General Anesthesia: Activating a Sleep Switch?

A widely used general anesthetic directly depolarizes sleep-promoting cells in the brain at doses that produce unconsciousness. The activation of an endogenous sleep pathway likely plays a role in general anesthesia.

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General anesthesia is a reversible, drug-induced state characterized by amnesia, unconsciousness, analgesia (loss of pain sensation), and lack of movement in the setting of stable physiological systems [1]. In the United States alone, more than 100,000 people receive general anesthesia every day, and most of us will experience it at some point in our lives. Since the first successful public demonstration of general anesthesia in 1846, physicians and scientists have sought to understand how anesthetics work. In this issue of *Current Biology*, Moore and colleagues [2] add another piece to the puzzle: they have discovered that the commonly used general anesthetic isoflurane directly activates a subset of cells in the ventral lateral preoptic area (VLPO) of the brain that promote natural sleep.

From Molecules to Neural Circuits

A variety of chemical compounds including ethers, alkanes, phenols, and even the noble gas xenon are capable of inducing the state of general anesthesia. In the late 1800s, Claude Bernard proposed a unitary mechanism for general anesthesia, and soon afterwards, a strong correlation between anesthetic potency and hydrophobicity was reported [3]. This led to the widespread acceptance of unitary, lipid-based theories of general anesthesia that dominated thinking in the field for almost a century.

By the mid-1980s, it was apparent that proteins, not lipids, are the likely targets of general anesthetics [4]. Still strongly guided by the principles of classical pharmacology and the notion of a unitary mechanism, efforts in the field were refocused towards identifying *the* protein target responsible for general anesthesia. A plethora of data emerged describing anesthetic effects on putative sites of action such as neuronal receptors and ion channels [5].

It soon became evident that anesthetics bind to (and alter the function of) a wide variety of relevant proteins at clinical concentrations. In most cases, general anesthetics enhance inhibitory ion channels such as γ-aminobutyric acid type A ($GABA_A$) receptors, inhibit excitatory ion channels such as glutamate receptors, or both [6]. Although genetically altered mice have been used successfully to demonstrate that the intravenous general anesthetics propofol and etomidate produce unconsciousness via actions at $GABA_A$ receptors [7], no such data exist for other anesthetic drugs. Proving a causal relationship between anesthetic effects observed at the molecular level and the specific behaviors that define general anesthesia remains a major challenge.

In the early 1990s, it was discovered that general anesthetics ablate movement in response to pain by inhibiting neurons in the spinal cord, rather than the brain [8,9]. This important finding proved that general anesthetics produce specific behaviors at distinct neuroanatomical sites. That is, the endpoints that define general anesthesia (amnesia, unconsciousness, analgesia and lack of movement) are created in different parts of the central nervous system (such as lack of movement in the spinal cord). To better understand their complex actions on the central nervous system, recent work has focused on studying the actions of anesthetics at the level of neural circuits and systems.

General Anesthesia and Sleep

Sleep is a comforting metaphor that is often used by anesthesiologists to describe the state of general anesthesia to patients, although in reality general anesthesia is more akin to a coma [1]. Although sleep and general anesthesia are both states that involve unconsciousness, sufficient stimulation will wake up any sleeping animal, whereas by design in a state of general anesthesia even severely noxious stimulation will not induce an arousal response. However, because it is now evident that individual behaviors are induced by anesthetics at distinct neuroanatomical sites, efforts to understand the specific endpoint of unconsciousness have focused on sites in the brain that regulate sleep and wakefulness.

There are numerous sites and neurotransmitters in the brain that promote behavioral arousal, including acetylcholine-releasing cells in the pedunculopontine and laterodorsal tegmental areas, histamine-releasing cells in the tuberomammilary nucleus, norepinephrine-releasing cells in the locus ceruleus, and others [10]. Arousal-promoting neurons activate the thalamus and cortex to maintain wakefulness, and also inhibit sleep-promoting neurons [11]. There is accumulating evidence that these arousal-promoting pathways are important for emergence from general anesthesia [12]. For instance, patients suffering from narcolepsy (a rare sleep disorder caused by the loss of wake-promoting orexinergic neurons) are abnormally slow to wake up from general anesthesia [13], and orexin-deficient mice show similar traits [14]. Conversely, activation of cholinergic [15] and dopaminergic [16] arousal pathways can induce emergence from general anesthesia in animals.

During natural sleep, neurons in the VLPO and median preoptic area are active and release the neurotransmitters GABA and galanin to inhibit arousal-promoting neurons [11,12,17]. Earlier work showed that anesthetics increase activity in VLPO neurons [18,19], but it was unclear whether anesthetics directly activate VLPO neurons, or indirectly activate them by inhibiting arousal-promoting neurons. The latter hypothesis has been favored, since general anesthetics are usually thought to produce their effects on the central nervous system through neuronal inhibition, not excitation.

Direct Activation of a Sleep Pathway

Moore *et al.* [2] used *c-Fos* expression as a marker of neuronal activity to show that VLPO neurons are more active in mice during both natural sleep and isoflurane anesthesia, compared to the awake state. Isoflurane activation of VLPO neurons was dose-dependent

and, more importantly, correlated with behavior, as a low dose insufficient to induce unconsciousness also failed to significantly increase VLPO activity. In addition, VLPO lesions in mice produced an acute resistance to isoflurane-induced unconsciousness, further suggesting that VLPO neurons are a target for isoflurane. However, while *c-Fos* and lesion data may be suggestive of a mechanism, they are not definitive.

The highlight of this paper [2] is the electrophysiological work using hypothalamic slices. The authors report that isoflurane selectively depolarized NA(–) cells, the subtype of VLPO neurons thought to promote sleep. Surprisingly, the excitatory effects of isoflurane were extrasynaptic. In other words, rather than indirectly activating VLPO neurons through synaptic inputs, isoflurane *directly* depolarized VLPO neurons. The authors go a step further and show that closure of a background potassium conductance is the likely mechanism.

The vast majority of electrophysiological work to date has found that anesthetics enhance inhibitory receptors or inhibit excitatory receptors, and in either case the common end result is neuronal depression. Although direct activation of central respiratory chemoreceptor neurons by isoflurane has been previously reported [20], direct anesthetic-induced depolarization of neurons is a highly unusual finding. More importantly, the discovery that isoflurane selectively activates sleep-promoting NA(–) neurons in the VLPO supports an emerging concept that anesthetics contribute to unconsciousness through specific effects at discrete neural circuits that regulate sleep and wakefulness.

Future Directions

The mechanisms of general anesthesia have evolved from an elusive pharmacology puzzle to a tractable neuroscience problem. Despite considerable advances in our knowledge of the molecular effects of anesthetics, much remains to be learned about the profound and complex changes that occur at the level of neural circuits and systems during general anesthesia. With our current knowledge of the molecular mechanisms of general anesthesia and the neurobiology of arousal, it has become feasible to apply a systems neuroscience analysis to the problem [17]. Such analyses will provide a framework to explore and better understand the mechanisms of general anesthesia.

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Plant Development: How Long Is a Root?

The plant hormone cytokinin controls root growth by balancing the division and differentiation of stem cells. But what controls accumulation of cytokinin? A new study has identified a regulatory loop between a transcription factor, *PHABULOSA*, and cytokinin biosynthesis that creates robust domains of cytokinin activity.

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Root growth is a highly variable process that is dependent on environmental stimuli. It is coordinated by a small population of undifferentiated stem cells close to the root tip (known as the meristem). These cells undergo several rounds of cell division before they elongate and finally differentiate [1]. The rate of root growth and the size of the meristem are