



RESEARCH HIGHLIGHT

Norepinephrine from the Locus Coeruleus Regulates Microglia Dynamics During Wakefulness

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Microglia, the brain's “busy bees”, continuously survey the microenvironment by extending and retracting their ramified processes to maintain brain homeostasis [1, 2]. Upon disease or injury, microglia quickly transform their morphology and extend their processes towards the disease/injury sites to clear damage [2]. The mechanisms underlying the high motility of microglial processes and the rapid morphological transformation of microglia have been extensively investigated. However, studies on microglial dynamics *in vivo* have predominantly been carried out in anesthetized animals, and how microglia behave under awake conditions remained unknown. Using two-photon microscopy to track microglia dynamics in awake mice, two independent studies published recently [3, 4] have demonstrated that during wakefulness, microglia exhibit shorter arborization, reduced motility, and diminished responsiveness to injury compared to those under anesthesia. Moreover, both studies showed that norepinephrine (NE), a neurotransmitter released from the axon terminals of locus coeruleus (LC) neurons, directly regulates microglial motility through β_2 -adrenergic receptor (β_2 -AR) signaling in the awake condition [3, 4].

To determine whether microglial dynamics differ in the awake and anesthetized conditions, Stowell *et al.* [3] and Liu *et al.* [4] carefully analyzed microglial dynamics under

both conditions, using the CX3CR1^{GFP/+} transgenic mouse line in which microglia are fluorescently labeled by GFP. Surprisingly, both groups found that microglia in the awake mice survey a smaller territory with shorter arbors, reduced motility, and attenuated responsiveness to acute injury than those in brains anesthetized with isoflurane or a fentanyl cocktail [3, 4]. To exclude the possibility that general anesthesia-related circulatory and respiratory effects influence microglial activity, Liu *et al.* [4] tracked microglial responses using different anesthetics such as ketamine/xylazine and urethane. In parallel, since the fentanyl cocktail produces a slow-wave-dominated state with both analgesic and sedative effects, Stowell *et al.* [3] recorded the microglial dynamics in mice anesthetized with the sedative-only dexmedetomidine (DEX). Consistently, both groups reported enhanced velocity and surveillance of microglial processes within 10 min–15 min of anesthesia induction, regardless of the type of anesthetic, suggesting that anesthesia may relieve an inhibitory signal affecting microglial dynamics during wakefulness.

Given that general anesthesia reduces neuronal activity, Liu *et al.* [4] investigated whether the enhanced microglial surveillance during anesthesia results from the suppressed neuronal activity. They imaged the dynamics of microglial processes in the barrel cortex of mice with unilaterally trimmed whiskers. Thirty minutes after trimming, the neuronal activity in the contralateral barrel cortex was substantially reduced based on Ca^{2+} imaging from neurons expressing GCamp6 (a fluorescent sensor reflecting the intracellular Ca^{2+} level). Meanwhile, microglia also robustly increased their process areas and surveyed greater territories in the contralateral but not in the ipsilateral barrel cortex. To directly address whether neuronal activity regulates microglial process dynamics, Liu *et al.* [4] suppressed the neuronal network activity with muscimol

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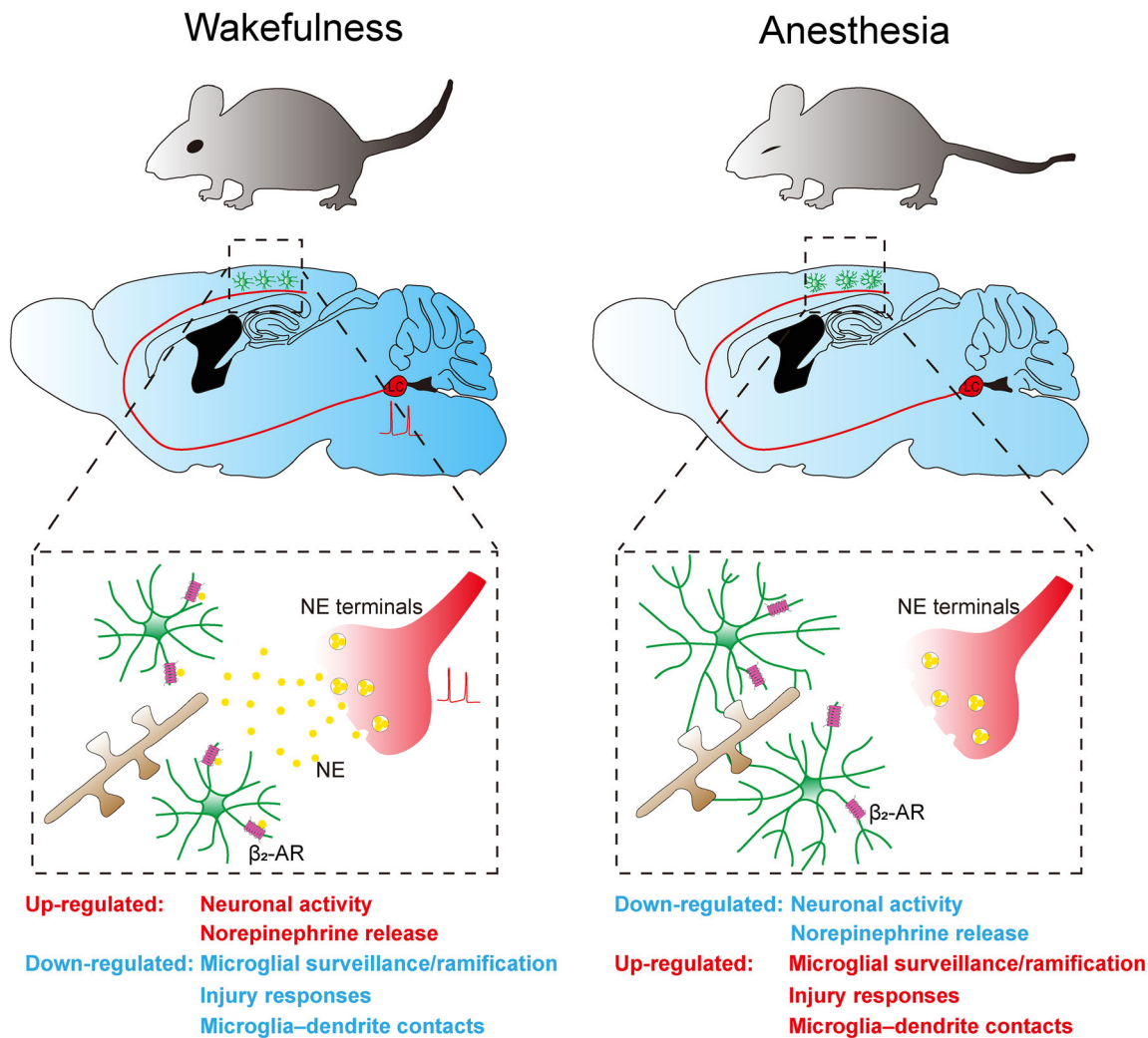


Fig. 1 Regulation of microglial dynamics by norepinephrine (NE)- β_2 adrenergic receptor (β_2 -AR) signaling. During wakefulness, tonic activation of NE neurons in the LC releases NE in the brain. Binding of NE to the β_2 -ARs on microglia restricts microglial surveillance and ramification, injury responses, and microglia-dendrite contacts.

(a GABA_A receptor agonist), tetrodotoxin (an inhibitor of voltage-gated Na⁺ channels), or optogenetic activation of GABAergic neurons. They found that reduced neuronal activity was accompanied by increased process dynamics and surveillance of microglia, reminiscent of the changes during anesthesia, suggesting that reduced neuronal activity triggers enhanced microglial dynamics and surveillance.

To reveal the mechanisms that underlie the differences between microglial dynamics in the awake and anesthetized states, both studies tested whether P2Y₁₂ and fractalkine-CX3CR1 signaling, important pathways known to regulate microglial dynamics [2], are involved and found that neither contributed to the changes. Since NE is an important mediator of wakefulness and DEX is known to reduce NE release from the LC, Stowell *et al.* [3] tested whether NE regulates microglial responses. They ablated

Under anesthesia, reduced NE neuronal activity decreases the levels of NE in the brain, releasing the inhibition of microglia by β_2 -AR signaling, thereby enhancing microglial surveillance and ramification, injury responses, and microglia-dendrite contacts.

NE neurons in the LC using the neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) and found that pre-ablation of LC projections eliminated the DEX-elicited increased arborization and surveillance of microglia. Conversely, optogenetic stimulation of NE release from LC axons reduced microglia arborization and surveillance in DEX-treated animals, suggesting that endogenous NE release from the LC inhibits microglial dynamics and surveillance during wakeful states. Liu *et al.* [4] reached the same conclusion by applying a wide range of neurotransmitters known to be reduced during anesthesia, including acetylcholine, dopamine, NE, and serotonin to the imaging area. They found that only NE prevented the anesthesia-enhanced microglial process surveillance. On the contrary, inhibition of NE release from NE neurons in the LC by chemogenetic manipulation in awake mice

enhanced the microglial process dynamics, further supporting the hypothesis that NE is the inhibitory signal that limits microglial surveillance during wakefulness.

NE exerts diverse actions through a wide range of receptors. Interestingly, microglia express much higher levels of β_2 -ARs than other brain cell types [5] and previous studies *in vitro* have suggested that β_2 -AR signaling inhibits microglial chemotaxis towards ATP [6]. Stowell *et al.* [3] then treated anesthetized mice with a selective β_2 -AR agonist, clenbuterol, and found significantly reduced microglial motility and persistent process retraction, mimicking the changes in the awake condition [3]. By contrast, both studies showed that antagonizing β_2 -ARs with ICI-118, 551 in awake mice produced changes that resemble the anesthetized state with enhanced microglial motility and parenchymal surveillance [3, 4]. Moreover, the effects of clenbuterol were absent in β_2 -AR-ablated microglia [3], suggesting that endogenous NE acts directly on β_2 -ARs to inhibit microglial ramification and process motility during wakefulness.

Microglia shape neuronal circuits by modulating synapse formation, plasticity, and pruning through process arborization and surveillance [7]. Notably, Liu *et al.* [4] reported that enhanced microglial process surveillance increases microglia–dendrite interactions under anesthetized conditions, indicating that changes in microglial surveillance may result in modifications in synaptic plasticity. To determine whether NE– β_2 -AR signaling regulates microglia-mediated synaptic plasticity, Stowell *et al.* [3] examined ocular dominance plasticity (ODP) after 4 days of monocular deprivation when β_2 -AR was pharmacologically activated or inhibited. Interestingly, chronic activation of β_2 -ARs by clenbuterol impaired ODP; however, chronic blockade of β_2 -ARs by ICI-118, 551 had no effect on ODP, suggesting that sustained stimulation of β_2 -ARs interferes with microglia–dendrite contacts, but β_2 -ARs may not be required for ODP. These data provide functional significance for β_2 -ARs-regulated microglia dynamics in the modulation of synaptic plasticity (Fig. 1).

Although microglial dynamics have been described for more than a decade, these two studies are the first to uncover the different behaviors of microglia under anesthetized and awake conditions. Despite the fact that microglia express a wide range of neurotransmitter receptors [8], these studies are the first to reveal how microglia tune their dynamics *in vivo* in response to the release of a neurotransmitter, NE. Together, these results highlight the importance of real-time imaging in awake animals and

reveal the impact of neuronal activity on microglial dynamics in different states. These studies also raise important questions regarding whether the loss or gain of NE signaling in different contexts contributes to the aberrant microglial functions implicated in multiple diseases. For example, loss of NE neurons in the LC, an early sign in Alzheimer's and Parkinson's diseases [9], may enhance microglial dynamics and surveillance, whereas chronic stress or sleep deprivation may inhibit microglial surveillance by increased NE release [10]. Whether and how NE– β_2 -AR signaling contributes to pathology through aberrant regulation of microglial activity in these conditions needs further attention and investigation.

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