



# Unexpected role of microglia and P2Y<sub>12</sub> in the induction of and emergence from anesthesia

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## Article summary

General anesthetics act on neurons to cause overall suppression of neuronal activity in the brain leading to loss of consciousness that renders a patient unarousable to painful stimuli. General anesthesia is reported to act on various ion channels, such as GABA<sub>A</sub>, glycine, nicotinic acetylcholine, and NMDA receptors expressed on neurons [1]. Typically, general anesthetics potentiate the activation of inhibitory postsynaptic channels or inhibit the activation of excitatory synaptic channels. In a recent publication in *eLife*, He et al., have shown that microglia, immune cell in the central nervous system (CNS), play an essential role in the sensitivity of mice to anesthetics [2]. They found that microglia-depleted mice required a longer time for loss of righting reflex (LORR) and a shorter time for recovery of righting reflex (RORR) induced by multiple anesthetics indicating that microglia are required for the induction and maintenance of anesthesia. Microglial repopulation reversed these effects. They further showed that genetic knockout or pharmacological inhibition of microglial P2Y<sub>12</sub> or receptors or the downstream rise in cytoplasmic Ca<sup>2+</sup> level, rendered mice more resistant to general anesthesia. The study highlights the essential role of microglia in the induction and emergence of anesthetics and expands the non-immune function of microglia in brain.

## Commentary

Communication between neurons occurs at tiny gaps called synapses, a specialized area where the presynaptic and postsynaptic neurons come within nanometers of one another to allow for signal transmission. The presynaptic neuron releases a neurotransmitter that is received by the postsynaptic neuron's neurotransmitter receptors. Neurotransmitters act on two types of neurotransmitter receptors, ligand-gated ion channels (LGIC) and G-protein-coupled receptors (GPCR). Depending on their functions, neurotransmitters are classified as excitatory neurotransmitters or inhibitory neurotransmitters. Excitatory neurotransmitters, for example glutamate and acetylcholine, act on LGIC receptors to induce the flow of cations (mainly Na<sup>+</sup>) across the plasma membrane, causing the membrane potential to depolarize, bringing it nearer to the action potential threshold. Conversely, inhibitory neurotransmitters, such as GABA, act on LGIC receptors to induce the flow of anions (mainly Cl<sup>-</sup>) to hyperpolarize the membrane potential. It is thought that the action of anesthetics and the various behavioral response patterns that they induce are due to the modulation of LGIC in neuron [1, 3, 4]. Typically, anesthetics potentiate the activation of inhibitory LGIC or inhibit the activation of excitatory LGIC to induce unconsciousness.

Microglia are the innate immune cells of CNS and constitute about 10–15% of all glial cells [5]. They derive from the primitive yolk sac, populate the brain rudiment of the developing embryo by embryonic day 8 (E8) and are embryologically distinct from the monocyte/macrophage system [6]. These cells participate in CNS development, homeostasis, and serve as an important connection between neurological and immunological activity in the CNS. Microglia constantly survey their surrounding microenvironment through the extension and retraction of their highly motile processes, interact with neurons and are involved in control of neuronal activity [7, 8].

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Previous studies have shown that microglial surveillance is increased in anesthetized mice [9, 10]. In this report He et al., found that microglia play essential role in the sensitivity of mice to anesthetics. Using behavioral (LORR and RORR) and electrophysiological (EEG and EMG) approaches, they showed that when microglia are depleted using CSF1R antagonist, PLX5622, mice exhibited delayed anesthesia induction and early emergence from anesthesia induced by a GABA<sub>A</sub> receptor agonist or a NMDA receptor antagonist [2]. They also showed that microglial repopulation restored the times taken for LORR and RORR induced by anesthetics. PLX5622 can ablate both brain microglia and peripheral macrophages [11]. To determine which of these cells is responsible for the observed effects, they used the blood–brain barrier-impermeable CSF1R inhibitor, PLX73086, to ablate peripheral macrophages without affecting brain microglia and found that it had no effect on the sensitivity to anesthetics indicating that the depletion of microglia is essential for the observed effect. In general, using multiple experimental approaches, they uncovered the essential role of microglia in facilitating and stabilizing the general anesthesia response.

Different brain regions regulate anesthesia induction and emergence from anesthesia [3, 4]. Based on neuronal c-Fos upregulation using immunohistochemistry and RNA in-situ hybridization, they showed that microglial depletion decreased neuronal activity in two regions associated with induction of general anesthesia, lateral habenula and supraoptic nucleus (SON) and increased neuronal activity in two regions associated with emergence of paraventricular thalamus, locus coeruleus (LC). They substantiated their immunohistochemistry findings with electrophysiological experiments and found that the excitatory/inhibitory (E/I) ratio was also significantly decreased in the SON of microglia-depleted mice, indicating decreased neuronal excitability. Furthermore, they also found that the E/I ratio in the LC was significantly enhanced, indicating increased neuronal excitability. Their results indicate that microglia both negatively and positively regulate neuronal activity, depending on the brain regions studied.

The metabotropic P2Y<sub>12</sub> receptor is expressed in microglia, but not macrophages and purinergic signaling is a key mechanism that regulates dynamic interactions between neurons and microglia [12, 13]. In the CNS, ATP released from neurons is enzymatically degraded to ADP and activates microglial P2Y<sub>12</sub> receptors [14]. To investigate the underlying mechanism, He et al., used pharmacological inhibition and genetic deletion of microglial P2Y<sub>12</sub> receptors. They found that both methods delayed LORR and accelerated RORR. To further validate this finding, they replaced microglia with bone marrow-derived cells that mature into P2Y<sub>12</sub> receptor-negative microglia-like cells. These microglia replaced mice showed similar outcomes to

those observed after P2Y<sub>12</sub> receptor-knockout. The P2Y<sub>12</sub> receptor is a GPCR and its activation leads to a rise in the concentration of Ca<sup>2+</sup> inside microglia [15, 16]. To further determine whether microglia-mediated modulation of the anesthesia response depends on downstream Ca<sup>2+</sup> signaling, they utilized designer receptors exclusively activated by designer drugs (DREAADs) to selectively elevate the intracellular Ca<sup>2+</sup> level in microglia. Under these conditions, they found that LORR was accelerated and RORR was delayed. Further, they genetically deleted STIM1, an endoplasmic reticulum Ca<sup>2+</sup> sensor, in microglia to disrupt intracellular Ca<sup>2+</sup> signaling and found that this was sufficient to delay LORR and accelerate RORR. These data convincingly show that microglial purinergic receptor and intracellular Ca<sup>2+</sup> signaling, are essential for regulating anesthesia in mice. However, the molecular mechanism through which microglial Ca<sup>2+</sup> affects neuronal activity remains unknown. Alongside another recent publication which also revealed that microglia modulate response to general anesthesia through P2Y<sub>12</sub> receptors [17], the findings of He et al., provide a new perspective of how microglia regulate anesthesia.

Although the authors have not provided evidence for how microglia control neuronal activity, it has also recently been shown that microglial depletion enhances neuronal activity in the striatum. ATP released by neuron triggers microglial adenosine production, which regulates neuronal responses via neuronal adenosine A<sub>1</sub> receptor [18]. A similar mechanism may be responsible for the observed increased neuronal activity in the LC of microglia-depleted mice. Additionally, in a recent publication, Haruwaka et al., reported that microglia transiently increase neuronal activity, after cessation of isoflurane anesthesia by shielding axosomatic GABAergic synapses onto excitatory neurons during the anesthesia phase thereby decreasing inhibitory inputs to excitatory neurons in the somatosensory cortex [19]. A similar mechanism maybe responsible for decreased neuronal activity in the SON of microglia depleted mice.

To conclude He et al., showed that microglia play an essential role in the sensitivity of mice to anesthetics. Microglia are activated in various neurodegenerative disorders and in aged mice. Further investigation studying the effect of anesthesia induction and post anesthesia recovery in mice with activated microglia can lead to a more comprehensive understanding of the how communication between neurons and microglia within the brain occurs in physiological and pathophysiological conditions and will have important clinical implications.

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## Declarations

**Ethics approval** This article does not contain any studies with human participants or animals performed by the authors.

**Informed consent** Not applicable.

**Competing interests** The authors declare no competing interests.

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