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Towards a comprehensive understanding of anesthetic mechanisms of action: A decade of discovery

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Abstract

Significant progress has been made in the 21st century towards a comprehensive understanding of the mechanisms of action of general anesthetics, coincident with progress in structural biology and molecular, cellular, and systems neuroscience. This review summarizes important new findings that include target identification through structural determination of anesthetic binding sites, details of receptors and ion channels involved in neurotransmission, and the critical roles of neuronal networks in anesthetic effects on memory and consciousness. These recent developments provide a comprehensive basis for conceptualizing pharmacologic control of amnesia, unconsciousness, and immobility.

Keywords

ion channel; memory; hippocampus; cortex; hypothalamus; consciousness

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Progress on defining the molecular targets and sites of general anesthetics

Defining the actions of general anesthetics at the molecular, cellular and neuronal network levels is widely regarded as the *sine qua non* for understanding their complex set of desirable clinical effects: unconsciousness, amnesia, analgesia, and immobility. Since our last review of this topic in 2005, which focused on necessary criteria for identifying molecular targets [1], scientific advances in biophysics, physiology, and neuroscience have facilitated our understanding of anesthesia at the systems level. Below we highlight progress in identifying anesthetic binding sites in critical targets using structural approaches, novel roles for synaptic and extrasynaptic γ-aminobutyric acid type A receptors (**$GABA_A$-Rs**) (see Glossary) in memory impairment (both intended and pathologic), and network level mechanisms regulating loss and recovery of consciousness. The goal is to provide an overview (and sampling) of impactful advances in the field that range from anesthetic actions on single proteins to synchronized brain rhythms.

There are broadly four approaches to identify important anesthetic molecular targets (reviewed briefly in Box 1). By combining these approaches, several general principles governing anesthetic:target interactions have emerged:

Promiscuity:

A complex **photolabeling** (see Glossary) milieu has revealed that 10–15% of detectable proteins can be considered anesthetic binding targets. These percentages translate to over 300 proteins, and the true number might be even 10-fold greater when considering undetected proteins. Evidence from photolabeling protection experiments indicates that most of these targets are pharmacologically “specific” [2]. However, because many *in vitro* and *in vivo* “sensitivity” targets (Box 1) are nonabundant, there has been little overlap in the yields of these approaches. **Click-chemistry** enrichment strategies [2] have confirmed anesthetic binding to the low abundance protein targets [3], including $GABA_A$-R subunits and voltage-gated channels. Even if only a fraction of the targets revealed by photolabeling contribute to anesthetic action, it is clear that general anesthetics are promiscuous ligands, and many anesthetic:protein interactions may not have relevant functional consequences.

Site character and selectivity:

The chemical character of typical anesthetics implies that their macromolecular binding sites are small and hydrophobic [4, 5]. However, anesthetics first must achieve a sufficient concentration in water in order to occupy these sites. Thus, in addition to hydrophobicity, anesthetics must possess a degree of polarity or polarizability. The protein features that best fit these general criteria (small size, hydrophobicity, modest polarity) are internal cavities or structural pockets (wherein an internal cavity has no access to a solvent larger than a water molecule, whereas a pocket does). The degree of selectivity for such general sites has been surprising. Ligand-gated ion channels, such as the $GABA_A$-R or *Gloeobacter* Ligand-gated Ion Channel (**GLIC**) for example, show considerable selectivity for sites – [3, 6–9] – despite producing similar changes in activity (Figure 1). Even volatile anesthetics may exhibit a degree of non-overlapping site occupancy as suggested by recent photolabeling studies [3]. The basis for this surprising selectivity must derive from weak van der Waals forces, since

volatile anesthetics are uncharged and have no H-bond capability. In the case of injectable anesthetics, however, the presence of an H-bond donor/acceptor may be important. For example, replacement of the propofol hydroxyl with an H-bond null fluorine completely removes both $GABA_A$-R binding, potentiation, and anesthetic activity [2, 10].

Functional alignment.—Functional alignment is the relationship between the valence of the functional effect produced by binding to different sites. Functional alignment occurs if ligand occupancy at each site produces the same functional valence on a channel (resulting in either additivity or synergy), whereas functional misalignment occurs when ligand binding to the different sites results in opposing (*i.e.*, antagonistic) effects. There is no *a priori* reason that all targets and sites contribute equally to any given drug action or behavioral effect. For example, the excitement phase that precedes “anesthesia” may not necessarily be transduced by the same target(s) as the neurodepressive phase that includes hypnosis and immobility. Even within a single drug target, functional misalignment may occur. For example, the pore site for propofol in GLIC transduces inhibition of ion channel function, whereas both transmembrane (TM) intersubunit and intrasubunit sites appear to transduce enhancement [11]. A further example of this misalignment might be the recent demonstration of sevoflurane binding to a novel binding site in the $GABA_A$-R γ-subunit TM bundle [3] as well as between α and β subunits, a region occupied by many potentiating anesthetics such as etomidate and propofol [12]. Variably occupied sites might underlie the commonly observed biphasic activity of anesthetics [11].

Recent advances in structural studies

The identification of prokaryotic pentameric ligand gated ion channel (pLGIC) homologs amenable to biochemical purification and subsequent crystallographic structural studies has provided a framework upon which decades of functional and photolabeling data can be mapped. Structures of prokaryotic pLGIC proteins in the presence of volatile anesthetics, propofol, anesthetic neurosteroids, and alcohols have been instructive in defining shared modulatory sites [13, 14]. While prokaryotic pLGICs do share many structural features with their mammalian counterparts, they are homopentamers that lack the complex and varied subunit distributions present in most human pLGICs. Structural studies of chimeric channels featuring prokaryotic extracellular domains fused to eukaryotic transmembrane regions have begun to shift our structural understanding of anesthetic binding into the eukaryotic realm [15–17]. The recent elucidation of structures of eukaryotic $GABA_A$ [18, 19], glycine [20], and $5HT_3$ [21] receptors represent major steps forward and enable generalization of findings in prokaryotic models to the clinically relevant human targets. Additionally, recent advances in cryo-electron microscopy have provided the first structural data for the most relevant human pLGIC target, the $GABA_A$-R [18, 19, 22] - setting the stage for future structures of ligand-gated, HCN, and K_2P channels in the presence of anesthetics.

Anesthetic modulatory sites defined by structural studies of pLGICs fall into three basic categories; intrasubunit, intersubunit, and pore-lining binding sites (Figure 1A). Through observations from functional, biochemical, and structural studies, it has become clear that anesthetic agents and other ligand-gated ion channel modulators can bind or act at multiple modulatory sites, resulting in mixtures of potentiating and inhibiting effects that combine to

produce the output of a given anesthetic modulator acting on a specific channel target [11, 23]. The intrasubunit cavity, confined within the TM domains of a single subunit, was the first structurally demonstrated site for anesthetic binding in the prokaryotic GLIC. This site was shown to accommodate binding of either propofol or desflurane (Figure 1C, 1D), both of which inhibited GLIC channel function [13]. Photolabeling studies in the nicotinic acetylcholine (nACh) receptor, a mammalian pLGIC that is similarly inhibited by propofol, identified a propofol binding site at the TM2 18' position within the intrasubunit cavity [24], supporting the generalizability of this binding site and its function across the pLGIC family.

All crystal structures of GLIC in the conductive conformation contain a co-purified structural lipid with its tail directed into the intrasubunit cavity (Figure 1A), thought to stabilize the conductive state on the channel [25, 26]. In the propofol-bound GLIC structure, propofol displaces this lipid to occupy the *intra*subunit cavity [13]. This suggests that anesthetic binding in this cavity could mimic or counter the actions of endogenous lipid modulators on pLGIC channels [27]. This assertion is supported by the finding that the intrasubunit cavity shrinks and the lipid is absent in a non-conductive GLIC structure [25], such that lipid (or anesthetic) occupancy in this cavity could play a mechanistic role in stabilizing the conductive state of the channel [26].

An *inter*subunit anesthetic site buried within the core of the pLGIC structure lies directly behind the pore lining TM2 helices of neighboring subunits (Figure 1A). In $GABA_A$-Rs, this intersubunit cavity features two residues, β3 N265 and β2 M286, that are important for modulating the *in vitro* and *in vivo* effects of anesthetics and ethanol [28, 29]. Co-crystallization studies of an ethanol-sensitized mutant of GLIC (TM2 F14'A) with either bromoform or ethanol demonstrate occupancy of the *inter*subunit site (Figure 1E, 1F), in close proximity to a GLIC residue that aligns with $GABA_A$ β3 N265 [14]. Interestingly, the TM2 F14'A mutation widens a hydrophilic passage between the intrasubunit and intersubunit cavities, suggesting a functional linkage between these two anesthetic binding regions. Recent structural evidence demonstrates binding of propofol to GLIC channels bearing mutations within the intersubunit site [11], and photolabeling studies show modification by barbiturates and isoflurane in this region [3, 7]. Additionally, a distinct intersubunit region at the cytoplasmic end of the channel has been implicated by structural and photolabeling studies as a binding site for anesthetic neurosteroids [16, 17], though neurosteroids have also been found to bind to an *intra*subunit neurosteroid bindings site at the extracellular end of GLIC [30, 31].

Inhibition of pLGIC activity *via* binding within the channel pore has been demonstrated for volatile anesthetics, propofol, barbiturates, neurosteroids, and xenon [16, 32–34]. These anesthetics have been demonstrated to bind at the TM2 6' position directly below the ion channel gate or at the TM2 13' position directly above the gate. It is posited that binding events within the channel pore are most physiologically relevant for channels like nACh that are inhibited by anesthetic modulators, though pore binding may play a role in modulating other pLGICs as well.

While the above described anesthetic binding sites all lie within TM domains of pLGIC's, endogenous ligands bind within extracellular (EC) domains (Figure 1B). Benzodiazepines

also bind to the EC domain of inhibitory pLGICs, albeit at a site that is distinct from the endogenous ligand binding site. Some anesthetics also bind at sites in the pLGIC EC domains. Co-crystallization studies of GLIC in the presence of ketamine identified a ketamine binding site in the EC domain that overlaps with the endogenous ligand binding site [35]. Functional and photolabeling studies suggest that alcohols, chloroform, and volatile anesthetics all target pLGIC EC domains for at least some of their modulatory effects [3, 23, 26].

Considerable recent progress has been made towards defining molecular targets of anesthetics, and rather than closing in on a unitary target, multiple approaches consistently reveal multiple targets, each with multiple intrinsic binding sites. Further understanding how this molecular complexity leads to the spectrum of events at the cellular, network, and behavioral levels remains a major unmet goal in anesthetic pharmacology research.

Extrasynaptic $GABA_A$ receptors and amnesia

While the molecular details of drug/receptor interactions play a large role in determining the behavioral responses induced by most inhaled and intravenous anesthetic drugs, receptor subtype composition and spatial distribution within the neuron are also important factors governing the effects of anesthetic drugs [1, 36, 37]. $GABA_A$-Rs form pentameric channels containing a variety of subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3), and are broadly categorized as either synaptic or extrasynaptic receptors [38, 39]. These two groups of $GABA_A$-Rs differ in their subunit composition, expression patterns, affinity for agonists, and pharmacological properties [40, 41]. While synaptic $GABA_A$-Rs generate fast, transient (phasic) inhibitory currents in response to the high concentrations of GABA released from presynaptic terminals, extrasynaptic $GABA_A$-Rs are high-affinity sensors that respond to low, ambient concentrations of GABA and produce a persistent (tonic) inhibitory current [38, 39, 42]. Extrasynaptic $GABA_A$-Rs frequently contain the α5 subunit, in combination with β3 and γ2 subunits, or the δ subunit combined with α1, α4, or α6 and β2 or β3 subunits [38, 39].

Expression levels of extrasynaptic $GABA_A$-Rs are thought to exceed those of synaptic $GABA_A$-Rs and activation of extrasynaptic $GABA_A$-R's by anesthetic drugs plays a role in a number of aspects of the anesthetized state [37, 39]. Amnesia is one of the most sensitive behavioral endpoints of anesthetic action, with memory blockade occurring at plasma concentrations that are considerably lower than those required for unconsciousness and immobility [36, 37]. The heightened affinity of extrasynaptic $GABA_A$-R's for GABA makes them logical targets for the low, amnestic concentrations of most anesthetic drugs. Extrasynaptic $GABA_A$-Rs containing the α5 subunit have been demonstrated to contribute to the acute, profound and desired amnestic properties of anesthetic drugs. Increased activity of α5 subunit-containing extrasynaptic $GABA_A$-Rs (α5$GABA_A$-Rs) reduces neuronal excitability, impairs synaptic plasticity and contributes to anesthetic-induced memory blockade caused by volatile anesthetics and intravenous anesthetics [36, 43]. Additionally, genetically-modified mice lacking α5$GABA_A$-Rs are resistant to anesthetic-induced memory blockade but not to loss of consciousness or immobility [36].

Encoding of memory depends on stimulus-enhanced coupling of neuronal assemblies located in different brain regions, most importantly the hippocampus and prefrontal cortex (PFC) [44, 45]. Computational studies suggest that increased extrasynaptic $GABA_A$-R activity disrupts the synchrony of memory-encoding rhythmic oscillations that underlie memory processes [46]. Increased tonic inhibitory current, in particular, has been shown to disrupt synchronized oscillatory rhythms [47]. Thus, up-regulation of extrasynaptic $GABA_A$-R function is thought to disrupt memory by acting locally on neuronal networks, disrupting memory-enhancing synchronized brain rhythms that connect different brain regions.

Persistent, undesired memory deficits result from altered trafficking of extrasynaptic $GABA_A$ receptors

In addition to their short-lived amnestic properties, general anesthetics can also trigger longer-term memory deficits that persist after the anesthetic has been eliminated from the body. Rodents treated with commonly used anesthetic drugs, including etomidate and isoflurane, exhibit anterograde deficits for spatial, fear and object recognition memory that last for hours to days [43, 48]. For example, a low, sedating dose of the injectable anesthetic etomidate causes subtle deficits in novel object recognition memory in mice for 72 hours, whereas a higher, immobilizing dose impairs memory for at least 1-week post-treatment [43].

In contrast to amnesia, which is linked to increased extrasynaptic $GABA_A$ receptor activity, long-term cognitive changes associated with anesthetic exposure have been tied to changes in extrasynaptic $GABA_A$-R surface expression; brief exposure to either etomidate or isoflurane triggers a sustained increase in the tonic inhibitory conductance in hippocampal neurons and impairs long-term potentiation (**LTP**) in brain slices [43]. These changes are attributed to altered receptor trafficking and expression of extrasynaptic $GABA_A$-Rs on the surface of neurons. Glia, particularly astrocytes, play a central role in increasing the expression of extrasynaptic $GABA_A$-Rs [43], and cross-talk between astrocytes and neurons has emerged as an attractive target for mitigating this post-anesthetic cognitive function. Dexmedetomidine, a sedative α_2-adrenergic receptor agonist, has been shown to reduce the incidence of delirium in patients [49]. Intriguingly, dexmedetomidine mitigates the anesthetic-induced increase in tonic current and prevents persistent behavioral deficit in a mouse model, through stimulation of the release of brain-derived neurotrophic factor [50]. Whether similar molecular mechanisms occur in humans remains an exciting area of future inquiry.

Anesthetic modulation of endogenous sleep circuits

Activation of endogenous sleep circuits

Despite considerable progress in identifying the molecular targets of general anesthetics, comparably less progress has been made in identifying the neuronal circuits involved in the actions of general anesthetics. Two landmark studies [51, 52] that initiated the ongoing search for the neuronal targets of anesthetic action indicated that anesthetic-induced loss of consciousness (also termed hypnosis) could arise from anesthetic actions on the neuronal

circuits regulating arousal. The GABA-facilitating anesthetics propofol and pentobarbital alter activity of neurons in ventrolateral preoptic (VLPO) and tuberomammillary (TMN) regions of the hypothalamus, brain regions that are enriched with sleep- and wake-active neurons, respectively. As positive allosteric modulators (PAMs) of $GABA_A$-Rs, it is plausible that anesthetics might inhibit firing of wake-active neurons, and it is perhaps not surprising that they might also cause disinhibition by activating sleep promoting neurons. However, despite isoflurane's potentiation of inhibitory GABAergic signaling affecting sleep-active neurons, the discovery that isoflurane nevertheless directly depolarizes sleep-active neurons in VLPO was unexpected; the net excitatory actions of isoflurane occurs selectively on sleep-active neurons through reduction of a background potassium conductance [53].

Propofol and dexmedetomidine, however, utilize two distinct mechanisms to indirectly excite these neurons [54, 55]. Propofol excites active VLPO neurons by increasing their excitatory glutamatergic afferent inputs. Like propofol, dexmedetomidine's depolarization of sleep-promoting VLPO neurons is also indirect and requires intact circuits, but additionally activates a wide variety of neurons across the ventrolateral, lateral, and medial preoptic hypothalamus [56]. With the notable exception of ketamine, direct or indirect recruitment of putative sleep-active neurons in VLPO appears to be a common mechanism for all anesthetics tested including hypnotic doses of propofol, barbiturates, chloral hydrate, dexmedetomidine, isoflurane, and halothane [51–53, 57]. Experiments to determine if anesthetic activation of sleep-active neurons causally contributes to the hypnotic state or is merely an epiphenomenon use complementary approaches. Chemical lesions that destroy both VLPO neurons and their neighbors produce acute partial resistance to induction of anesthesia with dexmedetomidine [52] or isoflurane [53]. Interestingly, following the acute effect, mice over time become hypersensitive to isoflurane, presumably due to compensatory adaptations [53].

More sophisticated genetic tagging of preoptic anterior hypothalamic neurons, which include the sleep-promoting neurons in the VLPO as well as in lateral and/or medial preoptic hypothalamus, lends convincing support for anesthetic hypnosis proceeding in part through activation of preoptic area neurons [56]. Labeling of dexmedetomidine-depolarized preoptic area neurons allowed for subsequent reactivation of these neurons in the absence of dexmedetomidine to produce a hypnotic, non-rapid eye movement (NREM) sleep-state [56]. Activation of putative sleep-promoting neurons by anesthetics is not unique to the VLPO, nor to dexmedetomidine. Exposure to hypnotic doses of isoflurane produces immunohistochemical evidence for activation of putative sleep-promoting neurons in the median preoptic area [58]. Blockade of glutamatergic outflow from the lateral habenula not only fragments NREM sleep but also diminishes the hypnotic potency of propofol [59].

Infusion of the GABA synthesis inhibitor 3-mercaptopropionic acid (that effectively reduces local GABA levels) into the brain stem **pontine reticular formation** promotes sleep and facilitates induction of unconsciousness by propofol or isoflurane. Conversely, infusion of the GABA uptake inhibitor nipecotic acid (that effectively increases GABA levels) in the pontine reticular formation promote wakefulness and retards induction of propofol or isoflurane anesthesia [60]. However, the most direct evidence for subcortical sites mediating

anesthetic-induced loss of consciousness comes from the observation that localized injections of GABAergic anesthetics into the mesopontine tegmentum can induce a complete state of anesthesia [61], while lesions to this region produce insomnia [62].

Inhibition of endogenous circuits that promote wakefulness and anesthetic induction

Monoaminergic neurons such as cholinergic neurons of the brainstem and basal forebrain, adrenergic locus coeruleus neurons, histaminergic tuberomammillary neurons, serotonergic dorsal raphe nuclei neurons, and orexinergic neurons of the lateral, perifornical, and posterior hypothalamus all show state-dependent firing patterns. Monoaminergic and orexinergic neurons display greater activity during the awake state (or the transition to the awake state), and lower activity during states of sleep. In a manner complementary to their activating actions on the sleep-promoting neural systems, most anesthetics also exert inhibitory actions on arousal promoting-systems [51, 52, 57, 63, 64]. Lesions to cholinergic basal forebrain neurons increase the anesthetic potency of propofol and pentobarbital [65, 66]. Mutations that disrupt the biosynthesis of adrenergic transmitters are but one example that both impair wake-promoting noradrenergic signaling and produce hypersensitivity to anesthetic induction [67]. The causal contribution of shutting down other wake-active neurons on the state of anesthetic-induced unconsciousness is less clear, as highlighted by the histaminergic system [68, 69]. Moreover, anesthetic modulation of the **orexin/hypocretin system** as well as lesions of dopaminergic neurons in the **ventral tegmental area** (**VTA**) appear to have no effect on induction but can uniquely affect emergence (*i.e.*, restoration of consciousness) from anesthesia [64, 70].

While the neuronal sites of action underlying anesthetic-induced unconsciousness remain debated [71, 72], anesthetic effects extend beyond systems regulating sleep and wakefulness.

Anesthetic-induced loss of consciousness in humans

Functional connectivity and consciousness

Anesthetic-induced unconsciousness remains a major unsolved problem, perhaps because of fundamental limitations in establishing the mechanistic basis for consciousness *per se*. Different brain regions are functionally connected in real-time, and different patterns of connectivity are relevant to specific tasks. An important network of connections is the default mode network (DMN; Box 2, and Box 2, Figure I), which is the pattern of connections observed in individuals not engaged in externally focused, goal-directed tasks [73]. A common feature of anesthetic-induced unconsciousness is a progressive functional disconnect between the thalamus and all other regions in the DMN, most prominently the cortex (Figure 2). Across different states of arousal, however, nonspecific thalamocortical (TC) functional connectivity is reduced to a greater extent than is specific TC connectivity [74].

That the thalamus disconnects from the rest of the DMN is of fundamental relevance to anesthetic-induced unconsciousness as it (notably the pulvinar – a “higher-order” thalamic nucleus that predominantly receives input from the cortex rather than the periphery) synchronizes activity between interconnected cortical areas according to attentional

allocation [75, 76]. Such a “synchronizing” role is consistent with the observation that the PFC is routinely observed to disconnect from either the thalamus or the posterior cortex during anesthetic-induced unconsciousness (as measured during functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) studies - [77] and references therein). However, it should be noted that the sevoflurane-induced “disconnect” in TC connectivity shown in Figure 2A (**fMRI**) was at concentrations associated with immobility in response to noxious stimulation (2%) and blunted autonomic responsiveness (3–4.4%), which are greater than those required to produce loss of directed responsiveness routinely considered the hallmark for loss of consciousness [78–80]; at a concentration associated with loss of consciousness (1–2%), however, such uncoupling is still present. Accompanying TC disengagement, there is a corresponding reduction in anterior-to-posterior **symbolic transfer entropy (STE)** and global permutation entropy (where entropy is a surrogate marker of information transfer) as measured by EEG (Figure 2B), consistent with reduced information processing in the cortex [81]. The loss of connectivity along the anterior-posterior axis in response to an appropriate dose/concentration of a general anesthetic, along with the accompanying loss of information transfer along that axis, both feedforward and feedback - ([82] and references therein), is associated with loss of consciousness. Whether the loss of such connectivity is necessary and sufficient to produce unconsciousness is an open question, hopefully one that can be answered given limits on appropriate assays and definitions of consciousness itself.

Anesthetic-induced unconsciousness and disruption of information transfer

Recent work by Mashour and colleagues shows that: “1) estimates of **Φ** alone are insufficient to differentiate between pharmacologically-induced unconsciousness produced by different anesthetics, 2) four additional parameters related to Φ and EEG connectivity are required to differentiate such states, and 3) while patient/subjects are equally unaware/unresponsive (and superficially similar to an external observer), the Φ and EEG relationships associated with anesthetic-induced unconsciousness/responsiveness are not identical across anesthetics” [83]. This latter point raises an interesting question: at comparable degrees of unresponsiveness (to the external observer), is the loss of consciousness produced by general anesthetic “A” neurophysiologically identical to that produced by general anesthetic “B”? Indeed, one may have a conscious experience while remaining behaviorally unresponsive, but the converse is also true – one may become unresponsive prior to anesthetic-induced unconsciousness [84, 85]. The existence of multiple unconscious states, identical to the outside observer but neurophysiologically unique, is implied by the observation in rats that there are distinct connected metastable “nodes” of neuronal activity (as measured by multiple simultaneous linear microarray recordings) through which a subject must transit on the path to recovery of consciousness from isoflurane anesthesia [86].

EEG patterns of activity as biomarkers for anesthetic-induced unconsciousness

How then, do we distinguish between unconsciousness and mere unresponsiveness? Is the use of a quantifiable biomarker as can be obtained from the EEG informative [87–89]? Phenomenologically, there is a pronounced shift in the α frequency component of the EEG (*i.e*, that portion of the signal occurring a frequency of 8–12 Hz) from the occipital to frontal cortex (anteriorization) with increasing levels of sedation (Figure 3A–B; [90–93]). Modeling

studies suggest that at the molecular and cellular levels, anteriorization arises from an increase in a $GABA_A$-R-mediated conductance in cortical pyramidal neurons coupled with a decrease in the **pacemaker current**, I_h, in high threshold TC projection neurons (HTC cells), a specialized subset of TC neurons that exhibit intrinsic rhythmic burst firing at α frequencies (Box 3, Figure I) [92].

An important limitation of EEG-guided assessment of anesthetic-induced unconsciousness is suggested by the observation that despite the clear presence of a frontal α−δ frequency EEG pattern following induction of general anesthesia (as seen in Figure 3B and D), ~7% of subjects demonstrate volitional (*i.e.*, intentional, as might be observed in response to verbal commands) responsiveness (but without recall) as assessed using the isolated forearm technique [94]. Similarly, subjects who appear “unconscious” to observers can partially process spoken words [95] and reliably report dreaming [84, 96]. Collectively, these results serve to reinforce the point that unresponsiveness is not equivalent to unconsciousness [78, 85]. There is strong evidence of correlation between the pattern of EEG oscillations and anesthetic-induced unconsciousness [97], whether anteriorization of the α signal is causal *per se* with regards to anesthetic-induced unconsciousness is still subject to debate. The frontal EEG as a marker for anesthetic-induced loss of consciousness is called into question by the provocative argument that the PFC, which is the source of electrical activity measured by the frontal EEG, is not relevant to most basic aspects of consciousness despite its fundamental contributions to high-level tasks associated with executive function [98, 99], so any signal originating there might not be relevant.

While clearly observed with PAMs of $GABA_A$-Rs such as propofol and volatile anesthetics (for example, sevoflurane), the α−δ pattern is not readily discernible with the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine or the α_2-selective agonist dexmedetomidine (Figure 3D). Despite non-uniformity of the signal change on the EEG (raw and processed, Figures 3C and D, **respectively**), ketamine [100] and dexmedetomidine [101, 102] clearly disrupt DMN thalamocortical connectivity at plasma drug levels associated with loss of consciousness. Across all classes of anesthetics (GABAergic, adrenergic, glutamatergic), however, there is a pronounced EEG δ component (Figure 3D). Changes in the δ signal may be a more reliable predictor of anesthetic-induced unconsciousness than any other EEG feature [103] particularly if coupled with **power spectral analysis** [87].

Recovery of consciousness

It was previously assumed that emergence from anesthetic-induced unconsciousness represented a pharmacokinetic process, *i.e* induction of general anesthesia but in reverse, but demonstration of hysteresis (also termed neural inertia) between the awake and anesthetized states suggests that this is not the case [64, 104, 105]; further, hysteresis may be anesthetic agent-specific, at least in humans [106]. Central to the return of consciousness is restoration of patterns of connectivity associated with the waking state. While reconnection is a necessary precondition, only a discrete number of pathways exist through which recovery of consciousness is possible [86]. Despite the apparent similarity of patterns of connectivity

during wakeful, conscious activity, and loss thereof during periods of unconsciousness, each individual is unique, and each may have a distinct connectome “fingerprint” [107, 108].

Delirium is a disturbance in attention with reduced ability to direct, focus, sustain, and shift attention and awareness and reduced orientation to the environment that develops rapidly - usually hours to a few days; it represents an acute change from baseline attention and awareness that can fluctuate in severity. Postoperative delirium is commonly seen following general anesthesia, particularly in the elderly [109]. Results from the ENGAGES study indicates that use of intraoperative EEG monitoring (which results in a modest, but significant reduction in anesthetic exposure) does not decrease the incidence of delirium in adults older than 60 years undergoing major surgery as compared to non-EEG guided usual care [110]. The results of the ENGAGES study are in contrast to those of the STRIDE study which found that in elderly (age >65 years) subjects with a low burden of comorbidities undergoing nonelective hip fracture repair, there was significantly less delirium in subjects lightly sedated with propofol as compared to those who were deeply sedated [111].

Postoperative delirium is an important clinical problem, one associated with significant morbidity, mortality, and cost (personal, societal, and economic) [112]. To date, a clear mechanistic link between anesthetic administration and delirium has not been identified; whether delirium results from a delay in establishing the native connectome, residual drug effect, surgical-induced inflammation/neuroinflammation, or a combination thereof, is an open question requiring further investigation.

Ascending arousal pathways and emergence from general anesthesia

Multiple pathways and neurotransmitters promote wakefulness. There is accumulating evidence that ascending reticular-activating pathways are involved in emergence from general anesthesia. From a clinical perspective, understanding these mechanisms is important to optimize recovery of consciousness and cognition in anesthetized patients. Conversely, general anesthesia provides a valuable tool to identify and characterize the fundamental neural circuits necessary for consciousness.

The two main *cholinergic arousal pathways* are (1) neurons in the laterodorsal tegmentum and pedunculopontine tegmentum projecting to the thalamus, basal forebrain and cortex, and (2) basal forebrain neurons projecting primarily to the cortex [113]. Microinjection of nicotine in the central medial thalamus restores consciousness in rats anesthetized with sevoflurane [114], suggesting that cholinergic pathways projecting to the thalamus promote anesthetic reversal. Microdialysis of a cholinergic agonist to the PFC in rats also restores consciousness during continuous sevoflurane anesthesia [115]. This is accompanied by EEG evidence of arousal and an increase in local acetylcholine concentration, suggesting that cholinergic basal forebrain neurons projecting to the PFC are involved in restoration of consciousness [116].

In rats, microinjection of histamine into the basal forebrain decreases time to emergence from isoflurane anesthesia, increases respiratory rate, and shifts EEG activity from **burst suppression** to δ oscillations (a relatively more active physiologic state that is still

associated with NREM sleep and anesthesia) [117], suggesting that enhanced histaminergic neurotransmission to the basal forebrain facilitates anesthetic emergence. Because arousal-promoting cholinergic neurons reside in the basal forebrain, it is possible that histaminergic stimulation produces arousal, at least in part, by activating cholinergic basal forebrain neurons.

Dopamine neurons are mainly found in the ventral tegmental area and substantia nigra pars compacta. Although dopaminergic neurotransmission has been studied primarily in the contexts of reward, cognition, and locomotion, recent findings demonstrate that dopamine neurons are also involved in promoting wakefulness [118]. Electrical stimulation of the ventral tegmental area (but not the substantia nigra) restores consciousness in rats anesthetized with isoflurane and propofol [119]. Consistent with this finding, selective **optogenetic activation** of dopamine neurons in the ventral tegmental area restores consciousness during continuous isoflurane anesthesia in mice [120].

Chemogenetic activation of *noradrenergic neurons* in the **locus coeruleus** produces EEG evidence of arousal and accelerates emergence in rats anesthetized with isoflurane [121]. Pharmacological blockade of norepinephrine reuptake, however, does not restore consciousness in rats under continuous sevoflurane anesthesia [122], nor does microdialysis delivery of norepinephrine to the PFC [115]. These findings suggest that while norepinephrine release may facilitate emergence when the brain anesthetic concentration is falling, enhanced noradrenergic neurotransmission by itself is insufficient to restore consciousness.

Genetic and pharmacological ablation of orexinergic signaling delays recovery of consciousness after general anesthesia in mice, although the anesthetic dose requirement for inducing unconsciousness is unchanged [64]. Accordingly, chemogenetic activation of orexin neurons promotes emergence from isoflurane anesthesia in mice [123]. These reports suggest that orexinergic neurons are important for anesthetic emergence, but not induction.

In summary, recent studies indicate that ascending arousal pathways are critical for emergence from general anesthesia. However, activating different arousal pathways during general anesthesia produces distinct states of arousal [124], encouraging further work to better understand how individual pathways and neurotransmitters are involved in anesthetic emergence.

CONCLUSIONS AND FUTURE PERPSECTIVES

Since our last review of emerging anesthetic mechanisms in 2005 [1], significant progress has been made in identifying and characterizing targets of general anesthetics at a molecular level and the impact of anesthetics on neurophysiological processes critical to consciousness. Structural approaches have resolved anesthetic binding sites in relevant target ion channels with increasing resolution, and the resulting biophysical and neurophysiological impact of these drug-receptor interactions are being revealed using advanced systems-level neuroscience approaches. An understanding of the anesthetic mechanisms that bridge the gaps between reductionist molecular approaches, cellular and

pathway neuroanatomical mechanisms, and specific desired (amnesia, unconsciousness, immobility) and undesired (delirium, neurocognitive dysfunction) neurobehavioral endpoints now appears within reach.

So what is the future trajectory of research into the pharmacology of general anesthesia? Since the discovery of anesthesia over 170 years ago, we have made enormous progress in understanding many, but certainly not all, fundamental aspects of anesthetic mechanisms (see Outstanding Questions). Despite progress in elucidating the molecular and cellular bases of learning and memory, the mechanisms by which general anesthetics produce anterograde amnesia are still unclear. The observations that anesthetics potentiate inhibitory (as with propofol and volatile anesthetics) and/or depress excitatory (as with ketamine) neurotransmission does not fully address why such alterations prevent memory encoding. By analogy, whether we can establish a clear understanding of anesthetic-induced unconsciousness requires an *a priori* understanding of the mechanisms underlying consciousness itself, the proverbial “hard problem”. As we gain deeper insights into the mechanisms contributing to anesthetic-induced amnesia and unconsciousness, we move closer to solving fundamental problems in neuroscience.

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Glossary

Burst suppression
a pattern of activity seen on the EEG that consists of co-mingled large amplitude (*i.e.*, high-voltage) slow waves with fast-spiking sharp waves alternating with longer periods of near, or absolute, electrical silence. Burst activity can arise in either the cortex or the thalamus, and is thought to reflect a state of hyperexcitability resulting from a decrease in cortical inhibition in the setting of preserved glutamate-mediated excitation. Burst suppression is observed both in patients who are deeply anesthetized as well as in those with pathologic conditions (cortical deafferentation, cerebral anoxia/hypoxia, deep coma, infantile encephalopathy, terminal status epilepticus, hypothermia) [128].

Chemogenetic activation
a technique by which cells, commonly neurons, have been genetically modified to express a chemically engineered G-protein-coupled receptor (DREADD, designer receptor exclusively activated by designer drugs or RASSL: receptor activated solely by a synthetic ligand) that binds a synthetic ligand that is selective for that receptor but which is otherwise devoid of biological activity.

Click-chemistry

a modular method for the rapid synthesis of new compounds through heteroatom links (C-X-C) by attaching simple building blocks to a lead molecule of interest. These are typically simple reactions that easily produce purified products in high-yield to rapidly generate combinatorial libraries.

Cutoff effect
the loss of predicted anesthetic potency in a homologous series of molecules as molecular size increases [129, 130].

Diffusion tensor imaging (DTI) fiber tractography
a magnetic resonance imaging (MRI) technique which takes advantage of the fact that water does not have equal mobility in all directions in the regions of brain white matter (*i.e.*, fiber tracts) and allows one to ascertain patterns of 2- and 3-dimensional tract connectivity [131].

γ-aminobutyric acid type A receptor ($GABA_A$-R)
a member of the pentameric Cys-loop superfamily of ligand-gated ion channels (LGICs; also includes nicotinic acetylcholine (nACh), 5-hydroxytryptamine type 3 ($5HT_3$), and glycine receptors – [132]. Following binding of the neurotransmitter GABA, influx of the permeant ion Cl^- results in hyperpolarization of the neuronal membrane, an important source of inhibition (a reduction in the propensity of the neuron to generate an action potential). $GABA_A$-Rs are modulated by a wide variety of clinically relevant CNS depressants, including neurosteroids, benzodiazepines, ethanol, and both intravenous and volatile anesthetics [41].

***Gloeobacter violaceus* ligand-gated ion channel (GLIC)**
first identified in bacteria [133], GLIC is a proton-gated, cation-selective homolog of human ligand-gated ion channels [134] that provides a useful structural model for studying eukaryotic pentameric ligand-gated ion channels.

Locus coeruleus
norepinephrine (NE)-synthesizing (noradrenergic) neurons are concentrated in the locus coeruleus (LC), one of several noradrenergic cell groups in the brainstem. These neurons send diffuse projections throughout the CNS. The LC-NE system has a major role in arousal, attention and stress responses. NE may also contribute to long-term synaptic plasticity, pain modulation, motor control, energy homeostasis, and control of local blood flow [135].

Long-term potentiation (LTP)
LTP is the persistent strengthening of synaptic transmission based on recent activity [136]. It is an important feature that contributes to learning and memory formation [137]. Impaired LTP, both in *in vitro* and in *in vivo* model systems, is a paradigm for understanding disruptions in neurocognitive function, such as impaired learning and memory [138].

Optogenetic activation
a technique in which cells, commonly neurons, have been genetically modified to express membrane proteins that can function as ion channels when activated by specific wavelengths of light. Such channels are ion-selective, and when activated produce either depolarization or hyperpolarization of the plasma membrane [139].

Orexin/hypocretin system

orexin (also known as hypocretin) is a peptide synthesized in a cluster of hypothalamic neurons located in the perifornical lateral hypothalamus of the brain. These neurons project widely, with targets in the histaminergic tubomamillary nucleus (and other hypothalamic structures), the noradrenergic locus coeruleus, the serotonergic dorsal raphe nucleus, the dopaminergic ventral tegmental area, the cholinergic basal forebrain, and the mesopontine laterodorsal tegmental nucleus and pedunculopontine nucleus. These target-regions are associated with feeding, metabolism, arousal and reward, of which, the orexin/hypocretin system is a crucial upstream regulator [140].

Pacemaker current

the pacemaker current I_h is an inward mixed Na^+ and K^+ current that depolarizes the membrane potential. Initially described as a current responsible for Phase 4 spontaneous depolarization that precedes the cardiac action potential, I_h is now known to be widely distributed and to regulate rhythmic oscillations and sub-threshold excitability in the peripheral and central nervous system through control of neuronal resting membrane potential, input resistance, and synaptic integration. The current is generated by four closely related channel proteins, hyperpolarization cyclic nucleotide (HCN) gated channel 1–4, each of which is encoded by a specific gene [141].

Photolabeling

also known as photoaffinity labeling, is a chemical technique that involves placement of an inert chemical group onto molecule (an anesthetic, for example) which undergoes a chemical reaction when irradiated (typically with ultraviolet light) that results in formation of a highly-reactive intermediate that rapidly and covalently binds to proximate target sites.

Φ:

a term used in integrated information theory (IIT; a mathematical approach to characterizing consciousness); it is a measure of the information that is specified by a system (the brain, for example) that is irreducible to that specified by its parts [142].

Pontine reticular formation

a group of interconnected nuclei that are distributed throughout the brainstem (midbrain, pons, and medulla oblongata). The reticular formation includes cortical projections (*via* the ascending reticular activating system; ARAS) as well as projections to the spinal cord (*via* reticulospinal tracts). Neurons of the reticular formation, particularly those of the ARAS, play a crucial role in maintaining behavioral arousal and consciousness [135, 143].

Power spectral analysis

a mathematical technique that examines the relative contribution (or power) of each frequency component of the EEG signal (in Hz: α, 8−15; β, 16−31; γ, >32; δ <4; θ, 4−7) as a function of time [144].

Symbolic transfer entropy (STE)

a computational method to quantify the dominant direction of signal transfer between time series from structurally identical and nonidentical coupled systems. In STE theory, the signals reflect the activity of brain regions and STE serves as a surrogate for communication,

i.e., the exchange of information, between those brain regions. Note that STE does not measure information directly and that no formal causal relationship between the two signals can be definitively determined [145].

Ventral tegmental area (VTA)

one of the two largest dopaminergic areas of the brain (the other being the substantia nigra). This midbrain nucleus contributes to positive and negative reinforcement, decision making, working memory, incentive/stimulus salience, and aversion [146].

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Highlights

- Major inroads have been made in identifying additional anesthetic binding sites using a combination of techniques, both *in vitro* and *in vivo*
- Previously, gene deletion studies demonstrated important roles for both ligand- and voltage-gated ion channels in producing anesthetic-induced loss of consciousness. *In silico* modeling now provides evidence for simultaneous anesthetic modulation of both channel families in thalamocortical neurons as being necessary and sufficient to produce the EEG signature associated with such unconsciousness
- Distinct circuits and networks in the brain contribute to anesthetic-induced loss of consciousness while others govern emergence
- Anesthetic effects can persist beyond the immediate perioperative period with adverse impact on memory, learning, and possibly cognitive function

Outstanding Questions

- Have all pharmacologically relevant anesthetic binding sites been identified? How does anesthetic binding lead to changes in the activity/function of specific target proteins?
- How do anesthetic-induced changes in target protein activity/function observed *in vitro* translate to anesthetic endpoints *in vivo*? Which of the many know molecular interactions of general anesthetics are relevant to specific desired and undesired clinical endpoints?
- Have all major brain circuits that relate to anesthetic-induced loss of consciousness and emergence from anesthesia been identified?
- Will a deeper knowledge of the molecular and cellular events responsible for anesthetic-induced amnesia inform our understanding of learning and memory?
- Can our understanding of the mechanisms governing anesthetic-induced loss of consciousness lead to a mechanistic understanding of consciousness itself? Can a detailed understanding of anesthetic neuropharmacology lead to advances in “hard problems” in neuroscience such as the basis of consciousness, with implications for disorders of consciousness?
- Can we separate out the desirable properties of anesthetics (*e.g.* amnesia and unconsciousness) from their potential for producing persistent adverse effects (*e.g.* neurocognitive dysfunction, neurotoxicity)? Will further understanding of the neuronal circuits involved in the actions of general anesthetics facilitate innovative approaches to the control and monitoring of memory and consciousness?

BOX 1 –

Approaches to identify and verify molecular targets of general anesthetics

Approaches to identify and verify molecular targets of general anesthetics (both volatile and injectable) can be divided into four basic categories:

1. ***In vitro* sensitivity**. In this approach, a suspected molecular target is isolated or expressed, and then its activity tested *in vitro* in the presence of different concentrations of anesthetics. The underlying assumption is that activity will be influenced in a plausible direction at a concentration similar to that producing anesthesia *in vivo*. This approach has verified a large number of plausible targets, including, but not limited to: specific ligand-gated ion channels [147, 148], voltage-gated channels [149, 150], hyperpolarization-activated cyclic nucleotide (HCN)-gated and tandem pore potassium (K_2P) channels [151], and mitochondrial proteins [152]. Attempts to distinguish functional significance of these many targets has used sensitivity, stereoselectivity [153], alkanol **cutoff effect** [9, 154], and other phenomenological observations, some of which has caused further ambiguity. Nevertheless, this has been a powerful approach that has constrained the degree of potential complexity.

2. ***In vivo* sensitivity**. This approach assumes that manipulation of an *in vivo* target genetically or pharmacologically will alter sensitivity of the *organism* to some anesthetic endpoint(s). This approach has confirmed a number of potential targets based on the *in vitro* approach, including specific $GABA_A$ receptor ($GABA_A$-R) subunits [29, 155], HCN1 [151], K_2P [151], mitochondrial complex 1 [152], and syntaxin [156]. *In vivo* sensitivity verifies that a target can influence the chosen endpoint, but cannot confirm that it is a direct anesthetic target.

3. **Direct binding**. This approach assumes that anesthetic targets have binding sites that interact with the anesthetic in a way that alters target activity. Challenges include the difficulty of associating a change in target activity with a behavioral endpoint in an intact organism (Approach 2). Moreover, not all “binding” interactions change target activity. Because anesthetics are low affinity ligands, **photolabeling** [157] has allowed discovery of novel targets in complex milieu (*e.g.*, synaptosomes) and of specific amino acid residues contributing to binding sites within those targets. The highly dynamic nature of anesthetic binding has made high resolution structural approaches (x-ray crystallography, cryo-electron microscopy) challenging, but several successful examples exist [11, 158].

4. ***In vivo* regulation.** This exploratory approach assumes that targets that are most influenced by an anesthetic are modulated in an attempt to restore homeostasis. In contrast to Approaches 1 and 2, this discovery approach has the potential of identifying novel targets through genomic and proteomic techniques [159]. It has resulted in identification of either a large or very

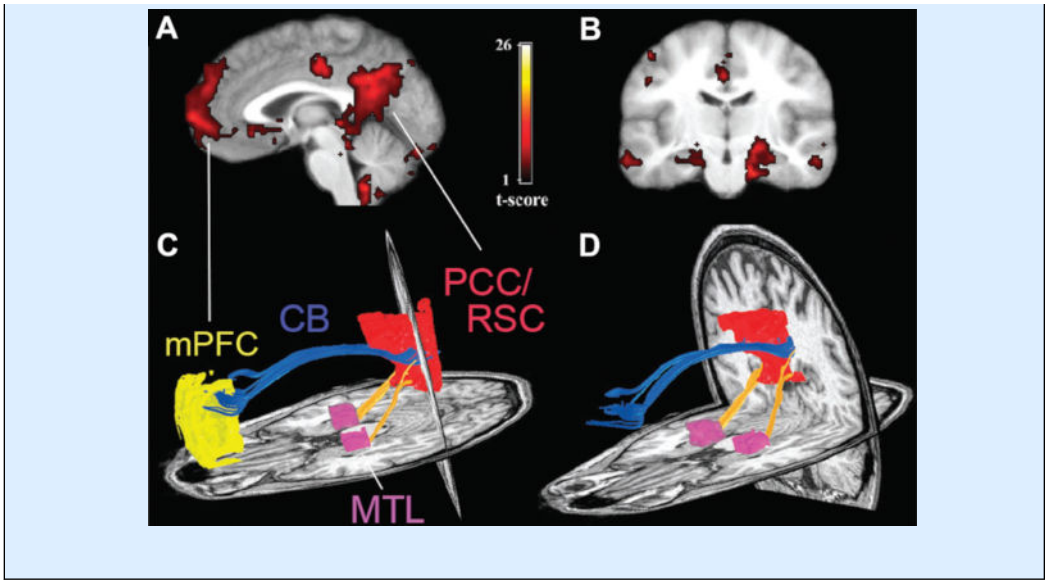
small number of targets regulated, depending on the statistical rigor employed. There has been little overlap with targets identified by other approaches, and uncertainty as to whether the regulated proteins are direct targets or downstream of direct targets.

BOX 2 -

Default mode network

The default mode network (DMN) is a *functionally* characterized pattern of connections, typically identified using magnetic resonance imaging (MRI), that includes voxels spanning the medial prefrontal cortex (mPFC), the dorsomedial (dm) PFC, rostral anterior cingulate, and parts of the anterior and ventral mPFC, the lateral frontal cortex (the superior frontal cortex and inferior frontal gyrus), medial parietal cortex (the posterior cingulate cortex (PCC) and retrosplenial cortex (RSC)), the medial temporal lobe (hippocampus and parahippocampal cortices), the lateral parietal cortex (spanning the angular gyrus and the posterior supramarginal gyrus/temporoparietal junction (TPJ), and the lateral temporal cortex (extending anteriorly to the temporal poles) (Box 2, Figure I). The DMN also includes large areas of the cerebellum and the striatum (the medial wall of the caudate and the posterior putamen) [160]. The thalamus is an integral component of the DMN and is considered critical to the genesis of consciousness [161, 162], and in pathologic states of altered consciousness (*i.e.*, minimally conscious and persistent vegetative states, coma), thalamic connectivity is markedly impaired [163].

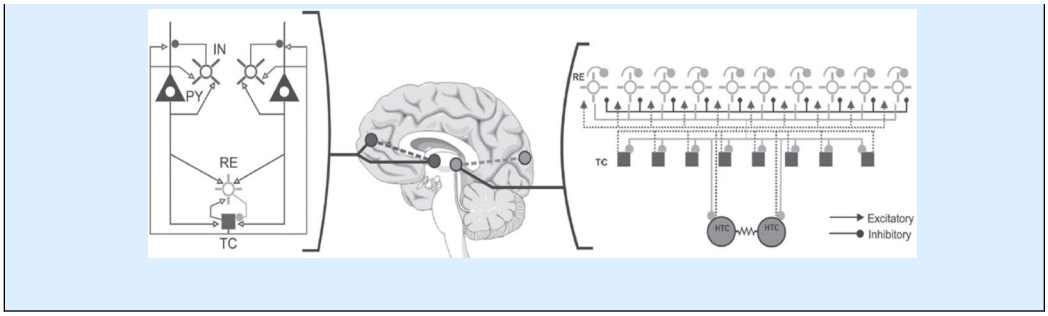
Recent work has demonstrated that functional connectivity correlates with defined anatomic pathways. Task-free, functional connectivity in the DMN is shown for a group of six subjects (Box 2, Figure I). The posterior cingulate cortex/retrosplenial cortex (PCC/RSC) and medial prefrontal cortex (mPFC) clusters are best appreciated on the sagittal view (**A**). Prominent bilateral medial temporal lobe (MTL) clusters (in pink in **C** and **D**) are seen on the coronal image (**B**, left side of image corresponds to left side of brain). (**C**) **Diffusion tensor imaging (DTI) fiber tractography** in a single subject demonstrates the cingulum bundle (blue tracts) connecting the PCC/RSC to the mPFC. The gold tracts connect the bilateral MTL to the PCC/RSC. Tracts from the mPFC enter the more rostral aspect of the PCC/RSC region of interest (ROI) corresponding to the PCC proper, whereas tracts from MTL enter the more caudal aspect of the PCC/RSC ROI, corresponding to the RSC proper. **C** and **D** show slightly different views of the same tracts to highlight the distinct entry points into the PCC/RSC. There were no tracts connecting the mPFC to the MTL. Legend and figure modified with permission from [164].



BOX 3 -

Simplified model for a frontal thalamocortical network

The frontal component consists of cortical excitatory pyramidal cells (PY) and inhibitory interneurons (IN) coupled to inhibitory thalamic reticular neurons (RE) and excitatory thalamocortical (TC) projection neurons (Box 3, Figure I **– left panel**). In this model [90], 10 TC neurons are reciprocally connected to 10 RE neurons. The RE cells provide inhibition to the TC cells, mediated by both $GABA_A$ and $GABA_B$ receptors, and also provide inhibition to each other, mediated by $GABA_A$-Rs. The TC cells in turn provide excitatory glutamatergic inputs to the RE cells. For the cortex, eight PY cells are connected to four IN. Excitatory connections from TC cells onto PY, and IN cells and reciprocal excitatory connections from PY cells onto RE and TC cells, form the TC loop. The posterior component [91] consists of TC cells, RE cells, and a specialized subset of TC cells (HTC) that are thought to generate the awake α rhythm (Box 3, Figure I **– right panel**). Modified with permission from [92].



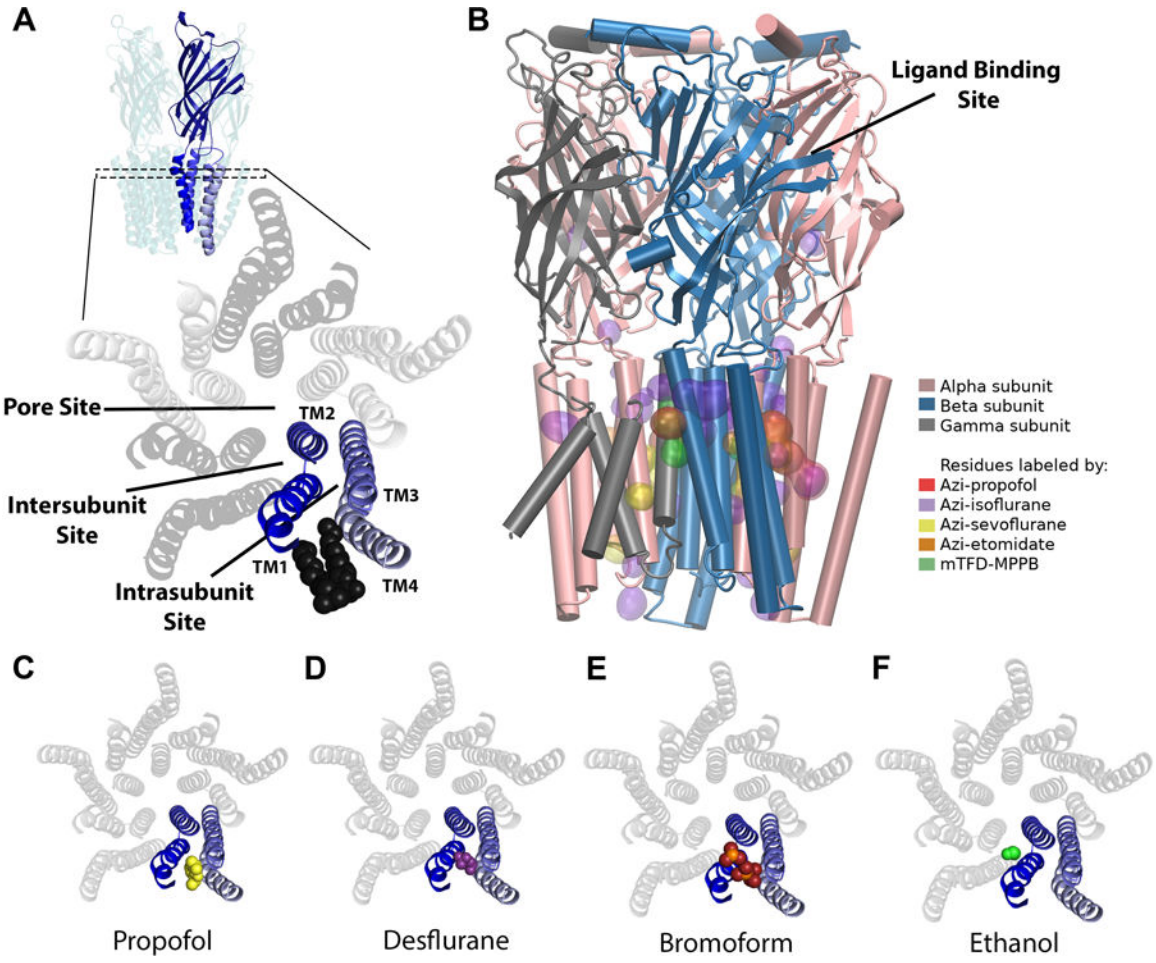


Figure 1. General anesthetics bind and alter $GABA_A$ receptor function at distinct sites.

(**A**) Structure of the homopentameric prokaryotic *Gloeobacter violaceus* ligand-gated ion channel (GLIC) in an ion conducting conformation determined by x-ray crystallography (PDB ID 3EAM) [13]. Shown in side-view (above) and in cross-section (below, enlarged), the locations of known anesthetic binding sites are labeled; these include a pore lining site, an intersubunit site located between neighboring subunits, and an intrasubunit site bounded by the transmembrane (TM), domains of a given subunit (shown in blue). Note that in the absence of anesthetic, a membrane lipid (black spheres) is present at the intrasubunit anesthetic binding site. Image created using PyMOL 1.5 (Schrödinger; New York, NY). (**B**) Homology model of the αβγ $GABA_A$ receptor, showing amino acid residues labeled by photolabel mimics of intravenous (Azi-propofol, Azi-etomidate, mTFD-MPPB) and volatile (Azi-isoflurane, Azi-sevoflurane) general anesthetics. Most residues are located in the more hydrophobic TM region of the receptor, and most are intersubunit residues. Note the multiplicity of sites and that different anesthetics label both distinct and overlapping residues, despite the fact that all of these anesthetics affect this receptor as either direct agonists or co-agonists. mTFD-MPPB (S-1-methyl-5-propyl-5-(m-trifluoromethyl-di azirynylphenyl) barbituric acid), is a photoreactive analog of the convulsant barbiturate S-1-methyl-5-phenyl-5-propyl barbituric acid (S-MPPB), which inhibits $GABA_A$-R responses at

the same concentration at which the anesthetic isomer, R-MPPB, potentiates responses. Image created using VMD software (available from: https://www.ks.uiuc.edu/) [125]. (**C–F**) Structures derived by x-ray crystallography of the conductive conformation of wild type GLIC in the presence of propofol (yellow, PDB ID 3P50), desflurane (purple, PDB ID 3P4W), or two molecules of bromoform (a brominated derivative of chloroform, red, PDB ID 4HFH), and of an ethanol sensitized GLIC F14'A mutant in the presence of ethanol (green, PDB ID 4HFE) [13, 14].

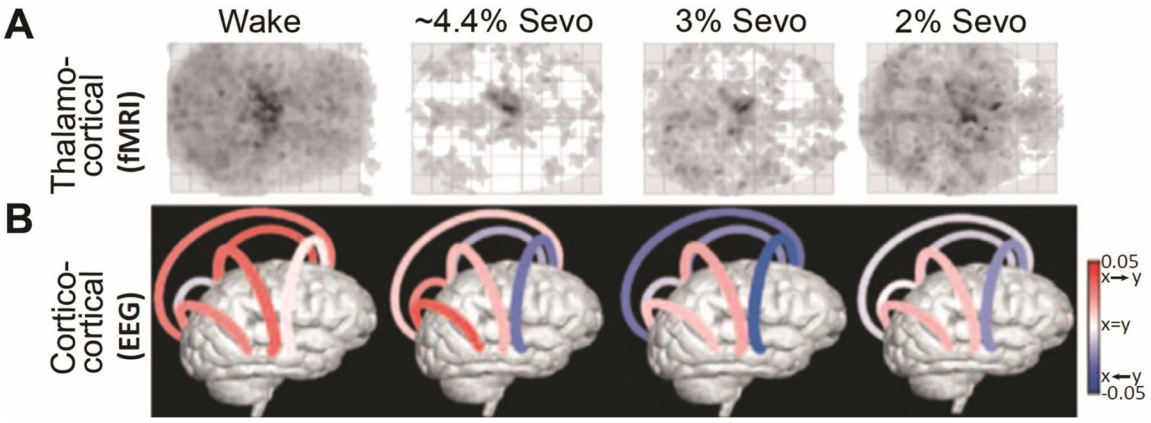


Figure 2. General anesthetics "disconnect" functional brain networks.

(**A**) Effect of sevoflurane anesthesia on functional connectivity. (Top) There is widespread thalamocortical (TC) connectivity as measured by functional magnetic resonance imaging (fMRI) during the waking state (gray voxels; horizontal plane, anterior to the right). Sevoflurane produces a pronounced reduction in connectivity, especially with frontal cortex (white voxels) at the highest sevoflurane concentration. There is a progressive increase in connectivity (gray voxels) as sevoflurane concentration decreases.

(**B**) Sevoflurane decreases directed connectivity as measured by EEG **symbolic transfer entropy (STE)**. Loop color encodes direction of information flow (red: rostrocaudal, blue: caudorostral); scale bar indicates degree of connectivity as measured by STE, where an STE value = 0 indicates balanced bidirectional flow of information. Legend and figure modified with permission from [126].

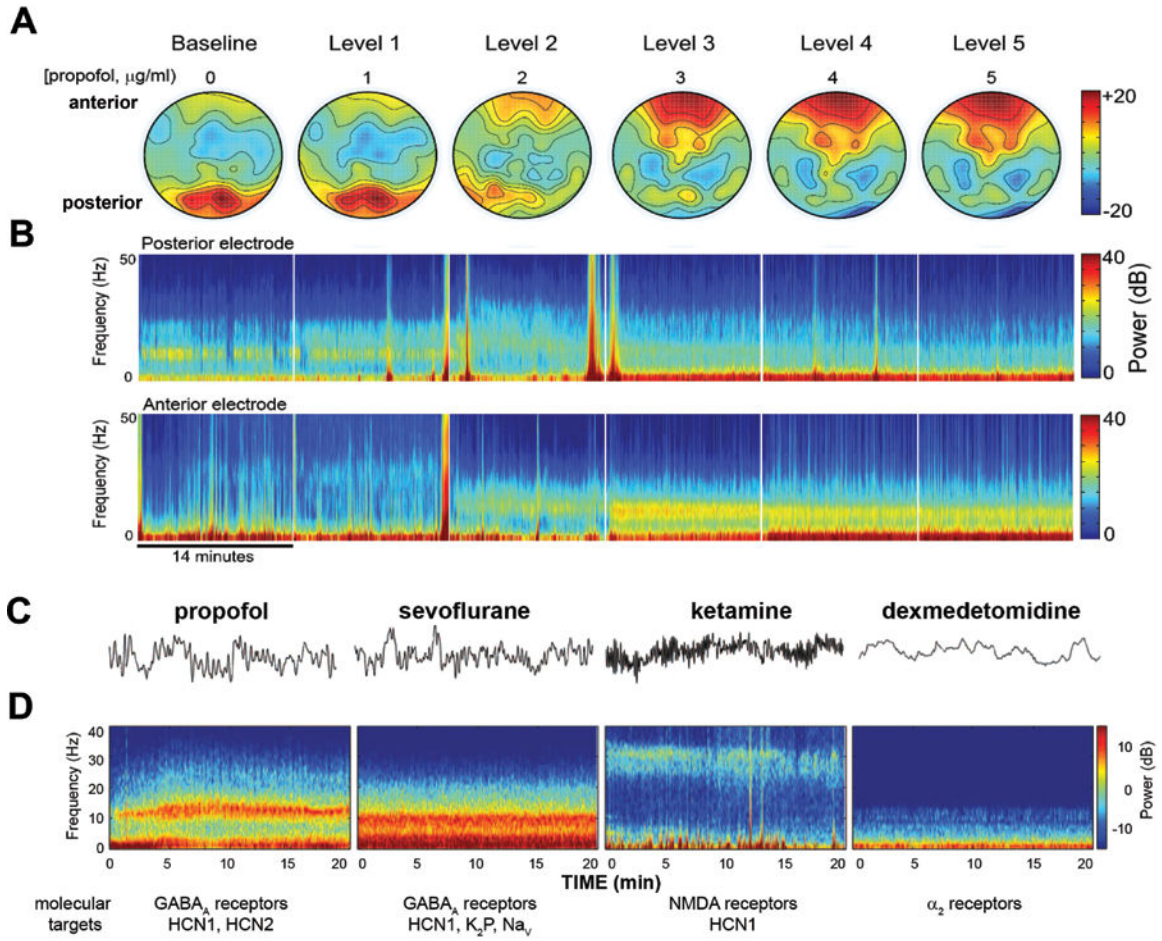


Fig 3. Different anesthetics produce different electroencephalogram signatures.

(**A**) Power maps of 10.3 Hz activity in a human subject, demonstrating “anteriorization” of the α component of the EEG spectrogram with increasing levels of propofol-induced sedation. Baseline indicates the subject is at rest and Levels 1–5 indicate increasing propofol concentrations (as shown). Here, anteriorization is demonstrated by the shift of the α signal (in red-orange) from posterior to anterior regions of the brain.

(**B**) Spectograms from posterior (top) and frontal (bottom) electrodes. With increasing propofol concentrations there is progressive loss of signal in the α bandwidth (8–12 Hz; in yellow) in the posterior electrode with its pronounced appearance in the anterior electrode. The δ signal (in red-orange) is initially present only in anterior brain regions, but as the level of sedation increases, both the α and δ signals are seen anteriorly. Scale bar is the power of the EEG signal, where power is in μV^2. **A** and **B** modified with permission from [92].

(**C**) In unprocessed electroencephalogram (EEG) waveforms, anesthetic-specific differences are slight. Sevoflurane is representative of volatile anesthetics, which have similar EEG patterns at equipotent concentrations.

x(**D**) Different anesthetics produce different spectrographic patterns. Electroencephalogram signatures can be related to the molecular targets and the neural circuits at which the anesthetics act to create altered states of arousal. Again, note the α-δ pattern of activity (in red-orange) for both propofol and sevoflurane. Molecular targets for which there are

compelling *in vivo* and *in vitro* data are shown. **C** and **D** modified with permission from [127].