Invited commentary

Nutrition and behaviour: the role of *n*-3 fatty acids in cognitive function

The lipid content of retina and grey matter in the mammalian brain is high in both arachidonic acid (AA, 20:4 *n*-6) and docosahexaenoic acid (DHA, 22:6 *n*-3). These long-chain polyunsaturated fatty acids are derived from their respective dietary essential fatty acid precursors, linoleic acid (LA, 18:2 *n*-6) and α-linolenic acid (LNA, 18:3 *n*-3), through a series of desaturations and subsequent chain elongations. In addition to their role as integral structural components of cell membranes twenty C fatty acids such as AA make an important contribution to regulatory function by serving as precursors for the eicosanoids, including prostaglandins (for review see Wainwright, 1997). AA and DHA accrue rapidly in the human brain during the third trimester and the early postnatal period, when the rate of brain growth is maximal and therefore vulnerable to the effects of nutritional deficiencies. There is controversy at present over whether the infant formulas that contain only LA and LNA are sufficient for optimum brain development, or whether additional pre-formed AA and DHA, as found in human milk, are also necessary. A specific dietary deficiency of *n*-3 fatty acids during development results in characteristic changes in brain fatty acid composition that include a decrease in DHA and a reciprocal increase in 22:5 *n*-6. Whether 22:5 *n*-6 serves as a functional substitute for DHA, or whether there are specific functions attributable to DHA is a question that remains unresolved. However, based mainly on studies that show that *n*-3 deficiency is associated with changes in the electroretinogram, as well as in some aspects of visual function in various species, including human infants (for review see Carlson & Neuringer, 1999), it has been suggested that DHA plays a unique role in the function of excitable membranes.

There are several published studies in which human infant subjects have been randomly assigned to be fed on formulas supplemented with DHA or with both DHA and AA, and assessed on cognitively-related measures, including visual recognition memory as assessed by the Fagan Infantest. Although there were no effects on visual recognition, preterm infants fed on DHA-supplemented diets showed shorter look durations (Carlson & Werkman, 1996; Werkman & Carlson, 1996). Interestingly, this effect has also been reported in rhesus monkeys, and it has been suggested that the longer look durations associated with lower DHA status may be due to an inability to shift attention from a visual stimulus (Reisbick *et al*. 1997). A recent study has shown improved problem solving in 10-month-old term infants fed on diets supplemented with AA and DHA compared with those fed on a control formula that was very low in the terms of *n*-3 fatty acid content (Willatts *et al*. 1998). In contrast, lower language scores have been reported in 14-month-old term infants fed on formulas supplemented with DHA (Scott *et al*. 1998). However, these effects appear to be transient, and the predictive validity of early language with respect to later cognitive function is controversial (Carlson & Neuringer, 1999). Also noteworthy with respect to the functional effects of DHA are reports of case studies where DHA supplementation has been shown to have a beneficial effect in patients with Zellwegger's syndrome, which is a peroxisomal disorder associated with severe retardation (Martinez, 1996).

Chronic dietary deficiency of LNA in animals has been associated not only with changes in retinal and visual function, but also with alterations in performance on various tests of learning and memory (for review see Reisbick & Neuringer, 1997; Wainwright, 1997; Carlson & Neuringer, 1999). Although an emphasis on learning is understandable in terms of a desire to identify nutritional factors that may have an impact on the development of human intelligence, there are various methodological reasons to be cautious in this regard. First, these findings are not consistent across laboratories. Second, some studies misinterpret main effects of diet on performance of such tasks. Specifically, learning is, by definition, change over time, and dietary-induced differences in learning ability can be inferred from different rates of change, i.e. diet$\times$time interactions, not main effects. Third, it would seem that implicit in much of this work has been the assumption that learning and memory are unitary phenomena in terms of brain function. However, there is evidence for the involvement of different neural systems in different types of memory, and experimental manipulations that impair performance on one type of learning task may actually lead to an enhancement on another (Everitt & Robbins, 1997). Thus, it is important to consider the functional domain that is being tapped by a particular task, and to assess the outcome over a variety of tasks before drawing the conclusion that overall behavioural adaptation is better or worse. Furthermore, it is important to realise that performance on cognitive measures (learning, memory) may be confounded by alterations in non-cognitive functions (emotionality, arousal) or by inadequate sensory and motor skills. This may not be viewed as a problem if one subscribes to the argument that, since it is performance that ultimately counts, to be able to demonstrate an effect on performance is sufficient. However, if one's objective is to elucidate mechanisms, it then becomes essential to include the control groups necessary to the identification of these potential confounds. For example, as discussed in the paper by Carrié *et al*. (2000) in the current issue of this journal, behavioural differences in the first session of testing on some behavioural tasks, such as those requiring an active avoidance response, are more likely to be indicative of the effects of factors such as

differences in arousal than differences in cognition. In addition, the findings of this paper illustrate the importance of considering not only multiple outcomes, but also multiple time points over the lifespan and in so doing, they support the contribution to be made by animal models to the resolution of such issues.

In general, there are two possible strategies in studying nutritional effects on behaviour. One approach is to use a 'top-down' approach, similar to that used in neurotoxicity testing, where batteries of tests are used to assess performance across a wide range of behavioural outcomes. In addition to tests of sensory capacity and emotional reactivity, these batteries include various measures of learning and memory, and may often also incorporate species-typical behavioural adaptations. Because the original intent of such test batteries was that they be used as screening tools, they are usually not hypothesis-driven with respect to specific mechanisms. This, together with the number of tests they encompass, does raise the spectre of false positive findings. Nonetheless, their overall pattern of findings may generate further hypotheses by directing attention to the possible neural systems involved.

The second approach is described as 'bottom-up', being based on the assumption that dietary-induced changes at a molecular or cellular level will necessarily have functional consequences. The challenge then becomes that of identifying the appropriate functional outcome to measure. Again, in the interests of hypothesis-driven enquiry, the best approach would be to measure an outcome that is known to be associated with the biochemical changes one finds. For example, there is evidence associating a dietary deficiency of *n*-3 fatty acid with changes in cortical dopaminergic function (Delion *et al*. 1994). The literature on the behavioural effects of dopamine suggests that measures of spatial learning ability that address working memory, i.e. memory for the specific details of a particular session (as opposed to reference memory, which refers to learning the rules associated with a task) might prove particularly informative (Murphy *et al*. 1996). Based on this, we recently conducted a study that showed that adult *n*-3-deficient rats were impaired on a working memory version of the Morris water-maze (Wainwright *et al*. 1998). However, in a subsequent study in which developing animals were fed on diets containing sufficient LA and LNA and supplemented with AA and DHA, we were unable to show a relationship between brain DHA levels and working memory performance (Wainwright *et al*. 1999). This suggests caution in generalizing from situations of dietary deficiency to those of dietary supplementation. Whereas a potential weakness with the 'top-down' approach is that of false positive findings, the corresponding weakness here is that of false negatives. This is because the plasticity of the brain may allow for considerable alteration in neurotransmitter levels before overt behavioural effects are manifest in certain domains.

Despite these complexities, the possible influence of nutritional factors such as dietary lipids on the functional properties of the nervous system has important implications. At the population level, a small decrease in terms of the mean performance on a particular cognitive test can translate into a large difference in terms of the numbers of individuals falling into the tails of the normal distribution, i.e. fewer classified as advanced and more as retarded. Within the last decade, nutritional neuroscience has emerged as a recognized discipline with the potential to make a significant contribution to our understanding of these relationships through the use of standardized nutritional manipulations and behavioural tests that are both sensitive and specific with respect to well-defined aspects of cognitive function. This is an enterprise from which much stands to be gained.

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