Mechanisms for Nutrient Effects on Brain Development and Cognition

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Introduction

Multiple studies over the past five decades have addressed the evaluation of the effects of malnutrition on central nervous system (CNS) development in experimental animals and humans. The results of these studies reveal that a reduction in the supply of energy and/or of several essential nutrients during the first stages of life has profound effects on nervous system structural and functional development. Malnutrition impairs brain development decreasing the number of cell replication cycles, reducing total brain DNA, restricting dendritic arborization thus reducing the connections between neurons. In humans, intrauterine malnutrition and early postnatal malnutrition specially affect brain cell number as measured by DNA content [1]. The development of the cerebellum is most affected by nutritional deprivation around the time of birth. Synaptic connectivity is particularly affected if malnutrition occurs after birth but before the third year of life. Alterations in dietary precursors may affect tissue levels of neurotransmitters (serotonin, norepinephrine, dopamine and acetylcholine) in specific brain regions [2]. Essential and nonessential lipid supply affects the structural composition of the brain and of myelin sheaths. The functional correlates of these biochemical changes induced by malnutrition are alterations in the waking electroencephalographic (EEG) activity and in visual and auditory evoked responses; motor and cognitive development and social abilities are also affected. Sleep-wake cycle organization as well as neurovegetative activities during sleep are perturbed by early human malnutrition [3]. Most of these effects are potentiated by
other environmental deprivations, which interact with poor diet in defining the adverse consequences.

The linkage between altered brain development and retarded somatic growth is strong in some particular nutritional deficiencies such as protein-energy malnutrition (PEM) or iodine deficiency disorders (IDD) as documented by many experimental studies and epidemiologic evaluations. However, under other circumstances, somatic growth may proceed unabated while brain structure and function are significantly altered by nutritional deficiencies. The effect of early anemia on brain function is independent of somatic growth. The impact of taurine deficiency on retinal and brain development in human- and nonhuman primates is not associated with altered growth; moreover in this case it is independent of protein synthesis since this sulfonic amino acid is not incorporated in protein structures. The role of essential fatty acids as basic components of brain chemistry are not associated to effects on body growth but rather on modifying membrane function and electrophysiologic responses. Examples in the opposite direction, namely of poor somatic growth and normal mental development, are more difficult to find, suggesting that somatic growth is a necessary but insufficient condition to attain normal mental development. Early malnutrition secondary to treatable disease such as pyloric stenosis or cystic fibrosis illustrate the capacity of the brain to recover from PEM but are not sufficient to negate the effect of sustained nutritional deprivation on brain development. The traditional point of view that protein and energy deficits directly affects brain structural development and cognitive performance have been challenged since PEM coexists with other nutritional deficiencies and other elements of psychosocial deprivation that can also disrupt child development. This makes it particularly difficult to tease out the role of specific nutrients or even combined nutritional effects from multiple other deprivations that interact with nutrition in defining the final outcome of the developmental process [4].

We highlight the problems associated with oversimplifying the complex nutrition/mental development interactions by presenting two examples of adverse effects of increasing protein intake in an effort to prevent malnutrition in early life. Nutritional interventions in preterm infants in the late 1940s focused on supplying as much protein as required to optimize somatic growth, this led to the provision of as much as 6–8 g of protein per kg per day. Follow-up of this population to adult life demonstrated that these elevated protein intakes were associated to significantly lower IQ scores [5]. The classic Columbia study which provided a protein supplement to pregnant low-income mothers in an effort to improve birth weight, revealed that the higher protein intakes given to Harlem women at risk of delivering a LBW were associated to lower mental developmental scores at 12 months [6]. This should motivate us to develop a better understanding of the mechanisms by which nutrients affect brain development and cognition in an effort to define optimal nutrition in early life. Optimal in this case means the right amount and balance between
The significance of past research in this field cannot be summarized in this brief introduction, the topic can be reviewed in classic references and also in recent publications including the chapter by S.M. Grantham-McGregor. The purpose of this chapter is to explore and illustrate the mechanisms by which nutrients can modulate brain development and cognition. The selection of topics included reflect the interest of the coauthors and the need to avoid unnecessary repetition with material included in other chapters:

a) Gene/nutrient interactions and structural CNS development
   Molecular regulation of gene expression
   Nutrients and CNS structural development

b) Nutrient effects on neural structures and functional properties
   Nutrients and neurotransmitters [covered by J.D. Fernstrom]
   Nutrients and neural membranes, emphasis on polyunsaturated fatty acids

c) Temporal organization of neural development: Nutrient interactions
   Circadian rhythms/sleep-wake cycle development
   Sensory systems/nutrient effects on development

Gene/Nutrient Interactions and Structural CNS Development

Molecular Regulation of Gene Expression by Nutrients

Regulation of gene expression by nutrients can occur at multiple levels. The following listing summarizes the main sites for nutrient effects on gene expression. Figure 1A–D presents models of potential sites for nutrient effects on gene expression (see figure legends for detailed description).

- Nutrients can bind to nutrient response elements in the DNA affecting gene transcription.
- Nutrients can interact with nuclear proteins that in turn act as transcription factors regulating gene expression.
- Nutrients can affect post transcriptional processing; mRNA transport, turnover and stability.
- Nutrients can modulate mRNA translation and protein synthesis rate.
- Nutrients can affect gene expression at the posttranslational level by modifying the gene products formed.
- Nutrients can modify the turnover rates of proteins or enzyme activity level.

Nutrients, metal cations such as Fe, Zn and Cu bound to specific or nonspecific ligands can bind to nutrient response elements in specific DNA motifs usually present in the promoter region of the gene, affecting gene transcription.
Fig. 1. A Potential effects of nutrients on gene expression are described from bottom left to top right. Nutrients can bind to nutrient response elements (NRE) in the DNA affecting gene transcription. Nutrients can interact with nuclear proteins that in turn act as transcription factors (TF) regulating gene expression by binding to DNA-responsive elements (TFRE). Nutrients can affect posttranscriptional processing, mRNA transport, turnover and stability. Nutrients can modulate mRNA translation and protein synthesis rate. They can modify gene products posttranslationally, modifying the turnover rates of proteins or enzyme activity level. B Mechanism for transcriptional regulation of single nutrients, usually bound to specific or nonspecific ligands. Known coactivators

(Fig. 1B). Nutrients can interact with nuclear factors in modulating the activation of specific nuclear proteins that in turn act as transcription regulators. In this case, the nutrient is indirect since it is the activated transcription factor that binds cis-regulatory elements of DNA found in target genes (Fig. 1C, D). Other forms of nutrient ligand interactions include changes in phosphorylation
Fig. 1 (continued) and unknown (?) potential activators or inhibitors are shown. The up- or down-regulation of the gene product as shown by the dotted line serves as a regulatory response by affecting nutrient metabolism, storage, uptake or release. C, D The mechanism for transcriptional regulation of TR or PPAR system. TR corresponds to thyroid hormone nuclear receptors, PPAR corresponds to peroxisomal proliferator-activated receptor, both are nuclear proteins. TRE and PPRE are corresponding responsive elements in the DNA. RxR corresponds to the retinoic acid receptor. Known coactivators and unknown (?) potential activators or inhibitors are shown. In addition, fibrate drugs, fatty acids and eicosanoids can bind and activate PPAR expression regulating transcription.

Nutrient and Brain Development

mediated by the nutrient. At the posttranscriptional level, once mRNAs are formed, nutrients may act modifying native RNA processing, mRNA transport and stability, and breakdown rates. Nutrients may also modify the rate of mRNA translation by activation of protein synthesis in the polyribosomal complex. Nutrients can also affect gene expression beyond the synthesis of
protein at the posttranslational level by modifying the gene products formed. This is the case for vitamin K-dependent protein carboxylations or the case of nutrients which function as cofactors bound to proteins modifying their function. Finally, nutrients can modify the turnover rates of proteins including enzymes thus affecting their activity level [7–9]. In most cases the nutrient-induced changes in gene expression are part of the adaptive response to a given level of nutrient exposure. Thus, may affect the uptake, metabolism, storage or excretion of the nutrient that triggered the gene regulatory response.

**Mechanisms for Nutrient Effects on CNS Structural Development**

**Role of Nutrients in Brain Organogenesis (Folate and Iodine)**

The role of nutrients on brain organogenesis has received renewed attention. Potentially, deficits in any essential nutrient could condition abnormal organ development, if severe enough. Cell replication is dependent on a sufficient supply of all essential nutrients. In fact, abnormal embryogenesis is the hallmark of experimental tocopherol deficiency, this vitamin was appropriately named for this effect. Deficits in vitamin B12, retinol, pyridoxine, pantothenic acid, folic acid, tocopherol and zinc have all been demonstrated to induce CNS malformations in experimental animals [10].

The fact that the embryo is dependent on maternal nutrient supply serves to buffer the effect of acute nutrient deficits; for most nutrients, maternal stores permit adjustments in response to high or low dietary intakes. In theory, maternal stores need to be fully depleted or replete before embryogenesis is affected. In practice, this is not the case because not all nutrients are significantly stored by the mother or if stored cannot be mobilized effectively. For some nutrients, such as iron, maternal stores are insufficient to meet fetal needs beyond the first half of pregnancy. In addition, genetic polymorphisms that affect nutrient metabolism may determine higher nutrient needs in specific population subgroups or limit nutrient transport for cellular utilization. These individuals may have an apparently acceptable intake but may be depleted in critical tissues affecting embryonic development, for example folate and neural tube closure [11]. Conversely, these polymorphisms may determine that nutrients or their metabolic products may become toxic at exposures within the acceptable range for the general population, for example exposure to alcohol and fetal alcohol syndrome in subjects with poor capacity to metabolize it [12].

In the case of folic acid, supplementation in preventing not only the recurrence of neural tube defects (NTDs) but also reducing the occurrence of this major nervous system malformation by 50–70%. This malformation is caused by failure in the elevation of neural folds required for closure of the neural tube at different levels of the dorsum, giving different phenotypic expression depending on the area affected. Animal studies have revealed potential...
mechanisms that underlie defective elevation, these include: faulty apoptosis, premature differentiation before NT closure has occurred, disruption of actin function or abnormalities in microtubular proteins necessary for cell migration, abnormal activity of the telomerase complex altering cell cycle, or faulty pyrimidine synthesis affecting cell replication [13, 14]. Several of these phenomena are affected by the supply of key micronutrients that can act directly such as retinoic acid or indirectly by serving as essential components of key regulators such as the role of iodine in thyroid hormone synthesis. Knowledge on the molecular mechanisms of retinol(retinoic acid action clearly indicates that vitamin A supply is crucial for cell replication and differentiation, and thus affects normal embryonic development. Experiments with zebrafish embryos demonstrated that retinoic acid induced major CNS malformations including loss of the midbrain-hindbrain border and severe disruption of the rostral hindbrain. These studies documented the involvement of retinoic acid and its receptors in the direct control of Hox gene expression and the early patterning of the CNS [24–37].

Folic acid obtained from food sources but must be reduced to tetrahydrofolic acid (FH4) before it is metabolically active as a coenzyme for methyl transfer reactions. FH4 functions as a coenzyme in the biosynthesis of purine bases and nucleic acids as well as in the remethylation of homocysteine to methionine. Over the past decade, specific defects in methylenetetrahydrofolate reductase (MTHFR) have been characterized and two mutations associated to increased susceptibility for NTDs identified. The substitution of a cytosine by thymidine in codon 677 (C677T) in the MTHFR gene is now being considered as a useful marker for NTD risk. Elevated homocysteine and a lower plasma response to folate supplementation characterize this genotype. A second mutation changing an adenosine to cytosine in codon 1298 of the MTHFR gene has been reported. In a recent study of 47 mothers bearing children with NTDs, these two mutations were present in for 35–50% of the group, thus explaining in part the protective effect of folate supplementation. In addition, heterogeneity in receptor-mediated folate transport may explain individual susceptibility to NTDs unrelated to FH4 reductase activity [16].

Folate entry into cells is mediated by a receptor system that involves formation of caveolae and folate-specific binding proteins. Reduced folate carriers (RFCs) and folate binding proteins (Folbp-1 and -2) have been described, they display tissue-specific expression and binding constants for different folate compounds. They are responsible for an intracellular folate accumulation 100- to 1,000-fold greater relative to the extracellular concentration. The recent discovery of defective embryonic development including major CNS malformation in mice with mutations for an inactive form or lacking Folbps provides a valuable experimental model to evaluate the contribution of folate to normal embryogenesis. Mice lacking Folbp-2, which binds folate poorly, develop normally while mice carrying the Folbp-1 mutation have severe morphogenetic abnormalities including NT defects and most died in
Folate supplementation during early gestation prevented the pheno-
typic expression of malformations in the Folbp-1 mutant mice suggesting that
folate intracellular levels are critical for normal organ development. This is
an important step in identifying the molecular mechanisms for folate-related
CNS structural malformations [14, 17].

Additional evidence indicates that the folate receptor gene is also a target
for retinoic acid transcriptional regulation, providing a precise molecular
mechanism to explain the folate retinol interaction in determining NTDs. The
regulation of folate receptors by retinoic acid may explain the occurrence
of NTDs in association with low vitamin A intake or exposure to vitamin A
derivatives with elevated retinoic acid like activity [18]. In addition, recent
evidence indicates that retinoic acid regulates the developmental expression
of the dopamine D2 receptor as demonstrated in rat primary neuronal cultures.
The transcriptional activity of the rat D2 receptor gene promoter region,
which bears a retinoic acid-responsive element, was increased by retinoic acid
in transfected C6 glioma. Conversely, site-specific mutations of the retinoic
acid-responsive element inhibited the transcriptional effect of retinoic acid.
These results suggest that retinoic acid is a key factor in regulation of the
embryonic onset of the dopaminergic D2 receptor [19].

Iodine-dependent thyroid hormone production plays an important role in
organ growth and development as well as in the regulation of overall energy
metabolism. Human brain development requires thyroid hormone for normal
maturation, the critical period extends from fetal life to 3 years of age. Up to
a couple of decades ago, iodine deficiency was considered the most common
cause of preventable mental retardation, 800 million humans were considered
vulnerable to iodine deficiency disorders while 200 million were affected by
this condition. The successful implementation of iodization programs has
virtually eradicated the clinical forms of the disease, but the less severe forms
are still present in some areas of the world. A syndrome that includes severe
mental retardation, deafness, mutism, and spastic-rigid motor dysfunction
characterizes cretinism secondary to iodine deficiency [20]. Despite these
dramatic effects, the anatomical structural development of the brain including
the gyral pattern of the cortex is basically normal suggesting that the insult
occurs after the first trimester of human gestation. The neuropathological
picture that emerges based on the very limited available human data is one of
mild brain atrophy, with a thin cortex and reduction in total number of neurons,
enlarged ventricles and increased sulcal spaces. Histological studies indicate
a dendritic branching is markedly decreased especially in apical dendrites of
pyramidal cells. The compromise of the development of the cerebral cortex,
basal ganglia and cochlea suggest that these alterations are dependent of
the absence of fetal thyroid function which occurs after the first 12 weeks of
gestation. The timing of the neuropathological alterations is concordant with
insults between 10 and 18 weeks, indicating that maternal thyroid hormone
transfer during the first trimester has important functional effects protecting
early fetal brain development. In cases where maternal T₄ is lowest, CNS development is most compromised; moreover, when T₄ is extremely low, fetal loss and stillbirths are highest. The prevalence of neurological cretinism has been linked to the degree of maternal hypothyroidism as measured by T₄. Maternal transfer of T₄ may be important in congenital hypothyroidism, in fact CNS development in these infants is less affected than in mothers from areas of severe iodine deficiency. In these cases the limited iodine available is distributed between maternal thyroid and other maternal tissues and there is little or no iodine left for fetal thyroid function. Thus, even the protective effect of maternal thyroxine on fetal brain becomes compromised [10, 21].

The presence of nuclear T₃ and T₄ receptors in the human fetal brain clearly precedes the onset of fetal thyroid hormone production in accordance with the concept that maternally derived thyroid hormone is responsible for early brain development. Thyroid hormone metabolism matures during the second and third trimesters of gestation with a progressive increase in serum T₄, free T₄, T₃ free T₄ and increase in free T₄/TSH ratio. Present neonatal screening for congenital hypothyroidism has served to document the critical importance of early treatment. Fetal hypothyroidism is associated with an approximate loss of 10 IQ points. More recently a controlled study of premature infants born before 27 weeks’ gestation supplemented with thyroxine revealed a significant positive effect of 18 IQ points relative to those given placebo. In contrast, those born and treated after 27 weeks demonstrated a 10-point lower IQ at 2 years of life, suggesting a possible critical period at which thyroxine effect may be negative rather than positive [22]. The possibility of a detrimental effect of thyroid hormone effect on the brain can be sustained on the basis of the balance between cell replication and functional maturation which is critically dependent on timing. Recent evidence of tissue-specific activity and timing of deiodinase enzymes, which inactivate thyroid hormone, suggest that the action of the hormone is under active control and its effect is critically timed for each tissue in order to achieve normal development.

The molecular mechanisms for iodine-dependent thyroid hormone effects on brain development are beginning to be elucidated in animal models (Fig. 1C). A selection of studies relevant to this paper will be reviewed for additional information (see Oppenheimer [23]). Progress in our understanding of thyroid hormone action in brain development over recent years has been made possible by the recognition of the central role of triiodothyronine in mediating thyroid hormone action and the recognition of specific nuclear receptors in target tissues as demonstrated by displacement studies. The cloning of the receptors and receptor variants has enabled investigators to undertake detailed analyses of the biochemical events that underlie the physiological and pathological action of thyroid hormone (TH) [24–37].

TH (3,5,3’-triiodothyronine; T₃) is essential for normal development of the vertebrate brain, influencing diverse processes such as neuronal migration, myelin formation, axonal maturation, and dendritic outgrowth.
Several $T_3$-regulated genes have been identified in the developing rat brain, a basic transcription element-binding protein (BTEB) and a small GC box-binding protein. BTEB mRNA levels in cerebral cortex exhibit developmental regulation and TH dependence. Overexpression of BTEB in Neuro-2a cells dramatically increased the number and length of neurites in a dose-dependent manner suggesting a role for this transcription factor in neuronal process formation [24]. In addition, the reelin and dab1 genes necessary for appropriate neuronal migration and lamination during brain development are thyroid-dependent as demonstrated by in vivo and in vitro by hormone treatment of hypothyroid rats and organotypic cultures. The effects of TH on neuronal migration may be in part mediated through the control of reelin and dab1 expression during brain ontogenesis [25].

The effect of TH on the expression of neurofilament (NF) genes was examined using a hypothyroid rat model at 3–4 postnatal weeks. A large reduction (60–90%) in the expression of NF proteins was induced by TH deficit. A 2- to 3-fold increase in cerebral NF mRNAs was noted within 2–4 h of TH hormone injection [26]. In vitro transcription of nuclei isolated from brain of young normal and hypothyroid rats showed that transcription of $\alpha_1$ and $\alpha_3$ mRNA and 20–40% reduction of $\alpha_2$ mRNA in TH-deficient animals. At the posttranscriptional level, T3 enhanced the half-life of $\alpha_3$ mRNA 1.5-fold [27].

TH also plays a critical role in normal cerebellar development. However, the molecular mechanisms of TH action in the developing cerebellum are not fully understood. This action could be exerted in part through brain-derived neurotropic factor (BDNF), as cerebellar BDNF messenger RNA (mRNA) expression is lower, and replacement of BDNF partially reverses the abnormal neurogenesis observed in the hypothyroid rat. These results indicate that TH regulates BDNF gene expression in a promoter-, developmental stage-, and brain region-specific manner. This may play an important role in region- and stage-specific regulation of brain development by TH [28].

Studies in weanling rats reveal that the cerebellar Purkinje cell-specific PCP-2 gene is transcriptionally activated by TH during the 2nd and 3rd weeks of postnatal life in the rat while it has no detectable effects on PCP-2 expression in the fetal rat. Immunohistochemical studies reveal that a transcription factor (COUP-TF) that represses $T_3$-dependent transcriptional activation of PCP-2 is specifically expressed in the immature fetal and early neonatal Purkinje cell. COUP-TF expression diminishes coincident with TH induction of PCP-2 expression. These findings are consistent with the hypothesis that the presence or absence of inhibitory proteins bound to the TH response element of $T_3$-responsive genes governs the responsivity of these genes to TH during brain development [29].

During the late fetal stage in the rat, the developing brain appears to be unresponsive to TH despite the presence of TH receptors suggesting the
presence of factors that suppress a precocious response to TH or the absence of cofactors essential for such a response [30]. Treatment with thyroid-blocking agents (53% fall in fetal brain T₃) or administration of excess T₄ (2- to 3-fold increase relative to in adult brains) modified the expression of the myelin basic protein (MBP), PCP-2 or calmodulin kinase IV genes. Transient transfection experiments in differentiating oligodendrocytes in culture showed that T₃ regulates MBP expression at the transcriptional level, but only for a limited period during differentiation [31].

TH is an important epigenetic factor in brain development, acting by modulating rates of gene expression. The active form of TH T₃, is produced in part by the thyroid gland but also after 5'-deiodination of thyroxine (T₄) in target tissues. In brain, approximately 80% of T₃ is formed locally from T₄ through the activity of the 5'-deiodinase type 2 (D2), an enzyme that is expressed mostly by glial cells, tanycytes in the third ventricle, and astrocytes throughout the brain. Type 1 and 2 deiodinases generate T₃ from T₄, while deiodinase type 3 (D3) transforms T₄ and T₃ to inactive metabolites. D2 activity is an important point of control of TH action because it increases in situations of low T₄, thus preserving brain T₃ concentrations. The expression of D2 by assessed by quantitative in situ hybridization in hypothyroid animals during postnatal development revealed that D2 mRNA concentration was increased severalfold in relay nuclei and cortical targets of the primary somatosensory and auditory pathways. The results suggest that these pathways are specifically protected against thyroid failure and that T₃ may have a role in the development of these structures [32].

Coordination of the expression and activity of iodothyronine deiodinases is postulated to play an important role in physiology and development, making it possible that individual cells and tissues regulate the concentrations of the active hormone according to specific needs. At postnatal day 0 in the rat brain, D3 transcripts were found to be selectively expressed in areas involved in sexual differentiation of the brain. Expression in these areas was transient and was no longer observed on day 10, suggesting that D3 expression may be linked to the imprinting of sexual function and behavior [33].

Although the role of the three functional TH receptor isoforms (TR β₁, TR β₂, and TR α₁) remains unclear, some studies have suggested that restriction of TR β₂ messenger RNA (mRNA) to rat pituitary could reflect a specific regulatory role in the pituitary [34]. Nuclear receptors play key roles in anterior/posterior (A/P) axis formation during vertebrate embryogenesis. Within this gene family, TH receptors are expressed during early periods of development, long before the establishment of the thyroid gland, and are able to interact with retinoic acid receptors and retinoic acid itself. TR α₁ effects on early embryonic development were demonstrated by mRNA injection of either repressor or activator forms of the TR. Overexpression of either the TR α₁ or a constitutive repressor form, v-erbA, caused a swelling in the rostral hindbrain. These defects were associated with disorganization and loss of rhombomere
borders as well as an increase in the number of acetylcholine esterase-positive cells. Overexpression of TR α1 during zebrafish embryogenesis disrupted hindbrain patterning suggesting an interaction with retinoic acid receptors in the control of hox gene expression. Injection of either TR α1 or v-erbA mRNA repressed a reporter gene that contained a retinoic acid response element, demonstrating the ability of the TH α1 to repress retinoic acid signaling [15].

Ethanol selectively reduced the expression of TR α1 mRNA in the neocortex and hippocampus on G21, compared with pair-fed and control fetuses. These data support the hypothesis that ethanol may interfere with TH action during fetal brain development. Some of the developmental defects characteristic of congenital or experimental hypothyroidism are also observed in children or experimental animals prenatally exposed to ethanol, suggesting that a subset of neurological defects attributable to ethanol exposure are produced by interfering with TH action [35].

TH also regulates myelination in the mammalian brain. TH action is mediated by interactions with TR that function as ligand-regulated transcription factors. Two genes, α and β, encode different isoforms, of which only the β and α1 isoforms are authentic nuclear T3 receptors (NT3R). In agreement with the important role of T3 on myelination and oligodendrocyte generation, the presence of NT3Rs has been reported in oligodendrocytes and their precursors. TH receptor isoforms are sequentially expressed in oligodendrocyte lineage cells during rat cerebral development [36]. The expression of the MAL gene is down-regulated by hypothyroidism and up-regulated by hyperthyroidism in the myelinated regions of the brain. The MAL (MAL, MVP17, VIP17) proteolipid, an integral membrane protein, has been proposed as a component of the machinery necessary for myelin formation during oligodendrocyte maturation [37].

**Lipid Effects in Retinal and Brain Development**

LCPUFAs and derived eicosanoids are involved in the regulation of cell growth, differentiation by modulating gene expression. For example the effect of DHA on the functional maturation of the retina observed in several animal species including primates can now be traced to a direct effect on photoreceptor differentiation. Studies using primary culture of retinal neuronal cells have revealed potential mechanism by which this conditionally essential nutrient affects gene expression critical to photoreceptor growth and to retinal function. A recent study in rats demonstrated that DHA significantly increases in rod outer segment apical process differentiation, this is the locus for rhodopsin- and opsin-dependent light transduction. This was paralleled by an increase in opsin expression and content in the rod photoreceptor apical processes (Fig. 2). The molecular mechanisms underlying these effects have not been fully clarified but these data suggest an effect of DHA on opsin gene expression and possibly on other proteins required for the assembly of disc membranes. Recent studies from Bazan’s group [38, 39],
have demonstrated that opsin and rhodopsin transport to the apical process via post-Golgi membranes is coupled to DHA transport. The close molecular interaction between this key photoreceptor protein and DHA suggests that DHA influences retinal photoreceptor structural development as well as function [38, 39].

Regulation of gene expression by LCPUFAs occurs at the transcriptional level and is mediated by transcription factors which bind cis-regulatory elements found in target genes. These transcription factors, which are activated by fatty acids, have a structure which is similar to the steroid-thyroid supergene family of nuclear receptors that includes the steroid hormone receptors, glucocorticoid receptor, vitamin D receptor, thyroxine receptor (TR) and the retinoic acid receptor (RxR) [40, 41]. PUFA-responsive transcription factors have been recently characterized, the peroxisome proliferator-activated receptor (PPAR) activated by the peroxisome proliferators (clofibrac acid, nafenopin and WY14,643) named accordingly were discovered by Isseman and Green [41]. Figure 1D provides a schematic representation of the regulation of the PPAR system. Ligands determine the dimerization of the receptors, specifying homodimer or heterodimer formation. Recent studies have identified a number of proteins, coactivators that interact with nuclear receptors playing a role in the regulation of transcriptional activity. The formation of the binding site for the coactivator in the nuclear receptor is ligand-dependent.

The activation of PPAR by fatty acids was first characterized in *Xenopus laevis*; α, β and γ isoforms are able to respond to fatty acids with overlapping
specificity [42, 43]. However, few studies have systematically explored the differential activation of PPARs by fatty acids of different chain length, degree and type of unsaturation. Yu et al. [43] compared the ability of fatty acids to activate the different PPAR isoforms using chimeric constructs. Whereas, the tetR/PPARα chimeric receptor was activated to almost the same extent by linoleic acid and by DHA, the tetR/PPARγ receptor was activated by DHA but not by LA and the tetR/PPARβ receptor was responsive to DHA and to a lesser degree, to LA. PPARα is apparently also activated by medium and long chain unsaturated fatty acids. This evidence has been used to support the notion that dietary n-3 and n-6 PUFA-induced reduction of hepatic expression of lipogenic enzymes is mediated by PUFA-activated PPARα [44]. In this chimeric PPAR expression model, DHA appears to be most active while saturated myristic acid induces the lowest activation. The net effects of PPARs on cellular processes and metabolism include enhanced peroxisomal proliferation, increased fatty acid oxidation, decreased fatty acid synthesis and enhanced glucose oxidation [45–47].

Additional work will be necessary to better characterize the intracellular fatty acid metabolites that regulate transcriptional activation mediated by LCPUFAs and other factors that may interact to determine the responsiveness of target genes critical for retinal and brain development.

**Nutrient Effects on Neural Structures and Functional Properties**

*PUFAs and Neural Membranes*

Lipid compounds such as various phospholipids and cholesterol serve as components of specialized cell membranes and organelles. The overall quantity and relative composition of these lipid species may affect membrane fluidity and protein/lipid interactions that result in changes in overall cell function. Fatty acid composition of structural membrane lipids can affect membrane function by modifying overall membrane fluidity, membrane thickness, lipid phase properties, membrane microenvironment, or by interaction of fatty acids with membrane proteins [48–50]. These effects may modulate receptor activity, transport in and out of cells, hormonal and other signal transduction processes.

Most dietary n-3 fatty acid-induced membrane changes are not reflected by an overall change in membrane fluidity, but result in selective changes in membrane microenvironment affecting specific domains. The replacement of DHA by DPA observed in n-3 deficiency results in very similar overall lipid unsaturation level since only one double bond has been lost. Thus, membrane fluidity on average remains unchanged. Furthermore, the major changes in the physical state, induced by the fatty acid composition of lipid bilayers, occur after the first and second double bonds are introduced, namely when a saturated fatty acid such as stearic acid (18:0) is replaced by oleic acid (18:1 n-9) or by linoleic acid (18:2 n-6) [50, 51]. Others have suggested that DHA
supply modifies the phospholipid molecular species present in neural tissues, thus possibly affecting overall function [52].

One of the most significant membrane effects of DHA is its role in photoreceptor signal transduction process. Recently, Litman and Mitchell [53] have reported that LCPUFAs present in membrane phospholipid molecular species have profound effects on rhodopsin activation and related structural modifications. Rhodopsin is a membrane protein present in rod outer segment disk membranes accounting for 90% of the protein content. It functions as a photon receptor coupled to a G protein. The light-induced conformational change of rhodopsin triggers a biochemical cascade finally leading to an increase in phosphodiesterase activity and decrease in cGMP that closes sodium ion channels in the photoreceptor disk membrane. The result is a hyperpolarization, increasing the negative charge of the plasma membrane, which is followed by a depolarization. The resulting signals correspond to the ‘a’ and ‘b’ waves of the electroretinogram. Membrane fatty acid composition affects the ability of photons to transform rhodopsin to the activated state [53–55].

The rhodopsin activation in response to light involves a transformation of metarhodopsin I (MI) form to the MII form. Figure 3 depicts results of Litman and Mitchell that demonstrate the effect of lipid microenvironment, studied in artificially reconstituted membranes, on MI ⇔ MII equilibrium. The equilibrium constant is 6 times higher with di-DHA acylated phosphatidylcholine (PC)

![Fig. 3. Effect of fatty acid composition of reconstituted phospholipid membranes on the equilibrium constant (K_eq) for metarhodopsin activation. Di-DHA phosphatidylcholine (PC) has the highest K_eq and is similar to reconstituted native disk membranes. Di-myristic acid 14:0 h.s the lowest constant. *p < 0.05 [data adapted from 53].](image)
than with di-myristic (saturated C14:0) PC. The di-DHA PC has an equilibrium constant that is almost identical to that of native rod disks. The effect is mostly explained by the increase in membrane free volume. This greater mobility of rhodopsin within the lipid microenvironment most likely explains the change in G protein activation and the corresponding enhanced signal transduction to photon stimuli [54]. The corresponding physiologic phenomenon is the increase in retinal sensitivity to light associated to DHA supply in the diet.

The role of membrane lipid composition in determining the electrical properties of cultured neuronal cells exposed to exogenous fatty acids has also been investigated [56, 57]. Both n-3 and n-6 fatty acids induced slower rates of rise, and to a lesser extent, lower amplitude of Na$^+$ action potentials. The opposite effects were observed when saturated or transmonoenoic fatty acids were added. These effects are likely mediated by a change in the number of active Na$^+$ channels. A change in membrane composition or altered fatty acid availability to the cells may explain this effect [57]. Free LCPUFAs modulate the inactivation of calcium and sodium channels [58]. In addition to the antiarrhythmic consequences for cardiac myocytes, there are also changes in cation currents in hippocampal neurons [59] and a higher seizure threshold in rat cortex [60]. These effects appear to depend on free extracellular LCPUFA concentration and not on membrane phospholipid composition [60]. The responsiveness of free LCPUFAs to dietary interventions, which alter tissue composition, remains unclear. The release of free LCPUFAs from membranes could have widespread effects on neurosensory organ function.

At the CNS level, interest in the effect of essential fatty acids on the maturation of visual function is based on their role as key structural components of cell membranes and highly enriched in visual and neural structures. Figure 4 demonstrates the effect of the early diet on fatty acid composition of phospholipids obtained from the cerebral cortex of infants who died suddenly within the first 6 months of life [61]. These data demonstrate that breast-fed infants who receive DHA have a higher content of this fatty acid in their brain cortex while infants fed formula have a lower DHA content and a rise in the corresponding n-6 derivative. n-3 fatty acids have been demonstrated as essential for retinal and brain function in animals and more recently for humans. DHA and AA are present in human milk and to date are absent from infant formulas, thus the issue of functional effects of deficiency for these nutrients has been raised. Neuringer et al. [62] have contributed in establishing the need for n-3 FAs in the diet utilizing infant rhesus monkeys as a model system for n-3 FA deficiency. Following prenatal (maternal) and postnatal diets deficient in n-3 FAs, the DHA concentrations in both the occipital cortex and the retina were reduced to 20% that in control monkeys. The n-3 FA deficiency also impaired visual acuity as measured by preferential looking techniques. By 12 weeks of age, the deficient monkeys presented Snellen acuities of about 20/125 vs. 20/50 in controls (20/20 is the average adult acuity; 20/50 is average for 12 weeks of age). In addition, the b-wave
**Fig. 4.** Effect of type of feeding on fatty acid composition of brain cortex in infants who died suddenly; 10/1 represents a formula with that n-6/n-3 ratio, this is closer to human milk; similarly 40:1 h.s that n-6/n-3 ratio, means excess n-6 fatty acids. Mixed feeds infants received human milk and formula. AA, DHA and DPA correspond to arachidonic acid (20:4 n-6), docosahexaenoic acid (22:6 n-3), and docosapentaenoic acid (22:5 n-6) respectively [data from 61].

Amplitudes of ERGs were reduced by 30% in the n-3 FA-deficient rhesus monkeys [62].

We have demonstrated that retinal function is significantly affected by n-3 deficiency in human preterm infants. There are higher rod threshold and lower maximal amplitude values for the b wave in the infants receiving n-3-deficient corn oil diet relative to controls fed n-3 fatty acids. The higher rod threshold and lower maximal amplitude values indicate that the sensitivity and gain, respectively, of the rod photoreceptors of the n-3 FA-deficient infants were significantly reduced. Thus, a greater illuminance was necessary for the rod response to reach a specific amplitude in the n-3 FA-deficient infants [63]. The results of dietary FA modification on the function of the visual cortex as measured by pattern reversal visual evoked potentials (VEP) and behaviorally by the forced-choice preferential looking (FPL) visual acuity response demonstrated that infants in the human milk and marine oil groups (both receiving DHA) had improved visual function relative to infants fed formulas devoid of DHA (corn and soy oil groups). Visual acuity tests measure
the integrity of the neural pathway from the retina to the occipital cortex. Our results and similar studies reported by other investigators, support an essential role for n-3 FAs in normal eye and brain development [63, 64]. To date, the data suggest that DHA, as present in human milk and in the experimental supplemented formula, is required for optimal visual development in the human. Figure 5A corresponds to results of studies of LCPs supplementation of infant formula comparing red cell DHA content in control formula (containing LA and LNA but no LCPs) or formula with DHA alone or DHA + AA. Figure 5B provides visual acuity results using VEP relating these two diet treatments. These results from a randomized study demonstrate a causal link between DHA supplementation and enhanced maturation of the visual cortex [65]. Further studies will serve to delineate the best and safest form to supplement infants. The reversibility of the changes in human infants cannot be fully answered from present data since most studies are terminated by 12 months. However, the primate studies have demonstrated that the reduced ‘a’ wave of the ERG and the abnormal visual acuity found in the rhesus monkey in response to dietary 18:3 n-3 deficiency does not recover fully after n-3 fatty acids are added back to the diet.

Several potential mechanisms by which early dietary essential fatty acid supply may affect visual and brain maturation and long-term function can be outlined based on the available experimental data. The potential role of DHA as a modulator of membrane properties can be supported by the in vitro studies of membrane fluidity and transport in neural cells modified in their membrane fatty acids. The putative role of DHA in amplifying the phototransduction cascade is supported by the electrophysiologic findings in animals and humans. Decreased retinal rod cell threshold means that less light is required to trigger a response, higher maximum amplitude means that more signal is being transmitted to the visual pathway. Moreover, the discovery of biochemical differences in phosphorylated microtubular associated proteins in neurons from the visual cortex of light-deprived animals during early development provide a mechanism for the classical observations by Hubel and Wiesel [65, 66]. Microtubular proteins play a key role in the dendritic arborization and interconnections in the cortex, darkness inhibited the expression of this gene product [67, 68]. Thus the role of light-mediated stimuli in triggering cortical differentiation offers a plausible explanation for the phenomenon of a critical period for ocular dominance that has a biochemical basis as well as structural and functional correlates [69–70]. We speculate that similarly DHA by affecting light transduction early on in life may have long-lasting effects on the organization and function of the visual cortex. The fact that human milk-fed infants exhibit more mature stereoacuity at 3 years relative to formula-fed infants is indicative that this phenomenon may be relevant to the human [71].
**Fig. 5.** (A) Fatty acid composition of red blood cells (RBC) in term infants at age 4 months measured as µg per ml of packed cells in relation to type of feeding at 6, 17 and 52 weeks of life AA and DHA correspond to arachidonic acid and docosahexaenoic acid respectively. (B) Visual acuities of term study infants measured using sweep visual evoked potential (VEP) at 6, 17 and 52 weeks of life in relation to type of feeding. Larger log minimum angle of resolution (log MAR) visual acuity values are associated with poorer visual acuity. The control formula had ample α-linolenic acid but no LCPs. An asterisk within a measure indicates significantly different from human milk mean values using ANOVA. *p < 0.05 [data adapted from 64].
Temporal Cycles and the Functional Integration of Nervous System Development (Circadian Rhythms/Sleep-Wake Cycle Development)

As the earth circles the sun it rotates on its axis with the consequence that half is in light at all times. This continuous, 24-hour cycle of light and dark is the most prominent recurring environmental stimuli. All forms of life have developed timing mechanisms expressed in cycles with a period of around 24 h. These oscillations termed circadian rhythms are an integral component of how living organisms adapt to the most pervasive stimuli facing all forms of life, that is the solar cycle. This anticipatory homeostasis in mammals, including humans, is expressed as the sleep-wake rhythm. Biological clocks have been demonstrated in organisms ranging from single-cell algae to mammals. In humans, circadian timing is a function of the nervous system. Thus, circadian timing is an integral part of neural function and as such is set by neural structures whose function is the generation and regulation of these rhythms. The circadian timing system has three principal elements: (a) pacemakers; (b) entrainment pathways which set the timing of the pacemaker in response to environmental stimuli, and (c) output from the pacemaker that controls function in other systems. It is now well established that circadian rhythms are regulated by a master pacemaker located in the suprachiasmatic nuclei of the hypothalamus. In this context, the endogenous circadian rhythm and the sleep-wake cycle are integral components of functional integration and brain development. Thus, nutritional influences on hormonal balances, metabolic responses and on function of the developing nervous system should be examined within this framework.

Fetal motor activity patterns, one of the earliest expressions of early neural CNS function, appear between 8 and 12 weeks’ gestation; movements are episodic, that is, cycles of activity are interspersed with periods of quiescence [72, 74]. The duration of the cycles increases with advancing gestation from the mid-trimester onwards [75, 76]. The increasing synchronization of cyclic motor activity with periodic changes in heart rate and eye movements with advancing gestation is considered an important milestone in CNS development [77, 78]. Behavioral states in this context will serve to describe patterns of brain-controlled physiologic activity overtly manifested by the various sleep-wake states that recur in time. The definition proposed by Prechtl [78] has been particularly useful: ‘by the term state, one tries to describe constellations of certain functional patterns and physiological variables which may be relatively stable and which seem to repeat themselves’. Behavioral states are not merely a convenient way of descriptive categorization of an ongoing stream of behaviors, but rather they have specific characteristics that reflect a particular mode of neural activity and CNS functioning. For example, it is now widely accepted that the characteristics of physiologic variables comprising cardiac, respiratory and motor activities, eye movements, EEG patterns, tonic activity
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of certain muscles, oxygen consumption, cerebral blood flow, temperature, endocrine secretion, change as a function of sleep-wake states. These states are well described in the human neonate, infant and adult. Neonatal states include quiet sleep, deep sleep, active sleep (rapid-eye-movement sleep); indeterminate sleep; and awake states. The differentiation of states progresses in a systematic way. First, all physiological variables have independent recurring cycles of quiescence and activity; then two or more variables initiate synchronous cycling; finally this synchronization recruits more variables and consolidates in an organized state as illustrated in Figure 6. The progressive changes in state proportion and the sleep-wake organization during the 24-hour cycle occur in a very organized pattern, suggesting that sleep-wake states are an expression of functional and developmental integrity of the CNS [77]. The development of fetal states and the organization of the respective defining parameters, modulation, disruption by disease is a potentially powerful way to assess CNS development.

Active sleep (AS) is a state characterized by spontaneous, intense, generalized neuronal firing in most areas of the brain [79, 80]. The CNS displays an increased level of endogenous neuronal activation during this state even in very immature animals. This suggests a key role for AS during ontogenesis of the nervous system. Roffwarg et al. [80] were the first to propose that the primary purpose of AS was to act as an inducer of CNS development in the fetus as well as the neonate. They proposed that AS provided endogenous stimulation to the sensory processing areas in the CNS and cited the early myelinization of these areas as evidence of the maturational effect of

**Fig. 6.** Illustration of the early temporal organization of sleep development. First, all physiological variables have independent recurring cycles of activity (more/less, pattern A/pattern B, activity/quiescence); then two or more variables initiate synchronous cycling; finally this synchronization recruits more variables and consolidates in an organized state.
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early stimulations. The pharmacological inhibition of AS induces structural and functional disturbances during development that become apparent in the adult animal. AS-deprived animals have a reduced brain size, hyperactivity, anxiety, attentional and learning difficulties, increased voluntary alcohol consumption and reduced masculine sexual behavior. Furthermore, while environmental enrichment has been shown to enhance cortical maturation, this is no longer possible in the AS-suppressed rats [81–84].

Several studies have shown that the endogenous (internal) activation that occurs during AS is essential for CNS maturation. This is particularly relevant at the onset of wakefulness (WA), characterized by the neonate's limited ability to profit from exogenous (external) stimuli. For instance, the threshold of different sensory systems is high and sensitivities are low, reducing thus the capacity of the neonate to process information during WA. It is well accepted that the maturation of sensorimotor systems is dependent on the activity induced by exogenous stimulation. The activity originating from endogenous stimulation during AS may have a similar or greater influence on CNS maturation. In this respect, Roffwarg et al. [80] noted that visual pathways were activated during periods of AS. They surmised that a process of intense information processing was stimulating the visual system from within, suggesting that AS served to functionally stimulate immature neurons and synapses 'internally' at an age when infants were in a state of partial sensory deprivation during WA. More recently, spontaneous retinal activation has been described in animals during early development. Ganglion cells of neonatal mammalian retina produce spontaneous bursts of activity in the absence of light stimulation. The assessment of the relationship between retinal electrical activity and sleep states may provide new insights in this respect. Supporting this concept, it has recently been shown that AS deprivation aggravates the anatomical and functional deleterious effects of monocular deprivation on lateral geniculate nucleus in kittens. Furthermore, as shown in Figure 7, the effect of AS deprivation in unoccluded kittens resulted in an impact of higher magnitude than the one provoked by monocular deprivation [84]. In a recent study we demonstrated the amplitude of electroretinographic responses was greater during AS; higher amplitude indicates increased maturation of the retina and probably affects structures within the visual cortex and its functional development [85]. There is also evidence from our results that the functional association between retinal activity and sleep states is attenuated at 4 months of age. Concomitantly the quantity of WA increases and quality improves, paralleling the decrease of the amount of AS during the daytime. The intrinsic activation provided by AS is first essential and then complementary to the stimulation obtained from extrinsic sources [85, 86]. The ability to maintain the normal progression in sleep-wake maturation is critical for brain development and may serve to assess how environmental factors including essential nutrient supply affect CNS development.
Observations from the literature indicate differences in the early functional organization of the CNS between breast-fed and bottle-fed infants. Nocturnal sleep-wake states organization at 4 months of age show that breast-fed infants spend a higher percentage of time in quiet sleep (QS) and a lower percentage of time in AS as compared to formula-fed infants [87]. Furthermore, heart rate values were lower in all sleep-wake states in breast-fed infants [88]. Even though total energy expenditure during AS and QS did not differ between feeding groups, the different organization of sleep states throughout the night might account for differences in energy expenditure between feeding groups [86, 87]. Findings from our laboratory indicate a more organized heart rate pattern in human milk (HM)-fed infants. As a whole, these infants present lower heart rate in different sleep states and better heart rate variability (HRV) patterns indicating a more mature pattern of CNS development. The effect is observed in all frequency components but is more marked in the high-frequency component suggesting a higher parasympathetic vagal tone in the HM-fed group. The mixed formula/human milk-fed group behaves similarly to the group fed HM exclusively. These differences are not observed during wake periods.

Breast-fed neonates, even at the age of 2 days’ postpartum, are better organized, physiologically, than bottle-fed neonates [88, 89]. They exhibit
slower overall mean heart rate in both AS and QS. They also present a higher incidence of low-frequency HRV, greater HRV and elevated vagal tone, indicating as a whole, more mature HRV patterns. These results suggest that alterations in maternal/environmental entraining factors including the supply of critical nutrients may underlie disturbances of sleep and feeding commonly experienced by infants.

Another example on a nutritional effect on sleep development is EFA deficiency in children given fat-free parenteral nutrition [90]. Studies in children maintained on fat-free parenteral nutrition for 2–6 months served to demonstrate consistently reduced duration of slow-wave sleep compared with children who received parenteral nutrition plus essential lipids. The main difference was a lower cumulative amount of slow-wave sleep time from the second hour of night-sleep onwards. These results suggest that early lipid nutrition may modulate sleep regulation and may have implications for whole body energy expenditure during sleep, since slow-wave sleep is associated with a lower metabolic rate than other stages of sleep [91].

Effects of Nutrients on Functional CNS Integration During Development

The necessity for sensory-driven activity has been widely recognized as crucial for normal infant brain development. Sensory deprivation induces anatomical and functional deficits in animals and humans. Neural activity is critical in the anatomical development of the intricate circuitry which connects sensory organs to relay centers, to primary cortical processing areas, to associative areas necessary for learning and memory. Figure 8 provides a scheme of this generalized model for sensory integration to CNS function and includes nutrients with proven effects on sensory organs and in cortical processing. The fetal nervous system develops pre- and postnatally from imprecise neuronal connections to a complex neuronal network with hundreds or thousands synaptic interactions. As described in the initial section, deficits in several key nutrients (protein energy, retinoic acid, folate, iron, iodine, zinc, essential fatty acids) can disrupt both the cell replication and/or dendritic arborization depending on the timing, severity and duration of the restriction. The evidence from animal and human studies provided here and in other chapters indicates that specific nutrient deficits affect auditory signals, retinal light transduction, somatosensory responses, taste and smell discrimination. The responses of sensory systems to either nutrient deficits or deprivation of environmental stimuli is in many way similar and potentially synergistic in their effects. The common response is an increased threshold to respond, prolongation of time to respond, a decrease in the amplitude of the response and a prolongation of its duration. The consequences of these changes in relay centers and cortical processing vary depending on the developmental stage of the particular function. All systems are more vulnerable during the periods of most rapid functional development, generally this implies younger
Fig. 8. Schematic representation for sensory organ integration to CNS function. Nutrients with proven effects on sensory organs and on cortical processing are included.

Ages. The combined effect of increased vulnerability due to developmental stage and greater severity of nutrient deficits in earlier ages make infancy a critical time for brain development. This has been well documented at this meeting. Cognitive processes, memory and learning, and all higher CNS functions are dependent on the integration of the input coming from different systems, in a time-precise and magnitude-dependent manner. Thus, a delay in millisecond delay auditory signal processing and a minute of arc increase in visual stereoacuity can act together in determining the delay of a child in acquiring the capacity to respond to a simple instruction, such as placing a blue cube on top of a red cube. This simplistic example illustrates how two or more sensory input processes interact in determining indices of cognition.

Normal infant development depends both on genetics and sensory input that provides appropriate patterns of neural activity to shape the developing brain. The requirements, however, are not only for sensory-driven activity but also for endogenous neural activity. Recent studies have revealed a similar requirement for endogenous neural activity generated by the nervous system itself, long before there is any sensory input. These patterns of sensory-driven and endogenously generated neural activity sculpt the precise circuits that are crucial to the many complex functions of the adult brain. This integrated view of functional brain development suggests that the ability to maintain the normal progression in sleep-wake maturation is an important index of brain development and may serve to assess how environmental factors including essential nutrient supply affect CNS development.
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References


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Discussion

*Dr. Fernstrom:* Monocular deprivation is, of course, a good example where you can see clear neuroanatomical changes in the cortex. But when you get to more subtle types of insult, it gets harder to understand the effects. In an area such as the polyunsaturated fatty acids – and the visual problems associated with deficiency – it occurs to me that quite by luck you’ve been looking at a sensory system where there is a well-known sensory input, and enough is known about it to allow it to be followed into the brain to different places. You have a neurophysiological correlate and this may be the best opportunity to start to untangle some of the structural changes that may be taking place further back in the brain. So it is a very good example to examine.

There are other nutritional insults that may not lend themselves to such easy examination. For instance, you can create an animal model that looks grossly abnormal by giving a high dose of monosodium glutamate and destroying part of the hypothalamus, but if you don’t look at the right moment after the administration of the insult, you won’t see an abnormal hypothalamus. Structurally it looks normal 10 days after the insult, but it doesn’t look normal 5 hours after the insult. It may be that by starting with a sensory input that’s very well defined you have the best chance of starting to see some of these structural changes.

*Dr. Uauy:* I think we’ll have to start working with a reductionist approach, trying to isolate a system working both in the animal and the cell culture model, looking at gene expression and the function related to those genes, and then starting to build the system up from scratch. I’m not saying that everything that has been done so far is wrong, but I’m just saying that if we’re going to start to have specific lesions and specific biochemical and functional markers, we’re going to have to refine our tools. Any time you see a cognitive test that has ±15% variability for 1 SD it means that there are many factors hidden in the abnormality.

*Dr. Woods:* I’m not a nutritionist but I was struck by the slide you showed where you could move IQ up or down depending upon the developmental age at which you gave a supplement to the thyroid system. It seems to me that there’s a profound message here for the other talks that we’ve heard about supplementing with iron or protein or whatever at different ages. Unless we know very specifically when to do it, there could be problems.

*Dr. Uauy:* I went to that thyroid paper [1] because the body of animal data is so impressive. I think there’s an emerging world where nutrients and nutrient-hormone relations will play a different role, especially when toxicants are involved as well. We’re going to have to redefine the effects of toxicants in all of the systems.

*Dr. Woods:* I think the timing point was very important.

*Dr. Grantham-MacGregor:* I think caution is a good message at this stage, rather than leaping in with lots of supplements. We’ve just done two zinc studies in Bangladesh, and we found that the children’s cognition was worse when we gave them zinc – not very much worse, and whether the reviewers will accept it I don’t know, but it’s a caution.
Dr. Uauy: Another concern is that we are supplementing people who are not deficient in that micronutrient. The present approach in some of the public health measures is to give vitamin A, zinc, iron to everybody.

Dr. Grantham-MacGregor: I think it’s frightening. We may be doing harm.

Dr. Cole: I was curious about DHA studies; did people add antioxidant supplements as well, considering they’re one of the most readily peroxidizable substrates? Especially if you have a program for feeding up the population.

Dr. Uauy: Yes, they did add antioxidants; secondly, the additions are all at the level found in human milk, so not very high. But antioxidants have been added and also vitamin E, and the peroxidative status of the infants has been followed.

Dr. Cole: So 0.3–0.6% DHA is more or less human milk level?

Dr. Uauy: Yes. The human milk model can be as low as 0.2% and as high as 0.8%, depending on where you are in the world.

Dr. Cole: Does the dietary intake of the mother regulate that?

Dr. Uauy: Yes. Mainly dietary intake of both precursor and product, because the formation of DHA from \( \alpha \)-linolenic acid is also regulated.

Dr. Cole: So maternal supplementation might affect the infant.

Dr. Uauy: Yes, people are doing that. Another issue is that growth outcome, which is usually an indicator of malnutrition, may not be the same as brain development outcome. The optimal mix for improved growth may not be the same as for optimal brain development. A lot of these nutrients have no effect on growth, or even a negative effect. So the way pediatricians use growth as an outcome measure may also need to be reassessed.

Dr. Crozier: I’d like to also address the question of what is optimal in terms of neurodevelopment. I think this is a really critical question. I was discussing this with Christina Williams, and she told me something about choline supplementation in animals. For example, you can regulate cholinergic neurons: if you give very little choline they will react very rapidly and very effectively; if you accustom them to a lot of choline they are able to function for longer; however, if you then deprive the neurons of choline after adaptation to a high choline they no longer work as effectively as those that started out deprived. Extrapolate that to a infant situation: if we give all these supplements and we get the neurons or other physiological systems adapted to the enriched environment, then maybe they will no longer be adapted to the environment they will later inevitably find themselves in.

Dr. Uauy: Maybe the measure of optimal development is healthy aging. So this is a unique opportunity for people interested in development to listen to the aging side. My prediction is that optimal development is going to be based on healthy life years after 80 or whatever.

Dr. Grantham-MacGregor: I was concerned when you showed in your experiments that visual acuity was better with DHA supplemented formula than with breast-feeding.

Dr. Uauy: Well, breast-feeding with how much DHA? That’s still debatable, but I think it raises the question as to whether breast milk is perfect for long-term development.

Dr. Grantham-MacGregor: Is bigger always better?

Dr. Uauy: We’re charting a new course here, because breast milk was intended to take us to life expectancy of maybe 45 years.

Dr. Grantham-MacGregor: God may have had 100 years in mind!

Dr. Uauy: But the point is that even that may need to be reconsidered, especially in the light of the increasing presence of toxicants. Obviously we want breast milk free of toxicants.

Dr. Holm: Did I understand correctly that you measure visual acuity by evoked responses?
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*Dr. Uauy:* Pattern reversal evoked responses. This provides very good estimates of visual acuity measured neurophysiologically. When we do use behavioral tests, the results are more variable.

**Reference**