Electrical Stimulation of the Ventral Tegmental Area Induces Reanimation from General Anesthesia

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ABSTRACT

Background: Methylphenidate or a D1 dopamine receptor agonist induces reanimation (active emergence) from general anesthesia. The authors tested whether electrical stimulation of dopaminergic nuclei also induces reanimation from general anesthesia.

Methods: In adult rats, a bipolar insulated stainless steel electrode was placed in the ventral tegmental area (VTA, n = 5) or substantia nigra (n = 5). After a minimum 7-day recovery period, the isoflurane dose sufficient to maintain loss of righting was established. Electrical stimulation was initiated and increased in intensity every 3 min to a maximum of 120 μA. If stimulation restored the righting reflex, an additional experiment was performed at least 3 days later during continuous propofol anesthesia. Histological analysis was conducted to identify the location of the electrode tip. In separate experiments, stimulation was performed in the prone position during general anesthesia with isoflurane or propofol, and the electroencephalogram was recorded.

Results: To maintain loss of righting, the dose of isoflurane was 0.9% ± 0.1 vol%, and the target plasma dose of propofol was 4.4 ± 1.1 μg/ml (mean ± SD). In all rats with VTA electrodes, electrical stimulation induced a graded arousal response including righting that increased with current intensity. VTA stimulation induced a shift in electroencephalogram peak power from δ (<4 Hz) to θ (4–8 Hz). In all rats with substantia nigra electrodes, stimulation did not elicit an arousal response or significant electroencephalogram changes.

Conclusions: Electrical stimulation of the VTA, but not the substantia nigra, induces reanimation during general anesthesia with isoflurane or propofol. These results are consistent with the hypothesis that dopamine release by VTA neurons, but not substantia nigra neurons, induces reanimation from general anesthesia. (Anesthesiology 2014; 121:311-9)

CLINICAL problems related to emergence from general anesthesia can cause significant morbidity in surgical patients. Difficulty with emergence is a common cause of severe intraoperative airway or oxygenation problems,[1] and emergence delirium is estimated to occur in approximately 5% of adults[2] and up to 30% of children.[3] It was recently reported that cardiac surgical patients who experience emergence delirium have a higher incidence of postoperative cognitive dysfunction lasting up to 1 yr,[4] suggesting that the neural circuits underlying emergence from general anesthesia may also be involved in restoring cognitive function. Although general anesthetics with favorable pharmacokinetics are now widely used, delayed emergence still occurs, particularly when propofol infusions are used in obese patients.[5] Because the neural mechanisms underlying emergence are poorly understood, the available options to treat common clinical problems such as emergence delirium, delayed emergence, and postoperative cognitive dysfunction remain very limited. For example, the centrally active cholinesterase inhibitor physostigmine has been suggested as a

What We Already Know about This Topic

- Activation of dopaminergic neurotransmission leads to arousal from general anesthesia
- Unilateral electrodes were implanted in the two major dopaminergic pathways to test their roles in arousal from anesthesia in rats

What This Article Tells Us That Is New

- Electrical stimulation of the ventral tegmental area, but not of the substantia nigra, restored righting and activated the electroencephalogram during anesthesia with isoflurane or propofol
- Selective activation of the ventral tegmental area pathway resembled pharmacological activation of dopamine receptors in evoking arousal from anesthesia

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treatment for emergence delirium, but a recent randomized, double-blinded trial in children found it no more efficacious than placebo.[6]

In current clinical practice, emergence from general anesthesia is treated as a passive process dictated by the pharmacokinetics of anesthetic drug elimination. However, recent work suggests that ascending arousal pathways in the brain may be activated to promote emergence from general anesthesia. Arousal responses during general anesthesia have been elicited by pharmacologically activating cholinergic,[7,8] histaminergic,[9] and noradrenergic arousal pathways.[10] In addition, orexin/hypocretin neurons have been implicated in emergence from general anesthesia.[11] Although dopamine is also known to promote arousal,[12] the role of dopaminergic pathways in emergence has not been well characterized.

In mammals, the ventral tegmental area (VTA) and substantia nigra (SN) are the two major dopamine nuclei, both located in the midbrain. In Parkinson's disease, there is degenerative loss of SN neurons, and dopamine therapy is the mainstay of treatment.[13] Therefore, the SN is viewed as a critical area of the brain that controls movement, whereas the VTA has been studied extensively as a motivation and reward center.[14] Unlike other monoaminergic neurons that are active during the awake state and quiescent during sleep, dopamine neurons in the VTA and SN have stable firing rates across sleep–wake cycles.[15] These findings supported the notion that dopamine does not play a significant role in maintaining wakefulness and led to a diminished interest in studying the contributions of dopamine neurons to behavioral arousal. As a consequence, the dopaminergic arousal pathways in the brain remain poorly defined.

In adult rats during general anesthesia, methylphenidate (an inhibitor of the dopamine transporter) restores the righting reflex and other conscious behaviors such as kicking, clawing, and grooming and induces electroencephalogram changes consistent with arousal.[16,17] We term this active emergence process "reanimation," distinct from the passive emergence process in current clinical practice. A D1 dopamine receptor agonist also induces reanimation from general anesthesia,[18] providing further evidence for a dopamine-mediated arousal pathway. The current study was conducted to test whether electrical stimulation of the VTA or SN induces reanimation from general anesthesia.

Materials and Methods

Animal Care and Use

Animal studies were approved by the Subcommittee on Research Animal Care, Massachusetts General Hospital, Boston, Massachusetts. Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were housed using a standard day–night cycle (lights on at 7:00 AM and off at 7:00 PM). All experiments were conducted during the day time. During all experiments with general anesthesia, the animals were kept warm with a heating pad to maintain rectal temperature between 36.5° and 37.4°C. A minimum recovery period of 7 days was provided after surgical implantation of stimulation and electroencephalogram recording electrodes, and at least 3 days of rest were provided between stimulation experiments with general anesthesia.

Surgical Placement of Electrodes

When the rats weighed approximately 290 g, they were anesthetized with isoflurane (Henry Schein, Melville, NY) and placed in a stereotaxic frame (Model 962; David Kopf Instruments, Tujunga, CA). A microdrill (Patterson Dental Supply Inc., Wilmington, MA) was used to create a hole in the skull for VTA or SN electrode placement. A two-channel electrode with pedestal (MS303; Plastics One, Roanoke, VA) was inserted through the hole, using the following coordinates relative to Bregma: for the VTA, 4.80 mm posterior, ±0.90 mm lateral, and 8.35 mm ventral (n = 8 total, four on the left side and four on the right side), and for the SN, 5.00 mm posterior, ±2.50 mm lateral, and 8.35 mm ventral (n = 8, four on the left and four on the right). The electrode was coated with the lipophilic long-chain dialkylcarbocyanine tracer Di-I (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) to facilitate final histological analysis of the electrode tip location. Additional holes were drilled for extradural electroencephalogram electrodes (E363; Plastics One) at the following stereotactic coordinates relative to Bregma: 1.3 mm anterior, 2 mm lateral (ground); 8.7 mm posterior, 0 mm lateral (lead 1); and 2.7 mm posterior, ±3 mm lateral (lead 2, placed on the opposite side of the stimulation electrode). The electroencephalogram electrodes were brought together with a multichannel electrode pedestal (MS363; Plastics One). Dental acrylic cement was used to secure the screws, sockets, and pedestals. During the minimum 7-day recovery period, carprofen (5 mg/kg subcutaneously) was administered for postoperative analgesia as needed.

Stimulation during Continuous Isoflurane General Anesthesia

After inducing general anesthesia with 2 to 3% isoflurane in oxygen, the animal was placed supine in a custom-built cylindrical acrylic anesthetizing chamber with ports for electroencephalogram and stimulation cables. Gas was continuously sampled from the chamber on the opposite side from the fresh gas inlet. An Ohmeda 5250 anesthetic agent analyzer (GE Healthcare, Waukesha, WI) was used to determine the concentrations of isoflurane, oxygen, and carbon dioxide in the chamber.

The minimum dose of isoflurane sufficient to maintain loss of righting was established as previously described,[16] and this dose was administered continuously throughout the remainder of the experiment. Stimulation was performed using a Multichannel Systems stimulus generator (STG4004; ALA Scientific, Farmingdale, NY). Stimulation was initiated using a 100-Hz square wave with a current intensity of 30 μA for 30 s, followed by a 30-s rest period. If righting did not occur, stimulation was repeated at the same current intensity for two additional 1-min cycles (*i.e.*, 30 s on/30 s off). If righting did not occur after three cycles at 30 µA, the current intensity was increased by 10-µA increments every three cycles, until a maximum of 120 µA was reached. The experiment was concluded if the animal righted itself, or if three cycles at the maximum current intensity of 120 µA failed to restore righting. If maximal stimulation failed to restore righting during isoflurane general anesthesia, the animal was sacrificed and the brain was removed for histological analysis.

Stimulation during Continuous Propofol Anesthesia

In rats that exhibited righting with stimulation during isoflurane general anesthesia, an additional experiment was performed with propofol general anesthesia after a minimum recovery period of 3 days. Rats were briefly anesthetized with isoflurane before placement of a 24-gauge intravenous catheter in a lateral tail vein, after which the isoflurane was discontinued and the rats were placed in the supine position on a heating pad in room air. After full recovery from isoflurane general anesthesia, a continuous infusion of intravenous propofol (APP pharmaceuticals, Shaumburg, IL) was initiated, and the minimum dose of propofol sufficient to maintain loss of righting was established as previously described.[17] This dose was fixed for the remainder of the experiment.

Histological Analysis of Electrode Placement

After all stimulation experiments were completed, animals were deeply anesthetized with isoflurane and perfused with phosphate-buffered saline followed by formalin. Brains were removed and postfixed in formalin overnight. The brains were sectioned (50 µm) using a VT1000 S vibratome (Leica Microsystems, Buffalo Grove, IL). Sections were stained with 4',6-diamidino-2-phenylindole, a blue-fluorescent nuclear counterstain that preferentially stains double-stranded DNA, and then imaged using an AxioImager fluorescent microscope (Zeiss, Oberkochen, Germany). Stained sections were compared with a rat brain atlas,[19] and the brain region at the deepest point of the electrode was identified for each animal. We used the definition of the VTA given by Paxinos and Watson,[19] which includes the paranigral, parainterfascicular, and parabrachial pigmented nuclei, as well as the rostral part of the VTA, and extends from Bregma −4.56 to −6.84 mm.

Statistical Analysis of the Effect of Stimulation on Return of Righting Responses

Matlab (Mathworks, Natick, MA) was used for statistical analysis. To compare the effect of SN *versus* VTA stimulation on restoration of righting during continuous isoflurane general anesthesia, we used a Bayesian Monte Carlo procedure to compute Bayesian 95% (credibility) CIs to assess the effect of stimulation on return of righting during continuous isoflurane general anesthesia, as described previously.[16] The posterior density for each group is a β density,[16,20] and the posterior density for the difference in the proportion of animals that had return of righting between any two groups, such as between the VTA stimulation group and the SN stimulation group, was computed using standard Matlab simulation procedures. The simulation, or Monte Carlo procedure, entailed drawing a value of the righting propensity (probability) from the posterior density for each group, computing the difference between the propensities, and then repeating these operations 10,000 times. We computed the Bayesian 95% credibility (CI) intervals for the difference in righting propensity by finding the 250th and the 9,750th largest values of the differences in the sample of 10,000 draws from the Monte Carlo analysis.[16]

Instead of P values for the Bayesian analyses, we reported the posterior probability that the propensity to right is greater in one group compared with the other. That is, the probability that the righting propensity in the VTA group was greater than that in the SN group is the fraction of times out of 10,000 that the propensity drawn at random from the VTA group was greater than the propensity drawn at random from the SN group. This is also equivalent to the fraction of times that the differences in these propensities were positive. A result was significant if this posterior probability was 0.95 or greater.[16]

Electroencephalogram Recording and Spectral Analysis

After completing each behavioral experiment that tested whether electrical stimulation restores righting during general anesthesia, the animals were anesthetized at a slightly higher dose of the same anesthetic in the prone position. For isoflurane, the dose was increased by 0.2%, and for propofol, the target plasma dose was increased by 1.0 µg/ml. The increased anesthetic dose and the change in position allowed us to record the electroencephalogram with minimal motion artifacts, which is an approach that we have used previously.[16–18] This new dose of general anesthetic was kept constant for the remainder of the experiment. After a minimum equilibration period of 40 min for isoflurane and 15 min for propofol, a baseline recording was taken during steady-state general anesthesia, and then three stimulations were performed (30 s on, 30 s off) at the same settings that had restored righting in the preceding experiment. If righting had not been restored in the preceding experiment, the maximal current intensity (120 µA) was used.

The potential difference between electroencephalogram electrodes 1 and 2 (referenced to the ground electrode) was recorded using a Quad AC Amplifier System (QP511; Grass Instruments, West Warwick, RI) and a 14-bit data acquisition board (USB-6009; National Instruments, Austin, TX). Data were filtered between 0.3 and 100 Hz. No line filter was used. The sampling rate was 512 Hz.

Spectral analysis was performed using Matlab 7.11 (Mathworks) and the Chronux software (Cold Spring Harbor, NY)[21] as previously described.[16] Group power spectra were computed by first normalizing the power spectra for individual animals by their mean power and then averaging the power at each frequency across all the animals in the same group. These normalized and averaged power spectra from the periods before and during stimulation were compared using Kolmogorov–Smirnov tests.[22] To determine the difference between the two spectra, a two-sample Kolmogorov–Smirnov test[23] was performed on the spectral power as a function of frequency computed from the 22 windows in the prestimulation and intrastimulation periods. A Bonferroni correction was used to adjust the significance level for multiple hypothesis testing. To compute the statistical significance of the combined *P* values of every frequency across all rats of a single group (VTA or SN), Fisher combined probability test was used.[24]

Results

Histological analysis revealed VTA electrode placement in five of eight rats and SN electrode placement in five of eight rats (fig. 1). In all five rats with VTA electrodes, stimulation during isoflurane general anesthesia at an inhaled dose of 0.9 ± 0.1% (mean ± SD) induced a profound arousal response, and the righting reflex was restored in five of five rats at a stimulation intensity of 62 ± 34 μA (mean ± SD). At the lowest current intensity of 30 μA, some animals exhibited no discernible response, whereas others had a mild arousal response (*e.g.*, eye opening or head lift). As the current intensity was increased, all five animals exhibited increasingly vigorous movements. The movements were variable and included head movements, orofacial movements, kicking, clawing, and escape behaviors. These movements generally appeared purposeful in nature, and no tonic-clonic seizures were observed. We also observed that the movements decreased during the 30-s rest periods with no stimulation. In separate experiments using the same rats with propofol general anesthesia at a target plasma dose of 4.4 ± 1.1 μg/ml (mean ± SD), VTA stimulation induced a similarly vigorous arousal response and restored righting in five of five animals

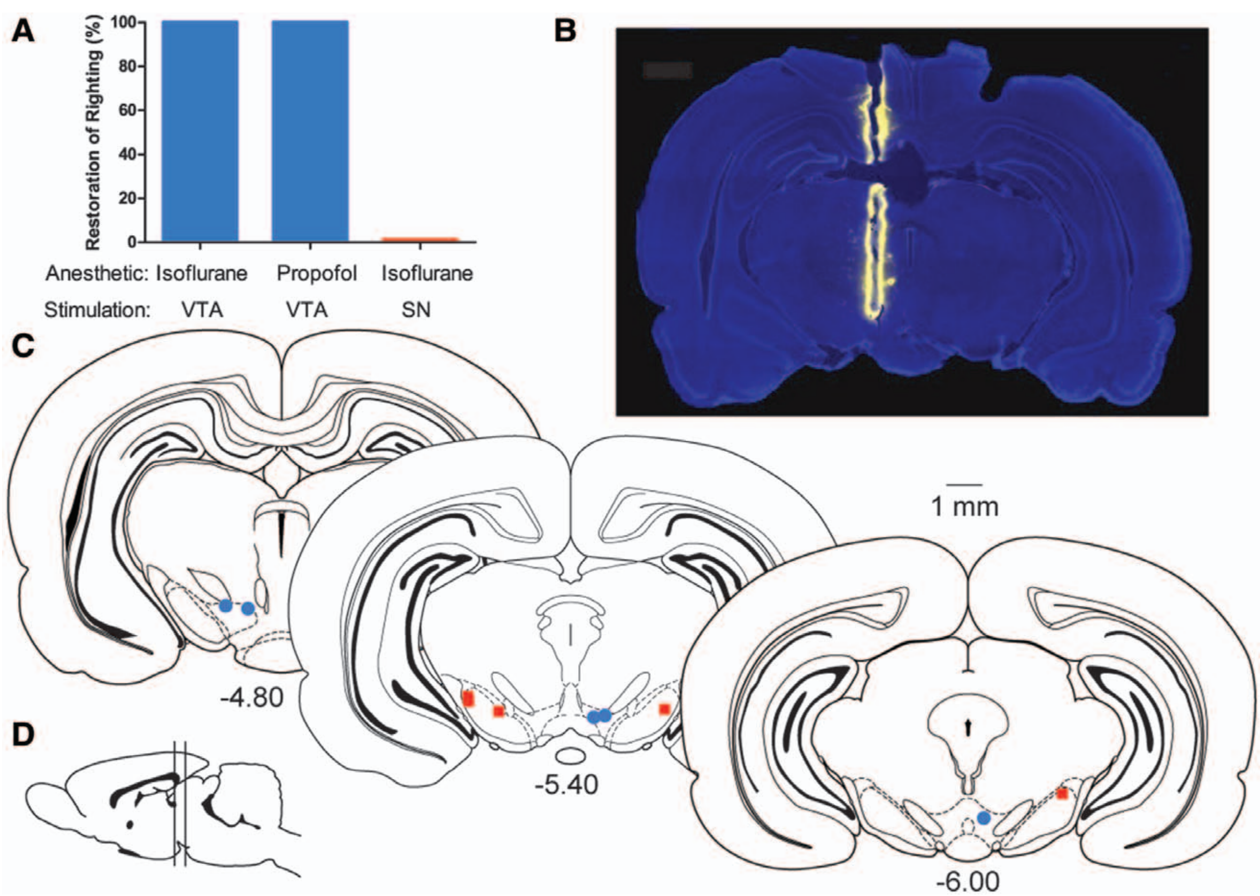

Fig. 1. (*A*) Summary of behavioral and histological data. All rats with confirmed ventral tegmental area (VTA) electrodes (*blue*) had restoration of righting with electrical stimulation during general anesthesia, whereas none of the rats with substantia nigra (SN) electrodes (*red*) exhibited an arousal response. (*B*) A representative coronal section shows placement of an electrode in the VTA. The section is stained with 4',6-diamidino-2-phenylindole, a blue-fluorescent nuclear counterstain that preferentially stains double-stranded DNA, and the electrode was coated with Di-I (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), a yellow, lipophilic long-chain dialkylcarbocyanine tracer. (*C*) Coronal sections from a rat brain atlas. Electrode tip placement in the VTA is shown in *blue circles*, and placement in the SN is shown in *red squares*. The number below each section indicates millimeter posterior to Bregma. (*D*) Sagittal section showing the anterior to posterior range of the coronal sections.

at a stimulation intensity of 48 ± 15 µA (mean ± SD). There was no difference in arousal response between animals with left-sided *versus* right-sided electrodes.

In contrast, none of five rats with SN electrodes exhibited an arousal response during isoflurane general anesthesia under identical experimental conditions, even with stimulation at the maximum current intensity of 120 µA. Bayesian 95% CI for the difference in probabilities of righting between rats in the VTA isoflurane group and SN isoflurane group was 0.30 to 0.96. Because zero was outside the CI and because the posterior probability that $p_{VTA} > p_{SN}$ was 0.9995, we concluded that there was a significant difference in arousal induced by electrical stimulation in the VTA compared with electrical stimulation of the SN.

In separate experiments, the animals were placed in the prone position at a slightly higher dose of general anesthetic to minimize motion artifacts, and the electroencephalogram was recorded. Representative 30-s electroencephalogram recordings are shown in figure 2. Figure 2A is a baseline electroencephalogram recorded during the awake state, and it shows dominant high-frequency activity. During isoflurane general anesthesia (fig. 2B), a rapid shift from low-frequency to high-frequency activity occurred with VTA stimulation (horizontal bar). A similar change occurred with VTA stimulation during propofol general anesthesia (fig. 2C). As shown in figure 2D, however, SN stimulation did not induce any obvious changes in the electroencephalogram pattern during isoflurane general anesthesia.

Figure 3 shows representative individual spectrograms computed from electroencephalogram recordings during general anesthesia. VTA stimulation rapidly shifted peak power from δ (<4 Hz) to θ (4–8 Hz) during isoflurane general anesthesia (fig. 3A). The θ-dominant electroencephalogram pattern induced by VTA stimulation was similar to the pattern induced by intravenous methylphenidate during reanimation from isoflurane general anesthesia.[16] As illustrated in figure 3B, VTA stimulation during propofol general anesthesia decreased power in the δ band and increased power in the θ and β (12–30 Hz) bands. In contrast, SN stimulation did not induce significant electroencephalogram changes (fig. 3C).

Figure 4 shows normalized and averaged power spectra computed from electroencephalogram recordings for all five animals in each group. At a 0.05 significance level, Fisher combined probability test rejects the null hypothesis at all frequencies except those marked with white squares. The averaged power spectrum during isoflurane general anesthesia before stimulation (fig. 4A, red) shows that power in the δ band is dominant. VTA stimulation (fig. 4A, green) shifted peak power from δ to θ, and induced statistically significant changes in power at most frequencies ($P < 0.05$, Fisher combined probability test). During propofol general anesthesia (fig. 4B, red), spectral analysis revealed that peak power was in the δ and α (8–12 Hz) frequency bands. VTA stimulation (fig. 4B, green) reduced δ and α power, whereas increasing θ and β (12–30 Hz) power, with statistically significant changes occurring at most frequencies ($P < 0.05$, Fisher combined probability test). However, as illustrated in figure 4C, SN stimulation during isoflurane general anesthesia induced no appreciable change in the power spectrum (white boxes represent no statistically significant difference between the two power spectra at a given frequency).

In six rats, the target was missed and the electrode was not implanted in the VTA or SN. Of these animals, only one of six exhibited a behavioral arousal response with

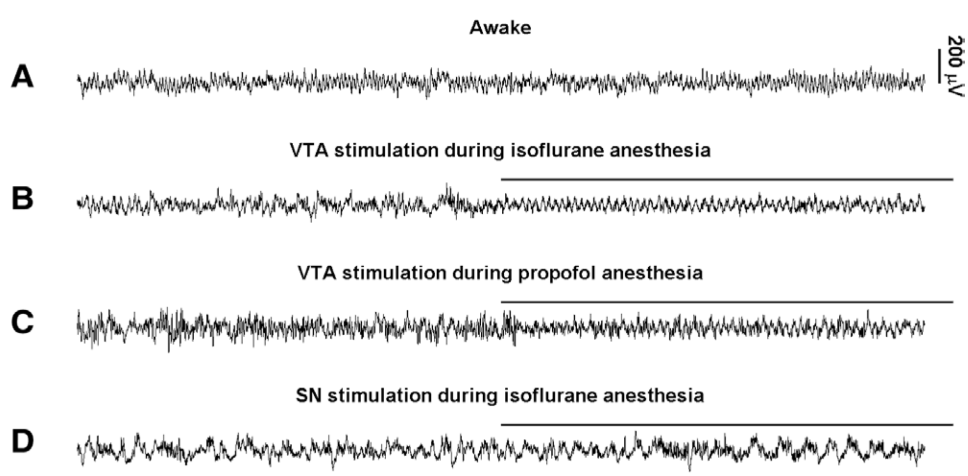

Fig. 2. Representative 30-s electroencephalogram recordings from individual animals. The *horizontal black bars* represent periods of electrical stimulation. (*A*) Electroencephalogram recorded from a rat with a ventral tegmental area (VTA) stimulation electrode during the awake state, before general anesthesia. (*B*) Electroencephalogram recorded from the same rat during isoflurane general anesthesia. VTA stimulation induced a prompt increase in electroencephalogram frequency. (*C*) Electroencephalogram recorded from a rat during propofol general anesthesia. VTA stimulation induced a prompt increase in electroencephalogram frequency. (*D*) Recording from a rat with a substantia nigra (SN) stimulation electrode during isoflurane general anesthesia. SN stimulation induced no obvious changes in the electroencephalogram.

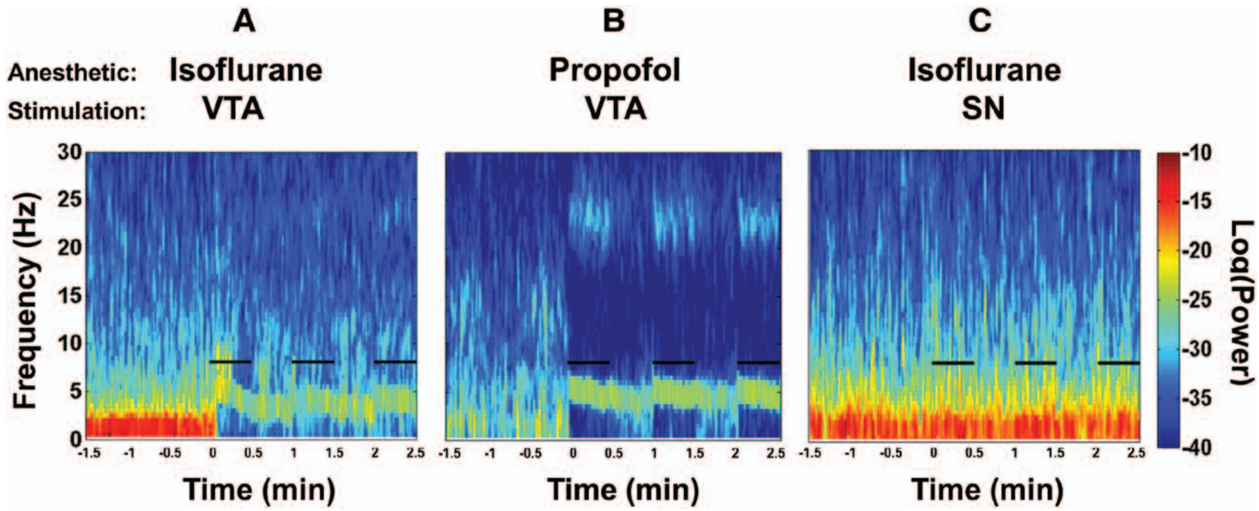

Fig. 3. Spectrograms computed from individual animals during general anesthesia. The *horizontal black bars* represent 30-s stimulation periods. (*A*) Ventral tegmental area (VTA) stimulation during isoflurane general anesthesia induced a rapid decrease in δ (<4 Hz) power and increase in θ (4–8 Hz) power. (*B*) VTA stimulation during propofol general anesthesia induced a prompt decrease in δ power and increases in θ and β (12–30 Hz) power. (*C*) Substantia nigra (SN) stimulation induced no appreciable changes in the spectral content of the electroencephalogram.

stimulation during isoflurane general anesthesia, and righting was restored at a stimulation intensity of 90 µA. This rat also exhibited an arousal response with stimulation during propofol general anesthesia, and righting was restored at a stimulation intensity of 110 µA. Histological analysis confirmed that in this one rat, the electrode tip was in the medial forebrain bundle. For the other five animals, there was no arousal response noted during isoflurane general anesthesia, even with stimulation at the maximum current intensity of 120 µA. These animals were found to have the electrode tip in the red nucleus (n = 2), cerebral peduncle (n = 1), internal capsule (n = 1), and medial amygdaloid nucleus (n = 1).

Discussion

In 1949, Moruzzi and Magoun[25] reported that electrical stimulation of the brainstem induces electroencephalogram changes consistent with arousal during general anesthesia in cats, suggesting that arousal pathways may be activated

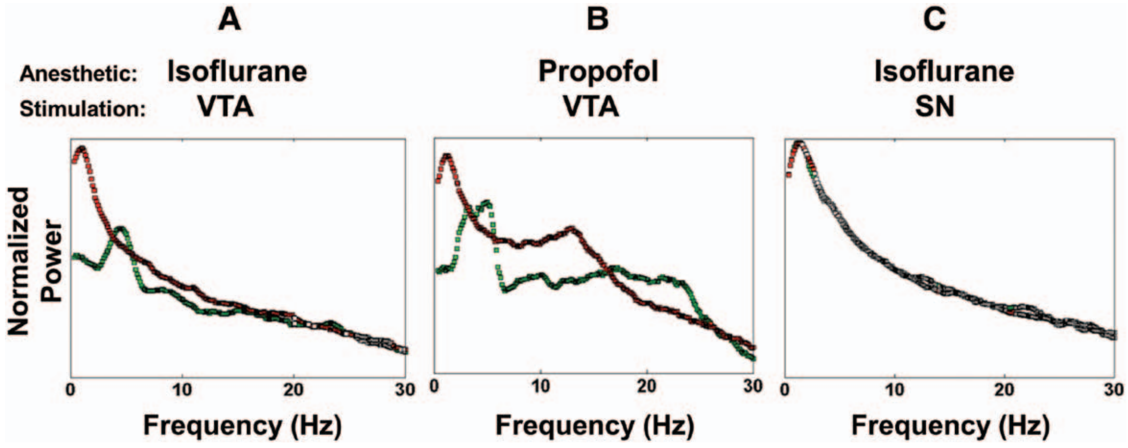

Fig. 4. Normalized power spectra averaged over all animals (n = 5 in each group), with results of Fisher combined probability test for the periods before (*red*) and during (*green*) stimulation. At a 0.05 significance level, the test rejects the null hypothesis at all frequencies except those marked with *white squares*. (*A*) Power spectra computed from animals with ventral tegmental area (VTA) electrodes anesthetized with isoflurane. Before stimulation, δ power was dominant (*red*). VTA stimulation (*green*) induced statistically significant changes at most frequencies and shifted peak power to θ frequencies. (*B*) Power spectra computed from animals with VTA electrodes during propofol general anesthesia. Before stimulation, δ and α power were dominant (*red*). VTA stimulation (*green*) shifted power to θ and β frequencies, and induced statistically significant changes at most frequencies. (*C*) Power spectra computed from animals with substantia nigra (SN) electrodes during isoflurane general anesthesia. Similar to *A*, low-frequency power dominates before stimulation (*red*). However, the presence of mostly *white squares* illustrates that SN stimulation (*green*) induced no statistically significant changes at most frequencies.

to reverse the state of general anesthesia. However, their experiments used the "encephale isole" technique to minimize motion artifacts on the electroencephalogram, which involved transection of the spinal cord at C1 before brainstem stimulation. Therefore, behavioral evidence of arousal was not observed. In the current study using intact and unrestrained rats, we found that stimulation of the VTA (a site in the midbrain distinct from the brainstem sites stimulated by Moruzzi and Magoun) reliably induced a robust behavioral arousal response in rats during general anesthesia, with return of righting and other conscious behaviors. The arousal response was not specific to one class of general anesthetics, as it was elicited during inhalational general anesthesia with isoflurane (an ether) and intravenous general anesthesia with propofol (a phenol). The stimulation current intensity required to restore righting varied considerably among rats with VTA electrodes, which likely reflects differences in electrode position within the VTA (fig. 1C).

The electroencephalogram changes induced by VTA stimulation during general anesthesia were similar to those induced by intravenous methylphenidate,[16,17] suggesting a common mechanism. Methylphenidate administration and VTA stimulation consistently decreased δ power and increased θ power during isoflurane and propofol general anesthesia, and increased β power during propofol anesthesia. However, methylphenidate sometimes increased β power during isoflurane anesthesia,[16] whereas VTA stimulation did not. These differences may reflect the different mechanisms by which methylphenidate and VTA stimulation induce arousal. Systemic methylphenidate administration inhibits dopamine and norepinephrine reuptake transporters in the entire brain, whereas electrical VTA stimulation activates a limited group of neurons in the vicinity of the electrode tip.

Electrical stimulation of the adjacent SN, as well as other brain sites close to the VTA, did not induce behavioral arousal during general anesthesia. This demonstrates that the effects observed with VTA stimulation were site specific and localized to a small region in the vicinity of the electrode tip. Although we did not measure dopamine release in our experiments, it has been shown previously that electrical stimulation of the VTA causes dopamine release.[26] It has also been reported that electrical stimulation of the SN induces dopamine release during general anesthesia, with maximal dopamine release occurring at a current intensity of 120 μA.[27] In our study, no arousal behaviors and no significant electroencephalogram changes were observed with SN stimulation at a current intensity of 120 μA during general anesthesia. Therefore, our findings are consistent with the hypothesis that dopamine release by VTA neurons, but not SN neurons, plays a role in reanimation from general anesthesia. An additional population of wake-active dopaminergic neurons was recently identified in the ventral periaqueductal gray area,[28] but these cells have yet to be further characterized, and we did not target them in our study. It is possible that these neurons are also important for emergence from general anesthesia.

One animal that exhibited an arousal response with stimulation was found to have the stimulation electrode implanted in the medial forebrain bundle, which contains projections from the VTA. Injury to the medial forebrain bundle causes akinetic mutism that improves with dopamine agonists,[29] suggesting that it contains a major dopamine-mediated arousal pathway. Although there was only one animal in our study with an electrode in the medial forebrain bundle, stimulation during general anesthesia produced a dramatic arousal response with restoration of righting which was indistinguishable from VTA stimulation. These results suggest that the medial forebrain bundle may contain a major arousal-promoting projection from the VTA.

Historically, dopaminergic VTA neurons have been studied mainly in the contexts of reward (mesolimbic pathway) and cognition (mesocortical pathway), whereas SN neurons are known to be important for movement (nigrostriatal pathway). Dopaminergic neurons in the VTA and SN send projections to key arousal-promoting brain regions including the dorsal raphe, locus ceruleus, pedunculopontine and laterodorsal tegmental areas, basal forebrain, tuberomamillary nucleus, and the perifornical area of the lateral hypothalamus, and in turn, these arousal-promoting centers also send inputs to the VTA and SN.[12] In addition, dopamine may promote arousal by activating the thalamus.[30] However, the specific contributions of dopaminergic pathways to behavioral arousal have been controversial.[31]

There are several lines of evidence that support the idea that dopaminergic neurotransmission projecting from the VTA is important for maintaining arousal. It was reported that lesions in the VTA lead to a coma-like state almost entirely devoid of arousal,[32] and mice with selective loss of dopamine in the brain appear hypoactive and apathetic, suggesting that dopamine plays a critical role in maintaining consciousness.[33] Recently, a D1 dopamine receptor–mediated arousal pathway that regulates wakefulness was also identified in *Drosophila*.[34] In Parkinson's disease, there is degenerative loss of VTA neurons in addition to SN neurons,[13] and excessive daytime sleepiness is a common symptom of this disease.[35]

The current findings and previous work using methylphenidate[16,17] and a selective D1 receptor agonist[18] together support the hypothesis that dopamine release by VTA neurons plays a role in reanimation from general anesthesia. However, more selective techniques are necessary to identify the specific neuronal populations underlying reanimation, because nondopamine neurons in the VTA that promote arousal may have been activated in these experiments. It is also possible that VTA stimulation induced reanimation through projections to other arousal centers in the brain.

Proposed mechanisms for anesthetic-induced unconsciousness include impairment of intracortical communication,[36] thalamocortical oscillations,[37] slow oscillations,[38] inhibition of ascending arousal pathways,[39] and activation of sleep-promoting pathways.[40] Recovery from general anesthesia requires the restoration of motor, sensory, and cognitive functions in addition to arousal. Through extensive projections, VTA stimulation may cause simultaneous activation of the cortex, thalamus, and other arousal centers. Targeting this single site in the brain may be an efficient method to promote recovery from general anesthesia.

In summary, electrical stimulation of the VTA restores conscious behaviors and electroencephalogram changes consistent with arousal during general anesthesia, whereas SN stimulation does not. Our findings suggest that arousal-promoting projections from the VTA may be exploited to induce reanimation from general anesthesia in surgical patients. Activating this pathway at the end of surgery may provide a novel approach to hasten recovery from general anesthesia, and treat or obviate emergence-related problems such as postoperative delirium and cognitive dysfunction.

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Competing Interests

The authors declare no competing interests.

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