Investigating the effects of general anesthetics on cortical network activity using \textit{in vitro} preparations

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Abstract

General anesthetics have been used to ablate consciousness during surgery for more than 150 years. Despite significant advances in our understanding of their molecular-level pharmacological effects, comparatively little is known about how anesthetics alter brain dynamics to cause unconsciousness. Consequently, while anesthesia practice is now routine and safe, there are many vagaries that remain unexplained. In this paper we review the evidence that cortical network activity is particularly sensitive to general anesthetics — and suggest that disruption to communication in and/or among cortical brain regions is a common mechanism of anesthesia that ultimately produces loss of consciousness. We review data from acute brain slices and organotypic cultures showing that anesthetics with differing molecular mechanisms of action share in common the ability to impair neurophysiologic communication. While many questions remain, \textit{ex vivo}, and \textit{in vivo} investigations together suggest that a unified understanding of both clinical anesthesia and the neural basis of consciousness is attainable.

Introduction and background

Since the inception of clinical anesthesia in the mid-19\textsuperscript{th} century, scientists have greatly advanced our fundamental understanding of modern anesthetics. In particular, there is now consensus that their molecular targets are proteins that control neuronal excitability and connectivity, including ligand- and voltage-gated channels \textsuperscript{1,2}. Anesthetic interactions at the GABA\textsubscript{A} receptor are best understood — where they bind to receptor subunit interfaces to potentiate the effect of GABA binding and prolong the resulting increase in chloride conductance \textsuperscript{3}. This explains why many anesthetics broadly depress brain activity. However, understanding how molecular-level effects translate into macro network-level phenomena underlying unconsciousness has proven problematic. This is where our understanding of anesthetic action falters — and bridging this gap is an important focus for ongoing research efforts.

The aim of this review is to highlight the insights into network-level mechanisms of anesthesia derived from experiments utilizing \textit{ex vivo} brain slice preparations. We begin
with a brief introduction into the methodology of these preparations. Next, we briefly review our still rudimentary understanding of anesthetic effects at the network-level, focusing particularly on evidence that cerebrocortical effects are central. Finally, we review data from ex vivo models supporting the hypothesis that a breakdown in cortical functional connectivity is a unifying explanation for anesthetic effects on consciousness. Anesthetic agents are diverse in terms of their chemical structure and molecular targets. We focus primarily on the effects of classical sedative/hypnotic agents such as volatile anesthetics, propofol, etomidate and barbiturates, occasionally referencing studies on the dissociative agent ketamine to point out some of its unique actions. We also draw on studies employing benzodiazepines, agents that are sedative and amnestic, and, at sufficient doses, can cause hypnosis, but are not used as general anesthetics in patients. These agents are highly specific for GABA_A receptor-mediated inhibition and thus offer mechanistic insights that are not readily available from studies relying on less specific agents.

Ex vivo/slice preparations of the brain

Brain slice preparations have been a staple of in vitro neurophysiological research for decades 4. The acute brain slice method has been embraced by anesthesiologists for investigating anesthetic drug effects on neural networks, with the advantage that drug actions can be examined in isolated, locally connected networks under highly controlled but flexible conditions. Acute slices are obtained by swiftly, yet carefully dissecting the brain out of an animal's skull and cutting off slices of 300-700 μM thickness. The slicing angle must be chosen deliberately such that the structures of interest are preserved as best as possible (Figure 1). For example, in neocortex, one usually opts for a coronal or sagittal plane of section, both of which are parallel to the elongated apical dendrites of pyramidal cells, the dominant cortical cell type (Figure 1B). In slices meant to preserve synaptic connections between brain regions, e.g. thalamocortical slices, the plane of section must account for thalamocortical axonal pathways 5.

Slicing the brain is clearly a traumatic procedure, warranting a careful choice of conditions 6. Relatively recent successes at improving the viability of slices include new slicer designs 7, improvements of oxygen supply 8, and specific formulations of the bathing medium (artificial cerebrospinal fluid, ACSF) for slicing or immediate post-slicing recovery 9,10. Thus, after decades of procedural optimization, brain slices offer a number of attractive features to experimentalists. They provide comparatively easy access to even the deepest brain regions, and single regions or subnetworks of the brain can be studied in isolation. Combined with a tight control over the environment of the neural tissue, including ionic composition of the ACSF and concentrations of drugs of interest, they are ideal experimental objects for a reductionist approach to neuroscience research. However, our interpretation of past and present findings of brain slice research must take into consideration a specific bias inherent to these preparations: in brain regions like neocortex with prominent long-range connections, the majority of which are excitatory, slicing results in a dysbalance of viable excitatory to inhibitory synaptic connections 11. In order to mitigate these and other side-effects of excising neuronal tissue, ‘thick’ slices (1 mm; 12) and in vitro preparations of entire hippocampi 13 have been devised.
An alternative to acute ex vivo preparations of the brain are organotypic slice cultures, essentially slices from neonatal or embryonic animals, cultivated for days to months. Although they have been in use for electrophysiological experiments for an equally long time, they are not nearly as widely used as acute slices. As has been detailed elsewhere, there is a tradeoff: high levels of spontaneous activity, the possibility to design a wide range of brain sub-networks not feasible in acute preparations, and fast diffusion of drugs into the thin tissue have to be weighed against a less faithful neuronal morphology and synaptic architecture.

In order to illustrate the important contribution ex vivo models have made to our understanding of anesthesia mechanisms, we next present the context of related and equally important in vivo experimental and clinical investigations. With a spotlight on ex vivo models, we have endeavored to integrate a broad knowledge base into a coherent picture of cortical anesthetic effects.

Cortical versus subcortical anesthetic action

While cerebrocortical networks are central to consciousness, there remains contention as to whether the behavioral effects of anesthetics are better explained by direct action on the cortex — or via subcortical effects on the thalamus or on natural sleep and arousal pathways. The potential influence of these neurophysiologic details on the quality of anesthetic induction, maintenance and emergence highlights the importance of this research avenue.

The subcortical and cortical areas involved in anesthetic action are not easily functionally separated when considering the patient as a whole. It can be helpful to conceptually divide general anesthesia appropriate for surgery as targeting two aspects of consciousness: level of consciousness, including overall arousal and responsiveness to external stimuli, and content of consciousness, including interpretation of the saliency of these signals as sensations that could be harmful or warrant decisive action, as well as mentation that may be unrelated or only tangentially related to the sensory environment. This functional separation can be used as a framework for understanding the neuroanatomy of clinical anesthesia at the systems neuroscience level. Accumulating evidence suggests that general anesthetics produce some aspects of hypnosis by acting on sleep and arousal centers in the brain. For example, anesthetics promote sleep-like patterns of activity in the subcortical nuclei in hypothalamus and brainstem that control sleep, for example activating sleep-active neurons in the ventrolateral preoptic nucleus. These agents also depress activity in subcortical arousal centers, e.g. noradrenergic, cholinergic and dopaminergic neurons in the locus ceruleus, basal forebrain and ventral tegmental area, respectively. There is also extensive evidence for anesthetic actions on the thalamus. Thalamus is a critical hub in the ascending arousal system, in relaying sensory information to cortex, and in cortico-cortical communication. Activity in thalamus is suppressed by nearly all anesthetics, with the exception of ketamine. The functional roles of each of these anesthetic targets in producing and maintaining hypnosis and in emergence from anesthesia, can be defined within the dichotomy of level versus content of consciousness. Actions on sleep and arousal centers (including the arousal-promoting functions of thalamus) would control...
arousal, i.e. level of consciousness, whereas the content of consciousness would be due to direct actions on the cortico-thalamic network, and also secondary to actions on sleep and arousal centers. Understanding the relative contributions of each to the functional endpoint of clinical anesthesia is a formidable challenge — and may ultimately prove to be dependent on clinical context and individual-specific differences.

**EEG changes under general anesthesia**

The importance of subcortical targets for anesthesia is supported by similarities in the electroencephalogram (EEG) during general anesthesia and natural sleep. For agents that predominantly act by enhancing GABA_A receptor inhibition (for example, propofol, volatile agents etc), surgical anesthesia is characterised by heightened power in the alpha and delta bands, resembling non-REM Stage 2 and non-REM Stage 3 sleep, respectively [26–29]. Recent *in vivo* work in rats has demonstrated that the central medial thalamus may act as an initiation hub for natural sleep and propofol anesthesia, with changes in dynamics of high frequency bands occurring prior to similar changes in the neocortex [27]. Neuroimaging studies also support functional thalamic disconnection during anesthesia [30,31]. However, as reviewed by Antkowiak, most of the EEG field potential patterns indicative of general anesthesia (thalamocortical oscillations being the obvious exception) can be reproduced in isolated cortical networks in the absence of subcortical connections [32]. This suggests that an EEG pattern resembling that of NREM sleep does not prove subcortical causation. Furthermore, not all anesthetics depress the EEG, ketamine being an obvious case in point [33,34].

**Subcortical microinjection studies**

Subcortical action is further supported by microinjection experiments showing that highly localised drug application to some mesopontine/midbrain regions can, on their own and without direct cortical effects, cause an anesthesia-like state [18,35,36], as well as studies showing that stimulation of dopaminergic VTA can hasten arousal from anesthesia in rodents [37]. Devor and colleagues have shown that anesthetic microinjection into a highly localised region of the upper brainstem called the mesopontine tegmental anesthetic area (MPTA) induces a reversible state of unconsciousness [38], along with associated anesthesia-like changes in the electroencephalogram [35]. Investigation of fos expression suggests that general anesthetic cortical action may be attributable to effects at subcortical neuromodulatory sites [39]. It should be noted, however that these results have not been fully replicated elsewhere [40]. Also, because the extent of drug diffusion is difficult to estimate, the choice of drug concentration is not straightforward in these types of microinjection studies — and may result in a tissue concentration as much as 50x the cerebral tissue concentration required for thiopental anesthesia in rats [35,41,42]. Under the reasonable assumption that such concentrations incapacitate neurons in any brain region, these studies provide valuable information on the role of the subcortical site in question in consciousness. However, this should not be considered evidence of the sites’ pivotal role in bringing about unconsciousness by clinically relevant concentrations of anesthetics. It is also worth noting that the microinjection studies that document dramatic anesthesia-like effects form a small minority of a large number of similar investigations. As summarised recently by Leung et al [43], these studies generally report an increase in anesthetic sensitivity, but not general...
anesthesia outright. At the very least, no one subcortical site seems to form a unifying "anesthesia-sensitive" area.

Pharmacologic investigations of a hyperpolarized thalamus

An idea that has remained prominent since its introduction by Angel in 1991 \(^{44}\), is that thalamic hyperpolarisation causes a transmission block to the passage of sensory information from periphery to cortex. The premise is that consciousness is unsustainable in the absence of a sensory substrate. While also appealing in its intuitive simplicity, there is now compelling experimental data refuting this hypothesis. Firstly, in keeping with its tendency to maintain cortical activity, ketamine at anesthetic concentrations neither suppresses the thalamus \(^ {45} \), nor blocks thalamo-cortical sensory transmission \(^ {46} \). Cortical sensory evoked potential studies also show that primary sensory cortical responsiveness is preserved, and can even be enhanced during anesthesia \(^ {47-50} \). It thus seems likely that unconsciousness occurs at the level of disruption to cortical information processing, not cortical sensory reception. Importantly, this does not preclude the thalamus as an essential anesthetic target, particularly in light of the role of thalamo-cortical relays in mediating cortico-cortical communication \(^ {51} \). Dexmedetomidine is reported to block resting state thalamo-cortical connectivity to a greater extent than cortico-cortical connectivity \(^ {52} \). Recent data also show that xenon anesthesia may be mediated by an HCN2-blocking action with effects on thalamo-cortical primary sensory relay pathways \(^ {53} \). The role of HCN channels in mediating anesthesia effects is not clear however. For example, targeted blockade of HCN channels with ZD-7288 is without effect on highly synchronised "seizure-like event" (SLE) activity in cortical slices \(^ {54} \) — while anesthetics such as propofol, etomidate and ketamine consistently reduce SLE activity in cortical slices \(^ {55} \).

Cortical versus subcortical anesthetic sensitivity

The cortical/subcortical debate hinges not so much on whether anesthesia can be induced by action at one or the other site — it seems that both are possible \(^ {17} \). Instead, the relative importance of one or the other depends on their relative anesthetic-sensitivity at clinically relevant concentrations. This distinction is not only of theoretical interest but is vital for understanding clinically relevant behavioral phenomena associated with anesthesia. For example, even though emergence from anesthesia may not be a simple passive reversal of the induction process \(^ {56} \), knowing the relative influence of dwindling anesthetic concentrations on the spiking activity at cortical vs subcortical sites is pivotal for understanding the nature of these transitions. If the subcortex is more sensitive than the cortex, emergence from anesthesia may be dominated by lingering anesthetic effects on subcortical structures (breathing, detection of sensory stimuli). Conversely, lingering cortical effects during emergence could result in a mismatch between subcortical sensory input mediating arousal and readiness to integrate complex information from different cortical areas. The arousal disturbance in children resulting in so-called "night terrors" may be a manifestation of this in natural sleep \(^ {57} \).

The balance of evidence seems to favour cortical over subcortical sensitivity, with the proviso that this remains an area of active research — and is perhaps even individual-specific. High frequency EEG power (> 14 Hz, beta and gamma waves) represent the
transfer of information among cortical regions during wakefulness and when present during clinical anesthesia for surgery, these features are associated with patient movement and heightened arousal. These "cortical arousals" can be pharmacologically induced and appear to hasten emergence from anesthesia in vivo. Furthermore, anesthetic reduction in cortical firing rates have been shown to be similar with or without subcortical connectivity.

Interactions among the thalamus and cortex

Thalamo-cortical slice studies add further support to the corticocentric view of anesthetic action. Although early reports provided evidence that anesthetics might suppress cortical activity via actions in the thalamus, more recent reports have demonstrated greater sensitivity of intracortical compared to thalamocortical signal pathways. By selectively activating cortico-cortical “top-down” and thalamo-cortical “bottom-up” pathways, Raz and colleagues showed that evoked cortico-cortical responses exhibited greater sensitivity to isoflurane compared to thalamo-cortical responses (Figure 2). In vivo, visual responses in A1, mediated at least in part by projections from V2, are blocked by anesthetics at doses that do not suppress auditory responses to pure tones. Consistent with these data, early- and mid-latency components of auditory evoked potentials are resistant to the effects of anesthesia, at least at doses causing loss of consciousness (LOC). These components most likely correspond to subcortical and thalamo-cortical synaptic potentials. At higher doses, corresponding to surgical levels of anesthesia, even these shorter components are suppressed by general anesthetics. By contrast, longer latency components, likely reflecting cortico-cortical signaling, are sensitive to even low doses of anesthetics. These data suggest that many of the effects on sensory evoked potentials during clinical anesthesia may be due to direct cortical effects rather than effects on thalamo-cortical afferents. In support of this, changes in cortical evoked potentials in isolated cortical slices closely resemble those observed in vivo for a range of anesthetic classes.

Imaging and EEG studies in primates and human subjects also point to cortico-cortical synaptic signaling as a critical locus for effects of anesthetics on consciousness. During midazolam-induced LOC and during slow wave sleep, cortical responses to TMS are enhanced locally but the spread of activity due to cortico-cortical interactions is reduced; similar effects are observed with the dissociative anesthetic ketamine. Feedback cortical connections are particularly sensitive to hypnotic doses of anesthetics, as they are in non-REM sleep and in disorders of consciousness. These data, which are conserved across species, suggest that LOC is accompanied by reduced cortical connectivity in the presence of maintained responsiveness to thalamic inputs in primary sensory cortex (Figure 3).

In the remaining sections we will explore the contribution that acute and organotypic slices can make to understanding and substantiating cortical anesthetic action. In particular, evidence suggests that cortical network activity may underlie bidirectional communication within the cortical hierarchy, a breakdown in which may disrupt the integrative processes central to conscious experience. While the cortex is central to the information that
mediates our conscious experience it should once again be pointed out that this does not preclude the involvement of thalamic and other subcortical nuclei 95,96.

**Anesthetic effects on physiological cortical network activity**

Are effects on cortical networks consistent with a corticocentric view of anesthetic action? At the very least, there is strong data to indicate that the cerebral cortex is an important direct target of anesthetic drugs. Accordingly, models utilising isolated cortical networks provide a valuable tool for investigating anesthetic mechanisms of action. Many have approached this by examining signature electrical patterns in cortical networks that are relevant to anesthetic mechanisms causing unconsciousness.

**Network activity in organotypic cultures inform anesthesia research**

A commonly reported effect of anesthetics consists of a reduction of network activity. Evidence that this effect is mediated at least in part by direct actions on cortical circuits is derived from experiments on neocortical organotypic cultures, where spontaneous activity rates are very sensitive to virtually all classes of anesthetics and sedatives 97. Diazepam was shown to depress spontaneous firing rates in a biphasic concentration-dependent manner, highlighting the different roles of classical and non-classical benzodiazepine binding sites of GABA(A) receptors 98. Analysis of activity rates and patterns in the same model system allowed disentangling the effects of midazolam from its main metabolite, suspected to have potent depressive actions on neuronal activity as well 99. In vivo, however, the picture is more complex. While a reduction in cortical neuronal firing (with accompanying changes in the EEG) does reliably accompany slow-wave sleep 100 and anesthesia 61,101–104, dissociative anesthetics such as ketamine maintain or even increase cortical activity 105. In addition, depression of cortical activity by anesthetics can be unrelated to LOC per se 106–108. Therefore, we posit that although changes of activity rates in cortical networks ex vivo are a very useful approximate marker of the potency/efficacy of anesthetics, there is no straightforward causal relation between their change and the state of consciousness.

**Anesthetic effects on cortical network activity and sensory information processing in acute slices**

Emerging evidence suggests that information transfer within the cortical network may occur via all-or-none responses in cortical ensembles rather than stochastic firing of individual cells 92,109–111. In auditory cortex, network bursts are triggered by sensory stimulation and contain specific spatio-temporal spike sequences (‘packets’) whose organization is stimulus-specific and thus may underlie a population-based encoding process 112,113. Importantly, these network bursts and the network bistability that underlies them are observed in waking conditions 114–116, suggesting an important role in sensory awareness. Spontaneous and induced network activity similar to that recorded *in vivo* during sensory processing can readily be observed in acute slices of rodent neocortex 117–120. The occurrence and propagation of this activity in cortex is modulated by volatile anesthetics, which can both promote its occurrence by synchronizing network activity and disrupt its propagation by interfering with cortico-cortical communication. Indeed, we have shown that in murine auditory thalamo-cortical brain slices, nearly all spiking activity in response to stimulation
of thalamo-cortical afferents occurs in the context of network bursts. In acute slices, which dwell primarily in quiescent ‘Down’ states, spontaneous and induced network bursts are suppressed by the volatile anesthetic isoflurane at moderate doses (Figure 4A). Importantly, spiking that is monosynaptically-driven by thalamo-cortical afferents is significantly more difficult to block by isoflurane than reverberant activity generated within the burst by local cortico-cortical connections (Figure 4B,C). These results are consistent with a model in which anesthetics, at doses causing LOC and suppression of sensory awareness, act directly on circuits intrinsic to cortex rather than on pathways carrying information to cortex from the periphery.

Sharing of information between local cortical networks is central to supporting consciousness. A major pathway for this integration process is via cortico-cortical connections between cortical columns, which form functional units for processing sensory information. Disruption of these connections by anesthetic agents would likely contribute to cortical disintegration during loss of consciousness. Experiments in brain slices support this model. Network activity propagates ‘horizontally’ in cortex from column to column via local connectivity, especially among infragranular pyramidal cells. In thalamo-cortical brain slices, isoflurane suppresses inter-columnar propagation of network activity, either spontaneous or induced by thalamo-cortical or cortico-cortical afferent stimulation (Figure 5A,B). These effects are consistent with a model in which isoflurane suppresses the activation of local networks by the propagating wave. Anesthetic interruption of propagating network activity is also seen in cortical slices activated by removal of artificial cerebrospinal fluid magnesium (Figure 6A,B). In this model spontaneous SLE activity spreads freely across the full extent of the cortex, even across hemispheres. While strictly an epileptiform model, correlated network activity in neocortex has also been described in the context of synchronized brain states that occur under surgical levels of anesthesia and during slow wave sleep. Furthermore, anesthetic effects on SLE activity directly correlates with in vivo anesthetic hypnotic potency. Two main conclusions can be drawn from recent experiments. Firstly, agents of different classes, including ketamine, etomidate and propofol, constrain the spatial extent of SLE spread from the event initiation source. When multiple initiation locations are evident in the same slice, the effect is identical to isolating one source from the other by physically sectioning the tissue. Thus, the anesthetic effect seems to equate to a functional disconnection. Secondly, the population events recorded near the source of SLE generation have shorter rise times and higher amplitudes during anesthetic exposure, suggestive of enhanced local network synchrony. The dual effects of enhanced local response combined with reduced activity spread bears striking resemblance to the effect of midazolam on TMS responses in anesthetised human subjects.

**Hippocampal slice preparations and their role in anesthesia research**

Much of the original work with brain slice techniques focused on the hippocampus, a specialized archecortical structure in the temporal lobe that is conserved in form and function across mammals. The hippocampal slice has led to many great advances in understanding learning and memory and anesthesiology researchers have used this system extensively to better understand the amnesic effect of anesthetic drugs.
However, even with its classic and well described role in learning a memory consolidation, it’s an over simplification to exclude the hippocampus from a discussion on the hypnotic effect of anesthetics. The hippocampus is exquisitely sensitive to anesthetics and a case can be built for it subserving a critical role in binding episodic memory formation to our experience of the external world. It has been argued that the neural processes underpinning episodic memory could actually be regarded as indistinguishable from those underpinning consciousness. The neuroanatomical connectivity of the hippocampus to some extent supports this. Although not spatially arranged exactly like those from the neocortex (i.e., motor and sensory cortices), the hippocampus does have many reciprocal connections with corticothalamic networks.

Outright seizures are a rare but important complication of anesthesia. The hippocampal slice has also been used in combination with pharmacological manipulation of the GABA(A) receptor for mechanistic investigations of seizure. For example, propofol and isoflurane have differential excitatory effects on cortical and hippocampal networks. Propofol, in particular, has a propensity to induce ictal-like events in hippocampal slices. Interestingly, recent evidence suggests that inhibition of GABA signalling by the bath application of the macrolide antibiotic clarithromycin does not cause seizure-like events when administered alone to slices, but increases the frequency of these events when given in combination with a solution (high potassium) known to hyperexcite. Human studies link clarithromycin with improved vigilance in patients with hypersomnia, highlighting a potential avenue for hastening recovery from general anesthesia without hypoactive delirium in the recovery room.

**Functional implications and future directions**

While we present for actions of anesthetics at multiple locations in the brain, each contributing to specific anesthetic endpoints, our emphasis has been on cortical actions that likely impact content of consciousness. That is, while actions on brainstem and midbrain nuclei play important roles in arousal and in the transitions into and out of awareness, there is substantial evidence that consciousness itself is a cortico-thalamic phenomenon. Within such a highly recurrent network, there may be multiple hubs and pathways that play critical roles, any one of which may represent the primary or ‘first’ site of anesthetic action depending on the agent, context and organism of interest. The greatest current gap in our understanding of loss and recovery of consciousness under anesthesia lies at this network level. Because monitors of brain activity in clinical settings invariably sample activity averaged over large numbers of cortical cells, improvements in assays of depth of anesthesia will derive from studies at this spatial scale. Herein, we have reviewed some of the evidence that cortical network activity is particularly sensitive to general anesthetics, but many questions remain. Primary on this list is the mechanism underlying this sensitivity. Is the disruption of cortical network activity a function of the structure of the network, i.e. sparsely-firing cells densely interconnected via synaptic connections with low release probability, balanced excitation and inhibition, and columnar stratification of external connectivity, or do cortico-cortical synapses or cortical pyramidal cells possess intrinsic elements that are specifically sensitive to anesthetics, properties not found at thalamo-cortical synapses or in membranes of subcortical neurons? A second critical question is why...
disruption of network activity is so deleterious, given that sensory stimulation still drives activity in sensory cortex under anesthesia and gross features of tuning are preserved \[143\text{-}145\]. Evidence suggests that cortical network activity may underlie bidirectional communication within the cortical hierarchy \[92\] that is critical for information integration and predictive coding \[93,94\], processes central to construction of self, mind and experience. Investigations in \textit{vivo} and \textit{ex vivo} aimed at understanding how the transfer of information packets \[109\] between cortical regions occurs and how this process is disrupted by general anesthetics will contribute to our understanding of both anesthesia in a clinical setting and to the neural basis of consciousness itself.

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References

1. Franks NP: Molecular targets underlying general anaesthesia. Br J Pharmacol 2006; 147:S72–81 [PubMed: 16402123]
2. Hemmings HC: Molecular Targets of General Anesthetics in the Nervous System. Suppressing the Mind. Edited by Hudetz AG, Pearse R. Springer, 2010, p DOI 10.1007/978-1-60761-462-3_2, doi: 10.1213/ANE.0b013e3181f6d954
3. Garcia PS, Kolesky SE, Jenkins A: General anesthetic actions on GABA(A) receptors. Curr Neuropharmacol 2010; 8:2–9 [PubMed: 20808541]
4. Dingledine R, Dodd J, Kelly JS: The in vitro brain slice as a useful neurophysiological preparation for intracellular recording. J Neurosci Methods 1990; 2:323–62 [PubMed: 6106092]
5. Cruikshank SJ, Rose HJ, Metherate R: Auditory thalamocortical synaptic transmission in vitro. J Neurophysiol 2002; 87:361–84 [PubMed: 11784756]
6. Aitken PG, Breese GR, Dudek FF, Edwards F, Espanol MT, Larkman PM, Lipton P, Newman GC, Nowak TS Jr., Panizzon KL, et al.: Preparative methods for brain slices: a discussion. J Neurosci Methods 1995; 59:139–49 [PubMed: 7475244]
7. Geiger JRP, Bischofberger J, Vida I, Frobe U, Pfitzinger S, Weber HJ, Haverkampf K, Jonas P: Patch-clamp recording in brain slices with improved slicer technology. Pfugers Arch 2002; 443:491–501 [PubMed: 11810221]
8. Hajos N, Mody I: Establishing a physiological environment for visualized in vitro brain slice recordings by increasing oxygen supply and modifying aCSF content. J Neurosci Methods 2009; 183:107–13 [PubMed: 19524611]
9. Rice ME: Use of ascorbate in the preparation and maintenance of brain slices. Methods 1999; 18:144–9 [PubMed: 10356344]
10. Ting JT, Daigle TL, Chen Q, Feng G: Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. Methods Mol Biol 2014; 1183:221–42 [PubMed: 25023312]
11. Stepanyants A, Martinez LM, Ferencskó AS, Kisvárday ZF: The fractions of short- and long-range connections in the visual cortex. Proc Natl Acad Sci U S A 2009; 106:3555–60 [PubMed: 19221032]
12. Wu C, Luk WP, Gillis J, Skinner F, Zhang L: Size does matter: generation of intrinsic network rhythms in thick mouse hippocampal slices. J Neurophysiol 2005; 93:2302–17 [PubMed: 15537814]
13. Khalilov I, Esclapez M, Medina I, Aggoun D, Lamsa K, Leinekugel X, Khazipov R, Ben-Ari Y: A novel in vitro preparation: the intact hippocampal formation. Neuron 1997; 19:743–9 [PubMed: 9354321]
14. Crain SM: Development of “organotypic” bioelectric activities in central nervous tissues during maturation in culture. Int Rev Neurobiol 1966; 9:1–43 [PubMed: 5337726]
15. Gahwiler BH, Capogna M, Debanne D, McKinney RA, Thompson SM: Organotypic slice cultures: a technique has come of age. Trends Neurosci 1997; 20:471–7 [PubMed: 9347615]
16. Drexler B, Hentschke H, Antkowiak B, Grashoff C: Organotypic cultures as tools for testing neuroactive drugs - link between in-vitro and in-vivo experiments. CurrMedChem 2010; 17:4538–50
17. Mashour GA, Hudetz AG: Bottom-Up and Top-Down Mechanisms of General Anesthetics Modulate Different Dimensions of Consciousness. Front Neural Circuits 2017; 11:44 [PubMed: 28676745]
18. Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M: The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway.[see comment]. Nat Neurosci 2002; 5:979–84 [PubMed: 12195434]
19. Moore JT, Chen J, Han B, Meng QC, Veasey SC, Beck SG, Kelz MB: Direct activation of sleep-promoting VLPO neurons by volatile anesthetics contributes to anesthetic hypnosis. Curr Biol 2012; 22:2008–16 [PubMed: 23103189]
20. Han B, McCarren HS, O’Neill D, Kelz MB: Distinctive recruitment of endogenous sleep-promoting neurons by volatile anesthetics and a nonimmobilizer. Anesthesiology 2014; 121:999–1009 [PubMed: 25057841]
21. Brown EN, Purdon PL, Dort Van CJ: General anesthesia and altered states of arousal: a systems neuroscience analysis. Annu Rev Neurosci 2011; 34:601–28 [PubMed: 21513454]
22. Franks NP: General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 2008; 9:370–86 [PubMed: 18425091]
23. Guillery RW, Sherman SM: Branched thalamic afferents: What are the messages that they relay to the cortex? 2011; 66:pp 205–19
24. Saalmann YB: Intralaminar and medial thalamic influence on cortical synchrony, information transmission and cognition. Front Syst Neurosci 2014; 8:83 [PubMed: 24847225]
25. Alkire MT, Miller J: General anesthesia and the neural correlates of consciousness. Prog Brain Res 2005; 150:229–44 [PubMed: 16186027]
26. Kokkinos V, Koupparis A, Stavrinou ML, Kostopoulos GK: The hypnospectrogram: an EEG power spectrum based means to concurrently overview the macroscopic and microscopic architecture of human sleep. J Neurosci Methods 2009; 185:29–38 [PubMed: 19747945]
27. Baker R, Gent TC, Yang Q, Parker S, Vyssotski a. L, Wisden W, Brickley SG, Franks NP: Altered Activity in the Central Medial Thalamus Precedes Changes in the Neocortex during Transitions into Both Sleep and Propofol Anesthesia. J Neurosci 2014; 34:13326–35 [PubMed: 25274812]
28. Pal D, Silverstein BH, Lee H, Mashour GA: Neural Correlates of Wakefulness, Sleep, and General Anesthesia: An Experimental Study in Rat. Anesthesiology 2016; 125:929–42 [PubMed: 27617688]
29. Iber C: The AASM manual for the Scoring of Sleep and Associated Events : Rules Terminology and Technical Specifications. Westchester, IL : American Academy of Sleep Medicine, 2007
30. Schrouff J, Perlberg V, Boly M, Marrelec G, Boveroux P, Vanhaudenhuyse A, Bruno M-A, Laureys S, Phillips C, Pétegrini-Issac M, Maquet P, Benali H: Brain functional integration decreases during propofol-induced loss of consciousness. Neuroimage 2011; 57:198–205 [PubMed: 21524704]
31. Alkire MT, Haier RJ, Fallon JH: Toward a unified theory of narcosis: brain imaging evidence for a thalamocortical switch as the neurophysiologic basis of anesthetic-induced unconsciousness.[see comment]. Conscious Cogn 2000; 9:370–86 [PubMed: 10993665]
32. Antkowiak B: In vitro networks: cortical mechanisms of anaesthetic action. Br J Anaesth 2002; 89:102–11 [PubMed: 12173223]
33. Maksimow A, Sarkela M, Langsjo JW, Salmi E, Kaisti KK, Yli-Hankala A, Hinkka-Yli-Salomaki S, Scheinin H, Jaaskelainen SK: Increase in high frequency EEG activity explains the poor

Anesthesiology. Author manuscript; available in PMC 2019 June 01.
performance of EEG spectral entropy monitor during S-ketamine anesthesia. Clin Neurophysiol 2006; 117:1660–8 [PubMed: 16807101]

34. Tsuda N, Hayashi K, Hagihira S, Sawa T: Ketamine, an NMDA-antagonist, increases the oscillatory frequencies of alpha-peaks on the electroencephalographic power spectrum. Acta Anaesthesiol Scand 2007; 51:472–81 [PubMed: 17378787]

35. Devor M, Zalkind V: Reversible analgesia, atonia, and loss of consciousness on bilateral intracerebral microinjection of pentobarbital. Pain 2001; 94:101–12 [PubMed: 11576749]

36. Correa-Sales C, Rabin BC, Maze M: A hypnotic response to dexmedetomidine, an alpha2 agonist, is mediated in the locus coeruleus in rats. Anesthesiology 1992; 76:948–52 [PubMed: 1350889]

37. Solt K, Dort Van CJ, Chemali JJ, Taylor NE, Kenny JD, Brown EN: Electrical Stimulation of the Ventral Tegmental Area Induces Reanimation from General Anesthesia. Anesthesiology 2014; 121:311–9 [PubMed: 24398816]

38. Minert A, Yatziv S-L, Devor M: Location of the mesopontine neurons responsible for maintenance of anesthetic loss of consciousness. J Neurosci 2017; 37:9320–31 [PubMed: 28821646]

39. Abulafia R, Zalkind V, Devor M: Cerebral Activity during the Anesthesia-Like State Induced by Mesopontine Microinjection of Pentobarbital. J Neurosci 2009; 29:7053–7064 [PubMed: 19474332]

40. Voss LJ, Young BJ, Barnard JP, Sleigh JW: Differential Anaesthetic Effects Following Microinjection of Thiopentone and Propofol into the Pons of Adult Rats: a Pilot Study. Anaesth Intensive Care 2005; 33:373–80 [PubMed: 15973921]

41. Archer DP, Priddy RE, Tang TKK, Sabourin M a, Samanani N: The influence of cryogenic brain injury on the pharmacodynamics of pentobarbitol. Evidence for a serotonergic mechanism. Anesthesiology 1991; 75:634–9 [PubMed: 183993]

42. Myers RD: Injection of solutions into cerebral tissue: relation between volume and diffusion. Physiol Behav 1966; 1:171–4

43. Leung LS, Luo T, Ma J, Herrick I: Brain areas that influence general anesthesia. Prog Neurobiol 2014; 122:24–44 [PubMed: 25172271]

44. Angel A: The G. L. Brown lecture. Adventures in anaesthesia. Exp Physiol 1991; 76:1–38 [PubMed: 2015066]

45. Langsjo JW, Maksimow A, Salmi E, Kaisti K, Aalto S, Oikonen V, Hinkka S, Aantaa R, Sipiila H, Viljanen T, Parkkola R, Scheinin H: Ketamine Anesthesia Increases Cerebral Blood Flow in Excess of the Metabolic Needs in Humans. Anesthesiology 2015; 103:258–68

46. Schwender D, Klasing S, Madler C, Poppel E, Peter K: Mid-latency auditory evoked potentials during ketamine anaesthesia in humans. Br J Anaesth 1993; 71:629–32 [PubMed: 8251269]

47. Cavallari F, Massimini M, Sarasso S, Casali A, Riedner BA, Angelini G, Tononi G, Pearce PA: Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. Proc Natl Acad Sci U S A 2010; 107:2681–6 [PubMed: 20133802]

48. Imas OA, Ropella KM, Ward BD, Wood JD, Hudetz AG: Volatile anesthetics enhance flash-induced gamma oscillations in rat visual cortex. Anesthesiology 2005; 102:937–47 [PubMed: 15851880]

49. Raz A, Grady SM, Krause BM, Uhlrich DJ, Manning KA, Banks MI: Preferential effect of isoflurane on top-down vs. bottom-up pathways in sensory cortex. Front Neurosci 2014; 8:1–22 [PubMed: 24478622]

50. Voss LJ, Sleigh JW: Cortico-centric effects of general anaesthetics on cerebrocortical evoked potentials. Neurosci Bull 2015; 31:697–704 [PubMed: 26480876]

51. Guillery RW, Sherman SM: Thalamic relay functions and their role in corticocortical communication: Generalizations from the visual system. Neuron 2002; 33:163–75 [PubMed: 11804565]

52. Akeju O, Loggia ML, Catana C, Pavone KJ, Vazquez R, Rhee J, Contreras Ramirez V, Chonde DB, Izquierdo-Garcia D, Ararbas G, Hsu S, Habeek K, Hooker JM, Napadow V, Brown EN, Purdon PL: Disruption of thalamic functional connectivity is a neural correlate of dexamethasodime-induced unconsciousness. Elife 2014; 3:e04499 [PubMed: 25432022]

53. Mattuschi C, Kratzer S, Buerg M, Kreutzer M, Engel T, Kopp C, Biel M, Hammelmann V, Ying S-W, Goldstein PA, Kochs E, Haseneder R, Rammes G: Impact of Hyperpolarization-activated,
Cyclic Nucleotide-gated Cation Channel Type 2 for the Xenon-mediated Anesthetic Effect: Evidence from in Vitro and in Vivo Experiments. Anesthesiology 2015; 122:1047–59 [PubMed: 25782754]

54. Voss LJ, Karalus S, Englund V, Sleigh JW: Ketamine Action in the In Vitro Cortical Slice Is Mitigated by Potassium Channel Blockade. Anesthesiology 2018; 128:1167–74 [PubMed: 29509582]

55. Voss LJ, Harvey MG, Sleigh JW: Inhibition of astrocyte metabolism is not the primary mechanism for anaesthetic hypnosis. Springerplus 2016; 5:1041 [PubMed: 27462489]

56. Kushikata T, Hirot a K: Mechanisms of Anesthetic Emergence: Evidence for Active Reanimation. Curr Anesthesiol Rep 2014; 4:49–56

57. Blackburn L, Ottaway K, Anderson BJ: Night terrors and emergence delirium. Paediatr Anaesth 2014; 24:456–7 [PubMed: 24628929]

58. Melloni L, Molina C, Pena M, Torres D, Singer W, Rodriguez E: Synchronization of neural activity across cortical areas correlates with conscious perception. J Neurosci 2007; 27:2858–65 [PubMed: 17360907]

59. Whalin MK, Kreuzer M, Halenda KM, Garcia PS: Missed Opportunities for Intervention in a Patient with Prolonged Postoperative Delirium. Clin Ther 2015; 37:2706–10 [PubMed: 26492795]

60. Safavyna SA, Keating G, Speigel I, Fidler JA, Kreuzer M, Rye DB, Jenkins A, Garcia PS: Effects of \( \gamma \)-Aminobutyric Acid Type A Receptor Modulation by Flumazenil on Emergence from General Anesthesia. Anesthesiology 2016; 125:147–58 [PubMed: 27111534]

61. Hentschke H, Schwar z C, Antkowiak B: Neocortex is the major target of sedative concentrations of volatile anaesthetics: Strong depression of firing rates and increase of GABA\( \alpha \) receptor-mediated inhibition. Eur J Neurosci 2005; 21:93–102 [PubMed: 15654846]

62. Ries CR, Puil E: Mechanism of anesthesia revealed by shunting actions of isoflurane on thalamocortical neurons. J Neurophysiol 1999; 81:1795–801 [PubMed: 10200213]

63. Banks MI, Uhrl ich DJ, Smith PH, Krause BM, Manning KA: Descending projections from extrastriate visual cortex modulate responses of cells in primary auditory cortex. Cereb Cortex 2011; 21:2620–38

64. Howard MA, Volkov JO, Mirs ky R, Garell PC, Noh MD, Gran ner M, Damasio H, Steinschneider M, Reale RA, Hind JE, Brugge JF: Auditory cortex on the human posterior superior temporal gyrus. J Comp Neurol 2000; 416:79–92 [PubMed: 10578103]

65. Santarelli R, Arslan E, Carraro L, Conti G, Capello M, Plourde G: Effects of isoflurane on the auditory brainstem responses and middle latency responses of rats. Acta Otolaryngol 2003; 123:176–81 [PubMed: 12701736]

66. Liegeois-Chauvel C, Musolino A, Badier JM, Marquis P, Chauvel P: Evoked potentials recorded from the auditory cortex in man: evaluation and topography of the middle latency components. Electroencephalogr Clin Neurophysiol 1994; 92:204–14 [PubMed: 7514990]

67. Boston JR, Moller AR: Brainstem auditory-evoked potentials. Crit Rev Biomed Eng 1985; 13:97–123 [PubMed: 3905257]

68. Steinschneider M, Tenke CE, Schroeder CE, Javitt DC, Simpson G V, Arezzo JC, Vaughan HG Jr.: Cellular generators of the cortical auditory evoked potential initial component. Electroencephalogr Clin Neurophysiol 1992; 84:196–200 [PubMed: 1372236]

69. Schwender D, Klasing S, Madler C, Poppel E, Peter K: Depth of anesthesia. Midlatency auditory evoked potentials and cognitive function during general anesthesia. Int Anesthesiol Clin 1993; 31:89–106

70. Santarelli R, Carraro L, Conti G, Capello M, Plourde G, Arslan E: Effects of isoflurane on auditory middle latency (MLRs) and steady-state (SSRs) responses recorded from the temporal cortex of the rat. Brain Res 2003; 973:240–51 [PubMed: 12738068]

71. Heinke W, Kenntner R, Gunter TC, Samm ler D, Olthoff D, Koelsch S: Sequential effects of increasing propofol sedation on frontal and temporal cortices as indexed by auditory event-related potentials. Anesthesiology 2004; 100:617–25 [PubMed: 15108977]

72. Heinke W, Koelsch S: The effects of anesthetics on brain activity and cognitive function. Curr Opin Anaesthesiol 2005; 18:625–631 [PubMed: 16534303]
73. Koht A, Schutz W, Schmidt G, Schramm J, Watanabe E: Effects of etomidate, midazolam, and thiopental on median nerve somatosensory evoked potentials and the additive effects of fentanyl and nitrous oxide. Anesth Analg 1988; 67:435–41 [PubMed: 3364762]

74. Lewis LD, Weiner VS, Mukamel EA, Donoghue JA, Eskandar EN, Madsen JR, Anderson WS, Hochberg LR, Cash SS, Brown EN, Purdon PL: Rapid fragmentation of neuronal networks at the onset of propofol-induced unconsciousness. Proc Natl Acad Sci U S A 2012; 109:E3377–86 [PubMed: 23129622]

75. Purdon PL, Pierce ET, Mukamel EA, Prerau MJ, Walsh JL, Wong KF, Salazar-Gomez AF, Harrell PG, Sampson AL, Cimenser A, Ching S, Kopell NJ, Tavares-Stoeckel C, Habeek K, Merhar R, Brown EN: Electroencephalogram signatures of loss and recovery of consciousness from propofol. Proc Natl Acad Sci U S A 2013; 110:E1142–51 [PubMed: 23487781]

76. Massimini M, Ferrarelli F, Huber R, Esser SK, Singh H, Tononi G: Breakdown of cortical effective connectivity during sleep. Science (80-) 2005; 309:2228–32

77. Schroeder KE, Irwin ZT, Gaidica M, Bentley JN, Patil PG, Mashour GA, Chestek CA: Disruption of corticocortical information transfer during ketamine anesthesia in the primate brain. Neuroimage 2016; 134:459–65 [PubMed: 27095309]

78. Lee U, Arbor A, Ku S, Noh G, Baek S, Choi B, Mashour GA, Arbor A: Disruption of fronto-parietal communication by ketamine, propofol and sevoflurane. Anesthesiology 2013; 118:1264–75 [PubMed: 23695090]

79. Vilisides PE, Bel-Bahar T, Lee U, Li D, Kim H, Janke E, Tarnal V, Pichurko AB, McKinney AM, Kunkler BS, Picton P, Mashour GA: Neurophysiologic Correlates of Ketamine Sedation and Anesthesia: A High-density Electroencephalography Study in Healthy Volunteers. Anesthesiology 2017; 127:58–69 [PubMed: 28486269]

80. Peltier SJ, Kerssens C, Hamann SB, Sebel PS, Byas-Smith M, Hu X: Functional connectivity changes with concentration of sevoflurane anesthesia. Neuroreport 2005; 16:285–8 [PubMed: 15706237]

81. Alkire MT: Loss of effective connectivity during general anesthesia. IntAnesthesiolClin 2008; 46:55–73

82. Lee U, Kim S, Noh GJ, Choi BM, Hwang E, Mashour GA: The directionality and functional organization of frontoparietal connectivity during consciousness and anesthesia in humans. ConsciousCogn 2009; 18:1069–78

83. Ku S-W, Lee U, Noh G-J, Jun I-G, Mashour GA: Preferential Inhibition of Frontal-to-Parietal Feedback Connectivity Is a Neurophysiologic Correlate of General Anesthesia in Surgical Patients. PLoS One 2011; 6:e25155. doi:10.1371/journal.pone.0025155 [PubMed: 21998638]

84. Liu X, Lauer KK, Ward BD, Rao SM, Li SJ, Hudetz AG: Propofol disrupts functional interactions between sensory and high-order processing of auditory verbal memory. Hum Brain Mapp 2012; 33:2487–98 [PubMed: 21932265]

85. Boly M, Moran R, Murphy M, Boveroux P, Bruno MA, Noirhomme Q, Ledoux D, Bonhomme V, Brichtant JF, Tononi G, Laureys S, Friston K: Connectivity changes underlying spectral EEG changes during propofol-induced loss of consciousness. JNeurosci 2012; 32:7082–90 [PubMed: 22593076]

86. Imas OA, Ropella KM, Wood JD, Hudetz AG: Isoflurane disrupts antero-posterior phase synchronization of flash-induced field potentials in the rat. Neurosci Lett 2006; 402:216–21 [PubMed: 16678343]

87. Boly M, Garrido MI, Gossieres O, Bruno MA, Boveroux P, Schnakers C, Massimini M, Litvak V, Laureys S, Friston K: Preserved feedforward but impaired top-down processes in the vegetative state. Science (80-) 2011; 332:858–62

88. Strauss M, Sitt JD, King JR, Elbaz M, Azizi L, Buiatti M, Naccache L, Wassenhove van V, Dehaene S: Disruption of hierarchical predictive coding during sleep. Proc Natl Acad Sci U S A 2015; 112:E1353–62 [PubMed: 25737555]

89. Cohen D, Swinderen vab B, Tsuchiya N: Isoflurane Impairs Low-Frequency Feedback but Leaves High-Frequency Feedforward Connectivity Intact in the Fly Brain. eNeuro 2018; 5

90. Alkire MT, Hudetz AG, Tononi G: Consciousness and anesthesia. Science (80-) 2008; 322:876–80
91. Mashour GA: Top-down mechanisms of anesthetic-induced unconsciousness. Front Syst Neurosci 2014; 8:115 [PubMed: 25002838]

92. Yuste R: From the neuron doctrine to neural networks. Nat Rev Neurosci 2015; 16:487–97 [PubMed: 26152865]

93. Bastos AM, Usrey WM, Adams RA, Mangun GR, Fries P, Friston KJ: Canonical microcircuits for predictive coding. Neuron 2012; 76:695–711 [PubMed: 23177956]

94. Tononi G: An information integration theory of consciousness. BMC Neurosci 2004; 5:42 [PubMed: 15522121]

95. Mhuircheartaigh RN, Rosenorn-Lanng D, Wise R, Jbabdi S, Rogers R, Tracey I: Cortical and Subcortical Connectivity Changes during Decreasing Levels of Consciousness in Humans: A Functional Magnetic Resonance Imaging Study using Propofol. J Neurosci 2010; 30:9095–9102 [PubMed: 20610743]

96. Liang Z, King J, Zhang N: Intrinsic Organization of the Anesthetized Brain. J Neurosci 2012; 32:10183–10192 [PubMed: 22836253]

97. Antkowiak B: Different Actions of General Anesthetics on the Firing Patterns of Neocortical Neurons Mediated by the GABA, Receptor. Anesthesiology 1999; 91:500–11 [PubMed: 10443614]

98. Drexler B, Zinser S, Hentschke H, Antkowiak B: Diazepam decreases action potential firing of neocortical neurons via distinct mechanisms. Anesth Analg 2010; 111:1394–9 [PubMed: 20889946]

99. Balk M, Hentschke H, Rudolph U, Antkowiak B, Drexler B: Differential depression of neuronal network activity by midazolam and its main metabolite 1-hydroxymidazolam in cultured neocortical slices. Sci Rep 2017; 7:3503 [PubMed: 28615640]

100. Hyder F, Fulbright RK, Shulman RG, Rothman DL: Glutamatergic function in the resting awake human brain is supported by uniformly high oxidative energy. J Cereb Blood Flow Metab 2013; 33:339–47 [PubMed: 23299240]

101. Noda H, Adey WR: Neuronal activity in the association cortex of the cat during sleep, wakefulness and anesthesia. Brain Res 1973; 54:243–59 [PubMed: 4350811]

102. Gaese BH, Ostwald J: Anesthesia changes frequency tuning of neurons in the rat primary auditory cortex. J Neurophysiol 2001; 86:1062–6 [PubMed: 11495976]

103. Erchova IA, Lebedev MA, Diamond ME: Somatosensory cortical neuronal population activity across states of anaesthesia. Eur J Neurosci 2002; 15:744–52 [PubMed: 11886439]

104. Vizuete JA, Pillay S, Diba K, Ropella KM, Hudetz AG: Monosynaptic functional connectivity in cerebral cortex during wakefulness and under graded levels of anesthesia. Front Integr Neurosci 2012; 6:90 [PubMed: 23091451]

105. Patel IM, Chapin JK: Ketamine effects on somatosensory cortical single neurons and on behavior in rats. Anesth Analg 1990; 70:635–44 [PubMed: 2344058]

106. Dueck MH, Petzke F, Gerbershagen HJ, Paul M, Hesselmann V, Girmus R, Krug B, Sorger B, Goebel R, Lehre K, Sturm V, Boerner U: Propofol attenuates responses of the auditory cortex to acoustic stimulation in a dose-dependent manner: a FMRI study. Acta AnaesthesiolScand 2005; 49:784–91

107. Kerssens C, Hamann S, Peltier S, Hu XP, Byas-Smith MG, Sebel PS: Attenuated brain response to auditory word stimulation with sevoflurane: a functional magnetic resonance imaging study in humans. Anesthesiology 2005; 103:11–9 [PubMed: 15983451]

108. Plourde G, Belin P, Chartrand D, Fiset P, Backman SB, Xie G, Zatorre RJ: Cortical processing of complex auditory stimuli during alterations of consciousness with the general anesthetic propofol. Anesthesiology 2006; 104:448–57 [PubMed: 16508391]

109. Luczak A, McNaughton BL, Harris KD: Packet-based communication in the cortex. Nat Rev Neurosci 2015; 16:745–55 [PubMed: 26507295]

110. Castelijn C, Nunez A: Cortical Neural Computation by Discrete Results Hypothesis. Front Neural Circuits 2016; 10:81 [PubMed: 27807408]

111. Abeles M, Bergman H, Margalit E, Vaadia E: Spatiotemporal firing patterns in the frontal cortex of behaving monkeys. J Neurophysiol 1993; 70:1629–38 [PubMed: 8283219]
112. Sakata S, Harris KD: Laminar structure of spontaneous and sensory-evoked population activity in auditory cortex. Neuron 2009; 64:404–18 [PubMed: 19914188]
113. Luczak A, Bartho P, Harris KD: Gating of sensory input by spontaneous cortical activity. J Neurosci 2013; 33:1684–95 [PubMed: 23345241]
114. McGinley MJ, David S V, McCormick DA: Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection. Neuron 2015; 87:179–92 [PubMed: 26074005]
115. Poulet JFA, Petersen CCH: Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. Nature 2008; 454:881–5 [PubMed: 18633351]
116. Mochol G, Hermoso-Mendizabal A, Sakata S, Harris KD, la Rocha de J: Stochastic transitions into silence cause noise correlations in cortical circuits. Proc Natl Acad Sci U S A 2015; 112:3529–34 [PubMed: 25739962]
117. Sanchez-Vives M V, McCormick DA: Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 2000; 3:1027–34 [PubMed: 11017176]
118. Rigas P, Castro-Alamancos M a.: Thalamocortical Up States: Differential Effects of Intrinsic and Extrinsic Cortical Inputs on Persistent Activity. J Neurosci 2007; 27:4261–72 [PubMed: 17442810]
119. Krause BM, Raz A, Uhrlrich DJ, Smith PH, Banks MI: Spiking in auditory cortex following thalamic stimulation is dominated by cortical network activity. Front Syst Neurosci 2014; 8:170 [PubMed: 25285071]
120. Neske GT, Patrick SL, Connors BW: Contributions of diverse excitatory and inhibitory neurons to recurrent network activity in cerebral cortex. J Neurosci 2015; 35:1089–105 [PubMed: 2669625]
121. Hentschke H, Raz A, Krause BM, Murphy CA, Banks MI: Disruption of cortical network activity by the general anaesthetic isoflurane. Br J Anaesth 2017; 119:685–96 [PubMed: 29121295]
122. Alkire MT, Hudetz AG, Tononi G: Consciousness and anesthesia. Science (80- ) 2008; 322:876–80
123. Sato TK, Nauhaus I, Carandini M: Traveling waves in visual cortex. Neuron 2012; 75:218–29 [PubMed: 22641308]
124. Wester JC, Contreras D: Columnar interactions determine horizontal propagation of recurrent network activity in neocortex. J Neurosci 2012; 32:5454–71 [PubMed: 22514308]
125. Steriade M, McCormick DA, Sejnowski TJ: Thalamocortical oscillations in the sleeping and aroused brain. Science 1993; 262:679–85 [PubMed: 8235888]
126. Harvey M, Sleigh J, Voss L, Pruijn F, Jose J, Gamage S, Denny W: Determination of the Hypnotic Potency in Rats of the Novel Ketamine Ester Analogue SN 35210. Pharmacology 2015; 96:226–32 [PubMed: 26352278]
127. Jose J, Gamage SA, Harvey MG, Voss LJ, Sleigh JW, Denny WA: Structure-activity relationships for ketamine esters as short-acting anaesthetics. Bioorganic Med Chem 2013; 21
128. Voss LJ, Hansson Baas C, Hansson L, Steyn-Ross DA, Steyn-Ross M, Sleigh JW: Investigation into the effect of the general anaesthetics etomidate and ketamine on long-range coupling of population activity in the mouse neocortical slice. Eur J Pharmacol 2012; 689:111–7 [PubMed: 22705895]
129. Voss LJ, Baas CH, Hansson L, Li D, Sleigh JW: Investigation into the effect of the general anaesthetic etomidate on local neuronal synchrony in the mouse neocortical slice. Brain Res 2013; 1526:65–70 [PubMed: 23791920]
130. Bliss TV, Lomo T: Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973; 232:331–56 [PubMed: 4727084]
131. Pearce RA: Volatile anaesthetic enhancement of paired-pulse depression investigated in the rat hippocampus in vitro. J Physiol 1996; 492 (Pt 3):823–40 [PubMed: 8734993]
132. Benkwitz C, Liao M, Laster MJ, Sonner JM, Eger EI II, Pearce RA: Determination of the EC50 amnesic concentration of etomidate and its diffusion profile in brain tissue. Anesthesiology 2007; 106:114–23 [PubMed: 17197853]
133. MacIver I: Anesthetic Agent-Specific Effects on Synaptic Inhibition. Anesth Analg 2014; 119:558–69 [PubMed: 24977633]
134. Voss LJ, Andersson L, Jadelind A: The general anesthetic propofol induces ictal-like seizure activity in hippocampal mouse brain slices. Springerplus 2015; 4
135. Behrendt RP: Conscious experience and episodic memory: Hippocampus at the crossroads. Front Psychol 2013; 4:1–14 [PubMed: 23382719]
136. Modica PA, Tempelhoff R, White PF: Pro- and anticonvulsant effects of anesthetics (part 1). Anesth Analg 1990; 70:303–15 [PubMed: 2407150]
137. Modica PA, Tempelhoff R, White PF: Pro- and anticonvulsant effects of anesthetics (part 2). Anesth Analg 1990; 70:433–44 [PubMed: 2180345]
138. Becker K, Eder M, Ranft A, Meyer von L, Ziegglansberger W, Kochs E, Dodt HU: Low dose isoflurane exerts opposing effects on neuronal network excitability in neocortex and hippocampus. PLoS One 2012; 7:e39346 [PubMed: 22723999]
139. Bichler EK, Elder CC, García PS: Clarithromycin increases neuronal excitability in CA3 pyramidal neurons through a reduction in GABAergic signaling. J Neurophysiol 2017; 117:93–103 [PubMed: 27733592]
140. Trotti LM, Saini P, Freeman AA, Bliwise DL, García PS, Jenkins A, Rye DB: Improvement in daytime sleepiness with clarithromycin in patients with GABA-related hypersomnia: Clinical experience. J Psychopharmacol 2014; 28:697–702 [PubMed: 24306133]
141. Hernandez OH, Serrato J, Ramon F: Chronic recording of electrical activity from the brain of unrestrained crayfish: the basal, unstimulated activity. Comp Biochem Physiol 1996; 114A:219–26
142. Harris KD, Mrcic-Flogel TD: Cortical connectivity and sensory coding. Nature 2013; 503:51–8 [PubMed: 24201278]
143. Guo W, Chambers AR, Darrow KN, Hancock KE, Shinn-Cunningham BG, Polley DB: Robustness of cortical topography across fields, laminae, anesthetic states, and neurophysiological signal types. JNeurosci 2012; 32:9159–72 [PubMed: 22764225]
144. Hubel DH, Wiesel TN: Receptive fields of single neurones in the cat’s striate cortex. J Physiol 1959; 148:574–91 [PubMed: 14403679]
145. Durand S, Iyer R, Mizuseki K, Vries de S, Mihalas S, Reid RC: A Comparison of Visual Response Properties in the Lateral Geniculate Nucleus and Primary Visual Cortex of Awake and Anesthetized Mice. J Neurosci 2016; 36:12144–56 [PubMed: 27903724]
Figure 1. Ex vivo slice preparations of the brain and common recording configurations in neocortex.

(A) schematic illustrating preparation of brain slices. Brains are sliced in any desirable plane and orientation (shown is a coronal slicing plane) and, depending on subsequent use, the slices may be trimmed to include just the region of interest. The resulting slices are either allowed to recover 1-2 h and are then used for experimentation on the same day ('acute slice') or are placed on a substrate and cultivated in nutritional medium, resulting in an organotypic slice culture. Acute slices can be prepared from animals of any age (commonly juveniles) whereas for cultures neonatal animals or even embryos are often required. (B) sketch of a partial coronal brain slice including neocortex, thalamus and hippocampus. Blue dots illustrate commonly used orientations of multi-channel recording sites within neocortex. In the 'horizontal' orientation the sites are situated within one layer or along layer boundaries, allowing the recording of inter-areal propagation of neuronal activity. The 'vertical', cross-layer orientation, running parallel to pyramidal cells' apical dendrites, is usually chosen if the spread of activity within a cortical column/across layers is of interest, e.g. upon thalamic stimulation. Two pyramidal cells (magenta) are shown schematically to illustrate the orientation of the long apical dendrites.
Figure 2. Pathway specificity of isoflurane effects in auditory cortex.

(A, B): Current source density responses to cortico-cortical (A; CC; ‘top-down’) and thalamo-cortical (B; TC; ‘bottom-up’) synaptic responses in murine brain slices of primary auditory cortex in control (left column) and recovery (right column) and three doses of isoflurane (middle columns). In each panel, vertical axis corresponds to normalized cortical depth (pial surface at top, white matter at the bottom). Horizontal grey lines indicate cortical layer boundaries. Blue colors correspond to current sinks, i.e. excitatory synaptic currents flowing into cells.

(C): Magnitude of layer ¾ TC sink (red) and layer 1 CC sink (blue) from the data in A, showing greater suppression by isoflurane of CC responses compared to TC.

(D): Same as C but showing the 2-D cross-correlation between sink pattern at each drug condition with the pattern in control. Reproduced under the terms of the Creative Commons Attribution Licence from 49.
Figure 3. Summary of effects of general anesthetics on long range connectivity in the cortico-thalamic network.

Schematic showing major feedforward and feedback afferent pathways in the cortico-thalamic network. Under awake conditions (left), projections from ‘core’ cells (blue) in thalamus carry specific information to granular layers (L4) in neocortex, while ‘matrix’ cells (red) exert modulatory influences in supragranular and infragranular layers (L1, L5). Feedforward cortical projection cells (cyan) in supragranular layers (L2/3) project to higher order cortex, while feedback cortical projection cells (magenta) in infragranular layers (L5/6) project to lower order cortex (and subcortically; not shown). Under doses of anesthesia causing LOC (right), feedback cortico-cortical and matrix thalamo-cortical projections are suppressed relative to feedforward cortico-cortical and core thalamo-cortical projections.
Figure 4. Isoflurane depresses polysynaptic bursts more than monosynaptically driven activity.

(A) Current source density (CSD) plots of activity in a mouse auditory thalamocortical slice induced by electrical stimulation of thalamic afferents. The vertical extent of the plots spans the entire cortical depth. Arrowheads indicate the times of occurrence of the stimulation pulses (four pulses at 40 Hz). Cold colors represent current sinks, warm colors current sources. Brief monosynaptic responses (~10 ms) appear immediately after each stimulation pulse, whereas the much longer bursts arise after the third stimulation pulse and evolve over hundreds of milliseconds post-stimulus. Compare the almost complete depression of bursts by isoflurane to the moderate attenuation of the monosynaptic responses. (B) Depression of monosynaptically driven (‘early’) spiking activity in thalamocortical slices by isoflurane. Each point represents the integral of these early responses (see 121 for details) from a slice, normalized to the drug-free condition. TC denotes thalamocortical stimulation. (C) Integral of burst activity induced by TC and cortical layer 1 (L1) stimuli (same conventions as in B apply). Reproduced under the terms of the Creative Commons Attribution License. Panel A modified from 49; panels B-C slightly modified from 121.
Figure 5. Anesthetics slow and impair propagation of cortical activity in acute thalamo-cortical slices.

(A) Example of neocortical burst activity in a thalamocortical slice. Bursts were either induced by electrical stimuli (dotted lines) in auditory thalamus (TC) or in cortical layer 1 (L1) or arose spontaneously; they were extracellularly recorded from a linear 16 channel-array placed in layer 5 of neocortex. Gray traces are three representative trials; colored thick traces are averages. Note the speedy uni- or bidirectional burst propagation during control, and its impairment by a very small concentration of isoflurane. (B) Speed of burst propagation in various isoflurane concentrations, normalized to control. Each filled circle is
one slice. Modified and reproduced under the terms of the Creative Commons Attribution Licence from 121.
Figure 6. Anesthetics slow and impair propagation of SLE activity in acute cortical slices. Schematic (A) and recorded data (B) showing the effect of etomidate on the pattern of zero-magnesium seizure-like event (SLE) activity in the cortical slice. Shown is one hemisphere of a coronally cut slice with 2 recording electrodes (R1 and R2), with a hypothetical (but realistic) scenario of 2 independent sources of SLE activity (S1 and S2) — each of which initiate repeating waves of excitation that spread across the full extent of the cortex in opposite directions (A, left). Under this baseline (drug-free) condition, each event will be recorded by both electrodes, with small inter-electrode time-lags reflecting the speed of wave propagation. As such, each event will appear “synchronised” across both channels (B, left). A proposed explanation for the effect of etomidate is shown schematically (A, right). Propagation of some of the SLE wavefronts is curtailed such that some of the events initiated at S1 will not reach R1 and vice versa. Consequently, the recordings will take on a “desynchronised” appearance (B, right). Variations of this theme will be apparent from slice to slice, according to the number of SLE initiation sources present and where those sources are located relative to the recording electrode positions. Recorded data is from 129.