ANESTHESIOLOGY

Historical and Modern Evidence for the Role of Reward Circuitry in Emergence

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ABSTRACT

Increasing evidence supports a role for brain reward circuitry in modulating arousal along with emergence from anesthesia. Emergence remains an important frontier for investigation, since no drug exists in clinical practice to initiate rapid and smooth emergence. This review discusses clinical and preclinical evidence indicating a role for two brain regions classically considered integral components of the mesolimbic brain reward circuitry, the ventral tegmental area and the nucleus accumbens, in emergence from propofol and volatile anesthesia. Then there is a description of modern systems neuroscience approaches to neural circuit investigations that will help span the large gap between preclinical and clinical investigation with the shared aim of developing therapies to promote rapid emergence without agitation or delirium. This article proposes that neuroscientists include models of whole-brain network activity in future studies to inform the translational value of preclinical investigations and foster productive dialogues with clinician anesthesiologists.

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Emergence from general anesthesia is a dynamic time of transition from the anesthetized state to the awake state that continues to be an unpredictable and fragile period for patients in perioperative care.[1–3] Some evidence suggests that emergence is regulated by additional neural processes independent of drug clearance[4–6] and is not simply the reverse process of anesthetic induction, as described by models of anesthetic hysteresis.[7–9] Many surgical cases necessitate rapid arousal for assessment of patients' postoperative cognitive and motor abilities. Therefore, rapid and smooth emergence is desirable for both patient safety and perioperative efficiency. Why some patients emerge quickly from higher anesthetic concentrations than others and why subsets of patients undergo an agitated, combative state during the process of emergence is currently not well understood at multiple levels. Together, this unpredictability and the lack of an established therapy to facilitate emergence highlight the need for a better understanding of the basic neuropharmacologic mechanisms mediating emergence from anesthesia.

Emergence agitation can be dangerous, with patients manifesting combative behaviors that can result in self-injury, harm to providers, catheter removal, self-extubation, and airway obstruction. Postoperative delirium can also result in longer hospital length of stay and worsened clinical outcomes.[10–12] Efforts to mitigate adverse emergence phenomena, like agitation and delirium, are currently focused on avoiding inhalational anesthetics or supplementing inhalational agents with intravenous sedatives like the α2 receptor agonist dexmedetomidine.[13–18]

However, the results from these studies vary widely with patient population,[19,20] and the basic neuronal mechanisms modulating emergence under different anesthetic conditions are still unclear. Mechanisms of emergence from volatile or propofol anesthesia, which can directly bind to γ-aminobutyric acid (GABA) receptors, can be inconsistent with mechanisms found to mediate the effects of ketamine, which blocks glutamatergic neurotransmission.[21,22] However, common anesthetic substrates in the brain exist, such as the activation of hypothalamic neurons.[23] Here, we will focus our discussion on studies that use propofol, sevoflurane, or isoflurane for maintenance of general anesthesia, since they form much of the literature investigating mesolimbic circuitry in emergence. Further mechanistic basic science research is needed to examine whether findings hold true across disparate anesthetic conditions. There is a clinical need for therapeutic interventions targeting emergence and the postanesthetic period to improve the predictability, speed, and safety of anesthesia care. By prioritizing a translational and multidisciplinary approach, basic neuroscientists can help to uncover these gaps in knowledge.

Decades of accumulating literature support a role for dopaminergic signaling through the brain reward circuitry in promoting arousal (see reviews[24–26]). Here, we summarize key clinical and preclinical evidence supporting a central role for the brain's mesolimbic dopaminergic reward circuitry in modulating emergence from general propofol and volatile anesthesia, with a focus on the ventral tegmental area and

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the nucleus accumbens regions. The same neural circuitry may be important for the pathophysiology of emergence agitation, given the essential role of reward circuitry in regulating emotional and arousal-related behavioral states. We then discuss systems neuroscience approaches for bridging preclinical and clinical studies of brain reward circuitry in emergence to promote the therapeutic application of preclinical investigations (fig. 1).

Until recently, high-resolution approaches for careful examination within the intact brain did not exist to enable discrete cell type– and region-specific investigations of circuit dynamics. However, now we can harness viral-mediated and genetic approaches to deliver engineered photoactivatable compounds and perform whole-brain imaging at single-cell resolution. This more intricate systems neuroscience approach can be used to understand brain circuitry in preclinical models of awake, behaving rodents and even nonhuman primates.[27–30] To place the granularity of these investigations in a clinically useful framework, mechanistic cellular-level studies must then be examined in the context of changes to whole-brain activity.

While there is a broad and vast literature describing the role of dopaminergic circuitry in mediating arousal and reward-reinforcing behaviors, this review is limited to systems neuroscience studies of particular relevance to the clinical practicing anesthesiologist (table 1). We also refer

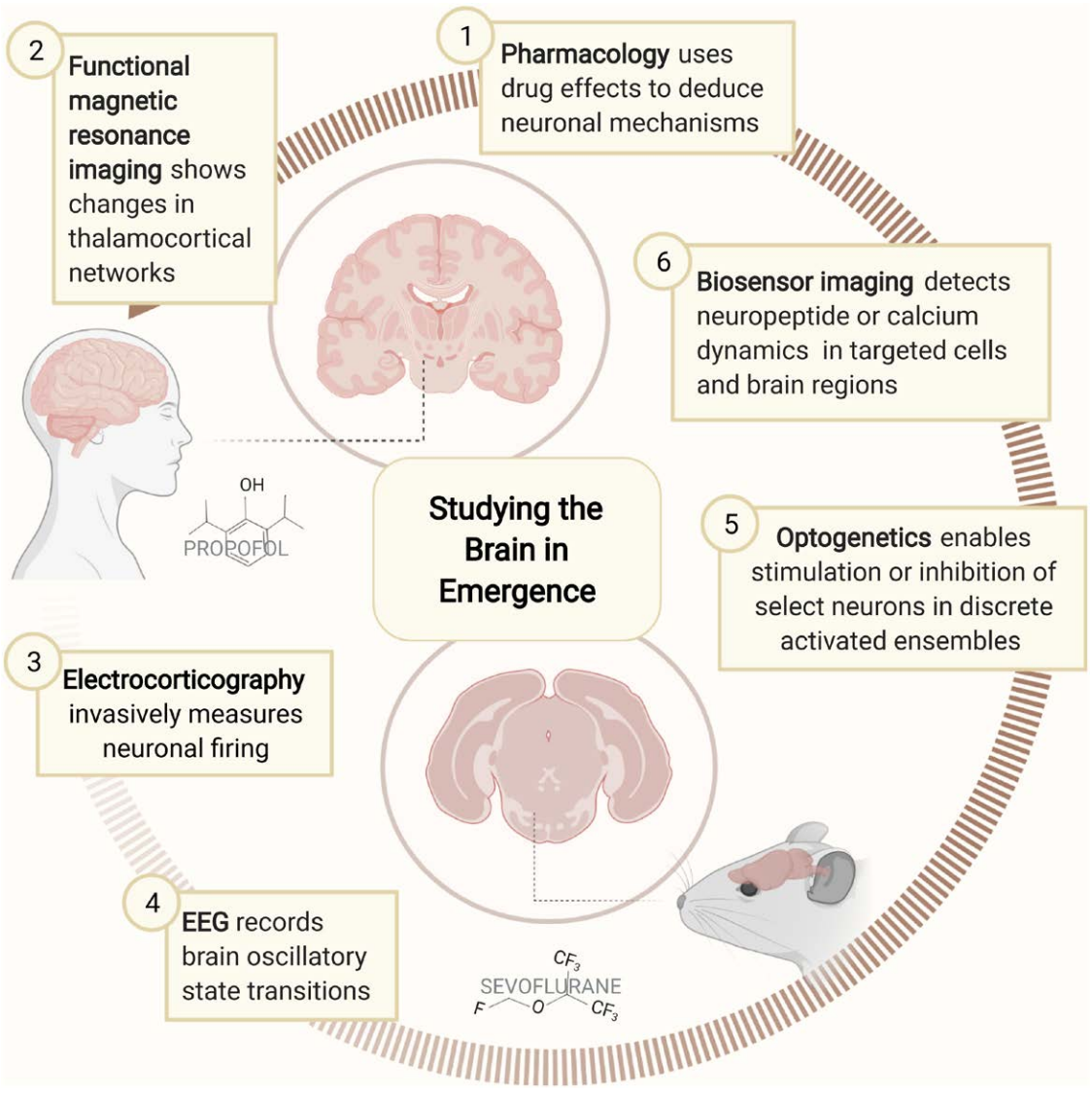

Fig. 1. Schematic highlighting the need to bridge the gap between preclinical and clinical studies of anesthesia emergence with translational research. The *numbered boxes* highlight common methodologies in either clinical or preclinical research. The *insets* show midbrain slices from the human and mouse brain, as well as the chemical structures of propofol and sevoflurane, two of the most common anesthetics under investigation. The figure was created with BioRender.com. EEG, electroencephalogram.

Table 1. Selected List of Historical References, Primary Preclinical Articles Investigating the Role of Mesolimbic Circuitry in Emergence, and Suggested Literature Reviews on Emergence

Citation	Brain Region/Neurotransmitter	Anesthetic	Species	Major Finding
Historical references				
Eckenhoff *et al.* (1961)[1]		Thiopental, halothane, ether, cyclopropane, nitrous oxide, spinal	Human	First descriptions of "emergence excitement" and differential effects of anesthetic drug treatment in 14,436 patients
Mantz *et al.* (1994)[31]	Striatum/dopamine	Halothane, isoflurane, thiopental, ketamine	Rat	Anesthetics significantly alter spontaneous and evoked dopamine release in striatal synaptosomes
Irifune *et al.* (1997)[32]	Nucleus accumbens/dopamine	Isoflurane	Mouse	HPLC assays show increased dopamine turnover, hyperlocomotion during emergence
Tsukada *et al.* (1999)[33]	Striatum/dopamine	Isoflurane	Rhesus monkey	Positron emission tomography and microdialysis show enhanced DAT inhibition and D2 receptor binding under isoflurane
Fiset *et al.* (1999)[34]	Medial thalamus, midbrain	Propofol	Human	Positron emission tomography shows cerebral blood flow changes in midbrain and thalamus
Brain region–specific manipulations				
Kelz *et al.* (2008)[35]	Hypothalamus/orexin	Isoflurane, sevoflurane	Mouse	Ablation of orexinergic neurons or an orexin-1 antagonist delays emergence, not induction
Mhuircheartaigh *et al.* (2010)[36]	Putamen, thalamus, cortex	Propofol	Human	Functional magnetic resonance imaging blood-oxygen-level–dependent imaging shows changes in subcortical connectivity
Shirasaka *et al.* (2011)[37]	Prefrontal cortex/orexin	Propofol	Rat	ICV injection of orexin speeds emergence, increases norepinephrine and dopamine release in the prefrontal cortex
Solt *et al.* (2014)[38]	Ventral tegmental area, substantia nigra/dopamine?	Isoflurane, propofol	Rat	Electrical stimulation of the ventral tegmental area speeds emergence
McCarren *et al.* (2014)[39]	Ventrolateral preoptic nucleus/norepinephrine	Isoflurane, dexmedetomidine	Mouse	Single-cell reverse transcription–polymerase chain reaction of VLPO neurons and role of adrenergic manipulation
Vazey and Aston-Jones (2014)[40]	Locus coeruleus/norepinephrine	Isoflurane	Rat	Chemogenetic activation of locus coeruleus neurons speeds emergence
Zhou *et al.* (2015)[41]	Ventral tegmental area/dopamine	Propofol, isoflurane, ketamine	Rat	Lesioning ventral tegmental area dopamine neurons with 6-hydroxydopamine prolongs emergence from propofol, not isoflurane
Taylor *et al.* (2016)[42]	Ventral tegmental area/dopamine	Isoflurane	Mouse	Optogenetic activation of ventral tegmental area dopamine neurons speeds emergence
Muindi *et al.* (2016)[21]	Parabrachial nucleus/glutamate?	Isoflurane	Mouse	Electrical stimulation of parabrachial nucleus speeds emergence
Fu *et al.* (2017)[43]	Central medial thalamus/norepinephrine	Propofol	Rat	Norepinephrine microinjection in central medial thalamus speeds emergence
Du *et al.* (2018)[44]	Locus coeruleus/norepinephrine	Propofol, etomidate	Zebrafish	Deletion of dopamine-β-hydroxylase in locus coeruleus neurons delays emergence from intravenous anesthesia
Yin *et al.* (2019)[45]	Ventral tegmental area, hypothalamus/GABA	Isoflurane	Mouse	Activation of ventral tegmental area GABA to hypothalamus slows emergence
Wang *et al.* (2019)[46]	Parabrachial nucleus/glutamate	Sevoflurane	Mouse	Activation of parabrachial nucleus glutamate neurons speeds emergence
Zhang *et al.* (2019)[47]	Reticular thalamus/norepinephrine	Propofol	Mouse	Locus coeruleus-to-TRN norepinephrine projections delay emergency by activating α1 adrenergic receptor
Torturo *et al.* (2019)[48]	Ventral tegmental area/dopamine	Isoflurane	Rat	Isoflurane inhibits exocytosis in cultured rat dopamine neurons by a distinct calcium-mediated mechanism
Li *et al.* (2019)[49]	Ventral tegmental area/orexin	Isoflurane	Rat	Microinjection of orexin in the ventral tegmental area promotes emergence by activating dopamine neurons
Zhang *et al.* (2020)[50]	Prefrontal cortex/acetylcholine, adenosine, norepinephrine	Isoflurane	Mouse	Microdialysis studies showing neurotransmitter roles in anesthetized-to-awake state transition
Luo *et al.* (2020)[51]	Basal forebrain/acetylcholine	Isoflurane, propofol	Mouse	Chemogenetic activation of cholinergic neurons speeds emergence
Gretenkord *et al.* (2020)[52]	Ventral tegmental area, prefrontal cortex/dopamine	Urethane	Rat	Stimulation of ventral tegmental area and D1 receptors in prefrontal cortex promotes arousal
Ao *et al.* (2021)[53]	Paraventricular thalamus/dopamine	Isoflurane	Mouse	PVT cFos activity increases after emergence, enhanced by a D2 agonist
Zhang *et al.* (2021)[54]	Nucleus accumbens shell/dopamine	Isoflurane	Mouse	D1 receptor agonist accelerates emergence in young but not aged mice
Bao *et al.* (2021)[55]	Nucleus accumbens/ dopamine	Sevoflurane	Mouse	Chemogenetic activation of D1 receptors delays induction and accelerates emergence
Recommended literature reviews on emergence				
Franks (2008)[56]			Human/rodent	Molecular targets of arousal
Brown *et al.* (2010)[57]			Human	Relationship of anesthesia to sleep and coma
Tarnal *et al.* (2016)[5]			Human	Hysteresis, neural inertia, and active emergence
Kelz *et al.* (2019)[4]			Rodent	Neurotransmitter modulators of emergence

DAT, dopamine transporter; GABA, γ-aminobutyric acid; HPLC, high-pressure liquid chromatography; ICV, intracerebroventricular; PVT, paraventricular thalamus; TRN, thalamic reticular nucleus; VLPO, ventrolateral preoptic nucleus.

the reader to comprehensive reviews of mesolimbic circuitry[58–62] and to articles discussing transcriptomic tools for studying the brain under anesthesia,[63,64] such as single-cell RNA sequencing and clustered regularly interspaced short palindromic repeats/Cas9 approaches[65–67] that are beyond the scope of this review.

Brain Reward Circuitry

The brain reward circuitry, also known as the mesolimbic dopamine system or mesolimbic circuitry, is composed of interconnected subcortical and cortical brain regions. This circuitry is evolutionarily conserved across mammals to mediate reinforcing behaviors important for survival, like sex[68,69] and food consumption.[70–80] The same brain regions also modulate sleep/wake transitions and states of arousal essential for executing these reward-related behaviors.[81–85] Dysregulation of reward seeking is concomitant with dysregulated arousal.[86] For example, disordered sleep is an important feature of illnesses characterized by anhedonia and dysregulated reward circuit functioning like depression, addiction, schizophrenia, and Parkinson's disease.[87,88]

Dopamine neurons in the ventral tegmental area project to the nucleus accumbens, forming a key projection in the mesolimbic dopamine system, the circuitry that guides reward-related behaviors and promotes arousal (fig. 2).[58,89–98] The nucleus accumbens is a central processing hub in ventral striatum that integrates inputs from the ventral tegmental area with inputs from myriad brain regions in the reward circuitry, which has been shown to guide a variety of behavioral responses and emotional states.[58,62,70,91,99–103] Distinct neurochemical markers in the striatum divide its neurons into either the direct ("go") or indirect ("no-go") pathways.[58] These markers include the dopamine receptors, which are G-protein–coupled receptors classified as either D1 (G_s-coupled) receptors that signal through the direct pathway or D2 (G_i-coupled) receptors that signal through the indirect pathway. The direct pathway, named for its direct projection to the ventral tegmental area in the midbrain, expresses the neuropeptides dynorphin and substance P, while indirect pathway peptide expression includes enkephalin and adenosine A2A receptors.[58] This region is very heterogenous in anatomical and functional properties and is enriched in numerous neuropeptides, and modulators include acetylcholine, among others.[58,70,97,99,100,104] The dorsal striatum expresses similar dopamine receptor subtypes as ventral striatum but projects to the substantia nigra, instead of the ventral tegmental area, to guide motor responses to stimuli. In reality, the canonical role of dorsal striatum as confined to pure sensorimotor processing and the role of ventral striatum as confined to emotional processing are less explicitly segregated than was once believed (see review[105]).

γ-Aminobutyric acid–mediated (GABAergic) medium spiny neurons are the principal neurons in the nucleus accumbens, comprising over 95% of the neuronal population, and form local inhibitory synapses between medium spiny neurons, as well as long-range GABA projections to other brain regions in mesolimbic reward circuitry (fig. 2). Approximately 3% of nucleus accumbens neurons are cholinergic interneurons releasing acetylcholine, while less than 2% are inhibitory interneurons releasing GABA with either somatostatin or parvalbumin.[58,106] As a result, the inhibitory GABA receptors are ubiquitously expressed in nucleus accumbens in addition to neuronal expression of dopamine receptors, μ-opioid receptors, glutamate receptors, enkephalin, dynorphin, and other neuropeptide signaling substrates. The mechanism of action of propofol and volatile anesthetics, like sevoflurane and isoflurane, is known to involve direct activation of GABA type A receptors (see review[107]). Binding of propofol or volatile anesthetic to GABA receptors presumably may occur at both the medium spiny neuron and interneuron, thus modulating dopaminergic signaling within the nucleus accumbens microcircuit in addition to affecting long-range GABAergic projections in mesolimbic circuitry. Dopamine signaling also changes calcium currents and *N*-methyl-D-aspartate–induced currents studied in striatal slices.[108–111] Changes in dopamine release, dopamine D1 receptor activation, and transcriptional activation of deltaFosB in nucleus accumbens are shown to be necessary for the behavioral and abuse liability properties of propofol administration.[112–115] Further research is needed to define GABA, glutamate, and dopaminergic interactions during behavioral emergence.

Ventral tegmental area dopamine neuron firing patterns signal errors in reward prediction, help to guide and modify behavior, and reinforce the motivation to seek rewards.[70–74,116–119] Ventral tegmental area dopamine neurons respond in two different modes: single-spike tonic firing to maintain dopamine tone[120–122] and phasic burst firing that is thought to signal an unexpected reward or salient event.[72,123–129] Dopamine neuron bursting activity increases during transitions from sleep to wakefulness.[81,82] Increased burst firing from ventral tegmental area neurons results in dopamine release to downstream interconnected brain regions, including the nucleus accumbens, prefrontal cortex, hypothalamus, and amygdala[94,98,130,131] (fig. 2).

Classically attributed to orchestrating dopamine signaling, ventral tegmental area circuitry is modulated by numerous other neuropeptides, as well as local and long-range GABAergic, glutamatergic, serotonergic, and cholinergic projections.[98,132,133] For example, ventral tegmental area GABA neurons send long-range projections to synapses directly on nucleus accumbens cholinergic neurons, forming one specialized circuit important for reward reinforcement[100] and associative learning.[134] Ventral tegmental area GABA neurons are also engaged in sleep arousal and modulated by anesthesia.[135–137] The role of ventral tegmental area GABA to nucleus accumbens cholinergic neuron projections in emergence is unclear. The ventral tegmental area connects directly with the thalamus, basal forebrain, orexinergic neurons in the hypothalamus, noradrenergic neurons

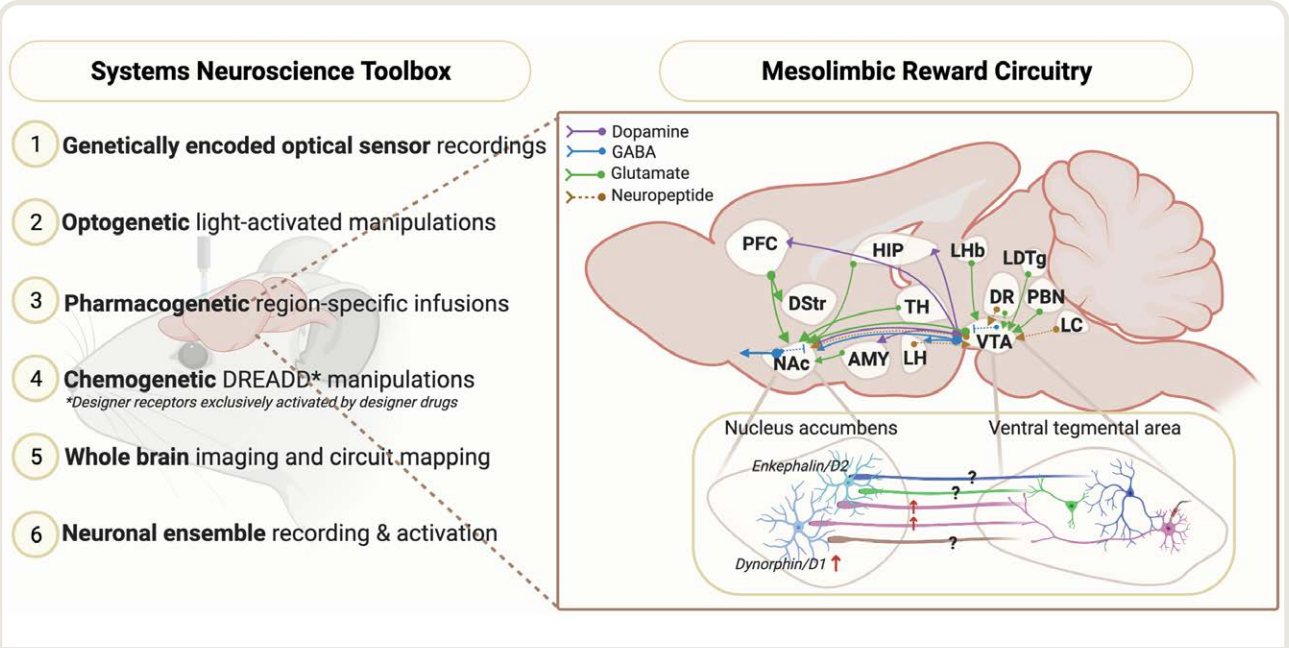

Fig. 2. (*Left*) Systems neuroscience toolbox for dissecting mesolimbic circuitry with *numbered boxes* highlighting key methodologies for circuit-level and whole-brain investigations. (*Right*) Highly simplified schematic of mesolimbic reward circuitry with relevant glutamate (*green arrow*), dopamine (*purple arrow*), γ-aminobutyric acid (GABA; *blue arrow*), and other neuropeptide (*dashed arrow*) projections shown. Serotonergic projections from the dorsal raphe (DR), noradrenergic projections from locus coeruleus (LC), and orexinergic projections from the lateral hypothalamus (LH) (not shown) are also important for emergence. The *inset* below shows a magnified view of the ventral tegmental area (VTA) to nucleus accumbens (NAc) projections with *upward arrows* indicating the increased activity of dopaminergic projections and D1 receptor activation in emergence. The role of the other VTA to NAc projections in emergence is unclear. The figure was created with BioRender.com. AMY, amygdala; DStr, dorsal striatum; HIP, hippocampus; LDTg, lateral dorsal tegmentum; LHb, lateral habenula; PBN, parabrachial nucleus; PFC, prefrontal cortex; TH, thalamus.

in the locus coeruleus, and serotonergic neurons in the dorsal raphe, each of which is individually important for mediating arousal and differentially affected by anesthesia.[4,138,139]

Importantly, the state of general anesthesia, a drug-induced reversible coma, is distinct from natural sleep (see comprehensive reviews on this topic[57,140]). While insight into brain reward circuitry is gained from studies of sleep arousal, the same mechanisms should not be expected to correlate directly with emergence from anesthesia. This important caveat must be considered when comparing studies of sleep arousal and anesthesia as reviewed in this article.

Human Studies of Reward Circuitry in Emergence

Pharmacologic, neuroimaging, and genetic manipulations support the role of monoaminergic circuits in both arousal from sleep and emergence from general anesthesia. Dopamine and norepinephrine are important neurotransmitter mediators of arousal, as evidenced by impaired arousal seen in mice missing the dopamine β-hydroxylase[26,141,142] and dopamine transporter[143] genes. In humans, single-nucleotide gene polymorphisms affecting the dopamine transporter and dopamine D2 receptor genes are associated with variations in self-reported sleep duration.[144] Treatment with a tyrosine hydroxylase inhibitor, with the end result of decreasing dopaminergic tone, increases sleepiness in studies of healthy adults.[145,146] Dopamine D2 receptor levels also decrease specifically in the human ventral striatum after sleep deprivation, as assayed by positron emission tomography imaging.[147]

In contrast, stimulants that increase dopaminergic and catecholaminergic tone have strong effects on arousal. Dopamine-enhancing medications heighten arousal and accelerate emergence, as shown by studies of D1 receptor agonist treatment.[148,149] In contrast, dopamine antagonism with droperidol slows emergence by deepening sevoflurane anesthesia.[150] Together, these studies indicate a role for dopaminergic tone in promoting arousal, with a specific role for activation of dopamine receptors in ventral striatum.

Human neuroimaging under anesthesia consistently demonstrates thalamic deactivation and disruption of thalamocortical connectivity in states of general anesthesia,[151–154] along with deactivation of the basal forebrain and basal ganglia,[36] important components of the brain reward circuitry. Studies using functional magnetic resonance imaging infer changes in brain activity by correlating changes in cerebral

blood flow. However, they are not able to directly measure neuronal activity, an important limitation when interpreting results of functional magnetic resonance imaging studies. Invasive electrocorticography can be used to obtain direct recordings from the cortex of patients undergoing neurosurgery for intractable epilepsy. These studies demonstrate thalamocortical suppression with induction of general anesthesia, while recovery from anesthesia reflects a progressive increase in cortical activity, a decrease in reticulothalamic activity, and a return of tonic activity in the thalamus.[155] General anesthesia also inhibits auditory processing in higher-order auditory association areas while maintaining local field potential neuronal activity in the primary auditory cortex, suggesting that anesthesia may selectively affect higher-order signaling to disrupt cortical circuits.[156]

Anesthesia research in both rodent models and the human clinical population utilize electroencephalogram (EEG) changes and perioperative EEG monitoring as a tool for monitoring the depth of anesthesia. General anesthetics are well known to produce distinct EEG patterns, with a shared pattern of increased delta oscillations (see a recent review[140]). Interestingly, a recent study links increased delta oscillations to dopamine depletion and loss of D2 receptor activation in mouse striatum independent of anesthesia exposure.[159] Studies of D1 dopamine receptor activation during emergence from isoflurane anesthesia in mice show reduced delta and increased gamma oscillations, accelerating emergence.[54,148] EEG changes do not always reliably correlate with behavioral arousal. For example, optogenetic stimulation of ventral tegmental area dopamine neurons promoted behavioral arousal with minimal change in EEG.[42]

EEG studies of the brain are useful as a noninvasive and easily translatable method but have limitations for interpretation. A recent multicenter study enrolled 60 healthy volunteers to evaluate frontal–parietal EEG dynamics in recovery from anesthesia, independent of surgery.[160] Results from this study support a model of early return of prefrontal cortical dynamics and executive function. However, EEG dynamics do not predict cognitive recovery after anesthesia. Burst suppression in the EEG is considered to reflect a very deep state of anesthesia that may be desirable to avoid.[158] In a study of 27 healthy human volunteers, EEG burst suppression does not change the time to emergence or affect the degree of cognitive impairment after isoflurane exposure, using a computational model to predict time of emergence.[161] The Electroencephalography Guidance of Anesthesia to Alleviate Geriatric Syndromes (ENGAGES) randomized clinical trial also finds that EEG-guided administration of general anesthesia does not reduce the incidence of postoperative delirium, compared to usual care, in adults aged 60 yr and older.[162] Thus, while EEG is a useful readout of brain oscillatory arousal states, it is only one tool for evaluating clinical effects of emergence in the perioperative setting. A multidisciplinary systems neuroscience approach, additionally informed by preclinical research, is needed for a holistic view of anesthesia emergence and postanesthetic cognitive sequelae.

Preclinical Studies of Reward Circuitry in Emergence

Current basic neuroscience understanding of arousal is derived primarily from rodent studies of sleep/wake states and general anesthesia.[35,42,46,49,52,85,135,137,138,163] Sleep studies in rodents consistently support a central role for dopaminergic signaling and specifically ventral tegmental area neuron activity in arousal.[26,85,135,137] Emergence from anesthesia, defined as arousal and return of awareness, is assayed behaviorally in the rodent by restoration of the righting reflex response, a reflex that develops shortly after birth to maintain the prone position. In these studies, the mouse or rat is turned on its back while anesthetized, and upon emergence, the animal will right itself to having its paws on the ground.[56,164–167]

Preclinical research supports an important role for dopaminergic signaling in mediating emergence from anesthesia. Isoflurane anesthesia inhibits synaptic vesicle exocytosis from dopamine neurons in cultured rat ventral tegmental area dopamine neurons.[48,168] Subanesthetic propofol exposure causes an increase in spontaneous ventral tegmental area dopamine neuron firing recorded from rat brain slices, and propofol potentiates evoked postsynaptic excitatory synaptic currents recorded downstream in the nucleus accumbens.[169] The stimulant drug amphetamine also causes presynaptic release of dopamine and inhibits dopamine reuptake in the striatum in recordings from striatal brain slices.[170] Systemic administration of methylphenidate and amphetamine, which increase catecholaminergic tone by inhibiting norepinephrine and dopamine reuptake, speeds emergence from both isoflurane and propofol general anesthesia in rodents, as measured behaviorally by restoration of the righting reflex.[171–174] Similarly, other reports show that intravenous caffeine administration also accelerates emergence from isoflurane general anesthesia in both mice and humans.[175–178] These indirect pharmacologic studies support a general role for increased dopaminergic tone influencing emergence.

Studies directly manipulating the ventral tegmental area under general anesthesia demonstrate the sufficiency of ventral tegmental area neuron activity in promoting emergence. Direct stimulation of ventral tegmental area neurons using an electrode inserted above the ventral tegmental area in the rat results in faster emergence from both isoflurane and propofol anesthesia.[38] Further, cell type–specific stimulation of only dopamine neurons in the ventral tegmental area using optogenetics in transgenic mice promotes emergence from isoflurane anesthesia.[42] Together, these findings support a working model of reduced ventral tegmental area dopamine neuron activity under general anesthesia, with emergence characterized by a resurgence of dopamine activity as brought about by direct neuronal stimulation or stimulant drug administration.

Multiple reports indicate a critical role for the engagement and activation of dopamine receptors during emergence. Early studies of phenobarbital anesthesia demonstrate a role for activation of both dopamine D1 and dopamine D2 receptors in promoting emergence using systemic receptor agonist treatment in rats[179,180] and rabbits.[181] Dopamine D1 receptor agonists promote emergence from isoflurane and propofol anesthesia.[148] Administration of A2A receptor agonist, which also activates medium spiny neurons expressing D2 receptors, modulates the depth of propofol anesthesia and activates the nucleus accumbens in mice as measured by increased cFos expression.[182] However, these studies all use systemic administration of dopamine receptor agonists, so the neural circuit mechanism and sites of their action in the brain are unknown. Direct microinjection of D1 receptor agonist or antagonist into the nucleus accumbens supports a bidirectional regulation of time to emergence with dopamine receptor activation specifically within the nucleus accumbens.[54] In addition, selective chemogenetic activation of nucleus accumbens D1 receptor–expressing neurons accelerates emergence and delays induction with sevoflurane.[55]

Together, these findings support a working model of the anesthetized state as marked by a reduction of dopaminergic tone, with emergence from anesthesia promoted by increased ventral tegmental area dopamine neuron activity, which subsequently causes activation of downstream D1-type dopamine receptors within the nucleus accumbens (fig. 2). It is unclear whether the increase in ventral tegmental area activity is driven by increases in tonic dopamine neuron firing or phasic discharge during emergence. While ventral tegmental area dopamine appears to be necessary for emergence, it is unclear whether ventral tegmental area stimulation alone is sufficient to drive emergence. Optogenetic studies of ventral tegmental area dopamine neurons in emergence used repeated stimulation for more than 30 min to increase the probability of righting.[42] Additional mechanisms may be engaged within ventral tegmental area circuitry with repeated stimulation over time. Ventral tegmental area dopamine neurons project to numerous target brain regions to form the mesolimbic reward circuit, as discussed previously. Outside of the ventral tegmental area, manipulations of the parabrachial nucleus, which directly projects to the ventral tegmental area, the locus coeruleus, and the thalamus, also promote emergence from general anesthesia.[40,183–186] It is possible that additional dopaminergic pathways are further engaged in these studies. In addition to dopamine, the ventral tegmental area contains numerous neuropeptide-containing neurons,[70] as well as GABAergic and glutamatergic cells that can send long-range projections. The effects of increased ventral tegmental area neuron activity during emergence on heterogeneous downstream circuitry remains to be fully described (fig. 2, *inset*). Many investigations of brain circuitry also largely ignore the contribution of nonneuronal cell types, while there is a new study indicating an important role for astrocytes in emergence.[187] Future research must be aimed at comprehensively evaluating all cell types in target brain circuit regions during emergence to form a complete mechanistic understanding and provide new therapeutic targets.

Preclinical Neuroscience Methods for Neural Circuit Investigation

Modern neuroscience tools enable a detailed dissection of neural circuits in the awake-behaving animal with high temporal and spatial resolution using optical manipulation and behavioral modeling. Neural circuit investigation is strengthened by an interrogation at multiple levels of analysis, from molecular/cellular to systems to behavioral. Beginning with the revolutionary introduction of optogenetics,[188–190] the optical tools available for interrogating brain circuit connectivity now extend from light-activated ion channels to optically active G-protein–coupled receptors, like parapinopsin[191] and the optogenetically activated μ-opioid receptor[69,146] and β_2 adrenergic receptor.[147] Genetically encoded fluorescent sensors of neuropeptide and neurotransmitter release, such as the dLight sensor, which detects dopamine release, and the GPCR activation-based norepinephrine sensor sensor, which detects norepinephrine release,[195] among many others,[196] are used together with calcium imaging to better elucidate the dynamics of neural circuit action during behavior. Optofluidic devices also enable the wireless light-evoked delivery of drugs into the brain for pharmacologic studies with high temporal and regional specificity.[197–200] Coupled to transcriptomic manipulations at the single-cell level, the investigation of novel receptor-mediated signaling mechanisms in specific brain circuits is possible with exquisite detail.[201]

Advances in microscopy now allow for imaging across the whole brain at single-cell resolution after brain clearing using light sheet microscopy.[202] Some studies of general anesthesia are beginning to take advantage of the whole-brain approach to investigating neural circuits, like the reticular activating system.[203] In addition, calcium dynamics can be imaged at the individual neuronal level within a specified circuit using *in vivo* two-photon microscopy in a head-fixed animal or *in vivo* one-photon imaging after implanting a miniature microscope (graded index lens) in freely moving mice.[204–207] Calcium imaging can then be paired with optogenetic studies to dissect the effects of circuit activation or inhibition on neuronal activity. These newer imaging modalities provide high single-cell and spatial resolution, enabling detailed cellular-level preclinical investigations, compared to approaches with poorer spatial resolution like functional magnetic resonance imaging and positron emission tomography.[208,209]

Multiregion, high-density recordings of neuronal activity using advanced physiology methods like implanted silicone probes, called Neuropixels (imec, Leuven, Belgium), can

be used to decipher circuit activity during emergence.[195] Neuropixels do not utilize genetically encoded sensors and thus lack cell-type specificity, as well as tracking of the same neurons across long-term temporal domains.[210] However, Neuropixels can be paired with optotagging, in which a neuron is optogenetically activated to determine its identity, and the high-density nature of Neuropixels recordings affords a more system-wide view of a given series of brain regions.

Neuronal recordings and optogenetic manipulation can then be paired with computational neuroethology for closed-loop stimulation or analytical studies.[211] Closed-loop deep brain stimulation therapy improved depression symptoms in one individual with major depression,[212] and similar approaches could be adopted for modifying emergence. Open source toolkits for high-throughput behavioral analysis using machine learning approaches, like DeepLabCut,[213] SimBA (https://github.com/sgoldenlab/simba, Golden Lab, University of Washington, Seattle, Washington),[214] or DeepSqueak,[215] can be applied to studying emergence from anesthesia. Machine learning approaches are useful for identifying previously unknown behavioral repertoires within simple behaviors, such as grooming[216] and subtle pharmacologic effects on behavior in rodents.[217] Behavioral models of emergence such as spontaneous restoration of the righting reflex are currently analyzed as a binary output interpreted by visual manual scoring (either positive when the rodent is aroused and upright or negative when the rodent is lying unconscious on its back). The binary restoration of the righting reflex model as currently analyzed is suggested to variably correlate with cortical signatures of arousal assayed by EEG and local field potential analysis.[218] Even a simple experimental model like restoration of the righting reflex presents an array of behavioral features (*e.g.*, whisker movement, tail curling, side rolling, increased chest movement, then righting). In-depth behavioral classification using pose estimation and machine learning classification methods helps to remove experimenter subjectivity and provides an automated analysis pipeline to facilitate data comparisons across experiments, investigators, and research centers. There are several studies applying machine learning approaches to assessing depth of anesthesia in human subjects.[219,220] These approaches may yield further mechanistic insights when also translated to the preclinical model for concurrent neural circuit interrogations.

Conclusions: Bridging the Preclinical–Clinical Divide

To develop a better understanding of anesthetic emergence and work toward new clinical strategies to promote smooth emergence, existing studies of network state changes in humans might be used as working templates for further mechanistic dissection of brain arousal circuitry in preclinical animal models. By layering relevant clinically translational endpoints onto preclinical models, such as EEG analysis and functional magnetic resonance imaging imaging to identify shared areas of activation, a comprehensive view of brain circuitry during emergence may develop. The preclinical model can then be used to develop a more granular mechanistic analysis of neuronal changes, taking advantage of high-resolution single-cell approaches in the context of whole-brain dynamics.

Emergence is likely a convergence of the activity of multiple distributed transmitters, receptors, and circuits, for example, a unification of orexinergic, dopaminergic, and noradrenergic systems.[4] Further investigations are needed to understand the effects of different anesthetic conditions, like ketamine as compared to sevoflurane or propofol, on neural circuits. To study emergence, the aggregate brain network must then be examined using network-wide manipulations. The patterns of neuronal circuit activity that regulate emergence can be directly controlled and modified by utilizing closed-loop approaches, as discussed in this article. Additional tools to elucidate behaviorally activated brain-wide circuits include utilizing transgenic mice, like Fos-CreER^{T2} [221], together with viral approaches to access activity-regulated neuronal ensembles[207,222,223] (see review[224]). At this level of whole-brain analysis and neuronal activity, it is then possible to generate neuronal decoders for predicting and modifying emergence. Overall, an improved understanding of brain circuitry changes during emergence will help to facilitate predictable transitions from the anesthetized state to the awake state that will in turn improve patient safety and satisfaction with anesthesia care.

While it is not easy to reconcile preclinical and clinical approaches, innovative new tools exist for studying brain circuitry that can be applied strategically to heighten the translational value of preclinical anesthesia investigations. We must build dialogue and collaborative studies between basic neuroscientists and clinician researchers, appreciate the limitations of each scientific approach, and compare parallel findings from the preclinical and clinical literature as guides for future shared investigation.

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Competing Interests

The authors declare no competing interests.

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