Behavioral methods used in the study of long-chain polyunsaturated fatty acid nutrition in primate infants

Susan E Carlson

ABSTRACT  Domains of behavior may be broadly categorized as sensory, motor, motivational and arousal, cognitive, and social. Differences in these domains occur because of changes in brain structure and function. Docosahexaenoic acid (DHA; 22:6–23) and arachidonic acid (AA; 20:4–26) are major structural components of the brain that decrease when diets deficient in the essential fatty acids (EFA) α-linolenic acid and linoleic acid are consumed. Early electrophysiologic and behavioral studies in EFA-deficient rodents showed behavioral effects attributable to lower-than-normal accumulation of DHA and AA in the brain. More recently, electrophysiologic and behavioral studies in EFA-deficient primate infants and analogous studies in human infants have been conducted. The human infants were fed formulas that could result in lower-than-optimal accumulation of long-chain polyunsaturated fatty acids (LCPUFAs) in the brain during critical periods of development. This article describes the behavioral methods that have been used to study primate infants. These methods may be unfamiliar to many physicians and nutritionists who wish to read and interpret the human studies. The behavioral outcomes that have been evaluated in LCPUFA studies represent only a fraction of those available in the behavioral sciences. Specific developmental domains have been studied less often than global development, even though studies of nonhuman primates deficient in EFAs suggest that the former provide more information that could help target the underlying mechanisms of action of LCPUFAs in the brain. Am J Clin Nutr 2000;71(suppl):268S–74S.

KEY WORDS  Infant behavior, sensory function, essential fatty acids, docosahexaenoic acid, arachidonic acid, attention, cognition, neural function, essential fatty acid deficiency, long-chain polyunsaturated fatty acids, LCPUFAs

INTRODUCTION  The central nervous system is highly enriched with long-chain polyunsaturated fatty acids (LCPUFAs), especially arachidonic acid (AA; 20:4n–6) and docosahexaenoic acid (DHA; 22:6n–3). Early research was based on the premise that because these fatty acids were present in the central nervous system, they likely had a role or roles in neural function. Caldwell and Churchill (1) first showed that diets deficient in n–3 and n–6 essential fatty acids (EFAs) influenced animal behavior. Others pioneered the study of the particular effects of diets deficient in n–3 fatty acids compared with n–6 fatty acids, specifically the effects on retinal and brain composition (2–4), behavior (5), and retinal physiology (6). For a more detailed evaluation of the literature related to LCPUFA accumulation and animal behavior, refer to the review by Wainwright (7). The present review of methods will focus only on those that have been used to study primate behavior in relation to LCPUFA status and have informed the human studies of LCPUFAs and behavior.

It has been suggested that, in addition to dietary deficiency of linoleic and α-linolenic acids, some physiologic conditions might lead to less-than-optimal AA and DHA accumulation despite the inclusion of EFAs in the diet. Examples of such situations are preterm birth and consumption of diets lacking AA and DHA in infancy. Most human studies were based on the theory that if DHA concentrations in infant brains were low, at least during some critical period of development, behaviors analogous to those of nonhuman primates deficient in EFAs would be influenced by this deficiency. Consequently, many studies designed to determine whether dietary DHA enhances function have used electrophysiologic and behavioral assessments analogous to those influenced by n–3 fatty acid deficiency in nonhuman primates. If experimental evidence were found that dietary DHA enhanced function, DHA would be considered a conditionally essential nutrient.

The domains of behavior can be broadly categorized as sensory, motor, related to motivation and arousal, cognitive (including information processing), and social (8). Because of the high concentrations of n–3 and n–6 LCPUFAs in neural tissue, all domains of neural and psychologic function are potentially influenced by LCPUFA status. In Table 1, some examples of behavioral tests developed to study these domains in primates are shown. Despite the many functional outcomes that could be studied, vision has been studied the most in relation to n–3 LCPUFA status in human infants. Methods for assessing visual function are discussed in this supplement by Neuringer (9). Only a few studies

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have measured attention, ability to learn, stereotyped behavior, or motor function in relation to LCPUFA status; these methods will be presented here with reference to the infant studies that have used them.

ATTENTION TO SENSORY STIMULI

During the first year of life, infants readily attend to environmental stimuli regardless of the sensory function required. Infant attention has been used extensively to infer development, and specific kinds of infant attention have been used to infer information processing and memory. Whereas any sensory function could be measured to study attention, most of the techniques available for assessing early processing and memory in primate infants measure visual attention.

Between 1 and 3 mo of age, human infants look at visual stimuli that are above their visual threshold (obligatory attention) and disengage from these same stimuli with difficulty (10). Older infants and children have obligatory attention as well, but their attention may be difficult to measure because they attend and disengage more rapidly than do younger infants. By \( \approx 3 \) mo of age, dendritic growth and myelination within the upper layers of the primary visual cortex and the frontal eye fields permit more complex patterns of looking at a visual stimulus (10).

Infants cannot look at a stimulus on command or provide verbal feedback about what they see, but the amount of visual attention they give to stimuli can provide important clues about how they process and remember information. Unlike visual grating acuity, a measure of sensory function, visual attention to stimuli can be used to infer information processing and involves the visual cortex as well as the parietal cortex, frontal eye fields, and dorsolateral prefrontal cortex.

If one is to infer that visual attention reflects information processing or an aspect of neurodevelopment other than sensory function, all aspects of visual function must be normal. In practice, this can be assessed by measuring attention to stimuli above the infant’s visual acuity threshold. The Teller Acuity Card procedure (11–13) is a simple test for measuring visual grating acuity that has been used in many infant LCPUFA studies. These studies have used grating acuity to measure sensory function (9) and ensure that sensory function is sufficient for performance on tests of visual attention. Visual grating acuity and attention have been measured both at simultaneous and sequential ages in several studies and these data have been used to evaluate whether visual attention is related to sensory function.

Habituation

Primate infants presented with any sensory stimulus will gradually habituate (attend or respond less) to the stimulus. Tests that measure habituation to a visual stimulus involve 2 components: 1) presentation of the stimulus for a long enough time that the infant reaches some criterion of decreased response, and 2) presentation of a post-habituation stimulus to ensure that the decrease in interest is due to true habituation rather than receptor fatigue or a change in state, such as sleepiness or hunger. The decrease in attention is observed as a reduction in look duration after the longest peak look or fixation during look to the stimulus. It is assumed that during the longest look, the infant is encoding the stimulus and the subsequent decrease in look duration implies that he has some memory of the stimulus. Infants whose peak fixation occurs within the first few looks in habituation trials (early peakers) are believed to have better attention control than do infants whose longest look occurs later (late peakers) (14, 15).

Habituation models can have a variety of different permutations. According to Olson and Sherman (16), “given the basic logic of the phenomena . . . any number of hybrids are possible if they should better suit the purposes of an experimental design.” Aspects of the procedure that have been varied include: 1) the type of stimulus (abstract patterns, letters, or, most often, faces), 2) the way the stimulus is presented (continuously or intermittently), 3) the look (criterion) considered evidence that the infant has habituated (frequently the first or second look that is less than half the duration of the longest look), 4) the presentation of 1 copy compared with 2 copies of the same stimulus (infants may habituate more rapidly with 2 stimuli instead of 1), and 5) use of a fixed interval of exposure to the stimulus (the infant may look all or only part of the time) compared with an infant-controlled procedure in which the stimulus is displayed only when the infant looks toward the location of the stimulus (in these paradigms, the infant may be required to look for a fixed amount of time or may be tested to a defined criterion).

### Table 1

<table>
<thead>
<tr>
<th>Functional outcomes</th>
<th>Nonhuman primates</th>
<th>Humans</th>
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<tbody>
<tr>
<td>Sensory</td>
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*Adapted and updated from references 7 and 8. MDI, Mental Developmental Index; IO, intelligence quotient; PDI, Psychomotor Developmental Index.*
look and interlook durations considered to be meaningful, and 7) the number of stimuli habituated (one or more).

Most studies of habituation are performed with 3–4-mo-old infants. Much younger infants may not attend and much older infants lose interest quickly. In one type of habituation trial that is fairly easy to do, the infant is placed in an infant seat facing a screen onto which the stimulus is projected. A video camera is placed well below the projected image on the screen and directly in front of the infant and the infant is taped while looking at the stimulus. Another camera simultaneously records the presence of the stimulus in front of the infant. These images (the infant and the stimulus) may be viewed as split-screen images on the tape. The procedure requires 2 individuals working in tandem during the test. One, often the mother, attends to the baby’s needs and another records his or her looking behavior. The videotape of the test session provides a permanent record that can be reanalyzed by the same or another investigator to ensure that interrater and intrarater reliabilities are acceptable.

A single video camera can be used to record the infant’s behavior, but without a second camera to simultaneously record the stimulus present during the same time frame, the test cannot be recoded later. It is also possible to measure visual attention without a videotaped record. This procedure is less expensive to set up, but it does not allow for the data to be analyzed again by the same or a different person. Because infants have been shown to remember visual stimuli for some time, it is not an option to retest infants when a problem occurs during administration of the test.

Generally, investigators are interested in determining 1) the duration of the longest look, 2) whether it occurs early or late in the habituation, 3) the number of looks or amount of looking time required to reach a defined criterion of habituation, and 4) the average duration of looks. Shorter look duration has been interpreted as evidence of more efficient information processing (17), enhanced ability to disengage from one stimulus and attend to another (18), or decreased reactivity (19, 20). When an infant’s longest look is after one or more shorter looks (late peak), the decision about which looks to average is open to interpretation. One may assume that the infant has been encoding information during the shorter, prepeak looks, in which case these looks should be included in the average. Alternatively, one may assume that the brief looks before habituation provided little or no information to the infant as suggested by Cohen and Gelber (15), in which case it would be better to average the duration of looks beginning with the longest look.

**Paired comparisons**

When a novel stimulus is presented after habituation to a previous stimulus, the average look duration increases compared with the look duration to the habituated stimulus. This increase in visual attention to an unfamiliar stimulus is reliable and is the basis for another model of visual attention, visual paired comparisons. Paired-comparison trials begin with familiarization, though not necessarily habituation, to one stimulus followed by a paired comparison of that familiar stimulus with a novel stimulus. During the paired-comparison test, the infant who retains a memory of the original stimulus will spend more time looking at the new stimulus. The total time spent looking at the novel stimulus is expressed as a percentage of the time that the infant looked at both stimuli. If the percentage of time spent looking at the novel stimulus exceeds chance (≥57% of the total time), an infant is said to have a novelty preference on that task. A consistent novelty preference during a series of paired comparisons indicates that the infant has visual recognition memory of the familiar stimuli.

The Fagan Test of Infant Intelligence (FTII; 21) is a standardized, paired-comparison test that has been used in studies of LCPUFA status. This test was developed to identify infants at risk for cognitive deficits (22). In the FTII, infants who do not spend significantly more time looking at the novel stimulus are considered at high risk for cognitive deficits because they apparently do not have recognition memory of the familiar stimulus after a fixed viewing interval. The interval provided for familiarization to the initial stimulus is long enough for unimpaired infants to remember the stimulus. Novelty preference with the FTII has moderate predictive validity for test scores in childhood, especially for tests designed to assess memory (23, 24).

For the FTII, the infant is seated on his or her mother’s lap in front of a stage and is presented with pictures of faces. The complete test uses a series of 10 familiarizations, each of which is followed by a paired-comparison trial in which the novel stimulus is presented first on one side and then on the other. In most cases, infants are presented with 2 identical pictures during familiarization. After the stimuli are presented to the infant, an investigator observing the infant through a peephole records the direction (right or left) and duration of each look. During each familiarization, the infant is required to look at the pictures for a defined amount of time. The test cannot be advanced to a paired-comparison trial until the requisite looking time has accumulated. The FTII was designed to be administered at 67, 69, 79, and 92 wk postmenstrual age (6, 6.5, 9, and 12 mo postterm). The required interval for familiarization decreases with increasing age, as does the time allowed for each paired-comparison test.

After the familiarization criterion is reached, the stimuli are removed from the infant’s visual field and one familiar stimulus is shown again, this time with a novel stimulus. Visual attention to the stimuli is recorded by keystroke for looks left or right. The results of all 10 paired comparisons are averaged to obtain the percentage of time spent looking at the novel stimulus. During the paired-comparison test, infants typically look at both the novel stimulus and the familiar stimulus, frequently by shifting attention directly from one to the other; however, looks at the latter are typically shorter.

As in tests of habituation, there are many possible variations of paired-comparison paradigms. The final novelty preference percentage will depend on the method. Only the FTII has been standardized to result in a significant novelty preference for infants at low risk for cognitive problems at all test ages. Furthermore, the test has been normalized so that the novelty preference across the full age range (6–12 mo) is similar. This was accomplished by making the test somewhat more difficult with each progressive age by decreasing the time allowed for familiarization. Without this reduction in the required familiarization time, all other things being equal, the novelty preference percentage would increase with age.

Another way to make a paired-comparison test more difficult is to require the infant to recognize as familiar a stimulus that has been presented to another sensory organ during the familiarization phase of the trial. Paired comparisons involving 2 senses are called cross-modal, because the infant must be able to recognize the familiar stimulus experienced previously by another sensory mode. For example, a toy that has been manipulated but not seen might be shown to the infant as the familiar stimulus, paired with
a novel toy. Some paired-comparison models have shown that term infants have better visual recognition memory than preterm infants (23, 25).

INFLUENCE OF LCPUFA STATUS ON HABITUATION AND PAIRED COMPARISONS

In addition to providing information about visual recognition memory (by measuring novelty preference), the paired-comparison test can also be used to measure look duration. In human infants, shorter look duration has been related to the ability to disengage attention (18) and to superior performance on tests designed to measure speed of processing in infancy (26) and childhood (27, 28). Primate infants with higher n−3 LCPUFA status were found to have shorter look duration during both habituation and paired-comparison trials (19, 29–31). Together, the evidence from studies of infant behavior and from LCPUFA trials involving behavior suggests that LCPUFA status affects concurrent as well as later function. Considering the number of options for measuring developmental function in infants and the few that have been used, it is remarkable that 3 independent groups measured duration of visual attention. Moreover, each observed shorter look duration with higher n−3 LCPUFA status. This general observation is strengthened further by the fact that the studies used different methods (habituation or paired comparisons), different species (human and monkey), and infants born at different gestational ages (term and preterm).

Forsyth et al (29) used a conventional habituation trial to measure the effects of supplementation with n−6 and n−3 LCPUFAs in 3-mo-old human infants born at term. The criterion look was defined as the first look that was half the duration of the longest, or peak, look. Although there were no overall differences in habituation, peak look duration, or average look duration between the diet groups, the investigators noted that term infants who had early peak looks (early peakers) were significantly larger at birth than those who had later peak looks (late peakers). Half of the infants in each diet group were early peakers and half were late peakers. This permitted an exploratory analysis of variance by birth weight and occurrence of the peak look. The results of that analysis suggested that dietary LCPUFA supplementation, including both AA and DHA, reduced look duration but only in the subgroup of later peakers. As mentioned previously, a late peak look during habituation has been regarded as evidence of poor attentional control. These investigators concluded that term infants with poorer intrauterine growth could have more efficient information processing if supplemented with LCPUFAs (29).

Because the supplemented diet contained both n−3 and n−6 LCPUFAs in the study by Forsyth et al (29), it was not possible to determine whether the effect on look duration was due to higher status of n−3, n−6, or both fatty acid types. However, higher n−3 LCPUFA status has been related to shorter look duration during paired-comparison tests in several studies of monkey and human infants who were not fed n−6 LCPUFAs (19, 30, 31). Reisbick et al (19) studied infant rhesus monkeys deficient in α-linolenic acid. The deficiency of n−3 fatty acids was initiated in utero by feeding deficient diets to the mothers and it continued after birth. The control infants were born to mothers who were fed a standard diet during pregnancy; after birth the infants were fed formulas containing α-linolenic acid.

Monkey infants were tested at 2, 5, 9, and 13 wk of age. At each age they were shown a new set of stimuli, each with 6 pairs of patterns and 6 pairs of primate faces. During every familiarization phase, infants were required to look at the stimulus for 30 s before proceeding to the paired-comparison test. During the test, the familiar stimulus was presented simultaneously with a new one and the infant’s attention to the novel and familiar stimuli was monitored for 10 s on each side beginning with the infant’s first look (19). During the paired comparison, the total looks and the duration of looks to the novel and familiar stimuli were recorded as was the total time spent fixating the novel and familiar stimuli and the percentage of time spent looking at the novel stimulus. The main effect of higher n−3 LCPUFA status was a decrease in look duration during the paired-comparison tests. This effect on look duration was consistent across test sets and was found for both novel and familiar stimuli. However, n−3 deficiency did not appear to have any effect on visual recognition memory (19).

In supplementation studies analogous to the deficiency studies of Reisbick et al (19), preterm human infants were fed commercially available infant formulas containing α-linolenic acid or the same formulas supplemented with the n−3 LCPUFA (30, 31). In each of these randomized studies, the experimental group received 0.2% of total fatty acids from DHA (~0.1% of energy). Both novelty preference and look duration were measured with the FTII at 6.5, 9, and 12 mo past term. As in the study by Reisbick et al (19), higher n−3 LCPUFA status was associated with shorter look duration at each study age. The effect of higher n−3 LCPUFA status on look duration reached significance only during the paired-comparison trials, but a trend toward shorter look duration during familiarization was observed (P < 0.12). In agreement with the findings of Reisbick et al (19), visual recognition memory (novelty preference) was unaffected by dietary LCPUFAs or LCPUFA status. All groups in both studies had a significant preference for the novel stimuli (in this case, faces), regardless of diet or study age (30, 31). Thus, none of these studies suggest that n−3 LCPUFAs affect recognition memory, although they do suggest that lack of LCPUFAs has effects on attention that could be interpreted as slower information processing (17), higher reactivity (32, 33), or an inability to appropriately disengage from stimuli (18).

IMITATION AND ELICITED PLAY

The ability to reproduce an observed behavior is called imitation. Olson and Sherman (16) suggested that imitation is a potential tool for assessment of infant memory, but it has been used only infrequently for this purpose. Studies of imitation have not been done with infants who differ in LCPUFA status, although infants randomly assigned to diets with and without LCPUFA supplementation were studied with a global scale of development, the 12-mo Bayley Scales of Infant Development, which includes tasks that require imitation. It appears that more specific tests of imitation in older children would be worthwhile.

PROBLEM SOLVING AND DELAYED-RESPONSE TASKS

Problem solving and delayed-response tasks are tools for the assessment of infant memory and response that are appropriate for older infants. Willatts et al (34) reported on their use of an Infant Planning Test that involved an infant’s ability to solve a 2-step,
means-end problem. The infant was shown a toy for a defined amount of time. The toy was placed on a cloth out of the infant’s reach and was then covered. The object of the task was for the infant to retrieve the toy, which required that the cloth be pulled within reach and the toy uncovered. The investigators reported finding a relation between solving the toy problem at 8 and 9 mo and performance on tests of vocabulary (British Picture Vocabulary Test) and intelligence (British Ability Scales) at 3.25 y. Recently, this group of investigators tested 10-mo-old term infants fed regular formula or a formula supplemented with n–3 and n–6 LCPUFAs until 4 mo of age. They found that infants fed the supplemented formula early in infancy scored significantly better on the Infant Planning Test at age 10 mo compared with infants fed regular formula (34).

The A-not-B task is a modification of Jean Piaget’s object permanence task. A toy is hidden on the left or right side until the infant retrieves it twice. Then it is hidden on the opposite side. After a delay of 3–15 s, the infant is allowed to search for the toy. The object of the test is to ascertain the longest delay through which the infant can remember and reach to the correct location. Longer intervals result in the A-not-B error; ie, the infant continues to search for the object on the side where the toy was previously found. Another test useful for older infants is object retrieval. The infant can see a toy through a transparent Plexiglas box and the object of the task is to determine whether he or she can reach the toy by reaching around and through the one opening as opposed to attempting to reach directly through the plastic side. Performance on both of these tasks (A-not-B and object retrieval) is lower in infants with lesions in a particular region of the frontal cortex. The A-not-B task was suggested as a simple test that could be useful for studies of LCPUFA status (35). In subsequent studies, monkeys deficient in n–3 fatty acids had normal performance for both object retrieval and short-term or working memory (A-not-B task) in infancy and adulthood, which is evidence that severe depletion of neural DHA does not affect learning or memory (33).

ACTIVITY AND IMPULSIVITY

Reisbick et al (32, 33) have observed higher activity levels and the occurrence of more bouts of stereotyped behavior in monkeys deficient in n–3 fatty acids. Citing the observations of Delion et al (36), who showed lower dopamine concentrations and dopamine-receptor binding in the prefrontal cortex of rats deficient in n–3 fatty acids, Reisbick et al (33) suggested that stimulated behavior may be enhanced in n–3 fatty acid deficiency. Higher reactivity could also explain the increased number of aggressive and predatory behaviors in LCPUFA-deficient monkeys (33). This finding has subsequently been confirmed in nonhuman primates (37). The A-not-B test also appears to be an effective measure of LCPUFA status in monkeys (38).

LCPUFAs AND LANGUAGE ACQUISITION

Language development was measured in infants fed diets varying in LCPUFA content; they were tested with the MacArthur Communicative Development Inventory, but these data have appeared only in abstracts (37, 38). The test is a parental-report measure of early language comprehension and production for the study of infants and toddlers. When tested at 14 mo, term infants fed formula supplemented with n–3 LCPUFAs for the first 12 mo of life had lower scores on vocabulary production than did breast-fed infants (37). Lower scores were not observed in infants fed formula containing both n–6 and n–3 LCPUFAs, adding to the evidence that a proper balance between n–3 and n–6 LCPUFAs is needed in infancy (37). At 3 y of age, all diet groups had similar language scores (38).

SENSORY-EVOKED POTENTIALS

Regional and sensory-evoked neuronal activity can be determined with electrophysiologic measures such as sensory-evoked potentials. Except for visual-evoked potentials, addressed by Neuringer (9), the influence of LCPUFA status on electrophysiology has not been studied in infants. These techniques have potential usefulness, as do several behavioral tests, for studying the effects of LCPUFAs on neural function.

TESTS OF GLOBAL NEURODEVELOPMENT

The Bayley Scales of Infant Development and the Brunet-Lezine Test

Two tests of global neurodevelopment have been used in studies of LCPUFA status: the Bayley Scales of Infant Development (39), which was originally developed in the United States, and the Italian version of the Brunet-Lezine Test, which was originally developed in France and is commonly used in Western European countries where Romance languages are spoken (40). The Bayley Scales of Infant Development and Brunet-Lezine Test both have some elements that evolved from the Gesell Developmental Schedules, which first systematically assessed the behavioral development of infants and preschool children (41). The Gesell Developmental Schedules, Bayley Scales of Infant Development, and Brunet-Lezine Test are all standardized procedures that provide general indexes of mental and motor age relative to group norms. Like all global-development tests designed for administration in infancy, their primary use is to assess current ability rather than to predict long-term outcomes.

The Bayley Scales of Infant Development provides several tools for the assessment of development in children aged 1 mo to 2.5 y. Two of these tools are the Bayley Mental Developmental Index and the Bayley Psychomotor Developmental Index. For 12-mo-old infants, the mental scale is designed to sample perception, memory, learning, problem solving, vocalization, early oral communication, and abstract thinking, whereas the motor scale primarily measures gross visual-motor abilities and hand and finger manipulation. At later ages, there is a much greater emphasis on language in the Bayley Mental Developmental Index and fine motor development in the Bayley Psychomotor Developmental Index.

Normative data for the Bayley scales were based on 1262 children ranging in age from 2 to 30 mo who were chosen to be representative of US children; premature infants and institutionalized children were excluded from the sample. In the first edition, published in 1969, each scale was normalized to a standard score with a mean of 100 and a standard deviation of 15 (39). Mean scores in many populations have increased since that edition. For this and other reasons, the decision was made to renormalize the Bayley scales, and the second edition became available in 1993 (42). Among other changes, it extends the targeted age range to 1–42 mo. The format, rationale, and many of the items on the tests did not change. However, as investigators switch to the renormalized Bayley scales, mean scores for various populations will be lower. It is important to note which edition was used and to understand that data obtained with the 2 editions cannot be compared directly.
At 4 mo of age, the Brunet-Lezine Test provides a global measure of neurodevelopment. At later ages, the test evaluates four developmental areas: fine motor skills, social skills, language skills, and posture and gross motor adaptation.

The Bayley Scales of Infant Development and the Brunet-Lezine Test are the most widely used tools for studying infant development and are useful for comparing the maturity of infants within and between cultures. Despite this, they provide fewer clues about specific domains of development affected by n–6 and n–3 LCPUFAs than do the tests mentioned earlier in this article. For the Bayley scales and Brunet-Lezine Test, completion of the tasks requires competence in several developmental domains, eg, sensory, motor, motivation- and arousal-related, cognitive, and social. In the case of the Bayley scales, several large studies have attempted to circumvent this limitation by reducing the data for logically and procedurally interdependent items and determining the principal components of these items by various procedures (43).

**LCPUFAs and the results of the Brunet-Lezine Test**

Agostoni et al (44, 45) compared the psychomotor development of term infants randomly assigned to receive formulas with or without AA and DHA (0.44% and 0.3% of total formula fatty acids, respectively). The Brunet-Lezine psychomotor development test was administered at 4, 12, and 24 mo of age. The infants supplemented with n–3 and n–6 LCPUFAs and those in a reference group fed breast milk scored significantly higher than did infants who received the standard formula without AA or DHA. The higher scores were found at age 4 mo, an age when the test evaluates postural, motor, and early social performance. At 12 mo of age, however, no effect of LCPUFA supplementation was found with the Brunet-Lezine Test. At 24 mo of age, 81 of the original 90 infants were tested, again without evidence of an effect of early dietary LCPUFAs on performance. A post hoc analysis indicated that among the 20 infants with concurrent measures of development and red blood cell phosphatidylcholine fatty acids at 24 mo, AA and DHA concentrations in the latter explained 52% of the interindividual variance in performance (45). That observation suggested that LCPUFA status was related to neural development, but that factors other than early diet accounted for LCPUFA status at 24 mo of age.

**LCPUFAs and the results of the Bayley Scales of Infant Development**

The Bayley Mental Development Index was used in several studies that investigated the relation between LCPUFA supplementation and development of term and preterm infants. At this time, none of these reports have been published as peer-reviewed articles and the published abstracts from these studies did not report data with sufficient statistical power to evaluate global development. Even with adequate statistical power, the results of global tests would not be as easy to interpret as results of tests that evaluate specific domains of development. If the null hypothesis was accepted, was this because affected and unaffected domains were grouped together, thereby obscuring the actual effects? If the null hypothesis was rejected, was this because effects on specific domains led to secondary effects on global development?

**CONCLUSIONS**

This article focused on methods used to measure attention and global neurodevelopment in human and nonhuman primates with known or presumed differences in neural LCPUFA accumulation. The functional tests that have been used represent only a few of many possibilities. Ideally, these tests should be complemented with other worthwhile measures of outcome that have not been included in this article. It is useful to keep in mind that if LCPUFAs have a role in membrane structure or signaling, any number of the neural outcomes shown in Table 1 could be influenced by variations in LCPUFA status. From the limited number of studies that have been reported, the most consistent finding was that n–3 LCPUFA status affects look duration. Therefore, n–3 LCPUFAs appear to be important for information processing, the ability to disengage attention, or reactivity. Individual reports also suggested that LCPUFAs affect global performance and motor activity at different stages of development. In several studies, it was not possible to attribute these effects to n–6 LCPUFAs or n–3 LCPUFAs specifically, because both were provided in the diet simultaneously. The evidence suggesting that n–3 and n–6 LCPUFAs have different and specific roles in neural function and behavior provides another argument for considering many measures, from motor development to language acquisition, in future studies.

Most studies of how LCPUFAs affect perceptual and cognitive function have been limited to infancy, and the significance of findings from infancy with regard to later performance of school-age children has not been determined. Selecting the best methods for assessing later development is difficult, because it is not clear how a behavior in infancy (such as attention, which has been influenced by LCPUFA status) relates to behavior at school age. There is no evidence that behavioral outcomes typically measured at school age tap the same developmental domains as those that have been affected by LCPUFAs in primate infants. Even if the relations between methods for assessing a specific domain of development in infants and children were entirely clear, environmental variables intervening between infancy and school age could hide the extent of the relation. At best, only a modest correlation between scores on tests of development in infancy and in childhood can be expected.

**REFERENCES**


