Resting microglia respond to and regulate neuronal activity in vivo

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Microglia are the primary immune cells in the brain. Under pathological conditions, they become activated and participate in scavenging, inflammation and tissue repair in response to brain injury. While the function and underlying mechanism of activated microglia have been intensively studied in the past decades, physiological functions of resting microglia remain largely underestimated. In our recent work, by simultaneously monitoring both the motility of resting microglial processes and the activity of surrounding neurons in intact zebrafish optic tectum, we examined the interaction between resting microglia and neurons. Local increase in neuronal activity attracts resting microglial processes and drives them to contact neurons with high levels of activity. This process is mediated by neuronal release of “find-me” signals such as ATP via pannexin-1 hemichannels and requires small Rho GTPase Rac in microglia. Reciprocally, the microglia-neuron contact reduces both the spontaneous and visually evoked activities of contacted neurons. We here summarize and explain the key results in the context of our previous work.

Microglia, under physiological conditions, spend most of the time in a “resting” state, with highly motile processes surveying surrounding neural tissues.1–3 However, it is still unclear whether and how resting microglial processes find specific neuronal targets for making contacts and what the effect of such contact on neural functions is. Taking advantage of the larval zebrafish model with the transparency of its brain and the availability of genetic tools, we addressed these questions in the previous work.3

Demonstration of Contact between Resting Microglial Process and Neuronal Soma

Using in vivo time-lapse confocal and two-photon imaging of the visual center optic tectum in living larval zebrafish, we first monitored changes in the morphology of microglia under resting state. Similar to the typical morphological properties of resting microglia in the mouse cortex,1,2 resting microglia in zebrafish are also highly branched with dynamic processes ended with either stick-like or bulbous tips (Fig. 1). These two differently shaped tips can be inter-converted as the process moves around. The bulbous ending forms rapidly through expansion from a stick-like ending, and stalls on the contacted neuronal soma for several minutes before gradually shrinking back again. The existence of resting microglia-neuron contact is further confirmed by using 3-dimensional reconstruction and transmission electron microscopy technique.

Neuronal Activity Steers the Motility of Resting Microglial Processes and Induces the Formation of Microglia-Neuron Contact

In previous studies, global increase or decrease of neural activity in vivo by using
microglial bulbous endings wrapping neuronal somata, we next asked what might be mechanisms by which neurons with high activity “talk” to resting microglia. We found that this process requires the membrane depolarization-activated pannexin-1 hemichannels on tectal neurons and the ATP/P2 purinergic receptor signaling between neurons and microglia. Pannexin-1, a large pore-like hemichannel, is widely expressed in the central nervous system. The opening of pannexin-1 is gated by some cellular signals, such as membrane depolarization, intracellular calcium and so on. Small molecules, including ATP, NAD and PGE2, can be released through these hemichannels. Using whole-mount in situ hybridization and in vivo whole-cell recording, we found that functional pannexin-1 hemichannels are expressed in tectal neurons but not microglia. After impairing the function of pannexin channels by drug treatment or morpholino-mediated genetic downregulation, glutamate uncaging-induced orientated movement of resting microglia is markedly decreased. All these results further confirm an instructive role of neuronal activity in regulating the dynamics of resting microglial processes and the formation of microglia-neuron contact.

Molecular and Cellular Mechanisms of Neuronal Activity-Induced Changes in Microglial Dynamics

Having shown that local elevation of neuronal activity can induce the formation of microglial bulbous endings wrapping neuronal somata, we next asked what might be mechanisms by which neurons with high activity “talk” to resting microglia. We found that this process requires the membrane depolarization-activated pannexin-1 hemichannels on tectal neurons and the ATP/P2 purinergic receptor signaling between neurons and microglia. Pannexin-1, a large pore-like hemichannel, is widely expressed in the central nervous system. The opening of pannexin-1 is gated by some cellular signals, such as membrane depolarization, intracellular calcium and so on. Small molecules, including ATP, NAD and PGE2, can be released through these hemichannels. Using whole-mount in situ hybridization and in vivo whole-cell recording, we found that functional pannexin-1 hemichannels are expressed in tectal neurons but not microglia. After impairing the function of pannexin channels by drug treatment or morpholino-mediated genetic downregulation, glutamate uncaging-induced orientated movement of resting microglia is markedly decreased.
Collectively, our work brings forth and demonstrates a novel reciprocal regulation between resting microglia and neurons in vivo. We not only demonstrate an instructive role of neuronal activity in resting microglial motility, but also reveal, for the first time, a previously unappreciated function of microglia in homeostatic regulation of neuronal activity (Fig. 2).

Considering the bi-directional modulation between neurons and microglia, this study also represents a new perspective in understanding the physiological function of resting microglia.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Physiological Significance of Resting Microglia-Neuron Contact**

Considering the facilitated formation of microglia-neuron contact induced by neuronal activity increase, we wondered what the physiological function of such contact is. By simultaneously monitoring changes in both the morphology of microglial processes and Ca\(^{2+}\) activity of tectal neurons, we found that microglial processes preferentially contact with neurons that exhibit high levels of spontaneous activity before contact. We then mosaiically overexpressed the human inward rectifier K\(^{+}\) channel Kir2.1 (Kir), which is used to reduce the excitability of neurons, and a non-conducting mutant version of Kir2.1 (mKir) in tectal neurons.\(^{18,19}\) In comparison with mKir-expressing neurons, Kir-expressing neurons show a lower probability of microglial contact. These results further indicate that resting microglia preferentially contact neurons with higher activities, consistent with the data obtained with glutamate uncaging.

While accumulating evidence shows that resting microglia respond to neural activity, we surprisingly noticed that resting microglia can also in turn regulate neuronal activity. In the zebrafish optic tectum, we found that such resting microglia-neuron contact can downregulate both spontaneous and visually evoked activities in contacted neurons. Interestingly, the decreases in both the frequency and magnitude of spontaneous Ca\(^{2+}\) activity are positively correlated with the duration of microglia-neuron contact. What is the consequence on neuronal firing after loss of resting microglia? In response to two-photon laser-induced focal injury, some microglia quickly translocate to the injury site. Consistent with the speculation based on our previous findings, the spontaneous activity of neurons within microglia pre-existing territories is significantly elevated after the translocation of microglia to the injury site. Meanwhile, in other cases with the similar injury but no microglial translocation, the neuronal activity is not significantly changed. Thus, our study reveals a functional regulation of neuronal activity by resting microglia under physiological conditions, offering a new way for homeostatic regulation of neuronal activity.

Collectively, our work brings forth and demonstrates a novel reciprocal regulation between resting microglia and neurons in vivo. We not only demonstrate an instructive role of neuronal activity in resting microglial motility, but also reveal, for the first time, a previously unappreciated function of microglia in homeostatic regulation of neuronal activity in the healthy brain (Fig. 2). Considering the bi-directional modulation between neurons and microglia, this study also represents a new perspective in understanding the physiological function of resting microglia.

**Figure 2.** Working model. Highly active neurons attract resting microglial processes and induce the formation of microglia-neuron contact via ATP signaling (top). Such neuronal activity-driven microglia-neuron contact in turn reduces the activity of contacted neurons (bottom).
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