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Review

The Biology of General Anesthesia from Paramecium to Primate

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General anesthesia serves a critically important function in the clinical care of human patients. However, the anesthetized state has foundational implications for biology because anesthetic drugs are effective in organisms ranging from paramecia, to plants, to primates. Although unconsciousness is typically considered the cardinal feature of general anesthesia, this endpoint is only strictly applicable to a select subset of organisms that are susceptible to being anesthetized. We review the behavioral endpoints of general anesthetics across species and propose the isolation of an organism from its environment — both in terms of the afferent arm of sensation and the efferent arm of action — as a generalizable definition. We also consider the various targets and putative mechanisms of general anesthetics across biology and identify key substrates that are conserved, including cytoskeletal elements, ion channels, mitochondria, and functionally coupled electrical or neural activity. We conclude with a unifying framework related to network function and suggest that general anesthetics — from single cells to complex brains — create inefficiency and enhance modularity, leading to the dissociation of functions both within an organism and between the organism and its surroundings. Collectively, we demonstrate that general anesthesia is not restricted to the domain of modern medicine but has broad biological relevance with wide-ranging implications for a diverse array of species.

Introduction

One of the most fascinating questions in biology is why all living organisms can be anesthetized by the same simple chemical molecules — the volatile anesthetics. In November of 1846, just one month after the first public demonstration of painless surgery using ether, Oliver Wendell Homes coined the term ‘anaesthesia’. This term is derived from the Greek word for insensibility and signifies a state in which the organism is no longer susceptible to stimuli from the external world. In the 21st century, the anesthetized state is considered primarily in the surgical context, with therapeutic endpoints encompassing amnesia, analgesia, immobility, and unconsciousness. However, volatile anesthetics exert actions not only on human patients, but on species spanning the evolutionary tree of life [1–8] (Figure 1).

From bacteria to yeast, from worms to flies, from plants to poets, all of life’s creations show a similar disruption of function within a relatively narrow 10-fold volatile anesthetic range. Experiments in the 1800s on plants led Claude Bernard to speculate that one definition of life itself is the ability to be anesthetized by volatile anesthetics: ‘‘what is alive must sense and can be anesthetized, the rest is dead.’’ This remarkable conservation across diverse living organisms has sparked the theory that natural selection may have led to an evolutionarily conserved anesthetic responsiveness dating back to a common unicellular ancestor [7]. Although this might appear unlikely from the perspective of general anesthesia as an exclusively human invention in modern medicine, it is known that plants can emit anesthetic gases when stressed, possibly as a self-regulating feedback loop [9]. This is the kind of phenomenon outside of human biology that could be explored to assess whether the seemingly conserved susceptibility to anesthetic gases is epiphenomenal or is linked to evolutionary biology. In addition to the low interspecies variability, intra-species variability in volatile anesthetic responsiveness, attributable both to environmental and genetic factors, is also small — as evidenced by the extremely steep Hill coefficients of these drugs. For example, in mice the difference between the most and least sensitive strains is less than 40% [10].

The molecular targets of volatile anesthetics are numerous, as described below. Loss of function studies, in which putative molecular targets of volatile anesthetics have been altered or deleted from the genomes of worms, flies, and mice, all confirm a contribution of individual genes [11–14], but in accordance with the known promiscuity of volatile anesthetic binding, no single gene mutation fully ablates volatile anesthetic responsiveness. Hence, the reductionist notion that a single, conserved, anesthetic-responsive ‘receptor’, derived from a common one-celled ancestor, might underlie the observed evolutionary invariance remains dubious and without experimental evidence despite a four decade-long search. Nevertheless, given the common

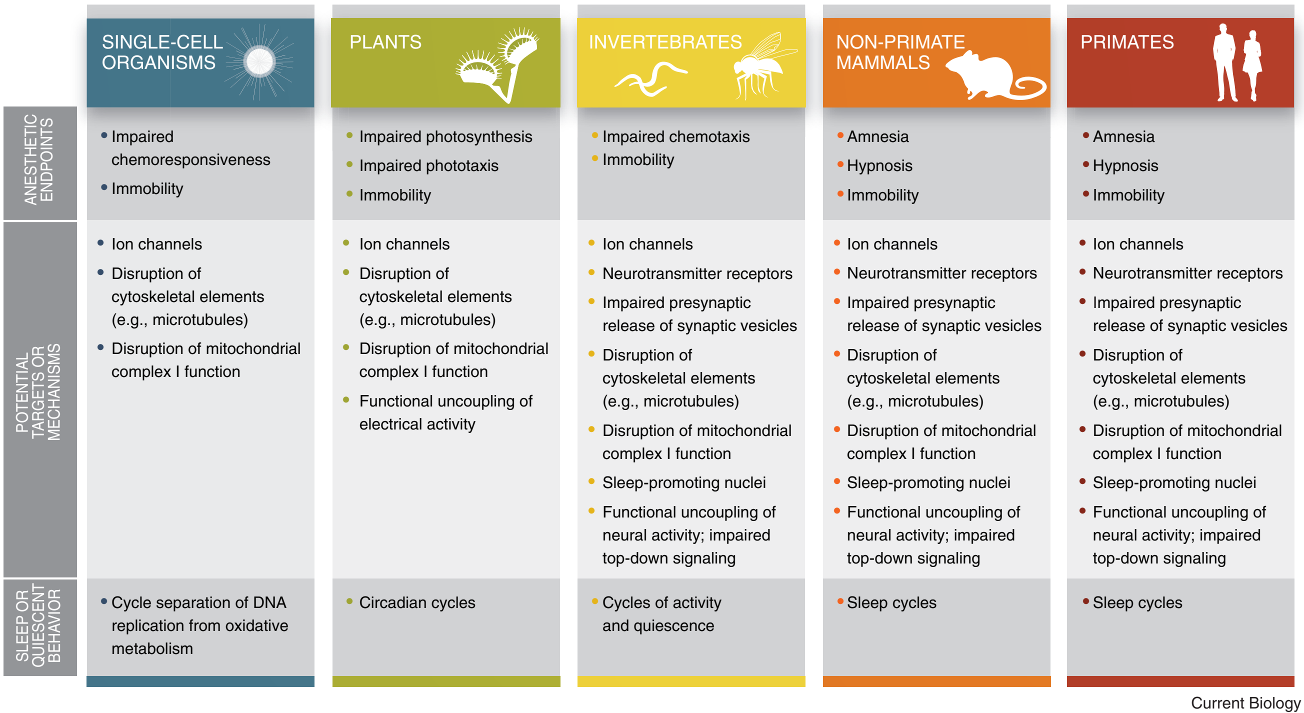

Figure 1. Anesthetic effects across evolution.
This figure highlights a select number of organisms, anesthetic endpoints, and potential anesthetic targets or mechanisms across species. Note that various endpoints and molecular substrates of anesthetic action are conserved from the single-celled organism to *Homo sapiens*.

responses across organisms separated by millions of years of evolution, some argue that it would be premature to exclude volatile anesthetic actions upon the lipid bilayer, which was considered the prime candidate for an invariant anesthetic target for the greater part of the 20th century [15,16]. Alternatively, evolutionary pressure could exist for some other biologic feature that is hijacked by volatile anesthetics. After all, it is difficult to fathom any possible evolutionary advantage conferred by conserved responses to anesthetic drugs that impair awareness of the external environment, retard movement, impede growth and procreation, and hinder self-defense [17]. Common features deemed essential for life that are known to be affected by anesthetics include mitochondrial energetics, cytoskeletal structure, as well as ion channel function. Hijacking of any of these core cellular properties, upon which biology depends, could thus arise with the lipophilic volatile anesthetics diffusing into hydrophobic cavities found in all proteins. Sparse packing of proteins enables the biophysical motion required for function. Hence, occupancy of sparsely packed lipophilic pockets is hypothesized to restrict a protein's conformational flexibility and reversibly impair its function [17,18]. This latter explanation highlights a theoretical resolution to the potential paradox of how drug responsiveness might persist in the absence of an obvious selection pressure.

Whether Earth's primordial environment created ether-like gasses mimicking volatile anesthetics (Figure 2) that placed a selective pressure on evolution, whether today's anesthetic vapors function through invariant cellular systems such as the lipid membrane, or whether the drugs' effect is mediated by percolation into sparsely packed cavities, there is no arguing that anesthetic responsiveness is pervasive across nature.

In parallel to, or possibly related to, volatile anesthetic action on all branches of life is the growing identification of sleep's phylogenetic ubiquity [19,20]. Like anesthesia, sleep is a state that at first glance should arguably have been selected against because it also incurs identically profound risks and opportunity costs. Alan Rechtschaffen, a pioneer in sleep neurobiology, wryly stated that "if sleep doesn't serve some vital function, it is the biggest mistake evolution ever made" [21]. Sleep has been recognized in each animal carefully scrutinized. From *Aplysia* to zebrafish, *Caenorhabditis elegans* to cockroaches, flatworms to fruitflies, brainless box jellyfish to single-celled *Saccharomyces cerevisiae*, behaviorally quiescent or sleep-like states may be as globally penetrant as susceptibility to volatile anesthetics [22–29].

The purpose of this article is to review key neuronal mechanisms of volatile anesthetic action, highlighting the known effects of these drugs on conserved molecular targets, on neural circuits regulating sleep and wakefulness, and on actions of the mammalian central nervous system that may corrupt information processing via multiple neural circuits. We also reformulate the meaning of 'the anesthetic state' beyond the current conception of unconsciousness (also known as anesthetic hypnosis) such that it can be applied across diverging species.

Conservation of Molecular Targets of Anesthetic Action Across Phyla

Considerable progress has been made over recent decades in identifying the molecular targets of anesthetic drugs [30,31]. For the majority of the 20th century, lipids featured as the

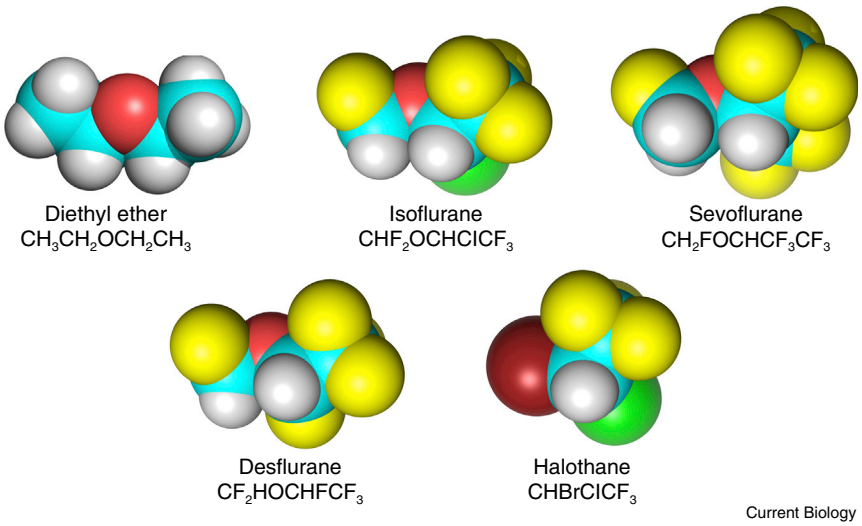

Figure 2. Chemical structures of the volatile anesthetics.
The volatile anesthetics include diethyl ether and its halogenated analogues in clinical use: isoflurane, sevoflurane, and desflurane plus the halogenated alkane, halothane. Atoms are color coded with cyan = carbon, gray = hydrogen, red = oxygen, yellow = fluorine, dark red = bromine; green = chlorine.

favored 'non-specific' molecular targets of anesthetic action. This prominence was based upon the Meyer–Overton relationship, which is a remarkable correlation between an anesthetic's lipophilicity and its potency that holds over six orders of magnitude. This relationship motivated the hypothesis that a neuron's lipid membrane was the hydrophobic target of volatile anesthetics. Subsequent recognition that distinct anesthetic drugs could similarly inhibit the protein firefly luciferase over an identical six orders of magnitude range in a cell-free environment, together with the discovery of stereoselective optical isomers of anesthetics with identical lipid solubility but reduced potency, called into question the non-specific lipid theory of anesthetic action [32]. A renewed search for molecular targets of anesthetics uncovered not only direct inhibition of firefly and bacterial luciferase, but a host of modulatory actions affecting ligand-gated neurotransmitter receptors, voltage-gated ion channels, and channels mediating leak currents, as well as intracellular effects on the cytoskeleton, intracellular signaling proteins, and mitochondria that are conserved across phylogenetic kingdoms.

Ligand-gated Ion Channels

With the recognition that general anesthetics could interact with protein targets, attention swiftly turned to pentameric ligand-gated ion channels (pLGIC) as these proteins convert chemical signals into electrical ones. The pLGIC family, which is conserved across both eukaryotes and prokaryotes, consists of both anion- and cation-conducting channels. Strong evidence indicates that anesthetic actions upon these channels contribute to the anesthetic state [33]. Direct evidence of anesthetic drugs binding to $GABA_A$ receptors, which are the most abundant inhibitory ligand-gated channel in the mammalian brain, has been obtained both with X-ray crystallographic and high resolution mass spectrometry studies [34–36]. Additional convincing evidence for a contribution of $GABA_A$ receptors to important anesthetic actions such as sedation, hypnosis, hypothermia, and immobility have come from experiments on knock-in mice, in which point mutations that abolish anesthetic binding sites also curtail the behavioral effects of several anesthetics [37,38]. Although these specific point mutations changed sensitivity to intravenous anesthetics, they did not alter potency of the volatile anesthetics. Nevertheless, as clinically relevant, low doses of all volatile anesthetics have been shown to potentiate $GABA_A$ receptor signaling [39], $GABA_A$ receptors are still considered to be important molecular targets [40]. Anesthetic actions on pLGICs are not limited solely to $GABA_A$ receptors [41–44] and the modulatory actions of anesthetics on pLGIC family members are not unique to mammalian systems [45,46].

Potassium Channels

General anesthetics modulate currents through other ion channels to alter a cell's membrane potential. Studies conducted in the freshwater snail *Lymnaea stagnalis* were amongst the first to document that volatile anesthetics could potentiate hyperpolarizing potassium currents flowing out of the cell [47]. Subsequent work led to the discovery of the tandem pore potassium channel (K_{2P}) family, which contains mammalian homologues that are activated by anesthetics [48] as well as those that are inhibited by anesthetics [49,50]. Additional support for a role of K_{2P} channels in contributing to the anesthetic state arises from murine gene knock-out studies. Deletion of TREK-1 in mice confers substantial resistance to five distinct volatile anesthetics [11]. Although deletion of TASK-1 and TASK-3 channels in mice is also associated with partial resistance to volatile anesthetics, the specific loss of TASK-3 not only reduces sensitivity to halothane, it also appears to stabilize the awake state and slows transitions into states of endogenous sleep [51,52].

Other potassium channel mutations are known to alter anesthetic sensitivity. Voltage-gated potassium channels play an important role in determining the level of neuronal excitability. Volatile anesthetics are known to modulate macroscopic conductance, opening probability, and likelihood of inactivation of voltage-gated potassium channels [53]. First identified in fruit flies, *Shaker* potassium channel mutants arise due to inactivation of the $K_v1.2$ subtype of voltage-gated potassium channels. These flies display a resistant, right-shift in their volatile anesthetic dose-response curves of roughly 25%–50% but, more remarkably, are capable of exiting states of anesthesia at doses under which their wild-type siblings remain fully anesthetized and immobile [54–57]. Anesthetic responses in loss-of-function *sleepless* mutants exhibit similar resistance to induction of and emergence from anesthesia, phenocopying responses of *shaker* mutant flies [58]. Although genetic studies assessing the anesthetic sensitivity of mice lacking $K_V1.2$ channels have not been conducted, these channels are extraordinarily sensitive to anesthetic drugs. Localized infusion of K_V1 channel inhibitors into the

central thalamus of rodents reverses ongoing sevoflurane or desflurane anesthesia at steady-state [59,60], arguing that these channels play an important role in establishing arousal thresholds across evolutionarily distant species.

Although not a selective potassium channel, hyperpolarization-activated cyclic-nucleotide gated (HCN) channels are nevertheless members of the potassium channel superfamily. HCN channels are weakly selective for potassium ions over sodium ions and conduct a mixed cationic current. These channels are sensitive to a wide variety of volatile and intravenous anesthetics in the clinically relevant dose range [61]. Anesthetics stabilize HCN channels in their closed conformation. Such actions, especially at HCN1 receptors, are thought to contribute to the hypnotic properties of volatile anesthetics [62]. HCN channels are expressed not only in mammals but also in invertebrates. Although there are four genes that encode mammalian HCN channel subunits, *Drosophila* have only a single HCN channel. Anesthetic sensitivity has not been studied in fruit flies harboring HCN channel mutations, but this genotype leads both male and female mutant flies to sleep less than their sibling controls [63].

Calcium Channels

Given the essential role calcium plays for intracellular signaling and neurotransmitter release, calcium channels are intriguing potential targets of anesthetic action. Amongst the many varieties of calcium channels, there is evidence for volatile anesthetics producing a dose-dependent depression of currents fluxing through high-voltage activated L-type and low-voltage activated T-type calcium channels [64]. T-type calcium receptors play an important role in cell excitability, are expressed in the mammalian brain in many regions (including the thalamus), and are especially sensitive to clinical doses of volatile anesthetics [65]. Electrophysiological recordings in the thalamus demonstrate that $Ca_V3.1$ T-channels are inhibited by volatile anesthetics [66]. Moreover, mice lacking $Ca_V3.1$ channels present with delayed induction of isoflurane anesthesia, though their steady-state anesthetic requirements remain unaltered [13]. This phenotype is similar to that observed in $Ca_V3.2$ T-channel global knock-out mice [67]. Volatile anesthetic-induced modulation of $Ca_V3.1$ channel function in midline thalamic nuclei alters thalamocortical rhythms in mice. Under identical isoflurane exposures, compared to sibling controls, $Ca_V3.1$ knock-out mice exhibit decreased slow-wave EEG activity, which is often associated with hypnosis, and increased EEG burst suppression, which typifies states of deeper anesthesia [68].

Sodium Channels

General anesthetics act both pre- and post-synaptically to affect neuronal function. Amongst their promiscuous actions, anesthetics have been found to inhibit neuronal voltage-gated sodium channels at presynaptic sites. Voltage-gated sodium channels are necessary for action potential propagation as well as dendritic integration and transient neuronal cell assemblies. Although clinical concentrations of volatile anesthetics do not markedly impair sodium currents in squid or crayfish giant axons, they significantly depress axonal conduction in mammalian, small unmyelinated hippocampal fibers [69]. Volatile anesthetics inhibit multiple mammalian sodium channel isoforms through a variety of mechanisms [70]. Similar inhibitory actions are also conserved in the smaller, yet homologous bacterial sodium channel, NaChBac [71,72]. Supporting a physiologically important role for modulation of voltage-gated sodium currents in arousal, mice with targeted knockdown of $Na_V1.6$ — the most abundant channel subtype in the CNS — exhibited a marked hypersensitivity to induction of both isoflurane and sevoflurane anesthesia when compared with their wild-type siblings [73].

Presynaptic Release Machinery

Additional support for presynaptic sites of anesthetic action was demonstrated using a forward genetic screen in *C. elegans*. Worms with altered sensitivity to volatile anesthetics were found to have mutations in syntaxin that could manifest either with resistance or hypersensitivity, depending on the mutated allele. Together with SNAP-25 and synaptobrevin, syntaxin forms the SNARE complex that regulates presynaptic neurotransmitter release. In general, mutations that enhanced presynaptic release led to volatile anesthetic resistance, whereas those that impaired release produced a hypersensitive phenotype [14]. The extraordinary power of syntaxin mutations to curtail the effects of isoflurane on neurotransmission has also been replicated in cultured cell lines [74,75]. Moreover, *in vivo* evidence highlighting presynaptic sites as modulating anesthetic sensitivity in worms has been confirmed by multiple independent lines of evidence in mammals. Volatile anesthetics have been shown to bind directly to syntaxin and the SNARE complex [76,77]. A number of studies point to an important role for anesthetics in modulating presynaptic neurotransmitter release as an intrinsic component of their actions [78–80]. Recovery from anesthetic-induced modulation of presynpatic function appears to play an even more important role in the exit from anesthetic states [81]. In yet another curious convergence, the use of a syntaxin gain-of-function mutation that increases synaptic activity in flies leads to volatile anesthetic resistance when targeted to wake-promoting neurons but anesthetic hypersensitivity when targeted to sleep-promoting neurons [82].

Cytoskeleton

Volatile anesthetics are known to impair chemoresponsiveness and motility in single-celled organisms. By definition, however, there is no neural network that can be modulated by the drug. This gave rise to the hypothesis that anesthetics might impair single-cell organismal function through modulation of the internal cellular network of cytoskeletal elements. Actin and microtubules form essential components of the cytoskeleton and are dynamically regulated to control a wide variety of intracellular and intercellular processes. Drug-induced modulation of the cytoskeleton, ultimately leading to impaired neurochemical signaling, was first hypothesized as a potential mechanism of general anesthesia in 1968 [83]. One example occurs with tubulin, an abundant protein that oligomerizes into microtubules to form critical components of cellular (and especially neuronal) scaffolding. Anesthetics bind to tubulin, causing microtubules to destabilize [84,85]. They can also cause microtubule-based molecular motors, such as kinesin, to reversibly fall off the microtubule lattice and thus disrupt transport of vesicles, proteins, and organelles to synapses [86]. The *in vivo* relevance of anesthetic action on the cytoskeleton has been validated in tadpoles that demonstrate increased anesthetic resistance in the presence of microtubule-stabilizing drugs and also suggested in humans [85,87]. Moreover, several theories now incorporate anesthetic effects on microtubule dynamics as being fundamental for loss of awareness [88].

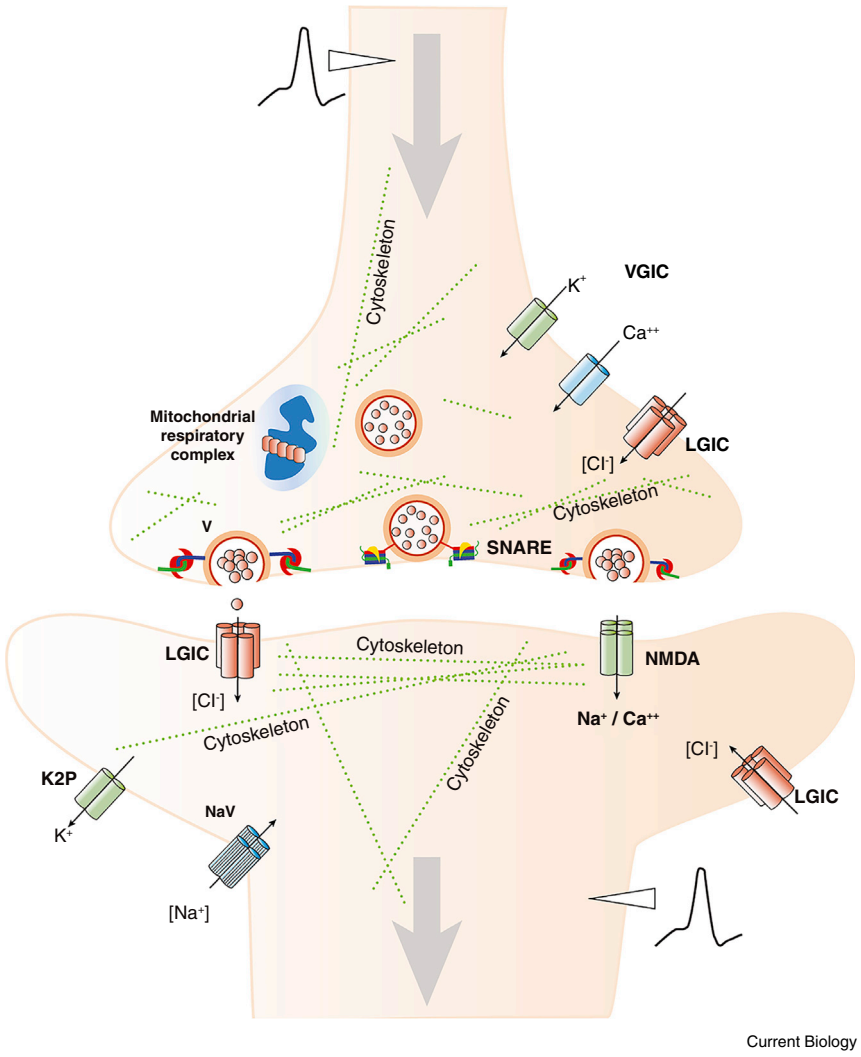

Figure 3. Molecular targets of anesthetics are predicted to affect synaptic function.
Anesthetics modulate pre-synaptic neurotransmitter release by binding to a variety of proteins that regulate resting membrane potential and calcium dynamics, including voltage-gated ion channels (VGIC), ligand-gated ion channels (LGIC), cytoskeletal elements (dotted green filaments), mitochrondrial respiration, and synaptic release machinery (SNARES). Similarly, binding to postsynaptic targets such as two pore potassium channels (K_{2P}) and extrasynpatic $GABA_A$ receptors will also affect signaling.

Through interactions with other scaffolding proteins, the cytoskeleton also offers additional opportunities to regulate synaptic function. Receptors such as NMDA, AMPA, and metabotropic glutamate receptors concentrate along the postsynaptic membrane based upon their interactions with scaffolding proteins. Hence, in addition to presynaptic mechanisms that reduce release of glutamate into the synapse, volatile anesthetics reversibly disrupt the interactions of NMDA and AMPA receptors with scaffolding proteins. This independently alters volatile anesthetic requirements. Thus, anesthetics disrupt glutamate receptor signaling through multiple pathways to modulate synaptic function [89,90].

Mitochondria

Mitochondrial complex I proteins are perhaps the best conserved example of molecular targets known to bind general anesthetic drugs. Mutations impairing mitochondrial complex I function affect species ranging from worms to flies to mice to humans. First identified in an unbiased genetic screen of volatile anesthetic sensitivity in *C. elegans*, the *gas-1* allele conferred profound anesthetic hypersensitivity, reducing effective doses of multiple anesthetic ethers by 25–80%. *gas-1* was found to be a hypomorphic allele of a highly conserved, nuclear-encoded subunit of complex I, exhibiting impaired ability to shuttle electrons into mitochondrial oxidative phosphorylation [91]. Mutations affecting complexes II–IV do not alter anesthetic sensitivity, raising the question of whether complex I function might specifically be essential to the mechanism of volatile anesthetics [92]. Mutations affecting complex I subunits produce homologous hypersensitivity to volatile anesthetics in mice as well as profound hypersensitivity to volatile anesthetic hypnosis in humans afflicted by complex I disorders. As is true in nematodes, only human patients with complex I disorders, in contrast to those with other mitochondrial respiratory deficits, are affected [93,94].

Although the array of potential molecular targets could account for the actions of an individual anesthetic drug, and although different anesthetic agents do utilize distinct subsets of molecular targets, all actions in multicellular organisms would appear to affect synaptic function (Figure 3). The identity of the 'relevant synapses' in applicable neural circuits underlying anesthetic loss of consciousness is the subject of the next section.

Neuronal Targets of Anesthetic Action

In addition to the conserved molecular targets across species that might mediate the effects of general anesthetics, there are also conserved networks in the brain that have evolved to control sleep–wake states. As the neurobiology of sleep–wake cycles became more systematically elucidated, a variety of neuronal candidate targets for general anesthetics emerged. Just as anesthetics are thought to achieve their therapeutic effects by either potentiating inhibitory transmission or attenuating excitatory transmission at the molecular level, so too might anesthetics act by either potentiating sleep-promoting neuronal subpopulations or attenuating the activity of wake-promoting neuronal subpopulations. In this section, we focus on these two major categories of subcortical nuclei, distributed throughout the brainstem and diencephalon.

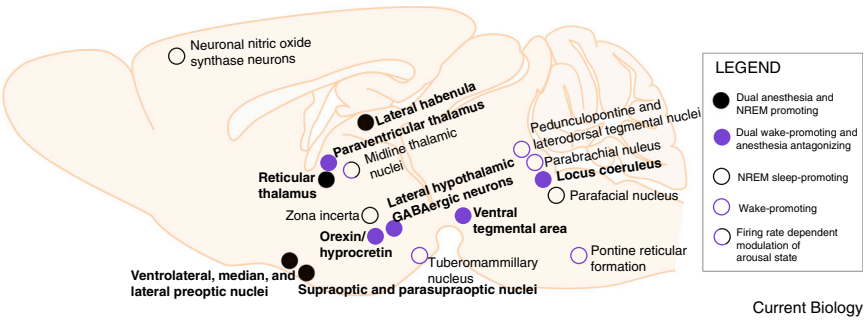

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Figure 4. Evidence for anesthetic utilization of endogenous sleep and arousal circuitry.
Sagittal section through the rodent brain revealing the approximate locations of nuclei with a known role in regulating NREM sleep or wakefulness that are also implicated in anesthetic hypnosis. Sleep-promoting nuclei are shown in black and wake-promoting nuclei in purple. Bolded names indicate structures for which anesthetic or experimental activation has resulted in deepening the anesthetic state (black) or enhancing exit from the anesthetic state (purple).

Anesthetic Actions on Endogenous Sleep-promoting Systems

Hypothalamic neurons in the preoptic area are thought to be important for both the generation and regulation of sleep [95]. In particular, the ventrolateral preoptic nucleus (VLPO) contains neurons that are sleep-active [96,97], which would be attractive candidates for the hypnotic effects of general anesthetics. Indeed, one of the first systems neuroscience approaches to anesthetic mechanism identified the metabolic activation of VLPO — as determined by c-Fos expression, a marker of antecedent cellular activity — in association with a variety of sedative-hypnotic drugs [98–100]. Subsequent studies determined that acute lesions of VLPO confer resistance to anesthetic effects, arguing for a role for VLPO in the induction of general anesthesia [99,101]. Even more compelling were the observations that sleep-promoting VLPO neurons were depolarized by isoflurane and other anesthetics [99,102]. General anesthesia is typically associated with a depression of neural spike activity — that a population of neurons whose putative function is to promote sleep would increase firing upon anesthetic administration is a remarkable finding that supports the hypothesis of shared circuitry between sleep and anesthesia. To date, with the exception of ketamine, systemic delivery of hypnotic doses of every general anesthetic examined recruits sleep-promoting neurons in the VLPO [98–102], highlighting a potentially convergent subcortical circuit mechanism to elicit unconsciousness. Anesthetics are able to recruit more sleep-promoting neurons in the lateral and medial preoptic hypothalmus than in the VLPO itself [103]. Volatile anesthetics also recruit neuroendocrine cells in the supraoptic and parasupraoptic nuclei. These neuroendocrine cells are depolarized by isoflurane as well as intravenous anesthetics (including ketamine) both *in vitro* and *in vivo* [104]. Moreover, recruitment of excitatory glutamatergic neurons in the lateral habenula appears as yet another example through which general anesthetics produce sedative and hypnotic actions by comandeering the endogenous machinery necessary for natural sleep [105]. This shared circuits hypothesis has been replicated across divergent species spanning invertebrates to mammals [54,58,82,98,99,101]. Additionally, multiple studies have identified the homeostatic interactions between states of sleep and general anesthesia, with the demonstration that inhaled anesthetics satisfy the need for slow-wave sleep in rodents that had undergone sleep deprivation [106–108]. Thus, the sleep–anesthesia connection appears to be linked across evolution to both the generation and regulation of sleep.

Activation of a subset of endogenous sleep-promoting neurons in the preoptic hypothalamus of warm-blooded animals causes body cooling [109]. Recent work suggests that general anesthetics coopt such ‘dual purpose’ warm-sensing and sleep-active subsets of neurons both to elicit hypnosis and to drive central cooling of body temperature, which occurs during anesthetized states [99,103,110]. In so doing, anesthetics may rely on molecular targets with intrinsic temperature-sensing ability such as the K_{2P} channels or mitochondrial targets in critical cellular populations to inextricably link reductions in body temperature with decreased metabolism as occurs with endogenous sleep [111].

Anesthetic Actions on Endogenous Arousal-promoting Systems

Neurons within the pedunculopontine and laterodorsal tegmentum (cholinergic), pontine reticular formation (unknown population), locus coeruleus (noradrenergic), parabrachial nucleus (glutamatergic), dorsal raphe (serotonergic), ventral tegmental area (dopaminergic), perifornical, lateral, and posterior hypothalamus (orexinergic), lateral hypothalamus (GABAergic) and tuberomammillary nucleus (histaminergic) are all capable of promoting the waking state [112]. Many of these nuclei have been explored as targets of the inhibitory effects of general anesthetics [113] (Figure 4). Although *in vivo* electrophysiologic recordings of activity are still needed, using c-Fos as a proxy for antecedent activity, neurons within many of these nuclei have been identified to be inhibited during general anesthesia [98,100,101,114]. Moreover, the causal reactivation of some wake-promoting neurons during emergence from the anesthetic state has been explored using approaches such as electrical stimulation, optogenetics, and chemogenetics [115–118] in rodents and suggested in humans [119]. It is important to note, however, that activation of wake-promoting neurons during emergence does not necessitate that inhibition of the same population occurred during induction of anesthesia, that potential inhibition was causal with respect to the induction of anesthesia, or that experimental reactivation recapitulates spontaneous changes in activity that occur during emergence.

Like many of the wake-promoting monoaminergic neurons, those in the locus coeruleus exhibit state-dependent firing patterns with highest levels occurring during wakefulness, reduced firing during NREM sleep, and virtual quiescence during REM sleep. Although distinct anesthetic drugs differentially affect locus coeruleus firing, volatile anesthetics such as isoflurane slow locus coeruleus discharge rates. Impairing adrenergic signaling from this nucleus increases the potency of volatile anesthetics — facilitating entry into and impairing exit from hypnotic states [120]. Exogenously activating the locus coeruleus primes

the brain for emergence from isoflurane anesthesia, without being able to antagonize a continuous isoflurane anesthetic [116].

Additional clusters of neurons in the pons play important roles in the regulation of arousal state and affect anesthetic hypnosis. Although neither the precise neurochemical identities nor the electrophysiological properties of wake-promoting pontine reticular formation neurons are known, the contribution of this region to sleep and wakefulness has long been recognized [121]. Localized microinjections of GABAergic anesthetic drugs into a cluster of several thousand mesopontine tegmental neurons in the pontine reticular formation induces a complete state of general anesthesia in rodents [122]. Lesioning neurons in this region also increases wakefulness in rats at the expense of NREM and REM sleep [123].

Subcortical–Cortical Connectivity during General Anesthesia

The preceding sections describe the subcortical networks that mediate sleep–wake behavior, or the *level of consciousness*. It is highly likely that thalamocortical and corticocortical networks mediate experience itself, or the *content of consciousness* [124]. In parallel, it has been argued that anesthetic actions map on to these two dimensions, with effects in subcortical systems depressing level of consciousness and effects in thalamocortical and/or corticocortical systems degrading or disrupting the content of consciousness [125].

The thalamus has long been considered a critical neuroanatomical target of general anesthetics in mammals and has been proposed as a 'switch' for anesthetic state transitions [126]. The original theory was motivated by the observation of metabolic depression of the thalamus by a broad range of anesthetics [127–129]. Earlier studies using positron emission tomography during exposure to inhaled anesthetics in humans revealed a disruption of thalamocortical functional connectivity in association with unconsciousness [130]. More recent studies have identified a specific disruption of functional connectivity between the thalamus and frontal cortex during sevoflurane anesthesia [131,132]. Furthermore, the thalamus has also been found to be activated during both spontaneous [133] and pharmacologically induced [134] emergence from anesthesia.

Disruption or functional disconnection of thalamocortical circuits is not the only effect of general anesthesia on subcortica–cortical connectivity. For example, a neuroimaging study of isoflurane in rats demonstrated functional disconnections between cortex and the striatum during general anesthesia [135]. Improved spatial resolution of 7T magnetic resonance imaging devices and templates for brainstem nuclei [136] promise to contribute to a better understanding of functional interactions between subcortical nuclei and other brain structures during general anesthesia [137].

Cortical Connectivity and Dynamics during General Anesthesia

The first wave of neuroimaging studies of the anesthetized state employed positron emission tomography and revealed regional patterns of metabolic depression in cortical areas, including frontal-parietal networks [138]. Based on fMRI, functional disconnections of frontal-parietal networks have been shown during general anesthesia in humans induced by structurally and pharmacologically diverse agents, including the volatile anesthetic sevoflurane [131,139–141] (Figure 2). These findings support the identification of depressed surrogates of information transfer between prefrontal and posterior parietal cortex as identified by electroencephalography [142]. It has been suggested that such disruption might be a critical mediator of general anesthesia [143]. Although no causal studies have demonstrated this, a recent study of nonhuman primates demonstrated that both volatile and intravenous anesthetics depressed functional connectivity patterns across prefrontal, posterior parietal, and cingulate cortices, restricting functional connections to brain regions with strong structural connections [144]. These particular brain regions are important because they are posited to constitute a global neuronal workspace that is critical to consciousness [145], the impairment of which can account for traits of the anesthetized state [146]. However, recent studies have demonstrated that corticocortical functional connectivity shifts dynamically during exposure to volatile anesthetics, even when controlling for pharmacokinetic stability and surgical stimulus [147,148].

Effects of General Anesthetics on Network Organization

Despite the significant effects of anesthetics on corticocortical and cortical-subcortical connectivity, there is not a complete breakdown of functional network organization in the brain during general anesthesia. Functional architecture associated with sensory, motor, and cognitive tasks in humans are preserved during isoflurane anesthesia in non-human primates [149]. There are also data in humans and animals, derived from both neurophysiology and neuroimaging, suggesting that the brain might reconfigure functional networks in order to adapt and maintain an optimal network configuration. For example, isoflurane anesthesia induces an organizational shift of particular networks in the rat brain, while maintaining certain global network features [135]. However, there are consistent findings of impaired network efficiency (which reduces the capacity for information processing) across various states of anesthetic-induced unconsciousness [150] as well as increases in modularity (which reduces the capacity for information integration [151]) observed in association with anesthesia, sleep, and pathologic unconsciousness [152]. Further work is required to link the known molecular events of anesthetic action with network events and with the state of information in the brain that ultimately determines the state of consciousness or anesthesia [153].

Network Effects of General Anesthetics across Species

The effects of general anesthetics on cortical networks and association cortex described in the last section present an ostensible paradox: how do we reconcile cortically mediated mechanisms of anesthetic-induced unconsciousness with the known susceptibility to general anesthetics across species, including those without a well-developed cortex? Indeed, this conflict arises even when considering subcortical mechanisms of general anesthesia, since organisms like *C. elegans* do not have a brain and organisms like the unicellular paramecia do not have a single neuron. These considerations suggest the need for a more generalizable definition of general anesthesia and a more fundamental formulation of anesthetic mechanism that might be common across species. Although general

anesthesia in humans is often defined as a complement of unconsciousness, amnesia, analgesia, and immobility, the more generalizable characterization is a *disconnection from the environment*, both in the receptive (e.g., sensation or experience) and expressive (e.g., motoric response) arms of the interaction. This definition is equally applicable to a single-cell organism such as the paramecium, which undergoes dose-dependent impairment of both chemoresponsiveness and motility due to anesthetics [154]. If a generalizable mechanistic framework is the goal, we are forced to dissociate anesthetic mechanism from the specific neural circuits that might be of more clinical relevance in the primate brain. Considering commonalities across species, anesthetic mechanisms must likely be reduced to principles involving action at molecular targets, resulting in network-level events, with a final common pathway converging on impaired information transmission and communication across the network. This is a biologically plausible approach because there are molecular targets that are conserved across animals and plants, and the properties of networks and information exchange hold independently of the particular physical instantiation (e.g., neural network of a mammalian brain versus electrical network of a plant).

There is evidence that the kind of informational or computational uncoupling observed during general anesthesia in humans, primates, and rodents extends to species such as *Drosophila melanogaster* and *C. elegans*. *Drosophila* exposed to isoflurane demonstrate an uncoupling of neural activity patterns from movement at lower doses and cessation of movement at higher doses that correlate with local field potential characteristics [155]. More recent work in *Drosophila* has focused on hierarchical brain organization, assessing the effects of isoflurane anesthesia on higher-order central structures and lower-order peripheral structures [156]. As in mammalian brain systems, there were faster frequencies associated with feedforward directed connectivity (from lower-order to higher-order) and slower frequencies associated with feedback directed connectivity (from higher-order to lower-order). Remarkably, and consistent with observed effects of general anesthesia in the mammalian brain, there was a selective suppression of feedback connectivity during general anesthesia.

The effects of general anesthetics in *Drosophila*, however, might be argued to relate to conserved arousal mechanisms that form a bridge to the mammalian phenotype [82]. However, anesthetic effects in *C. elegans* also result in a functional disconnection between neuronal activity. In a recent study using calcium imaging, isoflurane was found to induce desynchronization of neuronal dynamics and reduced (surrogates of) information exchange [157], which is strikingly similar to EEG-based phenotypes in humans. Furthermore, the preservation of local neuronal activity in the setting of functional uncoupling across the system is consistent with the predictions of the cognitive unbinding theory of general anesthesia [158], which was formulated in the context of mammalian anesthesia.

There is a known conservation of molecular targets for general anesthetics across mammalian systems, fruit flies, and worms. This could arguably be situated within a framework for the evolution of consciousness that originates in excitable membranes, in which the influx of positively charged ions to the alkaline milieu of the cytoplasm results in the most primordial form of 'sensation' [159]. Importantly, plants also exhibit depolarization of membrane potentials and electrical impulses that resemble neural signals. Thus, it is conceivable that there could be a common network-level effect of general anesthetics that reduces network efficiency, ultimately resulting in conditions that are inhospitable to information exchange. Such information exchange is critical to the coordination of events that allows both the sensation and response that is characteristic of a bidirectional relationship with the environment.

Conclusion

This review of the literature supports the 19th century hypothesis that the effects of volatile anesthetics are as biologically broad as life itself. Furthermore, impaired sensation and interaction with the environment appear to be common anesthetic endpoints from single-celled organisms, to plants, to primates. Common molecular or functional targets might account for these shared responses, with network-level effects representing a generalizable mechanistic framework across species. Further work is warranted on such a framework in order to inform the biological principles by which an organism responds to its environment as well as the evolution of consciousness itself [160,161].

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