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THEME ISSUE: MECHANISMS OF ANESTHESIA

Sleep and general anesthesia
Le sommeil et l'anesthésie générale

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Abstract

Purpose *The mechanisms through which general anesthetics cause reversible loss of consciousness are characterized poorly. In this review, we examine the evidence that anesthetic-induced loss of consciousness may be caused by actions on the neuronal pathways that produce natural sleep.*

Principal findings *It is clear that many general anesthetics produce effects in the brain (detected on electroencephalogram recordings) that are similar to those seen during non-rapid eye movement non-(REM) sleep. Gamma aminobutyric acid (GABA)ergic hypnogenic neurons are thought to be critical for generating non-REM sleep through their inhibitory projections to wake-active regions of the brain. The postsynaptic $GABA_A$ receptor is a major molecular target of many anesthetics and thus may be a point of convergence between natural sleep and anesthesia. Furthermore, we also present growing evidence in this review that modulating wake-active neurotransmitter (e.g., acetylcholine, histamine) release can impact on anesthesia, supporting the idea that this point of convergence is at the level of the brain arousal systems.*

Conclusions *While it is clear that general anesthetics can have effects at various points in the sleep-wake circuitry, it remains to be seen which points are true anesthetic targets. It will be challenging to separate non-specific effects on baseline arousal from a causal mechanism. Sophisticated experimental approaches are necessary to address basic mechanisms of sleep and anesthesia and should advance our understanding in both of these fields.*

Résumé

Objectif *Les mécanismes par lesquels les anesthésiques généraux induisent une perte de conscience réversible sont mal caractérisés. Dans ce compte-rendu, nous examinons les données probantes qui soutiennent que la perte de conscience induite par l'anesthésie pourrait être provoquée par des actions sur les voies neuronales qui produisent le sommeil naturel.*

Constatations principales *Il est clair que plusieurs anesthésiques généraux produisent des effets sur le cerveau (tels que détectés lors de l'enregistrement d'électroencéphalogrammes) qui ressemblent à ceux observés pendant le sommeil lent. On pense que les neurones hypnogènes GABAergiques sont des éléments cruciaux pour la génération du sommeil lent en raison de leurs projections inhibitrices dans les régions du cerveau actives à l'éveil. Le récepteur $GABA_A$ post-synaptique est une importante cible moléculaire de plusieurs anesthésiques et pourrait par conséquent constituer un point de convergence entre le sommeil naturel et l'anesthésie. De plus, nous présentons aussi dans ce compte-rendu des données probantes de plus en plus nombreuses qui suggèrent que la modulation de la libération des neurotransmetteurs actifs à l'éveil (par ex., acetylcholine, histamine) pourrait avoir un impact sur l'anesthésie, ce qui corrobore l'hypothèse selon laquelle ce point de convergence se situe au niveau des systèmes d'éveil du cerveau.*

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Conclusion *Alors qu'il est clair que les anesthésiques généraux peuvent avoir des effets sur divers points du circuit veille-sommeil, les points constituant de véritables cibles anesthésiques restent à être déterminé. Le défi sera de pouvoir distinguer les effets non spécifiques sur le réveil de base des mécanismes causaux. Il faudra utiliser des approches expérimentales perfectionnées pour aborder les mécanismes de base du sommeil et de l'anesthésie. De telles approches devraient faire progresser notre compréhension de ces domaines.*

Since the introduction of anesthetic drugs in the 1840's, sleep has been used as a metaphor for the state of general anesthesia. It is not difficult to understand why. Sleep is natural; it is evidently beneficial and restorative, and it is a state we enter willingly each day. Most patients are reassured by the metaphor, which is why anesthesiologists have persisted with its use in their day to day practice and in the logos and crests of their societies and associations. This first appeared with the introduction of the Greek God of Sleep, Hypnos, (Somnus in Latin) on the crest of the Canadian Anaesthetists' Society (now the Canadian Anesthesiologists' Society) in 1943 (Fig. 1). The crest was designed by the Canadian anesthesiologist, Wesley Bourne, one of the founders of the McGill Department of Anesthesia. Hypnos is shown holding a horn from which he is pouring a sleeping potion made from poppies (clutched in his other hand) onto a bunch of thistles that represent pain and arousal. Since ancient times, poppies (*Papaver somniferum* – the bringer of sleep) have been known to possess sleep-inducing medicinal properties. The Greek motto can be translated as, "We watch closely those who sleep".

In 1945, the theme of sleep and poppies was continued in the crest of the Association of Anaesthetists of Great Britain and Ireland with their motto, "Safe in Sleep", and with the depiction of poppy seed cases on the shield. In addition to Hypnos, the crest also incorporates the God of Dreams, Morpheus. The design of this crest strongly influenced the design of the Royal College of Anaesthetists' coat of arms fashioned in 1989. While any explicit mention of sleep is dropped in the design of the coat of arms, it retains its representation by featuring poppy seed cases on the shield.

Fig. 1 Crest of the Canadian Anaesthetists' Society (1943)

The purpose of this article is to examine whether the relationship between sleep and anesthesia is more than metaphorical by determining whether general anesthetics act on any of the neuronal pathways that are responsible for inducing or maintaining natural sleep.

Sleep and anesthesia – What do they mean?

The definition of sleep is relatively non-contentious, i.e., a naturally occurring, periodic state of rest during which consciousness of one's environment and responses to external stimuli are largely suspended. Further refinement of this definition is required when considering that two qualitatively different states occur during natural sleep. One state is characterized by a dimmed state of consciousness and brain activity with normal motor movement, while the other state is characterized by heightened brain activity accompanied by a paralysis of motor movement. This latter state occurs during dreaming, and the rapid eye movements that accompany it give it its name – rapid eye movement (REM) sleep. During REM sleep, the brain is alive with mental imagery, and descending atonia prevents any associated motor activity. In contrast, non-REM sleep is characterized by greatly diminished brain activity and lack of conscious awareness, and it is usually graded in terms of four levels of "depth".

Definitions of anesthesia and anesthetics have been more contentious. This controversy is mainly due to a desire to define a patient's ideal state of anesthesia (in terms of consciousness, immobility, analgesia, amnesia, and muscle relaxation) depending on the patient's medical status and the anesthesia requirements of the procedure in question. In reality, however, no single drug is used to achieve this state, because no single drug incorporates all of these theoretically ideal attributes. Although this explanation is somewhat circular, if we define anesthesia as the state induced by each of the drugs we currently classify as general anesthetics, then two common features emerge - loss of consciousness and loss of response to a painful stimulus. Thus, a commonsense definition of a general anesthetic would be a drug that produces a reversible loss of consciousness at low concentrations and a loss of response to a painful stimulus at higher concentrations.

In this article, our consideration of the similarities between natural sleep and anesthesia are focused entirely on the similarities between non-REM sleep and anesthetic-induced loss of consciousness. The clinical significance of trying to understand the basic mechanisms underlying anesthetic-induced loss of consciousness are obvious.

Assessing depth of anesthesia represents a major clinical challenge. The fearful consequences of awareness during anesthesia result from situations where the patient was not sufficiently deeply anesthetized. As relevant are the negative consequences of using excessively high doses of anesthetics. Understanding the neuronal mechanisms pertaining to the anesthetic concentrations where consciousness is lost or gained may be of central importance for the accurate titration of anesthetic drugs in the clinical setting. In addition, if sleep and anesthesia share common neuronal pathways, it may be possible to develop improved drugs that provide safer anesthesia and possibly even provide some of the restorative benefits of natural sleep.

How is sleep initiated and maintained?

The current generally accepted view that sleep is an active state can be traced back almost 100 years to the histological studies of the Viennese neurologist and psychiatrist, Constantin von Economo.[1] Post-mortem studies on patients suffering from *encephalitis lethargica*, a disorder of excessive sleep, showed massive cell loss in what are now known to be brain arousal regions, i.e., the posterior hypothalamus and the brainstem ascending reticular activating system (ARAS). Conversely, those suffering from pronounced insomnia showed lesions in regions, i.e., the preoptic hypothalamus and the basal forebrain, that have been characterized subsequently as hypnogenic.

However, for the ensuing 30 years, sleep was widely considered a passive state caused by the "functional deafferentation" of excitatory inputs to the forebrain. In truth, this was actually a theory of wakefulness, because the brain areas highlighted by various transection studies (e.g., Bremer's *encéphale* and *cerveau isolé* preparations)[2] went a long way towards defining arousal pathways such as the ARAS. In fact, the basic tenet that sleep results from the removal of such excitation is central to current thinking; however, early studies assumed a passive waning of activity rather than what is now known to be an active "off switch". The discovery of REM sleep,[3-5] characterized by the apparent paradox of behavioural sleep in the presence of an awake-looking cortex, helped to change this view. Evidence of a sleep "switch" first emerged from Moruzzi *et al.* in a series of experiments (reviewed by Bremer)[2] in which they reported a permanent state of wakefulness following transection of— and later barbiturate application to— the caudal brainstem (subsequently defined as within the nucleus of the solitary tract [NTS]) of sleeping rats, suggesting inhibition of a hypnogenic region.

Evidently, the NTS was not the hypnogenic centre predicted by the histological studies of von Economo. It is an understudied region with respect to sleep-wake control, but it does appear to contain spontaneously sleep-active neurons.[6] However, as suggested by von Economo, experiments performed by Sterman and Clemente in 1962 demonstrated that electrical stimulation of forebrain preoptic areas was able to rapidly and reversibly induce behavioural signs and electroencephalogram (EEG) correlates of sleep.[7,8] This result was further supported by the finding that lesions in this region produced insomnia.[9] In fact, such experiments had been performed previously (reviewed by Bremer)[2] but had not made an impact.

The forebrain preoptic area is not a homogenous brain nucleus but a collective term encompassing the preoptic area itself (including the medial preoptic nucleus), the median preoptic nucleus, the ventrolateral preoptic area (VLPO), and the lateral preoptic zone that extends into the magnocellular regions of the basal forebrain, an area differentiated by the presence of interspersed cholinergic neurons. The VLPO is found in the anterior hypothalamic region along the ventral edge of the brain between the optic chiasm and the diagonal band, and the median preoptic nucleus (MnPN) is a midline nucleus that caps the third ventricle.[10] The lateral preoptic and magnocellular preoptic regions are dorsolateral to the VLPO and extend rostrally from this level.[11]

The first nucleus to be definitively identified as containing sleep-active neurons was the GABAergic and galaninergic VLPO;[12] the number of c-Fos positive neurons was positively correlated with the amount of sleep prior to sacrifice. This pattern of c-Fos activation was mirrored in later unit recording experiments.[13] In fact, the predominant cell type (27% neurons) was wake-active, with the next most commonly occurring group (24% neurons) being non-arousal related, and with 22% being moderately wake-related. Of the sleep-related neurons, 16% were considered strongly related to sleep and 12% were considered moderately related.

The clear advantage of unit recording studies is to allow the activity of an individual neuron to be closely aligned with behavioural and EEG responses. Indeed, in the same study, VLPO neurons were found to begin their increased firing profile just prior to the onset of EEG synchronization, and they progressively increased their activity with sleep depth. The correlation with sleep depth was more pronounced following mild sleep deprivation, consistent with the idea that VLPO neurons actively cause natural and recovery sleep and do not represent sleep propensity. Ventrolateral preoptic area neurons were found to exhibit similar firing activity in non-REM and REM sleep.

Other studies have differentiated the VLPO into cluster and extended regions based on the observation that c-Fos in these regions was correlated with non-REM and REM sleep, respectively.[14] This was in agreement with earlier findings by this group that lesions of the extended VLPO were correlated with loss of REM sleep, and lesions of the cluster were correlated with the loss of non-REM sleep.[15]

Basal forebrain preoptic neurons also appear to exhibit differential sleep activity.[16] Similar to VLPO neurons, their firing is associated with sleep depth. However, unlike this nucleus, basal forebrain preoptic neuron firing rates are higher during non-REM sleep than during both REM sleep and waking. Basal forebrain preoptic sleep-active neurons are also GABAergic.[11]

The other clearly defined sleep-active nucleus, the MnPN, was found to contain 80% GABAergic neurons, comprising 58% sleep-active neurons that did not differentiate between non-REM and REM sleep.[10] However, as a whole, the MnPN also contained non-REM-selective (10%) and REM-selective (8%) neurons. Furthermore, the activity profile of MnPN sleep-active neurons was subtly different from the VLPO in that these cells increased their firing after sustained waking, which was maintained in the initial sleep period but dissipated as sleep continued.[17] Therefore, these neurons may be part of a sleep homeostat regulated by the amount of time spent awake.

These sleep-active nuclei generally target wake-promoting regions with some clear differences in bias. For example, the VLPO sends a major projection to the histaminergic tuberomammillary nucleus (TMN)[12,18] but sends a comparatively lower projection to other areas (e.g., locus ceruleus, dorsal raphe). In contrast, the MnPN sends only a minor projection to the TMN,[19] but sends an intense innervation to the lateral hypothalamic area (LHA) and has been shown to make contacts with orexinergic neurons in the perifornical (Pef) region.[10] Furthermore, warming of this region has been shown to inhibit wake-active Pef/LHA neurons.[20] These subtle differences may represent differing roles in sleep generation as suggested by the unit recording studies described above. However, the general pattern of regions innervated by VLPO, MnPN, and the lateral preoptic/basal forebrain indicate that gamma aminobutyric acid (GABA)ergic control of arousal centre firing is central to the control of sleep-wake states.

This hypothesis is supported by various functional studies. For example, slow wave sleep (SWS) was induced following muscimol injections into the posterior hypothalamus (which includes histaminergic and orexinergic neurons amongst others) of normal and insomniac cats.[21] More recently, it has been shown that local injections of muscimol directly into the TMN was sufficient to cause loss of righting reflex (LORR) in rats.[22] In addition, histamine levels in the brain are significantly higher during waking,[23] and all subdivisions of the TMN were shown to preferentially express c-Fos during the waking period.[24] Furthermore, their wake-related activity has been confirmed by unit recording studies showing that the TMN reduces firing activity prior to EEG synchronization, maintains this level during SWS, and ceases firing altogether during REM sleep.[25] In contrast, neither lesions to the TMN[26] nor removing the capability for histamine synthesis through knocking out the histidine decarboxylase (HDC) enzyme[27] produced dramatic changes to the normal sleep-wake cycle. However, this result could be indicative of compensatory changes within arousal networks.

Maintenance of wakefulness by histaminergic neurons appears to be reinforced by orexinergic excitation. Orexin peptides have been shown to directly depolarize histaminergic neurons[28] *in vitro*. In addition, orexinergic neurons have been shown to contain dynorphin. This endogenous opioid disinhibited TMN neurons by a presynaptic reduction of GABAergic inputs to this nucleus.[29] Consequently, if orexins and dynorphins were co-released, they would be expected to exert a powerful excitatory effect on the TMN.

Orexinergic neurons were first implicated in sleep-wake control by the sleep disorder, narcolepsy. This disorder is characterized in humans by reduced levels of orexin-A peptide in the cerebrospinal fluid (CSF),[30] which is thought to be due to loss of orexinergic neurons by an as yet undetermined mechanism.[31] A genetic version of the disorder has been linked to mutations in the orexin-2 receptor (OX_2R).[32] Although the symptoms of the two types of narcolepsy differ (cataplexy and wake-REM intrusions are more frequent with the peptide deficiency), they both have excessive daytime sleepiness in common.

Like other sleep-wake related neurons, orexinergic cell c-Fos expression changes in accordance with behavioural state, i.e., c-Fos was strongly associated with waking but negatively correlated with non-REM/REM sleep.[33] Later unit recording studies confirmed this finding,[34] i.e., consistent hypothalamic and basal forebrain levels of orexin-A were higher during wake than SWS.[35] However, these authors also found that orexin-A levels were high during REM sleep, which is at odds with c-Fos and unit recording studies. Additional evidence that orexins modulate arousal comes from the observation that intracerebroventricular administration of orexin-A induces wakefulness at the expense of both REM and non-REM sleep.[36] The effect on REM sleep has been attributed to the OX_1R-expressing locus ceruleus (LC).[37] Interestingly, the arousal effect of orexin-A was abolished in the H_1 receptor knockout mouse, suggesting that this effect is mediated by the histaminergic system.[38]

Though there are several independent arousal nuclei, wakefulness is reinforced by their interactions with one another. In addition, all arousal nuclei are hypothesized to

exert an inhibitory effect on the sleep-promoting system. This idea has been advanced by Saper *et al.* into what is now known as the "flip flop" model of sleep and waking.[39]

Although the neurotransmitters of arousal centres, such as the magnocellular basal forebrain (acetylcholine), the dorsal raphe nucleus (DRN) (serotonin), and the LC (noradrenaline), have been shown to hyperpolarize VLPO neurons, these cells may be insensitive to histamine.[40] However, TMN neurons have also been demonstrated to contain GABA and galanin,[41,42] which may be the mechanism of reciprocal inhibition of VLPO in these neurons.

Together, this complex network of sleep-promoting and arousal-promoting nuclei initiates and maintains sleep and wakefulness. Although it is still uncertain how circadian and homeostatic factors cause shifts between the two behaviours, the neuronal correlates of shifts in the network are becoming clearer; activation of the "sleep switch" in GABAergic preoptic (and brainstem) neurons inhibits the activity of defined arousal centres (Fig. 2). However, another missing piece is precisely how activity in arousal nuclei translates into the cortical activation that is characteristic of the waking brain.

One aspect that is clear, however, is the tendency for the reduction in arousal during sleep to shift both cortical and thalamic neurons from their tonic firing states towards their intrinsically hyperpolarized and bursting states. This, in turn, leads to thalamic and thalamocortical oscillations, which are the principal electrical signatures of sleep.[43,44] During non-REM sleep, the thalamic relay neurons switch into a burst-firing mode, which they adopt by default in the absence of external input.[44-46] Extensive connectivity among and between thalamic and cortical neurons leads to large populations of neurons firing in synchrony, and this is the origin of the slow "delta" waves at 1-4 Hz. During this burst-firing mode, ascending information through the thalamus is largely blocked. The transition from waking to sleeping also involves thalamic oscillations that lead to "sleep spindles" in the EEG. These are generated when a burst of spikes from a thalamocortical neuron impinges on a GABAergic reticular neuron (found in a shell-like structure that covers the anterior and lateral aspects of the dorsal thalamus) that then sends a robust inhibitory postsynaptic potential back to the same thalamocortical neuron. This action then hyperpolarizes the cell before it fires another barrage of spikes on rebound and an oscillation is established. The duration of the inhibitory potential (mediated by $GABA_A$ receptors) determines the time before another burst of spikes is generated by the thalamocortical neuron[44,45] and sets the frequency at $\sim$ 7-14 Hz.

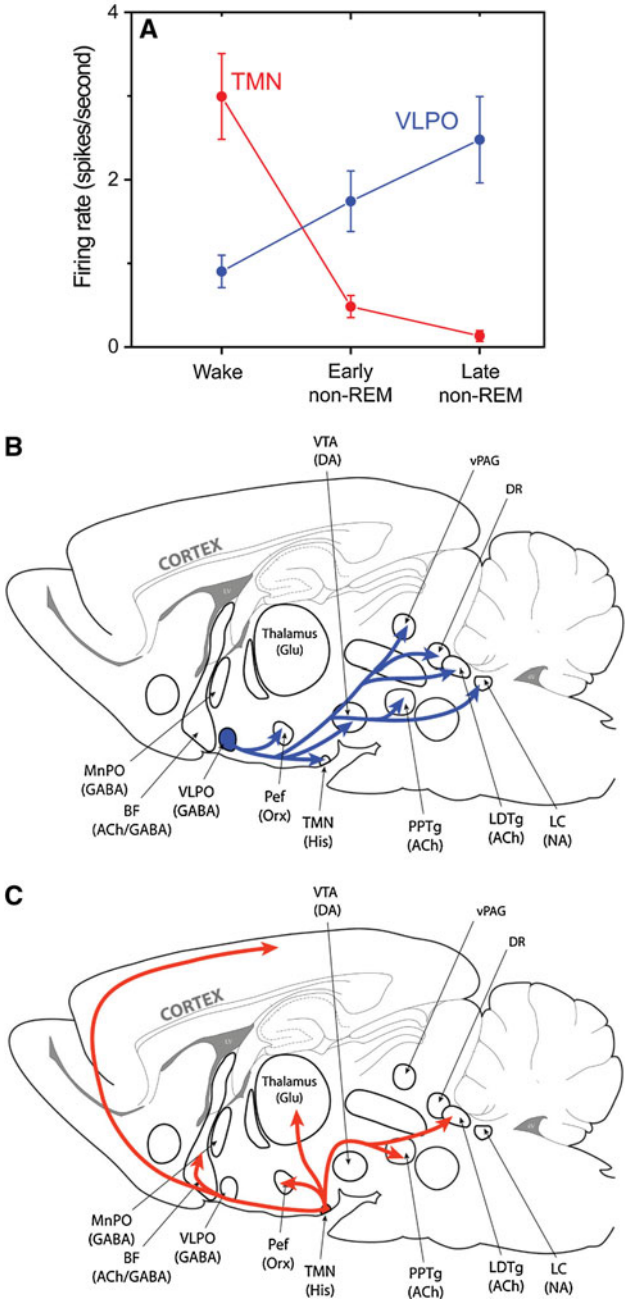

Fig. 2 Reciprocal activity of sleep and arousal areas. Panel A (redrawn)[83] shows the high firing activity of the histaminergic tuberomammillary nucleus (TMN, red line) during wakefulness, reducing during sleep. In contrast, the gamma aminobutyric acid (GABA)ergic ventrolateral preoptic area (VLPO, blue line) is more active during sleep than during wakefulness. Sleep-active VLPO neurons release GABA in various arousal regions (as indicated in panel B) just prior to sleep onset. The TMN is one such arousal pathway, and inhibition of histamine release (along with other arousal-related neurotransmitters) in cortical, subcortical, and thalamic sites (illustrated in Panel C) causes the switch from wakefulness to sleep

What is the evidence that general anesthesia resembles sleep?

There is no question that the state of natural sleep, even if one considers deep non-REM sleep, is not the same as the state of a patient or an animal anesthetized to the point of loss of consciousness. Other than the overall loss of postural control, body posture during anesthesia is clearly different to body posture during sleep, which is usually preceded by a defined behavioural choreography. However, the similarities in the state of the brain are much more striking than the differences.[47]

First, there are obvious similarities in the EEG in sleeping and anesthetized animals. Most general anesthetics (the main exceptions being nitrous oxide and ketamine) produce both spindles (brief 7-14 Hz bursts of activity) and delta waves (1-4 Hz), with the spindles generally occurring first, followed by sustained large amplitude delta oscillations at or beyond the point at which consciousness is lost.[48-52] As mentioned above, these same two features, spindles and delta oscillations, are the characteristic hallmarks of falling asleep and deep sleep. Figure 3 shows Power Spectra that illustrate the similarities between the EEG during non-REM sleep and dexmedetomidine-induced LORR. The large delta peak observed in both states contrasts markedly with the characteristic low voltage desynchronized EEG observed in a conscious animal and also contrasts with movement and REM sleep-associated theta. There are more subtle similarities when lower frequency (< 1 Hz) oscillations are compared. During both sleep and at least some general anesthetics, slow oscillations that are believed to originate in the cortex are also present. These slow oscillations may be important in shaping and organizing other neuronal oscillations. Cortical coherence also seems to be similarly compromised during non-REM sleep and drug-induced loss of consciousness.[53,54] Transcranial magnetic stimulation during wakefulness causes voltage deflections in parts of the cortex distal to the stimulation site. However, during non-REM sleep and midazolam anesthesia, the increase in cortical activity is confined to the stimulation site, indicating a breakdown in cortical connectivity in both of these states.

Second, the state of the brain when visualized using functional brain imaging is also similar in many ways during sleep and anesthesia. Most work has been done using positron emission tomography scanning and, despite certain technical limitations in the technique itself, comparisons between the two states can safely be made. In both sleep[55,56] and anesthesia,[57-60] activity is relatively more depressed in the thalamus, brainstem, basal forebrain, and basal ganglia, together with specific regions of the frontal and parietal cortices, particularly the anterior cingulate and orbito-frontal cortices and the precuneus/posterior cingulate. It should be stressed that these relative deactivations are in addition to an overall depressed level of neuronal activity, but, nonetheless, together with the EEG changes, point towards common neuronal pathways being involved. The pattern of cortical deactivation is particularly interesting, because it is clear that the polymodal cortical areas, such as the precuneus, are more sensitively affected than the unimodal cortical areas that respond to primary stimuli. Thus, the higher order processing that allows us to make sense of the world and our place in it, i.e., allows us to be conscious, is selectively dimmed during sleep and anesthesia, while primary sensory input is relatively spared. It is for these reasons that loud noises might register in a sleeping brain without conscious perception and why cortical activation by sensory stimuli[61] still occurs during profound anesthesia.

It is uncertain whether this pattern of cortical deactivation is caused by differential anesthetic effects in the cortex itself, whether it is mediated by the thalamus, or whether it is a combination of the two. Certainly, cortical neurons are sensitive to general anesthetics,[62,63] and cortical inhibition could influence thalamic activity via the descending corticofugal pathway.[64] A study that concluded that cortical changes in the EEG preceded changes in thalamic local field potentials at loss of consciousness supports this view but, interestingly, a recent study concluded the opposite during sleep onset.[65] Alternatively, anesthetics are known to hyperpolarize thalamocortical neurons,[66-70] and this could lead directly to the delta oscillations and spindle activity that are observed during anesthesia. Since the pattern of cortical deactivation that is seen during anesthesia resembles that of natural sleep, it seems more plausible to us that anesthetic effects at the level of the thalamus, or on the arousal pathways that modulate thalamic activity, are responsible for coordinating the overall network response.

Studies on a variety of brain arousal areas show that neurotransmitters released from these systems can have an impact on anesthesia. Experiments typically involve examining how systemic administration or a local injection of a neurotransmitter agonist or antagonist into a putative neuronal target affects either behaviour or some surrogate, such as the EEG. For example, activation of nicotinic acetylcholine receptors in the central medial nucleus of the thalamus can attenuate sevoflurane anesthesia[71] and activation of histamine receptors in the basal forebrain antagonizes that caused by isoflurane.[72] In the brainstem, application of GABAergic agents causes LORR and slow waves in the EEG.[73] Altering GABAergic tone in the TMN also has profound effects on anesthesia, e.g., muscimol (agonist) produced LORR when injected alone, whereas gabazine (antagonist) inhibited LORR duration with both

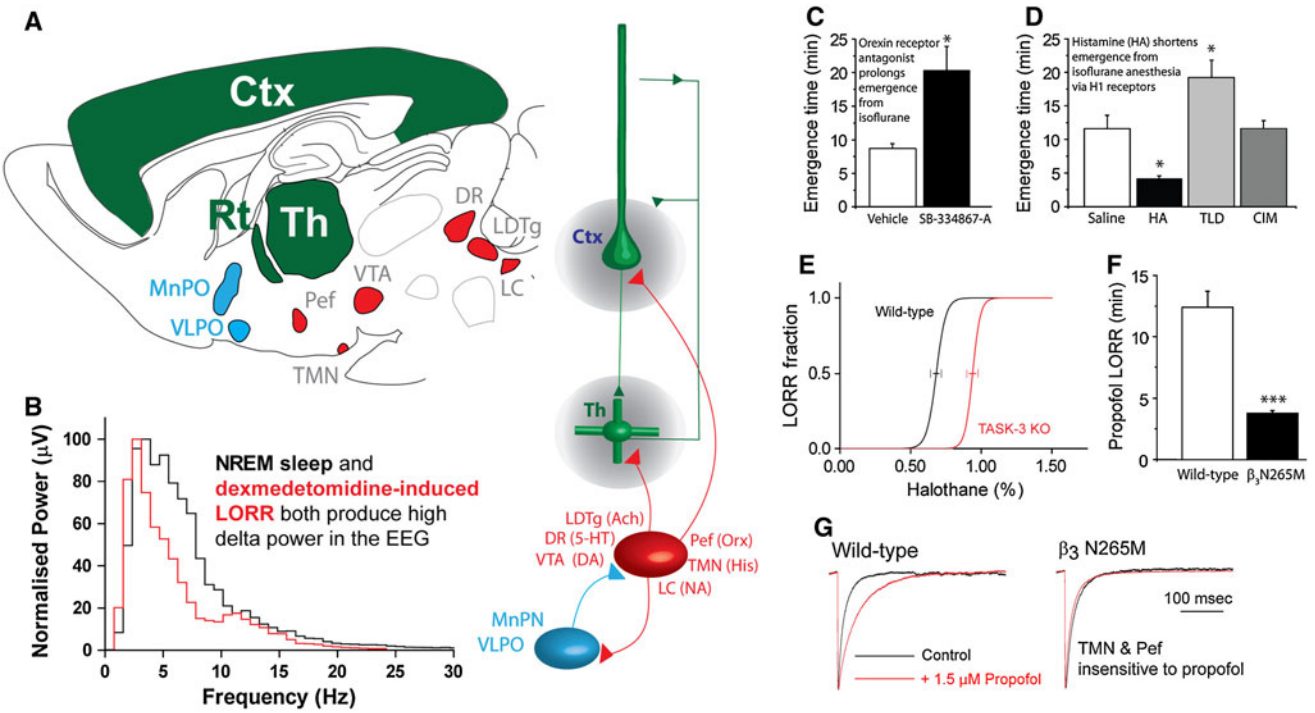

Fig. 3 Evidence for natural sleep pathways as anesthetic targets. Thalamocortical loops (A, green) are known to be involved in generating the characteristic electroencephalogram (EEG) patterns observed in the waking and sleeping brain. Wake-active regions (A, red) release neurotransmitters that generally depolarize these structures and enable tonic firing states and desynchronized activity. Sleep-active nuclei (A, blue) inhibit wake-active structures through the release of gamma aminobutyric acid (GABA), thereby removing this depolarization and favouring a burst-firing oscillatory state. Non-rapid eye movement non-(REM) sleep is characterized by such oscillations in the delta and spindle range (B, black). Anesthetics, such as dexmedetomidine (B, red) mimic this EEG pattern, indicating they may act on the brain structures that control natural sleep. Modulating neurotransmitter levels has been shown to create an impact on anesthetic parameters. For example, the orexin antagonist, SB-334867-A, (20 mg·kg^{-}1; C, redrawn)[75] has been shown to prolong isoflurane emergence time, indicating that this neuropeptide is necessary for awakening. Also, histamine (HA) reduces isoflurane emergence time by an H_1 receptor mediated mechanism; i.e., triprolidine (TLD) itself prolongs emergence (and prevents EEG activation by histamine), while cimetidine (CIM, H_2 antagonist) has no effect (D, redrawn).[72] Various receptors are known molecular targets of anesthetics (e.g., the expression of the two pore domain acid-sensitive potassium channel-3 (TASK–3) [E, redrawn][65] or the anesthetic-sensitive point mutation [N265M] in the β_3 subunit of the $GABA_A$ receptor [F, redrawn])[81] and modifying them both increases the anesthetic requirement *in vivo*. In the case of the point mutant, the reduced sensitivity has been mapped to at least the perifornical area (Pef) and the tuberomammillary nucleus neurons (G, redrawn),[77] but the extent of their involvement and other possible targets within and outside of the sleep circuitry have yet to be determined

propofol[22] and dexmedetomidine.[74] Inhibiting orexinergic activity was found to prolong emergence from isoflurane anesthesia,[75] but orexins may not be important for the actions of halothane[76] or dexmedetomidine.[77] As we have previously argued,[78] interpretation of such findings is fraught with difficulties due to the behaviourally activating/sedating effects of neurotransmitter agonists/antagonists, respectively. Clearly, there is a difference as to whether a particular neurotransmitter system can affect anesthesia (by altering the arousal baseline) and whether it is actually relevant to the mechanism of action of these drugs. The challenge is to demonstrate causality. Therefore, combining such observations with those that provide insight into what is actually happening in the anesthetized brain (such as microdialysis[79,80] and c-Fos[22,74] studies) can offer an idea as to which systems are really affected and can help to establish causality. For instance, orexinergic tone appears to be differentially inhibited by propofol and dexmedetomidine, but exogenous orexin can attenuate LORR caused by both.[77] Furthermore, the fact that both drugs appear to enhance VLPO activity and decrease TMN activity[22,74] indicates that studies examining the effects of drugs affecting the histamine system may be behaviourally relevant. Also, *in vitro* studies looking at neuronal responses to anesthetics can be useful, e.g., the β_3N265M knock-in mouse is behaviourally less sensitive to propofol and etomidate.[81] A necessary condition for the importance of a brain region as a mediator of anesthesia is that it too should behave differently in the knock-in animal. In the acute slice, GABAergic inhibitory post-synaptic currents (IPSCs) recorded in histaminergic and orexinergic neurons were insensitive to propofol in the knock-in animal, but IPSCs

recorded in the LC were still able to be prolonged by the anesthetic.[77] It would be interesting to see whether this test would support anesthetic actions in arousal pathways when applied to other knock-in (e.g., β_2N265S)[82] or knockout (e.g., two pore domain acid-sensitive potassium channel-3)[65] mice. Another exciting resolution to this problem may be the advance of cell-targeted genetic technologies, particularly those using viral delivery systems. Introducing anesthetic-insensitive receptors or modifying neurotransmitter action in the adult brain (thus bypassing much of the compensatory activity in arousal systems for acute experiments) would be a powerful tool.

In summary, it is clear that concentrations of general anesthetics that cause loss of consciousness induce many of the hallmarks of natural sleep. It remains to be seen whether these effects are driven top-down, bottom-up, or by simultaneous actions at various points in the neuronal network. Although it has been established that the neurotransmitters of sleep and arousal can influence the potencies of anesthetics, it remains a major challenge to distinguish between downstream effects from those on true anesthetic targets. We believe that a combination of selective genetic manipulation and systems analysis will be required for a comprehensive description of how anesthetics cause loss of consciousness.

Competing interests None declared.

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