

The 114th Abbott Nutrition Research Conference

April 8-9, 2013

# The 114th Abbott Nutrition Research Conference

April 8-9, 2013

Columbus, Ohio, USA

*Cognition and Nutrition*

# Welcome

At Abbott Nutrition, we are committed to scientific leadership in the emerging field of cognition and nutrition in discovering ways that nutrition can enhance brain structure and physiology, cognitive development, and learning and memory across the lifespan. The right nutrition at the right time is key to cognitive development early in life and to protect or enhance cognition in later years. We invite you to review the Proceedings from the *114th Abbott Nutrition Research Conference: Cognition and Nutrition* to learn about the newest developments in this exciting, rapidly changing field of science.

Cognitive development is critical in infants and children as the infant's brain triples in weight during the first 3 years of life. Specific nutrients support this rapid brain development. Investigating the role of lutein, docosahexaenoic acid (DHA), arachidonic acid, iron, folic acid, choline, and iodine in early neural development and cognition in the preconception, fetal and infant stages, and early childhood is an important strategy toward favorable long-term health outcomes.

A growing body of evidence suggests the potential for dietary lutein, DHA, flavonoids, and extracts from botanical sources along with physical activity and exercise for preserving and enhancing cognitive function during aging, while reducing the risk for cognitive impairment, Alzheimer's disease, and other dementias.

Translational and clinical research will help to identify those nutrients that influence cognition across the life cycle, including the type, form, dose, and timing of intake, as well as to understand the potential for synergistic effects with exercise. With advances in magnetic resonance imaging, we now can analyze the physiology of the brain, providing sensitive measures to assess the efficacy of nutrition interventions.

This publication offers 13 presentation summaries focusing on tools to assess brain structure and cognition, the effects of early nutrition interventions on cognitive development in infants and children, specific nutrients and exercise to help preserve cognitive function and reduce the risk for certain diseases in aging, and findings from animal models on how nutrition affects learning and memory.

We hope our conference proceedings spark your interest in the exciting area of nutrition and cognition and advance your knowledge of the importance of optimal nutrition to support cognitive development in the early years and also protect cognitive function throughout the adult years.

**Divisional Vice President ..... Gary Fanjiang, MD, MBA**  
**Abbott Nutrition Scientific and Medical Affairs**

**Senior Manager, Science Programs.....Rosemary Riley, PhD, LD**  
**Abbott Nutrition Health Institute**

**Section Head, Global Cognition Platform Lead ..... Matthew Kuchan, PhD**  
**Abbott Nutrition Discovery Research**



# Cognition and Nutrition

## Editors

Judith Gussler, PhD  
M. Ann Graham

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### 114th Abbott Nutrition Research Conference Faculty

*Left to right, front:* Elizabeth Johnson, Carol Cheatham, José Delgado-García, Andrew Scholey, Arthur Kramer. *Left to right, back:* Rodney Johnson, David Vauzour, Neal Cohen, Milagros Gallo, Billy R. Hammond, Cristina Campoy, Anne Marie Minihane, Bradley Sutton.

Abbott Nutrition  
Columbus, Ohio 43219  
Division of Abbott Laboratories, USA



# Cognition and Nutrition

The 114th Abbott Nutrition Research Conference was held in Columbus, Ohio, USA, on April 8-9, 2013. This Report contains summaries of presentations given by the following contributors.

## Assessing Cognition, Brain Function, and Efficacy of Nutrition Interventions

### *Assessing Cognition and Brain Function*

Neal J. Cohen, PhD  
University of Illinois at Urbana-Champaign, Center for Nutrition,  
Learning and Memory, Center for Lifelong Improvement of Minds & Brains  
Beckman Institute, USA

Cognition involves thinking and knowing, which are supported by acquiring, processing, and using information. These actions are driven by mental processes in different but interconnected brain regions that specialize in different functions. Dr Cohen describes “powerful” methods for identifying and characterizing multiple memory systems in the brain. He explains that the hippocampus and relational memory are highly susceptible to damage but also to enhancement by fitness and nutrition. Specifically, he cites potential benefits from antioxidants, omega-3 fatty acids, and flavonoids in the diet. He shares research on the potential benefits of exercise in reducing the negative effects of a diet high in saturated fat and refined sugar on memory performance.

### *Advances in MR Imaging and the Questions They Answer*

Bradley P. Sutton, PhD  
University of Illinois at Urbana-Champaign, Bioengineering Department  
Beckman Institute, USA

Magnetic resonance (MR) imaging provides many windows into the physiology of the brain, providing sensitive measures to follow nutrition interventions. Dr Sutton discusses three particularly promising techniques—magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), and magnetic resonance elastography (MRE)—that allow researchers to monitor changes in brain metabolism, neuronal connectivity, and tissue structure. He states that MRS can be used to examine localized brain chemistry and metabolism, DTI to assess white matter axonal connectivity and integrity of the membranes and myelin, and

MRE to provide information on the overall structural integrity of brain tissue. This multiparametric space, Dr Sutton says, allows for specific physiological questions to be asked with respect to the impact of a nutrition intervention on the brain.

## Impact of Nutrition on Cognitive Development

### *Early Programming of Brain Development*

Cristina Campoy, Prof, MD, PhD  
University of Granada, Department of Paediatrics, School of Medicine  
EURISTIKOS Excellence Centre for Paediatric Research  
Institute of Neurosciences, Spain

Epigenetic programming is increasingly recognized as an important mechanism underlying health and disease. Exposure to diet, drugs, and early life adversity during sensitive windows of life can lead to lasting changes in gene expression that contribute to the display of physiological and behavioral phenotypes. Diet is a potent modulator of epigenetic marks, especially during prenatal and early postnatal life. For example, it has been shown that diets high in choline, methionine, folate, and vitamins B<sub>6</sub> and B<sub>12</sub> increase DNA and histone methylation, alter gene expression, and can result in permanent changes in development. Prof Campoy describes studies that have identified some of the mechanisms associating early nutrition with later brain developmental outcomes. She concludes that understanding these mechanisms may have an enormous preventive potential, given the major public health implications, including opportunities for an improvement of cognition and an effective primary prevention of childhood and adult behavior and mental diseases.

### *Measuring the Impact of Nutrition on Cognitive Development*

Carol L. Cheatham, PhD  
University of North Carolina at Chapel Hill, Nutrition Research Institute, USA

Human brain development begins at conception. However, the influence of nutrition on brain development begins before conception and continues for many years. Dr Cheatham reviews the most important nutrients for brain development and discusses their cognitive effects. She outlines the rationale for studying the effects of nutrition on two specific cognitive abilities—memory and speed of processing. Dr Cheatham argues that the importance of nutrition to cognition in general cannot be overstated because memory is central to learning, and speed of processing underlies all cognitive abilities. She also illustrates behavioral and

electrophysiological methods of measuring the effects of nutrition on infant memory and speed of processing and states that nutrition researchers should work with developmental cognitive neuroscientists to use these methodologies to determine the effects of nutrition on brain development. Proper nutrition for fetuses, infants, and children can help ensure that children have a chance to achieve their cognitive potential.

## Lutein's Influence on Cognitive Development and Function

### *Emerging Science on Lutein in the Brain*

Elizabeth J. Johnson, PhD  
Tufts University, Jean Mayer USDA Human Nutrition Research Center on Aging, USA

Lutein is the predominant carotenoid in pediatric and adult brain tissue. Lutein in neural tissue has biological effects including antioxidant, anti-inflammatory, and structural actions. In infants' brains, the contribution of lutein to the total carotenoids is twice that found in adults, accounting for more than half the concentration of total carotenoids. In the adult, a variety of evidence supports a role for lutein in cognition. Therefore, Dr Johnson argues, the greater proportion of lutein in the pediatric brain suggests a need for lutein during neural development. Infant formula is not routinely supplemented with lutein, whereas breast milk is a highly bioavailable source of lutein. Dr Johnson suggests that further investigation of the impact of lutein intake on neural development is warranted. Given that the 1st year of life is a time of neural growth and development for which nutrition can have significant consequences, the addition of this dietary plant pigment to infant formulas could be an important strategy toward positive long-term health outcomes.

### *Lutein's Influence on Neural Processing Speed*

Billy R. Hammond Jr, PhD  
University of Georgia, Franklin College of Arts and Sciences  
Vision Sciences Laboratory, USA

Macular carotenoids, such as lutein, influence many aspects of central nervous system function. These effects extend from optical filtering within the eye to physiological activity of neurons within the brain. A growing body of evidence suggests that lutein can enhance neural processing speed. This is particularly important for the elderly since slowing appears to be a central feature of cognitive decline and impairment. Dr Hammond describes the mechanisms by which lutein

could produce these effects—eg, by reducing oxidative and inflammatory stress, improving neural collective processing, and preserving brain white matter. He concludes that lutein likely serves multiple functions within the central nervous system and that these functions seem optimally suited to the preservation and perhaps even enhancement of cognitive performance.

## Protecting Cognitive Function in Aging and Illness With Nutrition and Exercise

### *Nutrigenetics and Cognitive Health*

Anne Marie Minihihane, PhD  
University of East Anglia, Norwich Medical School  
School of Medicine and Health Sciences, UK

People are living longer than ever before and therefore are more likely to experience age-related diseases and conditions. However, living longer is not matched by an increase in healthy life expectancy. The aging population demographic is having a dramatic impact on dementia incidence worldwide, with prevalence approximately doubling every 20 years and estimated to increase to 115 million by 2050. Dr Minihihane reviews the acute and chronic impact of eicosapentaenoic acid and docosahexaenoic acid and the interaction with the apolipoprotein 4 (APOE-ε4) genotype.

### *The Role of Flavonoids in Preventing Neuroinflammation and Cognitive Decline*

David Vauzour, PhD  
University of East Anglia, Norwich Medical School  
School of Medicine and Health Sciences, UK

The potential of dietary flavonoids for aiding in the preservation of cognitive function during aging, while reducing the risk of Alzheimer's disease and other dementing disorders, has gained great interest in research literature during the past decade. Recent findings have suggested that in lower amounts, typical of those attained in the diet, flavonoids may exert pharmacological activity within the cells. Dr Vauzour discusses the impact of nutritional antioxidants on neuroinflammation and neurocognitive performance, and the role of flavonoids and their anti-inflammatory properties in cognitive protection. He also describes the effects of flavonoids on the vascular system, which may induce increases in cerebral blood flow capable of having an impact on acute cognitive performance or may lead to an increase in hippocampal vascularization capable of inducing new neuronal growth.



### ***Neurocognitive and Mood Effects of Nutrition and Nutraceuticals***

Andrew Scholey, PhD  
Swinburne University, Centre for Human Psychopharmacology  
NICM Centre for Natural Medicines and Neurocognition, Australia

Nutrients and extracts from botanical sources, unlike mainstream pharmacological agents, may contain many active components with a combination of properties that may affect multiple neuronal, metabolic, and hormonal systems with direct effects on cognitive processes. Dr Scholey describes the role of specific herbal extracts on adult cognitive function, such as ginseng and *Melissa officinalis* (lemon balm), and addresses techniques for mood and cognitive assessment, including magnetoencephalography, which measures changes in magnetic fields associated with postsynaptic potentials. He also discusses the need for future research to discover synergistic nutrition interventions to optimize day-to-day cognitive function, maintain psychological well-being throughout life, and even treat conditions where mental function becomes fragile, including dementia.

### ***From Inflammation to Sickness and Cognitive Dysfunction: When the Immune System Subjugates the Brain***

Rodney W. Johnson, PhD  
University of Illinois at Urbana-Champaign, Division of Nutritional Sciences  
Neuroscience Program, USA

Microglial cells, resident macrophages in the central nervous system, are relatively quiescent but can respond to signals from the peripheral immune system and induce neuroinflammation. In aging, microglia tend to transition to a proinflammatory state and become hypersensitive to messages emerging from immune-to-brain signaling pathways. Thus, in older individuals with an infection, microglia overreact and produce excessive levels of inflammatory cytokines causing behavioral pathology including cognitive dysfunction. Dr Johnson describes recent studies that indicate dietary flavonoids have anti-inflammatory properties and are capable of mitigating microglial cells in the brain of aged mice. Thus, he argues that dietary or supplemental flavonoids and other bioactives have the potential to restore the population of microglial cells in the aging brain to a more quiescent state.

### ***Exercise and the Aging Brain***

Arthur F. Kramer, PhD  
University of Illinois at Urbana-Champaign, Beckman Institute, USA

In 2008, the first comprehensive guidelines on physical activity based on extensive review of the scientific data were published by the US government. Dr Kramer's comments were organized around both animal and human research on physical activity and exercise. He described observational and randomized controlled human studies that have established the relationship between physical activity and cognitive maintenance in normal adults or in adults diagnosed with neurodegenerative diseases such as Alzheimer's or Parkinson's disease. Dr Kramer also cited more recent studies that have reported similar cognitive and brain benefits, as a function of exercise and physical activity, for children. Such findings are important to the understanding of lifestyle choices on cognitive and brain development as well as the impact of the increasing sedentary nature and levels of obesity observed for children in today's society. However, he said, many important questions remain unanswered, such as can a combination of nutrition and exercise bestow greater benefits to healthy minds and brains than either of these factors alone?

### ***Nutrition Effects in Learning and Memory in Animal Models***

#### ***Associative Learning and Long-Term Potentiation in Rodents: Effects of Nutrition***

José M. Delgado-García, MD, PhD  
National University Pablo de Olavide, Division of Neurosciences, Spain

For more than 60 years, acquired learning abilities have been assumed to be stored in the form of functional and/or structural changes in synaptic efficiency. Although there are many excellent studies in vitro of the electrophysiological processes and molecular events supporting activity-dependent synaptic changes, not much information is available on synaptic changes in strength during actual learning in behaving animals. Dr Delgado-García and his research team have shown that classical conditioning of eyelid responses in behaving mice increased the synaptic strength of the hippocampal CA3-CA1 synapse. He describes technical procedures used to study the firing and synaptic activities of selected brain sites during different types of associative learning tasks. He states that long-term potentiation evoked experimentally in laboratory animals shares some synaptic properties and

molecular mechanisms with learning-dependent changes in synaptic strength. Synaptic changes evoked by learning can be modified by environmental, social, and emotional factors, as well as by drugs and putative dietary ingredients.

***Taste Learning and Memory in Aging***

Milagros Gallo, PhD  
University of Granada, Institute of Neurosciences  
Centre for Biomedical Research, Spain

The effect of aging on taste memory is a complex mix of impaired, preserved, and enhanced functions. Research on safe taste recognition memory has pointed to the amygdala’s role in the taste neophobic response and its habituation when the taste is recognized as familiar and safe. However, the results are controversial regarding the impact of aging in taste neophobia, indicating a critical role of previous aversive experiences. Dr Gallo shares her research on the effect of aging in rodent taste memory and compares the brain mechanisms of taste and visual recognition memory. She concludes by explaining the need for further research involving the functional and anatomical dissociation among shared and independent recognition memory processes involving the temporal lobe and related areas.

Acronyms and Abbreviations

Aβ	.....	amyloid beta
AD	.....	Alzheimer’s disease
AHRQ	.....	Agency for Healthcare Research and Quality
AP-1	.....	activator protein 1
apoE	.....	apolipoprotein E
APOE-ε3	.....	apolipoprotein E3
APOE-ε4	.....	apolipoprotein E4
BDNF	.....	brain-derived neurotrophic factor
BMI	.....	body mass index
BSID	.....	Bayley Scales of Infant Development
C	.....	control
CARDIA	.....	Coronary Artery Risk Development in Young Adults
CB1	.....	cannabinoid (receptors)
CD	.....	cluster of differentiation
Cho	.....	choline
CI	.....	confidence intervals
CIBM	.....	Institute of Neurosciences, Center for Biomedical Research
CNS	.....	central nervous system
CR	.....	conditioned response
Cr	.....	creatine
CRP	.....	C-reactive protein
CS	.....	conditioned stimulus
CysDa	.....	cysteinyldopamine
D1	.....	dopaminergic (receptors)
DG	.....	dentate gyrus
DHA	.....	docosahexaenoic acid
DHBT-1	.....	dihydrobenzothiazine-1
DNMT-1	.....	DNA (cytosine-5)-methyltransferase 1
DTI	.....	diffusion tensor imaging
EEG	.....	electroencephalogram
EMG	.....	electromyographic

EPA.....eicosapentaenoic acid  
 ERPs.....event-related potentials  
 FADS1 .....fatty acid desaturase 1  
 FADS2 .....fatty acid desaturase 2  
 fEPSPs .....field excitatory post-synaptic potentials  
 HLE.....healthy life expectancy  
 HPA .....hypothalamic-pituitary-adrenal (axis)  
 IL.....interleukin  
 IL1/6 .....interleukin 1 and 6  
 IL-1 $\beta$  .....interleukin-1 beta  
 IQ.....intelligence quotient  
 ISCH.....ischemic  
 JNK.....c-Jun N-terminal kinase  
 LCPUFA.....long-chain polyunsaturated fatty acid  
 LE .....life expectancy  
 LPS.....lipopolysaccharide  
 LTP .....long-term potentiation  
 MAPK .....mitogen-activated protein kinase  
 MCI.....mild cognitive impairment  
 MEG .....magnetoencephalography  
 mGluR .....metabotropic glutamate receptor  
 MHC class II .....major histocompatibility complex class II  
 MMSE.....mini-mental state examination  
 MNG .....mangiferin  
 MOR .....morin  
 MPOD .....macular pigment optical density  
 MR.....magnetic resonance  
 MRE.....magnetic resonance elastography  
 MRI.....magnetic resonance imaging  
 MRS.....magnetic resonance spectroscopy  
 MTL .....medial temporal-lobe (structure)

MWM.....Morris water maze  
 NAA .....N-acetylaspartate  
 NF- $\kappa$ B .....nuclear factor kappa B  
 NHANES.....National Health and Nutrition Examination Survey  
 NIH .....National Institutes of Health  
 NMDA.....N-methyl-D-aspartate  
 NO .....nitric oxide  
 Nrf2.....nuclear factor erythroid 2-related factor 2  
 NUHEAL .....Nutrition and Health Lifestyle (study)  
 O.O. ....orbicularis oculi  
 OPAL .....Older People and n-3 Long-Chain Polyunsaturated Fatty Acids Study  
 ORs.....odds ratios  
 PI3K.....phosphoinositide 3-kinase  
 PPARG .....peroxisome proliferator-activated receptor gamma  
 PRh.....perirhinal cortex  
 pTfR .....placental transferrin receptor  
 RCTs.....randomized controlled trials  
 ROI .....region of interest  
 ROS.....reactive oxygen species  
 SGA .....small for gestational age  
 SNP .....single-nucleotide polymorphism  
 SOR.....spontaneous object recognition  
 St. ....stimulating (electrodes)  
 Sub .....subiculum  
 TLR.....toll-like receptor  
 TNF- $\alpha$  .....tumor necrosis factor alpha  
 US.....unconditioned stimulus  
 VBM.....voxel-based morphometry



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# Assessing Cognition, Brain Function, and Efficacy of Nutrition Interventions

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# Assessing Cognition and Brain Function

Neal J. Cohen, PhD

## Overview

Cognition and brain function can be assessed through a variety of powerful behavioral and cognitive neuroscience tools. In addition to assessment strategies that provide global measures of cognitive and brain health, it is possible to use more specialized tests to sample specific domains of cognition and brain function. In the work reviewed here, we target the assessment of memory specifically. But even the domain of memory is large, encompassing a range of capacities supported by multiple systems of the brain. The emphasis here is on one particular memory system, supporting relational (or declarative) memory, which depends critically on the hippocampus and related medial temporal-lobe (MTL) structures. The hippocampus and relational memory provide a favorable target for investigations of the effects of nutrition, because this memory system is highly modifiable, susceptible to damage or disruption, and also susceptible to enhancement by experience and by certain interventions, such as exercise or fitness. This paper highlights a few powerful and sensitive tests of relational memory and hippocampal function, and illustrates some findings that emphasize the modifiability of this system.

## Cognition and Brain Function

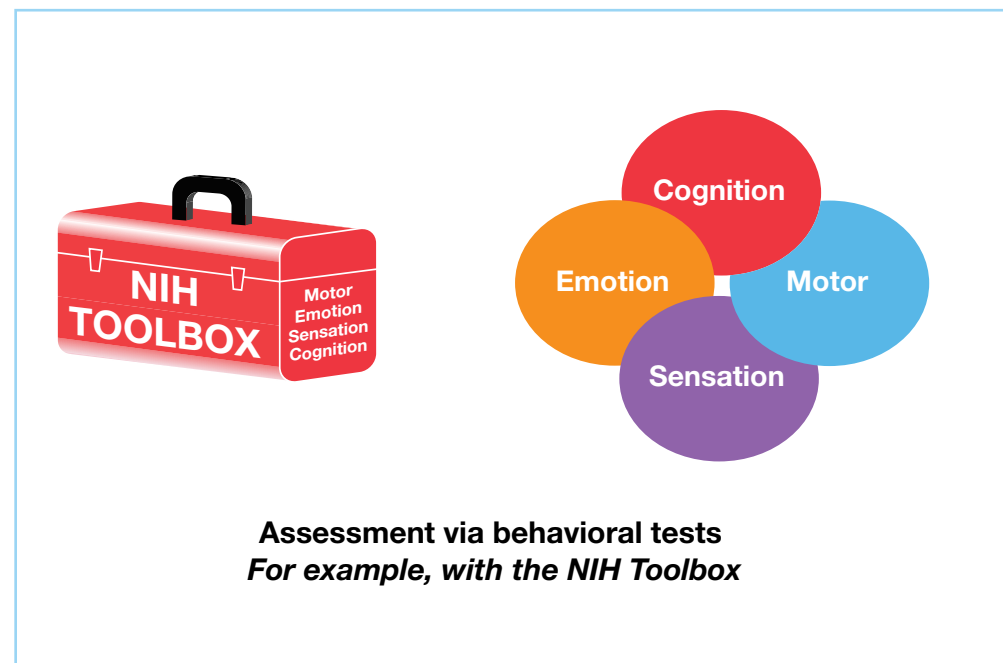
Assessment of the potentially beneficial effects of nutrition can be addressed using powerful assays of the status of cognition and brain function. Cognition refers to the abilities that derive from the acquisition, processing, and use of information or knowledge, and the mental processes that support them. Assessing cognitive function usually entails examining various behavioral performances and abilities. Cognitive functions are implemented in the biological processes of the brain: however, a fuller assessment of cognitive function also would include examination of the brain systems and brain mechanisms that underlie those mental processes, which is an approach favored in modern-day cognitive neuroscience.

## Assessment Strategies

The effects of various types of intervention, whether pharmaceutical, lifestyle choice (such as physical activity), or nutritional, may be assessed using very different approaches. Some intervention studies involve assessments conducted via purely

behavioral tests, while other assessments are directed at brain function more directly, through human electrophysiology and/or brain imaging. Another critical way in which assessment strategies differ is with regard to whether global measures of overall cognitive and brain health are used; or assessment of cognitive and brain function is broad and comprehensive, sampling across a range of different cognitive domains supported by disparate brain systems; or assessment is of a particular domain of cognition and brain function that is seen as an especially favorable target of the intervention.

Assessing multiple domains of cognition is often accomplished, when possible, with one or another ambitious battery of tests, each focused on one or another cognitive process or mental ability. One increasingly used example is the National Institutes of Health (NIH) Toolbox, which provides a computer-based examination of cognition that includes various measures of attention, episodic memory, working memory, language, executive function, and processing speed (Fig 1).<sup>1,2</sup>



**Fig 1. The National Institutes of Health (NIH) Toolbox is used to assess cognition and brain function.**<sup>1,2</sup>

Here, we focus specifically on memory, reflecting the emphasis of the Center for Nutrition, Learning, and Memory, University of Illinois at Urbana-Champaign (<http://cnlm.illinois.edu/>). In the remainder of this review we briefly consider the nature and organization of memory; introduce a particular form or type of memory and illustrate how to assess it with sensitive, targeted measures; and explain why it might be a favorable target for the beneficial effects of nutrition on brain and cognition.

### Multiple Memory Systems

Memory is itself a large domain, encompassing a collection of abilities that are supported by various brain mechanisms. Cognitive neuroscience research has shown that there are multiple memory systems in the brain.<sup>3,4</sup> Various taxonomies of memory have been proposed, but most investigators agree that important distinctions can be drawn between *declarative*, or *relational*, memory, and *procedural* memory. Declarative or relational memory refers to memory for facts and events, and the relations among them (eg, remembering the who, what, where, and when of experienced events, and the calendar of upcoming events; spatial layouts and maps; face-name pairings; and richly interconnected knowledge about various domains of interest, such as detective novels, or professional football, or Academy Award-winning movies, or nutritional supplements). Non-declarative or *procedural* memory refers to memory supporting the acquisition and expression of skills (eg, supporting ability in tennis, typing, dancing, wine tasting, dog judging, or detecting tumors in magnetic resonance imaging [MRI] scans).<sup>3,4</sup>

### Relational Memory

Relational memory depends critically on the hippocampus and related MTL structures. Why is this aspect or form of memory so important for our purposes? First, in supporting the ability to acquire, retain, retrieve, and flexibly use knowledge about facts and events, relational memory provides a critical foundation for many cognitive abilities, including aspects of language,<sup>5,6</sup> decision making<sup>7</sup> and other strategic behaviors,<sup>8</sup> inferential reasoning,<sup>9</sup> and creative thinking.<sup>10</sup> Accordingly, interventions that can alter relational memory will have broad consequences for cognitive function. Second, this memory system is highly modifiable, susceptible to damage or disruption in various neurological and psychiatric conditions, and also susceptible to enhancement by experience and by certain interventions, such as exercise or fitness. Accordingly, it may be a promising target for enhancement by nutrition.

## Assessing Relational Memory

We have developed several especially powerful and sensitive tests for assessing the status of relational memory and hippocampal function. In one, participants are shown a series of arbitrary face-scene pairings, and are subsequently tested with scenes on each of which are superimposed a test display with three equally familiar faces. The task is to identify the particular face that had been studied with that scene. Performance is assessed via both behavioral accuracy and eye movements. Tracking of eye movements has proven to provide a uniquely robust measure of memory.<sup>11</sup> The degree of preferential viewing of the “matching” face (the one that had been previously studied with that scene) relative to the other, equally familiar, faces in the test display constitutes the measure of relational memory.<sup>12</sup> Preferential viewing of the matching face occurs automatically and obligatorily early in viewing (within 500-750 msec)<sup>12</sup>; fails to occur in patients with amnesia following damage to the hippocampus<sup>12</sup>; is associated with hippocampal activity in functional MRI studies of normal participants, activity which predicts eye movements even when the behavioral response is inaccurate<sup>13</sup>; and is reduced in magnitude and delayed in onset in patients with schizophrenia,<sup>14</sup> a disorder associated with pathological changes of the hippocampus.<sup>15</sup>

A second test that has proven very sensitive to relational memory and hippocampal function involves spatial reconstruction of (even small) arrays of objects.<sup>16</sup> In this task, a particular type of memory error, involving the swapping of positions of pairs of objects (“swap errors”)—ie, failure to keep track of the arbitrary bindings of objects to their positions in the array—turns out to be diagnostic of hippocampal damage. Patients with amnesia following damage to the hippocampus made 40 times more errors of this type than did matched comparison participants, and the patients made swap errors even when only two objects had to be remembered across only a few seconds.<sup>16</sup>

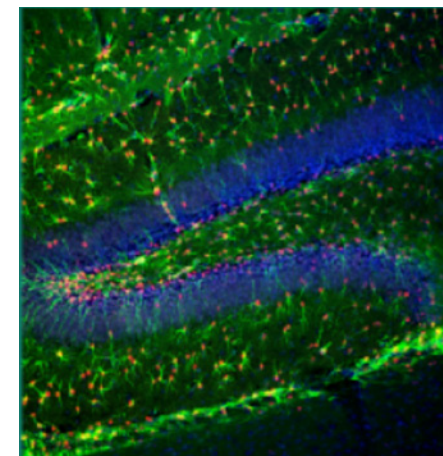
## Modifiability of the Hippocampus and Relational Memory

Hippocampal function and relational memory are highly susceptible to damage or disruption. In the examples above and in others,<sup>17,18</sup> damage to the hippocampus produced a deficit in memory that was highly selective, affecting memory for the relations among items while leaving intact memory for the items themselves tested individually.

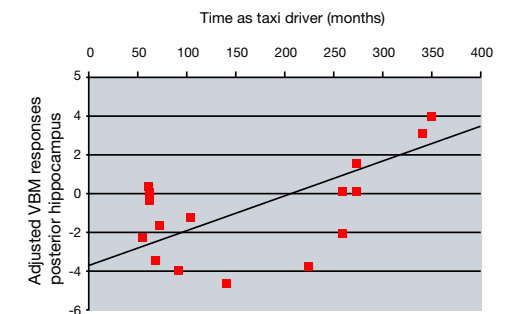
The modifiability of the hippocampus and relational memory also is seen in the opposite direction, with clear capability to show enhancement or growth. The hippocampus is one of only two structures in the brain that exhibit *neurogenesis*,

the addition of new neurons, in adulthood. This process is shown to occur even in humans.<sup>19,20</sup> In animals, neurogenesis is increased by exercise and by living in enriched environments, and has been shown to be associated with enhancement of synaptic plasticity and memory function.<sup>21</sup> In humans, the volume of the hippocampus likewise has been shown to increase in response to experiential factors and exercise or fitness. One well-known study found that the size of (posterior) hippocampus in London taxi drivers was proportional to the amount of experience they had in (months of) professional driving (Fig 2).<sup>22</sup>

### Changes in the Hippocampus



Visualizing creation of new neurons (*neurogenesis*) in the hippocampus



The volume of the hippocampus of London taxi drivers increased as a function of the number of years of experience driving

**Fig 2. Neurogenesis in the hippocampus of London taxi drivers.**<sup>22</sup>

VBM=voxel-based morphometry

**Source:** Maguire EA et al. Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci U S A.* 2000;97(8):4398-4403. © 2000 National Academy of Sciences, U.S.A.

Other studies show increased hippocampal volume in healthy elderly individuals who underwent an exercise intervention<sup>23</sup> and in higher-fit (relative to lower-fit) preadolescent children.<sup>24</sup> In the latter study, higher fitness and increased hippocampal volume were associated with better relational memory.



Finally, in rodents, there is promising evidence concerning the synergistic effects of nutrition plus exercise. Exercise reduced or overcame the negative effects of a diet high in saturated fats and refined sugar in the levels of brain-derived neurotrophic factors (BDNF) that are associated with synaptic plasticity, and the combination of exercise with dietary supplementation with docosahexaenoic acid (DHA) had stronger effects on spatial learning and on BDNF-mediated synaptic plasticity than did either factor alone.<sup>25-27</sup>

## Summary

Taken altogether, these studies suggest that the hippocampus and relational memory may provide a favorable target for investigations of the effects of nutrition such as dietary intake of omega-3 fatty acids, antioxidants, and flavonoids.<sup>26</sup> This memory system is highly modifiable, including in response to exercise or fitness interventions, and we have assessment tools powerful and sensitive enough to detect enhancement by nutrition.

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## Advances in MR Imaging and the Questions They Answer

Bradley P. Sutton, PhD (with Ryan Larsen, PhD, Joseph Holtrop, MS, and Curtis Johnson, PhD)

**M**agnetic resonance imaging (MRI) provides many windows into the physiology of the brain, providing sensitive measures to follow nutrition interventions. Three particularly promising techniques are discussed: magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), and magnetic resonance elastography (MRE). These techniques allow us to monitor changes in brain metabolism, neuronal connectivity, and tissue structure.

### MRS

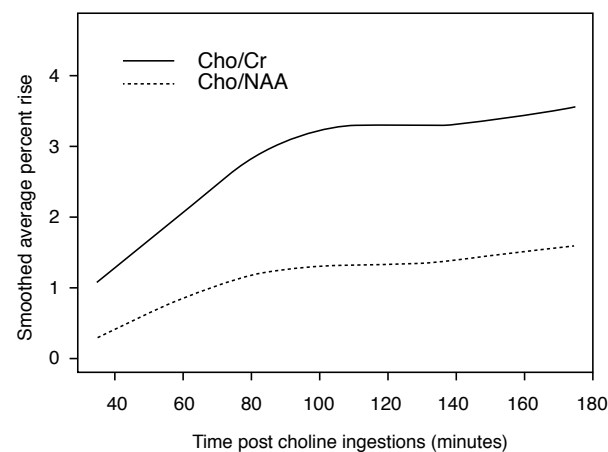
Nuclei with different chemical environments give off signals at different frequencies during an MRI experiment. In a normal MRI structural scan, the protons in water far outnumber other protons and the image mostly reflects water density, along with relaxation effects specific to different tissue compositions. However, with MRS, data acquisition occurs with water signal suppressed and a readout over time is obtained that is sensitive to these varying frequencies. With standard proton MRS, several metabolites are available that have sufficient concentration to be detected and quantified using their characteristic signatures in the frequency spectrum. These include N-acetylaspartate (NAA), glutamate, glutamine, creatine (Cr), choline (Cho), myo-inositol, along with a few others, depending on the experimental setup.<sup>1</sup>

MRS is sensitive to the functional state of the tissue, often demonstrating predictive power for future development trajectories or for future pathological deteriorations. In one study, infants were scanned at term with MRS, and the results indicated that cerebellar NAA/Cho ratio predicted performance on a cognitive test that was performed at 24 months.<sup>2</sup> In a study at the other end of the age-scale, Erickson and colleagues found that NAA levels declined with age in older adults, but that fitness compensated for the aging effect.<sup>3</sup> The researchers further found that NAA mediated the relationship between fitness and working memory performance.

During the long history of MRS, several nutrition studies have been conducted, demonstrating that MRS is sensitive to both acute and long-term dietary interventions. For example, Babb and colleagues performed a study in which healthy adults ingested Cho-containing compounds at a rate equivalent to 50 mg/kg.<sup>4</sup> Over the 3 hours of MRS scanning that followed the ingestion, Babb

et al found 6% increases in the Cho/Cr ratio and 3% increases in the Cho/NAA ratio (Fig 1). Over a longer term, Lyoo and colleagues had subjects ingest Cr monohydrate over the course of 2 weeks.<sup>5</sup> MRS of an axial slice of the brain demonstrated increases in brain Cr at scans after both 1 week and 2 weeks.

### Application to Nutrition: From Ingestion to the Brain



Small, but meaningful changes in brain concentrations need large, sensitive studies.

**Fig 1. Effects of choline ingestion on the brain.**<sup>4</sup> Subjects given equivalent of 50 mg/kg free Cho ingested orally. Magnetic resonance spectroscopy obtained 30 minutes to 3 hours post-ingestion. Six percent increase in Cho/Cr and three percent increase in Cho/NAA at peak.

Cho=choline, Cr=creatine, NAA=N-acetylaspartate

**Source:** Babb SM et al. Oral choline increases choline metabolites in human brain. *Psychiatry Res.* 2004 Jan 15;130:1-9. Reprinted by permission of Elsevier.

Current applications of MRS are limited by low signal-to-noise and low spatial resolution for resolving distributions of metabolites. Since specific regions of the brain often are associated directly with specific behavioral deficits, these limitations likely reduce the ability of MRS to detect functionally relevant changes. Several technologies hold great promise for increasing the spatial resolution of MRS to interrogate specific brain regions for functionally relevant measures of metabolism. These include advanced acquisition trajectories,<sup>6</sup> multinuclear acquisitions taking

advantage of other magnetic resonance-active nuclei, and denoising techniques based on prior spatial and temporal information.<sup>7,8</sup>

## DTI

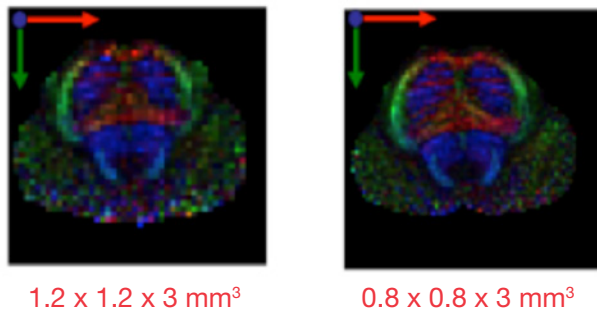
DTI acquisitions with MRI sensitize the image acquisition to small movements of diffusing water. With many barriers to water diffusion in a cell, including the cell membrane and myelin layers, the water diffusion in white matter is not isotropic. By examining this anisotropy, characteristics of neuron and myelin integrity can be examined.

Non-invasive measures of white matter integrity using DTI have seen increasing use in studies of age-related declines in cognitive behaviors.<sup>9-11</sup> These DTI measures have been verified in animal models showing that the diffusion properties in a neural fiber bundle give important information about the integrity and the myelination of brain fiber pathways.<sup>12,13</sup>

Although DTI measures very small displacements of water, it does so with a large volume averaging to summarize this information at the level of an imaging pixel. Sensitivity to fine structures of white matter is inhibited by large voxel sizes, requiring more complex acquisitions to estimate multiple axonal fiber types within a voxel. Our bioengineering group at the University of Illinois has developed high spatial resolution DTI acquisitions to try to resolve fine-scale structures to enable leveraging of simple models of the diffusion process (Fig 2).<sup>14</sup>

### High Spatial Resolution DTI

- Average diffusion properties within an imaging voxel—reduce partial volume effect
- Heterogeneity within a voxel: multiple fibers variation in direction, small lesions
- Precision depending on spatial resolution/ diffusion encoding scheme



**Fig 2. Some features of high spatial resolution diffusion tensor imaging (DTI).<sup>14</sup>**

**Source:** Karampinos DC et al. High-resolution diffusion tensor imaging of the human pons with a reduced field-of-view, multishot, variable-density, spiral acquisition at 3 T. *Magn Reson Med*. 2009 Oct;62(4):1007-1016. Reprinted by permission of John Wiley and Sons.

### MRE

The mechanical properties of biological tissues have been shown to vary greatly throughout tissues and in disease states. The shear modulus of brain tissue, in particular, varies significantly between and within white matter structures in the brain.<sup>15,16</sup> Recently, it was demonstrated through the use of MRE that the shear modulus of brain tissue decreases in neurodegenerative disease such as Alzheimer's<sup>17</sup> and normal pressure hydrocephalus.<sup>18</sup>

MRE uses slight mechanical actuation to induce small displacements in tissues.<sup>19</sup> The propagation of these displacements can be used to infer the mechanical stiffness of tissues, along with other mechanical properties. MRE of the brain requires high spatial resolution and high sensitivity to delineate wavelength effects in fine neural structures. We have developed techniques to enable localized MRE of white matter structures in the brain.<sup>20</sup> These developments will enable future studies of the impact of nutrition and brain chemistry on the structural integrity of specific brain areas.

### Conclusion

MRI provides many windows into the physiology of neural structures. MRS can be used to examine localized brain chemistry and metabolism, DTI to assess white matter axonal connectivity and integrity of the membranes and myelin, and MRE to provide information on the overall structural integrity of brain tissue. This multiparametric space allows for specific physiological questions to be asked with respect to the impact of a nutrition intervention on the brain.

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# Impact of Nutrition on Cognitive Development

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Carol L. Cheatham, PhD

# Early Programming of Brain Development

Cristina Campoy, Prof, MD, PhD

The concept of “programming” defines the role of environmental factors such as diet, socioeconomic status, environmental pollutants and toxins, personal lifestyle, and familial habits during early stages of life that influence optimal neurological,<sup>1</sup> psychological, and physiological development.<sup>2-4</sup> These programming mechanisms encompass the type of susceptibility to metabolic,<sup>2,5,6</sup> cardiovascular,<sup>7,8</sup> cancer,<sup>9</sup> bone,<sup>10</sup> and mental diseases.<sup>11</sup> Epigenetic programming is increasingly recognized as an important mechanism underlying health and disease. Exposure to diet, drugs, and early life adversity during sensitive windows of life can lead to lasting changes in gene expression that contribute to the display of physiological and behavioral phenotypes.<sup>12,13</sup> Diet is a potent modulator of epigenetic marks, especially during prenatal and early postnatal life. For example, it has been shown that diets high in choline, methionine, folate, and vitamins B<sub>6</sub> and B<sub>12</sub> increase DNA and histone methylation, alter gene expression, and can result in permanent changes in development.<sup>14</sup>

The human fetal brain grows to arrive at birth weighing 400 g. During the first 4 years of life, the brain continues to grow up to 1200 g (≈200 g less than an adult’s brain). During the next 10-15 years, brain growth continues, involving different brain compartments in a slightly different way. For example, the thickness of the different regions of the cerebral cortex changes between the ages of 5 and 18 years at different paces, with the regions important for reasoning, planning, and social communication maturing last.<sup>15</sup> Because the brain is one of the most sensitive organs to suffer malprogramming due to its long period of development and specialization, there are a number of critical windows. The consequences of early malprogramming of the brain affect its structure and the rest of body functions, because not only is the brain one of the most sensitive organs during development, it also is involved in the control of endocrine and inflammatory signaling from different brain-body axes, regulating all metabolic processes involved in growth and development.

Mothers may contribute to infant brain development and behavioral dispositions directly through milk constituents that build and fuel the brain (eg, long-chain polyunsaturated fatty acids [LCPUFAs]), or indirectly by providing the caloric



energy for infant activities and experiences that, in turn, shape brain development.<sup>16</sup> Recently, Herba et al<sup>17</sup> demonstrated that exclusive breastfeeding was associated with more optimal brain development than bottle feeding. Breastfed babies showed important structural differences in the brain and nonspecific differences in neural development compared to those who received infant formula. (This study, however, did not describe the type of infant formula used or indicate whether the formula contained supplemental LCPUFAs.)

### Brain Malprogramming by Early Malnutrition

It is well known that nutrients are vital to brain development, not only to the morphological development, but also to brain neurochemistry and neurophysiology. During late fetal and early neonatal life periods, regions such as the hippocampus, the visual and auditory cortices, and the striatum undergo rapid development characterized by the morphogenesis and synaptogenesis that make them functional.<sup>18</sup> Early malnutrition causes a decrease of cell proliferation, thereby affecting cell number,<sup>19</sup> volume, and width of the cerebral cortex.<sup>20</sup> Neurochemical alterations include changes in neurotransmitter synthesis, receptor synthesis, and neurotransmitter reuptake mechanisms.<sup>21</sup> Neurophysiologic changes reflect changes in metabolism and signal propagation.

Evidence from both epidemiological studies and animal models indicates that maternal diet and metabolic status play a critical role in programming the neural circuitry that regulates behavior. This happens directly by impacting the intrauterine environment and indirectly by modulating maternal behavior, resulting in long-term consequences for offspring behavior. Early malnutrition could predispose directly to externalizing behavior problems by impairing brain mechanisms such as those in the prefrontal cortex that are thought to regulate emotions and inhibit impulsive aggressive behavior.<sup>22</sup> Malnutrition also could predispose to externalizing behavior problems more indirectly by impairing cognitive functioning, which in turn predisposes to externalizing behavior problems.<sup>23</sup> Poor cognitive ability has been found consistently to predispose to externalizing behavior problems.<sup>24</sup> Malnourished children have less activity, more anxiety, and less imagination in solving a problem than well-nourished children.<sup>25</sup> Furthermore, malnourished children exhibit decreased exploration of the environment, as well as decreased verbal activity.<sup>26</sup> They are more deficient in arithmetic, standardized reading and vocabulary, as well as in literacy and general knowledge than well-nourished children.<sup>27,28</sup> Low-birth-weight children born to malnourished women show deficits in mental and psychomotor development indexes.<sup>24,29</sup>

Deregulation of the hypothalamic-pituitary-adrenal (HPA) axis and impaired stress response are common to different behavioral phenotypes such as depression and anxiety and visceral obesity.<sup>30</sup> Excessive release of steroids during vulnerable periods of life can be one of the mechanisms by which gut microbiota modulate HPA neuroplasticity,<sup>31</sup> and hence may enhance or reduce the risk of developing related disorders such as anxiety and depression later in adulthood. Offspring born to malnourished mothers also exhibit structural disorganization and malprogramming of the appetite-regulating system in the hypothalamus, central leptin resistance, and are predisposed to adiposity, displaying alterations in adipose tissue noradrenergic innervations and thermogenesis.<sup>32</sup>

Micronutrients play a determinant role in the development of brain substrates for language. In a recent study, thiamine deficiency during the 1st year of life was found to affect children's abilities selectively, yielding specific impairments in the language domains of syntax and lexical retrieval, without conceptual or general cognitive deficits.<sup>33</sup> Other nutrients such as iron have shown modest effects on psychomotor development in supplemented infants and toddlers <3 years of age,<sup>34</sup> although iron supplements do not seem to alter mental development or behavior.<sup>35</sup> Other studies have shown that perinatal iron deficiency produced an altered neurochemical profile of the developing hippocampus in children.<sup>36</sup> A recent study of the Avon Longitudinal Study of Parents and Children cohort within the NUTRIMENTHE EU Project showed that iodine deficiency was common (pregnant and breastfeeding women need 250 µg per day<sup>37</sup>), affecting two-thirds of women in the UK. Their children went on to have slightly lower IQs at the age of 8 years and worse reading ability at age 9. Three-point IQ differences were found between children who were born to mothers with low iodine in early pregnancy and children who were born to mothers above the cut-off. All these studies showed that an individual nutrient deficiency resulted in the impairment of multiple systems, and the development of the brain was influenced by various nutrients simultaneously.

Systematic reviews and meta-analysis have shown that the use of multivitamin-containing folic acid supplementation during pregnancy is associated with no benefit to the mental performance in children.<sup>38</sup> However, the Generation R study within the NUTRIMENTHE EU Project demonstrated that use of folic acid supplements protected from both internalizing and externalizing problems.<sup>39</sup> Low maternal folate status during early pregnancy was associated with a smaller head circumference, smaller transcerebellar diameter, and higher risk of emotional problems in the offspring.<sup>40</sup> In India, a recent study showed that low maternal vitamin B<sub>12</sub> and high folate status could contribute to the epidemic of adiposity and type 2 diabetes.<sup>41</sup>





Meta-analysis and meta-regression analysis have shown that the beneficial properties of LCPUFAs remain probable but not convincing for a robust effect on visual acuity, cognition, and mental performance.<sup>1,42</sup> The Nutrition and Health Lifestyle (NUHEAL) study<sup>43</sup> has demonstrated that arachidonic acid status at birth is a better predictor of visual development at 5.5 years of age than LCPUFA status. Folic acid status during pregnancy also is related to processing speed, working memory, and attention in children at 8 years of age. Recently, new confounder factors such as common polymorphisms of the genes fatty acid desaturase 2 (FADS2, encoding Δ-6 desaturase) and FADS1 (encoding Δ-5 desaturase) are being considered as important in the evaluation of long-term effects of early nutrition. These polymorphisms are found in about one quarter of the European population and are associated with markedly reduced plasma LCPUFA concentrations.<sup>44</sup> First results suggest marked effects of genetic variation in the FADS gene cluster on relevant clinical end points, including cognitive development.<sup>45</sup> Koletzko et al,<sup>46</sup> within the NUTRIMENTHE EU Project, showed a consistent significant association of rare single-nucleotide polymorphism (SNP) alleles with lower amounts of docosahexaenoic acid (DHA) in red blood cell phospholipids of pregnant women, which may be of major relevance for child outcomes. It is tempting to speculate that genetic heterogeneity in fatty acid metabolism may be one of the reasons for the apparent inconsistent results of different studies that investigated effects of DHA perinatal supply on developmental outcomes.<sup>1</sup>

The following table summarizes recently published systematic reviews and meta-analyses of early nutrition interventions on mental and motor development in infants and toddlers.

**Table. Early Nutrition and Mental and Motor Development: Recently Published Systematic Reviews and Meta-Analyses**

Intervention	Reference	Population	Conclusion
Breastfeeding	US AHRQ <sup>47</sup>	Term infants	NS
	US AHRQ <sup>48</sup>	Preterm infants	No definitive conclusion
	Herba et al, 2013 <sup>17</sup>	Term infants	Positive structural effects
LCPUFA	Simmer et al, 2008 <sup>49</sup>	Preterm infants	NS
	Smithers et al, 2008 <sup>50</sup>	Preterm infants	Findings varied according to whether BSID-I or BSID-II was used
	Simmer et al, 2008 <sup>51</sup>	Term infants	NS
	Campoy et al, 2012 <sup>1</sup>	Term infants	NS
	Gould et al, 2013 <sup>42</sup>	Term infants	NS
Iron	Logan et al, 2001 <sup>52</sup>	Infants & toddlers	Modest effect
	Sachdev et al, 2005 <sup>34</sup>	<3 years	No convincing evidence
	Szajewska et al, 2010 <sup>35</sup>	Infants & toddlers	Modest effect
Multiple micronutrients	Eilander et al, 2010 <sup>53</sup>	0-18 years	Fluid intelligence NS
	Skórka et al, 2012 <sup>38</sup>		Crystallized intelligence NS Other cognitive domains NS

AHRQ=Agency for Healthcare Research and Quality, NS=not significant, BSID=Bayley Scales of Infant Development

Brain Malprogramming by Mother’s Obesity

Obesity and associated comorbidities (eg, metabolic syndrome and diabetes) constitute a major health concern among diet-related diseases worldwide, with a prevalence of about 30% expected in the EU population aged 40-65 years by 2015, which is similar to the situation in the United States. Consequences of obesity for mental health and cognitive development are not established to the same degree as those for chronic diseases.

The proportion of reproductive-aged women who are obese in the EU and US populations is increasing sharply. Approximately 60% of women desiring pregnancy in the US are overweight, with the incidence rising exponentially over the last 15 years.<sup>54</sup> The percentage of pregnant women in EU countries such as UK, Italy, and Spain who are overweight or obese can be as high as 30%, rising

to nearly 50% in older women. This indicates that the obesity epidemic could become accelerated through different generations independently of other genetic or environmental factors.<sup>55</sup> Maternal obesity, a diet rich in calories, or excess gestational weight gain may lead to lifelong risk of obesity and related disorders in the child.<sup>56</sup> Overweight and obese women experience poorer reproductive outcomes than normal-weight women, including increased rates of infertility and pregnancy loss, as well as fetal and neonatal problems such as developmental delay and neurological deficits, respectively.<sup>57-60</sup> Obese women commonly deliver macrosomic infants, but it has been reported an overall incidence of small-for-gestational-age (SGA) births at 18%—significantly higher than 10% in the general population.<sup>61</sup> In addition, children born to obese mothers are more likely to acquire childhood obesity. There is also an increase in perinatal death rates, and babies who are large at birth have nine times higher risk of becoming obese as adults.

In relation to early programming of brain development, obese women have a significantly increased risk of neural tube defects, cardiovascular anomaly, septal anomaly, cleft palate and cleft lip and palate, anorectal atresia, hydrocephaly, and limb reduction anomaly.<sup>59</sup> Maternal obesity predisposes their infants to a greater risk of neurodevelopment delay<sup>62</sup> and atypical neurodevelopment<sup>63,64</sup> than those born to healthy, lean women. Offspring exposed to maternal obesity and high-fat diet consumption during development are more susceptible to developing mental health and behavior disorders such as anxiety, depression, attention deficit hyperactivity disorder, and autism spectrum disorders.<sup>65</sup> A recent review examining 12 studies concluded that the offspring of obese women may be at increased risk of behavior and cognitive deficits in childhood, as well as eating disorders in adolescence and psychotic disorders in adulthood.<sup>66</sup> In response to such findings, the Institute of Medicine has highlighted neurodevelopment as an important potential long-term consequence of gestational weight gain that needs further investigation.<sup>67</sup> Offspring of overfed or obese mothers also exhibit malprogramming of the appetite-regulating system in the hypothalamus, show central leptin resistance, and are predisposed to adiposity.<sup>30,32</sup>

In the PREOBE study (PREOBE Excellence Project - P06-CTS-02341) in Spain,<sup>68</sup> 51% of the obese mothers showed iron deficiency at delivery, compared to 25% of healthy, lean pregnant women. There was an association between iron deficiency and an increase of birth weight. The mechanisms underlying this effect are unknown, but there are several hypotheses: a) a greater demand for iron from a larger newborn,<sup>69</sup> b) an increase of placental vascularity determining an increase of nutrient transport,<sup>70</sup> and c) an increased incidence of newborns large for gestational age in anemic mothers.<sup>71</sup> The placental transferrin receptor (pTfR)

expression was not significantly related to maternal preconceptional body mass index (BMI), and was higher in iron-deficient women, independently of mothers' preconceptional BMI.<sup>72</sup> In placental tissue, the genes encoding important proteins such as peroxisome proliferator-activated receptor gamma (PPARG) and toll-like receptor 4 (TLR-4), involved in fatty acids transport and energy regulation, were overexpressed and DNA (cytosine-5)-methyltransferase 1 (DNMT-1), as a biomarker of DNA methylation, was higher in overweight/obese and diabetic mothers, indicating the adaptation to the metabolic environment and the presence of epigenetic effects. The follow-up of the PREOBE children showed that babies born to obese mothers at 6 months of age scored higher in cognition, communication and language skills (Bayley III) than those born to healthy mothers, but this effect disappeared by 18 months. An adequate weight gain during pregnancy was related to better psychomotor score at 6 months of age in the offspring.

The understanding of the mechanisms associating early nutrition and later healthy brain developmental outcomes may have an enormous preventive potential, given the major public health implications, including opportunities for an improvement of cognition and an effective primary prevention of childhood and adult behavior and mental diseases.

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## Measuring the Impact of Nutrition on Cognitive Development

Carol L. Cheatham, PhD

**H**uman brain development begins at conception. However, the influence of nutrition on brain development begins *before* conception and continues for many years. Certain nutrients are needed before a child is conceived if fetal development is to proceed in a typical manner. After a brief overview of a few of these nutrients, this summary focuses on the effects of nutrition on cognitive development. The rationale for studying the effects of nutrition on two specific cognitive abilities—memory and speed of processing—are outlined. The importance of nutrition to cognition in general cannot be overstated as memory is central to learning, and speed of processing underlies all cognitive abilities. Also, this summary illustrates behavioral and electrophysiological methods of measuring the effects of nutrition on infant memory (behavioral) and speed of processing (electrophysiological).

### Nutrition and Fetal Development

The best way to ensure a healthy pregnancy and good outcomes for the infant is to start with a healthy well-nourished mother. There is evidence that when mothers are underweight at conception they are at risk of having a baby who suffers from intrauterine growth restriction, even if they gain sufficient weight during pregnancy.<sup>1</sup> However, the opposite—overweight at conception—puts the mother at risk of gestational diabetes and preeclampsia and of having an overly large baby that requires a caesarian section.<sup>2,3</sup> However, there is no empirically derived recommendation for maternal weight for height at conception.

Malnourished women may experience difficulty getting pregnant as the body senses that the host is not prepared for gestation.<sup>4</sup> Those malnourished women who do get pregnant have increased risk of miscarriage or of giving birth to infants with developmental issues. One nutrient that has long been known to be important before conception is folate, which is related to neural tube closure. However, after 30 years of fortifying the food supply with folate, related birth defects are still occurring. New evidence indicates that choline, methionine, and betaine may also be important for neural tube development.<sup>5</sup> Ensuring that all these nutrients are in sufficient supply at conception may decrease the incidence of neural tube disorders.



Iodine is another nutrient important enough to ensure preconception sufficiency by fortifying the daily food supply (ie, salt). Iodine deficiency can result in cretinism. The sequelae of cretinism are due to the inability of the mother to produce enough thyroid hormone in those crucial first few months when the fetal thyroid is not yet functioning. Because thyroid hormones are involved in neurogenesis and neuronal migration, as well as in several other neuronal processes, the effects of iodine deficiency can be globally pervasive in the brain. Evidence indicates that sufficient iodine is important preconceptionally. In a study in Ecuador, one village was treated with iodine every 4 years and another was left alone. Intelligence quotients (IQs) of the children at 11 years of age were compared. Mean IQs were higher in the treated village, but interestingly, if the treatment occurred before pregnancy or in the 1st trimester, the difference in IQ was a full 11 points.<sup>6</sup> If treatment was administered later in pregnancy, there was little effect.<sup>7,8</sup>

Malnourished women who become pregnant are also at risk for preterm delivery due to iron deficiency.<sup>9</sup> In addition, iron deficiency is the leading cause of mental deficiencies worldwide. A fetus can become iron deficient in many different ways. If the fetus is experiencing intrauterine growth restriction, is small for gestational age, or if the mother develops gestational diabetes or preeclampsia, the fetus will suffer from iron deficiency.<sup>10,11</sup> Iron deficiency has been studied extensively, and we know that low iron in the last few months of pregnancy and just after birth is related to a disruption in recognition memory. If the deficiency happens later—around 9-10 months of age—it will disrupt myelination and speed of processing. Thus, timing of a deficiency is important.

Nutrition and Cognitive Development

From midgestation to 2 years after birth, the brain is developing rapidly. It triples in weight, and the 100 billion neurons that are present at birth each form 15,000 synapses (Figure).

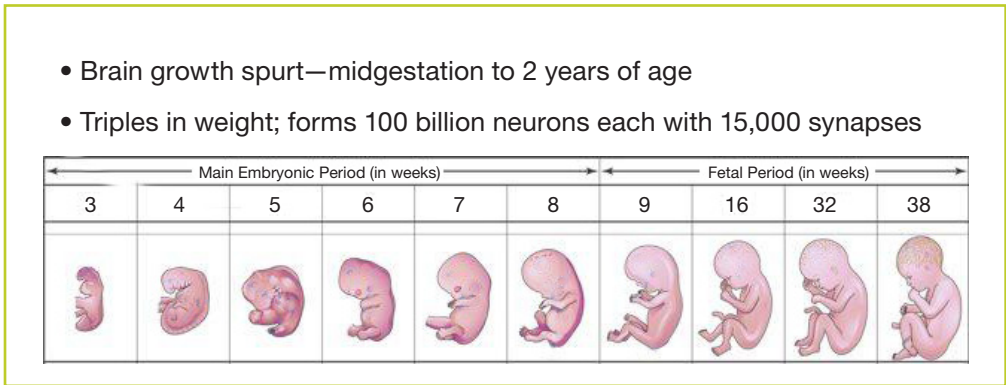


Figure. Critical periods in fetal and infant brain development.

Source (illustration only): <http://sara1hays.files.wordpress.com/2008/02/criticalperiodshumandevlopment1.jpg>.

The brain is most susceptible to insult when it is developing most rapidly and thus, as illustrated above, timing of the deficiency is central.<sup>12</sup> It follows, then, that during the prenatal months and the first 2 years of life, any nutrient deficiencies will have an enormous effect on the developing brain. Research with animals has shown that nutrient deficiencies rarely (except in the case of iodine) cause issues with the entire brain.<sup>13</sup> Some structures are affected permanently, and the hippocampus and cerebellum are the most vulnerable.<sup>13</sup> We would expect the behaviors subserved by the hippocampus, and the cerebellum to be affected; in the case of the hippocampus that would be memory functions, and in the case of the cerebellum, motor coordination and procedural learning. In the individual, malnutrition has been shown to have an acute effect on the actions of neurotransmitters and the functions of receptors.<sup>13</sup> At this cellular level, the link to receptors and neurotransmitters indicates that we would want to test speed of processing. Thus, from animal research of nutrient deficiencies, we see that memory and speed of processing are good candidate cognitive abilities to assess the effects of nutrition on human cognition.

Speed of processing can be implied from many behavioral assessments. However, by using an electrophysiology paradigm known as event-related potentials (ERPs), it is possible to document processing speed directly. ERPs are recordings of electroencephalograms (EEGs) that are time-locked to stimuli. The EEG is recorded and the differences in activity between the recording and a reference are extracted. The stimuli can be auditory or visual, and the number of recording electrodes varies from just a few to as many as 128 for an infant assessment. In the simplest of descriptions, the random background noise, which includes interference from



ambient electrical noise, is identified at the reference and is subtracted from the other leads, leaving the electrical potential of the brain that is related to the stimulus event. ERP has great temporal resolution, but we cannot assess individual trials. The segments of each condition within a participant are averaged together, and then data from the participants are averaged together to arrive at a grand mean. The resulting waveform includes information about latency to processing and processing time for each experimental condition (Table).

**Table. Extracting the Signal Using the Electrophysiology Paradigm Known as Event-Related Potentials (ERPs)**

- ERP generation is based on differences in voltage between electrodes and a reference
  - EEG background noise should average to zero
  - The residual activity represents brain activity to the event
  - Limitation: cannot access individual trials
- Compare grand means of conditions

The hippocampus-based cognitive ability known as declarative memory is supported by the temporal cortices and cortical areas, specifically the association areas and the frontal and prefrontal lobes.<sup>14</sup> The hippocampus and the surrounding cortices serve as a clearinghouse for memories, effectively interconnecting and consolidating information. In humans, hippocampal development starts around 16 weeks gestation and continues at least into the 3rd year of life,<sup>15</sup> not reaching adult morphology until the child is 5 years of age.<sup>16</sup> Hippocampal neurons change substantially in size and shape across the first year of life.<sup>15</sup> Also during the 1st year of life, there is evidence of the development of dentate connectivity<sup>16</sup> and neocortical connectivity.<sup>17</sup> The ability to differentiate familiar and novel stimuli (recognition memory) is evidenced in the first 6 months of life and is dependent on the integrity of the parietal lobe (attention) and the parahippocampal structures in the temporal lobes (recognition memory).<sup>18,19</sup> At 6 months, the hippocampus uses the same levels of glucose as an adult hippocampus.<sup>16</sup> The hippocampus proper and, more specifically, the dentate gyrus are crucial for encoding, storing, and recalling sequences of actions.<sup>20</sup> The hippocampus becomes functionally mature when it reaches peak synaptic density between 12 and 15 months of age.<sup>16</sup>

In the months prior to this (7-12 months of age), we would expect, and do indeed find, wide variation in longer-term declarative memory (ie, memory that is acquired rapidly, is subject to forgetting, is flexible, and can be articulated). A developmental shift occurs between the ages of 9 and 10 months in declarative memory abilities,<sup>21</sup> which coincides with the maturation of the dentate gyrus and the coalescing of the temporal-frontal circuit. Thus, cognitive development maps onto the development and functionality of the temporal lobes (hippocampus and surrounding cortices), parietal lobes, and frontal lobes, thereby supporting the structure-function relation.

How do we test the declarative memory abilities in infants who cannot offer verbal reports? Elicited and deferred imitation have become accepted as nonverbal assessments of declarative memory. That these paradigms measure declarative memory has been shown empirically. The definition of declarative memory is basically a memory that was acquired quickly, is subject to forgetting, and is flexible. A memory of an imitated event can be acquired in one trial.<sup>22</sup> Memories for the events fall out in a typical forgetting curve,<sup>23</sup> and they are flexible.<sup>24</sup> Finally, McDonough and colleagues have shown that adults with amnesia that affects declarative memory processes do, in fact, fail an age-appropriate analog.<sup>25</sup> So, we are confident that this behavioral measure actually is testing hippocampal function.

In an imitation protocol, 3-dimensional props are used to produce novel multistep sequences comprising multiple actions. Participants are tested for recall of specific sequences immediately (a measure of initial encoding), after a delay of a few minutes (a measure of the ability to successfully transfer information to long-term memory stores), and/or after a delay of weeks or months (a measure of the ability to maintain and retrieve a long-term mnemonic trace). The number of steps and the length of delays depend on the age of the participants<sup>26</sup>: the older the child, the longer the delay, and the higher the number of steps that can be tolerated. Administration of the task begins by offering the child the props that comprise one event to get a baseline measure of activity. This is followed by the demonstration of the target actions by the researcher and an opportunity for the child to imitate either immediately, after the prescribed delay, or both. Two dependent variables are determined from offline coding of the session video, the number of actions produced and the number of pairs of actions produced in the correct order. Recall of the proper ordering of the steps is the most challenging for the children.

ERP paradigms and imitation paradigms have been used in research with infants of mothers with diabetes who are inherently iron deficient and whose infants have received varying levels of the fatty acid docosahexaenoic acid (22:6n-3; DHA). ERP paradigms have proven to be sensitive to small fluctuations in nutrients

and subsequent cognitive development as early as the 1st week of life.<sup>27-30</sup> Imitation paradigms also differentiate between infants with different nutritional backgrounds.<sup>31-33</sup> Thus, nutrition researchers should work with developmental cognitive neuroscientists to use these methodologies to determine the effects of nutrition on brain development. Proper nutrition for fetuses, infants, and children can help ensure that children have a chance to achieve their cognitive potential.

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# Lutein’s Influence on Cognitive Development and Function

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## Emerging Science on Lutein in the Brain

Elizabeth J. Johnson, PhD

**L**utein and its isomer zeaxanthin are dietary carotenoids that are preferentially taken up into the central region of the retina, referred to as the macula.<sup>1</sup> In the macula, lutein and zeaxanthin are referred to as macular pigment and are believed to prevent damage that leads to age-related macular degeneration,<sup>2</sup> a leading cause of visual impairment and blindness in the United States.<sup>3,4</sup> The mechanisms by which lutein is thought to be protective are as an antioxidant and blue (visual) light filter.<sup>2</sup> Recently it has been suggested that lutein also may play a role in age-related cognitive function and early neural development.<sup>5</sup> The rationale behind this comes from the following observations: 1) lutein is the predominant carotenoid in human brain tissue,<sup>6,7</sup> 2) primate retinal lutein concentrations—ie, macular pigment—are related to brain lutein concentrations,<sup>8</sup> 3) macular pigment is related to cognitive function in adults,<sup>9</sup> and 4) lutein supplementation in adults improves cognitive function.<sup>10</sup>

### Lutein as a Component of Neural Tissue

Lutein and zeaxanthin are xanthophyll carotenoids commonly found in green, leafy vegetables and brightly colored fruits. These plant pigments are distributed ubiquitously in body tissues but tend to be the dominant carotenoids in central nervous tissues. For example, lutein and zeaxanthin are the sole carotenoids in the macula of the primate retina (macular pigment), where they exist in approximately 500-fold higher concentrations than in other body tissues such as serum and are believed to be protective through their roles as blue light filters and antioxidants.<sup>2</sup>

Lutein is also among the dominant carotenoids in human brain tissue, where it accounts for 30%–60% of total carotenoid concentration.<sup>6,7,11,12</sup> Cortical lutein and zeaxanthin are likely protective in nature and also may influence interneuronal communication and function via multiple mechanisms. Although the molecular basis of these neuroprotective effects of lutein remains unknown, several mechanisms have been proposed such as decreased oxidative stress, activation of anti-inflammatory pathways,<sup>13–16</sup> and modulation of functional properties of synaptic membranes along with changes in their physicochemical and structural features.<sup>17</sup> Lutein also has been shown to enhance gap junctional communication,<sup>18</sup> which in the retina is important for light processing and may be important for the development of neural circuitry in the visual system. Lutein, as macular pigment,

increases visual processing speed and reduces scotopic noise (noise associated with vision under dim light conditions).<sup>19-21</sup>

### Adults

Lutein plays a role in human health through its actions as an antioxidant, anti-inflammatory, and blue light filter. These characteristics provide biological plausibility to a relationship with many age-related diseases. The strongest evidence is in support of visual health. Given that the eye is an extension of the neural system, lutein may have a role in cognitive function.

Epidemiological studies indicate that dietary lutein and zeaxanthin may be of benefit in maintaining cognitive health. As stated previously, among the carotenoids lutein and zeaxanthin are the only two that cross the blood-retina barrier to form macular pigment in the eye.<sup>1</sup> They also preferentially accumulate in human brain.<sup>6,7,11,12</sup> Lutein and zeaxanthin in the macula were found to be significantly correlated with their levels in matched brain tissue.<sup>8</sup> Therefore, macular pigment can be used as a biomarker in brain tissue. This is of interest given that a significant correlation was found between macular pigment density and global cognitive function in healthy older adults.<sup>9</sup> Examination of the relationship between cognition and lutein levels in brain tissue of decedents from a population-based study of adults found that among the carotenoids, only lutein was consistently associated with a wide range of cognitive measures.<sup>11</sup> Furthermore, in a double-blinded, placebo-controlled trial of 4 months in women involving lutein supplementation (12 mg/d), alone or in combination with docosahexaenoic acid (DHA, 800 mg/d), verbal fluency scores improved significantly in the DHA, lutein, and combined treatment groups. Memory scores and rate of learning improved significantly in those in the combined treatment group, who also displayed a trend toward more efficient learning.<sup>10</sup> Given these observations, the idea that lutein can influence cognitive function is feasible.

### Infants

Current evidence supports a role for lutein in neural health (visual and cognitive function) in the adult. While it is unknown whether this role is specific to the adult, the proposed mechanisms by which lutein may exert its effect (antioxidant, anti-inflammatory, and structural) would apply to the infant. In fact, in a randomized, double-blind, placebo-controlled study of healthy term newborns, supplemental lutein was found to significantly increase serum measures of antioxidant activity.<sup>22</sup> This is particularly important in the early neonatal period when oxidative stress

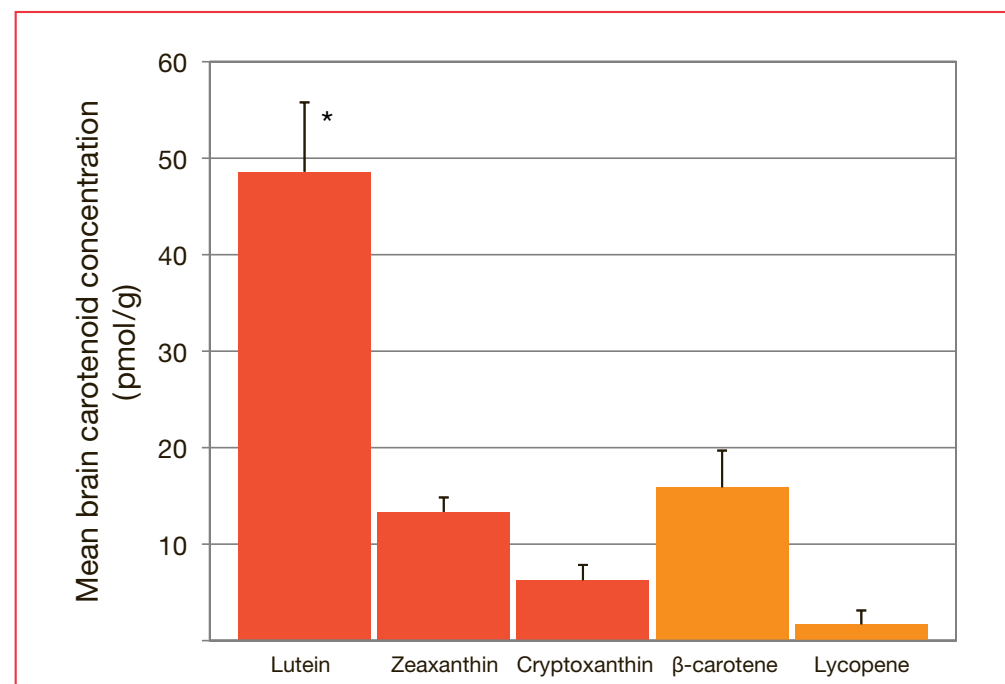
plays a crucial role in pathological conditions. Antioxidants are essential to the retina and brain, particularly because of the high metabolic rate of these organs. The human newborn brain has a relative deficiency of endogenous antioxidant enzymes.<sup>23</sup> In addition, neuronal membranes are rich in polyunsaturated fatty acids, which are highly oxidizable. Thus, the placement of an antioxidant in a highly oxidizable environment would be of benefit.

Studies in adults strongly suggest that macular pigment improves visual performance.<sup>24-28</sup> Optimal visual performance in early life could influence brain development, which is rapid in the 1st year.<sup>29</sup> Environmental enrichment, as would occur with visual cues, has long been investigated as an influence on brain structure and function. Morphological and functional effects elicited by environmental enrichment at the neuronal level have been reported to be accompanied by improvements in cognitive performance.<sup>30</sup> Lutein's role in early neural development has been a focus of recent research. The first step in addressing such a role is an assessment of infant brain concentrations.

### Distribution of Carotenoids in Pediatric Brain Tissue

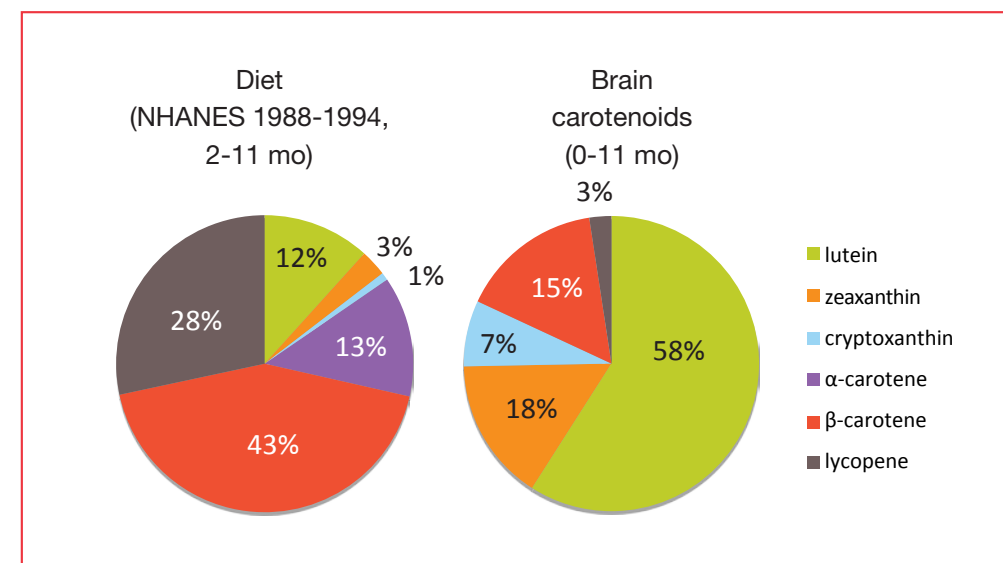
To assess brain concentrations of carotenoids, brain tissue from 30 subjects who died during the 1st year of life was assessed for carotenoids using standard methods.<sup>6</sup> Brain tissues (hippocampus, frontal, auditory, and occipital cortices) were obtained from a federally funded tissue bank. The cause of death was sudden infant death syndrome or another condition. There was significantly greater accumulation of xanthophylls (lutein, zeaxanthin, and cryptoxanthin) compared to carotenes ( $\beta$ -carotene and lycopene) in all four regions of the brain ( $P < 0.05$ ). Lycopene was detected in only two decedents. Alpha-carotene was not detected in any tissues. The average concentration of lutein in all four brain regions was significantly greater than that of the other dietary carotenoids (zeaxanthin, cryptoxanthin,  $\beta$ -carotene, and lycopene) (Fig 1).<sup>6</sup>





**Fig 1. Carotenoid concentration in the brain (mean of the four regions) from 30 infant decedents.**<sup>6</sup> \*Significantly different from all other carotenoids at  $P < 0.05$ . No  $\alpha$ -carotene was detected. Lycopene was detected in only two decedents.

As in the adult brain,<sup>11</sup> lutein was the major carotenoid in infant brains. However, the relative contribution of lutein to total carotenoids was approximately twice that of the adult (58% vs 31%, respectively), indicating a possible additional role of lutein in early neural development. Evaluation of dietary carotenoids in infants 2-11 months (National Health and Nutrition Examination Survey [NHANES] 1988-1994) shows a much different pattern, with  $\beta$ -carotene being the major dietary carotenoid (43% total) followed by lycopene (28%),  $\alpha$ -carotene (13%), and lutein (12%).<sup>31</sup> The respective values in infant brain tissue were 15%, 3%, 0%, and 58% (Fig 2) (Vishwanathan et al, unpublished data, 2013).



**Fig 2. Dietary carotenoid intake versus brain carotenoids (percent total carotenoids).**<sup>31</sup>

NHANES=National Health and Nutrition Examination Survey

Source: Vishwanathan R et al, unpublished data, 2013.

These data strongly suggest a preferential uptake of lutein into infant neural tissue. In part, this may be due to a preferential uptake of lutein into breast milk.<sup>32,33</sup> Further substantiating a preferential uptake of lutein into the infant brain is the observation that in two decedents lutein and zeaxanthin were the only carotenoids in brain tissue. None of the decedent brain tissues that had other carotenoids lacked lutein.

## Conclusion

Lutein is the predominant carotenoid in pediatric and adult brain tissue. Lutein in neural tissue has biological effects including antioxidant, anti-inflammatory, and structural actions. In infants' brains, the contribution of lutein to the total carotenoids is twice that found in adults, accounting for more than half the concentration of total carotenoids. In the adult, a variety of evidence supports a role for lutein in cognition. Therefore, the greater proportion of lutein in the pediatric brain suggests a need for lutein during neural development. Infant formula is not routinely supplemented with lutein, whereas breast milk is a highly bioavailable source of lutein.<sup>34</sup> The significantly higher scores for cognitive development in breastfed infants compared with formula-fed infants, as reported in a meta-analysis



of 11 studies, may be attributable in some part to the differential in carotenoid uptakes, including lutein uptakes, between the two groups.<sup>35</sup> Consequently, further investigation of the impact of lutein intake on neural development is warranted. Given that the 1st year of life is a time of neural growth and development for which nutrition can have significant consequences, the addition of this dietary plant pigment to infant formulas could be an important strategy toward long-term health outcomes.

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## Lutein's Influence on Neural Processing Speed

Billy R. Hammond Jr, PhD

**T**he macular carotenoids lutein and zeaxanthin influence many aspects of central nervous system function. These effects extend from optical filtering within the eye to physiological activity of neurons within the brain. Postreceptorally, they have been linked to faster visual processing speeds as assessed by numerous tasks. Macular pigment optical density (MPOD), for instance, is significantly ( $P < 0.05$ ) related to fixed and variable reaction time, coincidence anticipation errors (estimating the arrival of a stimulus at a target location moving at varying velocity), and balance ability.<sup>1</sup> Reduced processing speed is a central feature of cognitive decline, and current data suggest that higher MPOD (likely a biomarker for more central levels of lutein and zeaxanthin) is related to preservation of cognitive function.<sup>2</sup> Taken together, the multiple effects of lutein on nervous system function manifest throughout life and address vulnerabilities that also appear to change with age, eg, increased actinic stress to the retina in infants and the elderly.

### Overview

A growing body of evidence suggests that lutein and the zeaxanthins can enhance neural processing speed. This is particularly important for the elderly since slowing appears to be a central feature of cognitive decline and impairment. This summary focuses on the mechanisms by which lutein could produce such effects.

### Sensory Decline Is the Gateway to Cognitive Decline

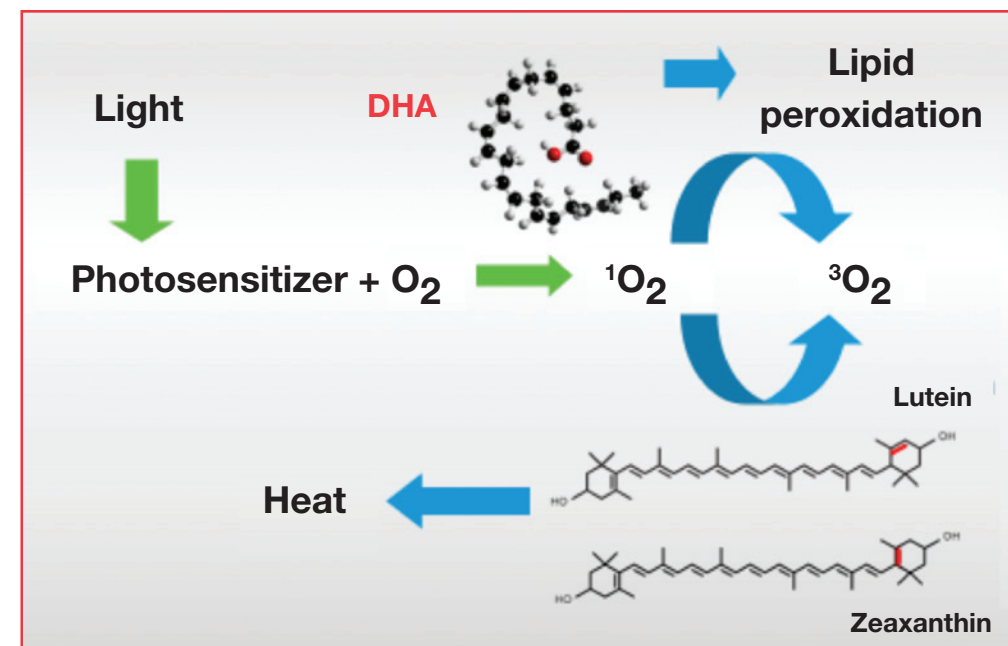
Numerous executive functions, especially those based on processing speed, decline with age. Salthouse et al originally argued that this decline is due to inefficient utilization of cognitive resources at the earliest stage of input, ie, sensory processing.<sup>3</sup> For example, older people with hearing loss have a rate of cognitive decline that is up to 40% faster than the rate in those with normal hearing.<sup>4</sup> This finding can be explained by the observation that such subjects both receive less information (eg, they periodically hear fewer words) and have a higher cognitive load (meaning they focus on trying to hear such that fewer resources are available for other cognitive processes). This suggests that preventing sensory loss would help reduce cognitive decline.

Lutein has been linked to preservation of sensory function with age<sup>5</sup> and may reduce the probability of age-related visual disease such as macular degeneration. In addition to preventing sensory loss, lutein appears to be palliative. Lutein levels in the retina (termed macular pigment) have been shown to be related to chromatic contrast enhancement, glare disability and discomfort, faster photostress recovery, and increases in visual range.<sup>6</sup> Preliminary unpublished data from our laboratory suggest that lutein may be related to improvements in audition and hearing measured under noise conditions in young healthy adults. Hence, lutein may be linked to reducing cognitive decline by improving sensory functions associated with vision and hearing.

### Lutein May Prevent Cognitive Decline by Reducing Oxidative and Inflammatory Stress

A large confluence of data exists, derived from both laboratory and epidemiological research, showing that damage due to reactive oxygen species (ROS) and chronic inflammation promotes cognitive decline. Since neurons in the brain do not undergo mitosis, much of this damage is cumulative over time. Lutein might help prevent this process by reducing oxidative and inflammatory stress.

Carotenoids are efficient lipid-based antioxidants. They function in this capacity due to their stable electron-rich chemical structure—carbon ring compounds linked to chains that possess alternating single and double bonds. This extended conjugated double-bond system allows these compounds to form extremely stable peroxy radicals. Consequently, carotenoids can tolerate the loss of an electron since this loss is distributed throughout the polyisoprenyl chain. The “excited” carotenoid easily can relax into its ground state by dissipating the excess energy as heat. Empirical data have shown that carotenoids do, in fact, markedly lower overall oxidative stress (Fig 1).<sup>7</sup>



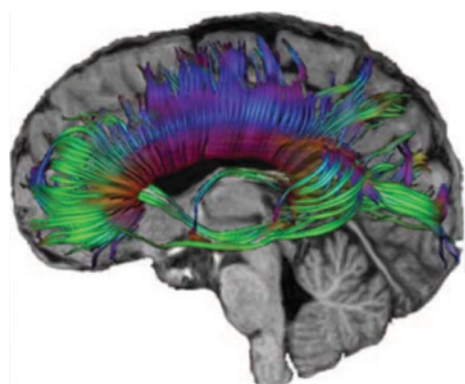
**Fig 1. The antioxidant effects of lutein.**

Serum carotenoids also have been related to lower levels of markers of inflammatory stress, including the inflammatory cytokine interleukin-6.<sup>8</sup> Quasim et al showed that circulating carotenoid levels are low in critically ill patients with high indices of inflammatory stress such as C-reactive protein.<sup>9</sup> An inverse relationship between markers of inflammatory stress and serum carotenoid levels—namely,  $\alpha$ -carotene and  $\beta$ -carotene, zeaxanthin/lutein, and  $\beta$ -cryptoxanthin—also was shown in the Coronary Artery Risk Development in Young Adults (CARDIA) study.<sup>10</sup> This is a large prospective (0-7 years) multicenter epidemiological study of young black and white men and women (N=4580).

Both oxidative and inflammatory stress tend to increase with age and neurodegenerative disease, creating a cascade that increases the need for antioxidant/anti-inflammatory protection. Lutein, a major food component that enters neural tissues, appears to be optimal for addressing this increased need of the elder brain.

### Lutein May Directly Influence Neural Activity

The idea that lutein may directly improve neural function within the brain was formally stated as the Neural Efficiency Hypothesis.<sup>11,12</sup> The hypothesis is based on several observations: 1) lutein and zeaxanthin are found throughout the visual pathway (eg, brain areas such as occipital and frontal lobes<sup>13,14</sup> [Fig 2]) in amounts that vary significantly across subjects; 2) ex vivo data show that lutein and zeaxanthin directly influence cell-to-cell communication (eg, enhancing gap junction communication, as has been shown in somatic cells<sup>15</sup>); and 3) empirical results indicate that macular pigment is related to temporal processing speeds, a visual measure known to be largely determined postreceptorally,<sup>12,16</sup> and cognitive function.<sup>2,17</sup>



**Fig 2. Diffusion tensor imaging. Reduced processing speed is a central feature of cognitive decline.**

How might lutein improve neural efficiency? One possibility is based on improving collective processing. One reason that processing speed is slowed in older adults is that more neural areas (eg, more cortical volume) must be recruited to achieve the same result—an increasing lack of functional differentiation. Distributing processing in this manner takes more time and hence slows processing speeds.<sup>18</sup> Less neural real estate is necessary to solve a given problem in younger brains, probably because of the higher density of cells in any given neural region.<sup>19</sup> If carotenoids induce neurons to connect laterally, as they presumably do through effects on connexin and gap junctions, they may facilitate the recruitment of adjoining areas during a processing task.<sup>15</sup>

Another possibility is based on the preservation of white matter within the brain. One hypothesis explaining cognitive decline and loss of executive skills with age is

the selective age-related loss of fiber tracts within white matter causing, essentially, a cortical disconnection.<sup>20</sup> These white-matter tracts form the essential pathways for much of the higher order reasoning that is facilitated by the neocortex. White matter (and myelin) has a much higher lipid content than gray matter,<sup>19</sup> and carotenoids in the brain associate more highly with white matter.<sup>13</sup> It is possible that lutein and zeaxanthin promote the preservation of white matter and the stabilization of informational tracts. This interpretation is consistent with the fact that lutein and zeaxanthin are known to stabilize membranes (the xanthophylls are sometimes described as transmembrane rivets spanning lipid bilayers) and bind to microtubules<sup>21</sup> in the cytoskeleton.

Lutein likely serves multiple functions within the central nervous system. In biology, a single molecular component often serves multiple purposes—eg, the actions of dopamine within the basal ganglia (motor) and limbic (emotional) system. These many functions seem optimally suited to the preservation and perhaps even enhancement of cognitive performance.

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# Protecting Cognitive Function in Aging and Illness With Nutrition and Exercise

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# Nutrigenetics and Cognitive Health

Anne Marie Minihane, PhD

Life expectancy (LE) continues to rise dramatically, as reinforced by the latest Global Burden of Disease Study statistics published in December 2012 in *The Lancet* (Table 1).<sup>1</sup> Between 1970 and 2010, LE increased in 179 of the 187 countries included, with LE at birth increasing 3 to 4 years per decade since 1970.

**Table 1: Worldwide Life Expectancy Data for Males and Females 1970-2010<sup>1</sup>**

	Male Life Expectancy					Female Life Expectancy				
	1970	1980	1990	2000	2010	1970	1980	1990	2000	2010
World	56.4 (55.5-57.2)	59.8 (59.2-60.2)	62.8 (62.3-63.3)	64.2 (63.6-64.6)	67.5 (66.9-68.1)	61.2 (60.2-62.0)	64.9 (64.3-65.4)	68.1 (67.6-68.6)	69.8 (69.3-70.2)	73.3 (72.8-73.8)

**Source:** Wang H et al. Age-specific and sex-specific mortality in 187 countries, 1970-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2071-2094. Reprinted by permission of Elsevier.

However, this apparent public health success story in increased LE is not matched by an increase in healthy life expectancy (HLE), with an estimated 0.8-year increase in HLE for every 1-year increase in LE. Therefore, although individuals are living longer, they are sicker for longer. This is very apparent when looking at US statistics, with increases of 4.2 years and 2.7 years in LE and HLE, respectively, in males between 1990 and 2010.<sup>2</sup>

Given the almost exponential association between age and cognitive decline, these aging population demographics are having dramatic impacts on dementia incidence worldwide, with the prevalence approximately doubling every 20 years and estimated to increase to 115 million by 2050.<sup>3</sup>

Existing drugs for Alzheimer’s disease (AD), the most common form of dementia, do provide medium-term symptomatic benefits, but currently no approved disease-modifying therapies are available.<sup>4</sup> Given the “explosion” in dementia incidence and the recent high-profile failures of various novel disease-modifying drugs in clinical trials, the development of effective lifestyle strategies to preserve cognition would prove extremely timely, providing enormous health, social, and economic benefits.

Data that allow an estimation of the public health impact of delayed disease onset in the area of dementia and AD are notably absent. However, available data suggest that at a population level, a modest 2-year delay in onset would reduce population incidence by 22% by 2050, resulting in 25 million fewer cases worldwide.<sup>5</sup> The

public health benefits are possibly even greater if effective lifestyle strategies are targeted at high-risk individuals, such as those with mild cognitive impairment (MCI) or an apolipoprotein E4 (*APOE*- $\epsilon$ 4) genotype.

### Omega-3 Fatty Acids and Cognitive Health

The cardiovascular benefits of the omega-3 fatty acids found in fish, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are well established. Typical current population recommendations of an intake of >0.5 g/day of EPA+DHA, increasing to >1 g/day in individuals with diagnosed cardiovascular disease, are based largely on these known benefits to the heart and systemic circulation. The brain is highly enriched in DHA, constituting 15% to 25% of total fatty acids, compared to <5% in most other body tissues. DHA performs numerous structural and metabolic roles, particularly at the neuronal synaptic region.

Although data from “fit-for-purpose” human randomized controlled trials (RCTs) are currently limited in the area of cognitive health, a relatively large body of animal and human cross-sectional and prospective data demonstrates that low dietary fish intake (EPA and DHA), and resultant low omega-3 fatty acid status, are associated with neurocognitive dysfunction and a greater risk of cognitive decline and dementia.<sup>6-8</sup>

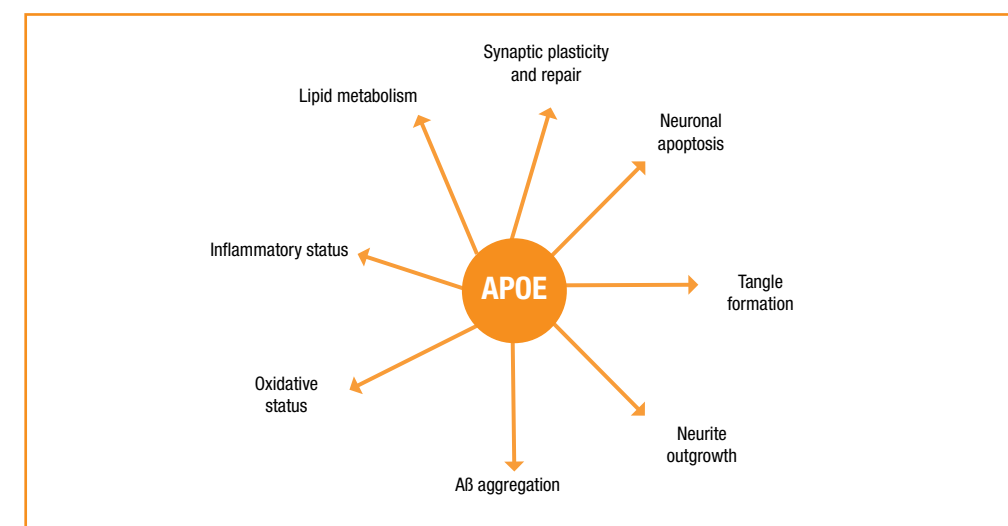
For example, in the Framingham Cohort, individuals in the top quartile of plasma DHA had a 47% reduced risk of developing dementia, compared to those in the bottom quartile.<sup>7</sup> In addition, lower plasma and red blood cell EPA and DHA levels are associated with smaller brain volumes and atrophy of regions associated with dementia, such as the medial temporal lobe.<sup>9,10</sup>

Findings from available RCTs are somewhat mixed, but they suggest the greatest benefit of fish oil (EPA+DHA) or DHA supplementation in individuals with MCI but without a clinical diagnosis of AD.<sup>11</sup> The lack of efficacy in the largest published RCT (UK-based Older People and n-3 Long-Chain Polyunsaturated Fatty Acids Study [OPAL] 2-year intervention, n=867) may be partly because of the inclusion of regular fish consumers, with 92% of participants consuming fish  $\geq$ 1 serving/week.<sup>12</sup> This pattern of fish consumption is atypical for a UK population, resulting in the overrepresentation of regular fish consumers. For these individuals, habitual omega-3 intake is perhaps close to optimal, making it difficult to achieve further benefits because the intervention dose was modest (700 mg/day).

Well-powered human RCTs that use sensitive state-of-the-art measures of cognitive function and brain imaging to assess efficacy are greatly needed in individuals with a low habitual EPA/DHA intake and compromised EPA/DHA status (majority of UK and US adults). These are the individuals most likely to respond and benefit.

### *APOE* Genotype and Cognitive Health

Originally described for its role in lipid transport (it is the almost exclusive lipid transporter in the brain and central nervous system), apolipoprotein E (apoE) is pleiotropic. Its role in brain and neuronal function is summarized in the following Figure.



**Figure. Role of apoE in neuronal function.**

A $\beta$ =amyloid beta, apoE=apolipoprotein E

*APOE*- $\epsilon$ 4 genotype is a highly significant genetic risk factor for AD, increasing risk  $\approx$ 4- and 15-fold in carriers of either a single (20% to 25% Caucasians) or double (1% to 2% Caucasians) copy of the risk allele, compared to *APOE*- $\epsilon$ 3 homozygotes (Table 2).<sup>13</sup> Importantly, an *APOE*- $\epsilon$ 4 genotype is associated with a 2-fold higher conversion rate from MCI to AD<sup>14,15</sup> and an earlier age of AD onset.<sup>16</sup> Therefore, in an era of a gradual move toward the provision of stratified preventative and therapeutic targets based on genetic make-up,<sup>17</sup> *APOE*- $\epsilon$ 4 carriers represent a large population subgroup that would particularly benefit from targeted strategies that may prevent or delay disease onset.



**Table 2. Impact of APOE genotype on Alzheimer’s Disease Incidence<sup>13</sup>**  
(data presented as odds ratios (ORs) with 95% confidence intervals (CI) in parentheses)

Study	ε3ε4 vs ε3ε3 (95% CI)	ε4ε4 vs ε3ε3 (95% CI)	ε2ε3 vs ε3ε3 (95% CI)	ε4 vs ε3 (95% CI)	Total sample size (number of independent samples)
<b>Farrer et al<sup>18,a,b</sup></b>					
Caucasian, clinic/autopsy	3.2 (2.8-3.8)	14.9 (10.8-20.6)	0.6 (0.5-0.8)	Not reported	6305 (31)
Caucasian, population-based	2.7 (2.2-3.2)	12.5 (8.8-17.7)	0.6 (0.5-0.9)	Not reported	4858 (10)
Asian (Japan)	5.6 (3.9-8.0)	33.1 (13.6-80.5)	0.9 (0.4-2.5)	Not reported	2313 (5)
<b>AlzGene<sup>c</sup></b>					
Caucasian, clinic/autopsy	4.3 (3.3-5.5) <i>P</i> <1 x 10 <sup>-16</sup>	15.6 (10.9-22.5) <i>P</i> <1 x 10 <sup>-16</sup>	0.6 (0.3-1.2) <i>P</i> =0.1	4.1 (3.5-4.8) <i>P</i> <1 x 10 <sup>-16</sup>	4946 (20)
Caucasian, population-based	2.8 (2.3-3.5) <i>P</i> <1 x 10 <sup>-16</sup>	11.8 (7.0-19.8) <i>P</i> <1 x 10 <sup>-16</sup>	0.3 (0.2-0.6) <i>P</i> =4.6 x 10 <sup>-7</sup>	3.2 (2.7-3.8) <i>P</i> <1 x 10 <sup>-16</sup>	2866 (8)
Asian (Japan)	3.9 (1.9-8.0) <i>P</i> =0.0002	21.8 (8.6-55.3) <i>P</i> =1.1 x 10 <sup>-16</sup>	0.7 (0.3-1.6) <i>P</i> =0.3	4.0 (2.9-5.5) <i>P</i> =1.1 x 10 <sup>-16</sup>	1541 (4)

<sup>a</sup>Based on pooled genotypes and adjusted for age and study. ORs and total sample sizes are taken from Table 3 of Farrer et al<sup>18</sup> and the number of independent samples from Table 1 of Farrer et al.<sup>18</sup>

<sup>b</sup>*P* values were not reported for the effect size estimates in Farrer et al.<sup>18</sup>

<sup>c</sup>Based on study-specific crude ORs, using random-effects models on published genotypes only. Studies, choice of ethnic group, and ascertainment scheme are based on information provided in Farrer et al.<sup>18</sup>

**Source:** Bertram L et al. Systematic meta-analysis of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet.* 2007;39(1):17-23. Reprinted by permission of Nature Publishing Group.

The Road Ahead: Nonreductionist Approach to Cognitive Health

Traditionally the reductionist approach in nutrition research has focused on establishing the impact of individual foods, food groups, or dietary components on specific health end points. Because the neural processes involved in cognitive health and decline are complex, a combination of nutritional compounds may prove most efficacious. However, few studies have adapted this approach. As recently reviewed,<sup>19</sup> accumulating knowledge on the physiological and molecular targets for individual dietary constituents provides strong justification for the cosupplementation of a number of components, which individually may have only modest benefits.

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## The Role of Flavonoids in Preventing Neuroinflammation and Cognitive Decline

David Vauzour, PhD

Significant advances in medical science during the last century have resulted in a gradual, but highly significant, increase in human life span. On the surface this is a great achievement, but as people age, their cognitive function is threatened by the normal aging process, as well as increasing risk for dementia.<sup>1</sup> The precise cause of the neuronal degeneration underlying these disorders, and indeed normal brain aging, remains elusive, although it is thought that several cellular and molecular events are involved, including increases in oxidative stress, impaired mitochondria function, activation of neuronal apoptosis, the deposition of aggregated proteins, and neuroinflammation.<sup>2</sup>

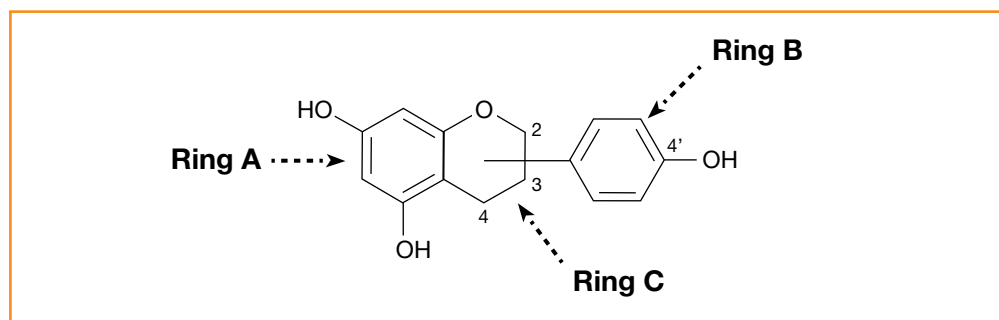
In most cases, neuroinflammation constitutes a beneficial process that ceases once the threat is eliminated and homeostasis is restored. However, sustained neuroinflammatory processes may contribute to the cascade of events culminating in the progressive neuronal damage observed in many neurodegenerative disorders.<sup>3</sup> For many years, a great deal of ongoing research has shown declines in both motor and cognitive functions, even in absence of neurodegenerative disease, in both animals<sup>4</sup> and humans.<sup>5</sup>

Alterations in cognition appear to occur primarily in secondary memory systems that reflect the storage of newly acquired information. For example, aging is associated with a decline in memory performance (eg, delayed recall of a story presented once),<sup>6</sup> processing, working memory, and executive function.<sup>7</sup> Furthermore, the use of nonsteroidal anti-inflammatory drugs, such as ibuprofen, is thought to delay or even prevent the onset of such neurodegenerative disorders,<sup>8</sup> and epidemiologic studies have indicated that the risk for developing Alzheimer's disease (AD) was reduced in regular anti-inflammatory drug users.<sup>9</sup> However, thus far, the majority of existing drug treatments for neurodegenerative disorders are unable to prevent the underlying degeneration of neurons, consequently creating a desire to develop alternative strategies capable of preventing the progressive loss of specific neuronal populations.

## The Role of Flavonoids in Preventing Neuroinflammation and Cognitive Decline



During the last decade, vast and growing research literature has focused on the potential of dietary flavonoids for aiding preservation of cognitive function during aging, while reducing risk for AD and other dementing disorders.<sup>10,11</sup> Flavonoids comprise the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. They consist of two aromatic carbon rings (A and B) that are bound together by three carbon atoms that form an oxygenated heterocycle (ring C) (Fig 1), and may be divided into six subgroups based on the degree of the oxidation of the C-ring, the hydroxylation pattern of the ring structure, and the substitution of the 3-position.



**Fig 1. General structure of flavonoids.**

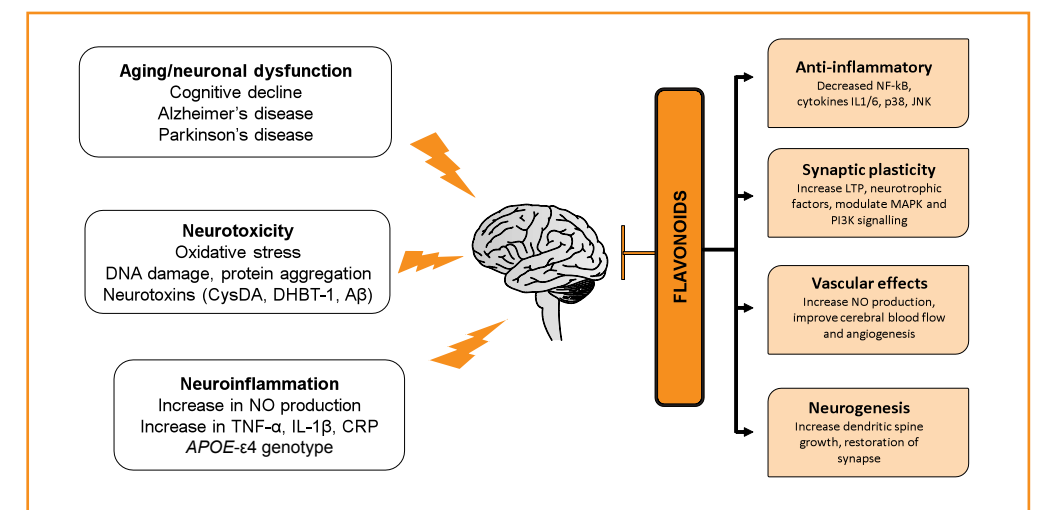
The main dietary groups of flavonoids are 1) flavonols (eg, kaempferol, quercetin), which are found in onions, leeks, and broccoli; 2) flavones (eg, apigenin, luteolin), which are found in parsley and celery; 3) isoflavones (eg, daidzein, genistein), which are mainly found in soy and soy products; 4) flavanones (eg, hesperetin, naringenin), which are mainly found in citrus fruit and tomatoes; 5) flavanols (eg, catechin, epicatechin, epigallocatechin, epigallocatechin gallate), which are abundant in green tea, red wine, and chocolate; and 6) anthocyanidins (eg, pelargonidin, cyanidin, malvidin), which are found in red wine and berry fruits.

Among berries, blueberries are particularly rich in flavonoids, with anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin), flavanols (monomers: catechin and epicatechin; oligomers: procyanidins B type), and flavonols (quercetin and myricetin) being the most represented.<sup>12</sup>

In general, it was assumed that the health benefits of flavonoids were linked to their capacity to directly scavenge free radicals and other nitrogen species in vitro.<sup>13</sup> However, it is not likely that the concentrations at which they exert such antioxidant activity are easily achieved in vivo, because many flavonoids have very limited bioavailability and are extensively metabolized, therefore reducing their antioxidant potential.<sup>14</sup>

During the last years, a new realization of how nutritional antioxidants may function has surfaced, and recent findings have suggested that in lower amounts, typical of those attained in the diet, flavonoids may exert pharmacological activity within the cells. Although the precise site of their interaction with signaling pathways is unclear, evidence indicates that they are capable of acting in a number of ways, including 1) the modulation of intracellular signaling cascades that control neuronal survival, death, and differentiation, 2) changes in gene expression, and 3) interactions with mitochondria.<sup>15,16</sup>

For example, flavonoids and their in vivo metabolites are shown to modulate signaling through tyrosine kinase, phosphoinositide 3-kinase, protein kinase C, and mitogen-activated protein kinase pathways.<sup>17</sup> These signaling cascades are also critical for the control of inflammatory processes in the brain, including the activation of microglia in response to cytokines and the induction of iNOS and nitric oxide production.<sup>18,19</sup> By affecting such pathways, flavonoids are not only suggested as novel dietary strategies for the reduction of the deleterious effects of neuroinflammation in the brain, but also as having a direct influence on memory acquisition, consolidation, and storage through the induction of new protein synthesis in neurons (Fig 2).



**Fig 2. Schematic representation of the possible effects of aging on cognitive performances and known effects of flavonoids in improving the cognitive decrements.**

Aβ=amyloid beta, APOE-ε4=apolipoprotein E4, CRP=C-reactive protein, CysDA=cysteinyl dopamine, DHBT-1=dihydrobenzothiazine-1, IL1/6=interleukin 1 and 6, IL-1β=interleukin-1 beta, JNK=c-Jun N-terminal kinase, LTP=long-term potentiation, MAPK=mitogen-activated protein kinase, NF-κB=nuclear factor kappa B, NO=nitric oxide, PI3K=phosphoinositide 3-kinase, TNF-α=tumor necrosis factor alpha



Alternatively, the well-established effects of flavonoids on the vascular system also may induce increases in cerebral blood flow capable of having an impact on acute cognitive performance, or may lead to an increase in hippocampal vascularization capable of inducing new neuronal growth.

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# Neurocognitive and Mood Effects of Nutrition and Nutraceuticals

Andrew Scholey, PhD

Cognitive processes involve multiple mechanisms that interact in complex and possibly idiosyncratic ways. This complexity likely underlies the general lack of meaningful impact of monopharmacological (or “magic bullet”) approaches to brain dysfunction, including cognitive decline and dementia. For example, the cholinesterase inhibitors used in the treatment of Alzheimer’s disease (AD) have a narrow therapeutic window and are effective only at certain stages of the disorder. This is perhaps unsurprising considering that the onset and progression of AD are influenced by numerous other processes, as well as cholinergic degeneration.

AD is a product of a pathological cascade, involving progressively accelerating neurotoxic interactions between oxidative stress, inflammatory responses, compromised hormonal pathology, compromised cerebral metabolism, neurofibrillary tangle generation, and  $\beta$ -amyloid deposition, among other processes, which include cholinergic degeneration (Fig 1).

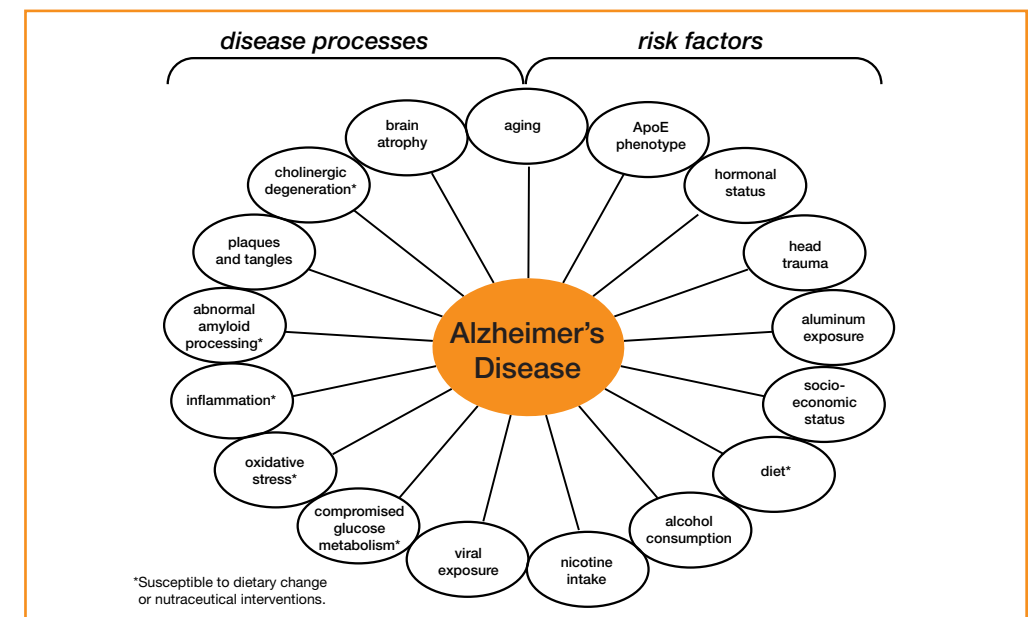
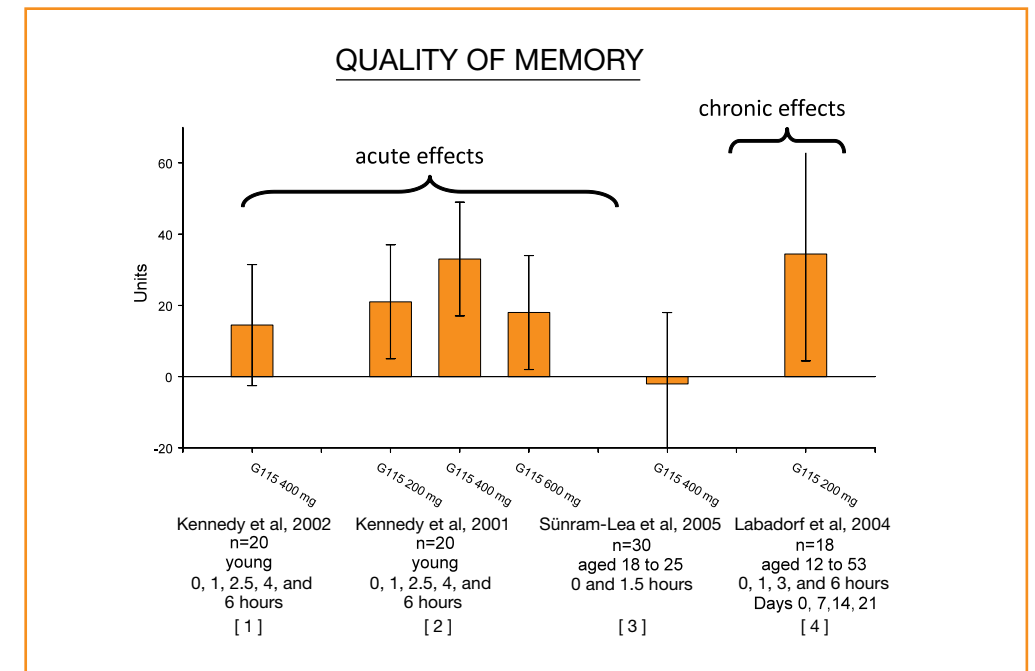


Fig 1. Processes involved in the risk and etiology of Alzheimer’s disease, with many also implicated in nonpathological aging.

Nutrients and extracts from botanical sources may offer a more promising approach by affecting multiple systems. Unlike mainstream pharmacological agents, nutrients and extracts from botanical sources may contain many active components. It appears that certain plants have evolved with a combination of properties which, in concert, may affect multiple neuronal, metabolic, and hormonal systems with direct effects on cognitive processes. In addition, numerous indirect physiological processes impinge on neurocognitive function (eg, glycemic control, vascular function, oxidative stress, inflammation, and psychological stress), all of which are possible to modify by appropriate dietary change.

Over the past decade or so, great progress has occurred in evaluating the efficacy of specific nutritional interventions, including dietary supplements, in the context of cognitive enhancement. Research on the biobehavioral effects of herbal extracts have included, but are not restricted to, randomized controlled trials using species of ginseng, *Melissa officinalis*, species of salvia (sage), *Ginkgo biloba*, valerian, guarana, ginkgo-phosphatidylserine, cocoa polyphenols, *Bacopa monnieri*, Pycnogenol®, Enzogenol®, tea catechins, soy isoflavones, and curcumin. This paper will use ginseng and *M officinalis* as examples.

For millennia, ginseng has seen use in traditional medicine systems and often is marketed as a “pick-me-up.” This and other properties relevant to neurocognitive function usually are ascribed to the ginsenosides, terpenoid saponins found only in ginseng. G115 is a standardized extract with an invariant 4% ginsenoside content. Several clinical trials have shown that G115 can acutely improve cognitive function and, in particular, memory (Fig 2).



**Fig 2. Effects of a standardized extract of Panax (G115) ginseng on an aggregate memory score in four studies.**<sup>1-4</sup> Bars represent improvement compared with placebo, with 95% confidence intervals.

Figure courtesy of Keith Wesnes, PhD, Bracket, UK; Swinburne University, Australia.

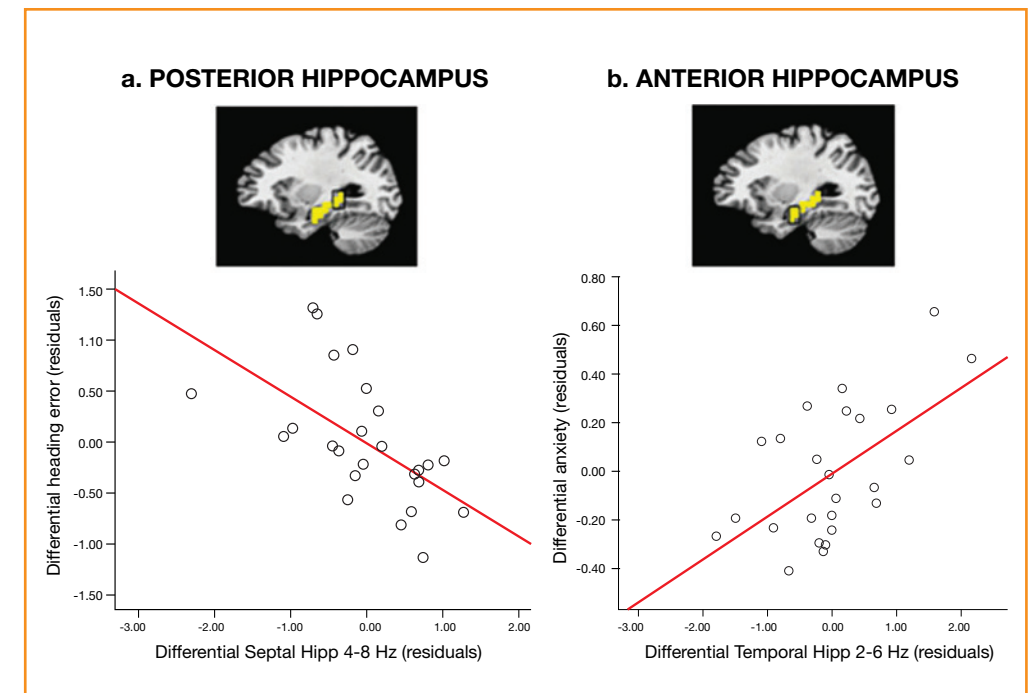
Like other nutraceuticals, ginseng can improve mood and cognitive function during heavily loaded cognitive processing (six repeated 10-minute cycles of serial subtractions and a vigilance/working memory task),<sup>5</sup> specifically improving Serial Sevens performance and reducing mental fatigue. More recent work has shown that an extract of American ginseng (*Panax quinquefolius*), which has a different ginsenoside profile, has replicable positive effects on working memory.<sup>6</sup>

In order to put these findings into perspective, effect sizes of ginseng were compared with those associated with the pharmacological cognitive enhancer du jour, namely modafinil.<sup>7</sup> The largest effect size in the healthy human modafinil literature was 0.77 (Cohen’s d for visuospatial working memory). Ginseng effects were more variable, including negative effects at some doses. Nevertheless, the highest effect sizes were 0.86 in the cognitive domain (reaction time) and 1.40 for mood (the aforementioned alleviation of mental fatigue). It is necessary to perform these comparisons in direct head-to-head clinical trials. However, they imply that polypharmacological approaches to cognitive enhancement may prove at least as fruitful as traditional pharmacological drug-discovery pipelines.

Other studies have focused on the mood effects of natural products. One example is *M officinalis* (lemon balm), with the plant and extracts thereof long considered as anxiolytic agents. In one study, the effects of two doses of a standardized extract of *M officinalis* on mood changes during a multitasking battery were evaluated. Subjects simultaneously performed four cognitive tasks with snapshots of mood taken immediately before and after the stressor.<sup>8</sup> The findings showed that compared with placebo, lemon balm was associated with decreased self-rated alertness and increased calmness during the stressor in a dose-dependent manner.

More recently this field has drawn on methodologies from the neuroimaging discipline. One such method is magnetoencephalography (MEG), which measures changes in magnetic fields associated with postsynaptic potentials. MEG has the advantage of other imaging techniques in that it has both extremely good spatial and temporal resolution. One exciting application is the use of a virtual water maze, which mimics the classic rodent Morris water maze (MWM). In the MWM, an animal finds a submerged platform in a large tank of opaque liquid using environmental or allocentric cues, which is a prototypical hippocampal task.

In the virtual version, humans navigate their way through a virtual pool to find a platform while in the MEG scanner. Like its rodent predecessor, successful completion of the task is dependent on hippocampal function<sup>9</sup> and specifically on the hippocampal theta waveform. More recently it was demonstrated that, during a version of the task where some trials were associated with electric shock, subfields of the hippocampus responded differentially to cognitive performance and anxiety,<sup>10</sup> with activity in the posterior third predicting cognitive performance and the anterior third associated with affect (Fig 3). These findings suggest the possibility of measurable and modifiable neural markers for cognition and mood, which may complement more established measures.



**Fig 3. Differential association between hippocampal region-of-interest (ROI) theta and navigation performance and anxiety level.**<sup>10</sup> Partial regression plots (with least square lines) show relationships between differential left hippocampal ROI high theta (4-8 Hz) and differential heading error (a), and between differential left hippocampal ROI low theta (2-6 Hz) and differential anxiety in the hidden platform condition (b).

**Source:** Cornwell BR et al. Distinct contributions of human hippocampal theta to spatial cognition and anxiety. *Hippocampus*. 2012;22(9):1848-1859. Reprinted by permission of John Wiley and Sons. Figure courtesy of Brian Cornwell, PhD, Swinburne University, Australia.

This work is in its infancy, but may uncover promising candidates with which to optimize day-to-day cognitive functioning, to maintain psychological well-being throughout life, and even to treat conditions where mental function becomes fragile, including dementias. However, it is important not to fall into the trap of attempting to mimic classic drug development pipelines. Specifically, nutrition interventions may rely on synergistic interaction between components, such that efficacy is possibly reduced by attempting to isolate individual active components.

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## From Inflammation to Sickness and Cognitive Dysfunction: When the Immune System Subjugates the Brain

Rodney W. Johnson, PhD

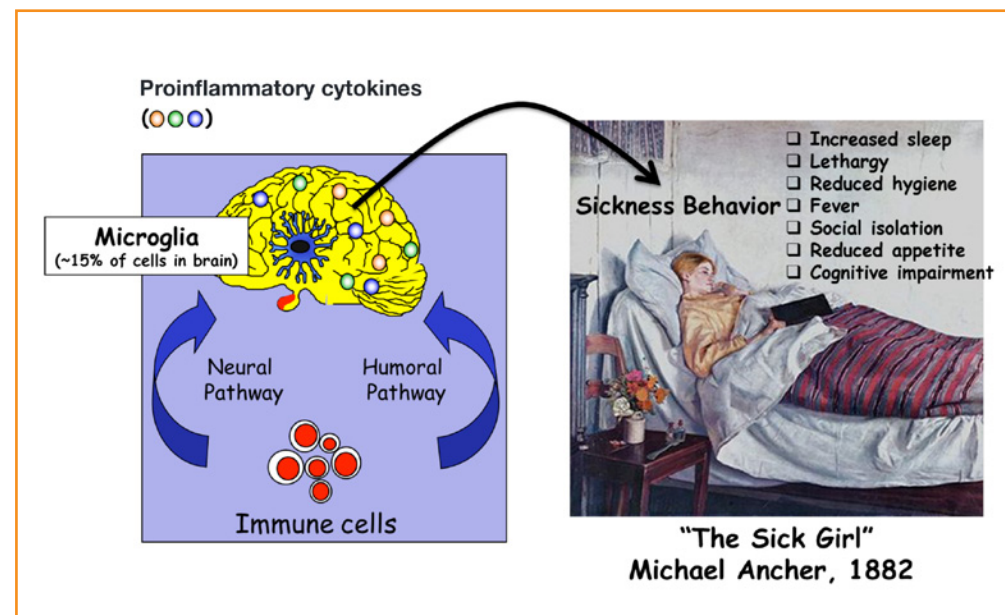
**M**icroglial cells, resident macrophages in the central nervous system (CNS), are relatively quiescent but can respond to signals from the peripheral immune system and induce neuroinflammation. In aging, microglia tend to transition to the M1 proinflammatory state and become hypersensitive to messages emerging from immune-to-brain signaling pathways. Thus, whereas in younger individuals in whom microglia respond to signals from the peripheral immune system and induce a well-controlled neuroinflammatory response that is adaptive (eg, when well controlled, fever and sickness behavior facilitate recovery from infection), in older individuals with an infection, microglia overreact and produce excessive levels of inflammatory cytokines, causing behavioral pathology including cognitive dysfunction. Importantly, recent studies indicate dietary flavonoids have anti-inflammatory properties and are capable of mitigating microglial cells in the brain of aged mice. Thus, dietary or supplemental flavonoids and other bioactives have the potential to restore the population of microglial cells in the old brain to a more quiescent state. The concept to constrain microglia through dietary intervention is significant because neuroinflammation and cognitive deficits are co-morbid factors in many chronic diseases. Controlling microglial cell reactivity has important consequences for preserving adult neurogenesis, neuronal structure and function, and cognition. This paper briefly describes the immune-to-brain signaling pathways, microglial cell activation, neuroinflammation, and, as one example, the potential of flavonoids to mitigate brain microglia and cognitive deficits induced by neuroinflammation.

### Lines of Communication: How the Immune System Says “Hello” to the Brain

Neurological and cognitive effects associated with influenza infection have been reported throughout history (eg, following the 1918 “Spanish flu”), as well as during the recent novel influenza A H1N1 pandemic.<sup>1-3</sup> The simplest explanation for these neurocognitive effects is that influenza virus makes its way to the brain,



where it is detected by neurons. However, most influenza strains, including those responsible for pandemics, are considered non-neurotropic,<sup>4-6</sup> suggesting that neurological symptoms associated with influenza infection are not a result of direct viral invasion into the CNS. Moreover, neurons do not have receptors to detect viruses (or other pathogens) directly. Cells of the immune system do, however, as the immune system's primary responsibility is to recognize infectious pathogens and contend with them. For example, sentinel immune cells such as monocytes and macrophages are equipped with toll-like receptors (TLR) that recognize unique molecules associated with groups of pathogens (ie, pathogen-associated molecular patterns). Stimulation of TLRs that recognize viruses (TLR3 and TLR7) and bacteria (TLR4) on immune sentinel cells can have profound neurological and cognitive effects (Fig 1), suggesting the immune system conveys a message to the brain after detecting an infectious agent. This message is cytokine based.



**Fig 1. Immune-to-brain communication pathways stimulate brain microglia, which can induce neuroinflammation and neurocognitive deficits.**

Macrophages and monocytes produce inflammatory cytokines (eg, interleukin [IL]-1 $\beta$ , IL-6, and tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ]) that facilitate communication between the periphery and brain. Early studies showing that infection-related neurocognitive changes could be induced in the absence of an infectious agent by injecting small quantities of recombinant IL-1 $\beta$  directly into a lateral cerebral ventricle indicated that inflammatory cytokines are essential to infection-related

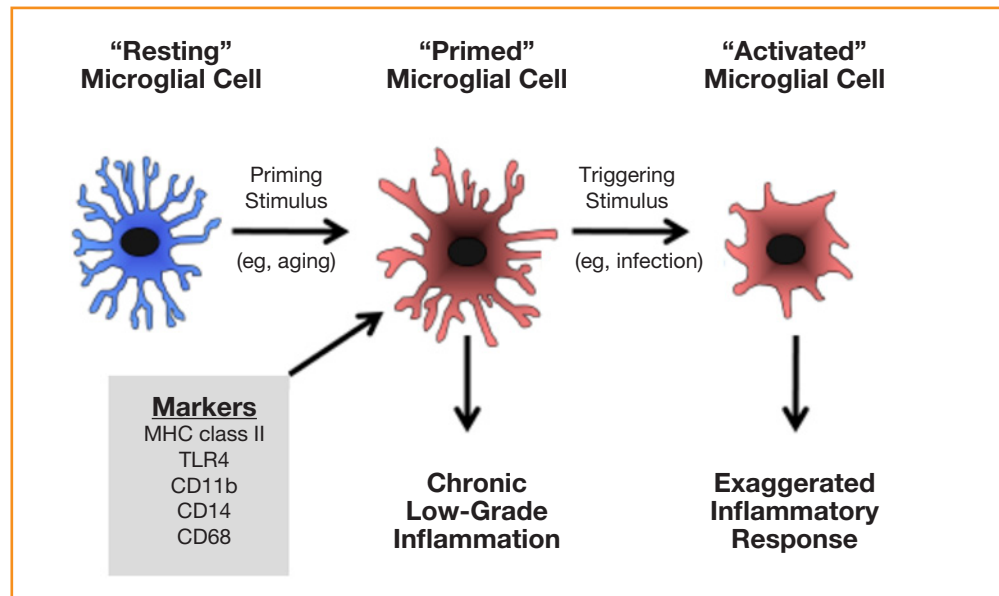
behavior and that they somehow transcend the blood-brain barrier. Several cytokine-dependent pathways that enable the peripheral immune system to transcend the blood-brain barrier have been dissected.

First, there is good evidence that inflammatory cytokines present in blood can be actively transported into the brain.<sup>7-11</sup> Cytokines produced in the periphery need not enter the brain to elicit neurocognitive changes. This is because inflammatory stimuli in the periphery can induce microglial cells to produce a similar repertoire of inflammatory cytokines. Thus, brain microglia recapitulate the message from the peripheral immune system.<sup>12,13</sup> Hence, in a second pathway, inflammatory cytokines in the periphery can bind receptors on blood-brain barrier endothelial cells<sup>14</sup> and induce perivascular microglia or macrophages to express cytokines that are released into the brain parenchyma.<sup>15,16</sup> Furthermore, in a third pathway, cytokines in the periphery convey a message to the brain via the vagus nerve. After immune challenge, dendritic cells and macrophages that are closely associated with the abdominal vagus have been shown to express IL-1 $\beta$  protein<sup>17</sup>; IL-1 binding sites have been identified in several regions of the vagus as well.<sup>18</sup> When activated by cytokines, the vagus can activate specific neural pathways that are involved in neurocognitive behavior. However, activation of the vagus also stimulates microglia in the brain to produce cytokines via the central adrenergic system.<sup>19-21</sup> Finally, a fourth pathway provides a slower immune-to-brain signaling mechanism based on volume transmission.<sup>22</sup> In this method of immune-to-brain communication, production of IL-1 $\beta$  by the brain first occurs in the choroid plexus and circumventricular organs—brain areas devoid of an intact blood-brain barrier. The cytokines then slowly diffuse throughout the brain by volume transmission, along the way activating microglia, neurons, and neural pathways that induce sickness behavior and inhibit cognition.

### What Are Microglia and What Do They Do?

A critical point is that the aforementioned communication pathways seem to have in common a need to activate microglial cells and induce neuroinflammation. An early definition of inflammation was based on four cardinal signs: dolor (pain), calor (heat), rubor (redness), and tumor (swelling); functio laesa (loss of function) was added later. While neuroinflammation can resemble its peripheral counterpart in circumstances such as viral and bacterial meningitis, head trauma, or autoimmune diseases of the CNS, the term neuroinflammation is increasingly used to identify a fundamentally different event that is exclusively driven by microglial cells and shows few if any of the cardinal signs described in the early definition.

Microglia account for 12%-15% of the cells in the brain. They originate from macrophages produced by primitive hematopoiesis in the yolk sac. The primitive macrophages migrate to the neural tube, where they give rise to microglia. Bone marrow-derived monocytes do not contribute to the mature microglia pool, suggesting microglia numbers are sustained by local progenitors. Under healthy conditions “resting” (quiescent) microglia are highly dynamic, randomly extending and contracting arms with filopodia-like protrusions to survey the microenvironment.<sup>23</sup> In response to insult, however, microglia become activated and proinflammatory (M1) (Fig 2). In this state, they can direct the movement of the protrusions toward the insult,<sup>23</sup> take on a de-ramified morphology that enables motility,<sup>24</sup> and/or express major histocompatibility complex class II (MHC class II) and other markers indicative of inflammation.<sup>25</sup>



**Fig 2. Priming of microglia (eg, with aging) and inflammatory response.**

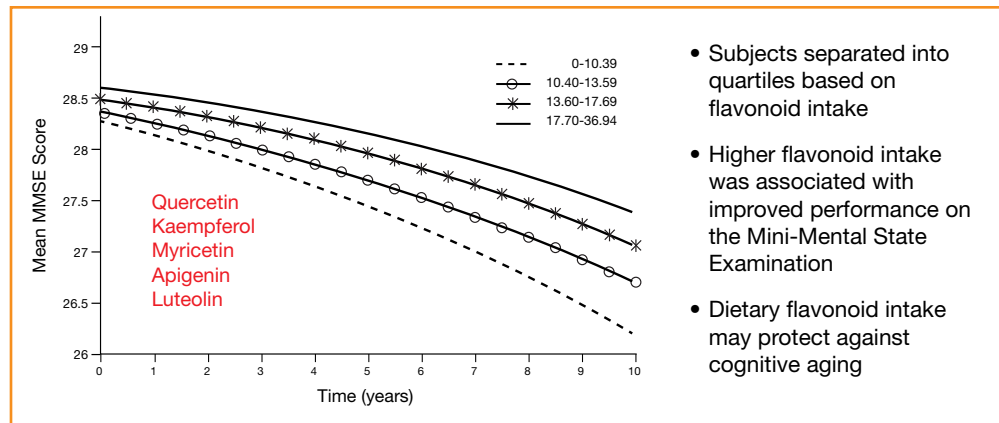
MHC class II=major histocompatibility complex class II, TLR=toll-like receptors, CD=cluster of differentiation

Notably, M1 microglia constitutively produce inflammatory cytokines and are hypersensitive to insults, including signals from the peripheral immune system.<sup>26,27</sup> Hence, microglial cell activation is frequently viewed to be tantamount to neuroinflammation. As neuroinflammation is deleterious to neurological function, mitigating microglial cell activity is vital for optimal brain health.

MHC class II frequently is used to identify activated microglia. During aging, the percentage of brain microglia that express MHC class II increases and signs of neuroinflammation emerge. For example, <3% of microglia isolated from the brain of healthy young adult mice stained positive for MHC class II.<sup>28</sup> This pales in comparison to the >25% of microglia from brains of old but otherwise healthy mice that were MHC class II-positive.<sup>28</sup> Most of the MHC class II-positive microglia from old mice were also IL-1 $\beta$ -positive.<sup>28</sup> This is consistent with a prior study in which the proportion of IL-6-positive microglia was markedly higher if the donor mouse was 22-24 months old compared to 6 months or 1 week old.<sup>27</sup> It is important to note that aging per se does not increase the number of microglial cells in the brain but rather it increases the proportion of resident microglia that are inflammatory and reactive to insults.<sup>29</sup> A recent study suggests that microglia from aged mice retain a prominent M1 profile and are less sensitive to the anti-inflammatory and M2-promoting effects of IL-4.<sup>30</sup> Reducing the proportion of microglia that are activated is a priority for reducing age-related neuroinflammation that may contribute to cognitive aging and be a predisposing factor for neurodegenerative disease.

### Can Flavonoids Reduce Neuroinflammation and Inhibit Cognitive Aging?

Flavonoids are naturally occurring polyphenolic compounds present in plants. The major sources of flavonoids in the human diet include fruits, vegetables, tea, wine, and cocoa.<sup>31</sup> Significant evidence has emerged to indicate that consuming a diet rich in flavonoids may inhibit or reverse cognitive aging. For example, in a prospective study of individuals aged 65 years or older, dietary flavonoid intake (ie, mg/d of five flavonoids—apigenin, kaempferol, luteolin, myricetin, and quercetin) was associated with improved cognitive function over a 10-year period (Fig 3).<sup>32</sup>



**Fig 3. Flavonoid intake (range 0-36.94 mg/d) and cognitive decline in people 65 years of age and older as shown by scores from the Mini-Mental State Examination (MMSE).<sup>32</sup>**

**Source:** Letenneur L et al. *Am J Epidemiol.* 2007 Jun 15;165(12):1364-1371. Reprinted by permission of Oxford University Press.

Furthermore, analyses of data from the Chicago Health and Aging Project—a cohort study of 3790 older residents residing on the south side of Chicago—suggested that adherence to a Mediterranean dietary pattern reduced the rate of cognitive decline.<sup>33</sup> Numerous other studies have yielded consistent results with older rats or mice, which show improved cognitive function when fed a flavonoid-rich diet.<sup>34-36</sup>

Flavonoids may improve cognition in the aged through a number of physiological mechanisms, including scavenging of reactive oxygen and nitrogen species<sup>37</sup> and interactions with intracellular signaling pathways.<sup>38</sup> Through these physiological mechanisms, flavonoids also impart an anti-inflammatory effect that may improve cognition. This seems likely for the flavone luteolin, which is most prominent in parsley, celery, and green peppers. Whereas luteolin inhibits several transcription factors that mediate inflammatory genes (eg, nuclear factor kappa B [NF- $\kappa$ B]<sup>39</sup> and activator protein 1 [AP-1]<sup>40</sup>), it is a potent activator of nuclear factor erythroid 2-related factor 2 (Nrf2), which induces the expression of genes encoding antioxidant enzymes.<sup>41</sup> A recent study of old healthy mice found improved learning and memory and reduced expression of inflammatory genes in the hippocampus when luteolin was included in the diet.<sup>34</sup> Thus, dietary luteolin may improve cognitive function in the aged by reducing brain microglial cell activity. Indirect support for a microglia-dependent mechanism comes from a recent in vitro study in which luteolin stimulated the formation of filopodia and caused ramification of BV-2 cells (a microglia cell line) even when they were activated with *Escherichia*

*coli* lipopolysaccharide (LPS).<sup>42</sup> Furthermore, supernatants from LPS-stimulated BV-2 cells caused discernible cell death in Neuro.2a cells even if Neuro.2a cells were incubated with luteolin; however, treating BV-2 cells with luteolin prior to LPS reduced neuronal cell death caused by conditioned supernatants.<sup>34</sup> Hence, the flavonoid luteolin is a naturally occurring immune modulator that may be effective in reducing inflammatory microglia in the senescent brain.

## Conclusion

In light of the recent evidence suggesting microglial cells become dysregulated due to aging and cause neuroinflammation, which can disrupt neural structure and function, it is an interesting prospect to think dietary flavonoids and other bioactives can be used to constrain microglia. But how can flavonoids impart this anti-inflammatory effect? Although in vitro studies clearly indicate that several flavonoids can act directly on microglial cells to restrict the inflammatory response, results from in vivo studies thus far do not prove that dietary flavonoids access the brain to interact with microglia in a meaningful way. This is a complicated question to dissect because flavonoids reduce inflammation in the periphery and microglia seem to act like an “immunostat,” detecting and responding to signals emerging from immune-to-brain signaling pathways. Thus, whether dietary flavonoids enter the brain and impart an anti-inflammatory effect on microglia is an interesting question but one that is more theoretical than practical because what is most important is how the immunostat is adjusted, whether that is via a direct or indirect route. However, because flavonoids are detectable in the brain,<sup>43-45</sup> they most likely affect microglia both directly and by dampening immune-to-brain signaling.



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## Exercise and the Aging Brain

Arthur F. Kramer, PhD

In 2008, the first comprehensive guidelines on physical activity were published by the US government. These guidelines, entitled *2008 Physical Activity Guidelines for Americans*,<sup>1</sup> were the result of an extensive review of the scientific data on physical activity and health performed by a group of 13 leading experts from the fields of exercise science and public health (Physical Activity Guidelines Advisory Committee, 2008). The report was unique in that the science base available at the time was used to formulate practical guidelines for physical activity from young childhood through old age and also included individuals with chronic medical conditions and physical disabilities. Recommendations were based both on the level of physical activity and its duration, across the course of a week and throughout most of the lifespan. Additionally, recommendations were formulated both in terms of aerobic and muscle-strengthening activities that were appropriate for different populations. More recently, these physical activity guidelines were extended to even younger children in the *Physical Activity Guidelines for Americans Midcourse Report: Strategies for Increasing Physical Activities Among Youth* (2012).<sup>2</sup>

The recommendations in these two reports were based on the large and growing body of studies that have examined the relationship between physical activity (and other factors) and health and disease. Lack of physical activity and exercise has been associated with increased risks for a number of diseases including type 2 diabetes, hypertension, colon and breast cancer, obesity, and coronary heart disease among others.<sup>3</sup> Indeed, lack of activity even has been associated with the onset of “adult” diseases such as diabetes and hypertension among children.<sup>4,5</sup> Finally, physical activity also has been found to result in increased life expectancy and a substantial decrease in medical expenditures across the lifespan.<sup>6,7</sup>

As described above, substantial data suggest that physical activity is important in reducing the risk of various diseases, including those diseases (eg, heart disease, stroke, and obesity) that have been associated with compromised cognitive and brain health and, in turn, independence and quality of life. The development of these diseases and their effects on cognition and brain occur over years and decades. This summary focuses on shorter-acting but equally important effects of physical activity and exercise. That is, effects that in nonhuman animal models can occur in weeks or months and substantially influence the molecular and cellular underpinnings of learning, memory, and other cognitive processes. In humans, such effects, characterized with sophisticated behavioral paradigms and neuroimaging

methodologies, take a bit longer to be realized, on the order of several months to a year or so.<sup>8</sup>

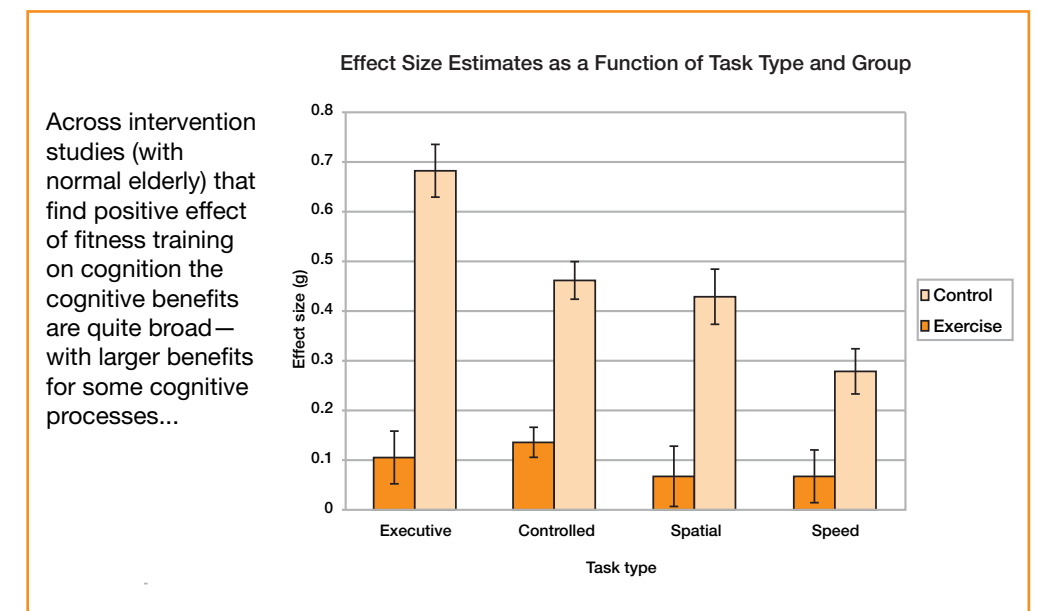
This summary is organized around both animal and human research on physical activity and exercise. The animal research potentially enables a low-level mechanistic understanding of how physical activity influences molecules, cells, and neurochemistry—and how such influences affect performance and cognition. On the other hand, while the ability to understand molecular and cellular changes is quite limited with humans, the ability to understand nuanced changes in general and selective aspects of perception, cognition, and action is quite possible. The development of new neuroimaging technologies also has enabled the field of cognitive neuroscience to begin to bridge the gap between animal and human studies of brain structure and function in physical activity and other types of interventions such as cognitive training, nutrition, and social interaction.<sup>9,10</sup> This review will describe both the animal and human literature on physical activity effects on brain and cognition, the overlap between studies with different species, and suggest how cross-species, multimethod research might further address important issues in the study of neural and cognitive plasticity, with a focus on physical activity and exercise.

A rapidly expanding animal literature suggests that increased exercise leads to the birth of new neurons in the hippocampus (a brain region responsible for important aspects of memory), increased connections among neurons throughout the brain, the development of new vasculature structure, increased production of neurotrophic proteins such as brain-derived neurotrophic factor, reduction of proteins associated with neurodegeneration in mouse models of Alzheimer's disease, and enhanced learning and memory.<sup>11</sup> Interestingly, such results have been found across the lifespan and in a multitude of species including rodents, dogs, and monkeys.

The increasing molecular and cellular knowledge of exercise effects with animals provides the basis for human studies of physical activity and exercise. Indeed, the human studies include both prospective observational or epidemiological studies and randomized controlled trials. The observational studies have established the relationship between physical activity (often self-reported) and cognitive maintenance in normal adults or adults diagnosed with neurodegenerative diseases such as Alzheimer's or Parkinson's disease.<sup>12</sup> For example, consider a relatively short-term study by Weuve et al.<sup>13</sup> The leisure-time physical activity and global cognitive function of 18,766 women from 70 to 81 years of age was assessed by self-report. During reassessment 2 years later, it was found that women who initially reported walking the most during initial assessment had a 20% reduced

risk of cognitive impairment compared to women walking the least. This kind of relationship has been reported in dozens of studies, with physical activity benefits ranging from 20% to 40%, in terms of maintained cognition, across 2 to 8 years. Although such results are both interesting and potentially important, they do not establish a causal relationship between physical activity and cognition. Hence, there is a need for randomized cognitive trials.

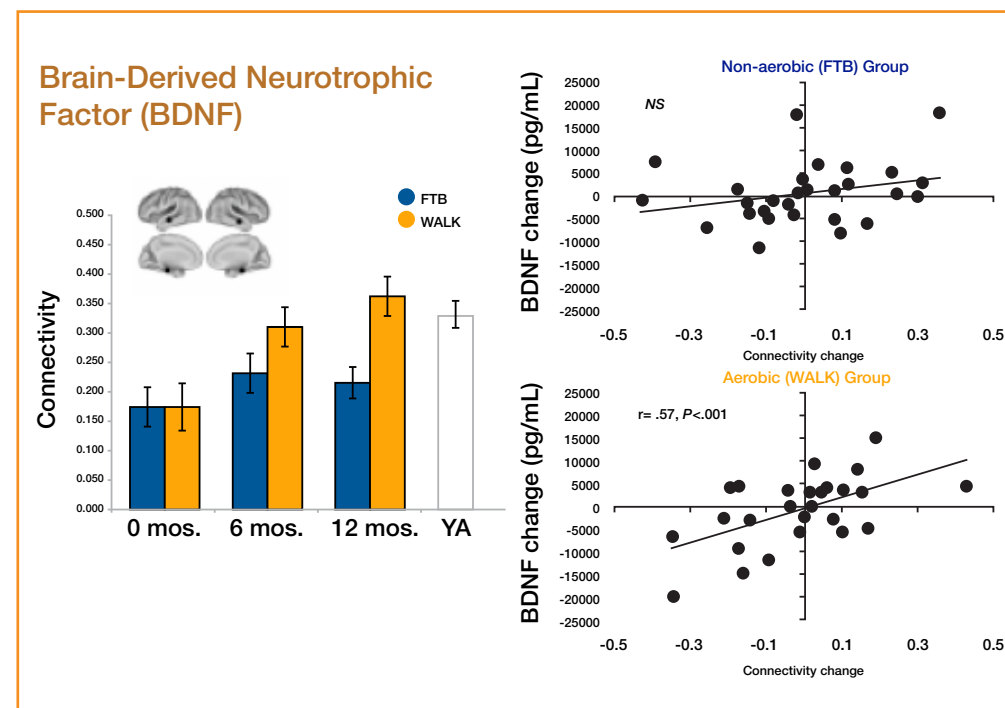
In a meta-analysis of 18 trials by Colcombe and Kramer,<sup>8</sup> a moderate effect size between exercise and cognition was reported. The effect size was moderated by the type of cognition being assessed, with executive control processes showing the largest benefit (Fig 1), females showing larger benefits than males, and exercise programs lasting 6 months or longer showing larger benefits for cognition than shorter exercise programs. Subsequent research has found similar benefits of exercise on cognition.



**Fig 1. Effect size between exercise and cognition in a meta-analysis of 18 trials.<sup>8</sup>** The effect size was moderated by the type of cognition being assessed, with executive control processes showing the largest benefit.

**Source:** Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci.* 2003 Mar;14(2):125-130. Published by Sage Publications, Inc.

More recently, human studies also have begun to include measures of brain function and structure along with behavioral measures of cognition. Thus, these studies might be viewed as a way to narrow the gap between the focus on brain function and structure with animals and the focus on sophisticated measurement of cognitive effects with humans. These human studies have reported that relatively brief fitness programs result in increased brain volume in a variety of brain regions such as the hippocampus that have shown declines during the normal course of aging<sup>14,15</sup> and increases in the integrity of white matter tracts<sup>16</sup> and patterns of brain activation, including measures of functional connectivity of different brain regions, which suggest more efficient memory, attention, and decision making (Fig 2).<sup>17,18</sup>



**Fig 2. Neurobiological mechanisms for exercise-induced brain plasticity.**<sup>16</sup>

FTB=flexibility, toning, balance (group), YA=young adult (comparison group)

**Source:** Voss MW et al. Neurobiological markers of exercise-related brain plasticity in older adults. *Brain Behav Immun.* 2013 Feb;28:90-99. Reprinted by permission of Elsevier.

Indeed, while the great majority of studies conducted in the past decade or so focused on adults, more recent studies have reported similar cognitive and brain benefits, as a function of exercise and physical activity, for children.<sup>19-21</sup> Such findings are important to understanding the effects of lifestyle choices on cognitive and brain development as well as the impact of the increasing sedentary nature and levels of obesity among children in today's society.

In summary, the last decade has led to a substantial increase in our knowledge concerning the influence of at least one lifestyle choice on healthy minds and brains—ie, physical activity and exercise. However, many important questions remain unanswered. What forms of exercise lead to the largest cognitive and brain benefits? What are the optimal doses of exercise (in terms of length and intensity of exercise programs) and to what extent does the definition of optimality differ with age, health, and other factors? Do other lifestyle choices show cognitive benefits similar to those of exercise that are based on similar mechanisms? How do lifestyle choices interact with our genome with regard to cognitive benefits? Finally, can a combination of nutrition and exercise bestow greater benefits to healthy minds and brains than either of these factors alone?

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## Nutrition Effects in Learning and Memory in Animal Models

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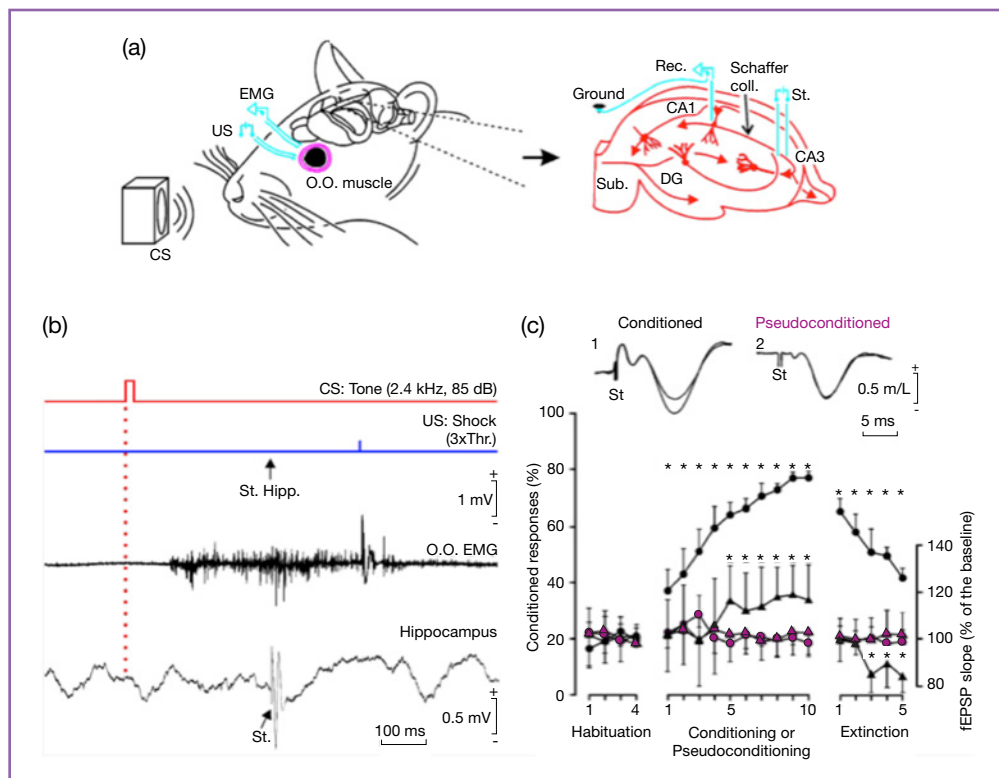
## Associative Learning and Long-Term Potentiation in Rodents: Effects of Nutrition

José M. Delgado-García, MD, PhD

### Long-Term Potentiation (LTP) and Learning-Dependent Changes in Synaptic Strength

Following the seminal proposal of Hebb<sup>1</sup> and many others, acquired learning abilities are assumed to be stored in the form of functional and/or structural changes in synaptic efficiency. Although there are many excellent studies in vitro of the electrophysiological processes and molecular events supporting activity-dependent synaptic changes, not much information is available on synaptic changes in strength (ie, synaptic plasticity) during actual learning in behaving animals. Functional changes evoked by learning should be susceptible to being detected at synapses relevant to the acquisition process. In 2006, our research group convincingly demonstrated that classical conditioning of eyelid responses in behaving mice was able to increase the synaptic strength of the hippocampal CA3-CA1 synapse (Fig 1).<sup>2,3</sup>





**Fig 1. Learning-dependent changes in the slope (volt/s) of field excitatory post-synaptic potentials (fEPSPs) evoked in the hippocampal CA3-CA1 synapse across a classical conditioning of eyelid responses in behaving mice.<sup>2,3</sup>** **A**, Experimental design. Stimulating electrodes (St.) were implanted on Schaffer's collaterals and recording electrodes were implanted in the hippocampal CA1 area. (DG=dentate gyrus, Sub=subiculum) **B**, Schematic representation of the conditioning paradigm. From top to bottom are shown the conditioned (CS, red) and unconditioned (US, blue) stimuli, the moment at which a single electrical pulse (100  $\mu$ s, square, biphasic) was presented to the CA3-CA1 synapse (St. Hipp.), the electromyographic (EMG) activity of the orbicularis oculi (O.O.) muscle, and the extracellular record of hippocampal activity. The illustrated records correspond to the 7th conditioning session. Note the fEPSP evoked by the single pulse presented to the CA3-CA1 synapse. **C**, At the top (i) are illustrated fEPSPs evoked in the CA3-CA1 synapse 300 ms after CS presentation. Records were collected from conditioned and pseudoconditioned animals during the 1st and 10th conditioning sessions. (ii) Evolution of the percentage (%) of conditioned responses (CRs) during the successive sessions for conditioned (control, dots) and pseudoconditioned (circles) groups. Mean % values are followed by  $\pm$ SD. Evolution of the fEPSP slope is also indicated for conditioned (black triangles) and pseudoconditioned

(open triangles) groups, expressed as the % change with respect to mean values collected during the habituation sessions.  $*P<0.001$ .

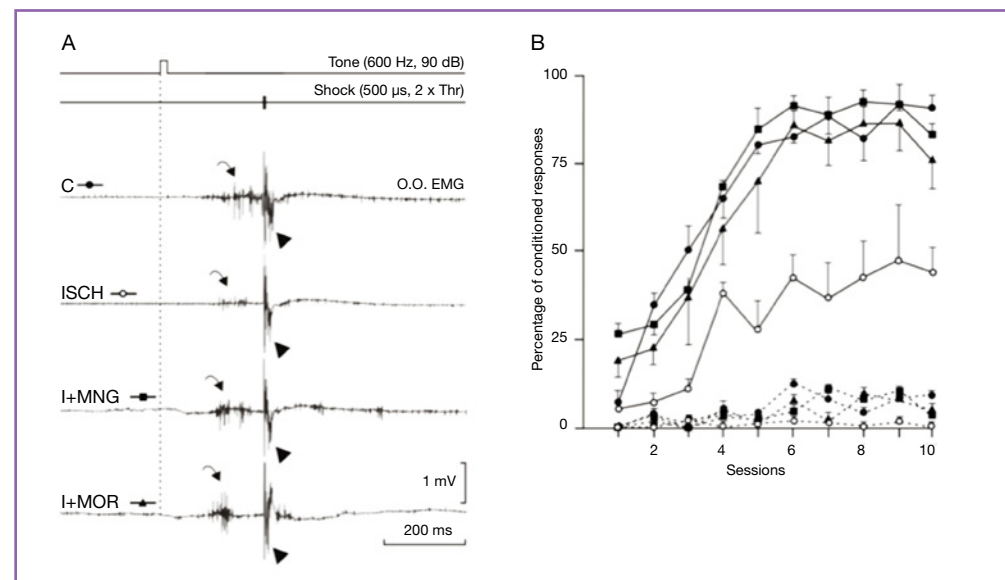
**Sources:** Delgado-García JM, Gruart A. Building new motor responses: eyelid conditioning revisited. *Trends Neurosci.* 2006 Jun;29(6):330-338. Reprinted by permission of Elsevier. Gruart A et al. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *J Neurosci.* 2006 Jan 25;26(4):1077-1087.

Another basic tenet of current neuroscience is that LTP could be the mechanism underlying certain forms of learning. LTP is evoked by high-frequency stimulation of selected afferent pathways, resulting in a long-lasting enhancement of synaptic efficacy. It was generally assumed that the experimental induction of LTP would disturb the synaptic changes taking place during associative learning in selected neural sites. Indeed, our research group and others have shown that LTP saturation of hippocampal circuits disrupts spatial and associative learning tasks.<sup>2-5</sup> It is important to point out that although LTP is evoked experimentally and activity-dependent synaptic plasticity takes place during actual learning, both plastic phenomena share some neural and synaptic properties, including the need for the proper activation of glutamatergic N-methyl-D-aspartate (NMDA) receptors at selected synaptic sites.<sup>2,3</sup> It should be kept in mind that LTP is evoked experimentally by high-frequency stimulation of selected synapses and that actual learning never evokes the huge increases in synaptic activity evoked by LTP. In this regard, LTP should not be used synonymously with synaptic changes evoked by actual learning and should not be confused with long-term memory (defined as lasting storage of acquired information). Nevertheless, the amount of LTP evoked, for example, in the hippocampus of an experimental group as compared with a control group of animals can give an idea of their relative learning capabilities.<sup>5-9</sup>

## Environmental, Social, Neuronal, and Nutritional Factors Involved in the Proper Acquisition of New Motor and Cognitive Abilities

During the last few years, research has shown that many different neuronal postsynaptic and presynaptic receptors, as well as neurotrophic factors and molecular intraneuronal cascades, are involved in the proper acquisition of new cognitive and motor abilities. For example, we have recently shown that metabotropic glutamate (mGluR), dopaminergic (D1), and cannabinoid (CB1) receptors are also involved in the acquisition and/or retrieval of associative learning tasks.<sup>6,7,9</sup> In addition, many other environmental, emotional, and social factors can modify learning capabilities.<sup>8</sup>

It also is feasible that selected dietary ingredients and nutritional factors can modify (at the least in the long run) learning and memory abilities of experimental animals and, by extension, of human beings. For example, we showed a few years ago that mangiferin and morin hydrate (two antioxidant polyphenols obtained from mango and mulberry fruits) prevent excitotoxic death and oxidative stress in cultures of rat cortical neurons and facilitate the recovery of associative learning capabilities in an in vivo model of stroke carried out in behaving rats (Fig 2).<sup>10</sup>



**Fig 2. A quantitative analysis of classically conditioned eyelid responses from control (C, and dots) and ischemic (ISCH, and circles) rats and from ischemic rats treated with mangiferin (I + MNG, and squares) or morin (I + MOR, and triangles).**<sup>10</sup> **A**, Electromyographic (EMG, in mV) recordings from representative animals of each of the indicated experimental groups collected during the 9th conditioning session. For trace conditioning, a tone (600 Hz, 90 dB) was presented for 20 ms as a conditioned stimulus (CS). The tone was followed 270 ms later by an electrical shock (500  $\mu$ s, 2  $\times$  threshold) presented to the supraorbital nerve as an unconditioned stimulus (US). Bent arrows indicate the presence of conditioned responses (CRs). Arrowheads indicate the appearance of unconditioned eyelid responses. **B**, Graphs of mean percent conditioned responses across the 10 conditioning sessions for the four experimental groups. Results collected from conditioned groups are indicated by continuous lines, whereas results corresponding to pseudoconditioned groups are indicated by discontinuous lines. Note the low learning curve corresponding to ischemic animals.

**Source:** Gottlieb M et al. Neuroprotection by two polyphenols following excitotoxicity and experimental ischemia. *Neurobiol Dis.* 2006 Aug;23(2):374-386. Reprinted by permission of Elsevier.

In addition, preliminary studies carried out in our laboratory indicate that the chronic administration of a human-milk derivate has a positive effect on LTP evoked in behaving rats as well as on their learning capabilities to solve Skinner box tasks.

## Cortical and Subcortical Circuits Involved in Learning and Memory Processes

The hippocampus has been traditionally implicated in a variety of learning paradigms, including spatial learning, object recognition, and classical conditioning of eyelid responses.<sup>2,3</sup> As a particular case, the cerebellum also has been related to the acquisition of the classical conditioning of eyelid responses using a delay paradigm. Nevertheless, our in vivo studies carried out in different experimental animal models (mice, rats, and rabbits) have shown convincingly that many other cortical (sensory, premotor, and motor) and prefrontal cortices seem to be necessary for the acquisition of complex cognitive and behavioral tasks.<sup>11-13</sup> For example, more than the hippocampus, prefrontal and striatal circuits seem to be involved in the acquisition of instrumental learning tasks.<sup>11</sup>

Interestingly, two recent reports from our laboratory<sup>12,13</sup> have opened a new experimental approach to the study of food as a natural reward and its putative effects in selected neuronal circuits. We have shown that appetitive behaviors (ie, to press a lever to obtain a food pellet) activate specific (CA3-CA1 synapse) hippocampal circuits, while consumatory behaviors (collect the pellet) depress it.<sup>12</sup> To our surprise, internal rewards as electrical self-stimulation of the medial septum by behaving mice placed in a Skinner box produce identical effects on hippocampal circuits.<sup>13</sup> These original experimental approaches open new possibilities for determining the rewarding effects of selected nutrients.

## Summary and Conclusions

Neural mechanisms underlying learning and memory processes have to be studied in alert behaving animals. Our group has developed different technical procedures for the study of the firing and synaptic activities of selected brain sites during the acquisition and recall of different types of associative (classical and instrumental) learning tasks. We also have shown that LTP evoked experimentally in laboratory animals shares some synaptic properties and molecular mechanisms with learning-dependent changes in synaptic strength. Synaptic changes evoked by LTP and/or

by actual learning can be modified by environmental, social, and emotional factors, as well as by many different drugs and putative dietary ingredients.

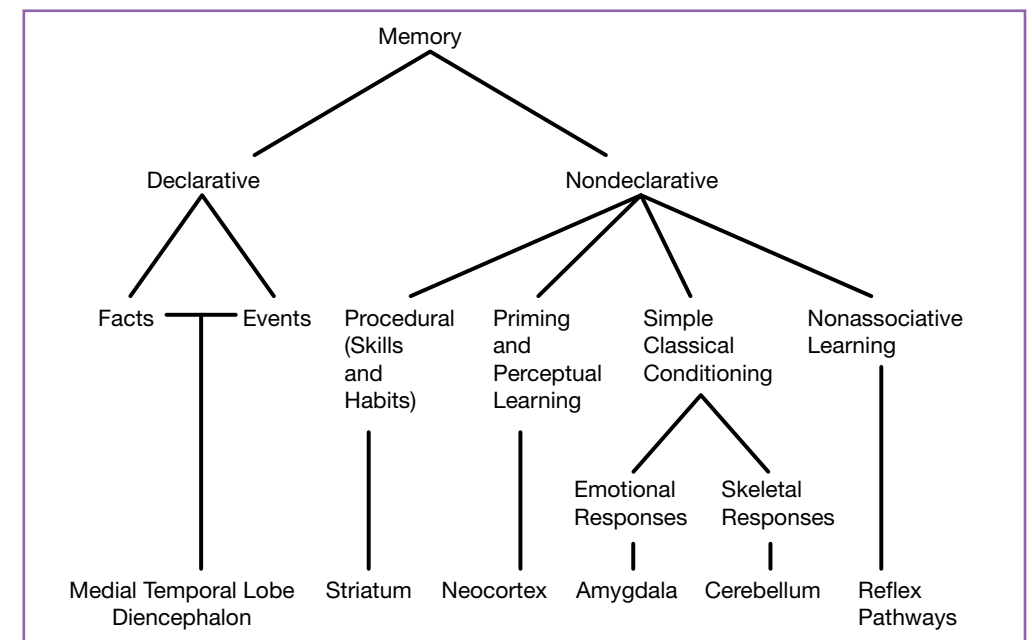
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# Taste Learning and Memory in Aging

Milagros Gallo, PhD

**D**eclarative memory includes semantic, episodic, and spatial memory, and in humans involves conscious recall.<sup>1</sup> Visual recognition memory is a type of episodic memory, while taste recognition memory leading to taste acceptance or aversion is conventionally classified as a type of nondeclarative memory not requiring conscious effort to recall (Fig 1). Dissociations between declarative and nondeclarative memory in rodents are based on the effects of aging and adult brain damage.



**Fig 1. Declarative and nondeclarative memory systems.<sup>1</sup>**

**Source:** Squire LR. Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem.* 2004;82(3):171-177. Reprinted by permission of Elsevier.

Performance in declarative memory tasks typically is impaired by aging and lesions of the hippocampal system, including the hippocampus and related cortical areas. In accordance with a nondeclarative memory classification of aversive taste recognition memory, previous animal studies have shown that taste aversion learning does not require the hippocampal integrity and is not impaired, but even improved, at advanced ages.<sup>2</sup> Nevertheless, conditioned taste aversion exhibits



learning and memory phenomena, such as blocking<sup>3,4</sup> and context-dependency<sup>5,6</sup> requiring an intact hippocampus in adults,<sup>7-10</sup> and decaying during aging.<sup>11,12</sup>

Research on safe taste recognition memory has pointed to the amygdala's role in the taste neophobic response and its habituation when the taste is recognized as familiar and safe. However, the results are controversial regarding the impact of aging in taste neophobia, indicating a critical role of previous aversive experiences.<sup>13</sup>

The Institute of Neurosciences, Center for Biomedical Research (CIBM), University of Granada, Spain, has used object recognition memory and habituation of taste neophobia tasks in order to compare the effect of aging and damage of the hippocampal system in visual and taste recognition memory, respectively. First, the spontaneous object recognition (SOR) memory task is based in the rodent's natural tendency to explore novel objects. It requires at least two sessions. The acquisition session in which the rat is allowed to explore two identical objects is followed after a variable interval by a similar retention session in which one of the objects is substituted by a novel one. The time that the rat spends exploring the novel vs the familiar object is used as an index of memory.

Second, taste neophobia is evidenced in decreased consumption of a novel taste solution compared to previous water intake during baseline and to later exposures as long as the taste becomes familiar. Habituation of taste neophobia refers to the increased intake of a familiar taste that was not followed by aversive consequences, thus showing safe taste recognition memory.

CIBM results in rats support a deleterious effect of aging, both in visual and taste recognition memory. Using a systematic approach with standard everyday objects to study the performance of aged rats in SOR memory tasks at various retention intervals, CIBM researchers identified age-related deficits at the 24-hour interval, but not at shorter intervals (10 seconds, 60 seconds, and 1 hour). A retention deficit in aged rats at the longer interval is consistent with most of the previous reports, but previous results were controversial regarding shorter intervals.

After discarding other potential explanations, CIBM explored the role of the object used, applying a 1-hour retention interval. In two different experiments, CIBM researchers dissociated the effect of object complexity, often related with ambiguity, and perceptual similarity. Thus, the pairs of objects used were either complex (ie, formed by 30 Lego® bricks of different colors, but very different in shape or very similar pyramids differing only in the number of planes [pentagon versus hexagon-based pyramids]). Surprisingly, old rats did not exhibit difficulties

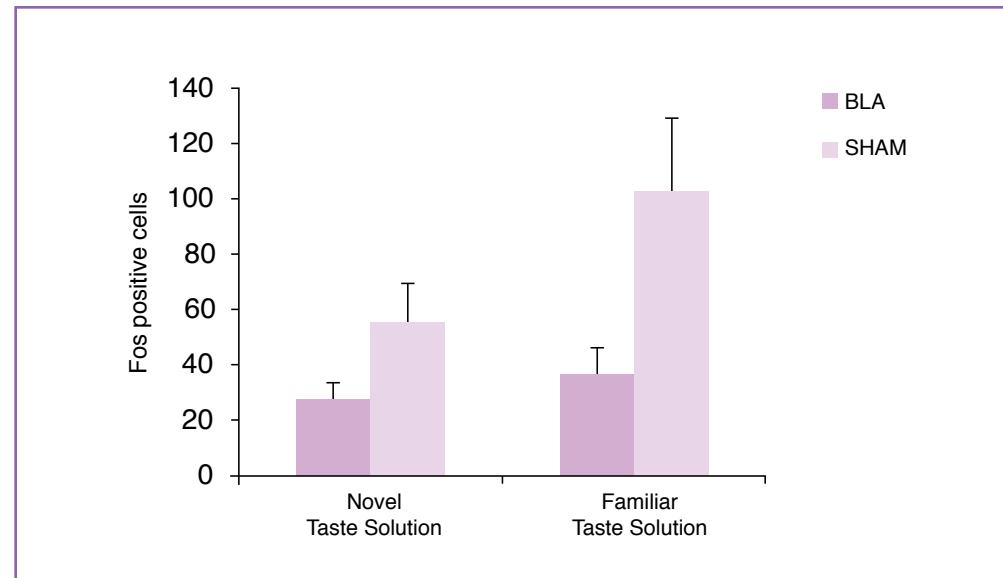
in recognizing simple geometric forms 1 hour after the acquisition, even if they were very similar, but evidenced recognition memory deficits using complex forms, even if they were very different.<sup>14</sup> This supports a selective age-related decay of memory processes involved in SOR memory.

Previous research on the effect of aging in safe taste recognition memory also yielded controversial results. CIBM researchers previously reported a reduced neophobic response and its habituation in old rats previously subjected to conditioned aversions to a different taste.<sup>13</sup> Later findings in naïve-aged rats indicated similar taste neophobia in old and younger adult rats. However, the habituation of taste neophobia is selectively impaired by aging. Thus, while younger adult rats recognize the taste as familiar and safe after one exposure, old rats require 4 exposure days. This indicates an age-related decay of taste recognition memory similar to that found in visual recognition memory.

Likewise, recognition memory seems to depend on temporal lobe areas, such as the perirhinal cortex (PRh), independently of the sensory modality. With respect to SOR memory, a growing number of studies point to the PRh as the critical brain area, although the hippocampus also is reported to have involvement. Consistently, laboratory results have shown that PRh neurotoxic lesions by N-methyl-D-aspartate (NMDA) interfere with object recognition memory, while dorsal hippocampal lesions selectively impair place-object recognition memory in adult rats. In fact, age-related deficits in SOR memory are attributed to decay of PRh function.

With respect to taste recognition memory, treatments that inhibit protein synthesis<sup>15</sup> or block cholinergic neurotransmission<sup>16</sup> in PRh interfere with the habituation of taste neophobia. CIBM also reported the first results indicating that a familiar taste solution induces increased c-Fos expression in the medial PRh,<sup>17</sup> thus supporting the involvement of the area in taste familiarity detection. Such an increase in PRh activity was abolished by amygdala lesions that impaired habituation of taste neophobia (Fig 2).<sup>17</sup> This suggests that the PRh might contribute to a widespread neural network involved in safe taste memory consolidation.





**Fig 2. Increased number of Fos positive cells in the perirhinal cortex induced by a familiar taste solution.**<sup>17</sup> Such increment was abolished by basolateral amygdala lesions.

BLA=basolateral amygdala (neurotoxic) lesion, SHAM=sham lesion

**Source:** Gómez-Chacón B et al. Basolateral amygdala lesions attenuate safe taste memory-related c-fos expression in the rat perirhinal cortex. *Behav Brain Res.* 2012;230(2):418-422. Reprinted by permission of Elsevier.

Although visual, but not taste recognition, memory in humans seems to rely on a conscious effort to recall and each memory modality depends on independent brain sensory systems, the results obtained in animal models point to shared brain processes that are similarly affected by age-related decay and that can result in functional improvement with choline dietary supplementation.<sup>18,19</sup> It is possible to propose the PRh as a shared component of the neural circuit required for taste and visual recognition memory, thus distinct mechanisms relying on PRh and the amygdala for safe taste memory and in PRh and the hippocampus for visual recognition memory are conceivable.

Therefore, functional and anatomical dissociation among shared and independent recognition memory processes involving the temporal lobe and related areas require further research. It is expected that bringing together memory tasks previously used in different and separate fields will prompt a new view to the concepts and classifications of memory.

The research reviewed is supported by research projects HUM 02763 (Junta de Andalucía, Spain) and PSIC2011-23702 (MINECO, Spain).

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# Conference Summary

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## Conference Summary

**T**he emerging field of cognition and nutrition explores ways that nutrition can enhance brain structure and physiology, cognitive development, and learning and memory across the lifespan. The right nutrition at the right time is key to cognitive development early in life and to the protection or enhancement of cognition in later years.

Dr Gary Fanjiang, Abbott Nutrition Divisional Vice President of Scientific and Medical Affairs, said in his opening remarks that nutrition and cognition are inextricably linked from preconception to old age. Cognitive development is especially critical in infants and children because the infant's brain triples in weight during the first 3 years of life. Specific nutrients support this rapid brain development. Investigations of the roles of lutein, docosahexaenoic acid (DHA), arachidonic acid, iron, folic acid, choline, and iodine in early neural development and cognition in the preconception, fetal and infant stages, and early childhood are important strategies toward favorable long-term health outcomes. A growing body of evidence also suggests the potential for dietary lutein, DHA, flavonoids, and extracts from botanical sources along with physical activity and exercise for preserving and enhancing cognitive function during aging, while reducing the risk for cognitive impairment, Alzheimer's disease, and other dementias.

The participants of the 114th Abbott Nutrition Research Conference described translational and clinical research that will help identify those nutrients that influence cognition across the lifecycle, including the type, form, dose, and timing of intake, as well as the potential for synergistic effects with exercise. With advances in magnetic resonance imaging, researchers now can analyze the physiology of the brain, providing a sensitive measure to assess the efficacy of nutrition interventions.

This publication offers 13 presentation summaries that address 5 key questions:

- How can we assess cognition, brain function, and efficacy of nutrition interventions?
- What is the impact of nutrition on cognitive development?
- What does emerging science tell us about the impact of lutein on the brain?
- How can nutrition and exercise help protect cognitive function in aging and illness?
- What can animal models tell us about nutrition effects on learning and memory?

## Assessing Cognition, Brain Function, and Efficacy of Nutrition Interventions

Science has long recognized the close relationships between cognition and brain function. The first two summaries lay the groundwork for the conference: The first explains the relationships between cognition, memory, and brain function; and the second describes the tools that are now available to measure brain structure and physiology and the effects of nutrition intervention on the brain.

### *Assessing Cognition and Brain Function*

“Cognition involves thinking and knowing, which are supported by acquiring, processing, and using information,” Dr Neal Cohen stated in his keynote presentation. He added that these actions are variously driven by mental processes in different brain regions. Different parts of the brain specialize in different functions, but the parts are extensively interconnected. Dr Cohen also described multiple memory systems in the brain and the brain regions in which those systems are expressed. He introduced the topic of nutrition and cognition by describing potential benefits from antioxidants, omega-3 fatty acids, and flavonoids in the diet. Furthermore, he presented research on the potential benefits of exercise in reducing the negative effects of dietary intake high in refined sugar and saturated fat on memory performance.

### *Advances in MR Imaging and the Questions They Answer*

Magnetic resonance imaging (MRI) techniques are used increasingly to study the brain, including investigations of how nutrition affects normal development and pathology across the lifespan. Dr Bradley Sutton discussed three particularly promising techniques—magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), and magnetic resonance elastography (MRE)—that allow researchers to monitor changes in brain metabolism, neuronal connectivity, and tissue structure. He stated that MRS can be used to examine localized brain chemistry and metabolism, DTI to assess white matter axonal connectivity and integrity of the membranes and myelin, and MRE to provide information on the overall structural integrity of brain tissue. These highly sensitive methods allow measurement of nutrition-associated changes that were not detectable with earlier imaging technologies or with assessments of cognitive behavior.

## Impact of Nutrition on Cognitive Development

Brain development occurs at a remarkable pace in the fetus and in infancy. In terms of energy utilization, 74% of a newborn infant’s energy intake fuels the brain and its growth, while just 23% of resting energy intake is consumed by the adult brain. Adequate energy and protein intake, as well as intake of other specific nutrients such as iron, folic acid, and long-chain polyunsaturated fatty acids are necessary to support this rapid structural growth of the brain.

### *Early Programming of Brain Development*

Prof Cristina Campoy advised that an infant’s brain triples in weight during the first 3 years of life—from 400 to 1200 grams. Exposure to diet, drugs, and adversity during such sensitive windows of early life can lead to lasting changes in gene expression that contribute to the display of physiological and behavioral phenotypes. Diet is a potent modulator of epigenetic marks, especially during prenatal and early postnatal life. Diets high in choline, methionine, folate, and vitamins B<sub>6</sub> and B<sub>12</sub> increase DNA and histone methylation, alter gene expression, and can result in permanent changes in development. Prof Campoy described studies that, using new and more sensitive measures, have identified some of the mechanisms associating early nutrition with later brain developmental outcomes. She concluded that understanding these mechanisms may have an enormous preventive potential, given the major public health implications, including opportunities for an improvement of cognition and an effective primary prevention of childhood and adult behavior and mental diseases.

### *Measuring the Impact of Nutrition on Cognitive Development*

Human brain development begins at conception. However, the influence of nutrition on brain development begins before conception and continues for many years. Dr Carol Cheatham reviewed the most important nutrients for brain development and discussed their cognitive effects. She outlined the rationale for studying the effects of nutrition on two specific cognitive abilities—memory and speed of processing. Dr Cheatham argued that the importance of nutrition to cognition in general cannot be overstated because memory is central to learning, and speed of processing underlies all cognitive abilities. Yet definitive data are lacking regarding roles, doses, and timing for intake of specific nutrients. Dr Cheatham concluded that nutrition researchers should work with developmental cognitive neuroscientists to use behavioral and electrophysiological methodologies to determine the effects of nutrition on brain development and help ensure that children have a chance to achieve their cognitive potential.

## Lutein's Influence on Cognitive Development and Function

New research is revealing the potential importance of dietary carotenoids to visual and brain development in infants and children. Lutein is a carotenoid that plays a key role in development and function of the human retina, especially in infants. The retina is a neural tissue; as such, retinal development may provide insights into development and function of the brain. Furthermore, lutein may protect the brain from cognitive decline related to aging.

### *Emerging Science on Lutein in the Brain*

Lutein is the predominant carotenoid in pediatric and adult brain tissue. Infants are born with carotenoids acquired during gestation, but because the body cannot make lutein, humans depend on dietary sources throughout life. Lutein in neural tissue has biological effects including antioxidant, anti-inflammatory, and structural actions. In infants' brains, the contribution of lutein to the total carotenoids is twice that found in adults, accounting for more than half the concentration of total carotenoids. In the adult, a variety of evidence supports a role for lutein in cognition. Therefore, Dr Elizabeth Johnson argued, the greater proportion of lutein in the pediatric brain suggests a need for lutein during neural development. Infant formula is not routinely supplemented with lutein, whereas breast milk is a highly bioavailable source of lutein. Given that the 1st year of life is a time of neural growth and development for which nutrition can have significant consequences, the addition of this dietary plant pigment to infant formulas could be an important strategy toward favorable long-term health outcomes.

### *Lutein's Influence on Neural Processing Speed*

Just as macular carotenoids such as lutein are important for infant vision and brain development, they also are important for the function of the adult brain and retina. Lutein, for instance, influences many aspects of central nervous system function. These effects extend from optical filtering within the eye to physiological activity of neurons within the brain. A growing body of evidence suggests that lutein can enhance neural processing speed. This is particularly important for the elderly because slowing appears to be a central feature of cognitive decline and impairment. Dr Billy R. Hammond Jr described the mechanisms by which lutein could produce these effects (eg, by reducing oxidative and inflammatory stress, improving neural collective processing, and preserving brain white matter). He concluded that lutein likely serves multiple functions within the central nervous system and that these functions seem optimally suited to the preservation and

perhaps even enhancement of cognitive performance. Thus, carotenoids are important to protecting both vision and cognition against age-related decline.

## Protecting Cognitive Function in Aging and Illness With Nutrition and Exercise

In 2010, there were 36 million people in the world living with dementia, and the number is expected to double every 20 years—reaching an alarming 115 million people in 2050. In the words of one conference participant, however, “By combining nutrition and exercise, we have a remarkable opportunity to preserve memory across the lifespan.” Use of bioactive ingredients such as flavonoids that have anti-inflammatory properties, combined with a program of physical activity, may help protect cognitive function during aging.

### *Nutrigenetics and Cognitive Health*

People are living longer than ever before and therefore are more likely to experience age-related diseases and conditions. However, living longer is not matched by an increase in healthy life expectancy. The aging population demographic is having a dramatic impact on dementia incidence worldwide, with prevalence approximately doubling every 20 years and estimated to increase to 115 million by 2050. In the context of these demographic changes, Dr Anne Marie Minihihane reviewed the acute and chronic impact of eicosapentaenoic acid and docosahexaenoic acid and the interaction with the apolipoprotein 4 genotype. According to Dr Minihihane, lifestyle, diet, and genetics interact to confer risk for cognitive decline with aging, and two of these factors are modifiable.

### *The Role of Flavonoids in Preventing Neuroinflammation and Cognitive Decline*

Plant-based compounds called flavonoids have powerful anti-inflammatory and anti-neurotoxic properties, which means they may help offset aging processes in the brain by preventing and repairing cellular damage. The potential of dietary flavonoids for aiding in the preservation of cognitive function during aging, while reducing the risk of Alzheimer's disease and other dementing disorders, has gained great interest in research literature during the past decade. Dr David Vauzour discussed the impact of nutritional antioxidants on neuroinflammation and neurocognitive performance, and the role of flavonoids and their anti-inflammatory properties in cognitive protection. He also described the effects of flavonoids on the vascular system, which may induce increases in cerebral blood flow capable



of having an impact on acute cognitive performance or may lead to an increase in hippocampal vascularization capable of inducing new neuronal growth.

### ***Neurocognitive and Mood Effects of Nutrition and Nutraceuticals***

Dr Andrew Scholey stated, “The list of factors contributing to dementia is long and complicated—including disease processes, specific risk factors, and lifestyle aspects.” Nutrients and extracts from botanical sources, unlike mainstream pharmacological agents, may contain many active components with a combination of properties that may affect multiple neuronal, metabolic, and hormonal systems with direct effects on cognitive processes. Dr Scholey described the role of specific herbal extracts such as ginseng and lemon balm on adult cognitive function, as well as techniques for mood and cognitive assessment—eg, magnetoencephalography, which measures changes in magnetic fields associated with postsynaptic potentials. He also discussed the need for future research to discover synergistic nutrition interventions to optimize day-to-day cognitive function, maintain psychological well-being throughout life, and treat conditions in which mental function becomes fragile, including dementia.

### ***From Inflammation to Sickness and Cognitive Dysfunction: When the Immune System Subjugates the Brain***

Dr Rodney Johnson reported substantial evidence for an association among infection, inflammation, and altered immune function, which is, in turn, associated with cognitive dysfunction. Microglial cells, resident macrophages in the central nervous system, are relatively quiescent but can respond to signals from the peripheral immune system and induce neuroinflammation. In aging, microglia tend to transition to a proinflammatory state and become hypersensitive to messages emerging from immune-to-brain signaling pathways. Thus, in older individuals with an infection, microglia overreact and produce excessive levels of inflammatory cytokines causing behavioral pathology, including cognitive dysfunction. Dr Johnson described recent studies that indicate dietary flavonoids have anti-inflammatory properties and are capable of mitigating microglial cells in the brain of aged mice. Thus, he argued that dietary or supplemental flavonoids and other bioactive compounds have the potential to restore the population of microglial cells in the aging brain to a more quiescent state.

### ***Exercise and the Aging Brain***

Dr Arthur Kramer summarized a wealth of information about how physical activity benefits cellular and molecular actions in the brain. He described observational and randomized controlled human studies that have established the relationship between physical activity and cognitive maintenance in normal adults or in adults diagnosed with neurodegenerative diseases such as Alzheimer’s or Parkinson’s disease. Dr Kramer also cited more recent studies that have reported similar cognitive and brain benefits, as a function of exercise and physical activity, for children. Such findings are important to the understanding of lifestyle choices on cognitive and brain development as well as the impact of the increasing sedentary nature and levels of obesity observed for children in today’s society. However, he said, many important questions remain unanswered, such as can a combination of nutrition and exercise bestow greater benefits to healthy minds and brains than either of these factors alone?

### ***Nutrition Effects in Learning and Memory in Animal Models***

Neuroscientists are looking closely at cellular, molecular, and electrophysiological mechanisms underlying learning and memory processes. Animal models provide a unique opportunity to examine complex details of the relationship between nutrition and learning.

### ***Associative Learning and Long-Term Potentiation in Rodents: Effects of Nutrition***

Many excellent in vitro studies describe the electrophysiological processes and molecular events supporting activity-dependent synaptic changes. However, little information is available on synaptic changes in strength during actual learning in behaving animals. Dr José Delgado-García and his research team have shown that classical conditioning of eyelid responses in behaving mice increased the synaptic strength of the hippocampal CA3-CA1 synapse. He described technical procedures used to study the firing and synaptic activities of selected brain sites during different types of associative learning tasks. He stated that long-term potentiation evoked experimentally in laboratory animals shares some synaptic properties and molecular mechanisms with learning-dependent changes in synaptic strength. Synaptic changes evoked by learning can be modified by environmental, social, and emotional factors, as well as by certain drugs and putative dietary ingredients.

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### *Taste Learning and Memory in Aging*

Dr Milagros Gallo and colleagues have studied taste aversion learning in rodents as a model for memory acquisition and its reorganization across the lifespan. The effect of aging on taste memory is a complex mix of impaired, preserved, and enhanced functions. Research on safe taste recognition memory has pointed to the amygdala's role in the taste neophobic response and its habituation when the taste is recognized as familiar and safe. However, the results are controversial regarding the impact of aging in taste neophobia, indicating a critical role of previous aversive experiences. Dr Gallo shared her research on the effect of aging in rodent taste memory and compared the brain mechanisms of taste and visual recognition memory. She concluded by explaining the need for further research involving the functional and anatomical dissociation among shared and independent recognition memory processes involving the temporal lobe and related areas.

### **Conclusion**

We hope this conference report encourages you to explore new strategies and tools to help ensure that your patients are receiving optimal nutrition that, combined with adequate physical activity, supports cognitive development in early life and protects cognitive function throughout the adult years.