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Review Article

A Possible Link between Microglial Process Dysfunction and Neuropsychiatric Disorders

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Abstract

Pío del Río Hortega first discovered microglia by histological staining with silver carbonate. He thought that microglia with highly branched fine processes in the healthy brain were quiescent and called these cells as resting microglia. After brain injury, microglia changes their morphology into activated type, which has phagocytic activity at the sites of neuronal damage and inflammation. At 90 years after the discovery of microglia, resting microglia in the healthy brain were found to be very dynamic, much more than any other cells in a live mouse brain using the two-photon scanning laser microscope. Beyond the roles as brain-resident macrophages, many lines of evidence have revealed that microglia have essential roles in the maturation and maintenance of neuronal circuits in the brain through both elimination and formation of dendritic spines through their processes. Furthermore, length and structural complexity of highly branched fine processes are regulated by microglial intrinsic molecular clock. Dysfunction of dendritic spine and disturbance of circadian clock system are widely accepted characteristic abnormalities in neuropsychiatric disorders. Therefore, the growing understanding of movement and functions of microglial processes may aid in the development of novel pharmacological interventions against neuropsychiatric disorders, which are associated with synapse loss and aberrant neuronal connectivity.

ABBREVIATIONS

CX_3CR1: Fractalkine Receptor; IDO: Indoleamine 2,3-Dioxygenase; NMDA: *N*-methyl-D-aspartate

INTRODUCTION

One hundred years ago, Santiago Ramón y Cajal proposed that the brain consists of three elements, the first element is neuron, the second element is astrocyte and the third element is a group of adendritic cells that seemed devoid of processes. In 1920, however, Pío del Río Hortega, a student of Cajal, found processes in third element by histological staining with silver carbonate. Furthermore, he divided the third element into two different cell types, microglia and oligodendrocytes, based on the number and feature of their processes. He published these findings without his teacher's permission. So, there was a long debate about the processes of Cajal's third element. Finally, Hortega's findings turned to be right, whereas existence of microglia in the brain was not widely accepted until the development of anti F4/80 antibody [1], because of a poor reproducibility of histological staining with silver carbonate.

Hortega called microglia with highly branched fine processes in the healthy brain as resting microglia. After brain injury, resting microglia change their morphology into activated type, which has phagocytic activity at the sites of neuronal damage and inflammation. At 90 years after the discovery of microglia, Axel Nimmerjahn tried to see microglia in a live mouse brain using the two-photon scanning laser microscope. He found that resting microglia are very dynamic, much more than any other cell in the adult brain [2]. Microglia are now being considered more active players in the normal healthy brain [2,3]. Furthermore, microglia with highly branched fine processes are associated with regulation of synapse functions [4-9]. Now, much attention has been paid on the movement and functions of microglial processes. It is considered that synapse loss and aberrant neuronal connectivity are important causative factors in neuropsychiatric disorders. Therefore, a possible link between microglial dysfunction and neuropsychiatric disorders is now one of most exciting topics of microglial research.

Synaptic stripping

Blinzinger and Kreuzberg first reported the involvement of microglia in synaptic functions. In response to facial nerve injury, microglia accumulate to spread on injured facial motoneurons to physically displace afferent synapses from cell bodies and dendrites through insertion of their processes [10]. This

phenomenon is referred to "synaptic stripping" and considered to play important roles in regeneration of facial nerve and synapse reorganization. Recently, we have proposed a new interpretation of synaptic stripping following nerve injury of motoneurons [11]. At an early phase after nerve injury, extracellular nucleotides including ATP and adenosine transiently inhibit the synaptic inputs through the activation of P2Y and A1 receptors localized on the presynaptic terminals before the opposition of microglia. In contrast, microglia spread on the surface of the injured motoneurons inhibit synaptic inputs through detachment of axon terminals at a later phase after nerve injury. Therefore, microglia induce blockade of synaptic transmission following nerve injury through two mechanisms: chemical blocking at an early phase and physical blocking at a later phase.

Synaptic pruning

During developmental stage of the brain, excess numbers of synapses are formed and then unnecessary synapses are eliminated. This phenomenon is referred to "synaptic pruning", because pruning is a horticultural practice involving the selective removal of parts of a plant, such as branches, buds, or roots. Synaptic pruning is a characteristic series of developmental events required for the formation of proper neural circuit. A recent study provides evidence the involvement of microglia in synaptic pruning during the postnatal developmental stage. Microglia eliminate synapses through fractalkine receptor (CX_3CR1), a microglia specific chemokine receptor, at P13-16 in the hippocampus [4]. Furthermore, CX_3CR1 deficient mice exhibit delayed maturation of thalamocortical synapse [12]. The importance of synaptic pruning for mature neuronal circuits is also demonstrated in the dorsal lateral geniculate nucleus of the thalamus. During the pruning period, microglia eliminate synapses with low synaptic activities in the retinal ganglion cells through recognition of complement component C3 [5]. Furthermore, the elimination of synapses by microglia is also regulated by the tumor growth factor-β signaling [8]. Not only postnatal developmental stage but also post-maturity, microglia are associated with synapse regulation. Constant darkness, shut off the visual information, initiated the elevation of synaptic element in the inclusions within the microglia [6].

Synaptic formation

Microglia have been reported to promote dendritic spine formation. Interleukin-10 from microglia promotes synapse formation through its receptor in hippocampal neurons [13]. However, the expression of interleukin-10 receptors in hippocampal neurons is limited in early developmental stage. More recently, the role of microglia in synapse formation has been demonstrated in young adult mice. During the late postnatal period or young adulthood, microglial depletion, which was accomplished by administration of diphtheria toxin in $CX_3CR1^{CreER/+}$:$R26^{iDTR/+}$ mice, caused the reduction of basal level of spine formation and elimination over 4 days in the motor cortex. Furthermore, microglia-depleted mice showed impairment of motor performance due to the reduction of motor-learning-related spine formation. Microglial depletion also causes the reduction of synaptic proteins, such as GluN2B and vGlut1, and miniature excitatory postsynaptic currents. These changes caused by microglial depletion were also observed in mice that are manipulated microglia-specific removable of brain-derived neurotrophic factor, which increases neuronal tropomyosin-related kinase receptor phosphorylation, a key mediator of synaptic plasticity [14]. Therefore, it is considered that microglia serves important physiological functions in learning and memory by promoting learning-related synapse formation. These observations further support the notion that microglia are active players in the healthy brain.

Circadian rythmicity

The sleep-wake cycle plays an important role in the determination of synaptic strength. Waking results in high synaptic density and strength, whereas sleep results in low synaptic density and strength. Although the sleep-wake cycle dependent synaptic strength is very important for the brain function, the precise mechanism remains unclear. Cajal proposed a possible role of astrocytes in the sleep-wake cycle. During sleep, astrocytes extend and insert their processes between axons and dendrites and disconnect synapses as a circuit breaker [15]. On the other hand, during awaking, astrocytes retract their processes, so axons can connect with dendrites.

Recently, we have found that microglia, instead of astrocytes, regulate sleep-wake cycle dependent changes in strength through extension and retraction of their processes [16]. We first noticed that the morphology of microglia is quite different between day and night. In both phase, microglia have highly branched morphology. However, total length of microglial processes was significantly longer and branch point of them was larger at night. Microglial represented highly complicated morphology at night. Interestingly, the contact ratio of microglial process and dendritic spine are higher at night. Microglia exhibited circadian rhythmicity, oscillating expression patterns of clock genes, which orchestrate the expression of $P2Y_{12}$ receptors. Inhibition of $P2Y_{12}$ receptors disrupted the rhythmic patterns of synaptic strength or spine density [16]. Upregulated $P2Y_{12}$ receptors in the microglia at night triggers the contact with dendritic spine sequentially initiate the reduction of synaptic strength or spine density at daytime. We could not detect phagocytic activity of microglia. Proteolysis or modulation of extracellular matrix with tissue-plasminogen activator or matrix metalloprotease 9 have critical role in the regulation of dendritic spine [17,18]. Among secreted proteases from microglia [19], cathepsin S, a microglia specific protease in the brain, is only regulated by clock genes. More interestingly, cathepsin S-deficient mice exhibit disrupted circadian oscillation patterns of synaptic strength and spine density. These mice exhibit higher locomotor activity and reduced sleep level [7].

Without microglia-synapse interactions, the synaptic strength may be kept at the high level. Decrease in the synaptic strength by microglia-synapse interactions may be necessary for the resetting the neuronal capacity for information processing. Therefore, microglia-synapse interactions regulated by the microglial molecular clock are important for the synaptic homeostasis in the healthy brain. Furthermore, dysfunction of microglial molecular clock may play a causative role in neuropsychiatric disorders including depression and cognitive deficits [20].

Synaptic transmission

After cellular activation, microglia secrete several molecules including low molecular mass (<500Da), glutamate, neurotrophic factor, and cytokine [21,22]. These molecules can regulate *N*-methyl-D-aspartate (NMDA) receptors, thus microglia involve synaptic plasticity. Actually, microglia-releasing factor enhance the formation of long-term potentiation in CA1 region. The factor was identified as glycine [23]. In contrast, excessive activation of NMDA receptor results in the neuronal death. Quinolinic acid is an endogenous modulator with agonistic properties on NMDA receptor, which was observed in microglia in the anterior cingulate gyrus of severely depressed and suicidal patients [24]. Interestingly, Indoleamine 2,3-Dioxygenase (IDO) and kynurenine monooxygenase, quinolinic acid biosynthesis enzyme, were increased in microglia from CX_3CR1 deficient mice [25]. Furthermore, blocking of IDO significantly inhibited the development of depressive-like behavior in CX_3CR1 deficient mice [26]. It indicated that dysfunction of microglia lead to abnormal neuronal activity. Abnormality in microglia has also proposed in the schizophrenia using positron emission tomography. The binding of (R)-[^{11}C]PK11195 to translocator protein, expressed in activated microglia, was increased in gray matter and hippocampus in the patient [27,28]. Interleukin-1β, an inflammatory cytokine, from activated microglia might possible candidate to cause abnormal neurotransmission in depression or schizophrenia.

Behavioral abnormality

Disruption of synaptic pruning by microglia might lead to neuropsychiatric disorders because of the positive correlation with abnormality of dendritic spine [29]. Besides the synapse dysfunction, aberrant behavior was observed by microglial dysfunction. *Hoxb8* mutant microglia results in the induction of excessive grooming behavior [30]. This behavior has similarity in obsessive-compulsive disorder, which is an anxiety disorder characterized by unreasonable thoughts and fears that lead

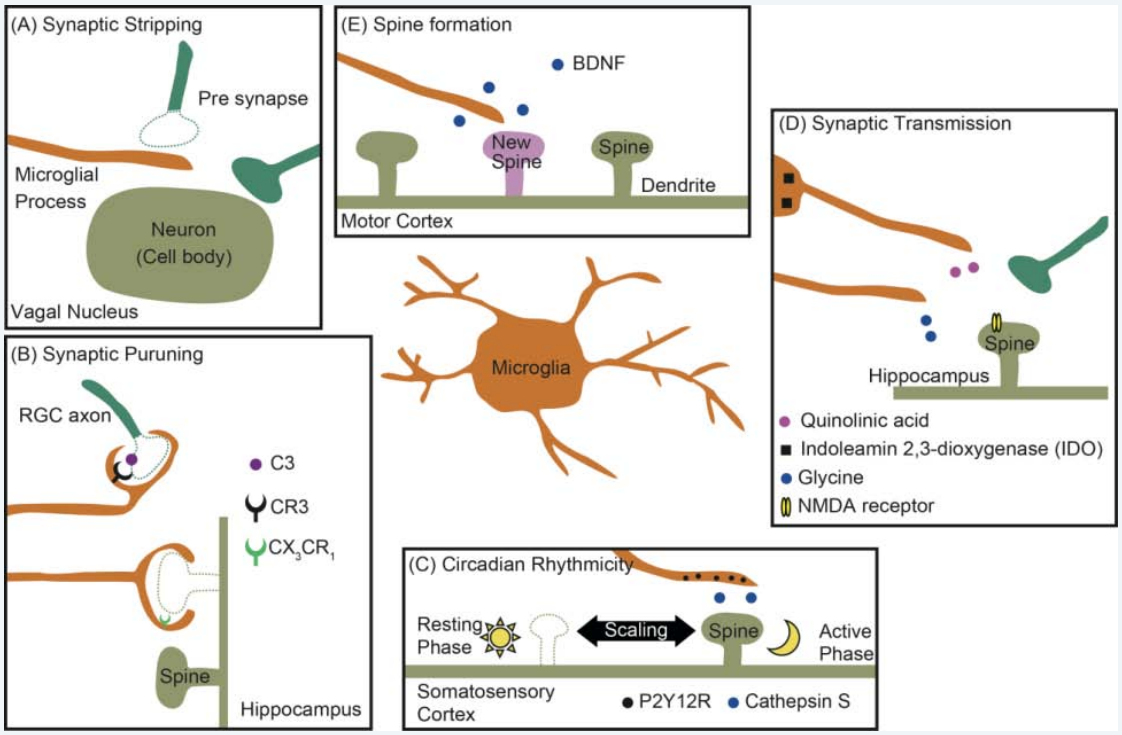

Figure 1 Schematic illustration of the regulation of dendritic spine by microglia.

A) Synaptic stripping. Microglia induce blockade of synaptic transmission following nerve injury through two mechanisms: chemical blocking at an early phase and physical blocking at a later phase (see text).

B) Synaptic pruning. Microglial processes engulf synaptic compornents, axon terminals and dendritic spines. Complementary component C1q and C3 cascade, which is recognized by microglial complementary 3 receptor (CR3), plays an essential role in normal pruning of retinal ganglion cell (RGC) axons in lateral geniculate nucleus. *Microglial processes also engulf dendritic spines in hippocampal neurons through microglial* CX3C chemokine receptor 1 (*CX_3CR1).*

C) Circadian rythmicity. During the active phase, molecular clock increases the expression levels of $P2Y_{12}$ receptors ($P2Y_{12}R$) and cathepsin S in microglia. Microglia recognize ATP released by synaptic sites of highly active neurons through $P2Y_{12}R$. Furthermore, microglia secrete cathepsin S from their processes extended towards synaptic sites of highly active neurons to degrade surrounding extracellular matrix molecules, which regulate maturation and elimination of dendritic spines. At the resting phase, microglia retract their process from synaptic sites of neurons with low synaptic activity.

D) Synaptic transmission. Activated microglia express Indoleamin 2,3-dioxygenase (IDO) which induces the production of quinolinic acid. Both of quinolinic acid and glycine bind to *N*-methyl-D-aspartic acid (NMDA) receptor subunit 1 to stimulate glutamatergic neurotransmission.

E) Spine formation. Brain Derived Neurotrophic Factor (BDNF) secreted from microglia promotes learning through dendritic spine formation.

you to do repetitive behaviors. Transplantation of wild-type bone marrow into *Hoxb8* mutant mice ameliorated pathological grooming in 4 weeks. It is considered that monocyte within transplanted bone marrow cell migrated into central nerves system and then differentiated into microglia [31]. *Hoxb8* is also found in the lamina I and II of spinal cord and dorsal root ganglia [32]. Further study using microglia specific mutation of Hoxb8 is desirable to understand significance of microglia in pathological grooming. Microglia are also involved in Rett syndrome, which is a devastating neurological developmental disorder that is seen in infancy and occurs almost exclusively in females. It is usually caused by a mutation of the *MECP2* gene on the X chromosome. Transplantation of wild-type bone marrow into *Mecp2*-mutant mice ameliorates Rett syndrome-like behaviors. In addition, pharmacologically inhibition of microglial phagocytosis by annexin V cancels the improvement of aberrant behavior with bone marrow transplantation [33]. More recently, positron emission tomography analyses have revealed that the number of microglia is increased in the autism patient [34]. These observations suggest that dysfunction of microglial functions including phagocytic elimination of synapses impairs normal maturation of the neuronal circuit, which in turn leads to neuropsychiatric disorders.

CONCLUSION

Beyond the roles as brain-resident macrophages, microglia have an essential function in the maturation and maintenance of neuronal circuits in the brain through elimination and formation of dendritic spines through their processes. However, the detailed underlying molecular mechanisms have not yet fully clarified. On the other hand, abnormalities in dendritic spine such as spine morphology and imbalance in spine formation and elimination are associated with neuropsychiatric disorders. Interestingly, these changes show layer-specific patterns in the cerebral cortex [29]. Although a link between microglial dysfunction and neuropsychiatric disorders remains hypothetical, the growing understanding of movement and functions of microglial processes may aid in the development of novel pharmacological interventions against neuropsychiatric disorders.

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