Explaining general anesthesia: A two-step hypothesis linking sleep circuits and the synaptic release machinery

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Several general anesthetics produce their sedative effect by activating endogenous sleep pathways. We propose that general anesthesia is a two-step process targeting sleep circuits at low doses, and synaptic release mechanisms across the entire brain at the higher doses required for surgery. Our hypothesis synthesizes data from a variety of model systems, some which require sleep (e.g. rodents and adult flies) and others that probably do not sleep (e.g. adult nematodes and cultured cell lines). Non-sleeping systems can be made insensitive (or hypersensitive) to some anesthetics by modifying a single presynaptic protein, syntaxin1A. This suggests that the synaptic release machinery, centered on the highly conserved SNARE complex, is an important target of general anesthetics in all animals. A careful consideration of SNARE architecture uncovers a potential mechanism for general anesthesia, which may be the primary target in animals that do not sleep, but a secondary target in animals that sleep.

Keywords:
Caenorhabditis elegans; *Drosophila*; general anesthesia; neuroscience; sleep; SNARE; syntaxin

Introduction

We all lose consciousness on a daily basis when we sleep, and most of us will be rendered unconscious at least once in our lives by general anesthetics, to allow surgery to proceed. Sleep and general anesthetics both produce a rapid, transient loss of awareness, as if a switch has been flipped in the brain [1]. The ability to render patients unresponsive under general anesthesia was one of the most important discoveries in medical history [2], yet the observation that sleep produces a similar outcome every night has always been evident. Research over the past decade supports the idea that general anesthetics act in part through endogenous sleep/wake pathways (Fig. 1A and B) [3–8], and a recent electrophysiological study has shown that the volatile anesthetic isoflurane activates sleep-promoting neurons in the mammalian brain [9]. These convergent effects may explain some similarities between sleep and general anesthesia, such as common molecular pathways [7, 10], interactions between sleep drive and sensitivity to general anesthetics [11, 12], and similar electrophysiological signatures during brain recordings [13, 14]. Nevertheless, it is also evident that drug-induced activation of sleep processes cannot be the whole explanation for general anesthesia, as general anesthesia is clearly more than just sleep. In addition to an enormous body of research pointing to a variety of clinically relevant protein targets unrelated to specific sleep circuits [15–19], research in model organisms such as the fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* highlights a problem with the relatively recent sleep-centric view of general anesthesia: all animals can be rendered unresponsive by similar concentrations of general anesthetics [20], but it is likely that not all animals sleep, at least during certain life stages [21, 22]. This suggests that other conserved processes (perhaps even more conserved than sleep mechanisms) must also be involved. In this review, we will investigate this conundrum, and propose a hypothesis that may provide an answer to the problem, with a focus on sleep and anesthesia research in invertebrate models.

General anesthetics activate sleep processes in different animals

Sleep and general anesthesia are defined by similar criteria pertaining to behavioral responsiveness in intact animals, which allows for informative

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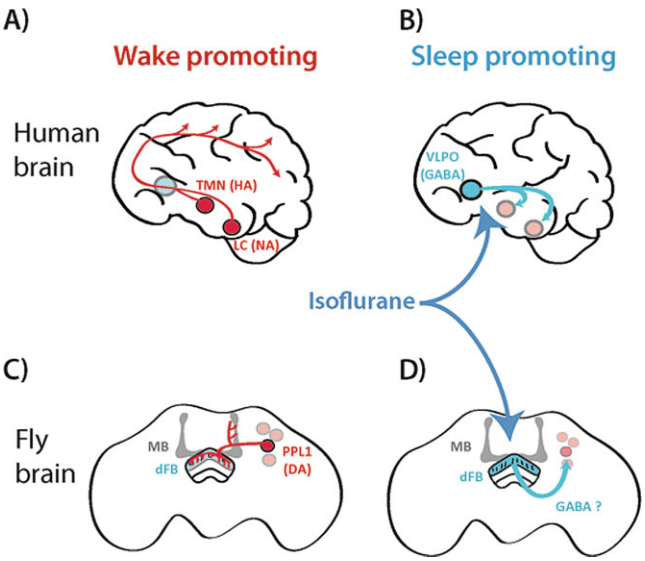

Figure 1. Sleep/wake circuits in the human and fly brain. **A:** Noradrenergic (NA) and histaminergic (HA) signaling (red lines) from the locus coreleus (LC) and the tuberomammillary nucleus (TMN) is wake-promoting in the human brain. **B:** Inhibitory GABAergic signaling (light blue arrows) from the vasolateral preoptic nucleus (VLPO) to the LC and TMN is sleep promoting. General anesthetics such as isoflurane and propofol potentiate the sleep-promoting pathway (dark blue arrow). **C:** In the fly brain, dopaminergic neurons in the PPL1 cluster (red cells) are wake promoting. Subsets in this cluster innervate both the mushroom bodies (MB, gray) and the dorsal fan-shaped body (dFB, blue). **D:** Activation of the dFB promotes sleep in flies. Analogy with the mammalian sleep/wake circuit predicts inhibitory (e.g. GABAergic) feedback to the PPL1 neurons (light blue arrow), and also predicts that general anesthetics such as isoflurane potentiate this sleep circuit (dark blue arrow).

comparisons to be made between both phenomena. The evidence that sleep processes (such as sleep deprivation) modulate general anesthesia is well established [4, 11], although evidence of the converse (that general anesthesia accomplishes some sleep functions) remains debated [12, 23, 24]. Patients addicted to certain arousal-inducing drugs, such as methamphetamines or cocaine, tend to be resistant to general anesthetics [25], suggesting that defined wake-promoting pathways might counteract the effect of general anesthetics. Recent work in rodent models suggests a role for mono-aminergic systems, such as dopamine, in mediating these effects: activating the dopaminergic system with methylphenidate, for example, can alter sensitivity to general anesthetics, and can even be used to acutely "awaken" rats under general anesthesia [26]. Similar evidence has recently been found in *Drosophila*, where genetic activation of mono-aminergic systems produced resistance to isoflurane [27]. In the nematode *C. elegans*, a recent study has shown that acute activation of cholinergic neurons can even reverse halothane-induced immobility [28]. These results from a wide range of model organisms suggest that a variety of circuits and endogenous molecules might directly modulate the potency of general anesthetics.

Most animals share a similar suite of neurotransmitters and neuromodulators, such as dopamine, acetylcholine, and GABA, that together control behavioral responsiveness [29]. This does not necessarily imply that all animals employ these systems in a sleep/wake pathway. The evidence for sleep in some "simple" animal models such as the fruit fly *D. melanogaster* is very strong [30]. Adult flies fulfill all of the historical criteria for sleep (transient quiescence, increased arousal thresholds, and homeostatic regulation [31, 32]), and a growing number of publications provide convincing support for the view that sleep in *Drosophila* has important similarities with mammalian sleep [30, 33, 34]. Recent studies have begun to describe a potential "sleep/wake circuit" in the fly brain (Fig. 1C and D), encompassing dopaminergic modulation of sleep-promoting neurons in the central brain [35–37]. A parallel with mammalian systems might predict a "sleep/wake switch" involving mutual inhibition between arousal centers and sleep-promoting neurons, perhaps analogous to the "flip-flop switch" that has been proposed to link sleep and wake centers in the mammalian brain [38]. Transient activation of sleep centers, in any sleeping animal from flies to humans, would thus inhibit arousal systems, with the net consequence that animals would become less responsive to external stimuli [3]. That this is associated with a loss of consciousness in humans could be incidental rather than germane to the evolutionarily conserved mechanisms involved in flies and humans [39].

Research in rodent models suggests that general anesthetics such as propofol and isoflurane trigger the sleep/wake switch in favor of sleep, by inhibiting wake-promoting neurons in the tuberomammillary nucleus (TMN) [4, 8], thereby assisting "normal" inhibitory input from the vasolateral preoptic nucleus (VLPO) of the hypothalamus [7] (Fig. 1B). Some VLPO neurons are GABAergic (thus inhibitory), and this hypothesis is consistent with a wealth of evidence that many general anesthetics potentiate ionotropic chloride channels, or $GABA_A$ receptors specifically [18, 40–44]. Together, these data support the view that the $GABA_A$ receptor is the primary target of general anesthetics, and that sleep-promoting GABAergic networks are activated by these drugs. Recent electrophysiological evidence in rodents showing that a subgroup of VLPO neurons are acutely activated (by firing more action potentials) under isoflurane exposure further supports this model for general anesthesia [9]. Similarly, acute activation of sleep-promoting neurons in *Drosophila* increases sleep and produces isoflurane hypersensitivity, whereas acutely blocking these fly neurons has the opposite effect [27]. Indeed, a strong correlation has been found between isoflurane sensitivity and sleep duration phenotypes in *Drosophila* [27, 45], further supporting a deep connection between these two processes in all animals that need sleep.

But not all animals apparently need sleep [21, 22]. It is unlikely that coral or

sponges sleep, although no thorough study seems to have been done, and it remains debatable whether jellyfish sleep [46], or even whether certain vertebrates sleep [47]. Although there is a strong interaction between circadian and sleep-homeostatic processes [48], diurnal rhythms in simple life forms, such as in yeast [49], are not sufficient evidence for sleep. The strongest evidence for sleep in any animal lacking a central nervous system comes from a handful of *C. elegans* studies and subsequent reviews [50–54]. This evidence is two-pronged. First, a brief quiescence and decreased behavioral responsiveness in molting larvae, termed lethargus, has been suggested to be a sleep-like state in this otherwise continuously moving animal. Second, developmental pathways linked to sleep functions in mammals have been found to control molting, suggesting that these conserved developmental programs have been co-opted to accomplish sleep functions. Some of these sleep functions, such as membrane homeostasis can be studied in cell culture [55], divorced from whole-animal behavior. However, while it is likely that sleep recapitulates key aspects of cell homeostasis and development (e.g. *notch* signaling [52]) that are conserved between nematodes and humans (and can thus be usefully studied in the former), it remains questionable whether adult *C. elegans* actually sleep; there is no evidence of extended quiescence, altered arousal thresholds, or sleep homeostasis in adult nematodes. Further, there is no clear evidence of any sleep-promoting circuit in adult *C. elegans*, as has been proposed for *Drosophila* [27, 35–37], (but see [56] for recent evidence in larvae), and no neural correlates of sleep or sleep stages have been identified in nematodes, as have been shown in flies [34, 57]. While such "absence of evidence" does not necessarily imply "evidence of absence", it is notable that circadian mechanisms exist in adult nematodes [58], and GABAergic plasticity mechanisms have been reported in the larvae [51]. While the question of sleep in adult nematodes remains unresolved, the likely absence of sleep in adult *C. elegans* would have profound implications for our understanding of mechanisms of general anesthesia.

Perhaps similar to adult *C. elegans*, there is also no evidence in the literature that the larval stage of *Drosophila* sleeps. Nevertheless, fly larvae display circadian rhythms [59, 60] and GABAergic plasticity mechanisms [61]. Perhaps consistent with the likely absence of sleep in fly larvae, circuits of the dorsal fan-shaped body in the fly brain (which are sleep promoting [27, 35–37]) only develop after the larval stage, i.e. during the pupal stage [62]. If general anesthetics produce their sedative effects only by targeting sleep/wake circuits, then adult nematodes (and fly larvae) should be insensitive to general anesthetics. However, they are not insensitive; surprisingly, these sleepless worms and maggots become unresponsive to general anesthetics at similar concentrations to those required to perform surgery in humans [63, 64].

How is loss of consciousness measured in simple animal models?

What is general anesthesia in a worm? Before discussing how *C. elegans* might be "rendered unconscious" if they lack sleep-promoting circuits, it is first necessary to outline how general anesthesia is measured in the nematode model. In most animals, clinically relevant concentrations of drug (e.g. 0.7 vol% isoflurane) induce immobility [20], although the relevance of the immobility endpoint for general anesthesia has been questioned [63, 65]. Instead, probing for responses to stimuli such as prods or pinpricks is most likely a more accurate measure of the depth of anesthesia in animals. *C. elegans* still move quite actively at anesthetic concentrations that would immobilize other animals, such as flies and humans. Therefore, general anesthesia in the nematode is perhaps better addressed by quantifying more complex behaviors, such as coordinated movement or responsiveness to a chemical gradient [63]; such behaviors are abolished by clinically relevant drug concentrations in nematodes, and it is interesting to note that the more complex the behavior (e.g. male mating coordination, versus defecation for example), the more sensitive it is to general anesthetics [63]. Similarly, more complex behaviors in *Drosophila* are also more sensitive to the effects of isoflurane [66], and short-term memory and consciousness in humans are more sensitive endpoints than responsiveness to pain [1, 65]. Taken together, the correlation between drug sensitivity and behavioral complexity in these diverse animals suggests a conserved set of targets for these drugs in all animals, whether or not they sleep. Behavioral characterization of mutant animals is one way of identifying such targets in genetic model organisms [63, 67–70].

Resistance to general anesthesia is often seen as a more convincing indicator of a potential target mechanism than hypersensitivity. This is because one might imagine a resistant animal if the main target protein or process is missing. Thus, a single point mutation in the $GABA_A$ beta3 subunit confers resistance to propofol in transgenic mice, strongly implicating the receptor subunit as a target site for this intravenous anesthetic [71]. Interestingly, this subunit does not appear as relevant for general anesthesia in *C. elegans*, and nematodes also seem less sensitive than mammals to GABAergic drugs such as propofol [72], which may be consistent with the probable absence of sleep-promoting circuits in this model organism. If sleep-promoting mechanisms are absent in *C. elegans*, and thus not a potential target, then mutations in other relevant drug targets might produce drug-resistant animals. Such mutants were found for *C. elegans* over a decade ago, in a screen for a motor coordination endpoint. A lesion in the syntaxin1A protein, a crucial component of the synaptic neurotransmission machinery in all animals [73], was found to make nematodes essentially insensitive to isoflurane [74]. Other mutations in syntaxin1A rendered the animals hypersensitive, suggesting that this molecule may be central to the effect of volatile general anesthetics in nematodes. A simple explanation for these anesthetic effects might be that anesthetic sensitivity is correlated with synaptic release levels, where mutants with decreased release are hypersensitive and mutants with increased release are resistant. This does

not appear to be the case for the *C. elegans* syntaxin1A mutants [74]. The mutation that conferred resistance produced a C-terminus truncation in the syntaxin1A molecule, which was co-expressed in addition to wild-type isoforms – producing a gain-of-function effect. Subsequent work in *C. elegans* confirmed a synaptic role for these effects [75], and other interacting synaptic machinery molecules also appear to be involved [76, 77]. Remarkably, the resistance-inducing effect of syntaxin1A truncations was recently replicated in PC12 cell culture experiments: the attenuating action of drugs such as isoflurane and propofol on neurotransmission could be completely blocked by co-expressing truncated syntaxin1A isoforms in the cultured cell lines [78, 79].

The likely absence of an endogenous sleep circuit in adult *C. elegans* (or in PC12 cells, for that matter) suggests a simple explanation for how such a high level of resistance to general anesthetics could be induced. If general anesthesia induction involves the activation of sleep-promoting circuits in most animals, from flies to humans, then the absence of such circuits in adult *C. elegans* might unmask any remaining relevant targets for the drugs. Interfering with this remaining target would thus render animals highly resistant (or hypersensitive) to general anesthetics, within the clinically relevant range (at higher doses, these drugs produce non-specific effects on various tissues, most likely irrelevant to the problem of general anesthesia [1]). As synaptic processes centered on syntaxin1A are extremely conserved across all species [73, 80, 81], this then suggests two primary targets in all animals that sleep, and only one primary target (at least for volatile anesthetics) in animals that do not sleep. The fact that different lesions in syntaxin1A can produce isoflurane resistance and hypersensitivity in *C. elegans* [74] strongly suggests that isoflurane targets syntaxin1A function at the synapse. This does not mean that the myriad of other protein targets are irrelevant; rather, these may broadly affect either of these two distinct processes: synaptic release dynamics in all animals, and local potentiation of sleep-promoting circuits only in animals that sleep.

Do general anesthetics target the synaptic release machinery?

At first glance, the evidence for the synaptic release machinery being a target of general anesthetics is not any more convincing than many other potential protein targets. A large number of cellular processes converge upon the inevitable communication bottleneck that is neurotransmitter release at the synapses of neurons, and it remains unclear which if any of these processes might be most relevant to general anesthesia. In vitro experiments have shown that general anesthetics can modulate neuronal excitability by their effects on a variety of membrane-bound proteins [82–85], especially members of the Cys-loop receptor superfamily, with greater specificity to $GABA_A$ receptors with the intravenous drugs [86]. How these diverse effects on protein functions translate to changes in synaptic release dynamics across an intact brain is a difficult question to address, because measures of synaptic release are most often confined to in vitro preparations examining neurotransmitter release from cultured cells [78, 79] or defined circuits [87, 88]. Thus, while the consensus is that general anesthetics do compromise synaptic release in these reduced systems, perhaps partially via their action on upstream ion channels [89, 90], these synaptic effects are generally small at clinically relevant drug concentrations, compared to the stronger, potentiating effects on post-synaptic chloride channels, for example [91].

The *C. elegans* and cell culture results described above, however, suggest a drug target that is much closer to syntaxin1A, the key membrane-bound component of the SNARE complex required for neurotransmission in all neurons, in all animals. Syntaxin1A is a highly promiscuous molecule with a large variety of identified binding partners [73, 92], thereby presenting an extensive list of potential interactions that could be disrupted by general anesthetics. Interaction with the exocytosis partner *unc-13*, for example, modulates anesthetic sensitivity in *C. elegans* [77]. Since *unc-13* alters the conformation of syntaxin1A [93], this suggests that an anesthetic target may be made less (or more) accessible depending on this protein interaction. While conserved N-terminal domains of syntaxin1A are important for transport, localization, and conformation of the molecule [94], the C-terminal domains that are required for promoting fusion of synaptic vesicles to the plasma membrane are also extremely conserved between all species of animals, with almost 100% identical amino acid residues in the key H3 alpha-helical domain in all animals from yeast to humans [81, 95, 96]. The syntaxin1A H3 domain interacts with an alpha-helical domain of the vesicle-bound synaptobrevin (also called VAMP) and two SNAP25 helices [97], to form a quadruple helix [98] (the "ternary" complex), one of the strongest protein complexes in biology [73, 99]. Formation of this protein complex leads to vesicle fusion with the plasma membrane (thus, neurotransmitter release) [100]. Mechanisms that *prevent* completion or "zippering" of the complex are key to fast neurotransmitter release [81, 101]. Docked vesicles are thus blocked from fusing with the plasma membrane by calcium-sensitive molecules such as complexin and synaptotagmin, primed for the calcium influx associated with neuronal activation [102]. The finely tuned choreography that is normal brain function depends largely on fast neurotransmitter release [103], which in turn depends on mechanisms *blocking* the SNARE complex from forming to completion. Could general anesthetics be compromising synaptic coordination in the brain, by interfering with the SNARE machinery? Even a modest decrease in synaptic release probability at every synapse might have drastic consequences when summed across all synapses the brain.

Nuclear magnetic resonance relaxation experiments [76], as well as recent cell culture [104] and photo-labeling experiments [105], suggest that isoflurane and propofol bind SNARE complexes, to potentially alter their three-dimensional structure and function. Recent crystal structure reconstructions of the SNARE-membrane interface suggest that the zippering domain of the complex associated with docked vesicles actually penetrates into the lipid bilayer of the plasma membrane [106], providing a possible

conduit between hydrophobic domains in the lipid bilayer and hydrophobic residues at the membrane interface of the SNARE zipper [107]. Since general anesthetics are hydrophobic molecules, and their potency is strongly correlated with their lipophilic qualities [82], this might suggest a mechanism whereby anesthetics could be interfering with vesicle fusion: via transient binding pockets at the C-terminus of the SNARE zipper domain (Fig. 2A), or even simply by interfering with membrane fusion. Either way, an increased concentration of general anesthetics around the membrane-bound SNARE machinery might decrease the probability of fast synaptic release following calcium influx.

The evolutionary requirement for almost total conservation of the structure of the ternary complex [80, 108], and in particular the syntaxin1A H3 domain [81], may be one reason why genetic analysis in different model systems has not readily uncovered this mechanism, and the SNARE complex in general, as a likely anesthetic target. However, *C. elegans* research has a distinct advantage in this regard over other model systems: since the nematodes are hermaphrodites, valuable yet potentially crippling mutations can still be propagated and studied. Thus, a variety of mutations in the syntaxin1A H3 domain have been found to produce a wide range of sensitivities to general anesthetics [74, 75]; in other animals, most of these mutations would probably be lethal. Interestingly, the neomorphic C-terminus truncation that produces hyper-resistance in worms has only minimal effects on native behavior [74]; animals still crawl around and feed, displaying sufficiently normal synaptic coordination for behavior and survival. This suggests that the mechanism whereby syntaxin1A modulates general anesthesia can be partially dissociated from the molecule's function for normal behavior, and that resistance may not merely reflect, for example, increased synaptic release. Indeed, a recent *Drosophila* study showed that increasing synaptic release across the fly brain did not produce isoflurane resistance [27], so a structural aspect of the synaptic release machinery may be more relevant to general anesthesia than synaptic release per se.

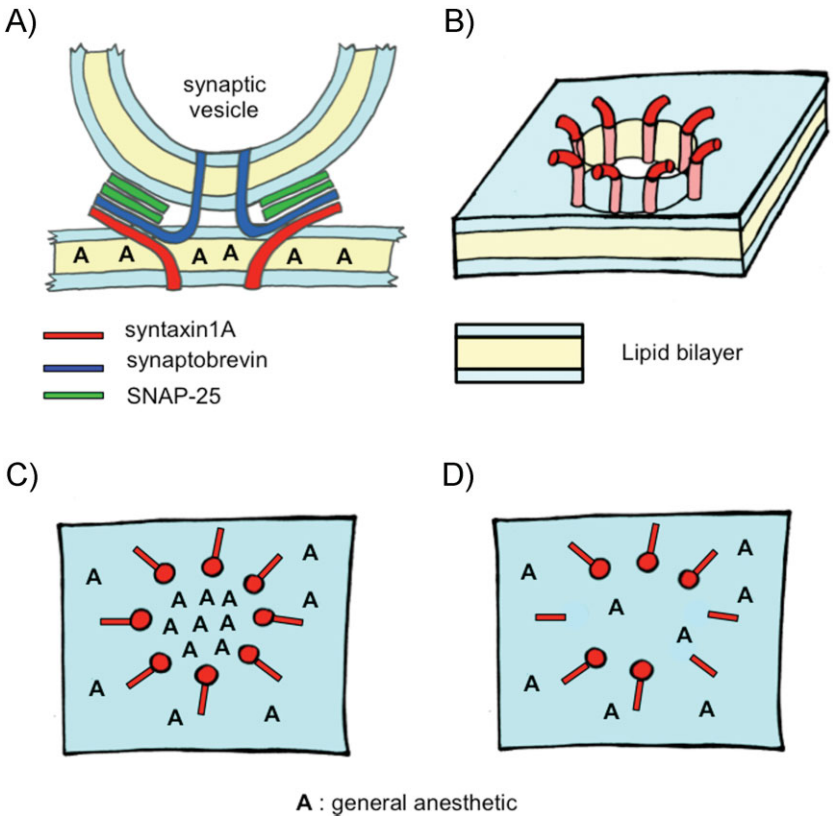

Figure 2. A synaptic hypothesis for general anesthesia. **A:** A cartoon (not drawn to scale) of the synaptic release machinery, with key SNARE components shown. Hydrophobic general anesthetics (A) are sequestered in the lipid bilayer and may interfere with synaptic vesicle fusion. **B:** SNAREs are likely to be arranged as a circular super-complex, or rosette, with different numbers of linked syntaxin1A elements (red) in different neurons. The super-complex is held together by a number of associated proteins, primarily SNAP-25 (not shown) which would bridge neighboring syntaxin1A elements. **C:** The super-complex architecture in the plasma membrane (a "barrel") might alter the local concentration of general anesthetic molecules, thereby interfering with membrane fusion mechanisms. Red circles represent the syntaxin1A trans-membrane (TM) domain, red bars represent the H3 domain emerging from the membrane. **D:** Co-expression of syntaxin1A isoforms missing their trans-membrane domain could still produce functional super-complexes. The altered microenvironment in these heterogeneous rosettes might not promote general anesthetic interference with membrane fusion events. Truncated syntaxin1A isoforms are represented by red bars, which include an intact H3 domain, still part of the ternary complex (not shown). The number of A's in the cartoon represents a probability distribution for general anesthetics around the syntaxin1A TM domains.

Since its first description a couple decades ago, the synaptic release machinery has been the subject of countless reviews, culminating in a Nobel Prize in 2013 for three key researchers in this field. Knowledge continues to grow about the workings of this amazing mechanism for fast cell-to-cell communication, upon which behavior and consciousness depend. The sequence of events from vesicle docking and priming to completion of the SNARE complex and eventual exocytosis following calcium influx has been repeatedly refined, and a growing number of molecular interactions involved in this process identified. For simplicity, most reviews illustrate the process in two dimensions, as a flat cartoon of sorts (e.g. Fig. 2A). Thus, one or two vesicle-bound synaptobrevins link up with one or two membrane-bound syntaxins, while other molecules such as SNAP-25 or *unc-13* hover in the vicinity. These are just simplified cartoons meant to educate, and it is important to try to imagine how these molecules might actually be arranged in a real 3-dimensional, cellular

environment. In reality, the interface between a sphere (the synaptic vesicle) and a surface (the cell membrane) is a circle, and it is likely that multiple SNARE complexes form along the perimeter of this circle (Fig. 2B). While it has been shown that only one complex is in principle sufficient for promoting vesicle fusion in reduced in vitro preparations [109], it is likely that multiple complexes are involved per exocytosis event [110], as formation of a SNARE complex might accelerate the formation of adjacent SNARE complexes [111]. These might self-organize into a ring of sorts, termed a SNARE super-complex [112]. The super-complex could vary in size in different synapses or neurotransmitter systems [113], with anywhere from a "tripod" of three to a ring of eight or more SNAREs.

The super-complex hypothesis provides a potential explanation for how general anesthetics might access the SNARE machinery. Multiple SNAREs organized in a circular arrangement would create a "barrel" through the membrane, with a circumference determined by the size of the super-complex (Fig. 2B and C). This unusually large, transient structure sitting in the plasma membrane could alter the concentration of general anesthetics coursing through the lipid bilayer [114], by for example changing the lipid microenvironment within (and around) the barrel. It is also interesting to consider the sizes of the molecules involved. Volatile general anesthetics range in size from 4 to 7 Å [42], and the grouped SNAREs at the membrane interface might be separated anywhere between 5 and 15 Å depending on the size of the complex [113], with slightly more space between the transmembrane (TM) elements within the lipid bilayer (e.g. 15–20 Å, Sergio Pantano, personal communication). Whether this transient structure in the plasma membrane alters the local concentration of small molecules should be testable with dynamic modeling techniques; an increased probability of anesthetic molecules within (or around) the super-complex might for example increase their interaction with the SNARE zippering mechanism (Fig. 2A and C), or increase the energy required for membrane fusion.

The super-complex architecture also suggests a potential mechanism for drug resistance: loss of some TM elements, as might occur when a truncated syntaxin1A is co-expressed, might sufficiently alter the lipid microenvironment to decrease the likelihood of anesthetics interfering with membrane fusion mechanisms (Fig. 2D). The super-complex, which is thought to be held together by a number of structural molecules, notably SNAP-25 [112], may still be anchored to the plasma membrane, and therefore functional, despite some syntaxin1A elements missing their TM domain [115], especially in larger complexes.

The super-complex model also provides a potential way to understand well-known idiosyncrasies, or side effects, among these diverse drugs. Differences in size of the super-complex at different synapses or in different neurotransmitter systems might determine the level of accessibility to the SNARE zippers for anesthetics of different size or structure. Along this line of reasoning, it is likely that the syntaxin1A complex might be irrelevant to the mechanism of action of some compounds, such as xenon, ketamine, or larger alkanes due to the considerable differences in size and chemical structures involved. Interestingly, co-expressing the truncated syntaxin1A isoform in PC12 cells does not produce resistance to etomidate [79], a larger compound compared to isoflurane and propofol.

Although the syntaxin1A hypothesis for general anesthesia remains pure speculation, it does not exclude a broader view that other anesthetic processes affecting synaptic release probability might also be relevant, such as the interactions among other SNARE-associated molecules [77]. However, several questions arise pertaining to the syntaxin-anchored super-complex hypothesis, and these are all testable, for example: Do tagged, truncated syntaxin1A isoforms get integrated into the SNARE? Do these isoforms co-localize to synaptic active zones? Do anesthetics concentrate preferentially to puncta-like domains in the active zones? If so, do these domains disappear when truncated syntaxin1A isoforms are co-expressed? Does the level of expression of these isoforms correlate with resistance to general anesthetics in an animal model, and in what neurons might this be most relevant? These questions are all addressable in genetic model systems such as *C. elegans* or *D. melanogaster*. In the light of common effects in *C. elegans* and cultured cell lines, a question that immediately arises is whether co-expression of truncated syntaxin1A isoforms in *Drosophila*, a sleeping animal, also produces resistance to general anesthetics.

Sleep and synaptic mechanisms together may explain general anesthesia

One way to reconcile both hypotheses for general anesthesia (potentiation of sleep pathways versus attenuation of neurotransmission) is that both are occurring, perhaps in succession. We propose that general anesthesia may be effectively described as a two-step process involving first the activation of endogenous sleep pathways, followed by a global attenuation of synaptic release across the brain (Fig. 3). Sleep induction via GABAergic circuits obviously involves neurotransmission (and thus syntaxin1A), so anesthetic effects on synaptic release would eventually supersede sleep-promoting effects. Accordingly, recovery from anesthesia would require re-establishing synaptic coordination across the brain first, before awakening through a reversal of the sleep/wake switch. This hypothesis might help to explain several curious observations about general anesthesia, in addition to the mystery of how such a high level of isoflurane resistance could be produced in nematodes. First, it is important to remember how general anesthesia is quite different from sleep: patients generally cannot be awoken while they are exposed to clinically relevant (i.e. surgical) doses of drugs. This already suggests that another process in addition to sleep must be involved. Also, only lower doses of drugs such as isoflurane or propofol produce slow-wave brain activity in mammals, as seen by electro-encephalograms (EEG) during slow wave sleep [1, 14]. Higher doses of these drugs, such as those required to perform surgery, are typically associated with entirely different kinds of EEG signatures, including burst suppression [116], and even higher concentrations

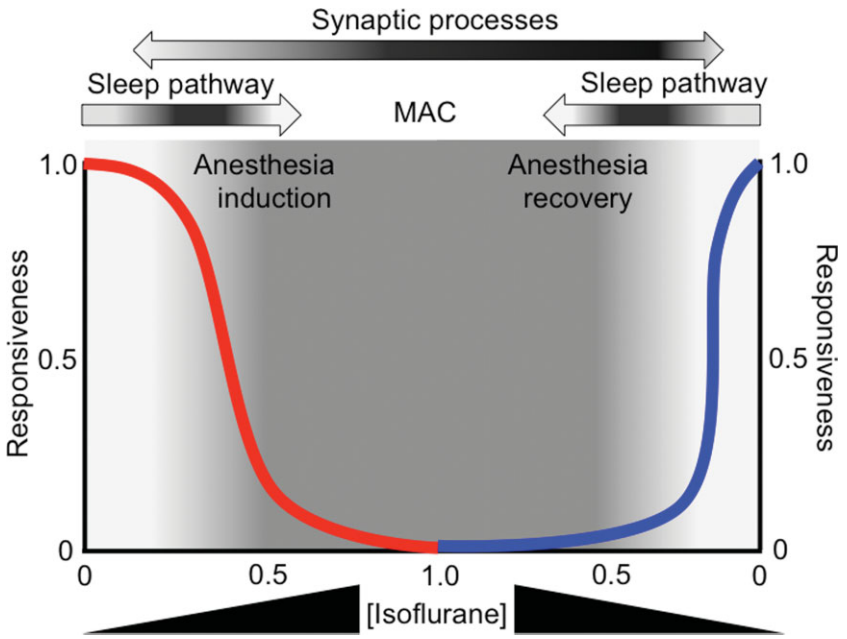

Figure 3. General anesthesia model. Induction of isoflurane anesthesia (red curve) might involve potentiation of an endogenous sleep pathway first, at lower drug concentrations ("Sleep pathway" arrow, darkness intensity represent maximal anesthetic effect, e.g. 0.3–0.5 vol% isoflurane), followed by non-specific attenuation of synaptic neurotransmission across the entire brain, at surgical (e.g. 0.7–1.0 vol% isoflurane) drug concentrations ("Synaptic processes" arrow, darkness intensity represents maximal anesthetic effect). Recovery from general anesthesia (blue curve) would first require recovery of synaptic coordination across the brain prior to awakening. These distinct effects on synaptic processes and sleep pathways could explain the inertia that is typically observed during recovery from general anesthesia. MAC, minimum alveolar concentration required for performing surgery.

produce isotonic activity that has been associated with loss of neuronal coherence [1, 14]. Another way that sleep and general anesthesia differ is in their dynamics: falling asleep and waking up seem to follow similar dynamics, whereas recovery from general anesthesia is characterized by a substantial inertia that has often puzzled anesthesiologists. If patients were simply being "put to sleep" with these drugs, then recovery and induction dynamics should mirror one another. This is not the case. Instead, recovery from general anesthesia appears to involve an entirely different, slower process than anesthesia induction [117]. The explanation for this inertia might be linked to synaptic coordination, if general anesthetics produce a loss of functional connectivity [118] across the brain (or across a neural network as in *C. elegans*). Recovering this capacity might involve slower pharmacodynamics, compared with anesthesia induction, which would be more like flipping a sleep switch.

The reason for which general anesthesia works so well may therefore be because it first activates an endogenous sleep pathway, at lower drug concentrations. However, the reason that it works at all for surgery may have less to do with sleep than with the lost capacity of a brain to support complex activity patterns [103, 118, 119], which should be highly sensitive to synaptic release dynamics and timing across the brain. Testing this hypothesis requires access to model systems where both processes (sleep and neurotransmission dynamics) can be acutely manipulated and studied separately, as exemplified recently in a *Drosophila* study [27]. Indeed, *D. melanogaster* might be the ideal genetic model in which to test this two-step hypothesis, because flies sleep in their adult stage, but probably not in their larval stage [33]. Thus, the effect of different classes of mutations might be more clearly assigned to either process, sleep or synaptic communication in general.

Our two-step hypothesis for general anesthesia unifies the more recent GABA-centric explanation with older hypotheses focused on the lipid bilayer of neurons. Over a century ago, two independent researchers (Hans Meyer and Ernest Overton) discovered that the potency of general anesthetics was highly correlated with their solubility in oil [120, 121], leading subsequent researchers to propose that these drugs affect neuronal function by primarily targeting the lipid bilayer of neurons [122–124]. This theory, underpinned by the "Meyer-Overton correlation", held for about 80 years, until it was refuted in the late 80s by elegant experiments indicating that the targets must involve proteins pockets because general anesthetics are stereospecific [125]. Since then, every molecular mechanism seems to have been proposed for general anesthesia, from nonspecific effects on various proteins to specific channel targets to gap junctions [126, 127] and microtubules [128, 129]. Accordingly, general anesthetics appear to impair the function of a wide range of molecules, as determined by in vitro assays [91], although it is now generally accepted that $GABA_A$ receptors represent a major target for most of these drugs [3, 42, 44]. Understanding general anesthesia, however, requires the investigation of clinically relevant behavioral endpoints in intact animals. It may therefore not come as a surprise that an understanding of general anesthesia first required a better understanding of sleep processes, which by definition requires working on intact animals that can sleep. Fortunately, evolution has provided us with approaches to uncover other target processes for these diverse drugs, in animals that may not sleep. One interesting prediction that emerges from our two-step hypothesis for general anesthesia (Fig. 3) is that specific sleep-promoting drugs such as isoflurane and propofol, which act on $GABA_A$ receptors at relatively low doses [7], will compromise neurotransmission at higher doses [78, 79, 104]. While sleep-related targets such as $GABA_A$ receptors may be stereospecific protein pockets, the SNARE-related target may be simply concentration-dependent. There is now good evidence that these two very different anesthetics,

isoflurane and propofol, both inhibit neurotransmitter release in cell culture assays, and that co-expression of syntaxin1A mutations in PC12 cells blocks this effect for both [78, 79]. Without any $GABA_A$ receptor present in the PC12 cell experiments, the conclusion that the synaptic machinery might be an important alternate target seems reasonable.

Conclusions

If synaptic release mechanisms are a relevant target for general anesthetics, why has this not been more evident in anesthesia research? Certainly, there has been an enormous focus on GABA, because the evidence for its role in general anesthesia is overwhelming: it is the primary inhibitory neurotransmitter system [130], it is part of the sleep pathway, and its receptors are targeted by general anesthetics. However, sometimes a large target can mask a smaller one that is equally important when considered in the context of the trillions of synapses that make up a human brain; drug effects that seem to have only a modest effect on synaptic release in vitro may become highly relevant when distributed across all of the synapses in an intact brain. Thus, the variety of general anesthetic targets that compromise synaptic function would comprise a diverse group of potential targets that all affect the same process, namely synaptic coordination or coherence of brain activity. The inevitable bottleneck for this process is the synaptic release machinery, and specifically, syntaxin1A-mediated fusion of synaptic vesicles with the plasma membrane. It is ironic but perhaps fitting that this alternate mechanism proposed for general anesthesia, involving the protein-mediated fusion of lipid membranes, brings to mind the original Meyer-Overton correlation, which pointed to the lipid bilayer of neurons as the obvious target of general anesthetics.

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References

1. **Brown EN**, **Purdon PL**, **Van Dort CJ.** 2011. General anesthesia and altered states of arousal: a systems neuroscience analysis. *Annu Rev Neurosci* **34**: 601–28.
2. **Snow SJ.** 2009. *Blessed Days ofAnaesthesia: How Anaesthetics Changed the World*. Oxford: Oxford Press.
3. **Franks NP.** 2008. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* **9**: 370–86.
4. **Nelson LE**, **Guo TZ**, **Lu J**, **Saper CB**, et al**.** 2002. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. *Nat Neurosci* **5**: 979–84.
5. **Nelson LE**, **Lu J**, **Guo T**, **Saper CB**, et al**.** 2003. The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. *Anesthesiology* **98**: 428–36.
6. **Tung A**, **Mendelson WB.** 2004. Anesthesia and sleep. *Sleep Med Rev* **8**: 213–25.
7. **Lu J**, **Nelson LE**, **Franks NP**, **Maze M**, et al**.** 2008. Role of endogenous sleep-wake and analgesic systems in anesthesia. *J Comp Neurol* **508**: 648–62.
8. **Luo T**, **Leung LS.** 2011. Involvement of tuberomamillary histaminergic neurons in isoflurane anesthesia. *Anesthesiology* **115**: 36–43.
9. **Moore JT**, **Chen J**, **Han B**, **Meng QC**, et al**.** 2012. Direct activation of sleep-promoting VLPO neurons by volatile anesthetics contributes to anesthetic hypnosis. *Curr Biol* **22**: 2008–16.
10. **Kelz MB**, **Sun Y**, **Chen J**, **Cheng Meng Q**, et al**.** 2008. An essential role for orexins in emergence from general anesthesia. *Proc Natl Acad Sci USA* **105**: 1309–14.
11. **Tung A**, **Szafran MJ**, **Bluhm B**, **Mendelson WB.** 2002. Sleep deprivation potentiates the onset and duration of loss of righting reflex induced by propofol and isoflurane. *Anesthesiology* **97**: 906–11.
12. **Pal D**, **Lipinski WJ**, **Walker AJ**, **Turner AM**, et al**.** 2011. State-specific effects of sevoflurane anesthesia on sleep homeostasis: selective recovery of slow wave but not rapid eye movement sleep. *Anesthesiology* **114**: 302–10.
13. **Brown EN**, **Lydic R**, **Schiff ND.** 2010. General anesthesia, sleep, and coma. *New Engl J Med* **363**: 2638–50.
14. **Murphy M**, **Bruno M-A**, **Riedner BA**, **Boveroux P**, et al**.** 2011. Propofol anesthesia and sleep: a high-density EEG study. *Sleep* **34**: 283A–91A.
15. **Talley EM**, **Bayliss DA.** 2002. Modulation of TASK-1 (Kcnk3) and TASK-3 (Kcnk9) potassium channels: volatile anesthetics and neurotransmitters share a molecular site of action. *J Biol Chem* **277**: 17733–42.
16. **Plested AJ**, **Wildman SS**, **Lieb WR**, **Franks NP.** 2004. Determinants of the sensitivity of AMPA receptors to xenon. *Anesthesiology* **100**: 347–58.
17. **Heurteaux C**, **Guy N**, **Laigle C**, **Blondeau N**, et al**.** 2004. TREK-1, a K+ channel involved in neuroprotection and general anesthesia. *EMBO J* **23**: 2684–95.
18. **Franks NP.** 2006. Molecular targets underlying general anaesthesia. *Br J Pharmacol* **147**: S72–S81.
19. **Brannigan G**, **LeBard DN**, **Henin J**, **Eckenhoff RG**, et al**.** 2010. Multiple binding sites for the general anesthetic isoflurane identified in the nicotinic acetylcholine receptor transmembrane domain. *Proc Natl Acad Sci USA* **107**: 14122–7.
20. **Humphrey JA**, **Sedensky MM**, **Morgan PG.** 2002. Understanding anesthesia: making genetic sense of the absence of senses. *Hum Mol Genet* **11**: 1241–9.
21. **Campbell SS**, **Tobler I.** 1984. Animal sleep: a review of sleep duration across phylogeny. *Neurosci Biobehav Rev* **8**: 269–300.
22. **Cirelli C**, **Tononi G.** 2008. Is sleep essential? *PLoS Biol* **6**: e216.
23. **Tung A**, **Lynch JP**, **Mendelson WB.** 2001. Prolonged sedation with propofol in the rat does not result in sleep deprivation. *Anesth Aanalg* **92**: 1232–6.
24. **Nelson AB**, **Faraguna U**, **Tononi G**, **Cirelli C.** 2010. Effects of anesthesia on the response to sleep deprivation. *Sleep* **33**: 1659–67.
25. **Hernandez M**, **Birnbach DJ**, **Van Zundert AA.** 2005. Anesthetic management of the illicit-substance-using patient. *Curr Opin Anaesthesiol* **18**: 315–24.
26. **Taylor NE**, **Chemali JJ**, **Brown EN**, **Solt K.** 2013. Activation of D1 dopamine receptors induces emergence from isoflurane general anesthesia. *Anesthesiology* **118**: 30–9.
27. **Kottler B**, **Bao H**, **Zalucki O**, **Imlach W**, et al**.** 2013. A sleep/wake circuit controls isoflurane sensitivity in Drosophila. *Curr Biol* **23**: 594–8.
28. **Singaram VK**, **Somerlot BH**, **Falk SA**, **Falk MJ**, et al**.** 2011. Optical reversal of halothane-induced immobility in *C. elegans*. *Curr Biol* **21**: 2070–6.
29. **Venter JC**, **di Porzio U**, **Robinson DA**, **Shreeve SM**, et al**.** 1988. Evolution of neurotransmitter receptor systems. *Prog Neurobiol* **30**: 105–69.
30. **Cirelli C.** 2009. The genetic and molecular regulation of sleep: from fruit flies to humans. *Nat Rev Neurosci* **10**: 549–60.
31. **Shaw PJ**, **Cirelli C**, **Greenspan RJ**, **Tononi G.** 2000. Correlates of sleep and waking in *Drosophila melanogaster*. *Science* **287**: 1834–7.
32. **Hendricks JC**, **Finn SM**, **Panckeri KA**, **Chavkin J**, et al**.** 2000. Rest in Drosophila is a sleep-like state. *Neuron* **25**: 129–38.
33. **Bushey D**, **Cirelli C.** 2011. From genetics to structure to function: exploring sleep in Drosophila. *Int Rev Neurobiol* **99**: 213–44.
34. **van Alphen B**, **Yap MH**, **Kirszenblat L**, **Kottler B**, et al**.** 2013. A dynamic deep sleep stage in Drosophila. *J Neurosci* **33**: 6917–27.
35. **Ueno T**, **Tomita J**, **Tanimoto H**, **Endo K**, et al**.** 2012. Identification of a dopamine pathway that regulates sleep and arousal in Drosophila. *Nat Neurosci* **15**: 1516–23.

36. **Liu Q**, **Liu S**, **Kodama L**, **Driscoll MR**, et al. 2012. Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. *Curr Biol* **22**: 2114–23.
37. **Donlea JM**, **Thimgan MS**, **Suzuki Y**, **Gottschalk L**, et al. 2011. Inducing sleep by remote control facilitates memory consolidation in Drosophila. *Science* **332**: 1571–6.
38. **Saper CB**, **Chou TC**, **Scammell TE.** 2001. The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci* **24**: 726–31.
39. **van Swinderen B.** 2005. The remote roots of consciousness in fruit-fly selective attention? *BioEssays* **27**: 321–30.
40. **Mihic SJ**, **Ye Q**, **Wick MJ**, **Koltchine VV**, et al. 1997. Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* **389**: 385–9.
41. **Belelli D**, **Lambert JJ**, **Peters JA**, **Wafford K**, et al. 1997. The interaction of the general anesthetic etomidate with the gamma-aminobutyric acid type A receptor is influenced by a single amino acid. *Proc Natl Acad Sci USA* **94**: 11031–6.
42. **Jenkins A**, **Greenblatt EP**, **Faulkner HJ**, **Bertaccini E**, et al. 2001. Evidence for a common binding cavity for three general anesthetics within the GABAA receptor. *J Neurosci* **21**: RC136.
43. **Bali M**, **Akabas MH.** 2004. Defining the propofol binding site location on the GABAA receptor. *Mol Pharmacol* **65**: 68–76.
44. **Olsen RW**, **Li GD.** 2011. GABA(A) receptors as molecular targets of general anesthetics: identification of binding sites provides clues to allosteric modulation. *Can J Anaesth* **58**: 206–15.
45. **Weber B**, **Schaper C**, **Bushey D**, **Rohlfs M**, et al. 2009. Increased volatile anesthetic requirement in short-sleeping Drosophila mutants. *Anesthesiology* **110**: 313–6.
46. **Seymour JE**, **Carrette TJ**, **Sutherland PA.** 2004. Do box jellyfish sleep at night? *Med J Aust* **181**: 707.
47. **Hobson JA.** 1967. Electrographic correlates of behavior in the frog with special reference to sleep. *Electroencephalogr Clin Neurophysiol* **22**: 113–21.
48. **Fisher SP**, **Foster RG**, **Peirson SN.** 2013. The circadian control of sleep. *Hand Exp Pharmacol* **2013**: 157–83.
49. **Eelderink-Chen Z**, **Mazzotta G**, **Sturre M**, **Bosman J**, et al. 2010. A circadian clock in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **107**: 2043–7.
50. **Raizen DM**, **Zimmerman JE**, **Maycock MH**, **Ta UD**, et al. 2008. Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature* **451**: 569–72.
51. **Dabbish NS**, **Raizen DM.** 2011. GABAergic synaptic plasticity during a developmentally regulated sleep-like state in *C. elegans*. *J Neurosci* **31**: 15932–43.
52. **Singh K**, **Chao MY**, **Somers GA**, **Komatsu H**, et al. 2011. *C. elegans* notch signaling regulates adult chemosensory response and larval molting quiescence. *Curr Biol* **21**: 825–34.
53. **Schwarz J**, **Bringmann H.** 2013. Reduced sleep-like quiescence in both hyperactive and hypoactive mutants of the Galphaq Gene egl-30 during lethargus in *Caenorhabditis elegans*. *PLoS One* **8**: e75853.
54. **Nelson MD**, **Raizen DM.** 2013. A sleep state during *C. elegans* development. *Curr Opin Neurobiol* **23**: 824–30.
55. **Hinard V**, **Mikhail C**, **Pradervand S**, **Curie T**, et al. 2012. Key electrophysiological, molecular, and metabolic signatures of sleep and wakefulness revealed in primary cortical cultures. *J Neurosci* **32**: 12506–17.
56. **Turek M**, **Lewandrowski I**, **Bringmann H.** 2013. An AP2 transcription dactor is required for a sleep-active neuron to induce sleep-like quiescence in *C. elegans*. *Curr Biol* **23**: 2215–23.
57. **Nitz DA**, **van Swinderen B**, **Tononi G**, **Greenspan RJ.** 2002. Electrophysiological correlates of rest and activity in *Drosophila melanogaster*. *Curr Biol* **12**: 1934–40.
58. **van der Linden AM**, **Beverly M**, **Kadener S**, **Rodriguez J**, et al. 2010. Genome-wide analysis of light- and temperature-entrained circadian transcripts in *Caenorhabditis elegans*. *PLoS Biol* **8**: e1000503.
59. **Kostal V**, **Zavodska R**, **Denlinger D.** 2009. Clock genes period and timeless are rhythmically expressed in brains of newly hatched, photosensitive larvae of the fly, *Sarcophaga crassipalpis*. *J Insect Physiol* **55**: 408–14.
60. **Keene AC**, **Mazzoni EO**, **Zhen J**, **Younger MA**, et al. 2011. Distinct visual pathways mediate Drosophila larval light avoidance and circadian clock entrainment. *J Neurosci* **31**: 6527–34.
61. **Larkin A**, **Karak S**, **Priya R**, **Das A**, et al. 2010. Central synaptic mechanisms underlie short-term olfactory habituation in Drosophila larvae. *Learn Mem* **17**: 645–53.
62. **Riebli N**, **Viktorin G**, **Reichert H.** 2013. Early-born neurons in type II neuroblast lineages establish a larval primordium and integrate into adult circuitry during central complex development in Drosophila. *Neural Dev* **8**: 6.
63. **Crowder CM**, **Shebester LD**, **Schedl T.** 1996. Behavioral effects of volatile anesthetics in *Caenorhabditis elegans*. *Anesthesiology* **85**: 901–12.
64. **Sandstrom DJ.** 2004. Isoflurane depresses glutamate release by reducing neuronal excitability at the drosophila NMJ. *J Physiol* **558**: 489–502.
65. **Aranake A**, **Mashour GA**, **Avidan MS.** 2013. Minimum alveolar concentration: ongoing relevance and clinical utility. *Anaesthesia* **68**: 512–22.
66. **van Swinderen B.** 2006. A succession of anesthetic endpoints in the Drosophila brain. *J Neurobiol* **66**: 1195–211.
67. **Nash HA.** 1999. General anesthesia. *Curr Biol* **9**: R83–5.
68. **Morgan PG**, **Sedensky MM**, **Meneely PM**, **Cascorbi HF.** 1988. The effect of two genes on anesthetic response in the nematode *Caenorhabditis elegans*. *Anesthesiology* **69**: 246–51.
69. **Allada R**, **Nash HA.** 1993. *Drosophila melanogaster* as a model for study of general anesthesia: the quantitative response to clinical anesthetics and alkanes. *Anesth Analg* **77**: 19–26.
70. **Campbell DB**, **Nash HA.** 1994. Use of Drosophila mutants to distinguish among volatile general anesthetics. *Proc Natl Acad Sci USA* **91**: 2135–9.
71. **Jurd R**, **Arras M**, **Lambert S**, **Drexler B**, et al. 2003. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J* **17**: 250–2.
72. **Bamber BA**, **Twyman RE**, **Jorgensen EM.** 2003. Pharmacological characterization of the homomeric and heteromeric UNC-49 GABA receptors in *C. elegans*. *Br J Pharmacol* **138**: 883–93.
73. **Sudhof TC.** 2004. The synaptic vesicle cycle. *Annu Rev Neurosci* **27**: 509–47.
74. **van Swinderen B**, **Saifee O**, **Shebester LD**, **Roberson R**, et al. 1999. A neomorphic syntaxin mutation blocks volatile-anesthetic action in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **96**: 2479–84.
75. **Hawasli AH**, **Saifee O**, **Liu C**, **Nonet ML**, et al. 2004. Resistance to volatile anesthetics by mutations enhancing excitatory neurotransmitter release in *Caenorhabditis elegans*. *Genetics* **168**: 831–43.
76. **Nagele P**, **Mendel JB**, **Placzek WJ**, **Scott BA**, et al. 2005. Volatile anesthetics bind rat synaptic snare proteins. *Anesthesiology* **103**: 768–78.
77. **Metz LB**, **Dasgupta N**, **Liu C**, **Hunt SJ**, et al. 2007. An evolutionarily conserved presynaptic protein is required for isoflurane sensitivity in *Caenorhabditis elegans*. *Anesthesiology* **107**: 971–82.
78. **Herring BE**, **Xie Z**, **Marks J**, **Fox AP.** 2009. Isoflurane inhibits the neurotransmitter release machinery. *J Neurophysiol* **102**: 1265–73.
79. **Herring BE**, **McMillan K**, **Pike CM**, **Marks J**, et al. 2011. Etomidate and propofol inhibit the neurotransmitter release machinery at different sites. *J Physiol* **589**: 1103–15.
80. **Fasshauer D**, **Sutton RB**, **Brunger AT**, **Jahn R.** 1998. Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. *Proc Natl Acad Sci USA* **95**: 15781–6.
81. **Lagow RD**, **Bao H**, **Cohen EN**, **Daniels RW**, et al. 2007. Modification of a hydrophobic layer by a point mutation in syntaxin 1A regulates the rate of synaptic vesicle fusion. *PLoS Biol* **5**: e72.
82. **Franks NP**, **Lieb WR.** 1994. Molecular and cellular mechanisms of general anaesthesia. *Nature* **367**: 607–14.
83. **Krasowski MD**, **Harrison NL.** 1999. General anaesthetic actions on ligand-gated ion channels. *Cell Mol Life Sci* **55**: 1278–303.
84. **Campagna JA**, **Miller KW**, **Forman SA.** 2003. Mechanisms of actions of inhaled anesthetics. *New Engl J Med* **348**: 2110–24.
85. **Rudolph U**, **Antkowiak B.** 2004. Molecular and neuronal substrates for general anaesthetics. *Nat Rev Neurosci* **5**: 709–20.
86. **Grasshoff C**, **Rudolph U**, **Antkowiak B.** 2005. Molecular and systemic mechanisms of general anaesthesia: the 'multi-site and multiple mechanisms' concept. *Curr Opin Anaesthesiol* **18**: 386–91.
87. **MacIver MB**, **Mikulec AA**, **Amagasu SM**, **Monroe FA.** 1996. Volatile anesthetics depress glutamate transmission via presynaptic actions. *Anesthesiology* **85**: 823–34.
88. **Sandstrom DJ.** 2004. Isoflurane depresses glutamate release by reducing neuronal excitability at the Drosophila neuromuscular junction. *J Physiol* **558**: 489–502.
89. **Westphalen RI**, **Kwak NB**, **Daniels K**, **Hemmings HC Jr.** 2011. Regional differences in the effects of isoflurane on neurotransmitter release. *Neuropharmacology* **61**: 699–706.
90. **Westphalen RI**, **Desai KM**, **Hemmings HC Jr.** 2013. Presynaptic inhibition of the release of multiple major central nervous

system neurotransmitter types by the inhaled anaesthetic isoflurane. *Br J Anaesth* **110**: 592–9.
91. **Franks NP**, **Lieb WR.** 1998. Which molecular targets are most relevant to general anaesthesia? *Toxicol Lett* **100–101**: 1–8.
92. **Rizo J**, **Sudhof TC.** 2012. The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices – guilty as charged? *Annu Rev Cell Dev Biol* **28**: 279–308.
93. **Richmond JE**, **Weimer RM**, **Jorgensen EM.** 2001. An open form of syntaxin bypasses the requirement for UNC-13 in vesicle priming. *Nature* **412**: 338–41.
94. **Fernandez I**, **Ubach J**, **Dulubova I**, **Zhang X**, et al**.** 1998. Three-dimensional structure of an evolutionarily conserved N-terminal domain of syntaxin 1A. *Cell* **94**: 841–9.
95. **Ferro-Novick S**, **Jahn R.** 1994. Vesicle fusion from yeast to man. *Nature* **370**: 191–3.
96. **Terrian DM**, **White MK.** 1997. Phylogenetic analysis of membrane trafficking proteins: a family reunion and secondary structure predictions. *Eur J Cell Biol* **73**: 198–204.
97. **Wu MN**, **Fergestad T**, **Lloyd TE**, **He Y**, et al**.** 1999. Syntaxin 1A interacts with multiple exocytic proteins to regulate neurotransmitter release in vivo. *Neuron* **23**: 593–605.
98. **Poirier MA**, **Xiao W**, **Macosko JC**, **Chan C**, et al**.** 1998. The synaptic SNARE complex is a parallel four-stranded helical bundle. *Nat Struct Biol* **5**: 765–9.
99. **Poirier MA**, **Hao JC**, **Malkus PN**, **Chan C**, et al**.** 1998. Protease resistance of syntaxin. SNAP-25.VAMP complexes. Implications for assembly and structure. *J Biol Chem* **273**: 11370–7.
100. **Wickner W**, **Schekman R.** 2008. Membrane fusion. *Nat Struct Mol Biol* **15**: 658–64.
101. **Jahn R**, **Scheller RH.** 2006. SNAREs – engines for membrane fusion. *Nat Rev Mol Cell Biol* **7**: 631–43.
102. **Sudhof TC.** 2013. A molecular machine for neurotransmitter release: synaptotagmin and beyond. *Nat Med* **19**: 1227–31.
103. **Alkire MT**, **Hudetz AG**, **Tononi G.** 2008. Consciousness and anesthesia. *Science* **322**: 876–80.
104. **Xie Z**, **McMillan K**, **Pike CM**, **Cahill AL**, et al**.** 2013. Interaction of anesthetics with neurotransmitter release machinery proteins. *J Neurophysiol* **109**: 758–67.
105. **Weiser BP**, **Kelz MB**, **Eckenhoff RG.** 2013. In vivo activation of azipropofol prolongs anesthesia and reveals synaptic targets. *J Biol Chem* **288**: 1279–85.
106. **Stein A**, **Weber G**, **Wahl MC**, **Jahn R.** 2009. Helical extension of the neuronal SNARE complex into the membrane. *Nature* **460**: 525–8.
107. **Khuong TM**, **Habets RL**, **Kuenen S**, **Witkowska A**, et al**.** 2013. Synaptic PI(3,4,5)P3 is required for Syntaxin1A clustering and neurotransmitter release. *Neuron* **77**: 1097–108.
108. **Antonin W**, **Fasshauer D**, **Becker S**, **Jahn R**, et al**.** 2002. Crystal structure of the endosomal SNARE complex reveals common structural principles of all SNAREs. *Nat Struct Biol* **9**: 107–11.
109. **van den Bogaart G**, **Holt MG**, **Bunt G**, **Riedel D**, et al**.** 2010. One SNARE complex is sufficient for membrane fusion. *Nat Struct Mol Biol* **17**: 358–64.
110. **Shi L**, **Shen QT**, **Kiel A**, **Wang J**, et al**.** 2012. SNARE proteins: one to fuse and three to keep the nascent fusion pore open. *Science* **335**: 1355–9.
111. **Bae W**, **Choi MG**, **Hyeon C**, **Shin YK**, et al**.** 2013. Real-time observation of multiple-protein complex formation with single-molecule FRET. *J Am Chem Soc* **135**: 10254–7.
112. **Montecucco C**, **Schiavo G**, **Pantano S.** 2005. SNARE complexes and neuroexocytosis: how many, how close? *Trends Biochem Sci* **30**: 367–72.
113. **Megighian A**, **Zordan M**, **Pantano S**, **Scorzeto M**, et al**.** 2013. Evidence for a radial SNARE super-complex mediating neurotransmitter release at the Drosophila neuromuscular junction. *J Cell Sci* **126**: 3134–40.
114. **Lenaz G**, **Mazzanti L**, **Curatola G**, **Bertoli E**, et al**.** 1978. A conformational model for the action of general anesthetics at the membrane level. II. Experimental observations on the effects of anesthetics on lipid fluidity and lipid protein interactions. *Ital J Biochem* **27**: 401–30.
115. **Zhou P**, **Bacaj T**, **Xiaofei Y**, **Zhiping PP**, et al**.** 2013. Lipid-anchored SNAREs lacking transmembrane regions fully support membrane fusion during neurotransmitter release. *Neuron* **80**: 470–83.
116. **Lipping T**, **Stalnacke J**, **Olejarczyk E**, **Marciniak R**, et al**.** 2013. Classification of EEG bursts in deep sevoflurane, desflurane and isoflurane anesthesia using AR-modeling and entropy measures. *Conf Proc IEEE Eng Med Biol Soc* **2013**: 5083–6.
117. **Friedman EB**, **Sun Y**, **Moore JT**, **Hung HT**, et al**.** 2010. A conserved behavioral state barrier impedes transitions between anesthetic-induced unconsciousness and wakefulness: evidence for neural inertia. *PLoS One* **5**: e11903.
118. **Ferrarelli F**, **Massimini M**, **Sarasso S**, **Casali A**, et al**.** 2010. Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. *Proc Natl Acad Sci USA* **107**: 2681–6.
119. **Casali AG**, **Gosseries O**, **Rosanova M**, **Boly M**, et al**.** 2013. A theoretically based index of consciousness independent of sensory processing and behavior. *Sci Transl Med* **5**: 198ra05.
120. **Meyer H.** 1901. Zur Theorie der Alkoholnarkose. Der Einfluss wechselnder Temperature auf Wirkungsstarke und Theilungscoefficient der Narcotica. *Arch Exp Pathol Pharmacol* **46**: 338–346.
121. **Overton C.** 1901. *Studien uber die Narkose zugleich ein Beitrag zur allgemeinen Pharmakologie Jena*. Switzerland: Gustave Fischer.
122. **Seeman P.** 1972. The membrane actions of anesthetics and tranquilizers. *Pharmacol Revi* **24**: 583–655.
123. **Miller KW**, **Paton WD**, **Smith RA**, **Smith EB.** 1973. The pressure reversal of general anesthesia and the critical volume hypothesis. *Mol Pharmacol* **9**: 131–43.
124. **Trudell JR.** 1977. A unitary theory of anesthesia based on lateral phase separations in nerve membranes. *Anesthesiology* **46**: 5–10.
125. **Franks NP**, **Lieb WR.** 1991. Stereospecific effects of inhalational general anesthetic optical isomers on nerve ion channels. *Science* **254**: 427–30.
126. **Wentlandt K**, **Samoilova M**, **Carlen PL**, **El Beheiry H.** 2006. General anesthetics inhibit gap junction communication in cultured organotypic hippocampal slices. *Anesth Analg* **102**: 1692–8.
127. **Jacobson GM**, **Voss LJ**, **Melin SM**, **Cursons RT**, et al**.** 2011. The role of connexin36 gap junctions in modulating the hypnotic effects of isoflurane and propofol in mice. *Anaesthesia* **66**: 361–7.
128. **Hameroff S**, **Nip A**, **Porter M**, **Tuszynski J.** 2002. Conduction pathways in microtubules, biological quantum computation, and consciousness. *Bio Systems* **64**: 149–68.
129. **Emerson DJ**, **Weiser BP**, **Psonis J**, **Liao Z**, et al**.** 2013. Direct modulation of microtubule stability contributes to anthracene general anesthesia. *J Am Chem Soc* **135**: 5389–98.
130. **Farrant M**, **Nusser Z.** 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* **6**: 215–29.