

The Influence of Neonatal Nutrition on Behavioral Development: A Critical Appraisal

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Specific nutrients appear to modify the metabolism of neurotransmitters, which are endogenous regulators of neurogenesis, neural migration, and synaptogenesis during both embryonic and early postnatal life. This has led to the question of whether, by affecting neurotransmission, malnutrition during the early neonatal period affects behavioral development. The literature based on animal models suggests that nutrient deficiencies during early life influence neurotransmission and, in some instances, also affect behavioral outcomes. A clear answer to the question, however, remains elusive. This can be attributed to the complexity of the process of brain development, where changes at a cellular level may not necessarily translate into changes at a behavioral level. Future investigations in this important area of research should work toward refinement of the design of behavioral experiments so that these studies can contribute to the understanding of the putative mechanisms involved.

Introduction

Although extensive research efforts have described the effects of poor nutrition in early life on brain development,¹ there has been renewed interest in the role of specific nutrients in brain and behavioral development. Interest in the influence of malnutrition on brain development has emerged from reconsideration of earlier work and findings in the current literature, recently reviewed by Levitsky and Strupp.² Although malnutrition (i.e., deficiencies of specific nutrients) and undernutrition (i.e., general caloric and nutrient deficiencies) profoundly affect the growth of diverse brain structures, these structural alterations eventually recover in many cases. It is possible, however, that more subtle, long-term effects, such as changes in evoked

neurotransmitter release and/or receptor sensitivity, may result from malnutrition and undernutrition, and that these may in turn lead to changes in behavioral outcomes.

In the adult central nervous system, neurotransmitters act as chemical mediators of intercellular communication by activating specific receptors and second messenger systems.³ The identification of neurotransmitters in the developing central nervous system, together with findings that pharmacologic agents, which act on neurotransmitters such as serotonin, dopamine, and acetylcholine, also interfere with the developmental progression of the brain, suggests that neurotransmitters may be endogenous signals that regulate neurogenesis, neural migration, and synaptogenesis.^{4,5} Although neurogenesis and the development of major brain structures in the human brain occur during the prenatal period, subsequent postnatal events include myelination of new axons, migration of neurons, and generation and modification of synaptic connections.⁶ Evidence from animal models shows that specific nutrients can modify the metabolism of neurotransmitters in the brain.^{7,8} This leads to the speculation that changes in neurotransmission as a result of malnutrition during early postnatal development may affect neural migration and synaptogenesis and thus the neuronal architecture and connectivity of the brain. These effects may subsequently alter the normal trajectory of brain and behavioral development.

Selective nutrient deficiencies can, theoretically, affect neurotransmission at many different sites within the biochemical pathways involved in the synthesis, release, and metabolism of neurotransmitters. Such effects may be either direct or indirect. For example, direct effects of nutrients on neurotransmission may include presynaptic effects, such as changes in the availability of precursors of specific neurotransmitters. Alternatively, nutrients may act postsynaptically by changing receptor concentrations or sensitivity, or by changing membrane fluidity, which could, in turn, affect signaling pathways.⁹ Dietary antioxidants, such as β -carotene and vitamins C and E, are examples of nutrients that may affect neurotransmission indirectly. Oxidative stress is proposed to be one of the mechanisms in the pathogenesis of Alzheimer's disease.¹⁰ Thus, di-

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etary antioxidant deficiencies, by increasing oxidative stress in the developing brain, may result in lipid peroxidation or apoptosis of neuronal cells,¹¹ which may then disrupt neurotransmission. It is also possible that nutrient deficiencies may alter the regulation of developmental processes that are responsive to growth factors. For example, it has been shown that retinoic acid (vitamin A) enhances neurogenesis and astrocyte differentiation by stimulating the proliferation of stem cells that are responsive to epidermal growth factor.¹² It is therefore conceivable that a deficiency will also be associated with alteration in brain cell growth and differentiation. However, this area has not been fully elucidated, and further research is necessary.

Nonetheless, identification of the factors that may mediate the relationship between changes at the cellular level and alterations in behavioral function is no easy task. When studying nutritional influences on behavior, there are two possible strategies.¹³ The first is the “bottom-up” approach, in which the assumption is that changes in essential molecules have an impact at all functional levels from synapse to behavior, and thus that changes at the cellular and molecular level will necessarily lead to behavioral changes. The behavioral tests most commonly used in this instance are those that are generally perceived to have some validity in terms of the neurotransmitter involved; for example, iron deficiency is associated with changes in brain dopamine D2 receptor density, and might therefore cause alterations in attentional functions.¹⁴ It would seem, however, that there is considerable plasticity in the neural systems that orchestrate behavior, such that it is likely for considerable alteration of neurotransmitter function to be present without overt behavioral changes. For example, the symptoms of Parkinson’s disease are related to a loss of the dopaminergic neurons in the substantia nigra, but these symptoms become evident only when this loss is in excess of 80%.¹⁵ It is therefore possible that compensatory mechanisms during development may act to obscure the impact of nutritional changes at the molecular or cellular level on functional outcomes, and that, in this instance, the “top-down” approach might prove more useful. This strategy is similar to that used in neurotoxicity testing, in which comprehensive test batteries are used to assess performance across a variety of behavioral outcomes.¹⁶ These include not only performance on various types of learning tasks, but also tests of sensory, motor, and motivational function, as well as species-typical adaptations, such as social behaviors. The purpose of these tests is to describe a pattern of behavioral findings that can then be interpreted in conjunction with the behavioral literature to infer the possible neural systems involved. This, in turn, should enable further mechanistic inquiries in appropriate directions, which may very often be different from those predicted by the “bottom-up” approach.

This paper reviews the literature using the “top-down” approach and, thus, it focuses largely on the question of how specific nutrients may affect neurotransmission with consequences for behavioral development. Furthermore, this review covers the literature on deficiencies in specific nutrients, rather than general undernutrition.

To investigate this question, only studies performed in laboratory animals are reviewed; human studies present several complications, including the difficulties in controlling confounding factors, as well as the limitations on experimental manipulations. Animal models make an important contribution to understanding mechanisms whereby specific nutrients affect neurochemistry and brain development. However, it is important to recognize that animal studies are not free from methodologic pitfalls,¹⁷ and that there are limitations to the interpretation of behavioral tests,¹⁸ which will be discussed later.

The evaluation of animal experiments requires an understanding of the similarities and differences between the brain development of humans and of animals, particularly the rat, which is one of the most commonly used animal models in the behavioral sciences. Dietary manipulations during gestation in rats are generally more representative of early gestation in humans. The period between birth and approximately 25 days postnatal age (around weaning) for the rat is equivalent to the human brain growth spurt, which occurs between the third trimester of pregnancy and 2 years postnatal age.¹⁹ Thus, for the purpose of investigating the effect of neonatal nutrition during this sensitive period of brain and behavioral development in the rat, dietary manipulations should be performed during the preweaning (lactational) period. Dietary manipulations after weaning in rats may not be as sensitive to alterations in behavior, because this represents the period following the brain growth spurt. Thus, to generalize the results of animal studies to humans, the timing of the experimentally induced malnutrition should be considered against a comparative schedule of development between species.

The next section addresses the effect of macronutrients (protein, carbohydrates, and essential fatty acids) on brain and behavioral development, followed by a discussion of the effects of micronutrients, specifically vitamins and the trace minerals zinc and iron.

Protein and Carbohydrates

Several specific amino acids act as precursors for neurotransmitters, such as tryptophan for 5-hydroxytryptamine (serotonin) and tyrosine for dopamine, norepinephrine, and epinephrine. Serotonin is synthesized and released by brain neurons depending on brain tryptophan concentrations, and brain levels of tryptophan are dependent on plasma concentrations and the rate of uptake across the blood-brain barrier membrane.²⁰ Tryptophan

crosses the blood-brain barrier via active transport and competes with other large neutral amino acids (LNAA).²¹ A meal high in protein has been shown to result in a sharper increase in plasma tyrosine than tryptophan and thus a greater tyrosine concentration in the brain. A meal high in carbohydrates, as an indirect consequence of insulin secretion, precipitates a rise in plasma tryptophan and a decrease in plasma LNAA, followed by a rise in brain tryptophan as well as serotonin.^{7,22} In young adult rats fed a high-carbohydrate diet over a 2-hour period, there were increased levels of the breakdown product of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), in the striatum and hypothalamus as monitored *in vivo* by the microdialysis technique.²³ The influence of feeding diets high in carbohydrates or protein on neurotransmitter metabolism and brain development during the preweaning period has not, however, been explored extensively.

In one such study it was demonstrated that when infant rats were fed different amounts of carbohydrate by manipulating the dams' diets during gestation and to 15 days of postnatal age, the increase in whole brain tryptophan and serotonin was greater in the first 24 hours postnatally in the rat pups whose mothers were fed a high-carbohydrate diet (12% and 24% glucose) compared with the offspring whose mothers received a control diet (0% glucose).²⁴ Also, at 15 days postnatal age, whole brain tryptophan, serotonin, and 5-HIAA were significantly higher in pups of mothers receiving a diet containing 60% glucose compared with pups whose mothers consumed 24% glucose.

It was suggested by the authors that the amount of carbohydrate in the diet might play a role in modulating serotonergic neurotransmitter levels during development. The question that needs to be addressed is whether changes in serotonin concentration in the brain during development translate into differences in brain development and behavior in later life. Particularly, the influence of serotonin on neuronal development has been studied in detail and has been summarized by Lauder.²⁵ Serotonin has been shown to have both inhibitory and stimulatory effects on neurite outgrowth, glial proliferation, and synaptogenesis. Serotonin appears to have differential effects, activating autoreceptors on serotonin neurons as well as postsynaptic-like receptors on glial and neuronal targets. Serotonin thus appears to be an important developmental signal during brain development and is postulated to act as a modulator of cell proliferation within the neuroepithelium.

The behavioral consequences of changes in serotonin concentrations in the brain during early development were investigated by Volpe et al.²⁶ The investigators produced a permanent reduction in serotonin neurons and serotonin concentration in the dorsal raphe nucleus and the hippocampus following treatment with 5,7-

dihydroxytryptamine in rat pups at 3 days of age. This was not associated with deficits in learning and memory in adulthood as measured by Morris water maze and 12-arm radial maze tasks. It has been demonstrated, however, that serotonin by itself is not an important modulator of place learning (i.e., learning the position of a hidden platform) in the Morris water maze,²⁷ but may be related to ancillary processes, such as sensory and motor processes and motivation, necessary for place learning.

Several neurotransmitter systems have been implicated in spatial memory performance. Studies have indeed shown that memory deficits result from combined alterations in both serotonin and cholinergic systems.^{28,29} It is therefore possible that changes in cholinergic systems may compensate for the deficits in serotonergic neurotransmission, particularly if the "insult" occurs during early development, when the brain may demonstrate considerable plasticity.

Performance on learning tasks will be influenced by motivation and emotion, which may in turn depend on concentrations of other neurotransmitters. Young rats receiving a tyrosine-enriched diet after weaning (after the completion of the brain growth spurt) displayed neither tail shock stress-induced depletion of norepinephrine in the hypothalamus, amygdala, and locus coeruleus, nor behavioral depression as measured by hole poking, standing on hind legs, and locomotion, when compared with animals fed a standard lab chow diet.³⁰ Thus, diet may also influence how an animal responds to a stressful event. As indicated by the studies described previously,^{7,22,24} a high-carbohydrate diet increases brain tryptophan and serotonin concentrations but reduces concentrations of circulating LNAAs, such as tyrosine. Reducing serum tyrosine concentrations in rats was accompanied by reduced brain tyrosine concentrations.³¹ Therefore, modulation of one neurotransmitter (e.g., serotonin) by the diet (e.g., rich in carbohydrates) might also affect other neurotransmitters (e.g., norepinephrine), which in turn might alter behavioral responses to a stressful event (e.g., the behavioral test itself). The possibility of intervening variables of this type, which are related indirectly to nutritional intervention, should be addressed when evaluating behavioral outcomes.

It has been demonstrated that dietary manipulations of protein change retinal and hypothalamic tyrosine and dihydroxyphenylalanine concentrations in adolescent rats.³² These changes might in turn affect dopamine and norepinephrine levels. Pappas et al.³³ demonstrated that when neonatal rats were depleted of dopamine by intraventricular administration of 6-hydroxydopamine after birth, learning and spatial memory deficits (as measured by elevated plus-maze, Morris water maze, and concentric ring maze tasks) were observed in later life. It is not known, however, whether dietary manipulations during the

preweaning period have an enduring influence on dopamine or norepinephrine release and turnover, and whether they affect behavior.

There are numerous other amino acids involved in neurotransmission in the nervous system. Substances such as γ -aminobutyric acid (GABA), glutamate, glycine, histidine, and aspartate are present in high concentrations in the central nervous system and constitute the major excitatory (glutamate) and inhibitory (GABA) neurotransmitter systems.³⁴ Furthermore, the central importance of the N-methyl-D-aspartate (NMDA) glutamate receptor for learning is strongly supported by the behavioral literature.³⁵ Because the efflux of glutamate and aspartate from the brain into the circulation is far greater than the influx of these amino acids from the circulation into the brain, it appears that the brain is capable of synthesizing aspartate and glutamate.^{34,36} It is therefore also unlikely that a high-protein diet would specifically influence these amino acid neurotransmitters. Little is known about the metabolism of glycine or histidine in nervous tissue and whether glycine is synthesized in the brain or taken up from the circulation.³⁴

Essential Fatty Acids

Dietary essential fatty acids, specifically the long-chain polyunsaturated fatty acids (PUFAs) arachidonic acid (AA) (20:4 ω -6) and docosahexanoic acid (DHA) (22:6 ω -3), are major constituents of the central nervous system and are functionally important components of membrane phospholipids (reviewed by Wainwright³⁷). AA is released directly from the membrane through activation of phospholipase A₂, or indirectly through the action of phospholipase C on the phosphoinositides. It has been suggested that AA, like nitric oxide, may function as a retrograde transmitter involved in synaptic plasticity.³⁸ In addition to acting as a second messenger, AA, together with the other 20 carbon fatty acids, serves as a precursor of the eicosanoids (prostaglandin, thromboxanes, leukotrienes) on which normal brain function depends. The prostaglandins are important contributors to regulatory functions in the brain and can influence neural activity by modulating neurohormones and neurotransmitters.³⁷ In the central nervous system, the primary prostaglandin, PGE₂, inhibits the release of norepinephrine and dopamine and may augment the release of serotonin.³⁹ Dietary fatty acids will change the fatty acid composition of membrane phospholipids in the central nervous system,⁴⁰ and it has been shown that eicosanoid production can be modified by changing the relative proportion of dietary ω -6 to ω -3 fatty acids in brain phospholipids.⁴¹ Eicosanoids may also be involved in storage of memory by influencing processes such as those related to long-term potentiation and depression.⁴² Changing the fatty acid composition of the brain may therefore affect neurotransmission indirectly

through changes in eicosanoid metabolism. There may also be effects related to changes in the physical properties of the membrane, such as fluidity, flexibility, and permeability, which will in turn influence the activities of membrane-bound proteins.⁴³

DHA levels in the retina and the gray matter of the brain are reduced by feeding developing animals diets deficient in ω -3 fatty acids. This reduction is accompanied by a reciprocal increase in levels of 22:5 ω -6, such that the overall chain length and degree of unsaturation are not altered drastically. Nonetheless, studies in several species have identified effects of ω -3 fatty acid deficiency on retinal function.⁴⁴ Furthermore, it has been shown in young and aged rats that chronic deficiency of ω -3 fatty acids alters dopamine and serotonergic neurotransmission by reducing dopamine receptor binding and increasing serotonin receptor density in the frontal cortex.^{45,46} Thus, a current question of great interest is whether there are also behavioral consequences of the biochemical changes induced by ω -3 fatty acid deficiency in the developing brain. It has been shown in animal models that essential fatty acid deficiency, i.e., deficiency of both ω -6 and ω -3 fatty acids, is associated with growth retardation and aberrations in cognitive development.⁴⁷ Wainwright et al.^{48,49} have addressed the specific effects of ω -3 fatty acid deficiency on behavior in both rats and mice; although effects of ω -3 deficiency on various types of learning were not seen in mice, significant deficits were observed in performance on a working memory version of the Morris water maze in rats fed a diet deficient in ω -3 fatty acids for two generations.⁴⁹

A current concern in the field of fatty acid nutrition as it relates to human infants is whether the content of linoleic acid (LA) (18:2 ω -6) and linolenic acid (LNA) (18:3 ω -3), which are the dietary precursors of AA and DHA, respectively, is sufficient for central nervous system development in early life, or whether preformed AA and DHA, as found in breast milk, are also necessary. Studies of piglets, rats, and mice have shown that dietary supplementation with AA and DHA will increase the concentrations of these fatty acids in brain phospholipids.^{48,50-52} There are reciprocal effects, however, between dietary DHA and AA, such that DHA will reduce brain levels of AA, and AA will reduce brain levels of DHA.⁵³ Thus, the ratio of dietary long-chain fatty acids (ω -3: ω -6) is also important for normal brain development. A considerable number of studies of both term and preterm human infants have addressed the influence of PUFAs on behavioral development,⁵⁴ but additional large-scale, randomized supplementation trials are needed to provide definitive answers to this question. Surprisingly few animal studies investigating the behavioral effects of supplementing the diet with long-chain PUFAs such as AA and DHA have been performed. Wainwright et al.⁵³ showed that supple-

menting pregnant mice with long-chain PUFAs with a ratio of ω -6: ω -3 fatty acids ranging from 0.3 to 49 did not affect spatial learning ability in offspring as measured by learning the position of a hidden platform (place learning) in the Morris water maze, or activity in a spatial open field. But the offspring of the mice fed the diet with the lowest ratio (i.e., very high levels of ω -3 as DHA and very low levels of ω -6) showed transient growth retardation and swam more slowly compared with offspring of mice fed diets with higher ratios of ω -6: ω -3 fatty acids. This result was offset when a portion of the ω -6 fatty acids was provided as AA,⁵³ thereby supporting the importance of AA for growth and development.

In studies that manipulate the maternal diet to alter the dietary intake of the offspring, a change in maternal physiology and behavior that may occur via the dietary manipulation could affect the development of the offspring indirectly, rather than directly, thus potentially confounding the results. An alternative approach that eliminates this possible confounding factor is the artificial-rearing model in rats. Such a study has been completed recently, in which the ratios of long-chain PUFAs (AA:DHA) were varied against a background of normal LA:LNA ratios.⁵⁵ Rats were fed artificial rat milk through gastrotomy tubes for 5–18 days postnatal age, then weaned onto diets of similar fat composition. Working memory and place learning in the Morris water maze at 6 weeks of age were not affected by experimental diets, but deficiencies in working memory were observed in a positive control group of animals fed saturated fat. Notwithstanding, no correlations were observed between working memory and brain phospholipid composition. As mentioned previously, the absence of a correlation between one specific aspect of brain biochemistry and a particular behavioral outcome does not necessarily mean that no nutritional effects are present. It remains possible that a wider range of behavioral tests in the artificially reared rat may be able to detect more subtle behavioral differences and thereby enhance our understanding of the functional role of long-chain PUFAs in the developing brain.

Trace Elements

Zinc and iron are the most prevalent trace elements in the brain.⁵⁶ They are distributed heterogeneously in the brain, and the concentration of certain trace elements correlates with particular regions of the brain, which suggests a role for trace elements in specific brain functions.

Zinc is retained primarily in the hippocampus, which also has the highest rate of turnover of zinc.⁵⁷ Zinc is important for normal morphogenesis of the central nervous system;⁵⁸ it also appears to play a role in regulating the release of neurotransmitters, such as GABA and glutamate, and has been implicated in the storage of histamine in the hippocampus.^{57–60} Marginal zinc deficiency

was shown to alter microtubule polymerization in the brain, which might affect migration of neurons during development.⁶¹

The presence of high concentrations of zinc in the hippocampus, together with its function in biochemical processes in the brain, might relate to observations of impaired learning, reduced activity, and poorer memory reported in many studies of zinc-deficient animals.⁶² Many of these studies have investigated the effects of gestational zinc deficiency. Studies evaluating behavior of zinc-deprived monkeys during gestation and postnatally revealed a range of behavioral alterations. These included reduced responses to environmental stimuli as a result of hypoactivity and impaired performance on cognitive tasks (visual discrimination learning).⁶²

Only a few studies have explored the effects of zinc deficiency during the neonatal period prior to weaning. The offspring of lactating mice fed a zinc-deficient diet from 5 days prior to birth to 15 days postnatal age exhibited poorer memory on a passive avoidance task compared with a control group fed ad libitum.⁶³ Although passive avoidance has been associated with hippocampal function,⁶⁴ the study by Golub et al.⁶³ did not demonstrate reduced zinc concentrations in the hippocampal region in the offspring of zinc-deficient mothers. The altered behavior, therefore, may not have been specific to zinc deficiency.

Halas et al.⁶⁵ demonstrated long-term memory deficits in adult rats that had experienced postnatal zinc deficiency. This study measured suppression of bar pressing during a tone that had been associated previously with an electric shock. General malnutrition, as determined in a paired control group, did not result in long-term memory deficits. The findings of these studies suggest that zinc deficiency interferes with mechanisms necessary for mediating long-term memory, but it is not known whether this effect is specific to zinc alone.

Iron is essential for central nervous system functioning.⁶⁶ It is involved in the production and maintenance of myelin.⁶⁷ It may also be necessary for normal dopamine metabolism, because it has been shown that iron deficiency blunts the dopamine reuptake mechanism in the brain.⁸ Several studies have associated changes in dopamine, serotonin, and GABA systems with iron deficiency.^{68,69} Furthermore, iron is a component of many of the enzymes involved in amine neurotransmitter systems.⁶⁶ Although altered behavior has been demonstrated in iron-deficient animal models,⁷⁰ few studies have evaluated the effects of iron deficiency during lactation on developmental processes. Earlier^{71,72} and more recent work^{73,74} demonstrated that iron deficiency in preweaning rats reduced brain iron as total nonheme iron and ferritin iron. These changes persisted even when hemoglobin concentrations and liver iron were restored postweaning.⁷¹ Weinberg et al.⁷² dem-

onstrated that these biochemical changes were associated with behavioral consequences. Rats fed an iron-deficient diet via manipulation of the maternal diet from birth to weaning were less responsive to a novel environment in an open field compared with rats fed a control diet. Furthermore, the iron-deficient animals demonstrated longer re-entry latencies during a passive avoidance learning task, which suggests that animals that were iron deficient postnatally were affected differently by shock stimuli. Deficits in noncognitive behavior, such as responsiveness, activity, or arousal, were also demonstrated by Felt and Lozoff.⁷⁴ In this study, anemia in the lactational period was related to delayed neurodevelopment as measured by home orientation at 8 and 12 days of age. Further cognitive deficits in iron-deficient rats were exhibited by a reduced ability to learn the location of a submerged platform (measured by distance swam) in the Morris water maze at approximately 3 months of age.

There are several issues to consider when evaluating behavioral findings in studies of iron-deficient animals. For example, iron deficiency anemia may increase maternal attentiveness to pups,⁷⁵ or the poor growth caused by iron deficiency anemia may affect motor development and thus performance on behavioral tasks. Thus, further research is necessary to elucidate more fully the effects of iron deficiency during lactation on brain and behavioral development.

Many other trace elements have been linked to various functions of the central nervous system.⁵⁶ Although nutritional deficiencies of copper have been observed in infants⁷⁶ and manganese toxicity has been shown to result in a central catecholamine depletion state in adults,⁷⁷ such nutritional deficiencies or excesses are rare and their effects on early brain development and cognition have not been explored.

Vitamins

Choline is a precursor for synthesis of the neurotransmitter acetylcholine. Choline is provided by the human diet, but *de novo* synthesis of choline via methylation of phosphatidylethanolamine also occurs.⁷⁸ The demand for choline by the human body is adapted to growth rate, as well as the interrelationships between choline, methionine, folic acid, and vitamin B₁₂. It is believed that dietary choline is essential to humans when methionine is not available in excess.⁷⁹

Acetylcholine neurotransmission is involved in attention and memory functions in humans and rodents.⁸⁰ Choline supplementation of rats prenatally and during lactation resulted in long-term enhancement of spatial memory capacity and precision compared with control animals receiving no choline, as determined by performance on tasks in an 18-arm radial maze.⁸¹ Russell et al.⁸² induced choline deficiency during the postweaning period (after comple-

tion of the brain growth spurt) by replacing dietary choline with N-aminodeanol. This resulted in persistent learning and memory impairments in rats as determined by conditioned avoidance; it also inhibited passive avoidance responses compared with control animals. These effects were present despite complete recovery of choline acetyltransferase activity in the striatum and hippocampus upon reintroduction of choline into the diet.

Although choline treatment enhances formation and release of acetylcholine in adult animals,⁸³ it may not do so in neonatal animals. When pregnant rats were supplemented with choline, fetal brain choline and phosphatidylcholine were not increased in the pups when compared with offspring of unsupplemented rats; rather, brain phosphorylcholine was increased.⁸⁴ Phosphorylcholine is involved in DNA synthesis in some cells and may thereby influence neuronal development.⁸⁵ Pyapali et al.⁸⁶ demonstrated that prenatal choline supplementation augmented spatial memory in adulthood by significantly altering the long-term potentiation threshold in hippocampal slices. This suggests that dietary choline enhances memory capacity by altering the cholinergic system. It is not known whether dietary choline improves spatial memory capacity by enhancing the formation and release of acetylcholine in neonatal animals.

There is some indirect evidence that other vitamins play a role in neurotransmission. Vitamin C is water soluble and capable of modulating the effects of dopamine in the mammalian forebrain, because it can inhibit binding of dopamine agonists.⁸⁷ One study reported that vitamin C potentiated amphetamine-induced conditioned place preference in adult rats, but only at a low dose of amphetamine.⁸⁸ Whether vitamin C affects brain development and behavior in neonatal animals is unknown.

Because there are interrelationships between choline, methionine, folic acid, and vitamin B₁₂ with respect to choline metabolism, it is possible that these nutrients may affect acetylcholine metabolism indirectly. In young adult rats, neurite outgrowth was significantly greater when the diet was enriched with a B vitamin complex.⁸⁹ It is therefore conceivable that nutrients related to choline metabolism modulate brain development, but no studies have investigated behavioral outcomes with regard to vitamin B₁₂, methionine, or folic acid status in early neonatal life.

Discussion

The studies reviewed in this paper indicate that nutrients can affect neurotransmission in several ways. Nutrients have the capacity to affect the concentration of neurotransmitters in the brain by serving as precursors for these compounds. For example, dietary protein and carbohydrates alter the bioavailability of the precursors tryptophan and tyrosine for the neurotransmitters serotonin, dopamine, norepinephrine, and epinephrine. Nutrients, such as

essential fatty acids, may also affect neurotransmission indirectly by affecting changes in second messenger systems, such as the eicosanoids. Furthermore, specific nutrients appear to act as modulators of neurotransmission, e.g., trace elements that appear to influence neurotransmission by altering neurotransmitter release, storage, and possibly sensitivity of receptors. Lastly, nutrients may act as neurotransmitters themselves, as do fatty acids and glutamate.

Nutrients clearly play a role in neurochemistry and neurotransmission, but the actual relationship between specific nutrients and behavioral outcomes is indirect. Although dietary manipulation may affect neurochemical and neurophysiologic function, behavioral changes will not necessarily follow. At present, a clear answer to the question of whether a deficiency of specific nutrients during early neonatal life influences behavioral development remains elusive, not only because few studies have addressed this question, but also because of the complexity of the question itself.

Neurotransmitters constitute highly complex and interactive systems. In this respect, it is important to consider that the relationship between precursors and product is not necessarily linear and may vary for different neurotransmitters. For example, acetylcholine synthesis demonstrates end-product inhibition by acetylcholine itself. Thus, an increase in plasma choline (e.g., through the diet) is not a sufficient condition for increased synthesis and release of acetylcholine. It appears rather that increased plasma choline concentrations will only amplify cholinergic function if cholinergic neurons are activated concurrently with the choline treatment.⁹⁰ Norepinephrine may be affected similarly; Lehnert et al.³⁰ suggested that increased availability of tyrosine influenced norepinephrine release only when catecholaminergic neurons were active. Serotonin synthesis, however, is not inhibited by its end product, and increased brain tryptophan concentrations will result in elevated serotonin concentrations regardless of activity of serotonergic neurons.²⁰

There may be considerable redundancy in some systems that allow the brain to maintain normal function despite considerable biochemical variation. Environmental factors will also influence behavioral outcomes. For example, it has been shown that environmental enrichment during the postweaning period eliminated spatial and learning memory deficits that were caused by neonatal dopamine depletion.³³ Furthermore, neurotransmitter systems are highly interactive and appear in the developing brain to demonstrate considerable plasticity, so that an insult to one system might be compensated for by changes in another. In this regard, the timing of the nutritional deprivation in relation to the schedule of brain development is another important consideration. It has been postulated that the brain will be most sensitive to insults during the

brain growth spurt—the so-called sensitive period. It is possible, however, that increased plasticity during this time will allow compensatory changes that offset the deleterious effects.

When studying the influence of malnutrition on brain development, it is often difficult to disentangle the effects of specific nutrients. For example, both zinc and copper deficiency have been shown to alter fatty acid metabolism; deficiencies resulted in lower concentrations of long-chain ω -3 and ω -6 fatty acids in the brain.^{91,92} Thus, the behavioral deficits observed with zinc deficiency may well have been caused by alterations in fatty acid metabolism. Such interactions should be taken into consideration in the study of the influence of specific nutrients on brain development and function. It is therefore important to measure a variety of biochemical changes in relation to behavioral measurements.

Another issue complicating the interpretation of developmental nutritional studies is that if the functional changes are subtle, current behavioral test batteries may fail to show long-term effects in animal and human studies owing to their lack of sensitivity and/or specificity. One of the most difficult challenges in behavioral neuroscience is that of translating changes in brain structure, neurotransmitter concentrations, or receptor activity into measurable behavioral outcomes. Measures of cognitive functions (learning, memory, intelligence) may be confounded by alterations in noncognitive functions (alertness, activity, arousal, responsiveness). For example, poor spatial memory as measured by the Morris water maze could be related to inability to see the spatial cues because of poor vision or inability to locate the hidden platform because of poor motor skills, rather than deficits in cognitive development. It is very important that study designs control for these potential confounders.

Furthermore, in animal studies that utilize multiparous species, an understanding of the appropriate methodology for developmental nutritional research is crucial. Many such studies have changed dietary intakes of offspring by manipulating the maternal diet. In this case, the unit that is randomly assigned to the treatment is the litter, not the individual pup, and this should be considered in the design of the study and statistical analysis of the data.¹⁷ In addition, changes in maternal physiology and/or behavior caused by the nutritional manipulation may affect the offspring indirectly, and thereby act as a potential intervening variable for nutritional effects. Newer animal models, such as the artificial rearing model, may be more appropriate to answer the questions related to the effects of early neonatal diets on brain and behavioral development. Furthermore, it may be useful for the study design to incorporate a positive control, particularly when nutritional intervention shows no effect. This control, which is commonly used in toxicologic studies, uses a group treated

in such a way that an effect on the dependent measure of interest is assured, and thereby supports its sensitivity with respect to the experimental manipulation. Other limitations of animal models must also be recognized. Because the behavioral repertoire of humans is more complex than that of animals, the use of animal models may underestimate the effects of nutrient deficiencies. Conversely, because rats are less mature at birth and place a greater burden on the dams both pre- and postnatally,⁷⁰ effects of nutrient deficiencies may be overestimated. Furthermore, because animals and humans differ in their rates of neuronal and biochemical development, it is important to ensure that a particular experimental procedure is appropriate for the species being studied.

The influence of early nutrition on cognitive development is an area of growing interest, particularly with respect to preterm infants. The possibility that nutrient deficiencies early in life have adverse effects on behavioral development must be accorded a high research priority, particularly if, as has been suggested by some studies, such adverse outcomes are irreversible despite nutritional recovery.

Acknowledgment. Dr. Wauben is supported by a Postdoctoral Fellowship Award from the National Institute of Nutrition, Canada.

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