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The aging brain, neuroinflammatory signaling and sleep-wake regulation

Giuseppe Bertini, Valeria Colavito, Cristina Tognoli, Paul F. Seke Etet, Marina Bentivoglio*

Section of Anatomy and Histology, Department of Neuroscience, University of Verona. * Corresponding author, Email: marina.bentivoglio@univr.it

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Summary

Tissues and organs change over time, regulated by intrinsic (genetic) determinants and environmental (and microenvironmental) adaptation. Brain changes during lifetime are especially critical, as the brain is the effector of cognition and the vast majority of neurons live throughout the life of the individual. In addition, brain aging mechanisms are especially critical for disease vulnerability, given the aging-related prevalence of pathologies that include neurodegenerative diseases. In this context, the present contribution concisely highlights data yielded by recent trends of research on the normal aging brain, and specifically: the occurrence of synaptic changes (rather than neuronal loss) and the altered regulation of adult neurogenesis (which represents a novel exciting field of knowledge); the development of a low-grade chronic inflammatory state which primes glial cells and may lead to changes in intercellular crosstalk, thus playing a potential role in the brain susceptibility to neurodegeneration; changes occurring in state-dependent behavior, sleep and wake, which are products of global brain functioning and underlie consciousness and cognitive performance; changes in the biological clock, the hypothalamic suprachiasmatic nucleus, which regulates sleep-wake alternation and other endogenous rhythms. Altogether, the present synopsis of recent studies at the molecular, cellular, and functional levels emphasizes the idea that the normal aging brain should be viewed as an example of adaptation and plasticity rather than as an obligatory decline.

Key words

Glia, synapse, cytokines

The aging brain: new trends in an old brain

By 2050, the world's population is expected to exceed nine billion people, and almost a quarter of them will be over 60 (2007 report of the United Nations Population Division). Given these worldwide demographic changes, the aging brain function and its vulnerability to age-related pathologies now represent a high-priority field of research.

Understanding brain changes during normal, "healthy" aging is an essential prerequisite for the study of its alterations caused by disease. Here, we will briefly highlight changes that occur in the aging brain at the cellular level, in relation to state-dependent behaviors, i.e. sleep and wake, which are strongly correlated to global brain functioning.

Morphological changes associated with aging have been repeatedly described in the central nervous system (CNS). Interestingly, it has now been established that,

contrary to previous beliefs, neuron loss is not a distinctive hallmark of normal brain aging. Rather, age-related changes tend to occur at the synaptic level (Hof and Morrison, 2004), with either loss of synaptic contacts (documented in many brain structures; see also further) or molecular changes at otherwise intact synapses. Thus, while neuron loss does not seem a solidly grounded feature to explain age-related cognitive decline, synapse loss and/or dysregulation potentially represent crucial correlates of functional decline.

Newborn neurons in the old brain: a slowing down process

In the context of brain senescence, we wish to briefly summarize the evidence, mostly accumulated over the last decade, that neurons are generated in the brain of mammals, including humans, throughout the entire life (Eriksson et al., 1998; Gage, 2002). Although debates are ongoing on the distribution of adult neurogenesis in different brain structures (especially upon different kinds of injury), a wealth of data has pointed to two neurogenetic niches in the adult brain in normal conditions: the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus (Lledo et al., 2006). Newborn cells in the rostral SVZ migrate through the rostral migratory stream to the olfactory bulb. In the hippocampal dentate gyrus (DG), newly generated neuronal cells migrate from the SGZ to the granular layer where they differentiate into neurons and extend axons to the Ammon's horn.

The biological significance of adult hippocampal neurogenesis depends on the extent to which newly born neurons can participate in signal processing. Although the precise function of neurogenesis during adulthood is still unclear, the process is considered a key participant in neuroplasticity (Lledo et al., 2006), with a role in modulating perceptive functions (i.e. olfaction) and cognitive processes (i.e. spatial memory). As it will be briefly dealt with further, interrelationships between adult neurogenesis and sleep have also been reported.

Normal aging is considered to be the strongest negative regulator of neurogenesis, being accompanied by a marked reduction in the number of new cells added to the DG and to the olfactory system across several species (e.g. Rao et al., 2005, 2006). In the DG of normal animals, the most dramatic decline occurs between youth and middle age, and seems then to slow down between middle and old age, leading not only to a reduction in the net number of newborn cells, but also to a decline in dendritic maturation (Rao et al., 2006). It has been proposed that the decline observed in neurogenesis during aging could represent a by-product of other changes occurring in the brain with age (Klempin and Kempermann, 2007), namely that the diminished neuronal proliferation could be linked to declining availability of cell proliferation factors, at least partially due to impairments of normal astrocytic function (Shetty et al., 2005).

It is also noteworthy that adult neurogenesis is greatly influenced by the interaction between cells of the adaptive immune system and CNS-resident immune cells (e.g. Ziv and Schwartz, 2008). This is also shown by data indicating that decreased (as in immune deficiency syndromes) or increased (as in severe inflammatory diseases) immune activity can lead to impaired hippocampal neurogenesis, which in turn may result in impaired hippocampal-dependent cognitive abilities. Such interaction is of special interest considering the aging-related alterations in inflammatory signaling outlined below, which need to be investigated also in relation to age-dependent decline of neurogenesis.

Intercellular crosstalk and brain senescence

Besides neurons (whether accompanying the individual throughout life or newly born even at an advanced age), experimental studies of the normal aging CNS have repeatedly demonstrated changes in non-neuronal cells, such as the presence of macrophages in the neuronal parenchyma and features of microglial activation (e.g. Perry et al., 1993; Hinman et al., 2004; Dilger and Johnson, 2008; Streit, 2006). Since microglia represent the resident immune cells in the CNS (Hanisch and Kettenmann, 2007), these findings indicate that the immune-related state of alert of the CNS varies during aging. Changes in the senescent brain also involve astrocytes, which become characterized by an early stage of reactive gliosis (e.g. Cotrina and Nedergaard, 2002).

In this context, of special interest is also the wealth of recent evidence indicating that normal aging is hallmarked by low-grade chronic inflammatory activity, with increased production of proinflammatory cytokines and decreases of anti-inflammatory mediators both peripherally and in the brain (e.g. Saurwein-Teissl et al., 2000; Bodles and Barger, 2004). This could result in a "priming" effect on glial cells (Dilger and Johnson, 2008) leading to an aging-related neuroinflammatory condition (Conde and Streit, 2006). Infiltration of T-cells and appearance of cells with a dendritic cell phenotype have also been described in the brain of mice older than 12 months (Stichel and Luebbert, 2007).

Aging is associated with a deterioration of the adaptive immune response, a state designated as immunosenescence (Miller, 1996), which causes a general decrease of the peripheral response to inflammatory challenges. Findings obtained in our and other laboratories indicate that responses to inflammatory stimuli in the brain are instead preserved with advancing age, and also include a gradual amplification in the responsiveness of astrocytes and microglia (Fig. 1), as well as increased T-cell recruitment in the brain parenchyma (Deng et al., 2006, 2010; Xu et al., 2010). The age-related increase of brain sensitivity to inflammatory stimuli could be involved in neuroprotective effects or in increased brain vulnerability to immune challenges during aging, which could play a role in neurodegenerative events.

In this context, it is worth mentioning that in the study of cell death regulation in the aging neocortex and hippocampus we found that upregulation of the anti-apoptotic Bcl-2 protein in response to cytokine exposure displays a strong age-dependent enhancement, whereas increase of the pro-apoptotic Bax protein does not show significant age-dependent variation (Xu et al., 2007). This indicates that the Bcl-2/Bax balance may be shifted towards neuroprotection in the brain during senescence.

Sleep-wake disturbances in the elderly

What changes in sleep-wake during senescence?

A puzzling aspect of aging is represented by the frequent changes in sleep structure and circadian rhythmicity (e.g Van Someren, 2000; Wu and Swaab, 2007). These alterations notably affect the quality of life of elderly individuals, with considerable societal impact.

Aging-related changes in human sleep-wake architecture (e.g. Dijk et al 2001; Vitiello, 2008) include repeated and frequent interruption of sleep by long periods of wakefulness, decreases in total sleep time and sleep efficiency, tendency to fall asleep and awaken earlier, early morning awakenings and undesired daytime sleepiness (with increased likelihood of napping or falling asleep during the day), decrease of tolerance of phase shifts in time of the sleep-wake schedule, such as those produced by jet lag and shift work. Concerning other physiological parameters linked to sleep, older adults are more easily aroused from nighttime sleep by auditory stimuli (e.g. Zepelin et al 1984), suggesting that they may be more sensitive to environmental stimuli, which could justify at least in part the increase of awakenings during sleep in the night. The phase advance in the normal circadian sleep cycle (Bonnefond et al., 2006, Monk, 2005) may justify why older people tend to go to sleep earlier in the evening and wake earlier.

It is also of interest that most of the sleep-wake cycle disturbances documented during normal aging in humans and experimental animals occur between early and middle adulthood (19 to 60 years in humans), and further age-related sleep changes are modest after age 60 (Vitiello, 2008).

Sleep, neurogenesis and depression: challenges for the old brain

Interestingly, sleep disturbances and affective disorders such as depression, are tightly linked by bi-directional, mutual relationships (Staner, 2010). In particular, depression is associated with sleep alterations, specifically difficulties in falling asleep, frequent awakenings during the night and early waking up. An interesting association has been postulated between the previously mentioned age-related decline of neurogenesis and the occurrence of depression, which is often observed in comorbidity with other illnesses during the elderly (Couillard-Despres et al., 2009).

Intriguing, in this context, are the data pointing to inter-relationships between sleep, depression and adult neurogenesis. An increased neurogenesis in the hippocampus could represent a major mechanism underlying the therapeutic effects exerted by antidepressant drugs. Specifically, the administration of antidepressants increases adult hippocampal neurogenesis, and the time course of this effect is consistent with the time course for the therapeutic action of antidepressants (Malberg et al., 2000). On the other hand, one night of sleep deprivation represents in humans a fast and effective treatment of depressive symptoms (Giedke and Schwarzler 2002). Interestingly, in rats "one night" (i.e. 12 hours) of sleep deprivation by gentle handling significantly stimulates neurogenesis in the hippocampus by increasing both cell proliferation and survival of newborn neurons (Grassi Zucconi et al., 2006), while prolonged sleep deprivation provokes a decrease in cell proliferation in the hippocampus (Meerlo et al., 2009).

In view of the above, a thorough exploration of the relationships linking aging-related sleep changes and depression, and neurogenesis and neuroinflammatory signaling represents a novel stimulating challenge in brain aging research, in which our laboratory is currently engaged.

Age-related changes in the biological clock

Alterations in sleep-wake during aging draw attention not only on the complex system of neural circuits and neurotransmitters that regulate these functions, but

also on the biological clock, the hypothalamic suprachiasmatic nucleus (SCN). This cell group notably plays the role of master circadian pacemaker for physiological and behavioral functions in the mammalian brain (Antle and Silver, 2005; Morin and Allen, 2006).

Studies on SCN size and volume, as well as cell counts in the nucleus, did not show changes in old versus young rodents. On the other hand, changes in spontaneous firing activity have been reported in SCN neurons of aged rodents, together with alterations in the expression of certain genes and selective alterations of peptides (Bentivoglio et al., 2006), as also observed in non-human primates (Cajetanot et al., 2005).

In investigating the old mouse SCN, we have documented a marked reduction of the synaptic network during senescence, including a decrease of GABAergic terminals (Palomba et al., 2008), which could underlie aging-related SCN dysfunction, including low amplitude output. Furthermore, we have observed aging-related changes in the response of SCN neurons (Sadki et al., 2007), as well as astrocytes and microglia (Deng et al., 2010) to an inflammatory challenge (Fig. 1), opening further key questions on the sensitivity of the biological clock to immune molecules.

Concluding remarks

It has become increasingly clear that brain aging should be viewed in terms of neuroplasticity and adaptation phenomena, and that mechanisms of regulation and functional outcome of intercellular dialogue in the brain can be substantially modified by aging. Sleep-wake changes associated with aging provide correlates of such adaptation, whose cellular and synaptic substrates need to be unraveled to provide a key for an understanding of brain changes over time.

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Figure

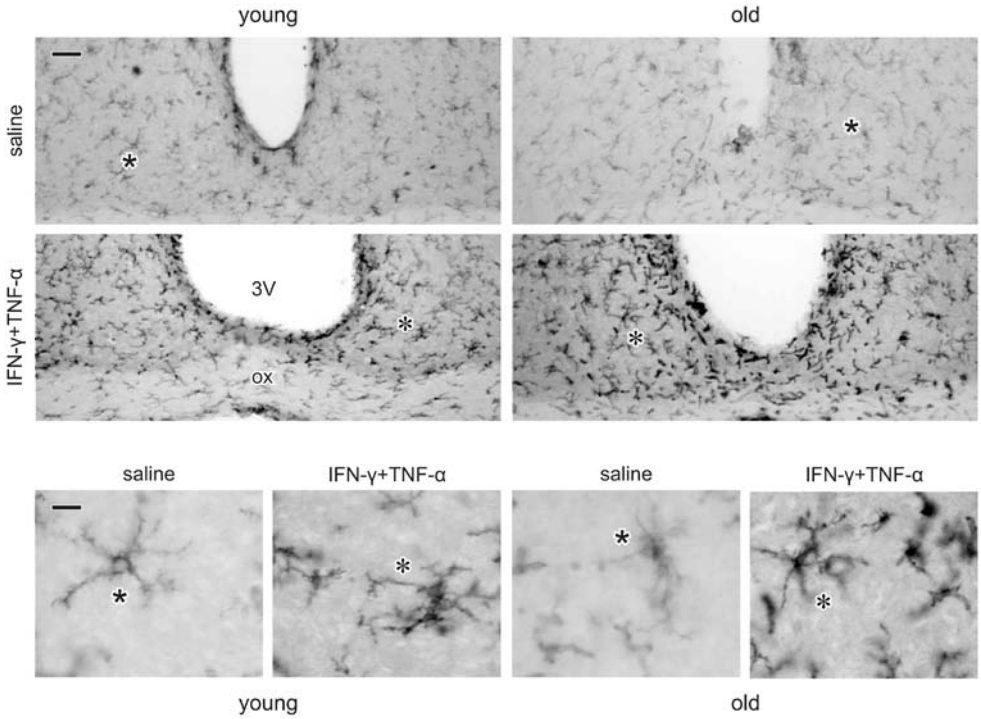

Fig. 1 – Distribution and phenotypic features of microglia (stained by means of F4/80 immunohistochemistry) in the hypothalamic suprachiasmatic nucleus of young (3 months of age; left panels) vs. old (22 months of age; right panels) mice, 2 days after intracerebroventricular injection of either saline, as control, or a mixture of the pro-inflammatory cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Higher magnification images illustrate cell morphology at the sites indicated by the asterisks as spatial reference. Note that cytokine-activated microglial cells appear as hypertrophic, rod-shaped, and macrophage-like cells, and that such features are more prominent in the aged than in young mouse, indicating an age-related amplification of the response to the inflammatory challenge. The images derive from the laboratory archival material. Labels: 3V, third ventricle; ox, optic chiasm. Scale bars: top panels, 50 μm; bottom panels, 12 μm.