Minireview

The aging brain – molecular and metabolic changes

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Summary. Aging is a complex set of events that involves the whole body. However, disruption of the central nervous system (CNS) function is the aspect of aging that elderly people worry about most. Aging has different effects on different aspects of neurological function. Our knowledge of the basic molecular mechanism of brain aging has significantly improved over the past few decades. The rate of aging is not fixed, but is plastic and subject to modifications. The environmental factor proven to be very potent in modulating aging is reduced dietary intake. Dietary restriction (DR) is a vigorous nongenetic and nonpharmacological intervention that is known to delay ageing and increase an active and healthy lifespan in diverse species, from yeast to mammals. Additionally, DR can improve various brain functions, including learning and memory, synaptic plasticity and neurogenesis.

Keywords: aging, brain, dietary restriction.

Introduction

According to the World Health Organization (WHO), the population of elderly people in the world in the last 50 years has tripled, and this trend will continue in the next 50 years. The old population is growing faster than the total population in almost all regions of the world, with a consequent increase in the incidence of age-related chronic diseases, including neurodegenerative disorders. As a result, the costs of long-term care are set to sharply increase (Figueira et al. 2016), becoming a tremendous social and political problem. On the other hand, there has been an expansion of research related to the aging process, as well as to the development of strategies that might slow aging and ensure a good quality of life for the elderly.

Aging is a phenomenon which is influenced by many factors, that happens at the molecular, cellular and tissue level cand represents a complex set of events (Cummings 2007), involving the whole body. However, disruption of the central nervous system (CNS) function is the aspect of aging that old people particularly worry about. It is believed that the brain represents one's essence, and losing a grip of it is rarely understandable and acceptable. Changes in the brain that occur during aging have been studied since the 19th century, but there is still a surprising amount of confusion as to what these changes are.

The term "normal brain aging" is used to describe the aging of the CNS in the absence of clinically diagnosed neurodegenerative or psychiatric disease or related pathologies. However, the molecular changes that occur during normal aging of the brain significantly overlap with those observed in many diseases.

Aging has different effects on various aspects of neurological function (Christensen 2001). Skills related to the speed of information processing, problem solving, inhibitory function, working memory, long-term memory and spatial orientation, all decrease with aging (Park and Reuter-Lorenz 2009). In addition, a variety of motor functions, including

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reaction time, speed of movement, hand and foot coordination, constantly decrease during the aging process. Changes in mood and perception of emotions are also present during the aging process. In contrast, abilities relating to general knowledge, implicit memory, understanding proverbs and professional expertise do not decrease with age, but over the years may even improve (reviewed in Christensen 2001; Park and Reuter-Lorenz 2009).

Our knowledge of the basic molecular mechanisms of aging has significantly expanded over the past few decades. Signaling pathways that appear to be the major regulators of aging have been identified. Recent studies have linked these signaling pathways to control of age-dependent brain pathologies, indicating that disrupted regulation of fundamental aging mechanisms may contribute to the pathogenesis of neurodegenerative disorders. Better insight into the biology of the aging brain is achieved by using advanced techniques. Microarray technologies have enabled a global analysis of gene expression in model organisms and humans, and have led to the identification of evolutionarily conserved changes during aging. At the same time, advanced brain imaging techniques have allowed insights into the cognitive networks of the aging human brain.

Macroscopic changes in the aging brain

Numerous postmortem studies of human brains have revealed a loss in brain weight of about 0.1% per year in people aged between 20 and 60 years. This loss is more severe after the age of 60 (Ho et al. 1980). Brain volume also decreases during aging (Pfefferbaum et al. 1994) and this reduction shows some regional differences. The frontal and parietal cortex is affected more than the temporal and occipital cortex. The striatum is also affected (Resnick et al. 2003). The reduction in brain volume increases by 0.1-0.2% per year between 30 and 50 years of age and 0.3-0.5% per year after the age of 70. The ventricular system expands and fills the space left by brain volume reduction. The leptomeningeal complex slightly widens, while the subarachnoid space increases.

Microscopic changes in the aging brain

Understandings of the microscopic basis for the macroscopic changes observed during brain aging have changed over the years. The main controversy relates to the loss of neurons. Initial studies that began in the 1950s examined the density of neurons in two-dimensional space and concluded that a substantial loss of neurons (10-60%) occurs with aging, depending on region and neuronal population. The neurons of the cerebral cortex (Henderson et al. 1980), the hippocampus (Ball 1977) and Purkinje cells of the cerebellum (Hall et al. 1975) were believed to be particularly affected by aging. Recent studies based on stereological studies of neurons in

three-dimensional space (Benes and Lange 2001) led to the conclusion that the loss of neurons in aging was undetectable, or relatively moderate (Hof and Morrison 2004). However, the size of neurons, reflecting the level of dendritic and axonal arborization, decreased during aging (Terry et al. 1987), which consequently led to synapse number reduction.

Cellular and molecular changes in the aging brain

The key factor that plays an important role in aging brain is the high rate of oxidative metabolism providing enough energy for neuronal functioning. Mitochondrial oxidative metabolism inevitable leads to the formation of free radicals capable of damaging proteins, lipids and nucleic acids. DNA damage leads to reduced gene expression and/or formation of abnormal proteins that have to be eliminated. It was shown that genes involved in synaptic function and plasticity, vesicular transport, mitochondrial function and calcium homeostasis exhibit reduced expression after the age of 40. Antioxidative enzymes and growth factors, which are expected to inhibit the effects of oxidative damage, are altered due to changes in the effectiveness of their signaling pathways or low production during aging (Mattson et al. 2004). Antioxidant defenses in the brain were, at best, relatively low. In contrast, the brain is rich in iron and unsaturated fatty acids, which are particularly susceptible to peroxidation (Xiong et al. 2002).

So far, the data have revealed the importance of neurotrophins in the treatment and prevention of numerous age-related neurodegenerative diseases. Amongst them, brain-derived neurotrophic factor (BDNF) is the best characterized as the modulator of normal brain aging (reviewed in Tapia-Arancibia et al. 2008). BDNF is a growth factor whose secretion is dependent on neuronal activity, and its level in the brain decreases with age. This factor is required for the change in density of dendritic spines that underlie learning and memory disorders, which are disturbed during senescence (reviewed in Tapia-Arancibia et al. 2008).

There is increasing evidence about the role of neurotransmitters, especially serotonin, glutamate and dopamine, in modulating normal brain aging. It is considered that serotonin plays a role in the normal aging brain, since its amount and the function of corresponding receptors are regulated by aging. In addition, serotonin and other age-regulatory molecules, such as BDNF and insulin growth factor 1 (IGF-1) share the same signaling pathways (reviewed in Mattson et al. 2004). However, its full role in the process of extending life and maintaining health has yet to be clarified.

Glutamate, the predominant excitatory neurotransmitter in the brain, is also a potential candidate as a brain-aging modulator. It facilitates the release of BDNF and is essential for long-term potentiation (LTP), synaptic plasticity, neurogenesis and neuronal survival depending on the activity and neural sprouting during development (reviewed in Mattson

2008). Through the increase in intracellular calcium and the effects of signal transduction pathways mediated by CREB and NFkB, glutamate modulates a rapid shifting of the dendritic architecture and long-term changes in the transcription of different genes, including neurotrophins, which probably contributes to the age-dependent morphologic changes (reviewed in Mattson 2008).

Dopamine is another neurotransmitter than can modulate brain aging. It is involved in several age-related diseases, including Parkinson's (PD) and Huntington's (HD) disease. Additionally, the dopamine transporter (DAT) and dopamine receptors D1 and D2 deteriorate with age (Backman et al. 2006).

Synaptic plasticity in the aging brain

Neuronal connections are not fixed, even in adulthood. They can change in response to both intrinsic and extrinsic stimuli. This plasticity allows the brain to compensate for injury and disease and to adapt to the changing environment, i.e. to learning processes and memory formation. Hence, it is the prerequisite for lifelong adaptive structural development (reviewed in Schaefers 2013).

Neuroplasticity involves several levels. Functional plasticity on the level of the synapse involves changes in the quantity of transmitter release or receptor densities. Structural changes lead to an enlargement or reduction of the synaptic contact area, the remodeling of whole synapses or even the retraction or extension of spines, branches, dendrites or axons. Finally, lifelong neurogenesis and synaptogenesis in the hippocampal dentate gyrus represent the most extraordinary forms of neuroplasticity. In the course of physiological aging, plastic processes continue to decline. The number of synapses decreases and the morphology of synaptic contacts alters (Hof and Morrison 2004; Morrison and Baxter 2012). Additionally, a reduction occurs in the synthesis, receptor densities and binding sites of several neurotransmitters, including dopamine, serotonin, GABA and glutamate (reviewed in Schaefers 2013).

Brain metabolism during aging

The brain comprises about 2% of body mass, but utilizes 25% of the total body glucose (Rossi et al. 2001). The greater part of glucose is used for energy generation through glycolysis and mitochondrial oxidative phosphorylation to support synaptic transmission. Neuronal glucose metabolism is complex. It includes mechanisms that control brain glucose uptake, such as insulin and the insulin signaling pathways. Brain glucose uptake comprises glucose transporter (GLUT)-dependent glucose transport across the endothelium into astrocytes via GLUT1 and its transfer into neurons, mostly via GLUT3 and GLUT4, and subsequent glucose involvement in the glycolytic pathway. The final step is the entry of glycolytic endpoints into the mitochondria that are further metabolized in the TCA cycle to generate ATP through oxidative phosphorylation.

Animal studies showed decreased brain glucose uptake with age along with a parallel decrease in neuronal GLUTs in Fischer 344 rats (reviewed in Yin et al. 2016). Aging induces changes in both glucose availability and the mitochondria energy-transducing capacity, including a decline in neuronal glucose uptake, decrease in electron transport chain activity, and increase in oxidant production (reviewed in Yin et al. 2016).

Insulin and IGF-1signaling (IIS) play a crucial role in regulating and maintaining brain metabolic and cognitive function (de la Monte et al. 2005). Some of the key metabolic characteristics of the human longevity phenotype are a preserved insulin sensitivity, low insulin levels and reduced flux through the IIS (Harper 2014; van Heemst 2010). Insulin signaling regulates mitochondrial function and its impairment underlies abnormalities in both mitochondrial function and biogenesis. Brain aging is associated with a reduced IIS pathway entailing inactivation of the PI3K/Akt signaling in rats (Jiang et al. 2013). Overall, one can conclude that aging is associated with an increased risk of deteriorating systemic control of glucose and of brain glucose uptake (Craft 2006). Several preclinical and clinical studies seem to support a role for lowered brain glucose uptake in age-associated cognitive impairment (Cunnane et al. 2011).

In addition to glucose, cholesterol is essential for proper brain functioning. The brain contains 5-10 times more cholesterol than any other organ. The blood-brain barrier (BBB) renders homeostasis of brain cholesterol independent of circulating cholesterol, and consequently the brain produces cholesterol by *de novo* synthesis. Cholesterol biosynthesis is a complex process. The first steroid intermediate in cholesterol synthesis, lanosterol, can be converted to cholesterol through two alternative pathways: the Bloch pathway via desmosterol, a direct cholesterol precursor, and the Kandutsch-Russell pathway via lathosterol. Being a multifaceted molecule, it performs a plethora of functions. Cholesterol is an indispensable membrane constituent, a cofactor for signaling molecules and a precursor for steroid hormones. The relationship between cholesterol and synaptic plasticity is firmly established (reviewed in Pfrieger and Ungerer 2011). A disturbance in cholesterol homeostasis underlies many neurodegenerative diseases, and with aging represents a major risk factor for the development of such pathologies. Data regarding brain cholesterol metabolism during aging are somewhat inconsistent (reviewed in Martin et al. 2010), suggesting that cholesterol metabolism in the different regions of the brain is not uniform. Our study revealed that cholesterol synthesis, assessed by measurements of the concentrations of its precursors (lanosterol, lathosterol and desmosterol), was increased with advanced age in extra cerebral tissues, while in the brain the rate of cholesterol biosynthesis was decreased

during aging. Major changes among brain regions were detected in the hippocampus (Smiljanić et al. 2013).

Environmental factors and aging brain

The rate of aging is not predestined, but is plastic and subject to modifications. Among others, an environmental factor proven to be very potent in modulating aging is diet. For decades it has been known that a reduced daily food intake significantly extends the life of various species (Katewa and Kapahi 2009).

Dietary restriction (DR) is a vigorous nongenetic, non-pharmacological intervention that is known to delay aging and increase the active and healthy lifespan of diverse species, from yeast to mammals (Katewa and Kapahi 2009). DR can improve various brain functions, including learning and memory, synaptic plasticity (Mladenović Djordjević et al. 2007, 2010; Smiljanić et al. 2015) and neurogenesis, to enhance the performance of rats and mice in various behavioral tasks and to counteract age-related molecular and cellular alterations that impair cognition (Duan et al. 2001). The protective role of reduced food intake may also be seen in Alzheimer's disease (AD) and PD, as well as in other neurodegenerative disorders (Mattson et al. 2002).

Dietary restriction and synaptic plasticity

Numerous clinical and epidemiological studies have confirmed the effects of DR on neuroplasticity and neuroprotection (for a review see Mattson et al. 2002). The impact of DR on age-associated alterations in gene expression is highly dependent on the class of gene and the brain regions (Park and Prolla 2005). DR induces transcriptional reprogramming of neural plasticity genes. Adams et al. (2008) found a significant increase in synaptophysin (SPH) in old animals that had been subjected to DR compared to *ad libitum* (AL) controls, indicating synaptic changes following DR. Results from our laboratory showed that long-term DR applied in 6-month-old rats can modulate age-related transcriptional changes of two presynaptic markers, GAP-43 and SPH, mostly in the cortex (Mladenović Djordjević et al. 2010). However, in the hippocampus lifelong dietary modulation increased the levels of these proteins whose expression significantly decreases during aging.

The beneficial effect of DR-promoted amelioration of age-related changes has been shown for other proteins associated with synaptic plasticity in the hippocampus (Park and Prolla 2005). Elevated GAP-43 and a-synuclein protein levels, in addition to SPH, suggest that a presynaptic compensatory mechanism is activated in the hippocampus during lifelong DR.

Dietary restriction and neurotrophins

One of proposed mechanisms accounting for the positive effects of DR includes enhanced neurotrophins expression. Literature data have verified the importance of BDNF in mediating DR-induced neuroprotective and metabolic effects (Duan et al. 2001). However, the data are inconsistent. While it has been reported that intermittent fasting (IF) increases BDNF levels in the rodent hippocampus (Duan et al. 2001), no significant alterations or even decreases were observed in a model of reduced daily food intake (Andrade et al. 2006). Furthermore, while in rodents long-term DR prevents age-related deficits in BDNF-triggered processes, i.e. LTP and memory consolidation (Okada et al. 2003), some studies have failed to reveal any influence of DR on learning capacity (Hansalik et al. 2006), whereas others have pointed to a DR-related worsening of cognitive functions despite increased longevity (Yanai et al. 2004). Our results demonstrated that long-term IF proved to be a potent modulator of the proBDNF/p75NTR and BDNF/TrkB systems in the rat cortex and hippocampus. It displayed both brain-region specific and age-dependent impacts on Bdnf transcription, BDNF protein synthesis/processing, TrkB receptor expression/glycosylation and p75NTR level (Smiljanić et al. 2015).

Dietary restriction and glucose metabolism

AMP-activated protein kinase (AMPK) is the principal energy sensor in eukaryotic cells and acts to maintain cellular energy homeostasis (Hardie et al. 2012), and therefore has a critical role in neuronal maintenance and survival. Dagon et al. (2005) showed that a moderate limited daily feeding (LDF, 60% AL) increased hippocampal AMPK activity, induced neurogenesis and improved cognition, but that severe LDF (40%) overactivated AMPK, reduced cognition and induced neural apoptosis. However, a study performed in our laboratory showed that an IF feeding protocol decreased the expression of both AMPK and its phosphorylated form in the cortex of both middle-aged (12-month old) and aged (24-month old) groups, while LDF had no impact (unpublished data). Hypoglycemia and activated AMPK were shown to regulate the expression of glucose transporters (GLUTs) in the brain (Cheng et al. 2003). Our study confirmed the influence of diverse DR approaches on AMPK expression in rat cortex and hippocampus. Consequently, the expression pattern of GLUT isoforms was affected following both IF and LDF. It should be emphasized that these changes strongly depended on the type of DR regime, as well as the age of the animals. Additionally, changes related to glucose metabolism following DR were more pronounced in the cortex compared to the hippocampus (unpublished data).

Dietary restriction and cholesterol metabolism

As mentioned above, the maintenance of brain cholesterol homeostasis is a prerequisite for the proper functioning of the CNS (reviewed in Pfrieger and Ungerer 2011). There is considerable evidence that cholesterol in the brain was influenced by various dietary regimes, although overall cholesterol content remained unaffected (Mulas et al. 2005; Fon Tacer et al. 2010). However, a significant decrease in the levels of cholesterol precursors was detected even after short-term (20 h) fasting (Fon Tacer et al. 2010). Our results showed that both LDF and IF lowered cholesterol synthesis, but their influence varied strongly, depending on the brain region and age. IF displayed a greater impact on cholesterol precursors in the group of middle-aged animals (12-month-old), while the influence of LDF was more prominent in old (24-month-old) animals. However, the most noteworthy difference between these feeding regimes was observed in the hippocampus of old rats in the case of the direct cholesterol precursor desmosterol. While LDF had no influence on desmosterol, IF promoted a pronounced increase in desmosterol concentration (unpublished data), The fact that desmosterol is a direct cholesterol precursor produced in the Bloch cholesterol synthesis pathway which prevails in young individuals, suggests a switch to a less energy-consuming synthesis pathway in old animals exposed to IF (Smiljanić et al. 2014). The data strongly pointed to the ability of IF, but not LDF, to promote an energy-saving mechanism, thus revealing the capacity of the hippocampus to an age-related adaptive response.

Dietary restriction and frailty

The current anti-aging strategies are switching their focus from suppressing to promoting successful aging, in order to counterbalance the lifespan with the health span, the length of time spent free of the costly and harmful conditions of old age. Thus, optimal aging depends on both stimuli that promote a longer life and stimuli that diminish disease and morbidity (Gallagher et al. 2011). This is where the concept of frailty comes into focus, as an important tool in predicting negative health outcomes during aging. Frailty is referred to as a "multidimensional syndrome in aging" and is based on the combination of genetic, biological, physical, psychological, social and environmental factors (Fulop et al. 2010); it can be evaluated using a frailty index (FI) (Parks et al. 2012). Frail individuals are at higher risk of accelerated physical and cognitive decline, disability and finally death (Fulop et al. 2010). In a recent study, our laboratory assessed the influence of diverse DR protocols (various duration and onset) on rat FI during aging. We observed that only early-onset, long-term DR decreased the FI, thus acting towards the improvement of the health span. These data clearly revealed the importance of the onset and/or duration of DR application

in order to achieve the most beneficial effects (Todorovic et al. submitted data).

Conclusion

Ageing is thought to be a stochastic process combining predictable and random effects that lead to the accumulation of unrepaired cellular damage, weakening cellular repair and compensatory mechanisms. Healthy aging depends on a combination of individual genetic factors, lifestyle and external environmental factors. Although brain cells are particularly vulnerable to the accumulated effects of ageing, the consequent changes are not inevitable. DR proved to be a noninvasive intervention that is able to attenuate age-related alterations and promote the plastic capacity of the brain. However, to achieve the beneficial effects of DR, one should have a good estimation of the protocol used, when to start and most importantly, what one wants to achieve with such an intervention.

Acknowledgments

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