The Role of Docosahexaenoic and Arachidonic Acids as Determinants of Evolution and Hominid Brain Development

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Lipids played a major, as yet unrecognised, role as determinants in evolution. Life originated 3 billion years ago during which time there was ample opportunity for DNA modification. Yet there was little change in the life forms for the first 2.5 billion years. It was not until about 600 million years ago when the oxygen tension rose to a point where air breathing life forms became thermodynamically possible, that a major change is seen in the fossil record. The sudden appearance of the 32 phyla in the Cambrian fossil record which flowed from this environmental change is referred to as the "Cambrian Explosion". It was also associated with the appearance of intracellular detail and cell differentiation. That detail was provided by cell membranes in which the lipids were structural essentials. Thus not just oxygen but also the lipids were drivers in the Cambrian explosion. Docosahexaenoic acid (DHA) provided the basic membrane backbone of the new photoreceptors that converted photons into electricity laying the foundation for the evolution of the nervous system and the brain. Although there are two closely related fatty acids with only one double bond different DHA was not replaced despite some 600 million years of genomic change. Whilst the marine food chain is rich in long chain omega 3 fatty acids, the land food web is dominated by omega 6 fatty acids. With the brain utilising omega 6 and 3 fatty acids in a ratio of between 1 to 1 and 2 to 1 the injection of the omega 6 through the

appearance of omega 6 rich protected seeds in the Cretaceous Period, would have played a critical role in the advance of brain evolution. This symbiosis between land and marine food chains, most likely created the condition that finally led to the cerebral expansion in human evolution. Lipids are still modifying the present evolutionary phase of our species with their contribution to a changing panorama of non communicable disease. The contemporary lipid malnutrition is most likely contributing to the rise in brain disorders which in the European Union has overtaken the cost of all other burdens if ill health at €386 billion for the 25 member states at 2004 price.

1. Introduction: The challenge of the rise in brain disorders

Brain disorders now account for the highest cost in the burden of ill health in Europe (Andlin-Sobocki *et al*. 2005). It follows the rise in death from cardio-vascular disease as predicted by Crawford and Crawford (1972). The cause is most likely nutritional with a similar background in the change in dietary fats which adversely impacted on cardio and vascular health that would logically lead to disorders of brain development and function. The reason for linking heart disease and brain disorders is that during early development, the brain relies heavily on an efficient placental vascular and the fetal cardio-vascular system. The fetal brain uses 70% of the energy transferred to the fetus from the placenta. The placenta itself is a rapidly growing vascular system which needs to be in place ahead of the fetal brain growth thrust of the last trimester. This paper raises several questions about the role of DHA in the brain, its extreme conservation in signalling systems with its possible relevance to human evolution. Importantly it raises a question on how to meet the challenge of human mental health in face of the problems facing aquatic food resources.

2. Docosahexaenoic Acid

Docosahexaenoic acid (all-*cis*-docosa-4,7,10,13,16,19-hexaenoic acid—$C22:6\omega3$, DHA) is a major, essential fatty acid constituent of the brain (Crawford and Sinclair 1972). DHA or its precursors have to be provided in the diet, hence the balance between the $\omega6$ and 3 fatty acids is important. There is a paucity of DHA in the land food chain which also contains competing fats. The brain first evolved using the marine food web some 500–600 million years ago and the richest source of DHA is the marine food chain. The movement in the 20th and 21st centuries away from historical use of sea foods and fish with an emphasis on land based food supply, is a likely cause in the rise in brain disorders now apparent (Hibbeln 1998). A better understanding of DHA and its function could help to motivate the required policy changes needed to meet this challenge.

Neural cells have a particularly high membrane content of DHA. In different mammalian species the profile with arachidonic acid and DHA does not vary: it is brain size that varies (Crawford *et al*. 1976, 1993) suggesting a high degree of evolutionary conservation of the neural lipid profile (Fig. 1). DHA is rapidly and selectively incorporated in neural membranes and is concentrated at synaptic signalling sites (Suzuki *et al*. 1997). It is the most unsaturated of cell membrane fatty acids (Jump 2002). DHA is synthesised from α-linolenic acid. However, the process is rate limited (Sprecher 1993; Sprecher *et al*. 1999) and moreover α-linolenic acid is oxidised at a rapid rate (Leyton *et al*. 1967).

In 1972 Crawford and Sinclair first published evidence that DHA itself, was an independent determinant of brain growth and evolution[1] (Broadhurst *el al*. 2002). Deficiency studies in rodents (Sinclair and

LIVER   BRAIN
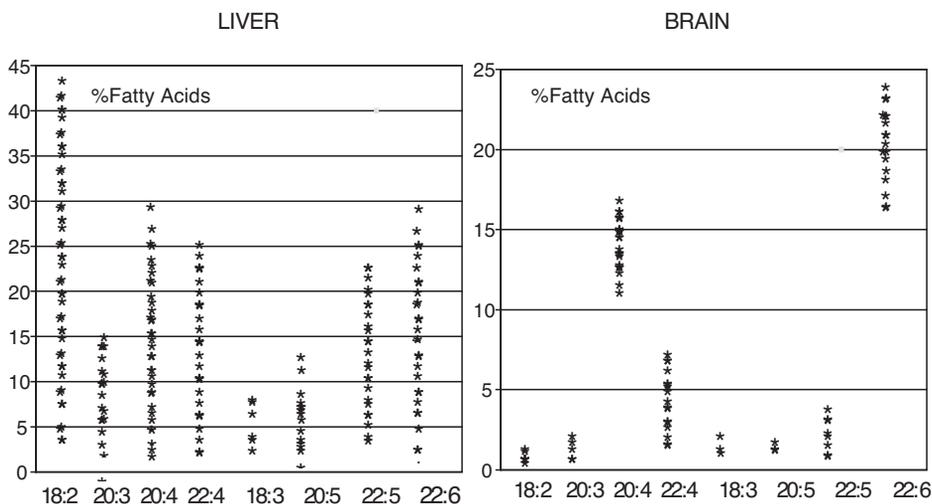

Fig. 1.   42 Species: inner membrane lipid varies widely in the liver but not the brain (Data from Crawford *et al*. 1976 and subsequent papers).

Crawford 1972; Benolken *et al*. 1973; Galli and Socini 1983; Weisinger *et al*. 1999; Catalan *et al*. 2002), chickens (Budowski *et al*. 1987), primates (Fiennes *et al*. 1973; Neuringer *et al*. 1986) and visual and cognitive trials in human infants (Carlson and Werkman 1996; Martinez and Vazquex 1998; Birch *et al*. 2000) have indicated that DHA is essential to brain development and function. Moreover, collaboration with the Hebrew University of Jerusalem (HUJ) we described competition existing between $\omega6/\omega3$ fatty acids and showed that their balance is critical for brain development and structural integrity (Budowski and Crawford 1985).

3. DHA Function—a question of liquidity?

Whilst the significance of DHA to brain function, is now recognised its mechanism of action is unknown. We have speculated that its unique, six methylene interrupted *cis*-double bond sequence may be responsible for its mechanism of action and conservation in neural tissues (Bloom *et al*. 1999).

The conventional view is that DHA provides for the high degree of liquidity needed by the brain. However, the notion that it is "needed" is teleological. In 1999 Bloom *et al*. discarded the idea of liquidity as an ex-

planation for its striking conservation in neural systems, on the grounds that the difference in liquidity between the $\omega3$-docosapentaenoic acid (all-*cis*-docosa-7,10,13,16, 19-pentaenoic acid C22:5$\omega3$, $\omega3$DPA) and DHA was marginal yet the $\omega3$DPA being more readily synthesized, less difficult to obtain from the food chain and less vulnerable to oxidative damage, does not seem to have replaced DHA in the visual and neural systems in the teleosts, elasmobranches, cephalopods, fish, amphibia, reptiles, birds or mammals. The $\omega6$ DPA, which also differs from DHA again by the absence of one double bond (between carbons 19–20) does not replace DHA except under extreme, artificial deficiency conditions in the laboratory and then the replacement is only partial and function is depressed. Nature's preference for DHA in the brain is strikingly demonstrated in large, vegetarian land mammals, in which DPA is the dominant $\omega3$ metabolite found in non neural tissues and thus abundantly available (Crawford *et al*. 1969). Yet neural membranes even in these mammals still conserved the DHA-rich composition. During the evolution of the land mammals,

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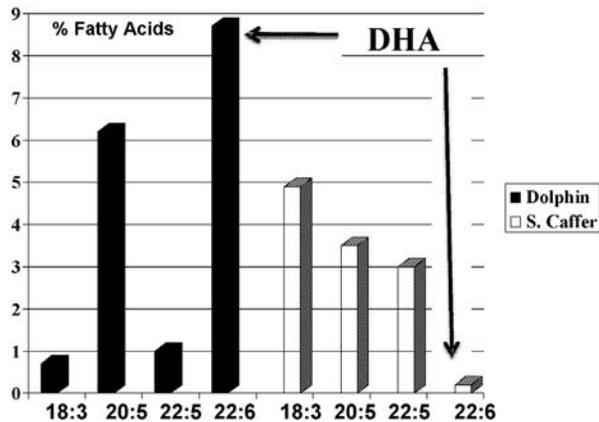[1] See *Science* 2002; **296** (April 12): 233, 340.

Fig. 2.   Liver ethanolamine phosphoglyceride from *Syncerus caffer* (Cape buffalo) and *Tursips truncatus* (dolphin).

this retention of composition in land mammals was associated with economy in brain size with a logarithmic reduction in relative brain size as they evolved larger bodies (Crawford *et al*. 1993).

4. Evolution of *Homo sapiens*

Certain mammals left the land to radiate into the marine habitat starting about 50 million years ago. With unlimited access to the DHA food web, the marine mammals retained a far better brain body weight harmony than is seen on the large land mammals. The dolphin for example has 1.8 kg brain which compares to little more than 350 g in a zebra which has a similar body weight and is also a non-ruminant (Fig. 2). A coastal ecological niche would have provided the rich source of DHA, iodine and other trace elements essential to the brain and in poor supply on land. Such a niche would have offered the evolutionary advantage compared to that of the land food chain and so avoid the loss of relative brain capacity on land.

The presence of DHA's full complement of six double bonds is for some reason an important priority in neural membranes and from the evolutionary record would seem to have been conserved in this capacity for 600 million years. The striking conservation of DHA in signalling systems implies that

biology is highly sensitive to the slight difference of the one double bond between DHA and DPA molecules. The reality is that all land mammals lost brain size as they evolved into larger body sizes demonstrating that different principles are involved in body as opposed to brain growth. In Fig. 3 we plot the approximate arithmetic decline in some land mammals.

Because of this discrepancy between body size and brain size some have used logarithmic plots to obtain straight lines to explore the relationship. This strategy of course means that one of the parameters is varying logarithmically to the other. In this case brain size diminishes logarithmically with body size. Even so *H. sapiens* and the marine mammals do not fall on the straight line. Of the large mammals, the Dolphin with about 1% of its body size, comes the closest to *H. sapiens*. At just under 2%,[2] *H. sapiens* has a brain body weight ratio which would be totally exceptional if considered as a land based mammal. Interestingly, *H. sapiens* has a smaller brain to body weight ratio than the squirrel. Indeed all the very small mammals have brain bodyweight ratios similar to or greater than *H. sapiens*.

The conclusion is that evolution on land resulted in diminishing relative brain size, a feature readily explained by the lack of DHA

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[2]   70 kg is considered a standard for men and at 1.3 kg brain the ratio is 1.86%, at 1.4 kg it is 2% (Blinkov 1968).
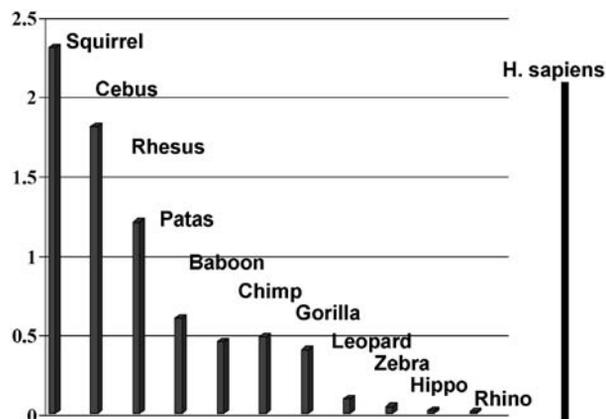
Fig. 3. Approximate brain weight as a proportion of body weight declines as body weight ingcreases.

in the land food web together with the rate limited synthesis being outstripped by the velocity of protein accretion and body growth. Note in Fig. 2 the buffalo liver lipid is quite rich in α-linolenic acid, EPA and even the ω3 DPA but despite this wealth of precursor, fails synthesise significant DHA. The contrast with the Dolphin lipids in this respect is striking.

However, both arachidonic and DHA are needed for the growth and development of the brain and its function (Crawford and Sinclair 1972). The difficulty the Dolphin and other marine mammals have is in obtaining arachidonic acid from the marine food chain to serve the brain. Hence arachidonic acid supply would be a constraint on brain evolution in the marine habitat although assumedly still required for mammalian reproduction (Williams and Crawford 1987). A littoral ecosystem would have provided and evolving primate with access to both arachidonic acid and DHA and hence would have had the best of both worlds.

This evidence puts the evolution of *H. sapiens* firmly at the marine and lacustrine coastlines with access to preformed DHA from the aquatic resources. Indeed, the concept that *Homo sapiens* actually went through an aqueous phase was put forward by Sir Alistair Hardy (1960) and followed up in several books written by Elaine Morgan (1995, 1997). For example, the loss of DHA

in the land based food web would lead to decline in relative brain capacity which in fact has happened. Conversely, the marine food web would be more likely to support brain capacity than that of the land which is also in evidence from the high relative brain capacity of the marine mammals versus the corresponding size in land mammals (Williams and Crawford 1987; Crawford *et al*. 1999; Broadhurst *et al*. 2002).

The evolution of humans at a coastal rather than a land based hunting system is now well explained by the evidence on omega 3 fatty acids and in particular on DHA in neural gene expression (Barcelo Coblijn *et al*. 2003a, b; Puskas *et al*. 2004; Kitajka *et al*. 2002, 2004). DHA is the dominant omega 3 fatty acid in the brain (Crawford *et al*. 1976). It has a far superior biological activity for brain growth, compared to its synthesis from plant fatty acids, even in rodents (Sinclair 1975). This evidence provides a simple mechanism and explanation as to why DHA and a marine food web would have supported brain evolution in an upward direction rather than downward.

Chris Stringer (2000) has suggested that *H. sapiens* populated the planet by migrating "out of Africa" around the coastlines. A coastal route would have certainly meant use of the marine food chain. There is fossil evidence of incontrovertible use of the marine food web dated to a time close to the biological

emergence of modern humans (Broadhurst *et al.* 2002; Marean *et al.* 2007). There is also contemporary evidence of fishing people in the Rift Valley of Africa with healthier cardio-vascular profiles than their inland cousins (Pauletto *et al.* 1996; Crawford *et al.* 1999), and contemporary evidence of the Moken and other sea dwellers living around the coast of Asia with a healthy, life style, possibly as a remnant of this migration (Gislén *et al.* 2003).

5. DHA in Neural Signalling Systems

Nuclear magnetic resonance (NMR) and fluorescence studies have attempted to differentiate the membrane properties conferred by PUFAs. Some of the constraints of such approaches have been discussed previously by Bloom *et al.* (1999). NMR investigations of the effects of polyunsaturation on lipid acyl chain orientational order, revealed significant changes as the number of double bonds increased from one to three (Holte *et al.* 1995; Soubias *et al.* 2006). Ehringer *et al.* (1990) directly compared the effects of 18:3 and 22:6 on membrane physical properties, and observed considerably higher permeability and perhaps vesicle fusability in the samples containing DHA. But again the differences are not of the order one would expect to be responsible for DHA to be chosen over 600 million years. A powerful reason is needed to explain why DHA was chosen and not its immediate precursor with only one double bond less.

Klaus Gawrisch and his colleagues have so far made the best attempt using solid-state NMR measurements and molecular simulations they portray an image of DHA (22:6ω3) as a highly flexible molecule with rapid transitions between large numbers of conformers on the time scale from picoseconds to hundreds of nanoseconds. The low barriers to torsional rotation about C–C bonds that link the *cis*-locked double bonds with the methylene carbons between them are responsible for this unusual flexibility. Both the amplitude and frequency of motion increase toward the terminal methyl group of DHA (Gawrisch *et al.* 2003; Mihailescu and Gawrisch 2006).

5.1. A special case for DHA as a receptor domain as targets for psychotropic drugs

Solid-state magic angle spinning (MAS) ^{13}C-NMR produces sharp resonances of C-atoms in the solid state, such as glycerophospholipids in dried liposomes (Underhaug Gjerde *et al.* 2004). Chlorpromazine (CPZ) is a cationic, amphiphilic psychotropic drug of the phenothiazine group that was the first drug used to treat schizophrenia and other psychiatric disease. When CPZ was included in liposomes of pure dipalmitoyl-phosphatidylcholine (DPPC), no alteration of the CH_2 resonances relative to liposomes without CPZ was found with MAS ^{13}C-NMR (Nerdal *et al.* 2000). However, when the liposome contained 30 mol-% pig brain phosphatidylserine (PBPS) together with DPPC, CPZ caused a large ($\approx$30%) low-field shift of the CH_2 resonances of 5–15 ppm at 37°C. This was interpreted as interdigitation of CPZ among the acyl chains of PBPS. This commercial phospholipid (from Sigma) was subjected to reverse phase HPLC that gave the separation of the molecular species, which revealed that the major species was 18:0/18:1 (63%) while the 18:0/22:6 species was next (24%). Later studies (Underhaug Gjerde *et al.* 2004) on liposomes with ^{31}P-NMR showed that CPZ interacted electrostatically with both the negative phosphate and carboxyl groups of PS and that PBPS strongly enhanced this interaction. Recently, similar studies (Chen *et al.* 2005) with pure 18:0/22:6n-3-PS in the DPPC-containing liposomes showed the same effects of CPZ with PBPS as described above, and T_1 relaxation measurements showed that CPZ reduced the mobility of the C_4 and C_5 atoms in DHA, which are attached to each other with a double bond.

It is therefore reasonable to assume that:

1) CPZ does not intercalate in liposomes containing the neutral PC.
2) CPZ intercalates in liposomes containing the acidic PS and neural PS is especially enriched with DHA.
3) The cationic CPZ binds electrostatically to the negative phosphate and carboxyl groups of PS.

4) The intercalation of CPZ in PS is affected by the unsaturatedness of acyls in PS with little intercalation in monounsaturated acyl and large intercalation in polyunsaturated acyls.

5) The double bond between C_4 and C_5 in DHA seems to be crucial for the strong intercalation of CPZ in DHA containing PS liposomes.

The small intercalation of CPZ in 18:0/18:1-PS (SOPS) and great intercalation in 18:0/22:6n-3-PS (SDPS) are important for psychotropic drug–membrane interaction. CPZ binds electrostatically to the negatively charged phosphate and carboxyl groups in SOPS through the positively charged tail amino group, but the phenothiazine moiety of CPZ is not intercalated among the acyl chains. In contrast, CPZ binds electrostatically to SDPS in the same way as in SOPS, but the lipophilic phenothiazine group is completely intercalated among the acyl groups and adjacent to the $C_4=C_5$ double bond. The apparent importance of this double bond should be tested in the future with 18:0/22:5n-3-PS, which should intercalate CPZ much less than SDPS.

The intercalation of psychotropic drugs into glycerophospholipid liposomes is not restricted to CPZ. Recently it was shown that the modern drug olanzapine also intercalates in both PC and PS liposomes (Song and Nerdal 2008), but these liposomes did not contain DHA. The intercalation of bulky molecules like CPZ in mono- or bilayers of phospholipids leads to increase of the intermolecular distances between the phospholipid molecules and alteration of the membrane structure. PS is exclusively in the inner leaflet of biological membranes. However, all pshycotropic drugs distribute between membranes and water with distribution coefficients in the range of 10,000 to 20,000 (for references, see Oruch *et al.* 2008). This suggests that the drugs will enter the membranes through the outer leaflet and diffuse through the acyl layer and be able to interact with the PS in the inner leaflet. One would assume that the structural changes caused by CPZ will affect the positioning of the proteins, such as membrane-bound enzymes and receptors and thereby alter their functions. Thus, in addition to act as antagonists for receptors, the drugs may also alter membrane protein activities.

DHA is very concentrated in nervous tissues, and in rat brain PS the major molecular species is 18:0/22:6n-3 (Bakken *et al.* 2006). Thus, brain PS may be the target for at least CPZ, and perhaps for other psychotropic drugs. This type of drug has been developed as brain receptor agonists. According to the discussion above, the drugs have membrane distorting activities in which DHA may play a central role. It has moreover practical relevance as it may be possible to modulate the DHA in the receptor-lipid domain in nerve cell membranes and so alter the efficacy of psychotropic drugs and neural membrane function despite its strong protection against external influences.

Added to this evidence on DHA-PS, Hee-Yong Kim has shown that neuronal apoptosis under adverse conditions is prevented by DHA enrichment in a PS-dependent manner. Moreover, the protective role of DHA enriched PS is not similar when DHA is deficient and there is an increase in the ω6DPA (Kim *et al.* 2003). They have also shown that DHA activates neurite outgrowth at low micromolar concentrations with a remarkable effect on morphological differentiation of hippocampal neurons which is achieved by increasing the population of neurons with more branches and longer neurites. This effect does not seem to be mediated by the expected nuclear receptor (retinoid X receptor) and may achieved by some function of DHA itself (Calderon and Kim 2007) again pointing to the significance of neural DHA rich PS and DHA itself.

5.2. Docosanoids

The example above is an entirely new role for DHA as a mediator of a receptor which is likely to be more widespread than just this example. The serine phosphoglycerides are known to be especially rich in DHA and are closely associated with membrane proteins. Added to this physico-chemical role of DHA in a receptor domain, Nicholas Bazan has discovered a striking anti-oxidant effect of derivative docosanoids from DHA: the

Neuroprotectins (NDP1). They claim that NPD1 acts against apoptosis mediated by A2E, a by product of phototransduction that becomes toxic when it accumulates in aging retinal pigment epithelial (RPE) cells. With DHA being selectively rich in neural systems, its neuroporotectins also protects against neural cell damage, most likely those associated with ageing, and Alzheimer's Disease (Lukiw *et al*. 2005; Bazan 2008). The design of DHA the polyenoic fatty acid most susceptible to peroxidation and located in regions of the most intense oxygen use, is a remarkable feat of Nature.

In addition to this neuroprotection role for DHA metabolites there is new evidence on the resolution of inflammation which has been shown by Charlie Serhan *et al*. (2008) to resolve inflammation through the action of biochemical processes that enable inflamed tissues to return to homeostasis. Following tissue injury it has been long thought that tissue injury, followed by inflammation, then repairs in good time, spontaneously. However, it now seems as though fatty acid derivates marshal the actors in the process of resolution and damage repair. Although it seems that resolvins can be derived from both EPA and DHA it is worth noting that human tissues, there is little EPA and the omega 3 family is mainly represented by DHA and some small amount of $\omega3$ docosapentaenoic acid. This focus by cell systems on DHA is especially pronounced in the brain and the testes.

5.3. The extreme conservation of DHA in neural signalling systems

A number of studies have been conducted on the physical effects of polyunsaturation on membranes, in which DHA has been compared to a range of other unsaturated chains having from one to five double bonds. Thus far, however, all differences that have been measured have been matters of degree, and none provide a compelling explanation for the striking specificity with which DHA is selected for membranes of the eye and brain over 600 million years of genomic change and evolution.

Where, then, can we hope to find an explanation of DHA's preferred status in neural membranes since the beginning of animal evolution in the Cambrian Era? An obvious starting point is that membrane protein interacts with the lipid in some way in which DHA favourably merges with the stereo and electro-chemistry of the protein of which the CPZ discussion above is an example. Such an effect could conceivably involve either an interaction with specific lipid molecular species, or modulation of bulk properties of the bilayer.

The conventional portrayal of proteins in lipid bilayers is of the lipid represented by a double row of soldier, standing to attention and the protein slipping in between them, so to speak, dissolved in the membrane. This cannot be a correct portrayal as otherwise lipid chemists would not need to use acid in extraction procedures.

Some believe specific binding interactions between lipid and protein molecules in a biological membrane are unlikely, since the membrane's fluid state means that individual lipid molecules will be undergoing rapid translational diffusion within the bilayer, and thus will never be in prolonged contact with any one protein. Furthermore, Brown's studies (1994) on the rod photoreceptor outer segment membrane revealed that specific chemical-type interactions could not be the cause of DHA's established role in supporting rhodopsin function. It was found that full rhodopsin efficiency could be obtained by substituting other lipid mixtures designed to mimic the bulk mechanical properties of the physiological, DHA-rich membrane. This gave rise to a model in which DHA's role was to promote mechanical conditions in the membrane suitable to stabilize certain critical conformational changes undergone by rhodopsin in the course of photoactivation. These models do not fully reconstitute the structure of the photoreceptor cell and its synaptic function, the ten thousand fold adaptive capability of which is still unexplained. However, should this model be valid to conditions *in vivo* it could potentially be extended to other G-protein systems elsewhere in the central nervous system (CNS).

6. A Hypothesis on the Molecular Dynamics and π-Electron Function in DHA

A more speculative, possibility is that DHA *in vivo* plays a more direct role in neuronal

signalling, in which some special properties conferred on the membrane by DHA chains exert an influence on membrane electrical phenomena (Bloom *et al*. 1999). These might include distinctive dielectric or polarizability properties arising from the unique periodic and symmetric arrangement of double bonds in the DHA chain. This arrangement is disrupted with the loss of the Δ4 double bond when the first seven carbons can occupy many more conformers than with the more ordered structure of the full sequence of six methylene interrupted double bonds.

It is conceivable that some polarization of π-electron clouds might occur in the DHA structure, and perhaps even be transmitted from one double bond to another, either within a given chain, or between neighbouring chains in the membrane. Our molecular dynamic calculations reveal that the π-electrons could come closer together in adjacent molecules than they are in the chain of a DHA molecule itself. In a similar vein, Penrose (1990, 2001) has postulated that some brain functionality may arise due to quantum coherence in the microtubules of neurones; it may be worthwhile to look for a similar phenomenon in signalling membranes containing DHA.

6.1. Nuclear overhauser enhancement

As a first step we have tested the possibility of electrical properties of DHA by examining its response to a magnetic field. In our Nuclear Magnetic Resonance (NMR) experiment, the magnetic moment is flipped [e.g. for ^{13}C] perpendicular to the magnetic field of the NMR magnet. The energy released to return to alignment with the magnetic field is then measured. The Nuclear Overhauser Enhancement (NOE) makes this process go significantly slower. If there is 2 s or 20 s between scans, a uniform molecule sees the same sea of magnetic moments in each scan. Spatially unequal concentration of magnetic moments as in $CH=CH–CH_2–$ or $–N–(CH_3)_3$ concentrates the magnetic polarization at these specific sites which is then detectable. Any response by any section of the molecule is then seen in a difference between the scans. Note that the proton magnetic moment four times greater than that of ^{13}C (Fig. 4).

It is important to note that polarization built up at the terminal methyl group as this would have potential to interact with adjacent molecules in the vertical plane of the bilayer.

These NMR experiments, which will be reported in detail elsewhere, demonstrate that the DHA molecule is subject to polarization in a magnetic field which is a signal of its potential to be electrically active. The final evidence comes from the expected polarization of the polar head group which contains a strong dipole moments. These are candidates for interaction with the aqueous phase and its ions.

6.2. The brain as an electrical machine

The conclusion from these studies is that DHA has the potential to act electrically. Further studies will be required to define the extent of this activity. However, molecular dynamic studies of the 2D electron distribution gives a clue as the special significance of DHA. It can be seen that the electron density map spreads across the whole molecule and even involved the aliphatic groups. This property is confirmed by the NMR experiment described above (Fig. 5).

In 1941 Albert Szent-Györgi wanted to know why electrons wandered from enzyme to enzyme in the electron transfer process of mitochondria. Hence the concept of electron involvement in biological processes is not new. Oleic acid in the membrane phosphoglycerides can increase membrane conductance, allowing the use of a voltage-clamp technique. Brunaldi *et al*. (2005) suggest that certain FAs increase proton transport across the lipid bilayer. In such studies, the membrane-unspecific or leak conductance contributes importantly to the measured conductance and constitutes a major source of indeterminacy.

Neural signalling is associated with the development of a potential difference across a lipid bilayer with subsequent depolarization. Whilst the action is considered to be down to large ions (e.g. sodium, potassium and calcium), it is difficult to imagine that the electrons are oblivious to the potential difference. The dumbbell shape of the π-electron clouds

20s recycle delay

2s recycle delay

Difference Spectrum
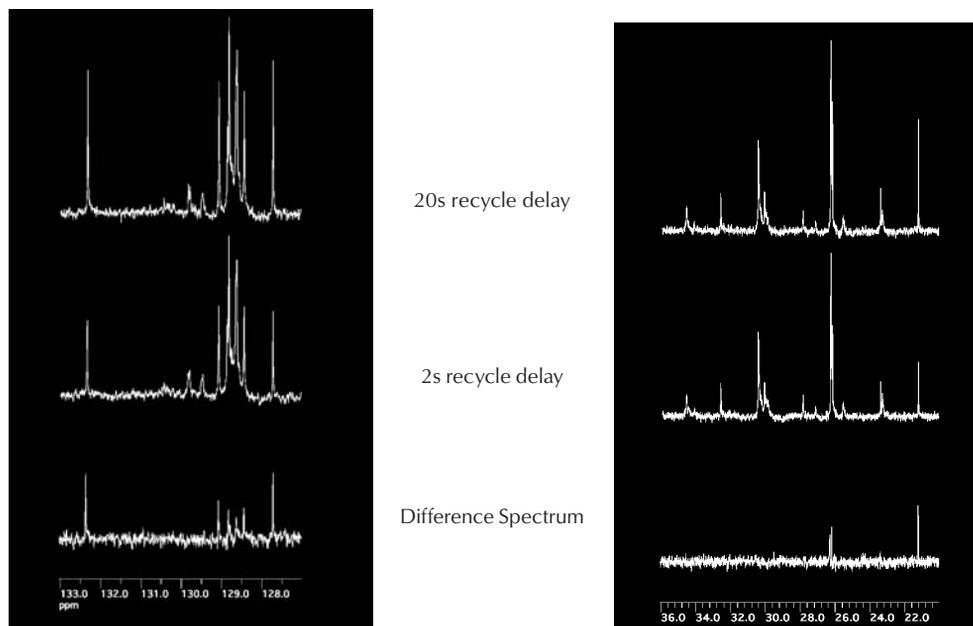
CH=CH region

Polarization with Nuclear Overhauser En-
hancement with DHA was seen to build
up at two CH=CH sites at 127.6 ppm,
and at 132.8 ppm as is seen opposite

Aliphatic CH$_2$ groups

NOE polarization did not build up at any
site specific CH$_2$ except at 21.05 ppm,
i.e. at a terminal –$\underline{C}$H$_2$–CH$_3$ group.

Small fractional increase in NOE at
26.05 ppm to aliphatic CH$_2$ backbone.

Fig. 4.   Alignment of DHA in a magnetic field: Nuclear Overhauser Enhancement.
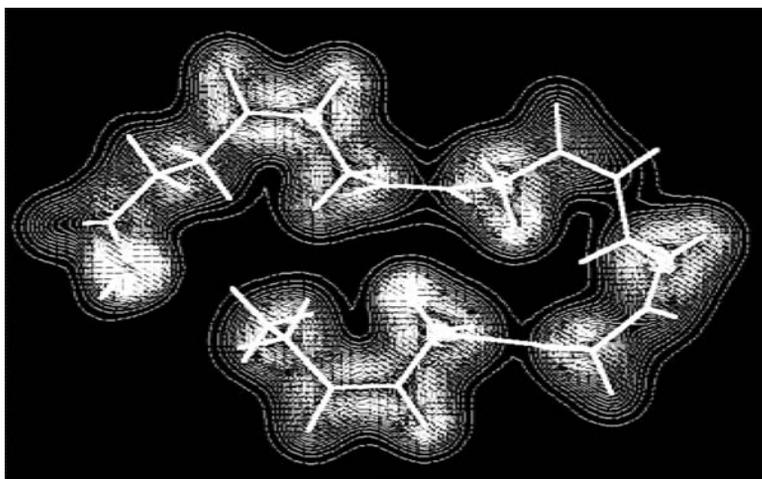

Fig. 5.   The 2D-charge density (3 double bonds coplanar).

would lean towards the positive charge and in consequence set up a differential charge across each double bond. With six in a row it would seem plausible that this arrangement would hold the potential for special conductivity.

Electron tunnelling (ET) would be one such mechanism whereby such conductivity could occur. It is precisely determined by quantum mechanics. Electron tunnelling is known to occur on proteins of the electron transport system (Yue *et al.* 2006; Moser *et al.* 2006). Rhodopsin is one of the best characterized trans membrane proteins involved at a site where large potential differences are created with subsequent depolarization to effect signal transduction in response to activation by a single photon which isomerizes the retinal receptor. Jin *et al.* (2006) have detected current flow through the (retinal-free) apo-membrane of bacteria rhodopsin (bR) which is approximately three orders of magnitude lower than was observed with native bR membranes. This result supports the idea that current flows dominantly through the bR proteins and that the retinal with its conjugated sequence of double bonds serves as a current transporter. Furthermore, the photoeffect observed with the native bR-containing membranes can be ascribed to the retinal rhodopsin.

Jin *et al.* (2006) conclude that transmembrane electron transport occurs essentially only *via* bR and not *via* the lipid bilayer and requires the presence of retinal or a similar conjugated π-electron system in the protein. The contribution of light-driven proton ejection to the steady-state photocurrents is negligible. Their result suggests that π-electron system in the retinal conjugated double bond sequence is acting as a copper wire with isomerisation acting as a switch to disconnect electron flow. So the dark current flows until stopped by a photon isomerising the retinal and changing its position relative to the aromatic amino acids from conduction to non conduction. They are concerned and ask why such π-electron transfer systems have not been described—are the "biological processes … hidden so well that we have not found them."

Whilst proteins contain aromatic rings ideal for electron transfer there is no such identity for the lipid bilayer. However, the calculated charge density of DHA is suggestive of a system which unlike retinal will not conduct electrons except under the special circumstance such as tunnelling. In tunnelling systems, transfer ability diminishes exponentially with distance. Hopfield estimated that an 8 Å edge to edge distance between ET parameters was about the limit. The distances in methylene interruption is <6 Å. In the case of the ω3 terminal end the distance for the first π-electron cloud is about 4.2 Å within the tunnelling range. However, remove one double bond and the distance jumps to over 8 Å implying that tunnelling would not work in the ω3DPA. This virtually complete uniformity of electron density in DHA is destroyed if one double bond was missing at either end of the molecule (Fig. 6).

Extended Huckel calculations based on the least occupied orbitals for DHA show as discussed above that the π-bonds have + and – lobes and that the + and – signs of orbitals of the two different hydrogens on the CH_2 groups also have + and – signs related to (typically opposite to) the signs of the adjacent π-bonds. This is a simple mechanism to explain electron coherence over a large distance, even though the double bonds are not extended resonance structures across a sequence of carbons with only single hydrogens.

This full, electron coherence does not seem to work with DPA which has five methylene groups following the double bond sequence. In DHA there are only two methylene groups sandwiched between the sequence and the carboxyl attached to the polar end group permitting cohesion throughout the whole molecule.

6.3. Is DHA a quantum gate to control transmission of electical information?

The methylene groups present an energy barrier to π-electrons. However, if one electron is removed from the end of the cohesive sequence in DHA it is then possible for an adjacent π-electron to tunnel through to the vacated level and so on down the sequence.

But electron tunnelling will only occur at the precise energy level vacated. This mechanism would provide an *absolutely precise*, quantum gate which would only open at a specific energy condition and then only permit a precise quantum of energy transfer (Table 1).
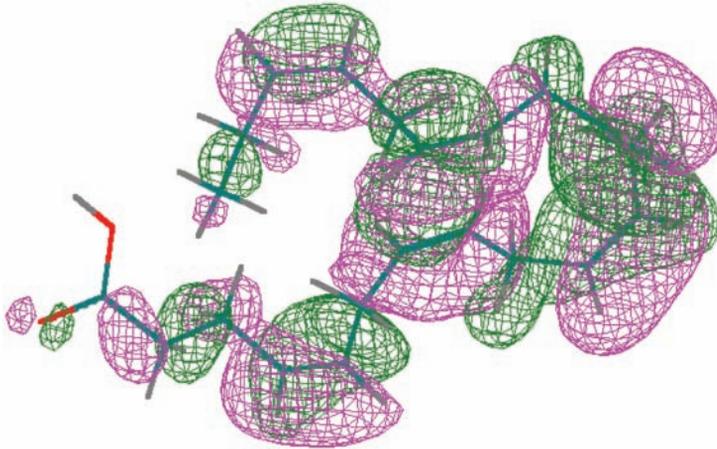
Fig. 6.   Electron distribution around the DHA molecule high and low alternating densities. (π-bond energy different above and below planes; green lower, mauve higher energy).

Table 1.   Electron tunneling edge to edge distances.

Vertical distances:
- Docosapentaenoic 22:5,n-6
  CH3/\/\/ᵃ 8.4 Å   ᵃ\/\CO–   4.2 Å

- Docosahexaenoic 22:6,n-3
  CH3/\＿ 4.2 Å   ᵃ\/CO–   4.2 Å

- Docosapentaenoic 22:5,n-3
  CH3/\＿ 4.2 Å   ᵃ\/\/CO– 8.4 Å

Horizontal minima distance between DHA double bonds 4 Å for 3 planar double bonds

Horizontal minima distance between DPA double bonds 7.6 Å for 2 planar double bonds

This precision is evident in photoreception but is so far difficult to explain (Rieke and Baylor 1996; Field and Reke 2002; Dunn and Rieke 2008). Although photoreception is one of the best described signalling systems, it is plausible that within it, DHA may be acting as a semi-conductor that will only allow electrons to pass at a specific energy level to contribute or control the depolarisation. This event would be consistent with the precision seen when the photoreceptor is activated by a single proton yielding the same energy regardless of energy input. The speculation is that as Jin *et al*. (2006) describe, the dark current would use retinal, isomerisation to disconnect the transmission of the dark current by the conjugated double bonds of the retinal, stop electron transfer leading to a build up of a potential difference. The ET properties of the DHA would ensure the potential difference continues to accrete until it reaches the level required for tunnelling at which point depolarization would follow. This speculation is not inconsistent with the present concept of photo transduction. The same principle could operate in synaptic transmission. It is already described in the electron transfer in the proteins of the cytochrome system (Gray and Winkler 2003). This potential is supported by the NOE studies indicating DHA will polarize in a magnetic field. As seen below with the edge to edge maximum distance for electron tunnelling to occur (about 8 Å) is exceeded if one double bond is removed from the DHA making such a process less likely.

Tunnelling may also operate in a cohesive manner collaborating with adjacent molecules in the bilayer with the potential for the very long chain (>26 carbons) contributing to connecting the outer and inner leaflets of the membrane. The coherence of DHA-ET is on the lines suggested for neural function by Penrose (1990, 2001).

7. A 600 Million Year Track Record in Neural Signalling

DHA was the only molecule so selectively used over 600 million years of evolution in

DHA   Omega 3 DPA
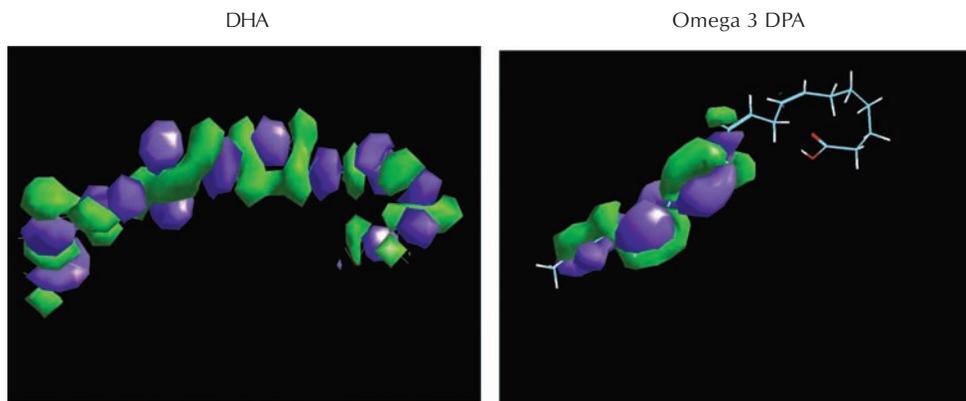

Fig. 7.   Electron density of higher energy state as might be expected with a strong positive negative charge across the membrane. The response of the electrons in DPA would make electron tunnelling unlikely along the full length of the molecule. The full length with alternating polarities is feasible with DHA.

the photoreceptor and synaptic junction: locations where there is the most intense oxygen utilization, a threat to such a highly, unsaturated molecule.

The precision of the energy output of the photoreceptor is not explained by an unquantized effect on the number of G-protein molecules activated. That does not mean to say that they do not contribute to the transduction process and signal amplification. However, photo-transduction has precision, which is a hall mark of quantum mechanics. Precision can be explained by tunnelling which can only take place at the precise energy and state of the first electron removed from the DHA methylene interrupted sequence.

Moreover, the same closeness in space that allows DHA orbitals to co-relate also enable them to co-relate in phospholipids. The results are remarkably simple: DHA–DHA allows at least 3 double bond pairs on each DHA molecule to be close enough in space to align with 3 on the other chain. And where does one find the Di-DHA phosphoglycerides but in the photoreceptor. Ergo, activation energy now is even simpler: head to foot/head to foot association in low energy [ground] state together with alignment of polarization head to head and foot to foot on individual chains.

In response to closure of the dark current and or the build up of a powerful potential difference across the lipid bilayer, which would create a relative negative–positive–negative–positive arrangement as seen in the LUMO molecular dynamic figures. The π-dipole can be visualised as a bar magnet in each of the DHA molecules in the phospholipid. So: in the ground state, the bar magnets align head to foot, head to foot. On activation by a signal (light in the case of the photoreceptor) in the presence of an appropriate, potential difference, the bar magnets align resulting in a force double the π–π force in each of the DHA molecules. Since the phospholipids align along the membrane: again, the dipoles align longer and longer, producing a greater and greater signal. On closure of the signal the dipoles slowly flop, back to head to foot, head to foot, no less the wear for their rearrangement (Fig. 7).

Independent support for an electron function for DHA comes from the early studies of Robert (Gene) Anderson on photo-transduction (Benolken *et al*. 1973). He had discovered that the rod outer segments of several species contained about >50% of its fatty acids as DHA He raised rats on a fat free diet and observed significant alterations in the electrinoretinogram (ERG) indicative of reduced A and B wave function.

The amount of rhodopsin and the shape of the absorption spectra and general bleaching characteristics were the same for rhodopsin

from EFA deficient and control groups of eyes. The density and packing of rods appeared normal in the deficient, test animals, and the ultrastructure of rod outer segments from these animals was preserved and indistinguishable from the ultrastructure of rod outer segments from control.

The A-wave of the ERG is a photoreceptor response function, while the B-wave is generated by electrical activity in other neural layers of the retina. The DHA content of the rod outer segments fell from 45.2% of the fatty acids to 19.0% in the EFA deficient rats. The loss of DHA was partially replaced by docosapentaenoic acid in the $\omega6$ family. This means the altered ERG was a function of the specific loss of membrane DHA. That in turn means that DHA is itself involved in the electrical response of photo transduction. The mechanism we propose here would enable DHA to act in a manner similar to a semi-conductor providing a quantum gate.

8. DHA and Neural Pathways?

A key characteristic of $\omega3$ deficiency is reduced learning capacity and behavioural pathology. We were the first to describe the behavioural pathology in an $\omega3$ deficient primate which is seen in Dr. Joseph Hibbeln's work at the NIH USA (Hibbeln *et al*. 2005, 2007). Suzuki *et al*. (1997) demonstrated the selective uptake by the synapse for DHADHA somewhat similar to that shown by Bazan and Anderson for the photoreceptor. The brain turns over its constituents rather than relying on imports. No recycling process is 100% efficient. Hence there will be continual loss which has to be replaced by some import.

Let us assume the letter A is seen on a teach-yourself typing screen. The response of putting the left hand's small finger on the second end key on the left of the 4th row of the PC keyboard requires the correct visualisation of A, its recognition as requiring a motor response, transmission to a motor section of the brain, identification of the hand and then the small finger, left hand and then the transmission of the message in 3D to the small finger. That neural pathway has to be learnt so that when the photoreceptors and then the brain calls for the letter A, the correct response is elicited. Learning requires repetition. In the rep-

etition process the synapses fire and reconstitute. With selective uptake of DHA the synapses in the pathway will be enriched. The more enriched the synapse the better its function which is the converse of the $\omega3$ deficiency experiment which depresses learning ability. Repetition will enrich a pathway and just as water takes the path of least resistance when flowing down a hill, so the signal A from the photoreceotors will take the least resistance DHA enriched pathway to the small finger on the left hand in time and space.

This concept of memory is not independent of other similar concepts of protein activation except the evidence on memory is mostly published with respect to $\omega3$ deficiency. One would expect the proteins which are encoded by DNA to be robustly built to the same specification. However, the lipids and lipid composition is subject to environmental inputs and variation. This proposed function of DHA would facilitate conduction of a signal and the establishment and function of a neural pathway.

9. Darwin and Conditions of Existence

Darwin in *The Origin of Species* (1868) stated there were two forces in evolution, *natural selection* and the *conditions of existence*. Of the two, he said, *the latter was the most powerful*.

However, Weismann (1893) rejected this view in the all sufficiency based on experiments in which he cut off the tails of breeding rodents and observed that subsequent generations still produced tails. That set in train the present paradigm of the *modern synthesis*, and genomic determinism within which the DNA is seen as the sole dictator of difference and evolution, and to the notion of the "Selfish Gene". Darwin spent much of the later part of his life searching for what he called "Pangenes" that were responsible for translated environmental influences. His failure served the all sufficiency of natural selection and excluded the conditions of existence. However, Darwin's "Pangenes" are now evident in the response of plasma membrane receptors responding to nutrients influencing gene expression (Chawla *et al*. 2001; Puskas *et al*. 2003, 2004; Anderle *et al*. 2004) and *vice versa* (Corella *et al*. 2005). Epigenetic effects consequent on manipulation of gene expression during early development,

were in evidence in the follow up of the Dutch food shortage in World War II. Low birthweight was transmitted to a second generation (Stein *et al.* 2006). Another example is prenatal programming (Barker 2004) resulting in adult risk to heart disease diabetes and stroke from poor maternal/fetal nutrition.

Darwin's original view is on conditions of existence are consistent with the remarkable conservation of DHA in signaling systems over 600–500 million years. That is despite wide ranging changes in the genetic code and the great evolutionary changes, DHA has been rigorously conserved. It is as though DHA has been instructing the genes to do its bidding rather than the conventional view which is the other way round. Apart from vindicating Darwin's concept and his superiority over Weismann and his all sufficiency, it raises basic questions in biology enhancing our understanding of the relationship between environment, the genes and function. The functionality of natural selection is readily identified in animal systems. It has one drawback in that it does not fit with degeneration. The loss of relative brain size in all land based mammals as they evolved larger bodies (Crawford *et al.* 1993) does not sit well with their loss of brain power and lack of ability to survive as witnessed in recent time. More importantly it does not predict as it is based on randomness. Darwin's conditions of existence offer predictive value which is a hall mark of science.

The evidence on omega 3 marine food consumption in pregnancy affecting childhood intelligence and behavior measured at 8 year of age acts as a reminder that *H. sapiens* is also subject to Darwin's conditions of existence (Hibbeln *et al.* 2007).

10. Reason for Concern on the Food System and the Brain

The 600 million year track record of DHA in neural systems is compelling evidence for its absolute requirement. We now wish to return to the evolutionary implications. The evidence for the evolution of *Homo sapiens* as a coastal dweller, utilising the marine food chain is now very persuasive. The brain first evolved in the marine environment utilising marine nutrients of which clearly DHA was a key for neural systems. It still utilises DHA today. As Philip Tobias said at a lecture in London[3] "Wherever humans evolved, they had to have water to drink."

In human fetal growth the priority is brain development which receives 70% of the growth energy delivered from the mother. Maternal nutrition before and during pregnancy is an independent risk factor for low birthweight and poor pregnancy outcome (Doyle *et al.* 1989; Wynn *et al.* 1994; House 2000; Carlson 2001; Rees *et al.* 2005). Postnatal nutrition is also a priority to ensure good maternal nutrition for herself and for milk (Birch *et al.* 2007). Although the outcomes of supplementing preterm infants is accepted as beneficial to cognitive development, there is some variation in the human studies of term infants (Simmer 2000). However, in the human species, most brain cells divide pre-natally and the studies in preterm infants have been consistently positive (Fleith and Clandinin 2005). This variation is likely to arise from supplement type, dose and background diets. None the less, tissue DHA levels have consistently correlated with outcomes (Gibson and Makrides 2001).

Hence, poor maternal health and nutrition before and during pregnancy disadvantages fetal development with permanent mental and cognitive deficits (Litt *et al.* 2005) and behavioural dysfunction (McNamara and Carlson 2006; Hibbeln *et al.* 2007) with a risk of heart disease, diabetes and stroke in later life (Barker 2007). Poor neurodevel-opment restricts the individual's capacity to acquire numeracy and technical skills (Birch and Gussow 1970). In addition, to the $\omega3$ fatty acid protection against sudden death from heart disease (Marchioli 2002) evidence has accumulated on the competitive effect of fatty acids with the $\omega3$ fatty acids inducing behavioural pathology (Fiennes *et al.* 1973; Hibbeln *et al.* 2004). Deficits of marine fats have been linked to manic depression (Saugstad 2001, 2007; Young *et al.* 2005). Depressed $\omega3$ status has also been linked with

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Alzheimer's disease (Beydoun *et al*. 2007).

11. Implications

There are 1.6 billion people at risk to iodine deficiency, a sure progenitor for mental retardation but seldom seen in the fishing communities. In Europe, brain disorders have now overtaken all other burdens of ill health (Andlin-Sobocki *et al*. 2005) and mental ill health is predicted by the Global Forum of Health (www.globalforumhealth.org) to be in the top three burdens of ill health world wide by 2020. There is compelling evidence that the reasons are related to the loss of sea foods and their replacement by land foods (Hibbeln *et al*. 2002, 2004, 2007). To solve this problem and prevent further rise in disorders of the brain may well require a new paradigm in food with a focus on the nutritional requirements for the brain. This may well mean agriculturalising the oceans and enhancing the development, use and consumption of sea foods worldwide.

12. Conclusion

The use of DHA in neural signalling systems over a 600 million year stretch of evolution is compelling evidence for its essentiality. It is now known to be involved in neural receptor domains, gene expression with derivatives providing protection from oxidative stress in the brain and resolution of injury. We speculate that DHA uniquely contributes π-electrons to signal transmission providing quantum mechanical provision for precise, signal control and an explanation for the uniqueness of DHA in signalling systems.

The DHA is the most limiting biosynthetically of the brain specific fatty acids. It therefore needs to be best obtained preformed for human nutrition, especially during pregnancy and lactation when the new fetal and infant's brain is forming at high velocity. The DHA is poorly represented in the land food chain. The richest source is the marine food web where the brain first evolved. The implication of the conclusion on its essentiality, if correct, is important to the future of humanity.

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