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Review

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Microglia in the normally aged hippocampus

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The hippocampus plays important roles in the regulation and combination of short and long term memory and spatial navigation with other brain centers. Aging is accompanied by a functional decline of the hippocampus and degenerative disease. Microglia are major immune cells in the central nervous system and response to degenerative changes in the aged brain. In this respect, functional and morphological changes of the hippocampus have been closely related to microglial changes during normal aging with or without disease. Therefore, in this review, we discuss morphological and functional changes of the hippocampus and microglia in the aging brain.

Key words: Aging, brain, memory, immune cells

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Aging is accepted worldwide in social and political agendas as well as in research agendas. Functional declines in various organ systems including the central nervous system (CNS) are associated with normal aging processes. Aging in the brain is a major risk factor for increases in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [1,2]. Most functional changes in the CNS with normal aging are associated with a decline in learning and memory. It is well accepted that the hippocampus is a very important region related to learning and memory because the hippocampus is connected to other brain regions that are related to learning and memory [3-6]. The hippocampus is also known as the most vulnerable region affected by the internal and external changes of normal aging, and with strokes and other neurodegenerative diseases [7-10]. The functional decline of the CNS, including the hippocampus, with normal aging and degenerative processes is accompanied by changes in the number and function of neurons and glia, their volumes, and various factors such as neurotransmitters, hormones, oxidative stress and inflammation [2,11,12].

There is no doubt that microglial change in the CNS is associated primarily or secondarily with neurodegenerative diseases in the aged brain [13,14]. Microglia, which are immune cells, account for 5-20% of the total glial cell population in the CNS and are evenly distributed throughout the brain parenchyma. They respond rapidly to a variety of alterations in the microenvironment of the brain and act as a sensor for pathological events in the brain [15,16]. Numerous studies and reviews have reported that numerical, morphological and functional changes in microglia are apparently changed in the normal aging brain and in the aged brain with diseases [1,2,6,12,17-21]. In this review, we discuss the literature on age-related changes in microglia in the hippocampus.

Structure and Function of the Hippocampus

The hippocampus is one of the oldest brain regions phylogenetically. It consists of two major parts: the hippocampus proper (Ammon's horn or cornu ammonis, CA) and the dentate gyrus (DG). The hippocampus proper contains three sub-regions (regions CA1-CA3), and each subregion consists of 3 distinct layers: stratum oriens (SO), stratum

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pyramidale (SP) and stratum radiate (SR) (from the outermost to the innermost). Main excitatory output neurons referred to as pyramidal neurons are located in the SP [22]. The hippocampus is involved in various physiological functions such as olfaction, arousal, cognition, learning and memory [4,23,24].

Extensive work on hippocampal function associated with memory is still ongoing [25-28]. Although controversy on the exact functional role of the hippocampal sub-region is ongoing, the CA1 region appears to be related with the association and completion of temporal patterns as well as intermediate-term memory. The CA3 region mediates processes related with spatial pattern association and completion as well as short-term memory [4,26,28-31]. Although the DG is a main subregion of the hippocampus, its function also needs to be studied more; the DG is involved in metric spatial representation and spatial pattern separation [23,24,28].

Aging Hippocampus

Age-related losses of hippocampal volume and neuronal number in the aged hippocampus have been reported [7,18,32-35]. Early studies showed that a significant neuronal loss in the CA1 region is correlated with aging in the human hippocampus, not in other hippocampal regions [32,36]. In addition, pyramidal neurons are significantly decreased in the SP of the CA1 and CA3 regions of the hippocampus in both cognitively impaired and unimpaired rats [33]. Later, however, the same researchers and others reported that the number of hippocampal neurons with aging is relatively persistent in primates and rodents during normal aging using different methods [7,34,35]. These studies proposed that early studies, which showed that neuronal loss happens in the aged hippocampus, had some errors in their methods, sampling and pathological conditions. Many other researchers have confirmed that neuronal loss in the normally aged hippocampus is not characteristic of this brain region [5,37-39]. However, a marked neuronal loss in the aged hippocampus occurs in some degenerative conditions such as Alzheimer's disease and experimental autoimmune encephalomyelitis [5,7,10,32,40].

On the other hand, a reduction in hippocampal volume with aging has been found in primates and rodents using histological studies [30,31,41]. Recently, many researchers have confirmed age-related decreases in the volume of the hippocampus using magnetic resonance imaging in primates and rodents [42-44]. Nowadays, therefore, it is well accepted that normal aging is accompanied by hippocampal shrinkage, and hippocampal shrinkage is closely related to functional decline in learning and memory in the aged brain. Various groups have tried to elucidate substrates of age-related hippocampal learning and memory deficits and have focused on neurobiological alterations as follows.

One of major finding in the literature is a change in synaptic plasticity of hippocampal neurons that present as long term potentiation and long term depression. It has been reported that no significant changes in dendritic regression are observed in the CA regions and subiculum, and, in the DG, dendritic extent is increased in the aged rat and human [45-47]. In addition, the spinal density of hippocampal neurons in the DG and CA1 region is not significantly changed in the aged human and rat [46,48,49]. Furthermore, biophysical properties of CA pyramidal cells or DG granule cells are mostly preserved in the normally aging hippocampus compared to those in the young hippocampus [6,18,50]. However, perforating synaptic contacts, especially presynaptic fibers, decrease in the DG of aged rats [51,52]. In addition, the density of fragmented axons, which project from various brain regions into the hippocampus, increases in the aged hippocampus [53].

On the other hand, there are many studies that show that noradrenergic, dopaminergic, serotonergic and cholinergic projecting fibers decrease in the aging hippocampus [29,54-56]. Other studies have focused on changes in the aged hippocampus in cellular substrates such as ions, hormones, neurotrophic factors and biomolecules. A role for glucocorticoids in neuronal aging in the hippocampus (the glucocorticoid cascade hypothesis) has been suggested by some researchers [9,57,58]. Starting with these studies, extensive studies have shown that, in the aging hippocampus, glucocorticoids and stress contribute to learning and memory function deficits in the aging hippocampus [19,21,59].

Many researchers have found correlations of hippocampal functional decline with changes in ions such as calcium, potassium and magnesium conductance in aged neurons [60,61]. For example, calcium conduction and the number of L-type calcium channels increase significantly in hippocampal CA1 and CA3 neurons of the aged rat and rabbit [62-65]. Other studies have reported that magnesium deficiency in the aged hippocampus impairs learning and memory function [66-68]. These studies suggest that the dysregulation of cation homeostasis might be a major cause of deficits in learning and memory in the aged hippocampus.

Brain-derived neurotrophic factor (BDNF) is implicated in age-related hippocampal function [69]. Many studies have reported that BDNF and the BDNF-TrkB system decrease with aging in human, monkey and rodent hippocampus [70-72]. The decline of BDNF levels in the hippocampus can cause an impairment in long-term potentiation in the aged

rat [73].

Recently, a few studies on changes in cellular substrates in the aging hippocampus have been done using proteomic analysis [3,74,75]: Broad changes in proteins focused on various processes such as glucose metabolism, oxidative stress, signal transduction, protein folding and neurotransmitter release and synaptic signaling in the aged hippocampus have been conducted. These studies have provided evidence for functional declines in these processes with aging.

Microglia in the Hippocampus

In the CNS, microglia are classified into ameboid, intermediate, ramified (resting), activated and phagocytic, depending on their morphology under normal and disease conditions [76-78]. During early postnatal development, ameboid microglia migrate and proliferate in the brain parenchyma, and are transformed into ramified microglia in the adult brain by transforming into intermediate microglia with elongated process or pseudopodia. Ramified microglia are known as resting microglia and have a small oval soma with numerous branched processes. These spread throughout the entire brain and play an important role in brain homeostasis under normal conditions. Ramified microglia are transformed into activated microglia and/or phagocytic microglia via reactive or primed microglia in response to certain pathological conditions such as traumatic injury, ischemia and Alzheimer's disease (AD), which are accompanied by inflammation [8,13,77,79-82].

The hippocampus is one region of the brain where dense microglia present, like the olfactory bulb, telencephalon, basal ganglia and substantia nigra [15]. Studies on the regional distribution of microglia between various brain regions are limited, and results are controversial [15,83-88]. An early study reported that in the adult mouse hippocampus F4/80-immunoreactive microglia in the DG are more numerous than in Ammon's horn [15]. Recently, the microglial distribution in the hippocampus has been reported using different stereological methods [83-86]. These studies show that microglial density in the CA1 region is higher than in the DG, although the total microglial number is also different. Using immunohistochemistry with ionized calcium-binding adapter molecule 1, Jino *et al* (2007) found that in the mouse hippocampus microglial density in the CA3 region is lower than in the CA1 region and the DG, and the density of microglia in the CA1 is higher than in the DG [87]. They suggested that microglial density might be involved in site-specific vulnerability of the hippocampus, and that the heterogeneous distribution of microglia would participate in the modulation of hippocampal neuronal activity [88].

Microglia in the Aging Hippocampus

Only a small number of studies have focused on microglial distribution and total number in the hippocampus using stereological methods [89-92], although a large number of studies have been conducted in other brain regions under normal and abnormal conditions. No age-related differences in microglia were found in the aging hippocampus of male mice [89,90]. However, the same research team reported that the number of microglia is significantly increased in the hippocampus of the same mouse strain [90]. In contrast, some researchers found that the number of microglia is decreased in the aged hippocampal CA1 region of the ICR mouse [91,92]. This discrepancy may be due to different animals or strains, markers for microglia and stereological methods.

Although there is a lack of detailed studies on changes in microglial number with aging, the change must be related with functional changes with age. Actually, many studies show that morphology and/or antigen expression in microglia are changed in the aged hippocampus as well as in other brain regions [8,82,92-98]. It is well known that the resting form of microglia is transformed into the activated form of microglia with aging [96,97]. Many studies have shown that the activated form of microglia is increased in number in the aged monkey, dog and rodent hippocampus as well as in the human hippocampus [8,82,92-95]. These activated microglia show high elevations of various antigens such as major histocompatibility complex (MHC) antigens, interleukin-1α (IL-1α), MHC class II cell surface receptor, Iba-1 and lectin in the hippocampus [8,82,94,98,99].

Recently, a large number of studies on functional changes in adult and aged microglia have been conducted with primed microglia. The primed microglia, which was introduced by Perry, have shortened processes with surface antigens such as MHC II similar to those in activated microglia, and the primed microglia are devoid of the ability to secrete pro-inflammatory cytokines in the CNS [79-81]. Along with the primed microglia concept, it has been suggested that the functions of adult and aged microglia are different under normal and pathological conditions [17,20,79,100,101]. Microglia cultured from aged brains express high basal IL-6, and microglia more highly express IL-6 as well as IL-1β after lipopolysaccharide treatment compared to microglia cultured from adult brain [102,103]. In addition, aged microglia show an exaggerated response to systemic inflammation [79,100,101]: microglia in adult animal models of some types

of inflammation produce an increase in anti-inflammatory cytokines and fewer inflammatory cytokines. However, microglia in middle-aged animals show a reversed production of anti-inflammatory and inflammatory cytokines [103-105]. Consistent with these studies, emerging evidence suggests that hippocampal functions after systemic infection are accompanied by an increase in microglia activation, which are more easily disrupted in aged rodents [98,100,103,105, 106]. Nevertheless, many researchers agree we still need to examine further specific markers, methods and criteria to define primed microglia and activated microglia as well as resting microglia, because the morphology of primed microglia show some features similar to activated microglia. In addition, resting microglia and activated or phagocytic microglia also express MHC II and Iba-1 in normal and pathological conditions.

Microglial senescence represents a dystrophy with aging [107]. In an *in vitro* study, was reported that morphological degeneration of cultured microglia occurs after expose to amyloid beta protein [108]. Streit and his colleague reported that dystrophic microglia show a loss of fine branches (deramification), shortened tortuous processes or cytoplasmic fragmentation except for spheroid cytoplasm in the aged human brain. They suggested that microglial dystrophy is a sign of microglial senescence [107,109]. In addition, they suggested, based on their data, that neurodegeneration may occur secondarily after microglial senescence and that neurodegeneration is associated with a loss of microglial neuroprotective function. They also showed that dystrophic (senescent) microglia rather than activated microglia likely precede neurodegeneration in Alzheimer's disease [110]. In addition, abnormal and degenerating microglia are detected in other neurodegenerative diseases including amyotrophic lateral sclerosis, Creutzfeldt–Jakob disease, Huntington's disease and schizophrenia [111-113]. They also suggested that an increase in the number of activated microglia in the brain with aging and neurodegenerative disease must be reconsidered, because dystrophic microglia might have been misidentified as activated microglia in previous studies. Other groups have consistently provided evidence that microglial senescence produces a shortened telomere and a decrease in their activities in aging and neurodegenerative disease [114-117]. However, to the best of our knowledge, there is no study that has focused in detail on dystrophic changes of microglia in the hippocampal subregions with normal aging, although some studies have shown dystrophic changes in microglia in the parahippocampal cortex [107,110,115,117]. Further studies on the stereological classification of microglia combined with primed and dystrophic microglia could identify the region-specific functions of microglia in the aged hippocampus.

Conclusion

The most distinctive age-related change in the hippocampus is a decrease in its volume with a reduction in the number of projecting fibers from other related brain regions. No significant loss of hippocampal neurons occurs with aging. With the volume change, many cellular substrates also markedly change in the aging hippocampus. Stereological studies on the total or region-specific number of primed and dystrophic microglia should be done in the aged hippocampus. In addition, there are some controversies about on major microglial functions that are harmful or beneficial for the patient undergoing degenerative changes in the aged brain. However, in the aged hippocampus the majority of functional declines with aging are closely related to morphological and functional changes of microglia.

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