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REVIEW

Cognitive and behavioral evaluation of nutritional interventions in rodent models of brain aging and dementia

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Abstract: Evaluation of behavior and cognition in rodent models underpins mechanistic and interventional studies of brain aging and neurodegenerative diseases, especially dementia. Commonly used tests include Morris water maze, Barnes maze, object recognition, fear conditioning, radial arm water maze, and Y maze. Each of these tests reflects some aspects of human memory including episodic memory, recognition memory, semantic memory, spatial memory, and emotional memory. Although most interventional studies in rodent models of dementia have focused on pharmacological agents, there are an increasing number of studies that have evaluated nutritional interventions including caloric restriction, intermittent fasting, and manipulation of macronutrients. Dietary interventions have been shown to influence various cognitive and behavioral tests in rodents indicating that nutrition can influence brain aging and possibly neurodegeneration.

Keywords: calorie restriction, intermittent fasting, aging, memory, macronutrients

Introduction

The major translational goals in aging research are to extend healthy lifespan and to reduce chronic diseases of aging. Old age is the greatest risk factor for all major causes of mortality and morbidity: cardiovascular diseases, cancers, and in particular the neurodegenerative diseases including movement disorders and dementia.[1] Most research into dementia has focused on developing pharmacological interventions to treat and prevent cognitive impairment, but despite an extensive international effort, the only medications that are available are cholinesterase inhibitors and memantine, which have modest effects on symptoms but not disease progression.[2]

An alternative approach to prevent the onset of age-related neurodegenerative disease, while simultaneously improving overall health, is nutrition. Nutritional interventions such as caloric restriction, intermittent fasting (IF), and manipulation of macronutrients have often been reported to improve healthspan and lifespan in a variety of organisms and laboratory settings, with increasing evidence that they are effective in humans.[3–5] Nutritional interventions are readily translatable because they are available to entire populations and have less economic burden and adverse effects associated with prescription medicines.[6] Yet, the study of nutritional interventions in the context of neurodegenerative disease remains undeveloped.

Rodent models of cognition and behavior have various strengths and weaknesses, particularly in how they reflect human brain aging and cognitive deficits.

Here, we review the effects of manipulations of nutrient intake on cognition and behavior in rodent models of aging, brain aging and dementia.

Nutritional interventions in aging research

Three nutritional interventions have been at the forefront of aging research: 1) calorie restriction (CR) – a total reduction by 10%–50% in daily caloric intake without malnutrition; 2) IF – extended periods of food deprivation (typically 16–24 hours) with intervening periods of food intake; and 3) varying macronutrient ratios in ad libitum diets.[3,7,8]

The molecular mechanisms by which those interventions counteract aging are complex, and recent findings implicate nutrient-sensing pathways (eg, sirtuins [SIRT-1], insulin-like growth factor-1 [IGF1], peroxisome proliferator-activated receptor gamma coactivator 1-alpha [PGC-1α], mechanistic target of rapamycin [mTOR], and fibroblast growth factor 21 [FGF21]).[9,10] The plasticity of these pathways in response to nutritional changes result in reduced levels of inflammation, increased insulin sensitivity, efficient autophagy, and increased cellular stress resistance, all leading to lifespan extension and health benefits in a variety of species.[11,12]

While CR can extend lifespan and counteract many age-related diseases in a wide range of species, it is difficult to implement in humans with essentially unlimited access to calorie-rich food. Another criticism of CR is that health benefits may not be due to a reduction in total calories, but rather a reduction in one or several dietary macronutrients. This line of reasoning grew from the field of nutritional ecology, in which the ratios and amounts of macronutrients in foods, meals, and diets have been shown to exert a driving influence on animal physiology across numerous species.[13] We recently confirmed this in rodents using a systematic experimental design, where an ad libitum low-protein, high-carbohydrate (LPHC) diet was shown to be more effective than CR (by dilution with fiber) in improving lifespan and several markers of cardiometabolic health.[14] Conversely, high-protein, low-carbohydrate diets resulted in reduced lifespan and poorer cardiometabolic health. We hypothesized that reducing protein while simultaneously increasing carbohydrate in the diets functioned in a similar way to CR and IF by changing the underlying molecular pathways. Specifically, we suggested that high-protein intake coupled with low carbohydrate was detrimental to health possibly due to branched-chain amino acids (valine, leucine, and isoleucine) activating mTOR which subsequently mediated the response between healthspan and lifespan. The mTOR and other pathways, including pathways involving insulin, growth hormone and IGF-1, have been implicated in lifespan in a variety of organisms.[15] IF can extend lifespan and forestall many age-related disease processes in rodents with minimal overall reduction in caloric intake.[7,16]

Studies have investigated the roles of specific amino acids on healthspan and lifespan, with a focus on branched-chain amino acids, methionine (Met), and tryptophan (Trp). Met restriction has been shown to improve lifespan of yeast and mammalian cells through a mechanism involving reduced amino acid intake and suppression of the mTOR pathway, resistance to stress, and attenuated levels of inflammation.[17] In rodents, Met restriction reduces mitochondrial oxidative stress and improves insulin sensitivity, with subsequent studies showing lifespan benefits.[18] There has been limited research on dietary Trp reduction, with results pointing toward reduced inflammation and constraint of cell growth.[19]

While the role of LPHC diets in extending healthspan has begun to be elucidated, there is limited information on potential roles of LPHC diets, Met, and Trp on brain health during aging. Such animal studies manipulated one dietary macronutrient for a short period and then assessed performance in a cognitive task shortly thereafter. A recent study demonstrated that a large increase in only one macronutrient, while keeping the other two at low percentages, exacerbates cognitive decline in a mouse model of Alzheimer's disease (AD).[20] Similar studies have concluded that each macronutrient plays a critical role in brain health, but it is not known what ratios of macronutrients may be optimal for memory and cognition. However, such "one-nutrient-at-a-time" manipulations do not consider the interactive effects of single nutrient manipulations on balance with respect to other dietary components.[21] There is evidence that the brain, like other physiological systems, requires a target ratio of macronutrients to develop and function optimally, with this ratio changing across the life course and with aging.[22] The developing brain may be particularly sensitive to macronutrient intake, such that restriction of certain macronutrients during embryonic and early postnatal development can adversely affect cognitive outcomes.[23]

A key concept that has emerged from studies of macronutrient intake is that nutrient sensing pathways responsible for metabolic adaptations also affect brain functionality and the pathogenesis of many different neurological disorders.[24]

In the case of energy restriction, we reviewed the effects of CR and IF on cognitive health during aging in rodents.[22] In brief, CR and IF can increase dendritic spine density in hippocampal neurons, improve mitochondrial respiration, promote neuron viability/survival, enhance synaptic plasticity, and regulate genes involved in memory and cognitive function.[25] CR and IF have consistently been reliable and robust interventions to hinder neurodegeneration in mouse and rat models of Alzheimer's, Parkinson's, and Huntington's diseases and stroke.[26,27] A limited number of studies have also demonstrated neuroprotective effects of CR in non-human primates.[28–30]

Although there is strong evidence that CR and IF are two of the most effective non-pharmacological or non-genetic treatments to prevent neurodegeneration, there are no studies on the potential benefits of protein restriction (particularly LPHC diets) on brain health during the aging process. Given the robust evidence that long-term LPHC nutritional interventions are beneficial for improving lifespan and metabolic health in rodents, coupled with the fact that those treatments appear to activate similar nutrient response pathways as CR and IF,[14] it is plausible that LPHC diets may also be beneficial for preventing the onset of neurodegenerative disease.

Many tools and methods have been used to evaluate the effects of macronutrient intake on the aging brain. Here, we review studies in which the impact of CR, IF, and restriction of dietary protein and specific amino acids on cognition have been evaluated in rodents. A better understanding of how nutritional interventions affect aging brain biology might enable the development of specific macronutrient-based interventions to optimize brain health during aging.

Overview of the impact of aging on cognition in rodents

Extensive research about age-related changes in learning and memory, and underlying cellular and molecular mechanisms has accrued during the past three decades.[31–34] Memory tests in rodents ideally will parallel those tests commonly used in humans; however, this is not possible because of major species differences in memory and cognition. There are limited animal tests that serve as models for many complex manifestations of dementia such as emotional and language impairment, and executive function.[35] There are, however, a variety of behavioral assessments (Table 1) that have been developed which are meant to target the same declining memory processes as seen in humans during aging and with dementia diagnoses.[36]

The first types of memory to deteriorate during AD in humans are episodic and semantic memory, while working spatial memory closely follows. These forms of memory are usually affected because of early involvement of the hippocampus and associated long-term potentiation processes.[37,38] Episodic and semantic memory loss often coincides with lapses in spatial and navigational learning and memory. Spatial learning is a process that involves multiple cognitive and perceptual processes and refers to attaining and maintaining a certain trajectory from one place (or object) to another and is learned over time. Therefore, cognitive–behavioral tasks assessing spatial memory are particularly important in rodent research and are widely used in nutritional interventions. There are a variety of well-established memory-assessing cognitive–behavioral tasks, either at a specific point or over a time course in rodents (Table 2). It is important to assess rodent cognition using behavioral tests that are relatively simple and with low levels of inter-animal variability within a laboratory and high reproducibility between laboratories. However, some learning and memory deficits are subtle; therefore, cognitive tests that are more demanding are often required to discriminate differences between ages and experimental groups of animals.[39] Testing animals in both negative- and positive-reinforcement paradigms can reduce the effects of

Table 1 A selection of different types of memory common to both rodents and humans

Name of memory process (reference)	Examples in rodents	Examples in humans
Episodic memory[105]	Object recognition, target object recognition, IntelliCage	Mnemonic memory, remembering past experiences and analyzing possible future events, information about the order of events
Recognition memory[51]	Object recognition, touch screen	Recognition of faces or well-known objects
Semantic memory[45]	Morris water maze, Barnes maze, object recognition	Memory of facts and places
Spatial memory[40]	Morris water maze, Barnes maze, Y-maze, radial arm water maze	Navigating through the streets of a hometown, following directions, navigating around rooms of a house, remembering where items are placed
Emotional memory[106]	Fear conditioning	Learning to associate cues to negative outcomes, expressing emotional responses (ie, stress)

Table 2 Commonly used tasks to assess rodent memory acquisition

| Name of test (reference)	Purpose and type of memory	Methodology	Strengths	Weaknesses
Water maze[40]	Spatial memory acquisition, some working memory	4-day spatial memory acquisition followed by probe trial 24 hours later	Well-established test and used in laboratories across the world, easily reproducible, simple procedure	Can be stressful for rodents, expensive, time consuming, can only test one rodent at a time, necessary to have a drainage and fill system for water, necessary for video tracking software
Barnes maze[42]	Spatial memory acquisition, some working memory	4-day spatial memory acquisition followed by probe trial 24 hours later	Less stressful for rodents utilizing more natural memory processes, well established, used in laboratories across the world	Time consuming, can only test one rodent at a time, necessary to have a large room for the 1 m wide maze and video tracking equipment
Novel object recognition[50,51]	Associative memory, declarative memory, working memory	2-day protocol, replace old object with novel object, calculate "recognition index"	Well-established test, utilizes rodents' natural exploratory processes, cost-effective, time efficient	Does not assess spatial memory, possible experimenter bias in results analysis
Fear conditioning[107]	Associative memory	2-day protocol – teach rodents to associate a "tone" with a foot shock, learning measured by freezing behavior	Short-time commitment from researcher (2 days), well established in the literature, robust results	Stressful for rodents, pain factor involved, expensive equipment, does not exclusively involve hippocampus
Radial arm water maze[108]	Spatial and working reference memory	Learned location of one of the eight escape arms, multiple trials over several days	Well established, natural exploring tendencies, short-time commitments, short training protocol, no olfactory cues	Can be expensive, swimming may induce stress, difficult to set up
Y-maze[60]	Active working short-term memory	Two trials, "spontaneous alternation" is calculated by the number of entries into the novel arm, quantified as spontaneous alternation	Cost-effective, minimal training, not time consuming, natural exploratory behavior of the animal	Does not measure memory acquisition over long periods of time

confounding factors unrelated to memory, such as sensory or motor function, anxiety, or depression.

Behavioral tests of learning and memory in rodents

Several experimental tests have been utilized to study the effects of nutrients on cognition and synaptic plasticity (Figure 1).

Morris water maze (MWM)

The MWM (Figure 1A) tests spatial learning and memory retention in rodents and involves an acquisition phase where the animal learns the location of a "goal or escape platform" target over a period of multiple days.[40,41] In this test, rodents swim in a circular pool and learn to find a platform which is hidden 1–2 cm below the surface of opaque water. The rodents use cues placed around the room to orient and learn the location of the platform. Memory acquisition is typically measured by how quickly the animals learn to find the platform over designated number of training trials (memory acquisition), and then probe trials are performed (with
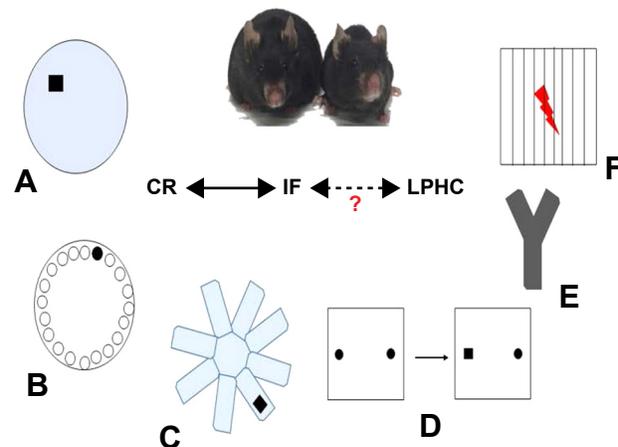
Figure 1 The most commonly used memory behavioral tests in rodents.
Notes: (**A**) Morris water maze: rodent learns to find submerged escape platform over time (black indicates escape platform); (**B**) Barnes maze: rodent learns to find escape box over time (black indicates escape hole); (**C**) radial arm water maze: rodent learns the location of the correct arm containing the submerged escape platform (black indicates escape platform); (**D**) novel object recognition: recognition index calculated as a percentage of time the rodent explores the novel object vs the old object on day 2 (black indicates objects, arrow signifies inter-trial interval); (**E**) Y maze: number of entries into novel arm quantified after brief training period; and (**F**) fear conditioning: freezing activity calculated after a brief conditioning phase (lines indicate wires, bolt indicates mild shock).
Abbreviations: CR, calorie restriction; IF, intermittent fasting; LPHC, low-protein, high-carbohydrate.

increasing lag times) in which the platform is removed from the pool and the amount of time the animal spends close to the former location of the platform is determined (memory retention). MWM probe trial results are most typically represented as the percentage of time the animal spends in the target quadrant and the number of entries to the previous location of the platform.

Barnes maze (BM)

The BM (Figure 1B) was developed based on the same principles as the MWM[42,43] but, in contrast to the MWM, imposes little or no stress on the animal because there is no swimming.[44,45] The test consists of a circular platform comprising 20 equally spaced concentric open holes. Under one of the holes lies a black escape box. Memory acquisition is measured by how quickly it takes the rodent to find the escape box over a 4-day period, and a probe trial is performed 1 day later to assess if the rodent actually learned the task. It is known that high-stress situations increase corticosterone levels in mice, which are inversely correlated with spatial memory acquisition.[46] For those reasons, the BM is recommended for the study of spatial memory acquisition.

Radial arm water maze (RAWM)

The RAWM (Figure 1C) test was primarily developed as a spatial and reference memory task, although it may also be used to evaluate working memory.[47] The RAWM is a popular test because it does not require extensive training and minimizes olfactory cues.[48] The basic premise of RAWM is that rodents learn to locate a hidden platform in one of the eight arms. After a brief training period, results are quantified by the number of errors (ie, incorrect arm entries) and the number of seconds the mouse takes to reach the escape platform.[49]

Novel object recognition (NOR)

One of the most popular memory tests in rodents is NOR (Figure 1D) because it parallels similar tests in humans. NOR in mice was predicated upon the primate and human preference to explore a novel stimulus rather than a known one.[50] This highlights the translational characteristics of the test and takes advantage of the natural tendency of rodents to explore a novel object rather than the one previously present.[51] The NOR testing protocol is relatively simple, cheap, and quick, thus widely utilized.[52] After a short period of habituation to the study arena (typically a box with opaque walls), the rodent is exposed to two similar objects. There is an inter-trial interval (24 hours) and one of the objects is replaced with a novel object. A "recognition index" is thus calculated as a percentage of time the rodent explores the new object compared with the familiar one.[53]

Y-maze

The Y-maze is a relatively simple behavioral test meant to quantify working memory and anxiety in rodents. Therefore, this assay might have an advantage in that dementia and anxiety are often comorbid.[54] Similar to the NOR task, the Y-maze test utilizes rodents' natural exploratory tendency and there is minimal human interaction with the animal. In keeping with its name, the Y-maze contains three identical arms with high walls (Figure 1E). In trial number 1, one arm is blocked (the novel arm) and the rodent explores for 5 minutes. In trial 2, the rodent freely moves among all the three arms. The general premise of the second trial is that mice will remember the previously explored arms while entering the novel arm a higher number of times, commonly termed "spontaneous alteration."[55]

Fear conditioning (FC)

FC (Figure 1F) is a widely used behavioral memory test in rodents because of its Pavlovian nature and its relative ease of performance. FC is in a general sense similar to the "eye-blink" associative learning paradigm seen in humans and primates, where adverse events are learned quickly and anticipated.[56] Studies have indicated that classical conditioning and amygdala-dependent FC are impaired in AD patients.[57] The basic FC protocol is to place rodents in an enclosed box on an electrified wire floor. After a brief exploration period, a series of audible tones/bright lights are presented and followed by mild foot shocks (pain). The rodents are then removed from the box for a certain time interval (usually 24 hours) and then placed back into the box. The same tone is presented and the measure of learning is quantified by how quickly, and to what extent, the rodent freezes after exposure.[58]

IntelliCage and touch screen

Novel behavioral assessing technologies are under development and therefore not currently used in the context of nutritional interventions. However, those technologies may be beneficial to further investigate the roles of nutrition in rodent memory. One tool is the rodent touch screen which is a useful tool for assessing working memory.[59] Another powerful tool is the IntelliCage which can be used for a variety of learning and memory paradigms.[60] After a time of extensive training, the touch screen and IntelliCage paradigms are heavily automated and therefore greatly reduce experimenter interference.

The impact of CR and IF on memory acquisition during aging in rodents

CR and IF positively affect MWM, BM, and RAWM performance under a variety of conditions and ages in rodents.[61] Recent studies have demonstrated that CR improved proximity and time to reach the escape platform in the MWM task highlighting improved spatial discrimination.[62] In another study, short-term CR (28 days) significantly decreased the time necessary to reach the escape platform, and animals spent significantly more time in the target quadrant during the probe trial.[63] Furthermore, it was demonstrated that exercise training plus CR enhanced rat spatial memory in the MWM, possibly due to upregulation of brain-derived neurotrophic factor (BDNF).[64] In a transgenic mouse model of AD, 1 year of 40% CR or every other day IF ameliorated spatial memory acquisition and retention in the MWM.[65]

In the BM, 11 months of IF significantly improved spatial acquisition and the time necessary to reach the escape hole when compared with those mice fed the same diet ad libitum.[66] In another study, short-term (6 weeks) IF did not improve RAWM memory acquisition in mice and did not significantly change hippocampal BDNF levels.[67] The results were similar to another study where starting CR at 6 months of age did not improve spatial memory in old rats.[68] Conversely, another study showed that CR significantly improved RAWM performance in rats possibly by the upregulation of BDNF.[69] Approximately, 40% CR for 3 months significantly improved RAWM performance in a rat model of cerebral ischemia.[70]

Spatial memory is one of the most widely studied forms of memory in animal models, although there are other forms of memory that must be taken into consideration to capture the full scope of memory loss that occurs during aging and neurodegenerative states. Associative and recognition memory, for example, are important to study as those forms of memory loss often occur either in conjunction with, or prior to, the loss of spatial memory in AD.[71] Familiar object detection, a form of recognition memory, is known to deteriorate in early stages of AD.[72] Patients may not recognize physical objects that they recently encountered, and in very late stages of the disease those "objects" include those experienced regularly throughout life, including the faces of family members and friends. This is often termed "associative memory," and it has been suggested that this effectively paired with working memory.[73] One form of associative memory has led to the paired associates learning task in humans, which tests the ability to remember the location of objects in the environment.[74] Those types of associative and working memory, often called "executive function," decline progressively in dementia.[75]

The Y-maze has been used to evaluate effects of nutritional interventions on working memory. One study demonstrated that 20% and 35% CR for 3 months significantly improved learning in male Kunmin mice.[76] In another study, a periodic diet designed to mimic fasting improved spontaneous alternation behavior in 23-month-old mice.[77] One study that investigated a 40% lifelong CR treatment in rats showed a decrease in NOR in the CR group, suggesting that CR treatment worsened NOR performance.[62] Another study demonstrated that long-term CR improved NOR memory in old LOU/C/Jall (LOU) rats, which are considered a "gold-standard" for aging research because they live much longer than other strains.[78]

There have been a variety of nutritional studies which utilized FC for assessing memory. One recent study suggested that hunger may play a critical role in the formation of fear-related contextual and cued memory by strengthening amygdala connections – more specifically the basolateral nucleus and central amygdala, both of which are involved in fear extinction learning. One recent study confirmed these results, where the deletion of the hunger *gene Y4* reduced appetite and impaired the FC response in mice. Interestingly, this result was reversed by fasting, as quantified by FC.[79] Other studies have confirmed those results, for example, CR significantly improved contextual freezing behavior in a mouse model of AD.[80] Another genetic study showed that CR ameliorated freezing behavior seen in presenilin-1 and -2 double knockout mice.[81] With regard to amino acids, one study looked at the effects of limiting dietary Trp intake for at least 1 month in young mice and assessing FC responses, concluding that Trp is essential in the formation of hippocampal contextual and cued memory formation.[82]

Influence of individual macronutrients on learning and memory in rodents

The limited number of studies looking at macronutrient ratios in mouse memory acquisition highlights the need for further investigation in that area. While there have been limited interventional studies in this regard, there is information on the general roles of single macronutrients on cognition.[83]

Protein and amino acids

Dietary protein restriction can extend lifespan in a range of organisms, including rodents.[84] Cycles of dietary protein restriction ameliorated working memory deficits evaluated in the Y-maze and lessened hyperphosphorylated Tau levels

without affecting Aβ levels.[85] Conversely, a high-protein diet worsened memory acquisition in male Wistar rats in the MWM.[86] Most of the studies on dietary amino acids and cognition have focused on Trp because it is a precursor to serotonin, which is a neurotransmitter that regulates learning and memory, and mood.[87] When rats were maintained on a Trp-deficient diet during the first 2 postnatal months, they exhibited impaired memory acquisition in the MWM.[88] BDNF likely plays a role in the beneficial effects of dietary Trp on cognition because serotonergic synaptic activity increases BDNF expression.[89] Indeed, it was recently reported that dietary Trp supplementation increases BDNF expression in the hippocampus and frontal cortex of aged rats.[90]

Fat

It is a well-established fact that excessive and chronic high-fat diet (HFD) hinders rodent memory performance and injures hippocampal neurons in many strains of mice and rats.[91,92] It was recently shown that an energy-dense diet worsens cognitive decline in a rat model of AD by increasing the levels of amyloid precursor protein and dysregulation of the sirtuin pathways.[93] In another study, a 3-month HFD period significantly increased the proinflammatory cytokine profile in mice and impaired NOR test performance.[94] A chronic HFD results in impaired short- and long-term memory in mice as measured by NOR and MWM, possibly due in part to impaired hippocampal insulin signaling. CR can attenuate the adverse effects of a HFD on cognition.[95]

Carbohydrate

Chronically excessive glucose or fructose intake impairs synaptic plasticity and cognition by mechanisms involving the production of proinflammatory factors, insulin resistance, and glucose metabolism dysregulation, possibly contributing to the development of AD and other neurodegenerative diseases.[96,97] Poor glycemic control and type-2 diabetes may accelerate the onset of dementia during aging.[98] IF is believed to enhance hippocampal synaptic plasticity and cognitive performance, in part, by elevation of the circulating ketone beta-hydroxybutyrate which is not only an energy substrate for neurons but also induces the expression of BDNF.[99,100] It was recently reported that a ketone ester diet can ameliorate learning and memory deficits and lessen anxiety in a mouse model of AD.[101]

Conclusion and future research

Nutrition influences brain aging. CR and IF are two robust interventions that positively impact the aging brain. Studies to date have not addressed the issue of dietary nutrient balance on the aging brain. There is mounting evidence that the ratios and amounts of individual macronutrients influence late-life cardiometabolic health and lifespan and do so via similar metabolic molecular pathways as CR and IF. Nutritional geometry offers an integrative framework for designing, interpreting, and implementing dietary studies that explore the main and interactive effects of different nutrients, and offers a powerful tool for future research into brain health.[102–104]

Regarding experimental tools, rodent behavioral testing provides a useful model for assessing the role of nutrition in brain health. There are a variety of behavioral assays available to assess those changes, each with their strengths and limitations. We suggest that the establishment of cooperative efforts and agreement among scientists with regard to rodent behavioral testing will help to "bridge the gap" in nutritional cognitive research. Those collaborative efforts will help establish similarities and differences in determining the effects of various nutritional interventions on rodent memory.

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Disclosure

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