REVIEW ARTICLE

Identifying c-fos Expression as a Strategy to Investigate the Actions of General Anesthetics on the Central Nervous System

Donghang Zhang[1,2], Jin Liu[1,2], Tao Zhu[1] and Cheng Zhou[2,*]

[1]*Department of Anesthesiology, West China Hospital of Sichuan University, Chengdu 610041, China;* [2]*Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, West China Hospital of Sichuan University, Chengdu 610041, China*

Abstract: Although general anesthetics have been used in the clinic for more than 170 years, the ways in which they induce amnesia, unconsciousness, analgesia, and immobility remain elusive. Modulations of various neural nuclei and circuits are involved in the actions of general anesthetics. The expression of the immediate-early gene c-fos and its nuclear product, c-fos protein, can be induced by neuronal depolarization; therefore, c-fos staining is commonly used to identify the activated neurons during sleep and/or wakefulness, as well as in various physiological conditions in the central nervous system. Identifying c-fos expression is also a direct and convenient method to explore the effects of general anesthetics on the activity of neural nuclei and circuits. Using c-fos staining, general anesthetics have been found to interact with sleep- and wakefulness-promoting systems throughout the brain, which may explain their ability to induce unconsciousness and emergence from general anesthesia. This review summarizes the actions of general anesthetics on neural nuclei and circuits based on a c-fos expression.

1. INTRODUCTION

General anesthetics are widely used in clinics to produce a state in patients characterized by amnesia, unconsciousness, analgesia, and immobility while maintaining vital physiological functions [1, 2]. The application of general anesthetics has revolutionized surgical procedures since the middle 19th century [2, 3]. More than 300 million patients worldwide undergo general anesthesia for medical procedures each year [4, 5]. Although thousands of studies have focused on the ways in which general anesthetics work, the exact molecular targets and neural circuits underlying the action of general anesthetics remain elusive [1, 2, 6-8].

Previous studies suggest that the modulation of general anesthetics on neural nuclei (*e.g.*, the thalamus and hypothalamus) and networks (*e.g.*, the thalamocortical loops and cortico-cortical loops) may be the key mechanism underlying the action of general anesthetics [2, 9, 10]. To investigate the modulation of general anesthetics on neural nuclei and circuits, a sensitive marker of neuronal activity is necessary. Numerous immediate early gene (IEG) products, such as c-fos, c-myc, Arc, Krox-24, fos-B, Egr1, Jun-D, Jun-B, and Jun-C, can serve as biomarkers of cell activity [11-14]. Among these IEG products, c-fos is the most commonly

used [15, 16]. To the best of our knowledge, except for c-fos, very few other IEG products have been used to detect neural activity to determine the neural basis of general anesthesia or sleep-arousal [17-19]. Therefore, in this review, we focused on studies that investigated the neural basis of mechanisms of action of general anesthetics based on a c-fos expression. Using this method, many neural nuclei and circuits have been identified as the possible neural targets of general anesthetics that induce their pharmacological effects, including induction of unconsciousness, emergence from general anesthesia, and analgesia (Fig. **1**).

In this review, we summarize the underlying neural substrates that mediate the action of general anesthetics, which were identified based on the strategy of c-fos staining. This review can potentially provide insights for future research on the neural basis of general anesthesia.

2. INDUCTION OF UNCONSCIOUSNESS BY GENERAL ANESTHETICS

Unconsciousness is the most pivotal pharmacological endpoint induced by general anesthetics. The mechanism by which general anesthetics induce unconsciousness remains unclear. Identifying the precise neural nuclei and/or circuits, especially the neuronal subtypes, that enable general anesthetics to induce unconsciousness will advance our basic understanding of general anesthesia. Neuronal activities can be primarily detected by c-fos expression in response to the induction of unconsciousness under general anesthetics. As a

*Address correspondence to this author at the Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, West China Hospital of Sichuan University, Chengdu 610041, China; Tel: +86-28-85164145; Fax: +86-28-85164039; E-mail: zhouc@163.com
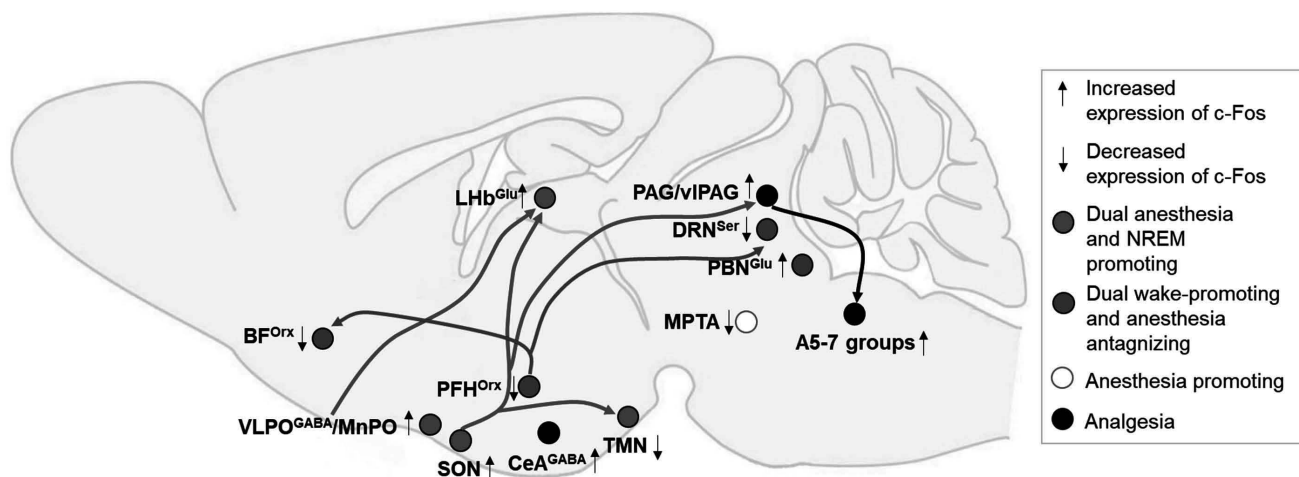
Fig. (1). The common brain nucleus, neural subtypes, and neural pathways for anesthetic actions and sleep-wakefulness behaviors based on changes in c-fos expression. The sagittal section through the rodent brain revealing that the brain nuclei related to general anesthesia-unconsciousness and emergence as identified by c-fos staining overlap completely with the known sleep-wakefulness nuclei. SON, supraoptic nucleus; TMN, tuberomamillary nucleus; LHb^{Glu}, glutamatergic neurons of lateral habenular nucleus; VLPO^{GABA}, GABA(γ-aminobutyric acid)-ergic neurons of ventrolateral preoptic nucleus; MnPO, median preoptic area; MPTA, mesopontine tegmental anesthesia area; PAG, periaqueductal gray; vlPAG, ventrolateral periaqueductal gray; A5, A6, A7 groups, the pontine noradrenergic cell groups; CeA^{GABA}, GABAergic neurons of central amygdala; PFH^{Orx}, orexinergic neurons of perifornical hypothalamus; BF^{Orx}, orexinergic neurons of basal forebrain; DRN^{Ser}, serotonergic neurons of dorsal raphe nucleus; PBN^{Glu}, glutamatergic neurons of parabrachial nucleus; increased expression of c-fos (⬆); decreased expression of c-fos (⬇). The arrows connecting any given regions A and B are neuronal pathways of the nucleus A on nucleus B. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

result, activation of sleep-promoting systems and inactivation of wakefulness-promoting systems may largely contribute to the induction of unconsciousness by both intravenous general anesthetics and volatile anesthetics.

The modulations of commonly used general anesthetics on c-fos expression, which have been demonstrated to be relevant to general anesthetic-induced unconsciousness, are summarized in Table **1**.

2.1. Identifying c-fos Expression in Putative Sleep-promoting Nuclei Associated with General Anesthetic-induced Unconsciousness

2.1.1. Ventrolateral Preoptic Nucleus (VLPO) and Median Preoptic Nucleus (MnPO)

The VLPO and MnPO are specifically active during sleep and contain GABAergic and galaninergic neurons that project many arousal-promoting nuclei [20, 21]. The recent study indicated that activation of VLPO galaninergic neurons promoted sleep and heat loss in mice, whereas inhibition of VLPO galaninergic neurons reduced sleep [21]. Identified by increased c-fos expression, many general anesthetics have been found to activate putative sleep-active neurons in VLPO, including propofol [22], barbiturates [22, 23], chloral hydrate [23], dexmedetomidine [24], isoflurane [23, 25], and halothane [25], suggesting that VLPO may be a common target for multiple classes of general anesthetics.

Isoflurane (1.2%, 2 h) and halothane (1.0%, 2 h) significantly increased the expression of c-fos in the GABAergic neurons within the VLPO and decreased c-fos expression in the non-GABAergic neurons, whereas the non-immobilizer 1,2-dichlorohexafluorocyclobutane (F6) failed to affect the

c-fos expression of VLPO, indicating that activation of GABAergic subpopulation neurons within the VLPO may be relevant to volatile anesthetic-induced unconsciousness [26]. Theoretically, the molecular targets that are sensitive to volatile anesthetics but not to the non-immobilizer, such as presynaptic voltage-gated sodium channel (Na$_v$) [27] and voltage-gated calcium channel (Ca$_v$) [28], are the underlying targets for volatile anesthetics to increase c-fos expression in GABAergic neurons of VLPO. This is likely due to presynaptic inhibition that increases glutamate release in the VLPO neurons. For the molecular basis, the volatile anesthetic sevoflurane should have bound multiple sites of Na$_v$ [29]. However, the increased c-fos expression in the GABAergic neurons in the VLPO was not prevented by pretreatment with tetrodotoxin, which impairs trans-synaptic neurotransmission, suggesting that such modulation may result from the neuronal intrinsic activity. Previous studies have reported that sodium leak channel (NALCN) [30] and tandem pore potassium (K2P) channels [31] are two major contributors to neuronal intrinsic activities; thus, they may be involved in such modulation.

In addition, another study showed that isoflurane (0.6% or 1.2%, 2 h) and halothane (1.0%, 2 h) increased the absolute number of c-fos positive neurons in the VLPO when mice were sedated or unconscious [25]. Destroying VLPO neurons opposed the isoflurane-induced hypnosis [25]. Their electrophysiological data further revealed that the neurons of the VLPO were depolarized by isoflurane, which was believed to be responsible for promoting sleep in previous studies, while the neighboring non-sleep-promoting VLPO neurons were unaffected by isoflurane. Similar to the above study, this depolarization is found not solely due to a presynaptic

Table 1. **The effects of general anesthesia on c-fos expression in brain nuclei related to anesthetic-induced unconsciousness.**

-	Isoflurane	Sevoflurane	Halothane	Pentobarbital	Propofol	Chloral hydrate	Dexmedetomidine	Ketamine
VLPO	+[23, 25, 26]	-	+[25]	+[22, 23]	+[22]	+[23]	+[24, 41]	–[23]
MnPO	+[26]	-	-	-	-	-	-	-
SON	+[18]	-	-	-	+[18]	-	+[18]	+[18]
LHb	-	-	-	+[23, 64]	+[49]	+[23]	-	+[23]
PFH	-[17]	–[17]	NA[77]	–[76]	–[76]	-	NA[76]	-
TMN	-	-	-	–[22]	–[22]	-	-	-
MPTA	-	-	-	–[19]	-	-	-	-

Abbreviations: +, increased expression of c-fos; **–,** decreased expression of c-fos; **NA,** no effects on c-fos expression.
LHb, lateral habenular nucleus; MnPO, median preoptic area; MPTA, mesopontine tegmental anesthesia area; PFH, periformical hypothalamus; SON, supraoptic nucleus; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus.

inhibition to increase glutamate release onto VLPO neurons as previously hypothesized, but also a direct postsynaptic effect on VLPO neuronal intrinsic excitability by a reduction of background potassium conductance. This was demonstrated by a significant reduction of inward current when VLPO neurons were held at the reversal potential for potassium [25]. These findings indicate that isoflurane may selectively activate a subpopulation of neurons in the VLPO to cause hypnosis via both pre- and postsynaptic mechanisms. This has been summarized in detail in previous reviews as follows: volatile anesthetics and other general anesthetics may modulate neural connectivity and synaptic transmission via both pre- and postsynaptic mechanisms [32, 33]. For the molecular basis, isoflurane was supposed to increase intrinsic neuronal excitability by inhibiting background potassium conductance. K2P channels are a group of background K^+ leak channels, including TASK, TREK, TWIK, TRESK, TALK, and THIK subfamilies, that regulate resting membrane potential (RMP) to control cellular excitability [34]. K2P channels are important contributors to the potency of volatile anesthetics. Isoflurane can induce unconsciousness via activation of TREK-1 and TASK [35, 36]. Moreover, K2P channels knockout animals exhibit resistance to volatile anesthetic-induced loss of consciousness [37]. Interestingly, recent studies have revealed the binding sites of isoflurane in the TREK1 K2P channel, including multiple neighboring residues on TREK1 TM2, TM3, and TM4 [38]. Another group of potassium channels, voltage-gated K^+ channels (K_v), are also modulated by volatile anesthetics and play important roles in general anesthesia [39]. Emerging studies have found volatile anesthetic-binging sites within K_v. For example, sevoflurane was reported to bind open and closed structures at multiple sites in K_v1.2 [40].

Unlike VLPO, the effects of isoflurane and halothane on MnPO are not directly parallel to the effects on the VLPO [26]. Isoflurane, but not halothane, significantly increased c-fos expression not only in the GABAergic neurons but also in non-GABAergic MnPO neurons. Such neuronal activation is disrupted by blocking synaptic transmission *ex vivo*, indicating that MnPO may be recruited indirectly by isoflurane either through disinhibition or VLPO-mediated secondary activation.

For other general anesthetics, systemic delivery of dexmedetomidine (100 mg/kg) for 2 h induces sedative and hypnotic effects in mice, accompanied by elevated c-fos expression in the neurons of the VLPO and reduced c-fos expression in the cerebral cortex and subcortical wake-promoting systems [41]. Although both dexmedetomidine [24] and isoflurane [25] can elevate c-fos expression in the neurons of the VLPO, dexmedetomidine can reverse the neuronal depolarization of VLPO neurons induced by isoflurane in brain slices *in vitro*, likely resulting from direct hyperpolarizing actions through α2 adrenergic receptors of the postsynaptic VLPO neurons. Moreover, direct injection of dexmedetomidine into the VLPO can oppose the activation of the VLPO induced by 0.8% isoflurane *in vivo*, thus promoting emergence from isoflurane anesthesia [42]. Given that dexmedetomidine is a selective α2 adrenergic agonist, it is possible that α2 adrenergic signaling in the VLPO is implicated in mediating the arousal state *via* inhibition of sleep-active neurons. Therefore, general anesthetics may have conflicting effects on neurons within a specific nucleus. However, systemic rather than local delivery of dexmedetomidine may induce hypnosis by indirect depolarization of VLPO neurons via a net consequence of silencing inhibitory input to the VLPO [24].

Unlike most general anesthetics that can increase neuronal c-fos expression in VLPO, the patterns of c-fos expression induced by ketamine in anesthetic doses are similar to those under wakefulness [23]. Ketamine at a dosage equal to or just below the dosage used in clinical rat anesthesia (~150 mg/kg) decreases c-fos expression in VLPO, increases c-fos expression in the central arousal system, and induces arousal behaviors. This may be due to the reduced excitatory transmission of glutamate through targeting of the N-methyl-D-aspartic acid (NMDA) receptor. At a high dose of 300 mg/kg, ketamine produces prolonged sedation and decreases c-fos expression in the cerebral cortex and arousal system [23]. Thus, the predominant effect of ketamine at anesthetic dosages (75-100 mg/kg) appears to cause arousal behaviors in rats. This may explain why ketamine is often administered in combination with other sedative drugs, such as xylazine and dexmedetomidine.

2.1.2. Supraoptic Nucleus (SON)

The SON is part of the hypothalamic-pituitary axis and is located in the anterior hypothalamus [18, 43]. The SON regulates many physiological functions related to osmotic balance and vasoconstriction [44]. Very few studies have explored the function of SON in sleep-wakefulness regulation; however, the number of damaged cells was increased in the SON after severe sleep deprivation [43]. Recently, it was reported that the hypnotic effect of thalidomide, a nonbarbiturate hypnotic agent, may be associated with the activation of vasopressin-containing neurons in SON due to the significantly increased c-fos expression [45]. Interestingly, a recent study reported that SON neurons are commonly activated by various classes of general anesthetics, including isoflurane (1%-1.2%), propofol (180 mg/kg), ketamine (100 mg/kg), and dexmedetomidine (100 μg/kg) determined by increased c-fos expression, recording of brain slices, and *in vivo* recording of multichannel electrophysiology [18]. In this study, a distinct cluster of SON neurons was identified with strong c-fos positivity in response to these general anesthetics. Activation of these anesthesia-activated neurons (AANs) by chemogenetic or optogenetic stimuli strongly promoted slow-wave sleep or enhanced isoflurane anesthesia, whereas conditional ablation and/or inhibition of these AANs led to significant loss of sleep and shortened the duration of isoflurane anesthesia. In slice recordings, these four chemically distinct general anesthetics all depolarized the membrane potentials of the AANs within SON, which suggested that AANs within SON are the common neural substrates for various general anesthetics; however, the underlying molecular mechanisms of the depolarization remain unclear. General anesthetics may activate some G protein-coupled receptors (GPCRs) in AANs, thus increasing intracellular calcium release, thereby facilitating neuropeptide and hormone release to induce depolarization. These results reveal that the neuroendocrine system plays a crucial role in the regulation of general anesthesia. At the circuit level, these AANs within the SON project to the septum, anterior thalamus, and several other brain areas involved in arousal control, including the posterior lateral hypothalamus (PLH) [46], tuberomammillary nucleus (TMN) [47], supramammillary nucleus (MM) [48], lateral habenula (LHb) [49], periaqueductal gray (PAG) [50], ventral tegmental area (VTA) [51], median raphe nucleus (MnR), pedunculopontine nucleus (PPTg), and laterodorsal tegmental nucleus (LDTg) [52]. However, the exact role of such projections has not yet been determined in general anesthesia, which may differentially contribute to various classes of general anesthetics to produce distinct pharmacological endpoints, even though they all activate the AANs in SON. It is also important to acknowledge that SON primarily consists of neuroendocrine cells; future studies should determine the role of endocrine regulation of SON in the action of general anesthetics.

2.1.3. Lateral Habenula (LHb)

The LHb is a glutamatergic hub [53, 54] that receives inputs from various forebrain regions (*e.g.*, prefrontal cortex, basal ganglia, preoptic, and lateral hypothalamus) [53-56] and projects to the rostromedial tegmental nucleus (RMTg) [57-59]. The LHb is involved in many neural functions such as depression, addiction, reward processing, motivation,

sleep, and circadian rhythm [60-63]. For general anesthesia, it has also been suggested that LHb neurons are anesthesia-activated neurons (AANs) [18]. Hypnotic doses of ethanol, chloral hydrate, barbiturates, and ketamine induce c-fos expression in the LHb in mice [23, 64]. Propofol (7 mg/kg) induces c-fos expression in the LHb, and blocking LHb glutamatergic output prevents propofol-induced loss of righting reflex (LORR) and reduces the propofol-induced enhancement in electroencephalogram (EEG) power, suggesting that the glutamatergic pathway of the LHb may play a pivotal role in regulating the hypnotic effects of propofol [49]. As mentioned above, since the LHb also receives inputs from the SON and preoptic hypothalamus (*e.g.*, VLPO), and these nuclei are the key nodes for anesthetic-induced hypnosis, SON/VLPO-LHb circuits may participate in the hypnosis induced by general anesthetics.

2.1.4. Nucleus Accumbens (NAc)

The nucleus accumbens (NAc), located within the ventral striatum, is known for its role in pleasure, reward, addiction, and pain processing [65]. The activity of the NAc also influences the effects of general anesthetics [66]. The adenosine A2A receptor (A2AR) in the NAc and the tuberomammillary nucleus has been reported to regulate physiological sleep [67, 68]. Systematic injection of a selective A2AR agonist significantly prolonged the duration of propofol-induced unconsciousness, accompanied by increased c-fos expression in the NAc [69]. However, the mechanism by which the A2AR agonist prolonged propofol-induced unconsciousness remains unclear. It is also unclear whether propofol anesthesia directly activates neurons in the NAc.

A recent study highlights the pivotal role of NAc in sevoflurane-induced unconsciousness [70]. Dopamine D1 receptor (D1R)-expressing neurons in the NAc were markedly inhibited under sevoflurane exposure (2%), evidenced by decreased Ca^{2+} signal intensity. However, the authors did not test the effects of sevoflurane on the c-fos expression in NAc D1R neurons. They further showed that activation of these neurons delays sevoflurane induction and induces emergence, whereas inhibition of these neurons accelerates sevoflurane induction and prolongs the emergence process. Their results provide the first evidence that NAc neurons directly modulate consciousness levels under unconsciousness induced by volatile anesthetics. Differential neural subpopulations within the NAc may contribute to the propofol- and sevoflurane-induced loss of consciousness; however, further confirmation is needed.

2.2. Identifying c-fos Expression in Putative Wakefulness-promoting Nuclei Associated with General Anesthetic-induced Unconsciousness

2.2.1. Tuberomammillary Nucleus (TMN)

The tuberomamillary nucleus (TMN) is the sole source of histaminergic neurons in the central nervous system (CNS), which commands the general state of consciousness [71]. The activity of TMN is high during wakefulness and low during sleep [71]. Intraperitoneal administration of GABAergic agonists or enhancers, such as muscimol (10 mg/kg), propofol (140 mg/kg), and pentobarbital (100 mg/kg), decreased c-fos expression in the TMN [22]. Accordingly, intracerebroventricular injection of muscimol into

the TMN produced dose-dependent sedation, suggesting that GABAergic sedative components may be mediated by GABA$_A$ receptors in the endogenous sleep pathway [22]. Conversely, gabazine, a GABA$_A$ receptor antagonist, attenuated the sedative effects of these GABAergic agents by direct microinjection into the TMN. At the circuit level, TMN receives inputs from SON; therefore, SON-TMN projections may be involved in GABAergic general anesthetic-induced sedation or unconsciousness. This modulation of the GABAergic agonist on TMN also indicates the underlying involvement of the histaminergic system in general anesthesia.

2.2.2. Hypothalamic Orexinergic Neurons

Neurons expressing orexin are primarily located in the lateral hypothalamus, especially in the perifornical area, and are widely projected throughout the brain [72]. Orexinergic neurons in the perifornical area of the hypothalamus are critical for normal sleep, and a disruption of this system is known to cause a sleep disorder called narcolepsy [73, 74]. The orexinergic neurons in the hypothalamus also participate in regulating the effects of general anesthetics [17, 75]. The orexinergic neurons are inhibited by GABAergic drugs, including muscimol (5 mg/kg), propofol (100 mg/kg), and pentobarbital (50 mg/kg), as identified by a marked reduction in c-fos expression during anesthesia [76]. Regulating the neuronal activity of orexinergic neurons in the perifornical area can affect the concentration of GABAergic drugs for LORR [76]. These results imply that hypothalamic orexinergic signaling participates in the unconsciousness induced by GABAergic anesthetics.

Isoflurane and sevoflurane are also known to decrease c-fos expression in orexinergic neurons of the perifornical hypothalamus (PFH) [17]. Interestingly, halothane-induced hypnosis occurred without inhibition of dorsomedial and perifornical orexinergic neurons in mice, identified by no change in c-fos expression and electrophysiological recording *in vivo* [77]. Indeed, emergence from halothane anesthesia is not affected in orexin/ataxin-3 transgenic mice (loss of orexinergic neurons) [77]. Therefore, hypothalamic orexinergic signaling may differentially contribute to the actions of volatile anesthetics, which may be due to differences in protein binding targets [78] or varied modulatory ability on ion channels [79] or GPCRs [80].

2.3. Identifying c-fos Expression in Non-putative Sleep-wakefulness Nuclei Associated with General Anesthetic-induced Unconsciousness

2.3.1. Mesopontine Tegmental Anesthesia Area (MPTA)

The mesopontine tegmental anesthesia area (MPTA) includes the sub-peduncular tegmental nucleus and the dorsomedial part of the pontine reticular nucleus pars oralis [81]. The MPTA is flanked by the ventrolateral periaqueductal gray (PAG), reticular tegmental nucleus of the pons, cholinergic pedunculopontine tegmental nucleus (PPN), and median and paramedian pontine raphe [82]. MPTA is sensitive to GABAergic anesthetics, including pentobarbital, propofol, and etomidate [81, 83, 84]. Systemic administration of the classical GABAergic anesthetic pentobarbital (50 mg/kg) can suppress c-fos expression in the MPTA, indicating that neuronal activity of MPTA may be inhibited during GABAergic agent-induced anesthesia [19]. These inhibitory

effects of classical GABAergic anesthetics may be due to the direct activation of postsynaptic GABA$_A$ receptors. Indeed, localized injections of pentobarbital into the MPTA can induce a complete state of anesthesia, which appears to be mediated by a circuit of dedicated axonal projections to the nearby arousal nuclei of the brainstem and distant targets in the forebrain and spinal cord [81, 84, 85]; meanwhile, lesions in this region lead to insomnia [86]. These results provide direct evidence for the role of subcortical regions in mediating GABAergic anesthetic-induced unconsciousness, and MPTA may serve as the on-off switch of GABAergic anesthetic-induced anesthesia. However, it is not clear whether MPTA is also involved in the action of volatile anesthetics or modulation of sleep-arousal behaviors.

2.4. Identifying c-fos Expression in Cerebral Cortex Associated with General Anesthetic-induced Unconsciousness

In addition to the subcortical brain nucleus, it is widely known that the neural activity of the cerebral cortex itself also plays an important role in the general anesthetic induced-unconsciousness. Previous studies indicated that systemic administration of pentobarbital, a classical GABAergic sedative, caused widespread suppression of c-fos expression in the cerebral cortex of both mice and rats [19, 23, 64, 87], suggesting the activity of cerebral cortex was suppressed in the anesthetic-induced unconsciousness. However, the precise mechanism of how general anesthetics interfere with activity and/or connectivity of the cerebral cortex to induce unconsciousness is not well known. Disruption of either cortico-cortical loops [88] or thalamocortical loops [6] is the two long-standing hypotheses. A recent and important study indicated that different classes of general anesthetics (isoflurane, ketamine/xylazine, and urethane) induced unconsciousness by decoupling the information flow between layer 5 pyramidal neuron dendrites and their cell bodies, thus causing the breakdown of both cortico-cortical and thalamocortical feedback loops [10]. This finding provides the cellular mechanism that reconciles the two distinct hypotheses of cortico-cortical loops and thalamocortical loops.

3. EMERGENCE FROM GENERAL ANESTHESIA

Rapid recovery from unconsciousness is the most important characteristic of general anesthesia compared to other disruptions of consciousness, such as coma. Inadequate emergence, including emergence delirium and delayed emergence, may occur despite a stable state during surgery and general anesthesia [89], which can lead to delays in the operating room, the overall increase in costs, coughing, potential intracranial or intraocular pressure rise, respiratory accidents, and hemodynamic instability [89]. Up to 20% of general anesthesia awareness with recall episodes is attributed to the awakening phase [90]. Therefore, the neural mechanisms of emergence from general anesthesia are critically important but remain unclear. Generally, emergence from general anesthesia is considered a slow and passive process with the elimination of general anesthetics from their targets of action, which is the inverse process of anesthetic induction [91]. However, several studies have also indicated that anesthetic induction and emergence from general anesthesia are not inverse processes [17, 92]. Multiple neural nuclei and

Table 2.   The effects of general anesthesia on c-fos expression in brain nuclei related to emergence from general anesthesia.

-	PFH Orexinergic Neurons	BF Orexinergic Neurons	DRN Serotonergic Neurons	PBN
Isoflurane	-[17]	-	-[112]	+[117]
Sevoflurane	-[17]	-	-	-
Propofol	-	-[93]	-	-

Abbreviations: -, decreased expression of c-fos under anesthetized status; +, increased expression of c-fos during emergence. BF, basal forebrain; DRN, dorsal raphe nucleus; PBN, parabrachial nucleus; PFH, perifornical hypothalamus.

circuits have been shown to influence anesthetic emergence by the c-fos expression [17, 93, 94]. A major challenge is that expressional changes in c-fos protein are difficult to detect during the emergence period, as their appearance is delayed after stimulation [95]. Moreover, the methods of execution and anesthesia in animals may influence c-fos expression during emergence from anesthesia. Therefore, caution should be exercised when interpreting c-fos results associated with emergence from general anesthesia. The effects of various general anesthetics on the c-fos expression of neural nuclei related to anesthetic emergence are summarized in Table **2**.

3.1. Orexinergic Neurons in Hypothalamus and Basal Forebrain (BF)

Orexinergic neurons within the hypothalamus and BF play a critical role in the promotion and maintenance of wakefulness [75, 93, 96-99]. We have described the role of orexinergic neurons in anesthesia induction above. In this section, we will separately discuss their contribution to the emergence from anesthesia. Intracerebroventricular injection of orexinergic agonists and antagonists can also potentially alter emergence from general anesthesia [75, 100]. Isoflurane (1.25%, 2 h) caused a 30% reduction in the number of c-fos-expressing neurons, and sevoflurane (2.14%, 2 h) resulted in a 50% reduction in the number of c-fos-expressing neurons in the PFH of adult mice. Genetic and/or pharmacological impairment of orexin signaling dramatically delayed emergence from anesthesia by isoflurane and sevoflurane but did not alter anesthetic induction, indicating that orexinergic neurons in the PFH specifically mediate emergence from volatile anesthesia [17]. This study further demonstrated that isoflurane and sevoflurane had no effect on c-fos expression in adjacent melanin-concentrating hormone neurons, suggesting that wake-promoting orexinergic neurons are the specific targets of these anesthetics [17].

Orexinergic signaling is also involved in emergence from general anesthesia by intravenous general anesthetics. Similar to the results of c-fos expression changes in orexinergic neurons of the hypothalamus, propofol anesthesia also reduced the number of c-fos immunoreactive orexinergic neurons within the BF in rats, and the activities of orexinergic neurons in the BF were restored when the rats emerged from propofol anesthesia. Indeed, microinjection of orexin-A into the BF had no effect on the induction time; however, it facilitated the emergence from propofol anesthesia. Conversely, microinjection of the orexin-1 receptor antagonist into the BF delayed emergence from propofol anesthesia. These findings indicate that orexinergic signaling in the BF may specifically regulate the emergence, but not induction, of propofol

anesthesia [93]. This also implies that emergence from anesthesia is not a simple converse process of anesthesia induction. Since hypothalamic orexinergic signaling appears to be involved in the unconsciousness induced by propofol, it would be interesting to determine whether induction and emergence of propofol anesthesia are mediated by orexinergic neurons of different neural circuits.

At the circuit level, a recent study found that optogenetic activation of orexinergic neurons in the perifornical lateral hypothalamic area or their terminals in the basal forebrain (BF) or locus coeruleus (LC) facilitated emergence from anesthesia by isoflurane, indicating that perifornical lateral hypothalamic area-BF/LC pathways are involved in the arousal from isoflurane anesthesia [101]. Other projects involving the perifornical lateral hypothalamic area must also modulate arousal from general anesthesia since it widely projects throughout the brain, such as the lateral hypothalamic glutamatergic projections to the LHb, which was recently shown to regulate anesthetic potency [102].

Cholinergic modulation in the BF also plays a pivotal role in emergence from general anesthesia. Acetylcholinesterase inhibitors may promote wakefulness partly by the direct activation of orexinergic neurons [17]. Moreover, inhibition of the excitatory nicotinic acetylcholine receptor (nAChR) is suggested to be responsible for the unconsciousness of general anesthesia [103]. At the molecular level, previous studies have identified the binding sites within the nAChR of several general anesthetics. Isoflurane persistently binds to three classes of sites in the transmembrane domain of nAChR [104], whereas propofol binds to an intra-subunit site in the transmembrane domain [105]. Using the bacterial homologue from Gloeobacter violaceus, researchers have revealed a common binding site for general anesthetics of propofol and desflurane, which pre-exists in the apo-structure in the upper part of the transmembrane domain of their protomers [103]. Collectively, these findings provide direct evidence that multiple ligand-receptors and ion channels are involved in the action of general anesthetics. Identification and specific modulation of the binding sites of general anesthetics will facilitate the exploration of the role of the brain nucleus under general anesthesia or sleep-arousal behaviors.

3.2. Serotonergic Neurons in the Dorsal Raphe Nucleus (DRN)

Orexinergic neurons project to the dorsal raphe nucleus (DRN) [72]. Serotonin (5-hydroxytryptamine, 5-HT) is the dominant monoamine neurotransmitter in the DRN [106] and plays a key role in the regulation of mood [107], appetite [108], sleep-arousal [109], memory [110], and learning [111]. Isoflurane (1.4%, 1 h) anesthesia reduced the number

of c-fos immunoreactive serotonergic neurons in the DRN. This inhibitory effect was partially reversed by the administration of orexin-A in DRN, which enhanced arousal behaviors, suggesting that orexinergic neurons facilitate emergence from isoflurane anesthesia, at least partially, by activating serotonergic neurons in the DRN in rats [112]. These results reveal that hypothalamic orexinergic neurons-DRN serotonergic neurons projection may be a potential circuit that mediates emergence from general anesthesia induced by isoflurane.

3.3. Parabrachial Nucleus (PBN)

The parabrachial nucleus (PBN) promotes arousal [113, 114]. The majority of neurons in the PBN are glutamatergic [115] and project to numerous areas, including the basal forebrain, hypothalamus, thalamus, amygdala, and cortex [116]. The PBN is also able to regulate the emergence from general anesthesia induced by isoflurane. Muindi *et al.* found a selective increase in c-fos expression in the PBN neurons during emergence from isoflurane anesthesia (2.5% for 20 min + 0.9 - 1.0% for 40 min). Indeed, electrical stimulation of the PBN induced arousal and restoration of the righting reflex during continuous anesthesia under isoflurane [117], suggesting that recovery from isoflurane anesthesia may be related to the enhanced neural activity of PBN. However, since most neurons in the PBN are glutamatergic, further studies are needed to determine whether glutamatergic neurons in the PBN and the related neural circuitry are the definitive targets that control arousal from general anesthesia.

3.4. Paraventricular Thalamus (PVT)

Clinical observations indicate that the paramedian region of the thalamus is a critical node for controlling wakefulness, compared to other sites of the thalamus, since lesions of the paramedian thalamus show disorders of consciousness ranging from hypersomnolence to sleep-like coma [118-121]. In rodents, the homologous area of the primate paramedian thalamus includes many nuclei, such as the paraventricular thalamus (PVT), nucleus reuniens, mediodorsal nucleus, and interanteromedial thalamic nucleus [122, 123]. These nuclei have distinct connections [123-125] and are implicated in various neural functions, such as stress and anxiety, feeding behavior, drug-seeking activities, memory generation, arousal, and awareness processes [122, 126, 127]. Ren *et al.* recently observed a higher level of c-fos expression in the PVT than in the other regions of the paramedian thalamus in mice after extended wakefulness, indicating that PVT neurons exhibit high activity during wakefulness [94]. Furthermore, they found that glutamatergic neurons, the primary neural types in the PVT, exhibited high activity during wakefulness by *in vivo* fiber photometry or multichannel electrophysiological recordings. Additionally, activation of PVT glutamatergic neurons induced a transition from sleep to wakefulness and promotion of emergence from isoflurane anesthesia [94], suggesting that glutamatergic signaling within the PVT may underlie emergence from isoflurane anesthesia, and isoflurane anesthesia and sleep may share a common neural basis within the PVT. Therefore, activation of PVT neurons may be sufficient to induce wakefulness from an unconscious state in patients with paramedian thalamic stroke and/or anesthesia. At the circuit level, the hypothalamic hypocretin neurons-PVT-NAc projection regulates wakefulness; how-

ever, the authors did not report whether hypothalamic hypocretin neurons-PVT-NAc projection regulates emergence from isoflurane anesthesia. Nevertheless, the authors did not test the c-fos expression under isoflurane anesthesia. Therefore, the exact change in c-fos expression in PVT glutamatergic neurons during emergence from isoflurane anesthesia is unclear.

In addition to glutamatergic signaling, dopaminergic signaling is another neuronal subtype of the PVT [128]. Ao *et al.* recently reported that activation of dopaminergic signaling in the PVT also facilitated emergence from isoflurane anesthesia [129]. The number of c-fos-positive neurons was significantly higher in the PVT of mice that underwent passive emergence from isoflurane anesthesia than in those who underwent isoflurane anesthesia or oxygen. Injection of the D2-like agonist quinpirole into the PVT shortened the emergence time from isoflurane anesthesia, while administration of a D2-like antagonist prolonged the emergence time. Indeed, the number of c-fos positive neurons was significantly higher in the PVT of mice receiving quinpirole than in those receiving saline. However, neither quinpirole nor raclopride affected the induction time of isoflurane. These results indicate that dopaminergic signaling in the PVT may be selectively associated with the emergence from isoflurane anesthesia. However, more specific manipulation of dopaminergic neurons is needed to determine its role in emergence from anesthesia. It would be interesting to compare the relative contribution of glutamatergic and dopaminergic signaling in regulating emergence from general anesthesia.

3.5. Locus Coeruleus Nucleus (LC)

The LC is the main site for synthesizing norepinephrine (NE) in the brain [130]. As a key arousal node, the LC receives inputs from arousal-related orexinergic and histaminergic neurons and provides widespread noradrenergic innervation to the cerebral cortex and wake-promoting brain regions [130-135]. The LC-NE system plays a critical role in regulating sleep and wakefulness, as well as in general anesthesia [136-138]. One study showed that dexmedetomidine-induced LORR was abolished by selective knockdown of α2 adrenergic receptors in the LC [139]. Unexpectedly, general anesthesia by isoflurane or pentobarbital increased c-fos expression in the LC [23], suggesting that this arousal system may be activated by certain general anesthetics. The evidence supporting the role of LC in sleep-wakefulness and general anesthesia is that optogenetic or pharmacological activation of LC neurons causes an immediate transition from sleep to wakefulness and facilitates arousal from isoflurane-induced unconsciousness in rodents [136-138]. However, the exact contribution of LC activity, associated neural subpopulations, and synaptic functions in the balance of hypnosis and emergence during general anesthesia are unclear.

4. ANALGESIA

Analgesia is another important endpoint of general anesthetics and is critical for surgeries and invasive medical treatments [140]. Different classes of general anesthetics induce unequal pharmacological actions. GABAergic agents (*e.g.*, propofol, etomidate, and barbiturates) produce profound hypnosis but weak analgesia [141]. In contrast, nitrous

Table 3.   The effects of general anesthesia on c-fos expression in brain nuclei or the spinal cord related to analgesia.

-	-	Pentobarbital	Chlorate hydrate	Ketamine	Isoflurane	Propofol	Halothane	Nitrous oxide
Supraspinal regions	PAG	+[163]	-	-	-	+[163]	-	-
	Vc	+[163]	-	-	-	+[163]	-	-
	A5, A6, A7 groups	NA[23]	+[23]	+[23]	NA[23]	-	-	-
	vlPAG	NA[23]	-[23]	NA[23]	-[23]	-	-	-
	CeA	-	-	+[18]	+[18]	-	-	-
Spinal cord	Superficial dorsal horn	NA[147]/-[151]	-	-	-[148-151]	-[147, 151]	NA[148, 150]	-[150, 151]
	Deeper layers of dorsal horn	NA[147]	-	-	-	-[147]	-[150]	-[150]

Abbreviations: **+**, increased expression of c-fos; **-**, decreased expression of c-fos; **NA**, no effects on c-fos expression.
A5, A6, A7 groups, the pontine noradrenergic cell groups; CeA, central amygdala; PAG, periaqueductal gray; Vc, trigeminal spinal nucleus caudalis; vlPAG, ventrolateral periaqueductal gray dopamine cells;

oxide, xenon, and ketamine produce analgesia but weak hypnosis [23, 141, 142]. Volatile agents (*e.g.*, isoflurane and sevoflurane) are notable for their efficacy in inducing both hypnosis and immobility in a predictable manner [141]. The exact neural targets of general anesthetics to produce analgesia remain elusive. Generally, the spinal cord is recognized as the main target for general anesthetics to produce immobility (no response to nociceptive stimuli) [143, 144]. However, emerging evidence demonstrates that supraspinal nuclei of the CNS are also involved in the analgesic effects of general anesthetics [23, 140, 145], despite the fact that the relative contribution of the spinal cord and supraspinal nuclei to the general anesthetic-induced analgesia is unclear. The effects of various general anesthetics on c-fos expression in the spinal cord and brain nuclei that are related to analgesia are summarized in Table **3**.

4.1. Identifying c-fos Expression in Spinal Cord Neurons Associated with Analgesia under General Anesthesia

The analgesic or immobility action of volatile anesthetics is largely induced by modulation of the spinal cord [143, 146]. Isoflurane (1.8%) and propofol (10 mg/kg), but not halothane (0.9%-1.5%) and pentobarbital (20 mg/kg), suppressed c-fos expression evoked by nociceptive stimuli in the spinal superficial layers [147-149], indicating that these anesthetics may inhibit the transition of nociceptive sensory signals from the periphery to the spinal cord. Another study indicated that nitrous oxide (40% or 70%) and halothane (0.5% or 1.5%) suppress c-fos expression in the deeper layers (laminae V-X), but not in the superficial layers of the spinal cord, suggesting that nitrous oxide and halothane may target motor neurons in the deeper layers of the spinal cord instead of the sensory neurons in the superficial dorsal horn, which directly receive noxious inputs [150].

The modulation of c-fos expression in the spinal cord also varies at different anesthetic concentrations/doses. Takasusuki *et al.* showed that fentanyl (30 μg/kg), propofol (100 mg/kg), pentobarbital (50 mg/kg), isoflurane (2.4%), and nitrous oxide (66%) could reduce c-fos expression in the superficial dorsal horn of the spinal cord [151]. Taken together, these results also suggest that general anesthetics may selectively modulate neural targets associated with primary afferent inputs and motor signal outputs within different laminae of the spinal cord; however, it is unclear whether these effects are sufficient for volatile anesthetics to induce immobility *in vivo*.

4.2. Identifying c-fos Expression in Supraspinal Regions Involved in Analgesia Under General Anesthesia

Although the analgesic targets of general anesthetics in supraspinal regions are less clear than in the spinal cord, it has been suggested that supraspinal brain regions may participate in the analgesic effects of general anesthetics [23, 140]. The supraspinal brain maintains strong descending inhibition to nociceptive neurons in the spinal dorsal horn [23, 152, 153]. The ventrolateral periaqueductal gray matter (vlPAG) is the first identified supraspinal site that participates in descending pain modulation [154]. Subsequently, the rostral ventromedial medulla (RVM) is found to relay the projection from the PAG to the spinal cord [155, 156]. In addition to RVM, three pontine noradrenergic cell groups (A5-7) also receive inputs from the vlPAG [157] and project to the spinal dorsal horn [158]. The A5-7 cell groups have been reported to modulate endogenous antinociception [159] and antinociceptive effects of morphine [160], nitrous oxide [161], and isoflurane [162], via α2-adrenoceptors of neurons in the spinal dorsal horn, indicating that supraspinal regions may be potential analgesic targets under general anesthesia. Additionally, it is important to clarify the role of these regions and their interactional neural pathways, neural subtypes, and the molecular basis of the analgesic effects of general anesthetics.

4.2.1. Periaqueductal Gray (PAG) and Trigeminal Spinal Nucleus Caudalis (Vc)

Pentobarbital and propofol can increase neuronal c-fos expression in the periaqueductal gray (PAG) and trigeminal spinal nucleus caudalis (Vc) in rats [163]. Lu *et al.* showed

that lesions of the ventrolateral PAG (vlPAG) attenuated the antinociceptive effects of both GABA$_A$-agents (*e.g.*, chloral hydrate 300 mg/kg, and ethanol 1.0 g/kg) and ketamine (60 mg/kg) on noxious thermal stimulation [23], suggesting that vlPAG may be implicated in the analgesic effects of GABA$_A$-agents and ketamine. However, one study found that c-fos expression was very low in vlPAG with these anesthetics. This is probably due to the fact that the analgesic effects induced by these anesthetics may be primarily due to the suppression of inhibitory circuits (*e.g.*, GABAergic signaling) within the vlPAG, which may fail to increase c-fos expression.

4.2.2. Noradrenergic A5-7 Groups (A5-7)

The analgesic effects of both classes of GABAergic agents and the NMDA receptor antagonist ketamine may be associated with endogenous brain systems [23]. Both GABAergic agents (*e.g.*, chloral hydrate 350 mg/kg, and muscimol 5 mg/kg) and ketamine (60-300 mg/kg) induce c-fos expression in the spinally projecting noradrenergic A5-7 groups [23]. Like the vlPAG, lesions of the A5-7 groups attenuated the antinociceptive effects of these anesthetics on noxious thermal stimulation, suggesting that these general anesthetics can produce analgesia via A5-7 groups. Since the vlPAG innervates the A5-7 cell groups [157], it is possible that these classes of general anesthetics may inhibit GABAergic signaling in the vlPAG, thus releasing descending noradrenergic inhibition of A5-7 groups to suppress pain.

4.3. Identifying c-fos Expression in Brain Nucleus Associated with Analgesia under General Anesthesia

4.3.1. Central Amygdaloid Nucleus (CeA)

Central nuclei and circuits have been recently identified as being responsible for pain suppression of low-dose general anesthetics [140]. The authors used c-fos staining to screen neurons that remained strongly activated under isoflurane exposure. Three clusters were identified: CeA, oval division of the bed nucleus of the stria terminalis (ovBNST), and supraoptic nucleus (SON). As mentioned above, activation of SON neurons is related to the sedative components of general anesthetics, including isoflurane (0.5% or 1.5%, 2 h), ketamine/xylazine (ketamine 100 mg/kg + xylazine 10 mg/kg), and dexmedetomidine [18]. Isoflurane or intraperitoneal administration of ketamine and xylazine induced c-fos expression in GABAergic cells of CeA (CeA^{GABA} neurons). Accordingly, optogenetic activation of CeA^{GABA} neurons significantly suppressed both pain-elicited and self-recuperating behaviors, and alleviated neuropathic pain-induced mechanical hyperalgesia. Conversely, inhibition of the activity of CeA^{GABA} neurons exacerbated pain, produced strong aversion, and eliminated the analgesic effects of low-dose ketamine. Therefore, CeA^{GABA} neurons may serve as a potential target for general anesthetics to produce analgesia. It is unknown whether other subsets of CeA neurons that are involved in pain perception [164], such as neurons expressing somatostatin that do not overlap with CeA^{GABA} neurons, contribute to analgesia under general anesthesia. However, the authors did not prove that the activity of CeA^{GABA} neurons is also required for the analgesic effects of isoflurane *in vivo* due to the difficulty in performing behavioral tests. We believe that more brain nuclei related to general anesthetic-

induced analgesia must exist and will be discovered in the future.

5. A BRIEF SUMMARY OF C-FOS EXPRESSION CHANGES IN OTHER NUCLEI UNDER GENERAL ANESTHESIA WHICH HAVE NOT BEEN DETERMINED TO BE ASSOCIATED WITH ANESTHETIC ENDPOINTS

A recent study indicated that pentobarbital produced a different influence on c-fos expression in subcortical regions of mice, among which 12 subcortical regions were activated during pentobarbital anesthesia [19]. For subcortical sites of rats, systemic pentobarbital anesthesia increased c-fos expression in the LHb; meanwhile, it reduced c-fos expression in the TMN, zona incerta (ZI), and nucleus raphe pallidus (Rpa) as compared to the awake group [64], which is not inconsistent with the results of Yatziv *et al.* [19]. Different exposure duration to general anesthetics may partially explain the inconsistent results of these two studies.

Sevoflurane anesthesia at a concentration of 2.9% ± 0.2% for 1 h induced a significant increase in c-fos expression in many brain regions in mice, most of which have not yet been proven to be associated with the action of general anesthesia [165]. C-fos was selectively expressed in GABAergic neurons in the lateral septal nucleus (LS), while no c-fos was expressed on GABAergic neurons of the thalamus [165]. Therefore, GABAergic neurons in the LS instead of the thalamus may be related to the unconsciousness induced by sevoflurane anesthesia. A brief summary of the c-fos expression changes in brain regions with undermined function under general anesthesia is presented in Table **4**.

6. THE USE OF OTHER IMMEDIATE EARLY GENE PRODUCTS IN THIS FIELD

Numerous IEG products, such as c-fos, c-myc, Arc, Krox-24, fos-B, Jun-D, Jun-B, and Jun-C, can be used as biomarkers for neuronal activities [11-14]. In most cases, expressional changes of different IEG show the same trend in response to stimuli. For instance, Chastain-Potts *et al.* showed that sevoflurane exposure induces upregulation of Arc and Jun-B mRNA expression in the subiculum, which is crucial for synaptic plasticity and normal neuronal development [11]. Etomidate reduces the protein expression levels of memory-associated IEG in the rat hippocampus, including Arc, c-fos, and Egr1 [13]. However, there is some evidence that reveals distinct expression patterns in IEG under the same stimuli. Propofol increases c-fos mRNA but decreases Jun-C mRNA in rat brains [12]. Marota *et al.* showed that pentobarbital and halothane had select effects on c-fos and Jun-B mRNA expression in rat brains [166]. The related function may determine the expressional changes under a specific stimulus. Indeed, phosphorylation of extracellular signal-regulated protein kinase (ERK) is rarely used as a marker of neural activation. However, numerous studies have demonstrated that phosphorylation of ERK can induce the expression of its downstream proteins c-fos and that ERK phosphorylation/c-fos signaling is involved in many biological processes [167-169], including general anesthesia [170]. Although a number of IEG products have been detected in multiple biological processes associated with stress [171],

Table 4.   **A brief summary of c-fos changes in brain regions with undetermined function under general anesthesia.**

-	Isoflurane	Sevoflurane	Pentobarbital	Chloral hydrate	Ketamine
ZI	-	-	+[19]/-[64]	-	-
CC	-	-	-[19, 23, 64, 87]	-	+[23]
Cg	-[23]	+[165]	-[23]	-[23]	+[23]
PVHN	-	+[165]	+[19]	+[23]	-
LS	+[174]	+[165]	+[19]	+[23]	-
E-W	+[23]	+[165]	+[23]	+[23, 174]	+[23]
DG	-	+[165]	+[19]	-	-
Rpa	-	-	+[19]/-[64]	-	-
DMHN	-	+[165]	-	-	-
HC (CA1)	-	+[165]	-	-	-
AHN	-	+[165]	-	-	-
MT	-	-	-	-	+[23]

+, increased expression of c-fos; **-**, decreased expression of c-fos; **NA**, no effects on c-fos expression.
AHN, arcuate hypothalamic nucleus; CC, cerebral cortex; Cg, cingulate cortex; DG, dentate gyrus; DMHN, dorsomedial hypothalamic nucleus; E-W, Edinger-Westphal nucleus; HC, hippocampus; LS, lateral septum; MT, midline thalamus; PVHN, paraventricular hypothalamic nucleus; Rpa, nucleus raphe pallidus; ZI, zona incerta.

memory [172], pain [173, 174], and general anesthesia [12], c-fos is still the most commonly used indicator of neural activation.

CONCLUSION AND FUTURE PERSPECTIVES

Despite a number of brain nuclei and neuron subtypes, as well as anesthetic binding sites, using a combination of techniques both *in vitro* and *in vivo* have emerged. The complex neuronal pathways in the CNS response to general anesthesia are less clear. Finding the link between molecular targets, neural circuits, and anesthetic-related endpoints remains challenging. Collectively, most evidence indicates that the brain nuclei identified by c-fos staining related to general anesthesia-unconsciousness and emergence overlap well with the known sleep-wakefulness nuclei, respectively. Previous work also suggests that comparisons with the features of natural sleep help to understand how general anesthetics affect neuronal pathways [2]. Our review provided a map of c-fos expression changes related to different components of action of various general anesthetics in the CNS and revealed several involved neuronal pathways, which may serve as a resource for future studies focusing on the neural basis of general anesthesia.

For future studies, unbiased screening of the brain regions throughout the brain under general anesthesia and sleep-wakefulness nuclei using c-fos expression is still helpful. Subsequently, it is possible to create a map of c-fos-related changes in all brain regions. Additionally, since c-fos protein expression is relevantly delayed, we also recommend timely detection of the dynamic changes of c-fos expression in brain nuclei, especially the neuronal type, throughout the brain to uncover the neural circuits underlying general anesthesia. In addition, multifaceted approaches that link molecular targets with neural subpopulations, neural circuits, and integrated endpoints are critical to determining the mechanism of action of general anesthetics.

LIST OF ABBREVIATIONS

A2AR	=	Adenosine A2A Receptor
A5, A6, A7 groups	=	The Pontine Noradrenergic Cell Groups
AANs	=	Anesthesia-Activated Neurons
AHN	=	Arcuate Hypothalamic Nucleus
BF	=	Basal Forebrain
Cav	=	Voltage-gated Calcium Channel
CC	=	Cerebral Cortex
CeA	=	Central Amygdala
Cg	=	Cingulate Cortex
CNS	=	Central Nervous System
D1R	=	Dopamine D1 Receptor
DG	=	Dentate Gyrus
DMHN	=	Dorsomedial Hypothalamic Nucleus
DRN	=	Dorsal Raphe Nucleus
ERK	=	Extracellular Signal-Regulated Protein Kinase
E-W	=	Edinger-Westphal Nucleus
F6	=	1,2-dichlorohexafluorocyclobutane
GABA	=	Gamma-aminobutyric Acid
GPCRs	=	G Protein-coupled Receptors
HC	=	Hippocampus

IEG	=	Immediate Early Gene
K2P	=	Tandem Pore Potassium Channels
Kv	=	Voltage-gated K^+ Channels
LDTMN	=	Laterodorsal Tegmental Nucleus
LDTg	=	Laterodorsal Tegmental Nucleus
LH	=	Lateral Hypothalamus
LHb	=	Lateral Habenular Nucleus
LORR	=	Loss of Righting Reflex
LS	=	Lateral Septum
MM	=	Supramammillary Nucleus
MnPO	=	Median Preoptic Area
MnR	=	Median Raphe Nucleus
MPTA	=	Mesopontine Tegmental Anesthesia Area
MT	=	Midline Thalamus
nAChR	=	Nicotinic Acetylcholine Receptor
NALCN	=	Sodium Leak Channel
Nav	=	Voltage-gated Sodium Channel
NE	=	Norepinephrine
NMDA	=	N-methyl-D-aspartic Acid
PAG	=	Periaqueductal Gray
PBN	=	Parabrachial Nucleus
PFH	=	Perifornical Hypothalamus
PFLH	=	Perifornical Lateral Hypothalamus
PLH	=	Posterior Lateral Hypothalamus
PPN	=	Pedunculopontine Tegmental Nucleus
PPTg	=	Pedunculopontine Nucleus
PVHN	=	Paraventricular Hypothalamic Nucleus
RMP	=	Resting Membrane Potential
RMTg	=	Rostromedial Tegmental Nucleus
Rpa	=	Nucleus Raphe Pallidus
RVM	=	Rostral Ventromedial Medulla
SON	=	Supraoptic Nucleus
TMN	=	Tuberomammillary Nucleus
Vc	=	Trigeminal Spinal Nucleus Caudalis
vlPAG	=	Ventrolateral Periaqueductal Gray Dopamine Cells
VLPO	=	Ventrolateral Preoptic Nucleus
VTA	=	Ventral Tegmental Area
ZI	=	Zona Incerta

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

[1] Brown, E.N.; Lydic, R.; Schiff, N.D. General anesthesia, sleep, and coma. *N. Engl. J. Med.,* **2010**, *363*(27), 2638-2650.
http://dx.doi.org/10.1056/NEJMra0808281 PMID: 21190458

[2] Franks, N.P. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.,* **2008**, *9*(5), 370-386.
http://dx.doi.org/10.1038/nrn2372 PMID: 18425091

[3] Robinson, D.H.; Toledo, A.H. Historical development of modern anesthesia. *J. Invest. Surg.,* **2012**, *25*(3), 141-149.
http://dx.doi.org/10.3109/08941939.2012.690328 PMID: 22583009

[4] Rose, J.; Weiser, T.G.; Hider, P.; Wilson, L.; Gruen, R.L.; Bickler, S.W. Estimated need for surgery worldwide based on prevalence of diseases: a modelling strategy for the WHO Global Health Estimate. *Lancet Glob Health,* **2015**, *3 Suppl 2*, S13-S20.
http://dx.doi.org/10.1016/S2214-109X(15)70087-2

[5] Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet,* **2017**, *390*(10100), 1260-1344.
http://dx.doi.org/10.1016/S0140-6736(17)32130-X PMID: 28919118

[6] Alkire, M.T.; Hudetz, A.G.; Tononi, G. Consciousness and anesthesia. *Science,* **2008**, *322*(5903), 876-880.
http://dx.doi.org/10.1126/science.1149213 PMID: 18988836

[7] Koch, C.; Massimini, M.; Boly, M.; Tononi, G. Neural correlates of consciousness: progress and problems. *Nat. Rev. Neurosci.,* **2016**, *17*(5), 307-321.
http://dx.doi.org/10.1038/nrn.2016.22 PMID: 27094080

[8] Rudolph, U.; Antkowiak, B. Molecular and neuronal substrates for general anaesthetics. *Nat. Rev. Neurosci.,* **2004**, *5*(9), 709-720.
http://dx.doi.org/10.1038/nrn1496 PMID: 15322529

[9] Allada, R. An emerging link between general anesthesia and sleep. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(7), 2257-2258.
http://dx.doi.org/10.1073/pnas.0711532105 PMID: 18272494

[10] Suzuki, M.; Larkum, M.E. General anesthesia decouples cortical pyramidal neurons. *Cell,* **2020**, *180*(4), 666-676.e13.
http://dx.doi.org/10.1016/j.cell.2020.01.024 PMID: 32084339

[11] Chastain-Potts, S.E.; Tesic, V.; Tat, Q.L.; Cabrera, O.H.; Quillinan, N.; Jevtovic-Todorovic, V. Sevoflurane exposure results in sex-specific transgenerational upregulation of target IEGs in the Subiculum. *Mol. Neurobiol.,* **2020**, *57*(1), 11-22.
http://dx.doi.org/10.1007/s12035-019-01752-0 PMID: 31512116

[12] Hamaya, Y.; Takeda, T.; Dohi, S.; Nakashima, S.; Nozawa, Y. The effects of pentobarbital, isoflurane, and propofol on immediate-early gene expression in the vital organs of the rat. *Anesth. Analg.,* **2000**, *90*(5), 1177-1183.
http://dx.doi.org/10.1097/00000539-200005000-00034 PMID: 10781476

[13] Li, X.; Lu, F.; Li, W.; Xu, J.; Sun, X.J.; Qin, L.Z.; Zhang, Q.L.; Yao, Y.; Yu, Q.K.; Liang, X.L. Underlying mechanisms of memory deficits induced by etomidate anesthesia in aged rat mod-

el: critical role of immediate early genes. *Chin. Med. J. (Engl.),* **2016**, *129*(1), 48-53.
http://dx.doi.org/10.4103/0366-6999.172570 PMID: 26712432

[14]   Lantéri-Minet, M.; Isnardon, P.; de Pommery, J.; Menétrey, D. Spinal and hindbrain structures involved in visceroception and visceronociception as revealed by the expression of Fos, Jun and Krox-24 proteins. *Neuroscience,* **1993**, *55*(3), 737-753.
http://dx.doi.org/10.1016/0306-4522(93)90439-M PMID: 8413935

[15]   Bullitt, E. Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. *J. Comp. Neurol.,* **1990**, *296*(4), 517-530.
http://dx.doi.org/10.1002/cne.902960402 PMID: 2113539

[16]   Morgan, J.I.; Curran, T. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. *Trends Neurosci.,* **1989**, *12*(11), 459-462.
http://dx.doi.org/10.1016/0166-2236(89)90096-9 PMID: 2479148

[17]   Kelz, M.B.; Sun, Y.; Chen, J.; Cheng Meng, Q.; Moore, J.T.; Veasey, S.C.; Dixon, S.; Thornton, M.; Funato, H.; Yanagisawa, M. An essential role for orexins in emergence from general anesthesia. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(4), 1309-1314.
http://dx.doi.org/10.1073/pnas.0707146105 PMID: 18195361

[18]   Jiang-Xie, L.F.; Yin, L.; Zhao, S.; Prevosto, V.; Han, B.X.; Dzirasa, K.; Wang, F. A common neuroendocrine substrate for diverse general anesthetics and sleep. *Neuron,* **2019**, *102*(5), 1053-1065.e4.
http://dx.doi.org/10.1016/j.neuron.2019.03.033 PMID: 31006556

[19]   Yatziv, S.L.; Yudco, O.; Dickmann, S.; Devor, M. Patterns of neural activity in the mouse brain: wakefulness *vs.* general anesthesia. *Neurosci. Lett.,* **2020**, *735*135212
http://dx.doi.org/10.1016/j.neulet.2020.135212 PMID: 32593772

[20]   Saper, C.B.; Scammell, T.E.; Lu, J. Hypothalamic regulation of sleep and circadian rhythms. *Nature,* **2005**, *437*(7063), 1257-1263.
http://dx.doi.org/10.1038/nature04284 PMID: 16251950

[21]   Kroeger, D.; Absi, G.; Gagliardi, C.; Bandaru, S.S.; Madara, J.C.; Ferrari, L.L.; Arrigoni, E.; Münzberg, H.; Scammell, T.E.; Saper, C.B.; Vetrivelan, R. Galanin neurons in the ventrolateral preoptic area promote sleep and heat loss in mice. *Nat. Commun.,* **2018**, *9*(1), 4129.
http://dx.doi.org/10.1038/s41467-018-06590-7 PMID: 30297727

[22]   Nelson, L.E.; Guo, T.Z.; Lu, J.; Saper, C.B.; Franks, N.P.; Maze, M. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. *Nat. Neurosci.,* **2002**, *5*(10), 979-984.
http://dx.doi.org/10.1038/nn913 PMID: 12195434

[23]   Lu, J.; Nelson, L.E.; Franks, N.; Maze, M.; Chamberlin, N.L.; Saper, C.B. Role of endogenous sleep-wake and analgesic systems in anesthesia. *J. Comp. Neurol.,* **2008**, *508*(4), 648-662.
http://dx.doi.org/10.1002/cne.21685 PMID: 18383504

[24]   Nelson, L.E.; Lu, J.; Guo, T.; Saper, C.B.; Franks, N.P.; Maze, M. The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. *Anesthesiology,* **2003**, *98*(2), 428-436.
http://dx.doi.org/10.1097/00000542-200302000-00024 PMID: 12552203

[25]   Moore, J.T.; Chen, J.; Han, B.; Meng, Q.C.; Veasey, S.C.; Beck, S.G.; Kelz, M.B. Direct activation of sleep-promoting VLPO neurons by volatile anesthetics contributes to anesthetic hypnosis. *Curr. Biol.,* **2012**, *22*(21), 2008-2016.
http://dx.doi.org/10.1016/j.cub.2012.08.042 PMID: 23103189

[26]   Han, B.; McCarren, H.S.; O'Neill, D.; Kelz, M.B. Distinctive recruitment of endogenous sleep-promoting neurons by volatile anesthetics and a nonimmobilizer. *Anesthesiology,* **2014**, *121*(5), 999-1009.
http://dx.doi.org/10.1097/ALN.0000000000000383 PMID: 25057841

[27]   Ratnakumari, L.; Vysotskaya, T.N.; Duch, D.S.; Hemmings, H.C., Jr Differential effects of anesthetic and nonanesthetic cyclobutanes on neuronal voltage-gated sodium channels. *Anesthesiology,* **2000**, *92*(2), 529-541.
http://dx.doi.org/10.1097/00000542-200002000-00037 PMID: 10691242

[28]   Recio-Pinto, E.; Montoya-Gacharna, J.V.; Xu, F.; Blanck, T.J.J. Isoflurane, but Not the Nonimmobilizers F6 and F8, inhibits rat spinal cord motor neuron CaV1 Calcium Currents. *Anesth. Analg.,* **2016**, *122*(3), 730-737.

http://dx.doi.org/10.1213/ANE.0000000000001111 PMID: 26702867

[29]   Barber, A.F.; Carnevale, V.; Klein, M.L.; Eckenhoff, R.G.; Covarrubias, M. Modulation of a voltage-gated Na$^+$ channel by sevoflurane involves multiple sites and distinct mechanisms. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(18), 6726-6731.
http://dx.doi.org/10.1073/pnas.1405768111 PMID: 24753583

[30]   Lu, B.; Su, Y.; Das, S.; Liu, J.; Xia, J.; Ren, D. The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. *Cell,* **2007**, *129*(2), 371-383.
http://dx.doi.org/10.1016/j.cell.2007.02.041 PMID: 17448995

[31]   Yekkirala, A.S.; Roberson, D.P.; Bean, B.P.; Woolf, C.J. Breaking barriers to novel analgesic drug development. *Nat. Rev. Drug Discov.,* **2017**, *16*(8), 545-564.
http://dx.doi.org/10.1038/nrd.2017.87 PMID: 28596533

[32]   Kelz, M.B.; Mashour, G.A. The biology of general anesthesia from paramecium to primate. *Curr. Biol.,* **2019**, *29*(22), R1199-R1210.
http://dx.doi.org/10.1016/j.cub.2019.09.071 PMID: 31743680

[33]   Hao, X.; Ou, M.; Zhang, D.; Zhao, W.; Yang, Y.; Liu, J.; Yang, H.; Zhu, T.; Li, Y.; Zhou, C. The effects of general anesthetics on synaptic transmission. *Curr. Neuropharmacol.,* **2020**, *18*(10), 936-965.
http://dx.doi.org/10.2174/1570159X18666200227125854 PMID: 32106800

[34]   Enyedi, P.; Czirják, G. Molecular background of leak K+ currents: two-pore domain potassium channels. *Physiol. Rev.,* **2010**, *90*(2), 559-605.
http://dx.doi.org/10.1152/physrev.00029.2009 PMID: 20393194

[35]   Patel, A.J.; Honoré, E.; Lesage, F.; Fink, M.; Romey, G.; Lazdunski, M. Inhalational anesthetics activate two-pore-domain background K$^+$ channels. *Nat. Neurosci.,* **1999**, *2*(5), 422-426.
http://dx.doi.org/10.1038/8084 PMID: 10321245

[36]   Pavel, M.A.; Petersen, E.N.; Wang, H.; Lerner, R.A.; Hansen, S.B. Studies on the mechanism of general anesthesia. *Proc. Natl. Acad. Sci. USA,* **2020**, *117*(24), 13757-13766.
http://dx.doi.org/10.1073/pnas.2004259117 PMID: 32467161

[37]   Heurteaux, C.; Guy, N.; Laigle, C.; Blondeau, N.; Duprat, F.; Mazzuca, M.; Lang-Lazdunski, L.; Widmann, C.; Zanzouri, M.; Romey, G.; Lazdunski, M. TREK-1, a K$^+$ channel involved in neuroprotection and general anesthesia. *EMBO J.,* **2004**, *23*(13), 2684-2695.
http://dx.doi.org/10.1038/sj.emboj.7600234 PMID: 15175651

[38]   Wague, A.; Joseph, T.T.; Woll, K.A.; Bu, W.; Vaidya, K.A.; Bhanu, N.V.; Garcia, B.A.; Nimigean, C.M.; Eckenhoff, R.G.; Riegelhaupt, P.M. Mechanistic insights into volatile anesthetic modulation of K2P channels. *eLife,* **2020**, *9*, 9.
http://dx.doi.org/10.7554/eLife.59839 PMID: 33345771

[39]   Barber, A.F.; Liang, Q.; Covarrubias, M. Novel activation of voltage-gated K$^{(+)}$ channels by sevoflurane. *J. Biol. Chem.,* **2012**, *287*(48), 40425-40432.
http://dx.doi.org/10.1074/jbc.M112.405787 PMID: 23038249

[40]   Stock, L.; Hosoume, J.; Cirqueira, L.; Treptow, W. Binding of the general anesthetic sevoflurane to ion channels. *PLOS Comput. Biol.,* **2018**, *14*(11),e1006605.
http://dx.doi.org/10.1371/journal.pcbi.1006605 PMID: 30475796

[41]   Feng, Z.X.; Dong, H.; Qu, W.M.; Zhang, W. Oral Delivered Dexmedetomidine Promotes and consolidates non-rapid eye movement sleep *via* sleep-wake regulation systems in mice. *Front. Pharmacol.,* **2018**, *9*, 1196.
http://dx.doi.org/10.3389/fphar.2018.01196 PMID: 30568589

[42]   McCarren, H.S.; Chalifoux, M.R.; Han, B.; Moore, J.T.; Meng, Q.C.; Baron-Hionis, N.; Sedigh-Sarvestani, M.; Contreras, D.; Beck, S.G.; Kelz, M.B. α2-Adrenergic stimulation of the ventrolateral preoptic nucleus destabilizes the anesthetic state. *J. Neurosci.,* **2014**, *34*(49), 16385-16396.
http://dx.doi.org/10.1523/JNEUROSCI.1135-14.2014 PMID: 25471576

[43]   Eiland, M.M.; Ramanathan, L.; Gulyani, S.; Gilliland, M.; Bergmann, B.M.; Rechtschaffen, A.; Siegel, J.M. Increases in aminocupric-silver staining of the supraoptic nucleus after sleep deprivation. *Brain Res.,* **2002**, *945*(1), 1-8.
http://dx.doi.org/10.1016/S0006-8993(02)02448-4 PMID: 12113945

[44]   Ohbuchi, T.; Saito, T.; Yokoyama, T.; Hashimoto, H.; Maruyama, T.; Suzuki, H.; Ueta, Y. Osmotic perception of GABAergic synap-

tic transmission in the supraoptic nucleus of rats. *IBRO Rep.,* **2020**, *9*, 58-64.
http://dx.doi.org/10.1016/j.ibror.2020.06.007 PMID: 32685762

[45] Hirose, Y.; Kitazono, T.; Sezaki, M.; Abe, M.; Sakimura, K.; Funato, H.; Handa, H.; Vogt, K.E.; Yanagisawa, M. Hypnotic effect of thalidomide is independent of teratogenic ubiquitin/proteasome pathway. *Proc. Natl. Acad. Sci. USA,* **2020**, *117*(37), 23106-23112.
http://dx.doi.org/10.1073/pnas.1917701117 PMID: 32848052

[46] Yamashita, T.; Yamanaka, A. Lateral hypothalamic circuits for sleep-wake control. *Curr. Opin. Neurobiol.,* **2017**, *44*, 94-100.
http://dx.doi.org/10.1016/j.conb.2017.03.020 PMID: 28427008

[47] Yu, X.; Ye, Z.; Houston, C.M.; Zecharia, A.Y.; Ma, Y.; Zhang, Z.; Uygun, D.S.; Parker, S.; Vyssotski, A.L.; Yustos, R.; Franks, N.P.; Brickley, S.G.; Wisden, W. Wakefulness Is Governed by GABA and Histamine Cotransmission. *Neuron,* **2015**, *87*(1), 164-178.
http://dx.doi.org/10.1016/j.neuron.2015.06.003 PMID: 26094607

[48] Pedersen, N.P.; Ferrari, L.; Venner, A.; Wang, J.L.; Abbott, S.B.G.; Vujovic, N.; Arrigoni, E.; Saper, C.B.; Fuller, P.M. Supramammillary glutamate neurons are a key node of the arousal system. *Nat. Commun.,* **2017**, *8*(1), 1405.
http://dx.doi.org/10.1038/s41467-017-01004-6 PMID: 29123082

[49] Gelegen, C.; Miracca, G.; Ran, M.Z.; Harding, E.C.; Ye, Z.; Yu, X.; Tossell, K.; Houston, C.M.; Yustos, R.; Hawkins, E.D.; Vyssotski, A.L.; Dong, H.L.; Wisden, W.; Franks, N.P. Excitatory pathways from the lateral habenula enable propofol-induced sedation. *Curr. Biol.,* **2018**, *28*(4), 580-587.e5.
http://dx.doi.org/10.1016/j.cub.2017.12.050 PMID: 29398217

[50] Weber, F.; Hoang Do, J.P.; Chung, S.; Beier, K.T.; Bikov, M.; Saffari Doost, M.; Dan, Y. Regulation of REM and Non-REM sleep by periaqueductal GABAergic Neurons. *Nat. Commun.,* **2018**, *9*(1), 354.
http://dx.doi.org/10.1038/s41467-017-02765-w PMID: 29367602

[51] Taylor, N.E.; Van Dort, C.J.; Kenny, J.D.; Pei, J.; Guidera, J.A.; Vlasov, K.Y.; Lee, J.T.; Boyden, E.S.; Brown, E.N.; Solt, K. Optogenetic activation of dopamine neurons in the ventral tegmental area induces reanimation from general anesthesia. *Proc. Natl. Acad. Sci. USA,* **2016**, *113*(45), 12826-12831.
http://dx.doi.org/10.1073/pnas.1614340113 PMID: 27791160

[52] Van Dort, C.J.; Zachs, D.P.; Kenny, J.D.; Zheng, S.; Goldblum, R.R.; Gelwan, N.A.; Ramos, D.M.; Nolan, M.A.; Wang, K.; Weng, F.J.; Lin, Y.; Wilson, M.A.; Brown, E.N. Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. *Proc. Natl. Acad. Sci. USA,* **2015**, *112*(2), 584-589.
http://dx.doi.org/10.1073/pnas.1423136112 PMID: 25548191

[53] Hikosaka, O. The habenula: from stress evasion to value-based decision-making. *Nat. Rev. Neurosci.,* **2010**, *11*(7), 503-513.
http://dx.doi.org/10.1038/nrn2866 PMID: 20559337

[54] Zhao, H.; Zhang, B.L.; Yang, S.J.; Rusak, B. The role of lateral habenula-dorsal raphe nucleus circuits in higher brain functions and psychiatric illness. *Behav. Brain Res.,* **2015**, *277*, 89-98.
http://dx.doi.org/10.1016/j.bbr.2014.09.016 PMID: 25234226

[55] Lazaridis, I.; Tzortzi, O.; Weglage, M.; Märtin, A.; Xuan, Y.; Parent, M.; Johansson, Y.; Fuzik, J.; Fürth, D.; Fenno, L.E.; Ramakrishnan, C.; Silberberg, G.; Deisseroth, K.; Carlén, M.; Meletis, K. A hypothalamus-habenula circuit controls aversion. *Mol. Psychiatry,* **2019**, *24*(9), 1351-1368.
http://dx.doi.org/10.1038/s41380-019-0369-5 PMID: 30755721

[56] Wang, D.; Li, A.; Dong, K.; Li, H.; Guo, Y.; Zhang, X.; Cai, M.; Li, H.; Zhao, G.; Yang, Q. Lateral hypothalamus orexinergic inputs to lateral habenula modulate maladaptation after social defeat stress. *Neurobiol. Stress,* **2021**, *14,*100298.
http://dx.doi.org/10.1016/j.ynstr.2021.100298 PMID: 33569507

[57] Jhou, T.C.; Geisler, S.; Marinelli, M.; Degarmo, B.A.; Zahm, D.S. The mesopontine rostromedial tegmental nucleus: A structure targeted by the lateral habenula that projects to the ventral tegmental area of Tsai and substantia nigra compacta. *J. Comp. Neurol.,* **2009**, *513*(6), 566-596.
http://dx.doi.org/10.1002/cne.21891 PMID: 19235216

[58] Gonçalves, L.; Sego, C.; Metzger, M. Differential projections from the lateral habenula to the rostromedial tegmental nucleus and ventral tegmental area in the rat. *J. Comp. Neurol.,* **2012**, *520*(6), 1278-1300.
http://dx.doi.org/10.1002/cne.22787 PMID: 22020635

[59] Laurent, V.; Wong, F.L.; Balleine, B.W. The lateral habenula and its input to the rostromedial tegmental nucleus mediates outcome-specific conditioned inhibition. *J. Neurosci.,* **2017**, *37*(45), 10932-10942.
http://dx.doi.org/10.1523/JNEUROSCI.3415-16.2017 PMID: 28986462

[60] Hu, H.; Cui, Y.; Yang, Y. Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.,* **2020**, *21*(5), 277-295.
http://dx.doi.org/10.1038/s41583-020-0292-4 PMID: 32269316

[61] Yang, Y.; Cui, Y.; Sang, K.; Dong, Y.; Ni, Z.; Ma, S.; Hu, H. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature,* **2018**, *554*(7692), 317-322.
http://dx.doi.org/10.1038/nature25509 PMID: 29446381

[62] Cerniauskas, I.; Winterer, J.; de Jong, J.W.; Lukacsovich, D.; Yang, H.; Khan, F.; Peck, J.R.; Obayashi, S.K.; Lilascharoen, V.; Lim, B.K.; Földy, C.; Lammel, S. Chronic stress induces activity, synaptic, and transcriptional remodeling of the lateral habenula associated with deficits in motivated behaviors. *Neuron,* **2019**, *104*(5), 899-915.e8.
http://dx.doi.org/10.1016/j.neuron.2019.09.005 PMID: 31672263

[63] Mendoza, J. Circadian neurons in the lateral habenula: Clocking motivated behaviors. *Pharmacol. Biochem. Behav.,* **2017**, *162*, 55-61.
http://dx.doi.org/10.1016/j.pbb.2017.06.013 PMID: 28666896

[64] Abulafia, R.; Zalkind, V.; Devor, M. Cerebral activity during the anesthesia-like state induced by mesopontine microinjection of pentobarbital. *J. Neurosci.,* **2009**, *29*(21), 7053-7064.
http://dx.doi.org/10.1523/JNEUROSCI.1357-08.2009 PMID: 19474332

[65] Harris, H.N.; Peng, Y.B. Evidence and explanation for the involvement of the nucleus accumbens in pain processing. *Neural Regen. Res.,* **2020**, *15*(4), 597-605.
http://dx.doi.org/10.4103/1673-5374.266909 PMID: 31638081

[66] Ma, J.; Leung, L.S. Limbic system participates in mediating the effects of general anesthetics. *Neuropsychopharmacology,* **2006**, *31*(6), 1177-1192.
http://dx.doi.org/10.1038/sj.npp.1300909 PMID: 16205783

[67] Hong, Z.Y.; Huang, Z.L.; Qu, W.M.; Eguchi, N.; Urade, Y.; Hayaishi, O. An adenosine A receptor agonist induces sleep by increasing GABA release in the tuberomammillary nucleus to inhibit histaminergic systems in rats. *J. Neurochem.,* **2005**, *92*(6), 1542-1549.
http://dx.doi.org/10.1111/j.1471-4159.2004.02991.x PMID: 15748171

[68] Zhang, J.P.; Xu, Q.; Yuan, X.S.; Cherasse, Y.; Schiffmann, S.N.; de Kerchove d'Exaerde, A.; Qu, W.M.; Urade, Y.; Lazarus, M.; Huang, Z.L.; Li, R.X. Projections of nucleus accumbens adenosine A2A receptor neurons in the mouse brain and their implications in mediating sleep-wake regulation. *Front. Neuroanat.,* **2013**, *7*, 43.
http://dx.doi.org/10.3389/fnana.2013.00043 PMID: 24409122

[69] Chen, L.; Li, S.; Zhou, Y.; Liu, T.; Cai, A.; Zhang, Z.; Xu, F.; Manyande, A.; Wang, J.; Peng, M. Neuronal mechanisms of adenosine A(2A) receptors in the loss of consciousness induced by propofol general anesthesia with functional magnetic resonance imaging. *J. Neurochem.,* **2021**, *156*(6),1020-1032.

[70] Bao, W.W.; Xu, W.; Pan, G.J.; Wang, T.X.; Han, Y.; Qu, W.M.; Li, W.X.; Huang, Z.L. Nucleus accumbens neurons expressing dopamine D1 receptors modulate states of consciousness in sevoflurane anesthesia. *Curr. Biol.,* **2021**, *31*(9), 1893-1902.e5.
http://dx.doi.org/10.1016/j.cub.2021.02.011 PMID: 33705720

[71] Haas, H.; Panula, P. The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat. Rev. Neurosci.,* **2003**, *4*(2), 121-130.
http://dx.doi.org/10.1038/nrn1034 PMID: 12563283

[72] Peyron, C.; Tighe, D.K.; van den Pol, A.N.; de Lecea, L.; Heller, H.C.; Sutcliffe, J.G.; Kilduff, T.S. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.,* **1998**, *18*(23), 9996-10015.
http://dx.doi.org/10.1523/JNEUROSCI.18-23-09996.1998 PMID: 9822755

[73] Mignot, E.; Lammers, G.J.; Ripley, B.; Okun, M.; Nevsimalova, S.; Overeem, S.; Vankova, J.; Black, J.; Harsh, J.; Bassetti, C.; Schrader, H.; Nishino, S. The role of cerebrospinal fluid hypocretin meas-

urement in the diagnosis of narcolepsy and other hypersomnias. *Arch. Neurol.,* **2002**, *59*(10), 1553-1562.
http://dx.doi.org/10.1001/archneur.59.10.1553 PMID: 12374492

[74]   Sakurai, T. The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat. Rev. Neurosci.,* **2007**, *8*(3), 171-181.
http://dx.doi.org/10.1038/nrn2092 PMID: 17299454

[75]   Kushikata, T.; Hirota, K.; Yoshida, H.; Kudo, M.; Lambert, D.G.; Smart, D.; Jerman, J.C.; Matsuki, A. Orexinergic neurons and bar-biturate anesthesia. *Neuroscience,* **2003**, *121*(4), 855-863.
http://dx.doi.org/10.1016/S0306-4522(03)00554-2 PMID: 14580935

[76]   Zecharia, A.Y.; Nelson, L.E.; Gent, T.C.; Schumacher, M.; Jurd, R.; Rudolph, U.; Brickley, S.G.; Maze, M.; Franks, N.P. The in-volvement of hypothalamic sleep pathways in general anesthesia: testing the hypothesis using the GABAA receptor beta3N265M knock-in mouse. *J. Neurosci.,* **2009**, *29*(7), 2177-2187.
http://dx.doi.org/10.1523/JNEUROSCI.4997-08.2009 PMID: 19228970

[77]   Gompf, H.; Chen, J.; Sun, Y.; Yanagisawa, M.; Aston-Jones, G.; Kelz, M.B. Halothane-induced hypnosis is not accompanied by in-activation of orexinergic output in rodents. *Anesthesiology,* **2009**, *111*(5), 1001-1009.
http://dx.doi.org/10.1097/ALN.0b013e3181b764b3 PMID: 19809293

[78]   Eckenhoff, M.F.; Eckenhoff, R.G. Quantitative autoradiography of halothane binding in rat brain. *J. Pharmacol. Exp. Ther.,* **1998**, *285*(1), 371-376.
PMID: 9536033

[79]   Correa, A.M. Gating kinetics of Shaker K+ channels are differen-tially modified by general anesthetics. *Am. J. Physiol.,* **1998**, *275*(4), C1009-C1021.
http://dx.doi.org/10.1152/ajpcell.1998.275.4.C1009 PMID: 9755054

[80]   Peterlin, Z.; Ishizawa, Y.; Araneda, R.; Eckenhoff, R.; Firestein, S. Selective activation of G-protein coupled receptors by volatile an-esthetics. *Mol. Cell. Neurosci.,* **2005**, *30*(4), 506-512.
http://dx.doi.org/10.1016/j.mcn.2005.08.012 PMID: 16185894

[81]   Devor, M.; Zalkind, V. Reversible analgesia, atonia, and loss of consciousness on bilateral intracerebral microinjection of pentobar-bital. *Pain,* **2001**, *94*(1), 101-112.
http://dx.doi.org/10.1016/S0304-3959(01)00345-1 PMID: 11576749

[82]   Leung, L.S.; Luo, T.; Ma, J.; Herrick, I. Brain areas that influence general anesthesia. *Prog. Neurobiol.,* **2014**, *122*, 24-44.
http://dx.doi.org/10.1016/j.pneurobio.2014.08.001 PMID: 25172271

[83]   Minert, A.; Baron, M.; Devor, M. Reduced sensitivity to anesthetic agents upon lesioning the mesopontine tegmental anesthesia area in rats depends on anesthetic type. *Anesthesiology,* **2020**, *132*(3), 535-550.
http://dx.doi.org/10.1097/ALN.0000000000003087 PMID: 31850942

[84]   Minert, A.; Yatziv, S.L.; Devor, M. Location of the mesopontine neurons responsible for maintenance of anesthetic loss of con-sciousness. *J. Neurosci.,* **2017**, *37*(38), 9320-9331.
http://dx.doi.org/10.1523/JNEUROSCI.0544-17.2017 PMID: 28821646

[85]   Minert, A.; Devor, M. Brainstem node for loss of consciousness due to GABA(A) receptor-active anesthetics. *Exp. Neurol.,* **2016**, *275*(Pt 1), 38-45.
http://dx.doi.org/10.1016/j.expneurol.2015.10.001 PMID: 26436687

[86]   Lanir-Azaria, S.; Meiri, G.; Avigdor, T.; Minert, A.; Devor, M. Enhanced wakefulness following lesions of a mesopontine locus essential for the induction of general anesthesia. *Behav. Brain Res.,* **2018**, *341*, 198-211.
http://dx.doi.org/10.1016/j.bbr.2017.12.035 PMID: 29288749

[87]   Takayama, K.; Suzuki, T.; Miura, M. The comparison of effects of various anesthetics on expression of Fos protein in the rat brain. *Neurosci. Lett.,* **1994**, *176*(1), 59-62.
http://dx.doi.org/10.1016/0304-3940(94)90871-0 PMID: 7970238

[88]   Boly, M.; Garrido, M.I.; Gosseries, O.; Bruno, M.A.; Boveroux, P.; Schnakers, C.; Massimini, M.; Litvak, V.; Laureys, S.; Friston, K. Preserved feedforward but impaired top-down processes in the vegetative state. *Science,* **2011**, *332*(6031), 858-862.
http://dx.doi.org/10.1126/science.1202043 PMID: 21566197

[89]   Cascella, M.; Bimonte, S.; Di Napoli, R. Delayed emergence from anesthesia: what we know and how we act. *Local Reg. Anesth.,* **2020**, *13*, 195-206.
http://dx.doi.org/10.2147/LRA.S230728 PMID: 33177867

[90]   Cascella, M.; Bimonte, S.; Amruthraj, N.J. Awareness during emergence from anesthesia: Features and future research directions. *World J. Clin. Cases,* **2020**, *8*(2), 245-254.
http://dx.doi.org/10.12998/wjcc.v8.i2.245 PMID: 32047772

[91]   Tarnal, V.; Vlisides, P.E.; Mashour, G.A. The Neurobiology of Anesthetic Emergence. *J. Neurosurg. Anesthesiol.,* **2016**, *28*(3), 250-255.
http://dx.doi.org/10.1097/ANA.0000000000000212 PMID: 26274626

[92]   Vanini, G.; Nemanis, K.; Baghdoyan, H.A.; Lydic, R. GABAergic transmission in rat pontine reticular formation regulates the induc-tion phase of anesthesia and modulates hyperalgesia caused by sleep deprivation. *Eur. J. Neurosci.,* **2014**, *40*(1), 2264-2273.
http://dx.doi.org/10.1111/ejn.12571 PMID: 24674578

[93]   Zhang, L.N.; Li, Z.J.; Tong, L.; Guo, C.; Niu, J.Y.; Hou, W.G.; Dong, H.L. Orexin-A facilitates emergence from propofol anesthe-sia in the rat. *Anesth. Analg.,* **2012**, *115*(4), 789-796.
http://dx.doi.org/10.1213/ANE.0b013e3182645ea3 PMID: 22798527

[94]   Ren, S.; Wang, Y.; Yue, F.; Cheng, X.; Dang, R.; Qiao, Q.; Sun, X.; Li, X.; Jiang, Q.; Yao, J.; Qin, H.; Wang, G.; Liao, X.; Gao, D.; Xia, J.; Zhang, J.; Hu, B.; Yan, J.; Wang, Y.; Xu, M.; Han, Y.; Tang, X.; Chen, X.; He, C.; Hu, Z. The paraventricular thalamus is a critical thalamic area for wakefulness. *Science,* **2018**, *362*(6413), 429-434.
http://dx.doi.org/10.1126/science.aat2512 PMID: 30361367

[95]   Hoffman, G.E.; Smith, M.S.; Verbalis, J.G. c-Fos and related im-mediate early gene products as markers of activity in neuroendo-crine systems. *Front. Neuroendocrinol.,* **1993**, *14*(3), 173-213.
http://dx.doi.org/10.1006/frne.1993.1006 PMID: 8349003

[96]   Kayaba, Y.; Nakamura, A.; Kasuya, Y.; Ohuchi, T.; Yanagisawa, M.; Komuro, I.; Fukuda, Y.; Kuwaki, T. Attenuated defense re-sponse and low basal blood pressure in orexin knockout mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **2003**, *285*(3), R581-R593.
http://dx.doi.org/10.1152/ajpregu.00671.2002 PMID: 12750151

[97]   Yoshimichi, G.; Yoshimatsu, H.; Masaki, T.; Sakata, T. Orexin-A regulates body temperature in coordination with arousal status. *Exp. Biol. Med. (Maywood),* **2001**, *226*(5), 468-476.
http://dx.doi.org/10.1177/153537020122600513 PMID: 11393177

[98]   Brisbare-Roch, C.; Dingemanse, J.; Koberstein, R.; Hoever, P.; Aissaoui, H.; Flores, S.; Mueller, C.; Nayler, O.; van Gerven, J.; de Haas, S.L.; Hess, P.; Qiu, C.; Buchmann, S.; Scherz, M.; Weller, T.; Fischli, W.; Clozel, M.; Jenck, F. Promotion of sleep by target-ing the orexin system in rats, dogs and humans. *Nat. Med.,* **2007**, *13*(2), 150-155.
http://dx.doi.org/10.1038/nm1544 PMID: 17259994

[99]   Shirasaka, T.; Nakazato, M.; Matsukura, S.; Takasaki, M.; Kannan, H. Sympathetic and cardiovascular actions of orexins in conscious rats. *Am. J. Physiol.,* **1999**, *277*(6), R1780-R1785.
PMID: 10600926

[100] Yasuda, Y.; Takeda, A.; Fukuda, S.; Suzuki, H.; Ishimoto, M.; Mori, Y.; Eguchi, H.; Saitoh, R.; Fujihara, H.; Honda, K.; Higuchi, T. Orexin a elicits arousal electroencephalography without sympa-thetic cardiovascular activation in isoflurane-anesthetized rats. *Anesth. Analg.,* **2003**, *97*(6), 1663-1666.
http://dx.doi.org/10.1213/01.ANE.0000089964.85834.EF PMID: 14633539

[101] Wang, D.; Guo, Y.; Li, H.; Li, J.; Ran, M.; Guo, J.; Yin, L.; Zhao, S.; Yang, Q.; Dong, H. Selective optogenetic activation of orex-inergic terminals in the basal forebrain and locus coeruleus pro-motes emergence from isoflurane anaesthesia in rats. *Br. J. Anaesth.,* **2020**, *126*(1), 279-292.
http://dx.doi.org/10.1016/j.bja.2020.09.037 PMID: 33131759

[102] Zhao, S.; Li, R.; Li, H.; Wang, S.; Zhang, X.; Wang, D.; Guo, J.; Li, H.; Li, A.; Tong, T.; Zhong, H.; Yang, Q.; Dong, H. Lateral hy-pothalamic area glutamatergic neurons and their projections to the

lateral habenula modulate the anesthetic potency of isoflurane in mice. *Neurosci. Bull.,* **2021**, *37*(7), 934-946.
http://dx.doi.org/10.1007/s12264-021-00674-z PMID: 33847915

[103] Nury, H.; Van Renterghem, C.; Weng, Y.; Tran, A.; Baaden, M.; Dufresne, V.; Changeux, J.P.; Sonner, J.M.; Delarue, M.; Corringer, P.J. X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. *Nature,* **2011**, *469*(7330), 428-431.
http://dx.doi.org/10.1038/nature09647 PMID: 21248852

[104] Brannigan, G.; LeBard, D.N.; Hénin, J.; Eckenhoff, R.G.; Klein, M.L. Multiple binding sites for the general anesthetic isoflurane identified in the nicotinic acetylcholine receptor transmembrane domain. *Proc. Natl. Acad. Sci. USA,* **2010**, *107*(32), 14122-14127.
http://dx.doi.org/10.1073/pnas.1008534107 PMID: 20660787

[105] Jayakar, S.S.; Dailey, W.P.; Eckenhoff, R.G.; Cohen, J.B. Identification of propofol binding sites in a nicotinic acetylcholine receptor with a photoreactive propofol analog. *J. Biol. Chem.,* **2013**, *288*(9), 6178-6189.
http://dx.doi.org/10.1074/jbc.M112.435909 PMID: 23300078

[106] Berger, M.; Gray, J.A.; Roth, B.L. The expanded biology of serotonin. *Annu. Rev. Med.,* **2009**, *60*, 355-366.
http://dx.doi.org/10.1146/annurev.med.60.042307.110802 PMID: 19630576

[107] Jann, M.W.; Slade, J.H. Antidepressant agents for the treatment of chronic pain and depression. *Pharmacotherapy,* **2007**, *27*(11), 1571-1587.
http://dx.doi.org/10.1592/phco.27.11.1571 PMID: 17963465

[108] Giorgetti, M.; Tecott, L.H. Contributions of 5-HT(2C) receptors to multiple actions of central serotonin systems. *Eur. J. Pharmacol.,* **2004**, *488*(1-3), 1-9.
http://dx.doi.org/10.1016/j.ejphar.2004.01.036 PMID: 15044029

[109] Seifinejad, A.; Li, S.; Possovre, M.L.; Vassalli, A.; Tafti, M. Hypocretinergic interactions with the serotonergic system regulate REM sleep and cataplexy. *Nat. Commun.,* **2020**, *11*(1), 6034.
http://dx.doi.org/10.1038/s41467-020-19862-y PMID: 33247179

[110] Kandel, E.R. The molecular biology of memory storage: a dialogue between genes and synapses. *Science,* **2001**, *294*(5544), 1030-1038.
http://dx.doi.org/10.1126/science.1067020 PMID: 11691980

[111] Roberts, A.J.; Krucker, T.; Levy, C.L.; Slanina, K.A.; Sutcliffe, J.G.; Hedlund, P.B. Mice lacking 5-HT receptors show specific impairments in contextual learning. *Eur. J. Neurosci.,* **2004**, *19*(7), 1913-1922.
http://dx.doi.org/10.1111/j.1460-9568.2004.03288.x PMID: 15078565

[112] Yang, C.; Zhang, L.; Hao, H.; Ran, M.; Li, J.; Dong, H. Serotonergic neurons in the dorsal raphe nucleus mediate the arousal-promoting effect of orexin during isoflurane anesthesia in male rats. *Neuropeptides,* **2019**, *75*, 25-33.
http://dx.doi.org/10.1016/j.npep.2019.03.004 PMID: 30935682

[113] Fuller, P.M.; Sherman, D.; Pedersen, N.P.; Saper, C.B.; Lu, J. Reassessment of the structural basis of the ascending arousal system. *J. Comp. Neurol.,* **2011**, *519*(5), 933-956.
http://dx.doi.org/10.1002/cne.22559 PMID: 21280045

[114] Kaur, S.; Pedersen, N.P.; Yokota, S.; Hur, E.E.; Fuller, P.M.; Lazarus, M.; Chamberlin, N.L.; Saper, C.B. Glutamatergic signaling from the parabrachial nucleus plays a critical role in hypercapnic arousal. *J. Neurosci.,* **2013**, *33*(18), 7627-7640.
http://dx.doi.org/10.1523/JNEUROSCI.0173-13.2013 PMID: 23637157

[115] Niu, J.G.; Yokota, S.; Tsumori, T.; Qin, Y.; Yasui, Y. Glutamatergic lateral parabrachial neurons innervate orexin-containing hypothalamic neurons in the rat. *Brain Res.,* **2010**, *1358*, 110-122.
http://dx.doi.org/10.1016/j.brainres.2010.08.056 PMID: 20735997

[116] Fulwiler, C.E.; Saper, C.B. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Res.,* **1984**, *319*(3), 229-259.
http://dx.doi.org/10.1016/0165-0173(84)90012-2 PMID: 6478256

[117] Muindi, F.; Kenny, J.D.; Taylor, N.E.; Solt, K.; Wilson, M.A.; Brown, E.N.; Van Dort, C.J. Electrical stimulation of the parabrachial nucleus induces reanimation from isoflurane general anesthesia. *Behav. Brain Res.,* **2016**, *306*, 20-25.
http://dx.doi.org/10.1016/j.bbr.2016.03.021 PMID: 26971629

[118] Percheron, G. The anatomy of the arterial supply of the human thalamus and its use for the interpretation of the thalamic vascular pathology. *Z. Neurol.,* **1973**, *205*(1), 1-13.
http://dx.doi.org/10.1007/BF00315956 PMID: 4126735

[119] Schmahmann, J.D. Vascular syndromes of the thalamus. *Stroke,* **2003**, *34*(9), 2264-2278.
http://dx.doi.org/10.1161/01.STR.0000087786.38997.9E PMID: 12933968

[120] Honig, A.; Eliahou, R.; Eichel, R.; Shemesh, A.A.; Ben-Hur, T.; Auriel, E. Acute bithalamic infarct manifesting as sleep-like coma: A diagnostic challenge. *J. Clin. Neurosci.,* **2016**, *34*, 81-85.
http://dx.doi.org/10.1016/j.jocn.2016.05.014 PMID: 27593970

[121] Hermann, D.M.; Siccoli, M.; Brugger, P.; Wachter, K.; Mathis, J.; Achermann, P.; Bassetti, C.L. Evolution of neurological, neuropsychological and sleep-wake disturbances after paramedian thalamic stroke. *Stroke,* **2008**, *39*(1), 62-68.
http://dx.doi.org/10.1161/STROKEAHA.107.494955 PMID: 18048862

[122] Vertes, R.P.; Linley, S.B.; Hoover, W.B. Limbic circuitry of the midline thalamus. *Neurosci. Biobehav. Rev.,* **2015**, *54*, 89-107.
http://dx.doi.org/10.1016/j.neubiorev.2015.01.014 PMID: 25616182

[123] Groenewegen, H.J.; Berendse, H.W. The specificity of the 'nonspecific' midline and intralaminar thalamic nuclei. *Trends Neurosci.,* **1994**, *17*(2), 52-57.
http://dx.doi.org/10.1016/0166-2236(94)90074-4 PMID: 7512768

[124] Jasper, H. Diffuse projection systems: the integrative action of the thalamic reticular system. *Electroencephalogr. Clin. Neurophysiol.,* **1949**, *1*(4), 405-419.
http://dx.doi.org/10.1016/0013-4694(49)90213-8 PMID: 18421831

[125] Krout, K.E.; Belzer, R.E.; Loewy, A.D. Brainstem projections to midline and intralaminar thalamic nuclei of the rat. *J. Comp. Neurol.,* **2002**, *448*(1), 53-101.
http://dx.doi.org/10.1002/cne.10236 PMID: 12012375

[126] Xu, W.; Südhof, T.C. A neural circuit for memory specificity and generalization. *Science,* **2013**, *339*(6125), 1290-1295.
http://dx.doi.org/10.1126/science.1229534 PMID: 23493706

[127] Van der Werf, Y.D.; Witter, M.P.; Groenewegen, H.J. The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. *Brain Res. Brain Res. Rev.,* **2002**, *39*(2-3), 107-140.
http://dx.doi.org/10.1016/S0165-0173(02)00181-9 PMID: 12423763

[128] Beas, B.S.; Wright, B.J.; Skirzewski, M.; Leng, Y.; Hyun, J.H.; Koita, O.; Ringelberg, N.; Kwon, H.B.; Buonanno, A.; Penzo, M.A. The locus coeruleus drives disinhibition in the midline thalamus *via* a dopaminergic mechanism. *Nat. Neurosci.,* **2018**, *21*(7), 963-973.
http://dx.doi.org/10.1038/s41593-018-0167-4 PMID: 29915192

[129] Ao, Y.; Yang, B.; Zhang, C.; Li, S.; Xu, H. Application of quinpirole in the paraventricular thalamus facilitates emergence from isoflurane anesthesia in mice. *Brain Behav.,* **2021**, *11*(1)e01903
http://dx.doi.org/10.1002/brb3.1903 PMID: 33128305

[130] Schwarz, L.A.; Luo, L. Organization of the locus coeruleus-norepinephrine system. *Curr. Biol.,* **2015**, *25*(21), R1051-R1056.
http://dx.doi.org/10.1016/j.cub.2015.09.039 PMID: 26528750

[131] Berridge, C.W.; Waterhouse, B.D. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res. Brain Res. Rev.,* **2003**, *42*(1), 33-84.
http://dx.doi.org/10.1016/S0165-0173(03)00143-7 PMID: 12668290

[132] España, R.A.; Schmeichel, B.E.; Berridge, C.W. Norepinephrine at the nexus of arousal, motivation and relapse. *Brain Res,* **2016**, *1641*(Pt B), 207-216.
http://dx.doi.org/10.1016/j.brainres.2016.01.002

[133] Sara, S.J. The locus coeruleus and noradrenergic modulation of cognition. *Nat. Rev. Neurosci.,* **2009**, *10*(3), 211-223.
http://dx.doi.org/10.1038/nrn2573 PMID: 19190638

[134] Song, A.H.; Kucyi, A.; Napadow, V.; Brown, E.N.; Loggia, M.L.; Akeju, O. Pharmacological Modulation of Noradrenergic Arousal Circuitry Disrupts Functional Connectivity of the Locus Ceruleus in Humans. *J. Neurosci.,* **2017**, *37*(29), 6938-6945.

http://dx.doi.org/10.1523/JNEUROSCI.0446-17.2017 PMID: 28626012

[135]   Hayat, H.; Regev, N.; Matosevich, N.; Sales, A.; Paredes-Rodriguez, E.; Krom, A.J.; Bergman, L.; Li, Y.; Lavigne, M.; Kremer, E.J.; Yizhar, O.; Pickering, A.E.; Nir, Y. Locus coeruleus norepinephrine activity mediates sensory-evoked awakenings from sleep. *Sci. Adv.,* **2020**, *6*(15)eaaz4232
http://dx.doi.org/10.1126/sciadv.aaz4232 PMID: 32285002

[136]   Vazey, E.M.; Aston-Jones, G. Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(10), 3859-3864.
http://dx.doi.org/10.1073/pnas.1310025111 PMID: 24567395

[137]   Carter, M.E.; Yizhar, O.; Chikahisa, S.; Nguyen, H.; Adamantidis, A.; Nishino, S.; Deisseroth, K.; de Lecea, L. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.,* **2010**, *13*(12), 1526-1533.
http://dx.doi.org/10.1038/nn.2682 PMID: 21037585

[138]   Carter, M.E.; de Lecea, L.; Adamantidis, A. Functional wiring of hypocretin and LC-NE neurons: implications for arousal. *Front. Behav. Neurosci.,* **2013**, *7*, 43.
http://dx.doi.org/10.3389/fnbeh.2013.00043 PMID: 23730276

[139]   Zhang, Z.; Ferretti, V.; Güntan, İ.; Moro, A.; Steinberg, E.A.; Ye, Z.; Zecharia, A.Y.; Yu, X.; Vyssotski, A.L.; Brickley, S.G.; Yustos, R.; Pillidge, Z.E.; Harding, E.C.; Wisden, W.; Franks, N.P. Neuronal ensembles sufficient for recovery sleep and the sedative actions of α2 adrenergic agonists. *Nat. Neurosci.,* **2015**, *18*(4), 553-561.
http://dx.doi.org/10.1038/nn.3957 PMID: 25706476

[140]   Hua, T.; Chen, B.; Lu, D.; Sakurai, K.; Zhao, S.; Han, B.X.; Kim, J.; Yin, L.; Chen, Y.; Lu, J.; Wang, F. General anesthetics activate a potent central pain-suppression circuit in the amygdala. *Nat. Neurosci.,* **2020**, *23*(7), 854-868.
http://dx.doi.org/10.1038/s41593-020-0632-8 PMID: 32424286

[141]   Solt, K.; Forman, S.A. Correlating the clinical actions and molecular mechanisms of general anesthetics. *Curr. Opin. Anaesthesiol.,* **2007**, *20*(4), 300-306.
http://dx.doi.org/10.1097/ACO.0b013e32816678a5 PMID: 17620835

[142]   Gorlin, A.W.; Rosenfeld, D.M.; Ramakrishna, H. Intravenous sub-anesthetic ketamine for perioperative analgesia. *J. Anaesthesiol. Clin. Pharmacol.,* **2016**, *32*(2), 160-167.
http://dx.doi.org/10.4103/0970-9185.182085 PMID: 27275042

[143]   Yang, J.; Chai, Y.F.; Gong, C.Y.; Li, G.H.; Luo, N.; Luo, N.F.; Liu, J. Further proof that the spinal cord, and not the brain, mediates the immobility produced by inhaled anesthetics. *Anesthesiology,* **2009**, *110*(3), 591-595.
http://dx.doi.org/10.1097/ALN.0b013e3181974bfd PMID: 19212265

[144]   Kungys, G.; Kim, J.; Jinks, S.L.; Atherley, R.J.; Antognini, J.F. Propofol produces immobility *via* action in the ventral horn of the spinal cord by a GABAergic mechanism. *Anesth. Analg.,* **2009**, *108*(5), 1531-1537.
http://dx.doi.org/10.1213/ane.0b013e31819d9308 PMID: 19372332

[145]   Stabernack, C.; Zhang, Y.; Sonner, J.M.; Laster, M.; Eger, E.I., II Thiopental produces immobility primarily by supraspinal actions in rats. *Anesth. Analg.,* **2005**, *100*(1), 128-136.
http://dx.doi.org/10.1213/01.ANE.0000139353.97950.FA PMID: 15616066

[146]   Jinks, S.L.; Dominguez, C.L.; Antognini, J.F. Drastic decrease in isoflurane minimum alveolar concentration and limb movement forces after thoracic spinal cooling and chronic spinal transection in rats. *Anesthesiology,* **2005**, *102*(3), 624-632.
http://dx.doi.org/10.1097/00000542-200503000-00022 PMID: 15731602

[147]   Gilron, I.; Quirion, R.; Coderre, T.J. Pre- *versus* postinjury effects of intravenous GABAergic anesthetics on formalin-induced Fos immunoreactivity in the rat spinal cord. *Anesth. Analg.,* **1999**, *88*(2), 414-420.
http://dx.doi.org/10.1213/00000539-199902000-00036 PMID: 9972767

[148]   Jinks, S.L.; Antognini, J.F.; Martin, J.T.; Jung, S.; Carstens, E.; Atherley, R. Isoflurane, but not halothane, depresses c-fos expression in rat spinal cord at concentrations that suppress reflex move-ment after supramaximal noxious stimulation. *Anesth. Analg.,* **2002**, *95*(6), 1622-1628.
http://dx.doi.org/10.1097/00000539-200212000-00028 PMID: 12456428

[149]   Sommers, M.G.; Nguyen, N.K.; Veening, J.G.; Vissers, K.C.; Ritskes-Hoitinga, M.; van Egmond, J. Suppression of noxious-induced c-fos expression in the rat lumbar spinal cord by isoflurane alone or combined with fentanyl. *Anesth. Analg.,* **2008**, *106*(4), 1303-1308.
http://dx.doi.org/10.1213/ane.0b013e3181678831 PMID: 18349210

[150]   Hagihira, S.; Taenaka, N.; Yoshiya, I. Inhalation anesthetics suppress the expression of c-Fos protein evoked by noxious somatic stimulation in the deeper layer of the spinal cord in the rat. *Brain Res.,* **1997**, *751*(1), 124-130.
http://dx.doi.org/10.1016/S0006-8993(96)01398-4 PMID: 9098575

[151]   Takasusuki, T.; Yamaguchi, S.; Hamaguchi, S.; Yaksh, T.L. Effects of general anesthetics on substance P release and c-Fos expression in the spinal dorsal horn. *Anesthesiology,* **2013**, *119*(2), 433-442.
http://dx.doi.org/10.1097/ALN.0b013e31829996b6 PMID: 23708866

[152]   Millan, M.J. Descending control of pain. *Prog. Neurobiol.,* **2002**, *66*(6), 355-474.
http://dx.doi.org/10.1016/S0301-0082(02)00009-6 PMID: 12034378

[153]   Lau, B.K.; Winters, B.L.; Vaughan, C.W. Opioid presynaptic disinhibition of the midbrain periaqueductal grey descending analgesic pathway. *Br. J. Pharmacol.,* **2020**, *177*(10), 2320-2332.
http://dx.doi.org/10.1111/bph.14982 PMID: 31971607

[154]   Yeung, J.C.; Yaksh, T.L.; Rudy, T.A. Concurrent mapping of brain sites for sensitivity to the direct application of morphine and focal electrical stimulation in the production of antinociception in the rat. *Pain,* **1977**, *4*(1), 23-40.
http://dx.doi.org/10.1016/0304-3959(77)90084-7 PMID: 927879

[155]   Fields, H.L.; Vanegas, H.; Hentall, I.D.; Zorman, G. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. *Nature,* **1983**, *306*(5944), 684-686.
http://dx.doi.org/10.1038/306684a0 PMID: 6656868

[156]   Mitchell, J.M.; Lowe, D.; Fields, H.L. The contribution of the rostral ventromedial medulla to the antinociceptive effects of systemic morphine in restrained and unrestrained rats. *Neuroscience,* **1998**, *87*(1), 123-133.
http://dx.doi.org/10.1016/S0306-4522(98)00119-5 PMID: 9722146

[157]   Bajic, D.; Van Bockstaele, E.J.; Proudfit, H.K. Ultrastructural analysis of ventrolateral periaqueductal gray projections to the A7 catecholamine cell group. *Neuroscience,* **2001**, *104*(1), 181-197.
http://dx.doi.org/10.1016/S0306-4522(01)00052-5 PMID: 11311541

[158]   Clark, F.M.; Proudfit, H.K. Projections of neurons in the ventromedial medulla to pontine catecholamine cell groups involved in the modulation of nociception. *Brain Res.,* **1991**, *540*(1-2), 105-115.
http://dx.doi.org/10.1016/0006-8993(91)90496-I PMID: 1711394

[159]   Martin, W.J.; Gupta, N.K.; Loo, C.M.; Rohde, D.S.; Basbaum, A.I. Differential effects of neurotoxic destruction of descending noradrenergic pathways on acute and persistent nociceptive processing. *Pain,* **1999**, *80*(1-2), 57-65.
http://dx.doi.org/10.1016/S0304-3959(98)00194-8 PMID: 10204718

[160]   Sawynok, J.; Reid, A. Effect of 6-hydroxydopamine-induced lesions to ascending and descending noradrenergic pathways on morphine analgesia. *Brain Res.,* **1987**, *419*(1-2), 156-165.
http://dx.doi.org/10.1016/0006-8993(87)90579-8 PMID: 3119144

[161]   Sawamura, S.; Kingery, W.S.; Davies, M.F.; Agashe, G.S.; Clark, J.D.; Kobilka, B.K.; Hashimoto, T.; Maze, M. Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of [alpha]2B adrenoceptors. *J. Neurosci.,* **2000**, *20*(24), 9242-9251.
http://dx.doi.org/10.1523/JNEUROSCI.20-24-09242.2000 PMID: 11125002

[162]   Kingery, W.S.; Sawamura, S.; Agashe, G.S.; Davies, M.F.; Clark, J.D.; Zimmer, A. Enkephalin release and opioid receptor activation does not mediate the antinociceptive or sedative/hypnotic effects of nitrous oxide. *Eur. J. Pharmacol.,* **2001**, *427*(1), 27-35.

http://dx.doi.org/10.1016/S0014-2999(01)01193-1 PMID: 11553360

[163]   Kubota, I.; Tsuboi, Y.; Shoda, E.; Kondo, M.; Masuda, Y.; Kitagawa, J.; Oi, Y.; Iwata, K. Modulation of neuronal activity in CNS pain pathways following propofol administration in rats: Fos and EEG analysis. *Exp. Brain Res.,* **2007,** *179*(2), 181-190.
http://dx.doi.org/10.1007/s00221-006-0779-x PMID: 17136530

[164]   Wilson, T.D.; Valdivia, S.; Khan, A.; Ahn, H.S.; Adke, A.P.; Martinez Gonzalez, S.; Sugimura, Y.K.; Carrasquillo, Y. Dual and opposing functions of the central amygdala in the modulation of pain. *Cell Rep.,* **2019,** *29*(2), 332-346.e5.
http://dx.doi.org/10.1016/j.celrep.2019.09.011 PMID: 31597095

[165]   Han, L.C.; Zhang, H.; Wang, W.; Wei, Y.Y.; Sun, X.X.; Yanagawa, Y.; Li, Y.Q.; Xu, L.X.; Wu, S.X. The effect of sevoflurane inhalation on gabaergic neurons activation: observation on the GAD67-GFP knock-in mouse. *Anat. Rec. (Hoboken),* **2010,** *293*(12), 2114-2122.
http://dx.doi.org/10.1002/ar.21113 PMID: 21046664

[166]   Marota, J.J.; Crosby, G.; Uhl, G.R. Selective effects of pentobarbital and halothane on c-fos and jun-B gene expression in rat brain. *Anesthesiology,* **1992,** *77*(2), 365-371.
http://dx.doi.org/10.1097/00000542-199208000-00021 PMID: 1642355

[167]   Li, T.; Li, Z.; Wan, H.; Tang, X.; Wang, H.; Chai, F.; Zhang, M.; Wang, B. Recurrence-associated long non-coding RNA LNAPPCC facilitates colon cancer progression *via* forming a positive feedback loop with PCDH7. *Mol. Ther. Nucleic Acids,* **2020,** *20*, 545-557.
http://dx.doi.org/10.1016/j.omtn.2020.03.017 PMID: 32330872

[168]   Kozinn, J.; Mao, L.; Arora, A.; Yang, L.; Fibuch, E.E.; Wang, J.Q. Inhibition of glutamatergic activation of extracellular signal-regulated protein kinases in hippocampal neurons by the intravenous anesthetic propofol. *Anesthesiology,* **2006,** *105*(6), 1182-1191.

http://dx.doi.org/10.1097/00000542-200612000-00018 PMID: 17122581

[169]   Miyashita, T.; Kikuchi, E.; Horiuchi, J.; Saitoe, M. Long-term memory engram cells are established by c-Fos/CREB transcriptional cycling. *Cell Rep.,* **2018,** *25*(10), 2716-2728.e3.
http://dx.doi.org/10.1016/j.celrep.2018.11.022 PMID: 30517860

[170]   Kidambi, S.; Yarmush, J.; Berdichevsky, Y.; Kamath, S.; Fong, W.; Schianodicola, J. Propofol induces MAPK/ERK cascade dependant expression of cFos and Egr-1 in rat hippocampal slices. *BMC Res. Notes,* **2010,** *3*, 201.
http://dx.doi.org/10.1186/1756-0500-3-201 PMID: 20637119

[171]   Schreiber, S.S.; Tocco, G.; Shors, T.J.; Thompson, R.F. Activation of immediate early genes after acute stress. *Neuroreport,* **1991,** *2*(1), 17-20.
http://dx.doi.org/10.1097/00001756-199101000-00004 PMID: 1768844

[172]   Brennan, P.A.; Hancock, D.; Keverne, E.B. The expression of the immediate-early genes c-fos, egr-1 and c-jun in the accessory olfactory bulb during the formation of an olfactory memory in mice. *Neuroscience,* **1992,** *49*(2), 277-284.
http://dx.doi.org/10.1016/0306-4522(92)90095-J PMID: 1279452

[173]   Lantéri-Minet, M.; Weil-Fugazza, J.; de Pommery, J.; Menétrey, D. Hindbrain structures involved in pain processing as revealed by the expression of c-Fos and other immediate early gene proteins. *Neuroscience,* **1994,** *58*(2), 287-298.
http://dx.doi.org/10.1016/0306-4522(94)90035-3 PMID: 8152540

[174]   Smith, M.L.; Li, J.; Cote, D.M.; Ryabinin, A.E. Effects of isoflurane and ethanol administration on c-Fos immunoreactivity in mice. *Neuroscience,* **2016,** *316*, 337-343.
http://dx.doi.org/10.1016/j.neuroscience.2015.12.047 PMID: 26742790