Brain Energy Metabolism and Effects of Aging: Do We Become What We Eat?

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Dogs as potential models for human cognitive change, and the benefits to dogs from what we have learned about humans: Although rats and mice are more commonly used, the domestic dog may be a better model for complex genetic traits such as those involving behavioral disorders, including brain aging[1]. The dog has several important advantages over rodents as a model for complex behaviors, among which is the shared evolutionary history of dogs and humans emphasized here. In addition, the enthusiasm and close cooperation of dog owners and breeders facilitate an ongoing interest in canine genetics within the dog community, and provide access to needed samples. Finally, breeds were developed through selection for specific types of tasks or work and most extant breeds are less than 150 years old, further reducing heterogeneity[2].

Glossary of Abbreviations

ALA: Alpha-Linoleic Acid
APOE: Apolipoprotein E
APP: Amyloid Precursor Protein
ARA: Arachidonic Acid
ATP: Adenosine Triphosphate
8-OHB: Beta-Hydroxybutyrate
BDNF: Brain-Derived Neuotrophic Factor
cAMP: Cyclic AMP/Cyclic Adenosine Monophosphase
CD: Cognitive Dysfunction
CDS: Cognitive Dysfunction Syndrome
CREB: Cytosolic Response Element Binding Protein
DHA: Docosahexanoic Acid
EPA: Eicosapentaenoic Acid
E-LTP: Early-Phase LTP (Protein Independent)
L-LTP: Late-Phase LTP (Protein Dependent)
LTM: Long-Term Memory
LTP: Long-Term Potentiation
MCT: Medium-Chain Triglycerides
NMRS: Nuclear Resonance Spectroscopy
PUFAs: Polyunstaturated Fatty Acids
RNA: Ribonucleic Acid
STM: Short-Term Memory
TCA Cycle: Tricarboxylic Acid Cycle [Krebs Cycle]
trkB: Tyrosine Kinase B

Recent advances in canine genomics have potentially facilitated efforts to map genes for complex behaviors[3,4], including some of those potentially involved in information processing and age-related effects on this. The work of Lindblad-Toh and colleagues is remarkable for its demonstration of extensive synteny — basically, exact similarity —- between the canine and human genomes, as well as the finding that, despite being more distantly related to humans than rodents[5], the dog shows more nucleotide homology with humans than do rodents. Again, such patterns are likely the result of a co-evolutionary process that still may be ongoing.

Recent data indicate that dogs are significantly more comparable to humans than are chimpanzees and wolves with regard to the complex social cognition involved in understanding long-distance signals that indicate where food is hidden. Dogs are further able to communicate this information to other dogs[6-10]. Dogs appear to have the ability to "fast map" — to make deductions about object class and name without having learned them — and to communicate this ability to humans[11].

Also, like humans, dogs suffer from what we recognize as maladaptive anxiety, which interferes with normal functioning and which was selected against during the co-evolution of dogs and humans[12]. Finally, when examining the rates of gene expression mutations in regional brain tissue, the only species studied to date that has comparable rates to those found for humans is the domestic dog[13]. Such data, when

taken together, strongly suggest that dogs can be excellent models for human brain aging, and that any data that accrue from studies of human brain function may be relevant for understanding canine brain function. Such syntenic patterns open an array of treatment and mechanistic modalities for those interested in brain aging.

Brain aging as a special case — newer challenges and findings: Given the previous evolutionary discussion, no one should doubt that canine brains age, and when they do, many of the dimensions of canine brain aging resemble those seen in humans. While humans are afflicted by numerous tauopathies, each of which may have defining cognitive and/or anatomical dimensions, emphasis in canines has been placed on a relatively nonspecific diagnosis of canine cognitive dysfunction (CD), sometimes also called cognitive dysfunction syndrome (CDS)[14].

In dogs, CD is usually diagnosed because of a history of disorientation, alterations in social/interactive behaviors, changes in locomotor behavior and sleep cycles, and what is often called "loss of housetraining." In early onset cognitive dysfunction, animals may have only slightly altered sleep cycles and appear more anxious. Alterations in social/interactive behaviors may manifest early in the condition as an increased neediness but change to a form of aloof disengagement in social interactions with all species.

We have chosen to treat such conditions with medications designed to address anxiety, panic and depression[15] because of the changes such medications cause at the receptor level. Oxidative damage to receptor systems has been at the core of much research on cascades that become damaged once free radicals are involved[16]. However, such treatments neglect other aspects that may affect how well learning occurs and how cognition can occur, including those aspects in providing power to the learning process.

Review of what happens in the dog's brain when it learns something: Behaviors are reinforced or learned best if every time they occur they are rewarded. At the cellular level, repeated reinforcement ensures better, more numerous and more efficient connections between neurons[17,18]. Stimulation is induced when a neurochemical in a synapse triggers a receptor to engage it. This stimulation of the receptor engages second messenger systems in the post-synaptic cell, usually cyclic AMP/cylic adenosine monophosphate (cAMP). The result is cellular memory or long-term potentiation (LTP). By itself, this initial process represents early-phase LTP (E-LTP) and short-term memory (STM). The process is short-lasting and RNA and protein-synthesis-independent, and the result does not persist or become self-potentiating unless the stimulus is consolidated into late-phase LTP (L-LTP), which is a more permanent form[19]. E-LTP can be induced by a single train of stimuli in either the hippocampus or the lateral amygdala.

In contrast, L-LTP and long-term memory (LTM) require repeated stimulation of cAMP, induction of cAMP response element binding protein (CREB, a nuclear transcription factor) — and are long-lasting, protein-synthesis dependent and RNA-transcription dependent[19]. When stimulation continues, brain-derived neuotrophic factor (BDNF) enhances neurotransmission and potentiates what is called activity-dependent plasticity at synapses (e.g., learning), particularly in the region of the brain most involved in learning, the hippocampus. This effect can also occur in the lateral amygdala and is one modality postulated to be involved in learned or conditioned contextual fear[19] (see Figure 1).

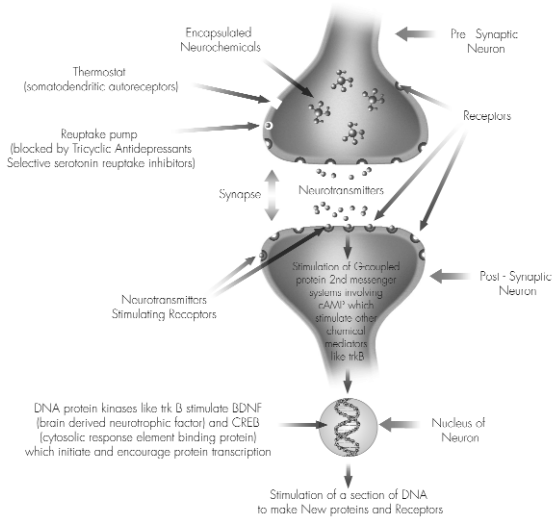

Figure 1: The stimulation of second messenger systems as a result of neurotransmission ultimately produces increases in BDNF and CREB, which are responsible for new protein formation as one mechanism by which molecular memory is produced. Note that many psychotropic medications affect this process, as may diet. All these activities require energy to be executed and completed.

Courtesy of Nestlé Purina PetCare Europe, Vevey, Switzerland.

What does the brain use for energy: Diet can affect behavior through chemical interactions between amino acids and by altering brain energy sources, allowing alterations in use of resources. Energy sources for the brain can actually be variable, and lactate, acetate and pyruvic acid are now considered viable energy sources in addition to what traditionally has been considered the main energy source, glucose.

Energy sources in the brain: Glucose is considered the common brain energy currency, but it is not stored. The stored form of glucose is glycogen. Glycogen is found mainly in astrocytes, and the amount of glycogen available is affected by glucose concentration and neurotransmitter presence and function[20]. During hypoglycemia, glycogen is converted to lactate via pyruvate (glucose → pyruvate → lactate). The lactate is then

transferred to adjacent neurons. This conversion and transfer allow the neurons to use a source of aerobic fuel.

Glycolysis can also be anerobic and is faster at producing energy than is oxidative phosphorylation[21]. In fact, glycolysis makes pyruvate faster than it can be oxidized: By converting glucose to lactate, adenosine triphosphate (ATP) is made twice as fast than would be the case were glucose oxidized completely[21].

Lactate:

The use of lactate in hypoglycemic events can extend axon functions for 20+ minutes[20]. This conversion of astrocyte glycogen to lactate also occurs during periods of intense neural activity, demonstrating the role of astrocytes as bankers of energy-conversion compounds.

Lactate is the preferred energy source for the human brain, after glucose[22], and there is no reason to assume that this may not also be an important pattern in dogs. The majority of lactate used as an energy source is thought to come from glycogenic processes because most lactate itself is too large a molecular to pass through the blood-brain barrier. However, blood lactate has been measured in oxidized form and may be a source of some energy for brain tissue[22]. In fact, some astrocytes appear to "prefer" to process glucose glycolytically into lactate[23]. Lactate can then be converted into pyruvate and enter the tricarboxylic acid (TCA) cycle, providing energy in the form of ATP.

Medium-Chain Triglycerides (MCT):

Ketone bodies and fatty acids have been proposed as alternate energy sources (see Figure 2) because of their modulating effects on hypoglycemia. In particular, 8-hydroxybutyrate (8-OHB) may be useful[24] for protecting hippocampal neurons from toxicity. In a placebo-controlled, double-blind study, Reger et al[24] found that mildly impaired Alzheimer's disease patients who were supplemented with MCT showed improvement in a number of pre-v. posttreatment cognitive test measures, and that such improvement correlated with 8-OHB increases. It should be noted that this result depended on whether there was an apolipoprotein E (APOE) genotype: Only patients without an APOE-epsilon4 allele responded to acute elevation of 8-OHB.

Fatty acid oxidation in the brain has been studied in rats using nuclear magnetic resonance spectroscopy (NMRS). One of the MCTs, octanoate, is thought to comprise up to 13% of the free fatty acid pool in humans. Because it readily crosses the blood-brain barrier, it's been studied in a variety of clinical and experimental settings. In a labeling study in rats subjected to NMRS, octanoate could contribute 20% of brain energy in an intact, physiological system[25]. The mechanism for this was likely incorporation into both glucose and ketones, and secondary effects on the metabolism of the excitatory neurotransmitter, glutamate.

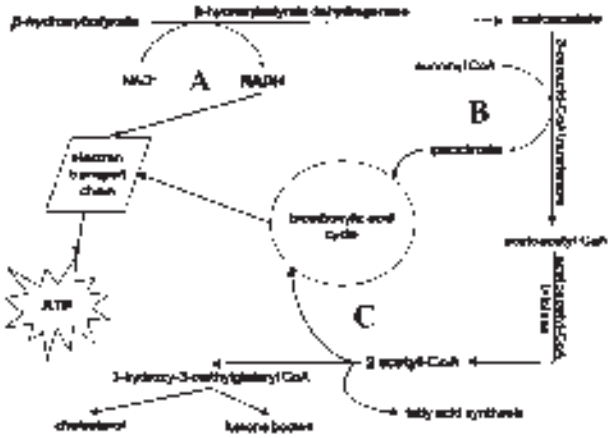

Figure 2: In this illustration showing ketone body catabolism, ATP is generated three ways. (1) NADH is produced from the dehydrogenation of 8-OHB, making electrons available for the electron transport chain (pathway labeled A). (2) CoA is transferred to acetoacetate, forming succinate, which then enters the TCA cycle (pathway labeled B). (3) Ketone bodies are converted to acetyl CoA, which then can also enter the TCA cycle (pathway labeled C). Source: Studzinski et al., *Brain Res.* 2008;1226:212.

In a study of 8 Beagles (4 control; 4 treatment) from 9 to 11 years of age, supplementation with MCT at a dosage of 2 g/kg/day resulted in improved mitochondrial function that was most pronounced in the parietal lobe[26]. Steady state levels of amyloid precursor protein (APP) also decreased in the parietal lobe after short-term supplementation leading the authors to conclude that short-term MCT supplementation can improve brain energy metabolism and also decrease APP levels in old dogs.

Taha et al.[27] have postulated that age-related cognitive decline in dogs may be associated with decreases in omega-3 PUFAs in the brain. Because MCT increase fatty acid oxidation, they may increase omega-3 polyunsaturated fatty acids (PUFAs) in the brain via metabolism of adipose tissue. In a two-month study of 8 Beagles (4 control; 4 treatment) fed an MCT-enriched diet, enrichment was shown to result in increases in brain phospholipid and total lipid concentrations.[27]

Factors Affecting Oxidative Stress — Roles for Neurotransmitters:

One of the major foci of age- and illness-related changes is the effect of a cumulative burden of oxidative stress over time. Increased oxidative stress is one of the most common topics examined in brain aging, and it appears to affect all major classes of molecules involved in neurotransmission. Development of oxidative stress may not be independent of energy source or use. Interestingly, intermittent fasting has been reported to induce the production of BDNF[28], which is associated with neurogenesis and molecular learning and memory, particularly in the hippocampus. Increases in BDNF affect numerous

signaling pathways involving tyrosine kinase B (trkB), which may directly or indirectly affect regional brain metabolism and function.

Astrocytes are responsible for de novo synthesis of two neurotransmitters: glutamate and D-serine[29]. Glutamate, the excitatory neurotransmitter that is responsible for an estimated 85% of synaptic activity, appears to also be essential in metabolic activity of the brain. Glutamate may be responsible for energy regulation by affecting neurovascular exchange[23]. Glutamate has as its signaling targets the synapse, astrocytes and intra-parenchymal capillaries.

In normal brain function, glutamate affects signaling by altering flow of calcium and sodium ions: post-synaptically it modifies the permeability of NMDA receptors to sodium and calcium, and the AMPA receptors to sodium, and presynaptically it affects NMDA receptors and metabotropic receptors via calcium. This interaction is what causes an excitatory postsynaptic potential (EPSP). Glutamate activity is also thought to be involved in pathological conditions where excitatory sensitivity has been implicated (e.g., strokes, impulsively aggressive states, cortical and hippocampal epileptogenic activity). In both normal and pathological conditions, glutamate's main effect is on excitability and synaptic plasticity.

Glutamate also affects astrocytes, which are non-neuronal cells[23]. Glutamate transporters appear to use the sodium gradient to facilitate glutamate uptake by astrocytes. Recent anatomical studies show that astrocytic processes ensheath intraparenchymal capillaries and synapses, and that many of these processes have receptors and reuptake sites for neurotransmitters. It is these findings that allow glutamate to act as a metabolic intermediary. In short, glutamate stimulates the conversion of glucose into lactate in astrocytes.

Interestingly, many pathways that affect glycolysis for brain energy are also adversely affected at some point by oxidative change. Many of these effects may be modulated by antioxidant or co-factor treatment, coupled with active behavioral interventions/enrichment. Alpha-enolase interconverts 2-phosphoglycerate and phosphoenolpyruvate. Alpha-enolase has been shown to be altered in canine models of neurodegenerative disorders and responds to treatment with antioxidants, mitochondrial co-factors (lipoic acid) and behavioral/ social/cognitive enrichment[30]. Decreased oxidation of alpha-enolase and GAPDH could improve glycolytic function, with a resultant increase in ATP production. Together, these alterations appear to lead to neuronal recovery and improved cognitive function in the canine model of human brain aging[30].

In a study of gene expression in brains of old dogs, the expression of genes involved in neurochemical signaling and synaptic transmission was decreased[31]. Particularly affected were levels of growth and transmission factors already discussed, including BDNF and trkB. These factors did not respond to antioxidant diet supplementation. Interestingly, in the same study, compounds like glutathione S-transferase — responders to oxidative stress — were also decreased in geriatric dogs. Such findings show the ultimate interrelatedness of available brain energy, neurotransmission and neuroregulator function and structural changes in aging dogs.

Structural components of neuronal membranes that may be important for use and transport of energy in the brain: Arachidonic acid (ARA), docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) are long-chain PUFAs that are essential for developing and maintaining the integrity of cells of the brain's membranes. These PUFAs are related by their synthetic sequence: linoleic acid (18:2 n-6) becomes ARA (20:4 n-6), which becomes docosapentanoic acid (22:5 n-6). Elongation of alpha-linoleic acid (ALA) by desaturation produces EPA (20:5 n-3), which can then be metabolized to DHA (22:6 n-3)[32].

All these PUFAs are essential for early brain development. ARA is thought to especially maintain hippocampal cell membrane fluidity and protect cells in the hippocampus from oxidative stress. The hippocampus is one of the main areas involved in LTP, a form of molecular learning, and is one of the main regions where associational learning takes place.

DHA may encourage development-stage specific associational learning, although the data are mixed. Supplementation with DHA and EPA affect concentrations of these substances in rat brains, but their distribution is not uniform. Diets deficient in ALA especially cause decreases of DHA in the frontal cortex — the part of the brain responsible for complex learning and integration of information and executive function. In dogs, low concentations of DHA during gestation and/or lactation depress the retinal sensitivity of puppies, which can have profound and complex behavioral outcomes. The current data support the need for DHA for optimal neurological development in puppies, and there are hints that it may improve both early- and long-term cognitive abilities, but the data are scant.

There has been some suggestion that PUFAs are also important in some canine behavioral conditions. In a study of German Shepherd Dogs with a history of aggressive behavior, aggressive dogs showed a significantly lower concentration of DHA (22:6 n-3) and a higher omega-6/omega-3 ratio when compared to unaffected dogs[33]. Plasma concentrations of ARA (20:4 n-6) and EPA (20:5 n-3) did not differ. These same animals showed reduced levels of cholesterol compared to control dogs. Similar nonspecific findings regarding cholesterol have been reported for aggressive dogs[34]. It is important to realize that the characterization of "aggression" in these studies is variable, and that such correlations say nothing about cause. Such findings could be the outcome of aberrant neurochemical function. However, one of the main roles of PUFAs appears to be maintenance of membrane fluidity and protection from oxidative stress, espe-

cially in the part of the brain essential to associational learning, the hippocampus.

Finally, in humans, the brain contains 600 g lipid/kg, with approximately equal amounts of ARA and DHA. It's been postulated that a dietary intake of 6 to 12% protein comprised of Rift Valley lake fish and shellfish provided sufficient DHA and ARA that allowed the early hominoid cerebral cortex to grow disproportionately without requiring an increase in body mass[35]. Any putative effects of these PUFAs on cognitive abilities are likely routed in this evolutionary history. Interestingly, PUFA levels in brains of young versus geriatric dogs, when measured, have not been shown to be different[31], but effects of varying amounts in different regions of the brain (e.g., the hippocampus, which is key to learning, and the frontal cortex, which is involved in learning and essential for executive function or application of that learning) in older animals has not been studied.

Summary

Our brains are doubtless shaped by what we eat, and so our dogs' brains are shaped by what we choose for them to eat. It would surprise no evolutionary biologist that alternative brain energy pathways exist and that they maintain healthy and active brain function and neurotransmission. But the effects of ebb and flow of food on these effects may suggest that our culture — and our dogs' culture — of constantly available food, rather than constantly available cognitive stimulation, has not adequately considered the extent to which this is a strategy that has not been chosen by the shared dog-human evolutionary history. Exploration of effects of diet on canine cognition and recovery from the dreaded effects of aging could enhance our understanding of the shared development of dogs and humans as inter-dependent, potentially co-evolved species.

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