.4.4. PUFA and transcriptional regulations in the CNS: the contribution of microarrays

Berger et al. studied the effect of dietary long-chain PUFA on the expression of 329 genes in the liver and 356 genes in the hippocampus of mice [144]. One-month-old mice received during 57 days, diets rich in long-chain PUFA (supplementation with fish oil rich in EPA and DHA or a fungal oil rich in AA, or a combination of both). The gene expression profile of these tissues was compared to that of a control diet containing only linoleic and α -LNA as PUFA. In the hippocampus, the long-chain n-6 and n-3 PUFA supplementation affected a category of genes controlling the transthyretin signalling pathway (a transporter of thyroxine in the cerebrospinal liquid), the liberation of serotonin, the functions relative to immunity (immunoglobulins) and the activation of transcriptional factors implicated in inflammation (NF κ B). By releasing their fatty acids, certain molecular species of ethanolamine-phosphoglycerides could be at the origin of transcriptional effects specifically induced by n-3 PUFA at the brain level [145–147]. These studies showed that among a panel of 3 200 genes screened in the rat brain, the supplementation in n-3

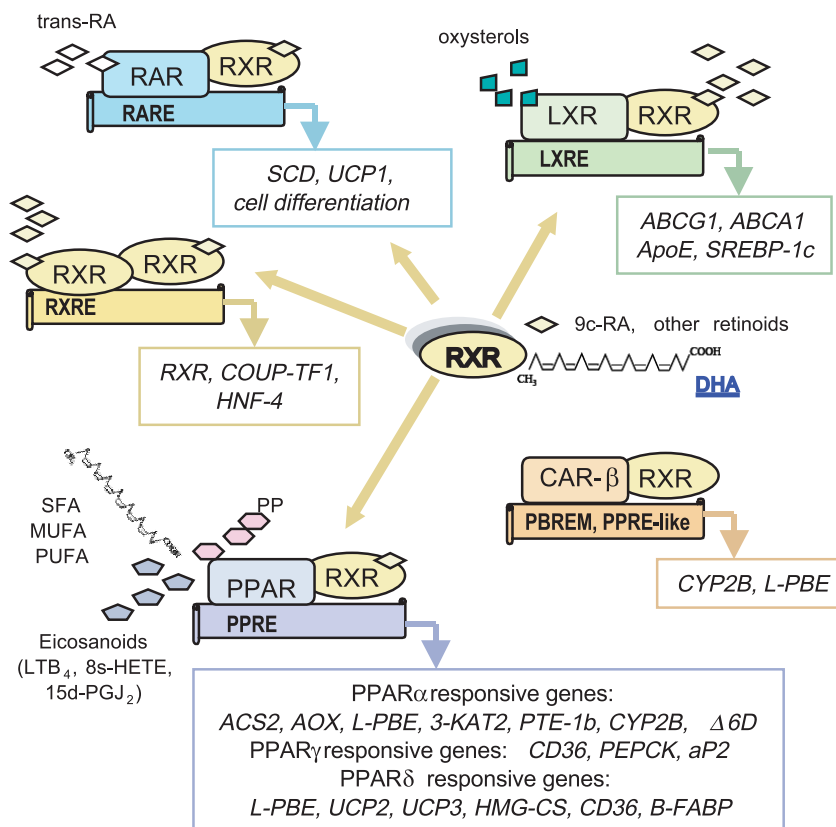


Figure 8. A schematic representation for the multiple gene transcriptions which are potentially activated by the dimer formed by the ligand-induced associations of RXR with one nuclear receptor. The liganded homo- or hetero-dimer binds to a specific DNA sequence, known as a responsive element (RE), i.e. RARE, LXRE, RXRE, PPRE and PBREM, located in the promoter region of the target gene. For simplification, tissue specificity is not considered, corepressors and coactivators of transcription are not represented, and only some examples of the target genes involved in the lipid metabolism are mentioned. By disrupting the interactions between RXR and other nuclear receptors, the ligand-activated PPARs can potentially interfere with the RXR-dependent transcription of multiple genes, notably those coding for lipogenic transcription factors. Fatty acids and some eicosanoids have been identified as natural ligands of PPARs in muscle, hepatic and adipose tissues. According to recent studies, DHA could be an endogenous ligand for RXR in different tissues, including the brain.

long chain PUFA increased the expression of 55 genes and decreased that of 47 genes. The genes affected are involved in synaptic plasticity, signal transduction, ion channel assembly, energetic metabolism and protein regulation. Applying microarrays to 2400 genes expressed in human retinal explants in culture, Rojas et al recently

found that the transcription of 14% of them was significantly increased when the explants were treated with 27 μ M DHA, whereas only 0.4% increased upon treatment with oleic acid [148]. Among the genes whose transcription of which was induced by DHA are those playing roles in neurogenesis, neurotransmission and intercellular connections.

To-date, the pathways of transcriptional activation induced by DHA have not yet been identified.

9. CONCLUSION

DHA, which is massively incorporated into the nerve cell membranes, exerts structural and neuroprotective roles that are favourable to the development and maintenance of cerebral and visual performances. EPA, although not stored in the brain cell membranes, can ameliorate diverse psychiatric disorders and probably has roles in brain function, possibly by counteracting the AA-mediated signalling. The mechanisms implicated are complex and multiple, reflecting the extraordinary diversity of the functions exercised by the PUFA, going from the modulation of dynamic properties of the membranes to the production of active mediators and the regulation of the expression of genes. The lipid nutrition of the brain, susceptible to influence each of the pathways at each step of life, is thus an essential element of its functioning.

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