MICROGLIA

Norepinephrine as a modulator of microglial dynamics

Two new studies demonstrate the importance of awake imaging to investigate microglia–neuron interactions. These studies show that microglial dynamics are influenced by neuronal activity, and they provide evidence that norepinergic signaling plays an important role in this effect.

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Microglia are highly motile, phagocytic, resident immune cells of the brain. In the normal physiological condition, they are highly ramified[1]; however, during disease or infection they become activated with shorter, thick processes[2]. Moreover, microglia are not only involved in the inflammation process but also have a continuous role in synaptic pruning and plasticity[3,4]. In this issue of *Nature Neuroscience*, Stowell et al.[5] and Liu et al.[6] use in vivo two-photon laser microscopy to show that microglia dynamics differ between awake and anesthetized mice and to highlight the roles of norepinephrine and β2-adrenergic receptors (β2-AR) in these states.

Until recently, studies on microglia activity have been mainly performed in anesthetized animals. However, microglia have close interactions with neurons, which are more active in the awake state than in the anesthetized state. It is therefore important to examine the neuronal communication with microglia in the awake state and to investigate microglial phenotypes under these conditions. In their papers, Stowell et al.[5] and Liu et al.[6] addressed this important question. They imaged CX3CR1^{GFP} mice (in which microglia express GFP) in anesthetized and awake conditions and, surprisingly, found that microglia present more complex arborization together with higher parenchymal surveillance in mice that were anesthetized with a fentanyl cocktail[5] or isoflurane[6] than in awake mice. Liu et al.[6] in addition imaged CX3CR1^{GFP}/THY1^{YFP} mice in order to investigate neuron–microglia interactions in the two conditions and observed that microglia processes spend a prolonged period of time near dendrites and have a greater contact area with dendrites during anesthesia compared with a wakeful state.

To take these findings further, Stowell et al.[5] and Liu et al.[6] compared microglial responses to an acute injury in the two different conditions. For this, they applied focal laser ablation and demonstrated that microglia had an enhanced injury response in anesthetized mice compared with awake mice. Stowell et al.[5] showed that the fentanyl cocktail put the brain in a slow-wave-dominated state. To eliminate possible misinterpretations due to the fact that a fentanyl cocktail has both sedative and analgesic effects, they repeated the experiments using dexmedetomidine (DEX), which does not have analgesic properties but is a sedative that reduces norepinephrine (NE) release from the locus coeruleus (LC). This showed that mice under DEX anesthesia also have increased microglial arborization and surveillance. Liu and colleagues[6] similarly tested other general anesthetics—ketamine/xylazine and urethane—to investigate whether the microglia were exhibiting the same behavior. Their results were consistent with the findings under isoflurane anesthesia, showing that microglia present higher process area and surveillance in anesthetized mice in comparison with awake mice.

Both groups[5,6] demonstrated that reduced neuronal activity due to anesthesia gives rise to enhanced microglial surveillance, with a negative correlation between neuronal activity and microglia dynamics. It is known

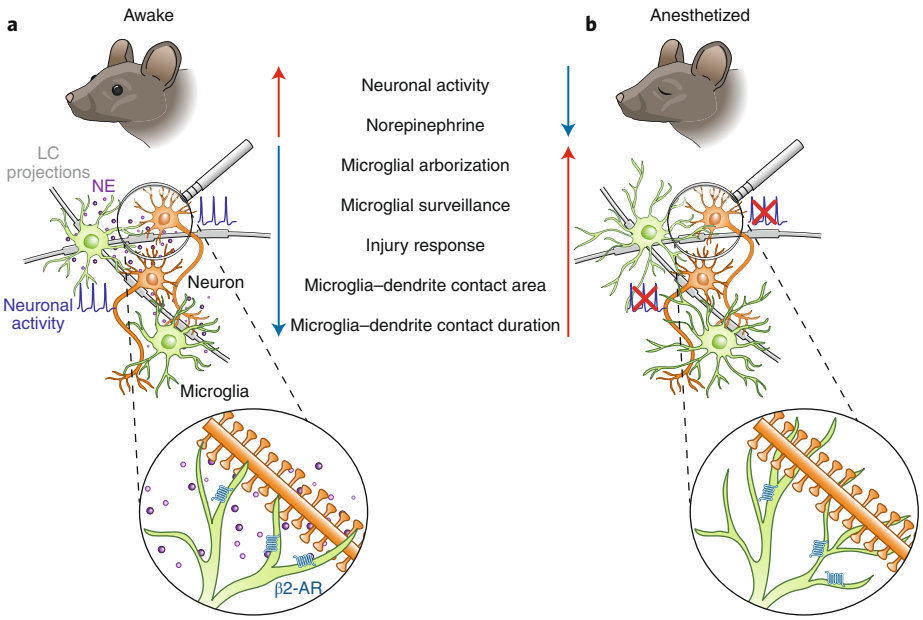

Fig. 1 | Schematic illustration of changes of microglial dynamics in awake and anesthetized mice. a, In the awake state, which is characterized by increased neuronal activity, LC terminals release NE. NE reduces microglial arborization, surveillance and response to injury via the β2-AR. In particular, NE reduces the contact areas of microglia with the neuronal dendrite as well as the time that they spend in direct contact. **b**, In contrast, anesthetics cause an increase of microglia arborization, surveillance and immune response.

that anesthesia affects the circulatory system (heart rate and blood pressure), which could in theory influence microglial surveillance. As another approach to reduce neuronal activity, Liu and colleagues[6] performed unilateral whisker trimming. Using an in vivo calcium imaging technique, they showed that neuronal network activity decreased in the contralateral barrel cortex after whisker trimming and that microglia displayed enhanced process area and surveillance in that same region. Lastly, the authors tested whether anesthesia had an add-on effect to the sensory deprivation induced by whisker trimming on microglia. Whisker trimming accompanied by isoflurane inhalation showed no further effect on microglial dynamics. These findings suggest that sensory deprivation and anesthesia may share the same mechanism and confirm that decreases in neuronal activity are inversely correlated with microglial dynamics.

It is known that the sedative effect of DEX is mainly achieved by inhibition of neurons in the LC, the main source of NE; DEX therefore decreases cortical NE release throughout the brain. Stowell et al.[5] asked whether changes in endogenous NE release were required for the observed effects of DEX. They injected the LC-selective neurotoxin DSP4 to induce degeneration of the LC axon terminals, and then analyzed the differences in microglial morphology in animals before and after DEX treatment. They found that DSP4 eliminated the effect of DEX on microglial branching and surveillance. In view of these important findings, they next examined whether optogenetic stimulation of the LC using channelrhodopsin was sufficient to reduce microglial process dynamics under DEX treatment. They indeed detected diminished process surveillance. The data indicate that NE release during the awake state gives rise to a decrease in both microglia processes and microglial surveillance. Liu et al.[6] reached the same conclusion using a different approach. As it has previously been reported that acetylcholine, dopamine, serotonin and NE levels are reduced during general anesthesia[7], Liu and colleagues[6] investigated whether these neurotransmitters are able to modulate microglial dynamics. The authors administered these potential neuromodulators intracerebrally in mice under isoflurane anesthesia and found that only NE was capable of preventing the anesthesia-induced effects on microglia.

NE has effects on numerous cell types via adrenergic receptors that belong to the G-protein-coupled receptor family, and these receptors have different expression profiles on different cells. Microglia express higher numbers of β2-ARs than any other cells in the brain[8]. To examine the direct effect of β2-ARs on microglial dynamics, Stowell and colleagues[5] applied the β2-AR agonist clenbuterol to fentanyl-anesthetized mice and found that this decreased microglial motility, arbor complexity and process coverage in the parenchyma.

Stowell et al.[5] and Liu et al.[6] also examined the effect of blocking β2-ARs in awake mice by treating mice with the β2-AR antagonist ICI-118,551. As expected, ICI-118,551 had the opposite effect of clenbuterol and increased microglial surveillance and motility. In addition, although clenbuterol did not alter microglial processes, ICI-118,551 enhanced microglial ramification. Taken together, the reported findings in awake and anesthetized mice suggest that during the awake state microglia have decreased ramification and motility due to elevated endogenous levels of NE. Treatment with a β2-AR antagonist blocks this effect and enhances microglial ramification and surveillance.

The studies by Liu et al. and Stowell et al.[5,6] unveil a new mechanism that underlies the critical difference in microglial dynamics in awake versus anesthetized states (Fig. 1). Their findings are very important because microglia, the brain's busy bees, are responsible not only for inflammatory responses in the CNS but also for maintaining brain homeostasis and have roles in brain development[9]. They regulate synaptic activity[10], stimulate new spine formation (especially during early stages of life)[11] and play a critical part in neurogenesis[12]. In addition, recent studies have revealed that microglia may also have a neuroprotective role in regulating neuronal hyperactivity[13]. Together with these previous reports, the findings from Liu et al. and Stowell et al.[5,6] suggest that microglia surveillance may have an important role in fine-tuning neuronal circuits.

LC neuronal loss is one of the earliest indicators of neurodegeneration in Alzheimer's and Parkinson's diseases[14]. It has been shown that reducing NE levels in the brain gives rise to decreased microglial phagocytosis and a recruitment to amyloid plaques in the APP-transgenic animal model[15]. By highlighting the impact of NE and β2-ARs on microglial dynamics, the studies by Liu et al. and Stowell et al.[5,6] together demonstrate the importance of using awake in vivo models, which will undoubtedly improve the research and development of treatment of strategies for such neurodegenerative conditions.

From a technical perspective, both manuscripts underline the fact that microscopy plays a critical role in uncovering new dimensions in modern science. Over time, microscopic techniques have improved remarkably, and we are now able to perform deep-tissue in vivo imaging with the help of high-power pulsed lasers. These instruments are particularly important for the field of neuroscience, as the brain is a very complex organ that harbors many different cell types including neurons, microglia and astrocytes; in vivo imaging gives us a great opportunity to understand the interactions between these cells and to visualize their physiological and pathological changes in different conditions. ❐

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Competing interests

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