

Nutrition and the development of cognitive functions: interpretation of behavioral studies in animals and human infants¹⁻⁴

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ABSTRACT

A rapidly accumulating body of evidence on the neural basis of cognition suggests that cognition is not a unitary function but rather depends on the functions of multiple and dissociable neural systems. The nonlinear interactions in the differing trajectories of these systems during development result in changing patterns of cognitive functions over time; they may also lead to paradoxical outcomes, for which enhancement of one function through dietary intervention may be at the expense of another. This emerging understanding has important implications for the design and interpretation of studies on the cognitive effects of specific nutrients during development. It is important that researchers move away from global tests of development and strive rather to ensure that their choice of behavioral task is based on specific hypotheses of the systems expected to be altered by a dietary manipulation and on an understanding of which behavioral tests are valid, sensitive, and reliable indicators of this disruption. Furthermore, to understand whether accelerated or delayed development related to a particular cognitive function is beneficial or problematic, it is important to study the entire behavioral profile over different time points, rather than relying on one outcome measured at one time point. It is also necessary to control for sensory or motivational differences that will affect performance on the behavioral tasks. Implementation of these methodologic recommendations may contribute to a deeper understanding of the mechanisms involved in the nutrition-associated changes in cognitive functions and thereby aid in the development of an appropriate population-based dietary policy. *Am J Clin Nutr* 2006;84:961-70.

KEY WORDS Cognitive development, nutrition, animal models, infants, behavioral assessment, neural systems, attention, learning and memory

INTRODUCTION

There is increasing interest in the role of nutrition in the development of the functions of the brain, with levels of analyses varying widely across studies. The topics of these studies range from the intricacies of regulation of gene expression at the cellular level to the equally complex regulation of the neural systems involved in an infant's or animal's behavioral interactions with its environment (1). A prominent focus of much of this behavioral work is on the higher-order functions that we typically characterize as comprising "cognition," many of which address capabilities in various forms of learning and memory. However,

measurement of cognitive behaviors in both animals and humans is not without its inherent challenges. Although terms such as learning and memory are often used to represent unitary processes in the brain, experimental evidence supports the existence of different forms of cognitive processing that reflect the operations of biologically distinct neural systems (2, 3). Neural systems that are involved in cognitive processing differ not only in their biochemical and functional properties but also in their developmental trajectories. Thus, these systems may be influenced differently by various nutritional manipulations. Furthermore, dysfunction in a particular neural system may be apparent on one type of learning and memory task but not another (4). It is therefore important that experimenters strive to ensure that their choice of behavioral task is based on specific hypotheses of the systems expected to be altered and on an understanding of which tests would be expected to be sensitive to this disruption. Moreover, because conclusions about functions such as learning and memory are based on inferences drawn from the observation of an animal's or infant's performance on a specific behavioral task, it is also incumbent on the researcher to validate such inferences by ensuring that there are no alternate explanations, such as other variables that may covary with either the treatment or outcome of interest (5). These variables may involve the sensory, motor, or motivational components of the task or they may also involve other aspects of cognitive performance, including the ability to focus or sustain attention or to inhibit competing behavioral responses. An additional consideration in developmental studies is that of identifying the most sensitive period for showing specific cognitive effects, including whether effects seen earlier are predictive of those occurring at a later time point or, alternatively,

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whether there may be effects at older ages although none were apparent earlier. Such concerns are important because, if ignored, they may result in misinterpretations of research findings that are counterproductive to the development of appropriate population-based nutritional interventions.

We recognize that the rapid growth of knowledge in relation to nutrition and the brain precludes a scientist in one area of research from being fully cognizant of all issues that pertain to a different area of research. Thus, not only do we advocate that nutritional researchers be informed on behavioral research, but also that behavioral researchers avail themselves of nutritional expertise when it comes to matters related to dietary formulation and the measurement of biochemical outcomes. Hopefully, increased dialogue and collaboration among researchers with different expertise will enable a deeper understanding of the important role played by specific nutrients in the development and maintenance of cognitive functions. Therefore, our intent in writing the present article was to enable such dialogue by providing to the nonspecialist an understanding of the ways in which behavioral scientists frame hypotheses and measure outcomes to ensure the validity of the inferences drawn from such studies. The article begins with a brief overview of contemporary understanding of the neural circuitry in the mammalian brain that is involved in information processing and decision making. The emphasis here is on the implications of incorporating a neural systems approach into the design of studies that address specific behavioral competencies. This information is then integrated with more general issues pertaining to the design and experimental validity of such studies, particularly how these relate to the role of nutrition in the development of cognitive functions in both animals and human infants.

NEURAL CIRCUITRY INVOLVED IN COGNITIVE FUNCTIONS

What do we mean when we refer to “cognitive functions?” One way in which such functions may be conceptualized is as those constituting the neural processes necessary to support the flexible use of information in the execution of adaptive, goal-directed behavior. Of the many different processes involved, learning and memory are the two that most readily come to mind. However, in addition to learning and memory, the ability to enable adaptive behavioral responses also depends on the capacity to focus and sustain attention on the relevant stimuli and to hold this information in working memory while choosing and executing the appropriate motor response. It also involves more complex information processing functions, such as the ability to abstract information and to establish a system of rules for responding appropriately in different contexts and to suppress other competing behavioral responses. In their discussion of behavioral flexibility, Atallah et al (6) proposed the following set of working criteria, which can be seen as a useful summary of the functional outcomes served by cognitive processes in general: “The ability to flexibly apply or generalize acquired knowledge in novel situations; the ability to flexibly switch between different behavioral tendencies, depending on the context of the environment; the ability to flexibly adapt to new situations and to change behavior with changing task demands” (6, p 259).

The neural circuits that have been implicated most prominently in mediating the information processing and decision-making processes of the mammalian brain comprise a series of

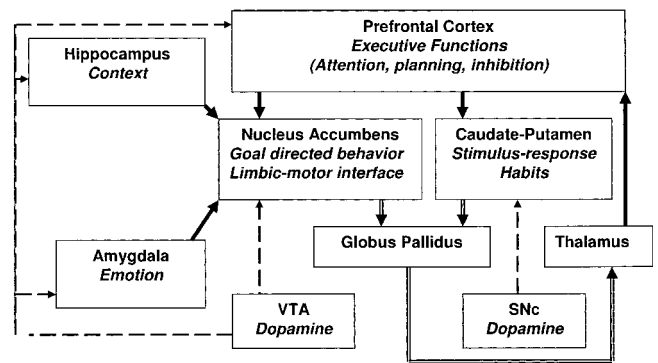


FIGURE 1. Cortical-subcortical circuitry involved in mediating cognitive functions, simplified to show only limbic input and modulation by mesocorticolimbic and nigrostriatal dopamine pathways. VTA, ventral tegmental area; SNc, striatonigral, pars compacta. Pathways with glutamate as the neurotransmitter are shown with a thick solid arrow, pathways with γ -aminobutyric acid as the neurotransmitter are shown with a hollow arrow, and pathways with dopamine as the neurotransmitter are shown with a dashed arrow [adapted from Everitt and Robbins (9)].

reciprocal cortical-subcortical loops. As shown in **Figure 1**, these circuits originate in the cortex; project to various subcortical structures including those in the ventral and dorsal striatum, such as nucleus accumbens and basal ganglia, respectively; and then return via the thalamus to the region of the cortex from which they originated (7).

These pathways do not operate autonomously; rather, they are subject to the influence of input from many other regions of the brain. For example, in addition to the prefrontal cortex, important sources of input to the nucleus accumbens are the hippocampus and amygdala (8). Recent evidence indicates that the functional interactions between these structures are integral to both behavioral and physiologic regulation, and this understanding has served to undermine the traditional distinction made between the cognitive and affective contributions to decision making (9). The hippocampus is necessary for the formation of long-term memories and contributes to higher-level decision-making processes by representing the relations between discrete stimuli, thereby providing information relating to the overall context (10). The amygdala is involved in processing the affective (ie, emotional) valence of sensory stimuli and relaying this information to the prefrontal cortex. The amygdala also plays an integral role in the initiation of the coordinated pattern of physiologic and behavioral changes that constitute the stress response to real or perceived dangers (11). This includes the release of glucocorticoid hormones, where appropriate concentrations of glucocorticoids are necessary for mnemonic functions to be accomplished effectively (12). The hippocampus in turn, together with the prefrontal cortex, is involved in the feedback regulation of these glucocorticoid concentrations (13). As part of the executive functions that are engaged during the process of decision making, the prefrontal cortex generates possible behavioral alternatives in response to the specific nature and emotional valence of sensory stimuli. This information is then relayed to the nucleus accumbens, where input from the hippocampus is thought to “gate” neural activity in such a way that the motor response chosen is that most appropriate to the overall context (14). In this way, the circuitry involving the hippocampus, prefrontal cortex, and nucleus accumbens plays a key role in enabling behavioral flexibility (6). This is in contrast to the circuitry that includes the basal ganglia

(dorsal striatum), which has been associated with the learning and implementation of the sequences of motor output that constitute habitual learned behavioral response patterns (6, 15). Which of these systems is engaged to support behavior depends on the demands of the situation; that is, it depends on the integration of these systems with other systems that can access the organism's goal in the context of the task at hand as well as the organism's past successes and failures in attaining such goals (16). Under routine and familiar conditions, habitual responses may be the most effective, but any unexpected change will necessitate a switch either from one set of learned responses to another or to an entirely new set of behaviors based on a novel combination of internal representations gained from previous experience.

The modulation of states that lends the organism to be receptive to stimulation (eg, alertness or arousal) is likely mediated by several ascending pathways, which are generally identified by their neurotransmitters: the dopaminergic pathways that originate in the midbrain; the cholinergic, serotonergic, and adrenergic pathways that originate in the brainstem; and the cholinergic pathways that originate in the basal forebrain (17, 18, 19). Although the specific functions in which these subsystems are involved are not yet fully understood, these pathways have widespread cortical targets, and substantial evidence suggests that their input can also be modulated by feedback from cortical sites. Two midbrain dopamine systems exist, with one pathway (nigrostriatal) projecting from the substantia nigra to the dorsal striatum and another pathway (mesocorticolimbic) projecting from the ventral tegmental area to the nucleus accumbens, limbic regions (including the hippocampus and amygdala), and the prefrontal cortex (20). Dopamine is thought to enable behavioral flexibility in these pathways by facilitating the learning and execution of adaptive behavioral responses (21). Note that the availability of some neurotransmitters can be influenced by dietary supply of their amino acid precursor; for example, tryptophan is the dietary precursor of serotonin and tyrosine is that of dopamine and norepinephrine (22). Many of these neurotransmitters serve a dual role, functioning also as growth factors that influence the intricate choreography of growth of neural systems in the developing brain (23, 24). Thus, one of the mechanisms whereby changes in the availability of nutrient supply may result in disturbances of specific brain and behavioral functions during development is through their selective effect on some of these systems and not others. In the next section, we discuss the importance of applying distinctions in cognitive systems to the design and interpretation of behavioral experiments.

MULTIPLE SYSTEMS IN COGNITION: IMPLICATIONS FOR RESEARCH

Perhaps it is a byproduct of the predominance of the concept of general intelligence in psychology, but one of the common assumptions about cognitive function held by nonpsychologists is that cognition is, in fact, a unitary function. As indicated above, however, research on the neural basis of cognition over the past several decades suggests that this is far from the case, and the presence of multiple and dissociable systems in cognition has important implications for the design and interpretation of studies of nutrition. In the sections that follow, we delineate the multiple and dissociable processes that contribute to two particular cognitive functions: attention and memory.

Attention

Attention is commonly considered to be a unitary construct, but research over the past several decades on the visual realm in the mammalian brain suggests that the construct actually comprises many dissociable systems, each mediated by its own pathway [reviewed by Colombo (25)]. Two low-order systems in visual attention branch from the visual cortex, and these generally code for the location and the object features of a given stimulus. The former system appears to be coded by a pathway that terminates dorsally in the parietal lobe but which interacts closely with the superior colliculus to holistically monitor the visual field and then move the eyes to the position in which a novel object appears. The latter system can be found along the ventral surfaces of the temporal cortex and appears to reconstruct the features of the object into a whole that can be subject to recognition.

The most colloquial conceptualization of attention comes in the notion described by such words as "focus," "concentration," or "attention span," and represents the purposeful or voluntary inhibition or deployment of attention, usually in the service of making some response with a goal in mind. We asserted elsewhere (26) that this last form of attention is mediated largely by the integration of low-order attentional systems with memory functions and is likely mediated by frontal areas. The presence of such a multidimensional framework suggests that different aspects of attention will be used by different tasks or measures. For example, measures of psychophysiological reactivity may be related to brainstem systems; measures of attentional engagement, disengagement, and shifting are likely related to dorsal pathway function; object recognition may be related to ventral pathway function; and persistence, inhibition, and resistance to distraction may reflect frontal integrative activity. The degree to which, for example, nutritional interventions may affect different brain areas would determine which of these tasks would most likely show significant effects, and the choice of appropriate measures is the obvious key to drawing the appropriate conclusion about the effect of a particular compound.

Memory

Much of the evidence on the different memory systems in the human brain derives from studying the effects of injury or stroke, where, depending on the location of the damage, some types of learning and memory will be impaired and others remain unaffected (2). Widely accepted distinctions have been drawn between explicit and implicit memory. Explicit memory (also called declarative memory) refers to the ability to remember unique events, facts, and people and depends on the integrity of the hippocampus and surrounding cortical areas, which are collectively called the medial temporal lobe. Typically, the information is acquired quickly and does not require a large number of multiple repetitions in the form of learning trials. This is in contrast to implicit memory, which accumulates more slowly as the result of repetition, as in the acquisition of the motor skills necessary to play a musical instrument or drive a car, and which has been related to the functions of the basal ganglia structures situated in the dorsal striatum.

These observations in humans have been supported by experimental research conducted in animals with lesions in specific brain regions (3, 4). Consider, for example, a rat foraging for food in a complex radial arm maze, with many possible start arms and

many food locations. After exploring the maze many times, the rat will learn the most efficient route to find the food, regardless of its start position. Thus, if a particular route is blocked or the food source is relocated, the rat will adjust its behavior accordingly. This shows that the rat has acquired knowledge of the overall spatial relations in the maze and is able to use this knowledge to reach its goal of obtaining food. This knowledge, which is acquired after a few trials, is dependent on the integrity of the neural circuitry that includes the prefrontal cortex, hippocampus, and nucleus accumbens. This is in contrast to learning a simple discrimination in which the rat is able to find the food by making the appropriate behavioral response to a distinctive visual cue in one of two arms of a T-maze, and where it learns the association between stimulus and reward over many repeated trials. This so-called procedural memory or habit-learning system is dependent on the integrity of the circuitry involving the dorsal striatum. The rat also develops a learned emotional response to the visual cue, which relies on circuitry that includes the amygdala. Thus, methodologic variations in animal studies influence which of these systems the outcome represents, and it is important for the nonpsychologist to appreciate this. In the Morris water maze, for example, escape from the water by locating a hidden platform from varying start positions involves spatial learning ability, whereas escape to a visible platform or from the same start position can be accomplished on the basis of stimulus-response learning. Similarly, the assessment of high-order cognitive functions may require more extensive testing than simple stimulus-response type tests. One such example is the formation of a high-order cognitive ability known as a "learning set," which has been related to prefrontal cortex function. The development of a learning set can be assessed through the use of a sequence of stimulus-response tasks, where each task requires the animal to learn a different association. If the later discriminations in the sequence are learned more rapidly than are those presented earlier, this transfer of learning from one task to the next is considered evidence of a learning set. This is nicely described in research on rats with neonatal hyperphenylalanemia (an animal model of phenylketonuria), where treated rats showed deficits on the transfer of learning and not in their initial ability to make olfactory discriminations. These studies have been previously described at length (27, 28, 29) and illustrate the importance of appropriate task selection in drawing inferences about behavioral capabilities. These references also provide a thorough discussion (highly relevant to the present context) of the issues associated with the evaluation of the behavioral effects of neurotoxicant exposure during development. [For the nonpsychologist interested in reading further on this topic, Eichenbaum (30) provides an accessible introductory level text. In addition, Whishaw and Kolb (31) and Cheatham et al (32) discuss conceptual and methodologic issues of the conduct of studies in animals and human infants, respectively.]

Another important consideration is how specific outcomes may be influenced by interactions among the neural systems involved. Although these systems are typically considered to operate in parallel, there is evidence that they can interact either competitively or cooperatively depending on the circumstances (33, 34). Paradoxically, what is also sometimes observed is that when one system is damaged, enhanced performance is seen in the other. Thus, rats with hippocampal damage often perform better on stimulus-response habitual type learning tasks than do

control animals, whereas those with damage to the caudate nucleus (part of the basal ganglia) are better on spatial learning tasks that involve the hippocampus. This suggests there is a balance between the activities of the two systems under normal conditions and that this balance is disturbed by injury. We reiterate here the importance of measuring performance on various tests that have been validated for particular neuropsychological functions and then interpreting the overall pattern of findings before drawing conclusions about the specific cognitive effects associated with a dietary treatment.

DISTINCTIONS BETWEEN LEARNING AND PERFORMANCE

The validity of the inferences drawn from experimental studies depends not only on the use of outcomes that are sufficiently reliable and sensitive to detect change, but also on construct validity, ie, the ability to eliminate factors related to either treatment or outcome that may be alternative explanations for the findings (sometimes referred to as "confounds"). Thus, in studies conducted on animals or human infants, it is important to make the distinction between specific effects on the construct of interest, such as learning and memory, and the more general effects on task performance. For example, if a dietary treatment markedly decreases the time it takes a rat to locate a hidden platform in a water maze, it is not clear that this change in behavior can be interpreted as clear evidence of its memory of the platform location. It is possible that another factor associated with the latency of the rat in finding the platform, such as swimming speed, is responsible for the observed improvement in the time taken to reach its goal. In this sense, an increase in swimming speed is a potential competing explanation for an interpretation that is based on spatial learning and memory. Thus, the preferred way of showing spatial learning in the water maze is to remove the platform and then measure the distance swum in the platform quadrant as an index of knowledge of the platform location in relation to extra-maze cues. In addition to effects on motor capacity, performance on cognitive tasks can also be affected by differences in affective or motivational states, including how stressful the procedure is or the time during the circadian cycle at which the measurement is taken. Thus, in addition to cognitive measures, additional information on the animal's affective state can be sought through activity in the open field (response to novelty) or time spent on the open arms of an elevated plus maze (anxiety). Cahill et al (35) discuss in more detail the distinction between learning and performance, which includes issues pertaining to studies conducted in humans.

The question often raised in an applied context is "if we can show an improvement in an outcome that we value, can we afford to wait until all the necessary studies have been completed to understand the mechanism?" The answer to this depends on one's objectives. Consider the hypothetical situation in which the aim is to improve the mental arithmetic scores of 8-y-old boys, ie, achieve an improvement in performance on this particular function. If one can show in a placebo-controlled study that drinking a nutritional supplement for breakfast accomplishes this end, this may be sufficient evidence to support a recommendation for the implementation of such an intervention. However, instead of wishing to improve only performance on this particular task, one may be interested rather in obtaining a precise understanding of the role of a specific dietary ingredient on working memory, ie,



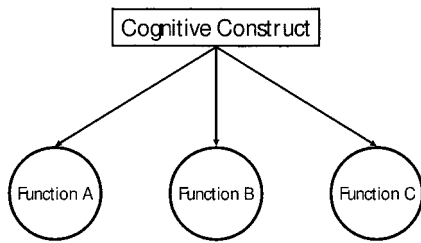


FIGURE 2. Schematic diagram reflecting the modular nature of a hypothetical cognitive construct. Here, the theoretical construct is actually composed of 3 distinct cognitive functions (A, B, and C).

the short-term memory store necessary to hold and manipulate information in working memory while solving a problem, of which mental arithmetic is one example (36). Then one would also have to obtain convergent evidence from working memory tests in other contexts, for example, recall or recognition of letters or words. It would also be necessary to obtain divergent evidence that could rule out a nonspecific effect, such as an increase in alertness due to increases in blood glucose concentrations, which may improve performance on many types of cognitive tasks and not only on those involving working memory. Furthermore, in light of the evidence described above that, in some cases, enhancement in one type of memory may be related to impairment in another, one may also want to ensure that the improvement seen is not at the expense of other cognitive capabilities. Moreover, to determine which component of the supplement is the “active” ingredient, it would be necessary to systematically vary the formulation of the supplement by using either the same group of people at different times or different groups of people as subjects. Only when these concerns are satisfied can one claim with some degree of confidence that there is support for the hypothesis of a specific effect on working memory of a defined ingredient in a dietary supplement. If there is one lesson to be learned from all of this, it is that there is seldom a “quick and dirty” behavioral assay when it comes to measures of cognition. Rather, various outcome measures may be necessary to deconstruct the experimental manipulations in light of plausible alternative explanations before concluding that the observed effects are attributable to a specific cognitive process.

DEVELOPMENTAL ISSUES

The point of the previous section was to show that common cognitive constructs are probably best represented by functions that are mediated by dissociable neural pathways. However, the fact that nutritional research is increasingly focused on developmental processes raises the difficulty of measuring cognition to another level entirely (37, 38, 39). This is because, in addition to their inherent complexity, such functions and pathways often have different and dissociable developmental courses that lead to the emergence of mature cognition through interrelated cascades of development. Most developmental scientists will recognize this passage as describing one of the archetypal scenarios of developmental systems theory (40, 41). The framework of this theory may be best presented visually. Three different subcomponents of a hypothetical cognitive construct are shown in a simple schematic in **Figure 2**; a construct of attention may be similarly represented by orienting, arousal, and persistence, and memory may be similarly represented by divisions of declarative,

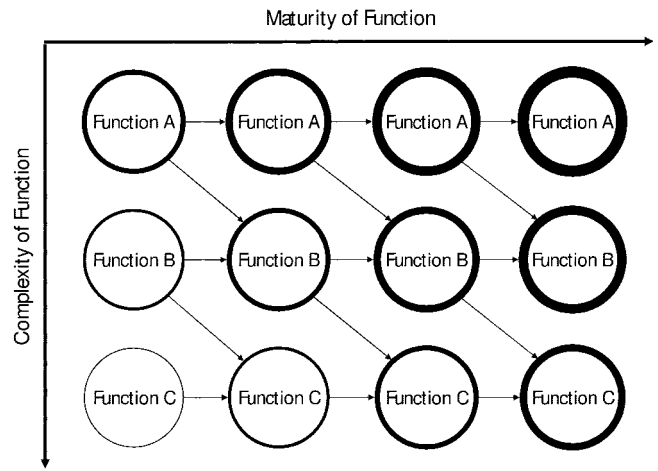


FIGURE 3. Cognitive functions arranged in order of complexity in the vertical plane, with the lowest-order function at the top and the highest-order function at the bottom. The schematic also shows their relative developmental course and the manner in which they relate to themselves and other functions across time. The functions’ maturity is reflected by the relative thickness of the circles (eg, Function A matures fully by Time 3). In this developmental cascade, the maturity of each function at any point in time is related to its own level of maturity at the immediate previous time point (horizontal arrows). However, the maturity of each of the higher-order functions at each time point is also driven by the developmental status of the lower-order function at the previous time point (diagonal arrows).

episodic, and procedural phenomena. The interrelated development of these 3 functions is represented in **Figure 3**; the functions are placed in the vertical plane to represent increasing levels of complexity, with the simplest or lowest-order function (A) at the top and the most complex and highest-order function (C) at the bottom. The horizontal plane shows that the functions mature at different rates, with the simpler functions reaching maturity (represented by the thickness of the circles) first. Note that each function relates both to itself across each point in time and to the immediate higher-level function at the next point in time. Finally, each subfunction eventually reaches some asymptote of maturity, although only Function A is shown in the thickest circle and represents full maturity at point 4.

The detailed description of such a cascade [examples of which abound in the cognitive developmental literature of attention, memory, and higher-order adaptive functions such as reading (eg, 42)] illustrates how the effects of an intervention (eg, a nutritional supplement or deprivation) will affect the system as a whole and how such an effect will manifest from the point of view of the investigator. Consider what happens if one intervenes with a nutritional supplement that is administered before the first time point represented in **Figure 3** and if that supplement significantly accelerates the development of Function A (see **Figure 4**). Because each subfunction is related to another over time, the effect of that intervention will ultimately be reflected in all 3 functions, although the effect will likely be weaker as it becomes progressively more distal from the application of the intervention. However, the developmental pattern shown over time for this effect will vary markedly, and it will be manifest in different ways, depending on the level or function of the construct that the investigator chooses to assess and the point in development at which the investigator assesses it.

First, assume that the investigator is well informed about the nature of the cognitive construct at hand and has resources that



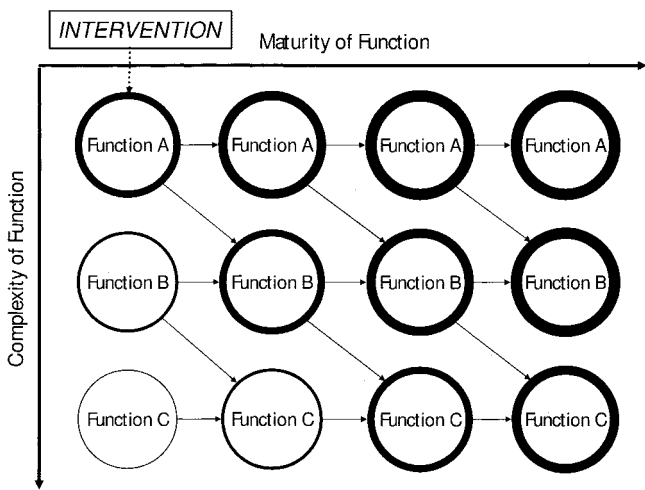


FIGURE 4. Figure 3 is repeated, except that an intervention (eg, nutritional supplementation or an experimental or natural deprivation) is depicted at Time 1. This intervention affects the developmental status of Function A at an early point in time and ultimately affects the developmental status of Functions B and C.

allow for the measurement of all of the functions involved in the construct at a sufficient number of longitudinal points. Furthermore, assume that the effects of the nutritional supplement can be contrasted with the presence of a placebo control. The outcomes are represented in the 3 graphs in **Figure 5**. The top left graph shows the effect of the intervention on function A; because all subjects will reach the asymptote of maturity by point 4, the intervention produces a transient acceleration in performance on function A that disappears by the end of the study. The top right graph shows the curious effect of the intervention on Function B; here, there is a transient effect that is present only at the middle 2 points of the study (because the Function is not yet mature to show intervention-related variation), nor at the end of the longitudinal sequence (because subjects in all groups have attained asymptotic levels of maturity). Finally, the effect of the intervention on Function C emerges only later in the developmental sequence, taking on the appearance of a classic “sleeper” effect. If the investigator had all 3 effects on hand, it would be possible to piece together a plausible scenario for a sophisticated scientific audience and successfully advocate for the intervention’s efficacy.

Now, however, consider that the investigator has chosen only one of the functions to measure, but has measured the chosen

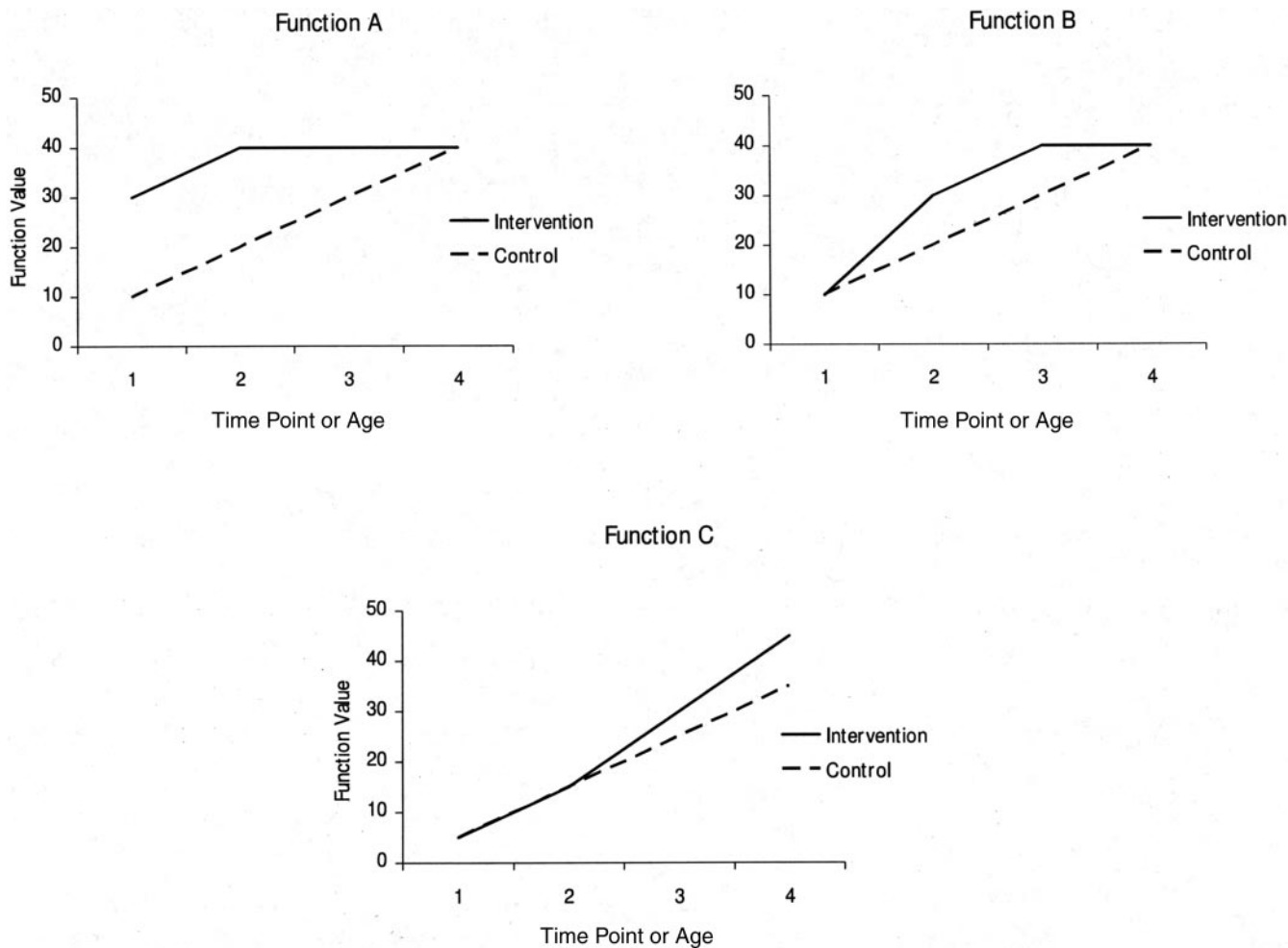


FIGURE 5. Relative effects in time for the intervention shown in Figure 4 for the 3 cognitive subfunctions. Function A shows a transient effect with early differences disappearing as the function attains maturity. Function B shows a transient acceleration, with differences emerging only at intermediate time points and then disappearing as the function reaches maturity. Function C shows a sleeper effect, with effects emerging only at the last assessment.

function in a developmental framework. Here, there is no luxury of having all the data presented in Figure 5. If the choice was made to measure Function A, the investigator may be tempted to conclude that the effect “washes out” over time, and may find it difficult to advocate for the intervention as having a meaningful or powerful effect. Taken in isolation, the profile for Function B is puzzling; the investigator would find this outcome even more difficult to promote as meaningful or powerful, and may actually be tempted to dismiss it as spurious. Finally, the choice of Function C may be the most convincing, because sleeper effects may be more readily accepted by a scientific audience. However, the investigator must have enough confidence in the effect to endorse it; given that the effect of the intervention has appeared only at perhaps 1 or 2 time points in the study, the investigator would not be unreasonable in questioning its reliability. Moreover, the presence of effects assumes that the investigator had the resources and patience to wait for its appearance. The interpretations of these outcome patterns are difficult even under the more optimal conditions, but consider finally what happens if the investigator chooses to conduct a “snapshot” study in which one function is assessed at one time point. An examination of the individual time points from any of the functions shown in Figure 5 indicates that the probability of concluding that the intervention has an effect is barely better than 50–50.

An additional and important consideration is that the example above was presented with respect to the precise measurement of specific measures of cognitive function. However, particularly in early development, assessment is commonly executed with the use of broad-band, standardized developmental tests. This raises the possibility of additional reductions in sensitivity and power for detecting effects of interventions. Tests such as the Bayley Scales of Infant Development, for example, were developed initially as screening tools in clinical situations (43). The infant’s developmental status is represented by an aggregate score that represents the average of the scores obtained across different developmental domains. Implicit in the use of this approach is the assumption that there is some general construct represented by this score, such as a “general developmental (or intelligence) quotient.” However, it is possible for different infants to obtain the same score with a measure such as this, each with a very different developmental profile in the overall pattern of scores. Moreover, although such scales have proved useful in identifying problems in clinical populations, there is no strong correlation between these scores and tests used later to assess intelligence in school-age children in a normal population. As we indicated previously, the brain is not a homogeneous structure, and different regions, including those involved in the different cognitive systems, mature at different rates (44). Furthermore, as discussed above, because of nonlinear interactions among developing neural systems, disruptions in one system may have long-term consequences in another in terms of cognitive functions. This is illustrated by the outcome of a study that compared the effects of hippocampal lesions in neonatal and adult rats. When the neonatally lesioned rats were tested as adults, they showed anomalies on a working-memory task that relies on the integrity of the prefrontal cortex, whereas the performance of the adult lesioned rats was unaffected (45). This suggests that some reorganization of function occurred in the early lesioned rats. Similarly, it has been argued that the developmental effects of genetic anomalies such as Fragile X syndrome, which is a leading cause of mental retardation, can only be understood within a

developmental context (46). Thus, recent developments in the field of infant cognition advocate alternatives to standardized scales, rather than relying on tests of overall function. These alternatives would address questions directed at more specific aspects of cognitive development that are related more directly to underlying neurobiological mechanisms and for which there may be better predictive ability [discussed in depth by Colombo (47) and McCall and Mash (48)].

GENERALIZATIONS FROM ANIMAL STUDIES TO HUMANS

Animal studies have made important contributions to understanding the role of nutrition in the development and operations of the brain by allowing manipulations and controls that would not be possible in similar studies conducted in humans. In this section, we address the benefits to be gained by the inclusion of animal models in a research program on cognitive outcomes in humans, particularly in relation to task selection. One concern in generalizing findings from animal studies to humans is that a task may show apparently flawless face validity in terms of the representation of similar psychological constructs (eg, attention, procedural memory) in rats and humans (or in infants and adults), but there may, in fact, be species differences (or age differences) in the neural systems involved in the task performance. In other words, task performance may be analogous between species or age groups (ie, it may serve the same end), but not homologous (ie, it may not be mediated by the same neural systems). This concern notwithstanding, recent technological advances that allow more sophisticated imaging of brain function in relation to behavior in both humans and animals have provided evidence that in many cases supports similarities in the neural substrates involved (49). Nonetheless, tasks may be structured differently to allow for differences in sensory or motor function. For example, the Morris water-maze, which has been used extensively to probe spatial learning in rats, was adapted to a land maze used to measure human navigational capabilities related to hippocampal function (50).

The obvious differences in cognitive capabilities that involve consciousness between humans and other mammals pose interesting research challenges. For example, the definition of working memory in both humans and animals describes a type of short-term memory where items held in memory are intermediate steps invoked during a specific problem-solving event and typically do not enter into long-term memory stores (36, 51). But definitions of working memory in humans include not only the short-term memory component that can be studied in animals, but also the conscious manipulation of this information. However, our inability to assess consciousness in animals (and, to some extent, in nonverbal human infants) notwithstanding, tasks involving delayed response have proved useful in probing the mechanisms of prefrontal function in both humans and animals. For example, in infants, the A-not-B task has been used to assess working memory and inhibitory control, both of which contribute to various types of executive function (52, 53). In the A-not-B task, the child is shown a desirable object hidden under a cloth or in a recessed well. After a brief delay, the child is allowed to search and find the object. After several successes finding the object at a particular location or hiding place, the object is then taken and, in full view of the child, is hidden at an alternate



location. Here, correct performance depends on the child's ability to retain the memory of the new hiding place as well as to inhibit the prepotent response of searching at the location where the object was previously found. Performance is dependent on several experimental parameters, such as the length of the delay, the distance between the wells, and the number of wells that might be searched (54). However, success at this task usually does not emerge until between 9 and 12 mo, when considerable maturation of the frontal association areas has taken place (55, 56); indeed, Diamond (57) has long contended that success at this task is linked to maturation of the dorsolateral prefrontal cortex.


A similar delayed response-type task was used in rats to assess functions related to the prefrontal cortex (58). For example, in a 2 choice situation, rats are provided with a food reward if they alternate goal arms, ie, select the goal arm not selected on the previous trial. Successful performance requires both working memory and the ability to apply the alternation "rule." If a rat can perform well at very short delays between trials, but is impaired as the delays are increased, this is taken as evidence of memory impairment, whereas impairment at all delays suggests an inability to either learn the rule or to inhibit a tendency to reenter the arm that was rewarded previously. It was also shown that performance on this task is related to the dopaminergic activity of the prefrontal cortex (59). Thus, although not entirely analogous to human working memory, this task can nonetheless be considered homologous to that used in humans because it depends on the integrity of similar neural systems. In the following section, we briefly describe the contribution made by such an animal model in the context of the pattern of cognitive impairments associated with early treated phenylketonuria in humans. For the interested reader, a more detailed discussion of these studies is provided by Strupp and Diamond (28) and by Diamond et al (60).

EXAMPLE OF SPECIFIC COGNITIVE DEFICITS ASSOCIATED WITH PHENYLKETONURIA

Phenylketonuria results from a recessive mutation in the gene coding for phenylalanine hydroxylase, the enzyme that converts dietary phenylalanine to tyrosine. The resulting excess in phenylalanine impairs cognitive development in infants and leads to severe mental retardation, which can be prevented by the provision of a diet low in phenylalanine started early in development. However, these early treated children initially had plasma phenylalanine concentrations that were 3 to 5 times normal. Furthermore, despite their overall cognitive improvement, they showed subtle deficits in the A-not-B task described above (60). Because amino acids compete for access to the brain through the blood-brain barrier, it was hypothesized that the residual phenylalanine-tyrosine imbalance in the treated children was sufficient to limit access of tyrosine to the brain. Because tyrosine is the dietary precursor of dopamine, it was further hypothesized that this would compromise the development of the dopaminergic pathways to the prefrontal cortex, which, because of their unique firing properties, would be particularly sensitive to this type of dietary insult. Evidence in support of this hypothesis was provided by a rat model of early treatment of phenylketonuria. Rats with moderately elevated phenylalanine concentrations were able to learn the task and perform well when there was no delay between trials, but these animals did show impairment in the presence of a delay. Moreover, task performance was

shown to correlate with prefrontal cortex concentrations of homovanillic acid, which is a dopamine metabolite. Thus, the animal model confirmed the mechanism of dysfunction in prefrontal cortex suggested by human evidence; these findings have, in turn, resulted in revised treatment guidelines for children with phenylketonuria so that phenylalanine concentrations are now better controlled from early diagnosis. This illustrates the knowledge to be gained through the use of a specific neurochemical hypothesis to guide the selection of cognitive tasks in a program of research that includes both human and animal models.

SUMMARY AND RECOMMENDATION

Our objective in writing the present article was to give scientists who are unfamiliar with behavioral methodologies an understanding of the basis on which behavioral researchers, who work on the development of cognitive functions in animal models and human infants, design studies and interpret the behavioral literature. We believe that this article is timely because a rapidly accumulating body of evidence on the neural basis of cognition suggests that cognition is not a unitary function but depends rather on the functions of multiple and dissociable neural systems. Furthermore, the nonlinear interactions among the different trajectories of these systems during development result in changing patterns of cognitive functions over time; they may also lead to paradoxical outcomes, where enhancement of one function through a dietary intervention may be at the expense of another. Two important messages emerge due to the methodologic implications of embracing a neural systems approach to developmental questions. One is the need for researchers in this area to move away from global tests of development and strive rather to ensure that their choice of behavioral task is based on specific hypotheses of the systems expected to be altered by a dietary manipulation and on an understanding of which behavioral tests are valid, sensitive, and reliable indicators of this disruption. The other is that it is important to study the entire behavioral profile over different time points, rather than relying on one outcome measured at one point in time, to understand whether accelerated or delayed development is beneficial or problematic. It is also necessary that studies control for confounders, such as sensory or motivational differences, that will affect performance on behavioral tasks. Furthermore, the use of appropriate animal models will enable the necessary research into underlying biological mechanisms. We recognize that the implementation of these recommendations can best be accomplished in the context of interdisciplinary research teams with expertise in both nutritional and behavioral research; this has important implications with respect to the levels of funding needed to provide the resources to support such large and labor-intensive research programs. We also believe that it is important to recognize that the complexity of these issues reflects the inherent complexity of the process of brain development itself and, as such, cannot be ignored. An in-depth understanding of the role of specific nutrients in the development of cognitive functions is essential to create appropriate population-based dietary policies for infant and child nutrition. 

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