General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal

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Abstract | The mechanisms through which general anaesthetics, an extremely diverse group of drugs, cause reversible loss of consciousness have been a long-standing mystery. Gradually, a relatively small number of important molecular targets have emerged, and how these drugs act at the molecular level is becoming clearer. Finding the link between these molecular studies and anaesthetic-induced loss of consciousness presents an enormous challenge, but comparisons with the features of natural sleep are helping us to understand how these drugs work and the neuronal pathways that they affect. Recent work suggests that the thalamus and the neuronal networks that regulate its activity are the key to understanding how anaesthetics cause loss of consciousness.

Every year, tens of millions of patients are exposed to general anaesthetics, drugs that remove the most precious human attribute — consciousness. The ability of the anaesthetist to induce safe and reversible loss of consciousness (LOC) in patients has proved to be of inestimable value; however, it has also posed one of the most long-standing and baffling pharmacological puzzles. How can such a structurally diverse group of drugs, ranging from simple inert gases such as xenon to complex barbiturates and steroids, produce this common end point? All general anaesthetics by definition cause LOC in humans (BOX 1), and anaesthetic potencies range over at least five orders of magnitude. However, for any given anaesthetic, the concentration at which consciousness is lost is extremely well defined (FIG. 1). For both the most potent intravenous anaesthetics, such as propofol, which is effective at submicromolar concentrations, and the least potent gaseous agents, such as nitrous oxide, which acts in the millimolar range, the switch from the conscious to the unconscious state occurs abruptly over a change in concentration of only approximately 0.2 log units (that is, a factor of less than 1.6). Are there common mechanisms for all general anaesthetics that can explain how they cause this sudden switch between the conscious and unconscious states? The molecular and neuronal mechanisms that underlie this remarkable phenomenon are the subject of this Review. I first summarize recent molecular data on the most important anaesthetic targets, then I review the functional evidence that reveals the similarities between anaesthetic-induced LOC and natural sleep and discuss how general anaesthetics might act on specific neuronal pathways.

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doi:10.1038/nrn2372

Molecular targets and anaesthetic determinants

The idea that general anaesthetics might act on specific neuronal pathways is difficult to entertain without first accepting that anaesthetics act selectively at the molecular level. The old idea that anaesthetics act by disrupting lipid bilayers or by some other 'nonspecific' mechanism has been discarded, and anaesthetics are now thought to exert their effects by binding directly to specific protein targets (BOX 2). Although over the years a large number of different ion channels, receptors, enzymes and other proteins have been investigated as putative anaesthetic targets[1–4], there is strong evidence for a direct involvement in anaesthetic action for only a handful of these. First I will highlight the three targets for which there is the most compelling evidence for a role in anaesthetic-induced LOC (FIG. 2); for these three, the *in vivo* effects can be convincingly attributed to a particular molecular target *in vitro*.

$GABA_A$ receptors. GABA (γ-aminobutyric acid) type A ($GABA_A$) receptors are found throughout the CNS, and their potential importance as an anaesthetic target has been appreciated for many years[5–7]. They are members of a superfamily that also includes receptors for acetylcholine, glycine and serotonin, several of which are anaesthetic-sensitive[1]. So far, 19 receptor subunits have been cloned, but the vast majority (>85%) of neuronal $GABA_A$ receptors

Box 1 | **General anaesthetic end points**

The chemical diversity of the molecules that cause general anaesthesia is matched only by the range of physiological effects that they can induce. Very few of these effects, however, are common to all agents. The most intriguing of those that are is the ability to cause a reversible loss of consciousness (LOC) and, at higher concentrations, a state in which a human (or any other higher animal) is unresponsive to a painful stimulus. When trying to understand fundamental mechanisms of action, these two end points have the advantage of being straightforward to define and relatively easy to measure. For humans at least, the failure to respond to a meaningful verbal command (such as "Open your eyes") is a satisfactory indication of LOC that avoids any complex philosophical discussions about the exact nature of consciousness itself. The failure to respond to a painful stimulus can also be determined reproducibly and with relatively little ambiguity. The extension of these end points to other animals is obviously problematic because we cannot ask an animal if it is conscious. Nonetheless, for over a century, loss of the righting reflex (LORR) in animals has been used effectively as a surrogate measure. The data in FIG. 1 show that there is an excellent correlation between LOC in humans and LORR in animals over a range of potency exceeding five orders of magnitude. Similarly, there is an equally good correlation between the concentrations that are required to prevent movement in response to a noxious stimulus in humans and those in rats and mice.

Many other anaesthetic end points have been investigated, primarily because of their clinical relevance. These include numerous effects on the cardiovascular and respiratory systems, emesis, analgesia, learning and amnesia. Only effects on amnesia seem to be a characteristic feature of all anaesthetics. However, effects on memory vary qualitatively between different anaesthetics and depend on which experimental paradigm is being used. Although these effects on memory are of considerable interest to both neuroscience and clinical medicine, they are poorly understood at present and will not be considered in this Review.

Tonic inhibition
Background inhibition resulting from persistent low-level activation of $GABA_A$ receptors. The main component of this current is thought to be mediated by extrasynaptic receptors with relatively high affinities for GABA.

Phasic inhibition
Inhibition resulting from the transient release of a high concentration of GABA from presynaptic terminals.

Sulphydryl reagents
Various chemical reagents that selectively react with SH groups and which are hence used to label the sulphur-containing amino acids (cysteine and methionine).

Photoreactive anaesthetics
Modified anaesthetics that contain groups (such as diazirines) that are converted into reactive species following stimulation by light; they are used to selectively and irreversibly label an anaesthetic-binding site.

Righting reflex
The postural response of an animal when placed on its back or side to reorient itself such that its paws or feet are oriented towards the ground.

are composed of $\alpha_1\beta_2\gamma_2$ (the most abundant subunit combination), $\alpha_2\beta_3\gamma_2$ or $\alpha_3\beta_{1-3}\gamma_2$ (REF. 8).

Almost all general anaesthetics have been found to potentiate GABA-induced Cl^- currents and, generally at higher concentrations, directly activate $GABA_A$ receptors in the absence of GABA. Only the relatively small and apolar anaesthetics such as xenon and cyclopropane have little or no effect on $GABA_A$ receptors[9,10]. The functional effects that general anaesthetics have on $GABA_A$ receptors can depend on the receptor's subunit composition (because of intrinsic differences in subunit sensitivity[11] or because of the different biophysical properties of the receptors) as well as on their distribution on the cell surface[8].

In addition to the $GABA_A$ receptors that are clustered at synapses, a large number are distributed extrasynaptically, where they are exposed to fluctuating but low concentrations of GABA; this population contributes to so-called tonic inhibition. At synapses, the predominant receptor subtype seems to be $\alpha_{1-3}\beta_{2/3}\gamma_2$, whereas receptors containing α_{4-6} ($\alpha_4\beta_x\delta$, $\alpha_5\beta_x\gamma_2$ and $\alpha_6\beta_x\delta$) are predominantly or exclusively extrasynaptic. Anaesthetics have substantial effects on these extrasynaptic receptors, which generally have higher affinities for GABA and show less desensitization[12–15]. The greater percentage potentiation of extrasynaptic currents compared with synaptic currents could be due to receptor subunit differences or differences in the ambient levels of GABA. However, the relative contributions of tonic and phasic inhibition to the *in vivo* actions of anaesthetics remain to be established.

A major impetus to explore $GABA_A$ receptors as anaesthetic targets came with the demonstration that specific mutations in the receptors could remove, or at least greatly reduce, their anaesthetic sensitivity[16,17]. An enormous body of literature now exists on the effects that specific point mutations have on the responses of $GABA_A$ receptors to general anaesthetic modulation. These studies are of particular interest because if a specific mutation that affects only anaesthetic modulation (and no other channel properties) is identified, it would be possible, by introducing such a 'silent' mutation into mice, to test the importance of the receptor to the *in vivo* anaesthetic phenotype[18–20].

Because anaesthetic binding potentiates the actions of GABA, any anaesthetic-binding site must also be closely coupled to receptor gating. Consequently, it is difficult to identify anaesthetic-binding sites by studying the effects of mutations[21,22]. Although it also requires mutations, one strategy involves measuring the reactivity of mutant cysteine residues to sulphydryl reagents in the presence and absence of anaesthetics[23,24]. This approach was used to show that Met286 is protected from labelling in the presence of propofol[24], hinting that Met286 might form part of the binding pocket. A more direct approach involves the use of photoreactive anaesthetics[25–27]. A photoreactive etomidate analogue labelled Met286 in the β subunit and Met236 in the α subunit, suggesting that there is an anaesthetic-binding site at the α–β subunit interface (an equivalent site for benzodiazepines is thought to exist at the α–δ interface)[28]. This is a promising but technically difficult approach owing to the small quantities of receptor that are available, the low levels of labelling that occur, and because the labelling can occur preferentially, with some amino acids reacting far more efficiently than others.

Several mutations in the $GABA_A$ receptor subunits have been found to modulate anaesthetic action (FIG. 2a). Those on the α subunit reduce or eliminate the effects of volatile anaesthetics[17,21,22,29–33] but have little influence on the effects of intravenous agents[29,33], whereas those on the β subunit can reduce the effects of both intravenous[16,21,29,34–37] and volatile anaesthetics[21,22]. The β3N265M mutation, for example, greatly reduces the ability of etomidate[18], propofol[18] and pentobarbital[38] to cause loss of the righting reflex (LORR) and essentially eliminates their ability to prevent the response to a painful stimulus, but it has little or no effect[18] on the actions of alphaxalone, halothane or enflurane. Subsequent work showed that the mutation has no effect on the reduction in motor activity that is caused by etomidate[39]. A similar study[19] in mice with the equivalent mutation in the β2 subunit (β2N265S) found that the mutation's largest effect was on the ability of etomidate to induce sedation and LORR; it had a relatively smaller effect on etomidate's ability to reduce the response to a painful stimulus. Interestingly, the β2N265S knock-in animals (and, to a lesser extent, the β3N265M knock-in animals[39]) were much less susceptible to etomidate-induced hypothermia[40]. Because the main neuronal regulator of body temperature is the anterior hypothalamus, an area that is also involved in the control of natural sleep, this might reflect anaesthetic effects on overlapping neuronal pathways (see below).

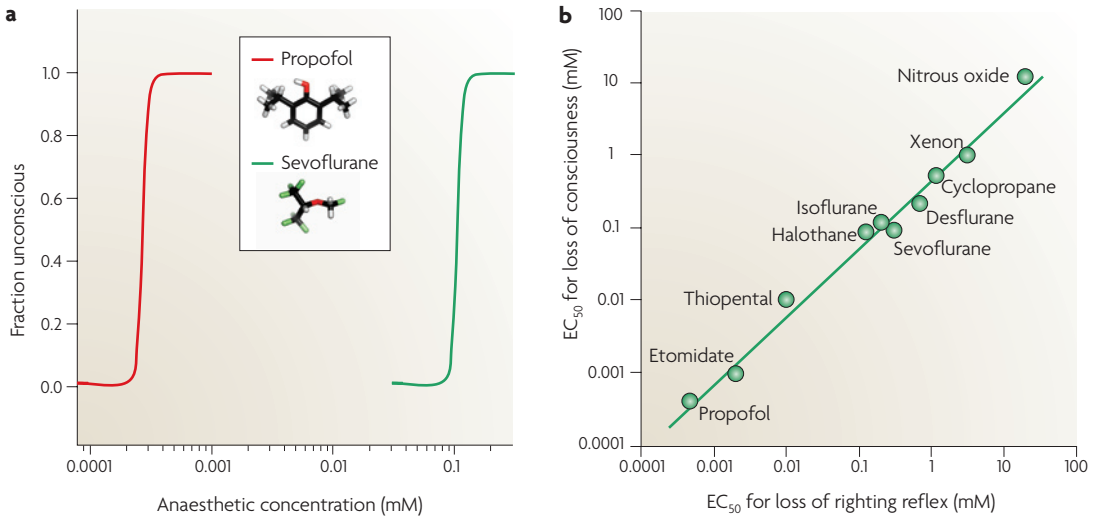


Figure 1 | **Loss of consciousness in humans occurs over a very narrow range of anaesthetic concentrations and correlates with loss of the righting reflex in rodents. a** | The concentration–response curves for anaesthetic-induced loss of consciousness are extremely steep. Many factors, including genetic variability, pharmacokinetics and age, tend to broaden population concentration–response curves; when age, in particular, is taken into account, the switch between the conscious and unconscious states can be seen to occur over a very narrow range of concentrations for a given anaesthetic. These data are from two studies in which patients were grouped according to age: the data for sevoflurane[219] are from patients aged 18–39 years; those for propofol[220] are from patients aged 17–49 years. **b** | The correlation between the anaesthetic concentrations that are needed to cause a loss of consciousness in humans (failure to respond to a verbal command) and those that are needed to cause a loss of the righting reflex in rats and mice. The concentrations that are given are millimolar concentrations in physiological saline at 37°C and, for the intravenous anaesthetics, they take protein binding into account (see Supplementary information S1 (table)). Graph in part **a** based on data from REFS 219,220.

Mice carrying a double knock-in in their α1 subunit (S270H and L277A) that was engineered to retain an unperturbed sensitivity to GABA showed altered LORR sensitivity to volatile agents, supporting a role for α1-containing receptors in mediating the effects of these anaesthetics[20]. The modified activity resembled that of α1-containing $GABA_A$ receptors *in vitro*.

Despite the inevitable concerns regarding compensation when using knockout animals, two recent papers using α4- and α5-knockout animals show interesting and clear-cut results[41,42] that strongly link particular anaesthetic or sedative phenotypes to extrasynaptic $GABA_A$ receptors. Mice lacking α4 subunits, which are thought to be found predominantly in extrasynaptic locations, lack the ataxic, sedative and analgesic responses to gaboxadol, a drug that selectively targets extrasynaptic $GABA_A$ receptors[41]. Mice lacking the α5 subunit, which is also thought to be mainly extrasynaptic, showed no alteration in the effect of etomidate on the LORR but did show some differences in the effects of etomidate on memory impairment (compared with wild-type mice)[42].

There is little doubt that $GABA_A$ receptors have an important role in anaesthetic-induced LOC, although they are clearly more important for some general anaesthetics than for others. The most persuasive data have come from experiments using genetically modified animals, and the close correspondence between the stereoselectivities seen in animals *in vivo* and those found *in vitro* with the $GABA_A$ receptor[39,43–46] also provides strong supporting evidence of a causal link.

Two-pore-domain K⁺ channels. The opening of K^+ channels by anaesthetics has long been considered to be potentially important[47], and there is growing evidence that K^+ channels mediate at least some of the effects of volatile agents. Anaesthetic-activated K^+ channels were first characterized in the mollusc *Lymnaea stagnalis*[48], and a novel class of K^+ channels was subsequently discovered in mammals[49,50]. The demonstration that some of these mammalian K^+ channels were activated by volatile anaesthetics established them as potentially key molecular targets[51]. Two-pore-domain K^+ (2PK) channels are thought to provide 'background' modulation of neuronal excitability. There are 15 different 2PK subunits, and functional channels are formed from dimers, which can be either homomeric or heteromeric. Five members of this channel family (TREK1, TREK2, TASK1, TASK3 and TRESK) can be directly activated by volatile general anaesthetics[51,52].

Anaesthetic sensitivity among these channels is not uniform, however. Although all five channels are sensitive to halothane, the effects of isoflurane and chloroform, for example, are variable, with TASK1 channels being barely affected by either[51,53,54]. Selectivity is also seen with the small anaesthetics xenon, nitrous oxide and cyclopropane, which activate TREK1 channels but have no significant effect on TASK3 channels[55]. Heterodimerization can provide additional variability to the anaesthetic sensitivity of 2PK channels[53]. No 2PK channels have been shown to be affected by clinically relevant intravenous anaesthetics.

Box 2 | **The molecular nature of anaesthetic targets**

To act as a general anaesthetic, a drug must cross the blood–brain barrier. This means that it must be relatively apolar. Thus, anaesthetics lack the functional groups that give most drugs their specificity, and only interact with their targets through weak polarization forces (mainly London dispersion forces) and some degree of hydrogen bonding[183]. These weak interactions inevitably lead to reasonably good correlations between anaesthetic potency and countless physical properties of the drug, which are determined by the same physical forces that mediate their interactions[184]. By far the most influential correlation has been that between anaesthetic potency and lipid partitioning — the famous Meyer–Overton correlation. This correlation was interpreted as meaning that anaesthetics acted by dissolving in the lipid bilayer of nerve membranes and modifying their properties. Many variants of this idea were subsequently proposed[185–187].

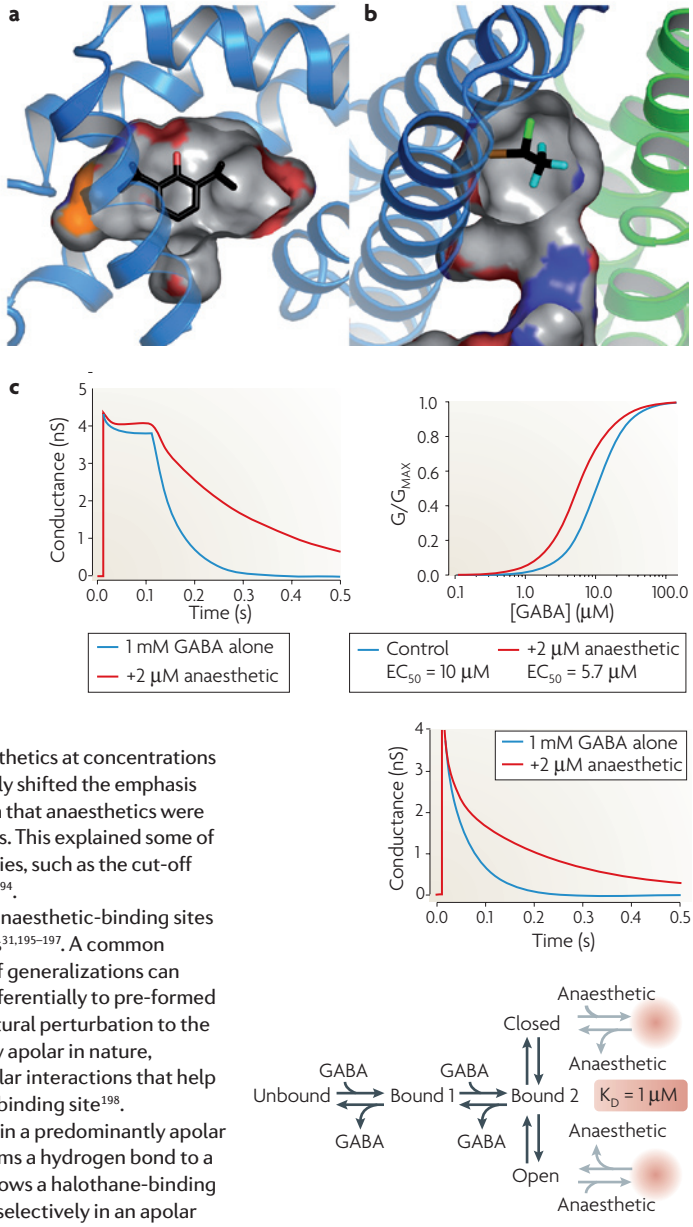
The problems with these theories are both quantitative and qualitative[1,188,189]. The discovery that a pure soluble protein, firefly luciferase, could be competitively inhibited by a chemically diverse range of simple anaesthetics at concentrations that closely mirrored animal potencies[190] firmly shifted the emphasis away from lipid theories and towards the idea that anaesthetics were acting by binding directly to sensitive proteins. This explained some of the qualitative difficulties with the lipid theories, such as the cut-off effect and their temperature dependence[191–194].

High-resolution structural information on anaesthetic-binding sites has, so far, been restricted to soluble proteins[31,195–197]. A common picture is emerging, however, and a couple of generalizations can already be made. First, anaesthetics bind preferentially to pre-formed cavities on proteins, causing little local structural perturbation to the protein. Second, although it is predominantly apolar in nature, anaesthetic–protein binding also involves polar interactions that help to stabilize and orient the anaesthetic in the binding site[198].

Part **a** of the figure shows propofol binding in a predominantly apolar pocket on serum albumin[195], where it also forms a hydrogen bond to a carbonyl oxygen on the main chain. Part **b** shows a halothane-binding site on apoferritin[196]. Halothane binds stereoselectively in an apolar cleft formed at the interface between two four-helix monomers. In both examples the anaesthetics cause negligible changes to the protein structure (Root Mean Square changes of α-carbon atoms are less than 0.15 Å). The images were calculated using PyMol and the crystal structures 1XZ1 for apoferritin–halothane and 1e7a for serum albumin–propofol.

The absence of significant structural rearrangements in the protein following anaesthetic binding is consistent with the weak interactions that are involved, and the highest-affinity anaesthetic-binding sites might be expected to be those where the anaesthetic displaces water from a predominantly apolar, pre-formed cavity. This suggests that anaesthetics do not induce new conformational states but rather only bind to pre-existing states. Thus, they could exert their effects simply by shifting the equilibrium in favour of those conformations that contain anaesthetic-binding sites. As shown in part **c** of the figure, this simple idea accounts for the effects of anaesthetics on a key target — the GABA (γ-aminobutyric acid) type A ($GABA_A$) receptor.

Kinetic schemes for the $GABA_A$ receptor (bottom panel in part **c** of the figure) almost invariably involve two molecules of GABA binding to the receptor before it moves to either an open state or a closed (desensitized) state. If anaesthetics bind to these two states with equal affinity, then all of the key effects of anaesthetics that are observed experimentally[199,200] are predicted. These effects are: a large prolongation in deactivation with little change in desensitization (top left graph); a parallel leftwards shift in the GABA concentration–response curve (top right graph); and a prolongation of the inhibitory postsynaptic current (bottom graph). (Although these changes can be mimicked by arbitrary adjustments of the control rate constants[199,200], such schemes have no physiological meaning without explicit anaesthetic-bound states; see, for example, REF. 201.)



Blood–brain barrier
A layer of tightly apposed endothelial cells that lines blood capillaries and forms a barrier to the diffusion of substances from the blood into the brain. Apolar molecules, such as general anaesthetics, however, readily permeate this barrier.

Anaesthetic activation of 2PK channels would generally inhibit neuronal activity by either hyperpolarizing the membrane and/or increasing the membrane conductance, thus reducing the effects of excitatory currents[48,56–58]. However, these channels are also found presynaptically, and here their activation[59] can be either inhibitory (at excitatory synapses) or excitatory (at inhibitory synapses).

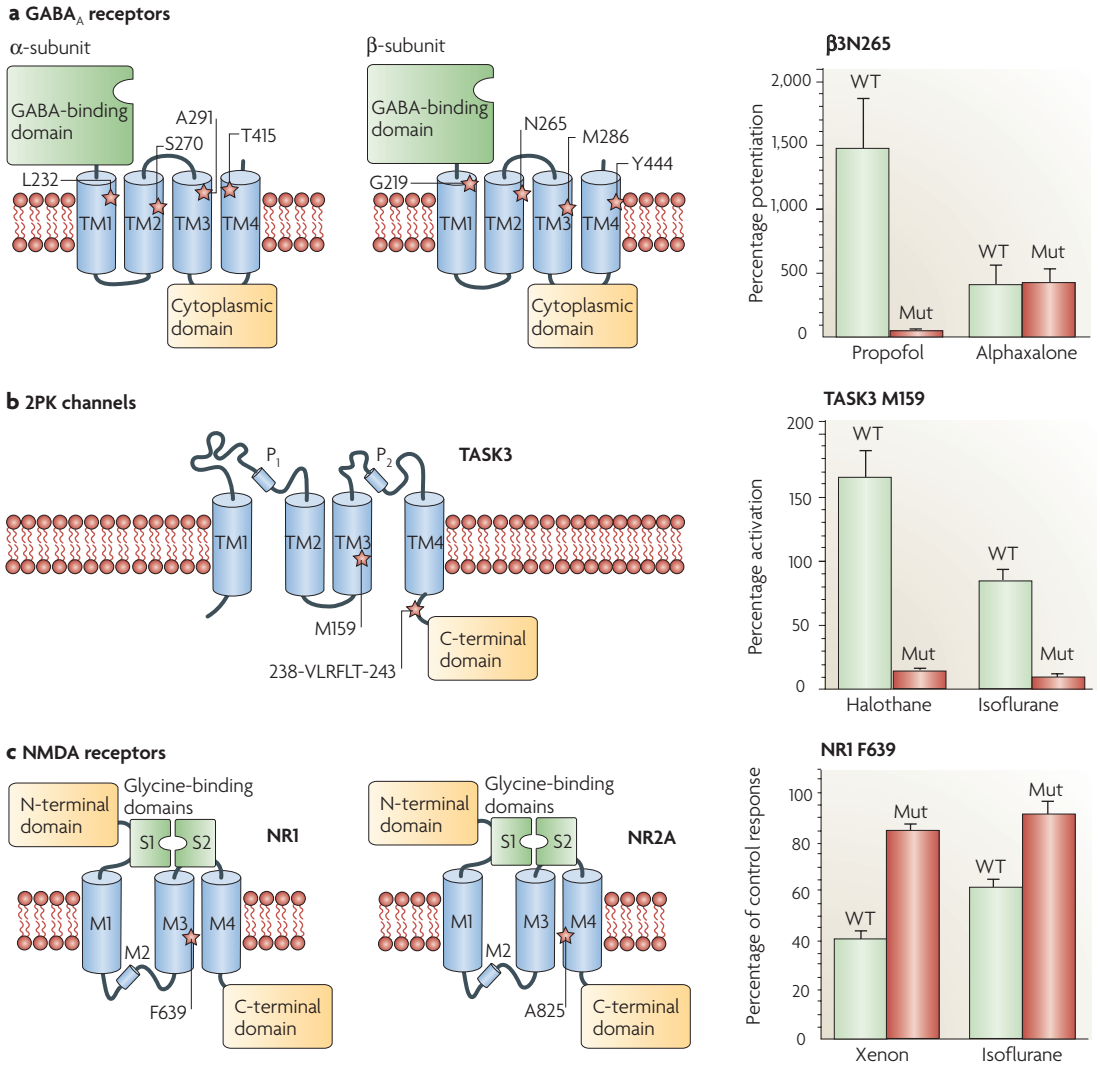


Figure 2 | **Three key molecular targets and some known determinants of their anaesthetic sensitivities. a** | The GABA (γ-aminobutyric acid) type A (GABA$_A$) receptor. Several mutations (indicated by stars) in the α subunit (left-hand panel) affect GABA$_A$ receptor sensitivity to volatile anaesthetics[17,21,22,29–33]; the side chains of the affected amino acids might form an anaesthetic-binding cavity[31]. Substitutions at Ser270, for example, decrease potentiation by isoflurane as the volume of the amino-acid side chain increases[22,30]. This is accompanied by a progressive decrease in the GABA EC_{50}. The eightfold reduction in the GABA EC_{50} that accompanies an increase in side-chain volume of ~150 Å^3 can be accounted for by a small change in free energy — much smaller than the changes that would be expected if the amino acid was buried in a tightly packed protein[221]. This implies that Ser270 might lie adjacent to a cavity. Mutations in the β subunit (middle panel) principally affect intravenous anaesthetics[16,21,29,34–37,222], but they affect some more than others. The mutation β3N265M, for example, eliminates potentiation by propofol but not by the neurosteroid alphaxalone[36] (right-hand panel). **b** | In the TASK two-pore-domain K^+ (2PK) channels, the linker region between transmembrane domain 4 (TM4) and the large carboxy-terminal cytoplasmic domain is critical for both activation by the anaesthetic halothane and second-messenger modulation[51,60]. However, it is probably important for signal transduction rather than anaesthetic binding (left panel). For TASK3 channels, a mutation in TM3 (M159A) essentially eliminates anaesthetic activation (right panel); this amino acid might form part of an anaesthetic binding site[54]. **c** | NMDA (*N*-methyl-D-aspartate) receptors. Mutations in membrane domain 3 (M3) and M4 that have been found to reduce alcohol-induced inhibition of these receptors (left-hand and middle panels)[223,224] also affect the receptor's sensitivity to inhalational anaesthetics (right-hand panel)[71]. Because isoflurane and xenon largely inhibit the receptors by competing with the co-agonist glycine[77], it is likely that these mutations exert their effects by increasing the receptor's apparent affinity for glycine[77]. Mut, mutant; WT, wild type. Graph in part **a** based on data from REF. 36. Graph in part **b** based on data from REF. 54. Graph in part **c** based on data from REF. 77.

Compared with $GABA_A$ receptors, little is known about the anaesthetic-sensitivity determinants of 2PK channels. For TREK1 channels, the large carboxy-terminal domain is clearly important for their anaesthetic sensitivity[51,60] (as it is for many other modulatory influences), although it has a less important role in TASK channels[60]. However, the region that links the transmembrane core of this channel to the large cytoplasmic C-terminal domain is also critically important. A six-amino-acid segment (238-VLRFLT-243) in this region was shown to be necessary for the anaesthetic activation of TASK channels[51] and was later shown to be equally important for the closing of these channels by neurotransmitter-mediated second-messenger modulation[60]. Similarly, Glu306, an amino acid that lies at the start of TREK1's C-terminal domain, has been found to be critical for TREK1 channel gating. Mutation of Glu306 leads to a constitutively active channel that is largely insensitive to modulatory control[61], including anaesthetic activation[55]. From the available evidence, it seems that the C-terminal domain and/or the short linker region that precedes it are important for transducing anaesthetic effects, but these regions are unlikely to be involved in anaesthetic binding. Recently, however, a mutation (M159A) in the third transmembrane domain (TM3) of human TASK channels has been shown to eliminate the effect of anaesthetics on channel activation[54] (FIG. 2b). The fact that the equivalent mutation in the *L. stagnalis* 2PK TASK channel also disrupted the stereoselective effects of isoflurane[62] suggests that this amino acid resides in an anaesthetic-binding site[54].

Direct evidence linking anaesthetic action on 2PK channels to effects in animals is limited but persuasive, particularly for TREK1. TREK1-knockout mice[63] display a marked reduction in the extent to which a range of volatile anaesthetics induce a loss of response to a painful stimulus and, to a lesser degree, LORR. A small reduction in anaesthetic sensitivity has also been observed in TASK1-knockout mice compared with wild-type mice[64]. Importantly, the TREK1-knockout mice showed no changes in sensitivity to the barbiturate pentobarbital (which does not activate TREK), implying that there was no significant compensatory upregulation of their $GABA_A$ receptors[63]. Nonetheless, functional compensation can always occur in knockout animals, and further evidence is required before the role of 2PK channels in anaesthesia can be established for certain.

NMDA receptors. Postsynaptic receptors at glutamatergic synapses fall into two broad categories: NMDA (*N*-methyl-D-aspartate) receptors and non-NMDA receptors[65]. The latter group (which is subdivided into AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors and kainate receptors) mediate the fast component of excitatory postsynaptic currents (EPSCs) and show relatively little anaesthetic sensitivity[9,66,67]. By contrast, NMDA receptors, which mediate the slow components of synaptic transmission[65] and which are also found both presynaptically and extrasynaptically, might be an important target for some anaesthetics[68]. NMDA receptors consist of an obligatory NR1 subunit and at least one of four NR2 subunits (A–D). Two NR3 subunits also exist, as do numerous splice variants. This heterogeneity confers a rich repertoire of kinetic, pharmacological and anatomical variability[65].

Most inhalational anaesthetics inhibit NMDA receptors to some extent, although estimates of these extents vary considerably[9,69–72]. There has been particular focus[9,69,71,73,74] on nitrous oxide and xenon, partly because these two anaesthetics have little or no effect on $GABA_A$ receptors and partly because they have some features in common (such as the ability to induce profound analgesic and psychotomimetic effects) with known NMDA receptor antagonists, such as ketamine. Both nitrous oxide and xenon potently reduce NMDA-receptor-mediated synaptic transmission in the spinal cord[75] and provide neuroprotection (another characteristic of NMDA receptor antagonists)[74,76].

Two mutations (F639A in NR1 and A825W in NR2A) that reduce the anaesthetic sensitivity of NMDA receptors have been described[71], but their effects vary for the different agents (FIG. 2c). It has been shown that xenon and isoflurane can compete for the essential co-agonist glycine (which binds to the NR1 subunit), and that the F639A mutation probably exerts its effects by increasing the receptor's apparent affinity for glycine[77]. This implies that anaesthetic inhibition of NMDA receptors will depend, at least in part, on local glycine concentrations and could explain some of the discrepancies in the literature regarding the anaesthetic sensitivity of these receptors.

Many selective NMDA receptor antagonists will, at high enough concentrations, cause sedation and then LOC or LORR[78]; however, supposedly selective drugs often recruit additional targets as their concentrations increase[79]. This might well be the case for ketamine, which is known to affect a number of other receptors; nonetheless, the correspondence between *in vitro* and *in vivo* stereoselectivities[1] suggests that the NMDA receptor is still likely to be an important target. Another point to bear in mind is that the LOC or LORR induced by NMDA receptor antagonists is usually preceded by many behavioural abnormalities[78]. In the case of ketamine, for example, the final state of LOC in humans is more akin to a cataleptic stupor, and is accompanied by a pattern of brain activity[80] that is quite unlike that induced by other general anaesthetics. Although it is likely that NMDA receptor antagonism has a role in the actions of xenon, nitrous oxide and, to some extent, other volatile agents, particularly for their analgesic effects, additional targets are required to account for their ability to cause LOC.

Other possible molecular targets. In addition to the three targets discussed above, there are a number of other plausible targets that are worth mentioning. First, glycine receptors, which are homologous to, and often colocalized with, $GABA_A$ receptors, are a potential anaesthetic target. Glycine receptors have an inhibitory role, particularly in the lower brainstem and spinal cord, where they might mediate the action of volatile anaesthetics[81–83]. Second, cyclic-nucleotide-gated (HCN) channels, which underlie the hyperpolarization-activated cation current,

Positron-emission tomography
(PET). A technique that uses positron emitters, such as ^{15}O, to measure local changes in cerebral blood flow, which can be interpreted as reflecting local neuronal activity.

I_h, are also potential anaesthetic targets. These channels are found throughout the brain, but their anaesthetic sensitivity has been relatively little explored; however, the available evidence shows that they do display some sensitivity to volatile agents in brainstem motor neurons[84,85] and considerable sensitivity to propofol in thalamocortical neurons[86]. Their inhibition by propofol in thalamocortical neurons[86] is considerably higher than it is in the hippocampus or in medullary neurons[87] (presumably as a result of different receptor-subtype sensitivities) and is of particular interest because of the critical role that the I_h current has in setting the frequency of thalamocortical oscillations (see later). Finally, although anaesthetic effects on most voltage-gated ion channels are small, presynaptic inhibition[88] of certain Na^+ channel subtypes[89,90] by volatile anaesthetics could explain some of the inhibition that is seen at glutamatergic synapses[88].

How do anaesthetic actions at the molecular level lead to LOC? This link can only ultimately be determined by understanding the functional consequences of the effects of anaesthetics on specific neuronal pathways at the cellular and synaptic levels. However, human brain-imaging studies, in which neuronal activity throughout the brain can be visualized while the subject moves from the awake to the anaesthetized state, are providing crucial clues about which pathways might be important, and comparisons between anaesthetic-induced LOC and natural sleep have revealed striking similarities.

Functional human brain imaging

None of the currently available imaging techniques can directly measure neuronal activity. Rather, they infer changes in activity from changes in, for example, blood flow, glucose metabolism or oxygen concentration. Because these surrogate measures might be affected by anaesthetics independent of any changes in neuronal activity (for example, anaesthetics might cause changes in vascular resistance), the interpretation of results has to be tentative. Nonetheless, important generalizations about the similarities between the sleeping and the anaesthetized brain can be made when the same techniques are compared.

Despite its technical limitations (in terms of temporal and spatial resolution), positron-emission tomography (PET) has shown that most anaesthetics cause a global reduction in cerebral blood flow (CBF) when consciousness is lost (ketamine is an exception[80]), although the extent of this reduction is variable[91–94]. More interestingly, the pattern across the brain is not uniform: certain regions are consistently more deactivated than others. Studies with propofol[91–93], sevoflurane[93] and xenon[94] showed deactivation of the thalamus and some midbrain structures that are associated with the ascending reticular activating system, together with varying degrees of deactivation of particular association cortices, such as the precuneus and the posterior cingulate cortex (in the parietal cortex), the cuneus (in the occipital cortex) and some localized regions of the frontal cortex (FIG. 3). Effects were also seen in the cerebellum, but these varied considerably between anaesthetics. Most studies compared the awake state with that following LOC, but a more powerful approach involves measuring changes in CBF as the dose of anaesthetic is increased[91,92]. Work with propofol showed that CBF changes that were induced by vibrotactile stimulation of the forearm were first reduced in the somatosensory cortices, but that LOC only occurred when CBF changes in the thalamus were abolished[92]. A series of imaging studies that measured changes in glucose metabolism[95,96] also highlighted the importance of thalamic deactivation during anaesthetic-induced LOC.

Sleep and anaesthesia. Imaging studies have shown obvious parallels (but also some differences) between the anaesthetized brain and the brain during deep non-rapid-eye-movement (NREM) sleep[97–99]. Comparisons between NREM sleep and the waking state characteristically show a marked deactivation of the thalamus, the brainstem, the basal forebrain and the basal ganglia, together with deactivation in regions of the frontal and parietal cortices, particularly the anterior cingulate and orbitofrontal cortices, and the precuneus and the posterior cingulate cortex. The precuneus and adjacent posterior cingulate cortex are the most active regions of the conscious, resting brain (along with the frontal cortex and the thalamus)[100]; thus, one needs to be cautious about the interpretation of 'absolute' changes. Interestingly, the polymodal association cortices tend to be affected more profoundly than the primary and secondary sensory cortices. This functional dissociation between unimodal and polymodal cortices implies that during sleep the brain can respond to external stimuli (such as loud noises) but lacks the higher levels of processing that are necessary to make meaningful sense of the input. The precuneus, for example, on the medial aspect of the parietal lobe, is thought to be involved in a range of highly complex tasks including the continuous monitoring of the world around us[101], and is profoundly deactivated during deep sleep and anaesthetic-induced unconsciousness. The message to be taken from this body of work on whole-brain imaging is that the final states of 'unconsciousness' during deep sleep or anaesthesia are remarkably similar.

Although the overall picture of anaesthetic-induced LOC might resemble deep sleep, what does this tell us about how anaesthetics exert their effects? For example, the patterns of neuronal activity during xenon and propofol anaesthesia are reasonably similar, yet, as discussed above, these two anaesthetics have different (probably mutually exclusive) molecular targets. They might, however, affect common neuronal pathways.

A common finding in all of the human imaging studies is that the thalamus is deactivated during anaesthesia. The thalamus is the major gateway for the flow of sensory information from the periphery into the cortex[102–104] and can switch between a state that allows the flow of ascending information and one that essentially isolates the cortex from the environment (FIG. 4). The disruption of thalamocortical connectivity, and hence of information transfer from the periphery to the cortex, might be an essential common feature of anaesthetic action[91,95,105].

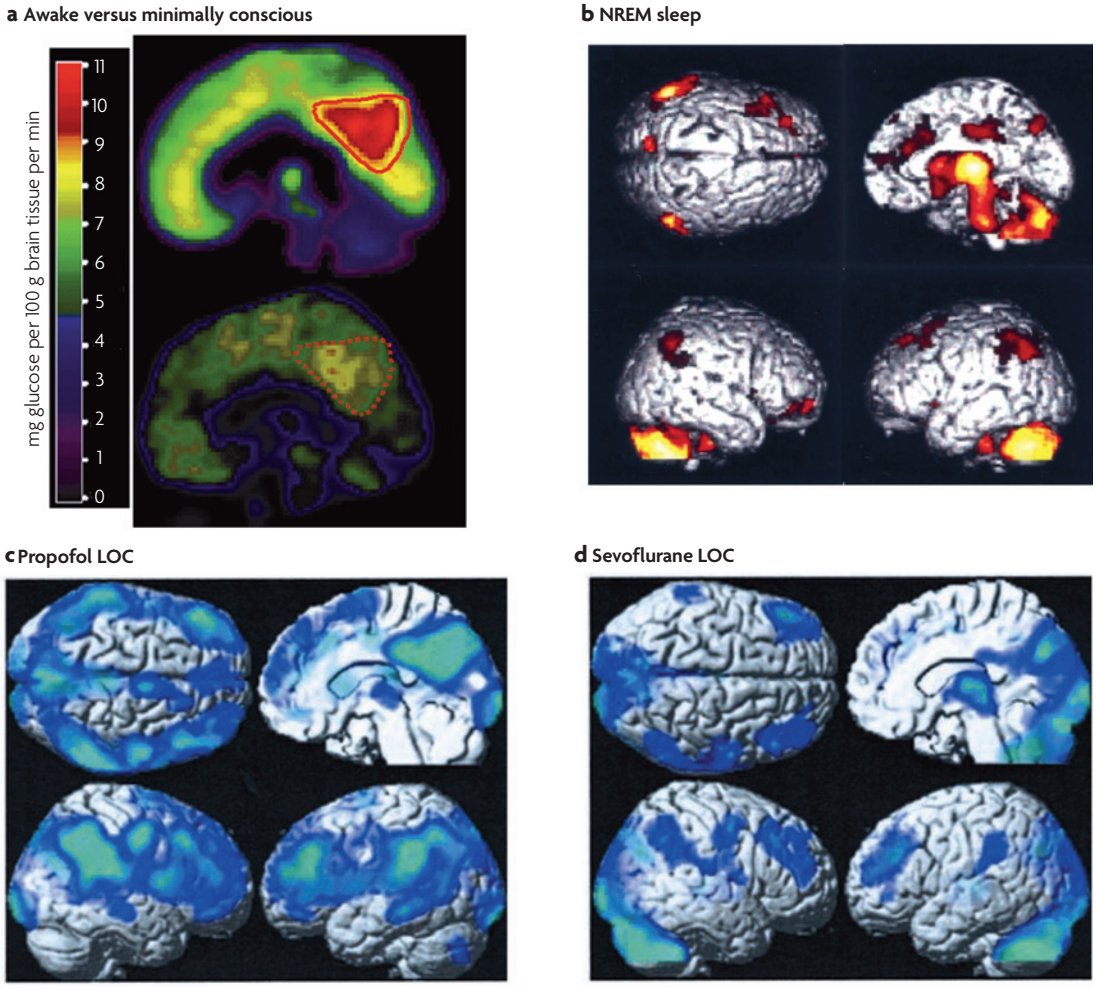


Figure 3 | **Functional brain imaging reveals similarities between anaesthetic-induced loss of consciousness and deep natural sleep. a** | The top image is of cerebral metabolism in an awake, conscious individual. The highest levels of activity are in the precuneus and the posterior cingulate cortex, but high activity is also observed in the thalamus and in other cortical areas[100]. The bottom image is of cerebral metabolism in a brain-damaged, minimally conscious individual. The lack of conscious awareness is reflected in the low metabolism, particularly in the precuneus and the posterior cingulate cortex[100]. **b** | The largest decreases in relative cerebral blood flow (rCBF; shown as a red–yellow scale) during deep non-rapid-eye-movement (NREM) sleep are in the thalamus, the brainstem and the basal ganglia. There is also deactivation in regions of the frontal and parietal cortices, particularly the anterior cingulate and orbitofrontal cortices, and in the precuneus and the posterior cingulate cortex[97,98]. **c** | Decreases in rCBF (shown as a blue–green scale) resulting from propofol administration at concentrations that induce loss of consciousness (LOC) (3.7 ± 0.7 μg/ml plasma). The major changes occur in the thalamus, the precuneus and the posterior cingulate cortex, the cuneus and the frontoparietal cortex[93]. **d** | Decreases in rCBF (shown as a blue–green scale) caused by sevoflurane administration at concentrations that induce LOC (end-tidal concentration of 1.5 ± 0.3% atm). The major changes occur in the thalamus, the precuneus and the posterior cingulate cortex, the cuneus, the frontoparietal cortex and the cerebellum[93] . Part **a** reproduced, with permission, from REF. 100 © (2004) Elsevier Science. Part **b** reproduced, with permission, from REF. 98 © (1999) Society for Neuroscience. Parts **c** and **d** modified, with permission, from REF. 93 © (2003) American Society of Anesthesiologists.

The thalamus and cortex in sleep and anaesthesia

The thalamic switch and the EEG. During wakefulness, excitatory input to thalamocortical (TC) neurons from the arousal nuclei causes a persistent depolarization, allowing sensory information to progress more-or-less faithfully to the cortex. During the transition from wakefulness to sleep, 'spindle waves' (oscillations at 7–14 Hz that occur every few seconds) appear in the electroencephalogram (EEG). These spindles are generated by the thalamus[102,103,106] and involve a reverberation between the TC neurons and inhibitory GABAergic reticular (RT) neurons that are found in a shell-like structure that covers the anterior and lateral aspects of the dorsal thalamus. Network effects govern the cortical synchronization of spindles and their waxing and waning. Spindling causes a decorrelation between sensory input to TC neurons and the neurons' output to the cortex[107]. As deep NREM sleep develops, thalamic neurons enter a persistent,

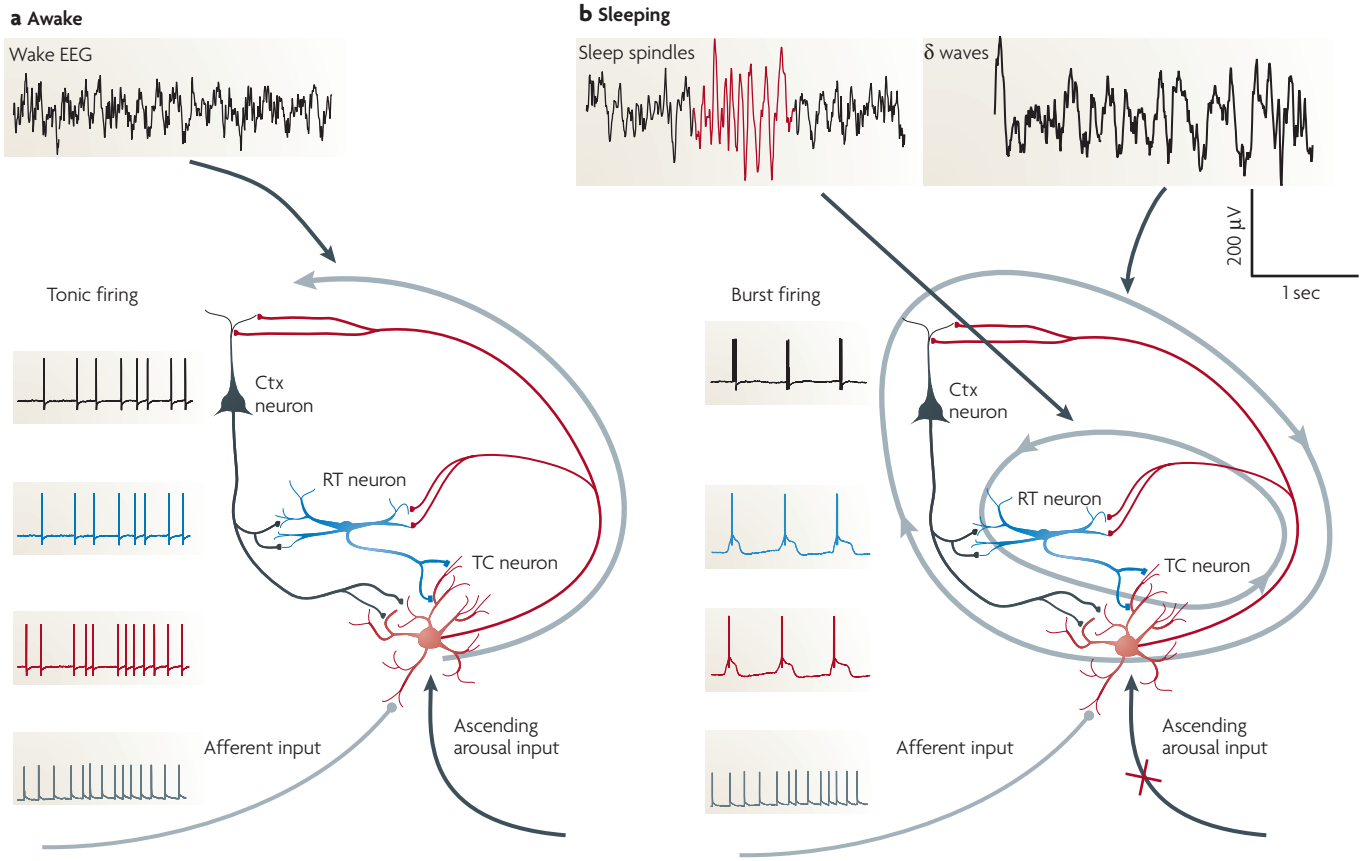


Figure 4 | **Thalamic oscillations. a** | During wakefulness, ascending excitatory input from arousal nuclei to thalamocortical (TC) neurons (red) provides a depolarizing drive that causes thalamocortical neurons and reticular (RT) neurons (blue) to exhibit single-spike tonic firing and allows a more-or-less faithful transfer of information from the periphery up to cortical (Ctx) neurons (black). During wakefulness there is also a descending depolarizing drive onto TC neurons from Ctx neurons. **b** | During deep non-rapid-eye-movement (NREM) sleep, the thalamic relay neurons switch into a burst-firing mode which they adopt by default in the absence of external input. The intrinsic ionic conductances of TC neurons favour a rhythmic burst-firing pattern, which is generated following a hyperpolarizing drive[102,103,106]. Because of the extensive connectivity that exists among and between thalamic and Ctx neurons, large populations of neurons are induced to fire in synchrony; this is the origin of the slow delta (δ) waves that are the electroencephalographic signature of deep sleep. During this burst-firing mode, ascending information through the thalamus is blocked. The transition from waking to sleeping also involves thalamic oscillations. In the electroencephalogram (EEG) these are called sleep spindles (highlighted in red on the left-hand EEG trace); they are generated when a burst of spikes from a TC neuron impinges on a GABA (γ-aminobutyric acid)-ergic RT neuron which then sends a robust inhibitory postsynaptic potential back to the same TC neuron. This hyperpolarizes the cell, which then fires another barrage of spikes on rebound, establishing an oscillation. The length of the inhibitory potential (which is mediated by GABA type A receptors) determines the time until another burst of spikes is generated by the TC neuron[103,106] and sets the frequency at ~7–14 Hz. Although the TC–RT loop is necessary for spindle oscillations, isolated RT neurons can also oscillate with a natural frequency in the same frequency range, and this property might aid spindle generation. (For a more complete description of the mechanisms that underlie thalamic oscillations, see REFS. 102,103,106.)

low-frequency bursting mode. Because of extensive lateral connectivity, this bursting pattern spreads to large groups of thalamic and cortical neurons and gives rise to the large-amplitude synchronous delta oscillations (with frequencies of ~1–4 Hz) that are characteristic of deep sleep. This sustained and widespread rhythmic activity essentially blocks ascending information flow. The bursting pattern is generated by a thalamocortical loop, with the frequency being set by the intrinsic conductances (I_T and I_h) present in the TC neurons[106]. The deactivation of the thalamus that is observed in human imaging studies during the progression from wakefulness to deep sleep is highly correlated with both spindle and delta waves in the EEG[108].

Anaesthetic actions on the thalamus. Other than the obvious similarity between sleep and anaesthesia — that is, that subjects are apparently oblivious to their environment — the most long-standing reason for believing that sleep and anaesthetic-induced LOC might share common neuronal mechanisms is the fact that most anaesthetics (ketamine is the most striking exception) produce EEG patterns that are reminiscent of both spindle and delta waves, the EEG landmarks of falling

Somatosensory-evoked potentials
Electrical signals generated by the nervous system following a mechanical or, more usually, an electrical stimulation in the periphery that reflect sequential activation of neural structures along the somatosensory pathways.

Inhibitory shunt
Inhibition of excitatory inputs owing to an increase in neuronal membrane conductance.

asleep and deep, dreamless NREM sleep, respectively. Generally, anaesthetics initially produce high-frequency oscillations followed by a lower frequency, higher amplitude EEG pattern at or beyond the point at which consciousness is lost[109–113].

The generation of these oscillations obviously suggests actions at, or at least involving, the thalamus. A substantial body of literature on the effects of anaesthetics on somatosensory-evoked potentials in humans supports the idea that information transfer through the thalamus is disrupted, with the 'nonspecific' nuclei being most sensitively affected. However, virtually all attempts to directly demonstrate in animals, using *in vivo* electrophysiology, that anaesthetics interfere with thalamic gating of ascending sensory information have first deeply anaesthetized the animals and then studied the effects of additional anaesthetic[105,114,115]. None of these studies addressed the question of how the animal was initially anaesthetized[115].

Nonetheless, thalamic neurons are almost certainly involved, because anaesthetics can generate spindles and delta waves in the EEG; however, these thalamic neurons must first be hyperpolarized[106]. Anaesthetics can directly hyperpolarize thalamocortical neurons by activating 2PK channels[56,116,117] and/or by potentiating $GABA_A$ receptors[15,118], and both effects might be important. Anaesthetic-activated TASK channels that are sensitive to volatile agents seem to be present in TC neurons[117], as are extrasynaptic $\alpha_4\beta_2\delta$ $GABA_A$ receptors[15,118], which are sensitive to etomidate[15] and gaboxadol[15,118]. Although the hyperpolarization of TC neurons can account for both the generation of delta oscillations and the consequent impairment of information transfer through the thalamus, the effects of anaesthetics on TC neurons (which cause inhibition of I_T[119] or I_h[86] or which enhance the membrane conductance[56] and cause an inhibitory shunt) can account for only the latter.

Anaesthetic actions on the cortex. The thalamus has many diverging projections to the cortex, with individual thalamic nuclei modulating the activities of specific cortical regions. However, the cortex also sends projections back to the thalamus (in the so-called corticofugal pathway[120]). These corticothalamic projections greatly outnumber the thalamocortical projections. Thus, when the brain is in an activated, wakeful state, the corticofugal pathway (which is exclusively excitatory) provides a tonic depolarization of the thalamocortical neurons, tending to prevent them from entering synchronized, oscillatory states. This provides some degree of positive feedback, because if the TC neurons enter into an oscillatory mode as a result of a diminished excitation of the arousal pathways, then the tonic corticofugal excitation will also be reduced, further favouring synchronous oscillation. Anaesthetics could act, at least in part, by inhibiting cortical neurons and thus favouring TC burst firing and a sleep-like state.

The major anaesthetic targets that have been discussed are all highly expressed in cortical neurons. Moreover, in both acute brain slices[121] and cultured organotypic slices[122], the firing of cortical neurons shows a degree of anaesthetic sensitivity that is comparable to that which is seen *in vivo*. However, the extent to which isolated *in vitro* preparations accurately reflect the true sensitivity of cortical networks remains uncertain. Although the thalamus might control the state of consciousness, processing in the cortex is responsible for the detailed content of consciousness, and during both deep sleep and anaesthetic-induced LOC the cortex is profoundly deactivated. Cortical inhibition could be driving anaesthetic-induced LOC as, in Parkinson's patients, changes in the cortical EEG were larger and more abrupt than those seen in a subthalamic electrode during the induction of anaesthesia with either propofol or sevoflurane[123]. This is an interesting observation, but the conclusion that the cortex is the key target is premature until the functional relationship between these two signals is better understood. To what extent cortical inhibition actually causes anaesthetic-induced LOC remains an open question.

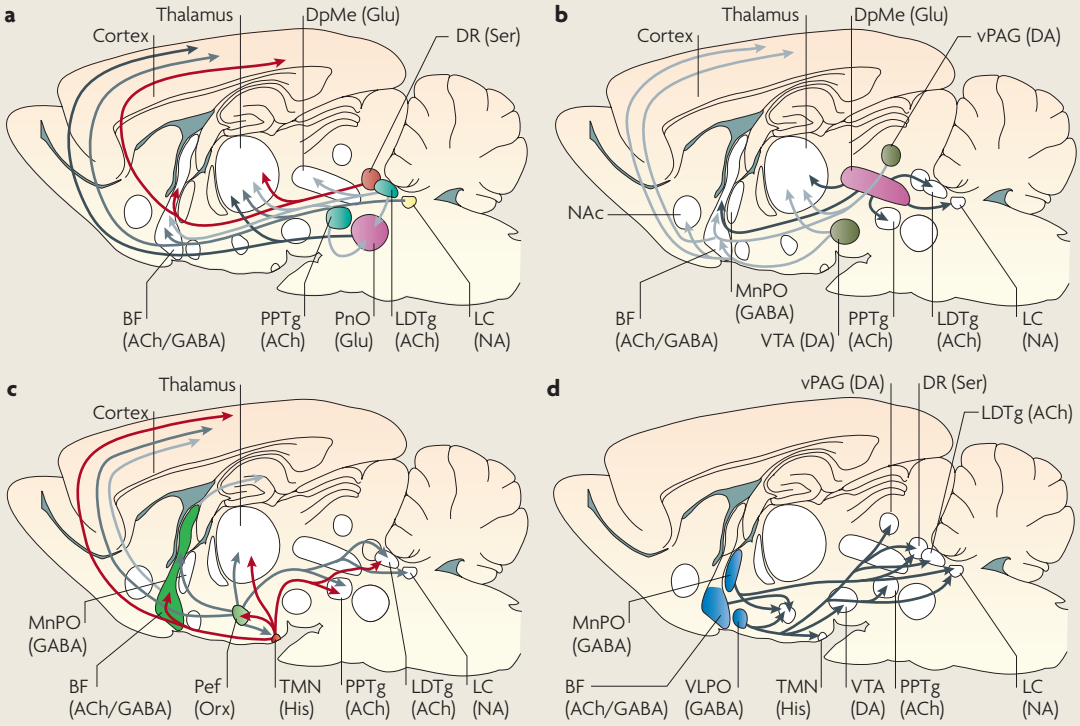
An alternative to anaesthetics acting directly at either cortical or thalamic neurons is the possibility that they inhibit excitatory arousal pathways or potentiate the sleep pathways that control them. Next I consider the evidence that supports both of these possibilities.

Arousal and sleep pathways

Because of the self-evident evolutionary importance of remaining alert during normal waking, several parallel ascending neuronal pathways have developed to promote and sustain cortical arousal, with ventral pathways through the hypothalamus and basal forebrain and a dorsal pathway through the thalamus (BOX 3). In addition, several neuronal populations have been identified as being sleep-active and are involved in promoting and maintaining natural sleep (part d of the figure in BOX 3). The first to be identified were neurons in the ventrolateral preoptic area (VLPO)[124,125], which provides inhibitory control of many of the arousal nuclei. These cells mainly release GABA, but some also release the small inhibitory peptide galanin. VLPO neurons increase their firing just before the onset of EEG synchronization and progressively increase their activity with sleep depth, a pattern that is consistent with them being involved in both causing and stabilizing natural sleep[126]. A second population of GABA-releasing neurons in the median preoptic nucleus (MnPO)[127] also displays enhanced firing during both REM and NREM sleep[128], with firing increasing in anticipation of sleep but then slowly declining during prolonged periods of NREM sleep, implying a role for these neurons in sleep initiation[129]. Another group of sleep-active GABAergic neurons[130] is interspersed among cholinergic cells in the magnocellular regions of the basal forebrain[131]. As with VLPO neurons, the firing of these neurons is associated with sleep depth, but in this case the firing rates are higher during NREM sleep than during REM sleep[132].

These findings suggest that sleep initiation and maintenance is an active process that exerts inhibitory control over the ascending arousal nuclei, predominantly through GABAergic inhibition from the hypothalamus and the basal forebrain. Importantly, arousal nuclei can also send reciprocal inhibitory feedback to the sleep-promoting

Box 3 | **Neuronal pathways of sleep and arousal**



Several neuronal pathways have evolved to maintain cortical activation and behavioural arousal during normal waking, and others have evolved to promote and maintain natural sleep by providing inhibitory control of this arousal network. The figure illustrates the most important arousal and sleep pathways in a representative parasagittal section of rat brain. Part **a** shows arousal nuclei in the pons. Cholinergic neurons in the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg)[141] display dense innervation[140, 202,203] of the nonspecific intralaminar and midline nuclei and the thalamic reticular nucleus. In addition to this thalamic pathway, brainstem cholinergic neurons also project to other arousal nuclei, including the deep mesencephalic reticular formation (DpMe), the oral pontine nucleus (PnO), regions of the prefrontal cortex and magnocellular cholinergic nuclei of the basal forebrain (BF)[204]. Another important arousal nucleus in the pons is the noradrenergic locus coeruleus (LC). In addition to causing excitation through α1 and β1 adrenoceptors, arousal is enhanced by inhibition of sleep-promoting neurons in the BF[130] and preoptic areas[135,205], through activation of α2 adrenoceptors. The LC also contributes to cortical activation through the dorsal thalamic route but, in addition to innervating the nonspecific intralaminar and midline thalamic nuclei, there is a complex pattern of noradrenergic innervation of specific and nonspecific thalamic nuclei[206–208]. The dorsal raphe (DR) in the medulla sends a large projection to the cortex and also sends projections to the basal forebrain and other arousal nuclei[209]. In addition, there are several projections to midline and intralaminar nuclei in the thalamus[209]. Part **b** of the figure shows arousal nuclei in the midbrain. The glutamatergic DpMe nuclei and the dopaminergic nuclei in the ventral tegmental area (VTA) and the ventral periaqueductal grey (vPAG) are the most important midbrain arousal nuclei. Dense projections from the DpMe excite thalamocortical neurons in the midline and intralaminar nuclei of the thalamus[208] and also innervate the basal forebrain[207]. Projections to the cholinergic PPTg and LDTg, the noradrenergic LC and the medullary/pontine reticular formations serve to reinforce arousal-promoting excitation[207]. Dopaminergic neurons in the VTA cause arousal through the thalamus, the basal forebrain, the nucleus accumbens (NAc) and the cortex, and a recently described[162] dopaminergic pathway from the vPAG also causes arousal by innervating the thalamus, the basal forebrain and the cortex (in addition to other nuclei)[162]. Part **c** of the figure shows arousal nuclei in the hypothalamus and basal forebrain. Orexinergic neurons in the perifornical area (Pef) of the lateral hypothalamus project diffusely to the entire neuroaxis, with projections to other arousal nuclei, the thalamus and the cortex[210]; orexins (Orx) excite nonspecific thalamocortical neurons[211]. A similarly diffuse pattern of innervation emerges from the histaminergic neurons of the tuberomammillary nucleus (TMN) and ascends through the dorsal and ventral pathways[212,213]. Several thalamic midline/intralaminar nuclei are densely innervated, as are the anterior nuclear group and the mediodorsal thalamic nucleus[212]. Neurons in the BF provide a complex pattern of innervation to the limbic system and the cortex[166]. In addition to the predominantly cortical innervation of BF cholinergic neurons, these cells also target select areas of the thalamus and provide a mainly excitatory drive through nicotinic acetylcholine (Ach) receptors and muscarinic M1 receptors. Part **d** of the figure shows sleep-promoting pathways. The major sleep-promoting centres lie in the anterior hypothalamus and the basal forebrain. The ventrolateral preoptic nucleus (VLPO) sends dense inhibitory innervation to the TMN and to most of the other arousal-promoting nuclei[125,214]. Efferents from the median preoptic area (MnPO) are less well described, but a major inhibitory projection to orexinergic neurons in the Pef has been demonstrated[128,215], as have projections to the LC and the DR[216]. GABA (γ-aminobutyric acid)-ergic neurons from the BF (perhaps those that are sleep-active[130,166]) also provide inhibitory input to the orexinergic neurons of the Pef[217] and to the midbrain and the brainstem[166,218]. DA, dopamine; Glu, glutamate; His, histamine; NA, noradrenaline; Ser, serotonin.

nuclei[133–136]. This means that when the arousal nuclei are inhibited this positive feedback disinhibits the sleep-promoting centres, further enhancing their firing. This results in a bi-stable system that can only flip-flop between sleeping and waking and cannot normally rest in some intermediate state[134].

Because anaesthesia, like sleep, induces a state of reduced arousal and responsiveness, it is a reasonable idea, *a priori*, that common neuronal pathways are involved. The importance of anaesthetic-sensitive $GABA_A$ receptors and 2PK channels in sleep and arousal pathways might account for some of the similarities between sleep and anaesthetic-induced LOC. The observation that sleep deprivation seems to enhance anaesthetic potency[137], the separate observation that it is relieved by prolonged anaesthesia[138] and the fact that both sleep and anaesthesia promote hypothermia[127] all add weight to the idea that sleep and anaesthesia have some common mechanisms[139]. Some direct evidence comes from work on specific neuronal pathways.

Arousal nuclei and anaesthesia. Since the classic work of Moruzzi and Magoun, brain stem nuclei have been known to be essential in maintaining cortical arousal (part a of the figure in BOX 3). Pontine cholinergic neurons in the pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus provide most of the cholinergic innervation of the thalamus and excite TC neurons either directly (through nicotinic and muscarinic M1 receptors) or indirectly by inhibiting GABAergic RT neurons (through muscarinic M2 receptors), leading to generalized cortical activation[140,141]. These cholinergic nuclei also innervate the glutamatergic oral pontine reticular formation (PnO) and the glutamatergic deep mesencephalic reticular nucleus (DpMe), both of which send excitatory input to the forebrain and the thalamus (parts a and b of the figure in BOX 3). These nuclei, together with the cholinergic input from the pons, have been described[142] as the backbone of the ascending reticular formation.

Evidence that these nuclei could be involved in anaesthetic-induced LOC comes from studies which show that halothane-induced EEG spindles are antagonized when carbachol is injected into the PnO[110]. Moreover, nicotine injected into the thalamic central medial nucleus can overcome sevoflurane LORR[143], and physostigmine (an acetylcholinesterase inhibitor) can reverse propofol LOC[144] (this can be blocked by the muscarinic antagonist scopolamine[144]). However, muscarinic receptors are insensitive to propofol[145], and although volatile agents sensitively inhibit neuronal nicotinic ACh receptors[146,147], blocking this receptor *in vivo* has no apparent effect on LORR[148]. Thus, although cholinergic pathways might be causally involved in anaesthetic-induced LOC, these experiments might only demonstrate that anaesthetic-induced LOC can be reversed by a sufficiently powerful activation of arousal mechanisms.

More direct evidence for the involvement of targets in the PnO and/or in the neighbouring DpMe comes from studies which showed that GABAergic agents microinjected into these regions cause LORR and EEG synchronization (as well as descending atonia)[149,150]. As with virtually all microinjection studies, however, high drug concentrations were used, and there are reports of bilateral lesions in this region causing irreversible coma following stroke[151]. Thus, the relevance of these findings to the actions of systemically administered anaesthetics remains to be seen.

The other key pontine nucleus that promotes cortical activation and behavioural arousal is the noradrenergic locus coeruleus (LC). Neurons in this region fire most frequently during waking, quieten during NREM sleep and are silent during REM sleep[152]. Stimulation of the LC leads to activation of the cortical and the hippocampal EEG [153], an effect that is probably mediated by noradrenaline exciting cholinergic neurons in the septo-hippocampal system[154] (an area that, when inactivated by local muscimol injections, greatly increases anaesthetic potency[155]). LC neurons are hyperpolarized by halothane[57] (owing to TASK-channel activation) and are also a major site of action for the selective α2 agonist dexmedetomidine[156], although recent evidence[157] suggests that some of its effects are mediated by hypothalamic sleep pathways (see below).

A medullary nucleus that has long been associated with sleep and arousal is the serotonergic dorsal raphe nucleus. Its actions are complex and somewhat controversial, but there is little doubt that one of its functions is to promote arousal through pathways to the thalamus, the basal forebrain and the cortex[158]. As with the LC, raphe neurons are inhibited by anaesthetic-activated TASK channels[58].

Midbrain dopaminergic nuclei (part b of the figure in BOX 3), such as the ventral tegmental area and the substantia nigra pars compacta, are also often thought to contribute to arousal, and both pathological and pharmacological disruption of dopaminergic pathways are known to have major effects on the sleep–wake cycle[159,160]; however, establishing a causal relationship with behavioural arousal has been problematic. Moreover, anaesthetic effects on firing rates are modest[161]. Recently, wake-active neurons in the ventral periaqueductal grey have been identified[162] that, when lesioned, cause a significant increase in total sleep; this brain area seems to provide a major ascending dopaminergic pathway for arousal. There are no reports of anaesthetic effects on this system.

The basal forebrain (part c of the figure in BOX 3) consists of a heterogeneous set of structures in the ventral forebrain that have mixed neuronal populations and diverse functions. Cholinergic neurons send monosynaptic projections to the entire neocortex and limbic system and are widely believed to produce cortical activation and behavioural arousal. Acetylcholine levels in the cortex are elevated during waking and REM sleep[163] (compared with NREM sleep), and stimulation of the basal forebrain causes an increase in cortical acetylcholine together with a desynchronization of the EEG[164,165]. Interspersed among the cholinergic neurons are several other cell types, including GABAergic neurons that are thought to be sleep-promoting[130, 166]. This heterogeneity of sleep- and wake-active neurons extends to the adjacent medial preoptic area[130], where injections of pentobarbital or propofol cause small but significant increases in NREM sleep[167,168].

Encephalitis lethargica
A condition that affected millions of people in the 1920s and which was first described by Constantin von Economo. It was characterized by a high fever, profound lethargy and many other neurological changes that, in extreme cases, led to irreversible coma. It was sometimes described as 'sleeping sickness'.

c-fos
An immediate-early-gene product that can be upregulated by various cellular stimuli, including neuronal excitability. It is sometimes used as a surrogate measure of neuronal activity.

The importance of the posterior hypothalamus (part c of the figure in BOX 3) in maintaining wakefulness was first postulated by von Economo, who noted lesions in this area in the brains of encephalitis lethargica patients. The key nucleus involved is the tuberomammillary nucleus (TMN)[169], which is the sole source of histamine in the CNS[170]. The importance of this neurotransmitter for arousal was originally noted because of the sedative side-effects of antihistamines. Histamine levels in the brain are elevated during waking[171], as is activity in TMN neurons[172,173]. The release of histamine causes neuronal excitation, mainly through G-protein-coupled receptors (H_1 and H_2). During sleep, firing in the TMN decreases markedly[172,173] owing to GABAergic inhibition by sleep-active VLPO neurons. It has recently been discovered that this same pattern of firing pertains during anaesthetic-induced LOC[157,174], consistent with the marked reduction in brain histamine levels that occurs during anaesthesia[175]. Bilateral microinjection of the $GABA_A$ receptor agonist muscimol into the TMN caused LORR, whereas injection of the $GABA_A$ receptor antagonist gabazine during anaesthesia antagonized LORR, suggesting that this pathway is causally involved[157, 174]. However, injection of propofol itself into the TMN caused sedation but not LORR, showing that other pathways must also be required.

One candidate is the orexinergic pathway from the lateral hypothalamus[176,177]. Orexins (A and B) are small peptides that are released exclusively from the lateral hypothalamus, particularly the perifornical area, and defects in this system are thought to have a pivotal role in narcolepsy[178]. Neuronal firing and c-fos expression in orexinergic neurons is high during wakefulness compared with during NREM or REM sleep[179,180]. These cells cause widespread excitation throughout the brain by acting on either one or both of the known G-protein-coupled orexin receptors (OX_1R and OX_2R), which would then reinforce wakefulness. Intracerebroventricular administration of orexin A induces wakefulness at the expense of both REM and NREM sleep[181]; however, arousal is probably mediated by the histaminergic system, because orexin A has no effect on H_1-receptor-knockout mice[182].

Conclusions

Progress at the molecular level in identifying a relatively small number of targets for general anaesthetics is beginning to influence ideas about how these drugs might act on neuronal pathways, and there is growing appreciation that specificity at the molecular level might extend to specificity at the level of neuronal networks. The clear similarities between anaesthetic-induced LOC and natural sleep are caused, in large part, by the deactivation of the thalamus that occurs in both conditions, leading to a similar pattern of cortical inhibition. There are several possible explanations for this thalamic deactivation, including direct actions on thalamocortical and/or cortical neurons, inhibition of arousal pathways and potentiation of sleep pathways. The relative importance of each of these mechanisms remains to be determined. An explanation for the sudden switch between consciousness and unconsciousness might lie in the intrinsic bi-stability of thalamocortical neurons, together with the reciprocal inhibitory connections that exist between hypothalamic sleep-promoting centres and the arousal nuclei in the midbrain and the brainstem, which would tend to favour rapid transitions between high and low states of ascending arousal.

Future work aimed at developing a better understanding of the fundamental mechanisms of natural sleep and arousal and how the relevant neuronal pathways are affected by general anaesthetics should lead to clearer answers to the question of how these unique drugs cause LOC.

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Acknowledgements
I thank S. Brickley and A. Zecharia for many helpful and stimulating discussions during the writing of this Review and their assistance in the preparation of the figures, P. Brick and S. Curry for help with the crystallographic images and C. Robledo for providing the EEG data in figure 4. I also thank the Medical Research Council UK for support.

DATABASES

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