Physostigmine and Methylphenidate Induce Distinct Arousal States During Isoflurane General Anesthesia in Rats

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BACKGROUND: Although emergence from general anesthesia is clinically treated as a passive process driven by the pharmacokinetics of drug clearance, agents that hasten recovery from general anesthesia may be useful for treating delayed emergence, emergence delirium, and postoperative cognitive dysfunction. Activation of central monoaminergic neurotransmission with methylphenidate has been shown to induce reanimation (active emergence) from general anesthesia. Cholinergic neurons in the brainstem and basal forebrain are also known to promote arousal. The objective of this study was to test the hypothesis that physostigmine, a centrally acting cholinesterase inhibitor, induces reanimation from isoflurane anesthesia in adult rats.

METHODS: The dose-dependent effects of physostigmine on time to emergence from a standardized isoflurane general anesthetic were tested. It was then determined whether physostigmine restores righting during continuous isoflurane anesthesia. In a separate group of rats with implanted extradural electrodes, physostigmine was administered during continuous inhalation of 1.0% isoflurane, and the electroencephalogram changes were recorded. Finally, 2.0% isoflurane was used to induce burst suppression, and the effects of physostigmine and methylphenidate on burst suppression probability (BSP) were tested.

RESULTS: Physostigmine delayed time to emergence from isoflurane anesthesia at doses ≥0.2 mg/kg (n = 9). During continuous isoflurane anesthesia (0.9% ± 0.1%), physostigmine did not restore righting (n = 9). Blocking the peripheral side effects of physostigmine with the coadministration of glycopyrrolate (a muscarinic antagonist that does not cross the blood–brain barrier) produced similar results (n = 9 each). However, during inhalation of 1.0% isoflurane, physostigmine shifted peak electroencephalogram power from δ (<4 Hz) to θ (4–8 Hz) in 6 of 6 rats. During continuous 2.0% isoflurane anesthesia, physostigmine induced large, statistically significant decreases in BSP in 6 of 6 rats, whereas methylphenidate did not.

CONCLUSIONS: Unlike methylphenidate, physostigmine does not accelerate time to emergence from isoflurane anesthesia and does not restore righting during continuous isoflurane anesthesia. However, physostigmine consistently decreases BSP during deep isoflurane anesthesia, whereas methylphenidate does not. These findings suggest that activation of cholinergic neurotransmission during isoflurane anesthesia produces arousal states that are distinct from those induced by monoaminergic activation. (Anesth Analg 2016;XXX:00–00)

Although emergence from general anesthesia is clinically treated as a passive process driven by the pharmacokinetics of drug clearance, agents that hasten recovery from general anesthesia may be useful for the treatment of delayed emergence, emergence delirium, and postoperative cognitive dysfunction.[1] Numerous ascending arousal pathways and their corresponding neurotransmitters have been identified in the brain,[2–4] and it is becoming increasingly evident that these pathways are important for emergence from general anesthesia.[4–8] The pharmacologic modulation of arousal states can provide considerable insight into the neuronal pathways and circuit mechanisms of general anesthesia. However, the brain states produced by activating arousal circuits during general anesthesia remain largely uncharacterized.

Methylphenidate (an inhibitor of dopamine and norepinephrine reuptake transporters) induces reanimation, or active emergence (behaviorally defined by restoration of righting in rodents), during continuous general anesthesia with isoflurane[9] or propofol.[10] Reanimation also occurs with the administration of dextroamphetamine[11] and a D1 dopamine receptor agonist,[12] as well as electrical stimulation of the ventral tegmental area (VTA), a major dopamine nucleus in the brain.[1] These findings suggest that a dopaminergic arousal pathway projecting from the VTA promotes active emergence from general anesthesia.

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Accepted for publication January 13, 2016.

JD Kenny and JJ Chemali were co-first authors.

Funding: This work was supported by National Institutes of Health grant numbers TR01-GM104948, DP1-OD003646, and K08-GM094394.

Conflict of Interest: See Disclosures at the end of the article.

This report was previously presented, in part, at the Association of University Anesthesiologists in Philadelphia, PA, on May 12, 2011.

Reprints will not be available from the authors.

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DOI: 10.1213/ANE.0000000000001234

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Arousal-promoting cholinergic neurons project from the laterodorsal tegmental area and pedunculopontine tegmental area in the brainstem to the thalamus, upper brainstem, midbrain, and regions of the prefrontal cortex and basal forebrain, whereas a separate group of arousal-promoting cholinergic neurons in the basal forebrain project mainly to the cortex.[13] Unlike neostigmine, physostigmine is a cholinesterase inhibitor that crosses the blood–brain barrier and therefore stimulates central cholinergic neurotransmission.[14] Some studies suggest that physostigmine may be clinically useful to enhance the recovery from general anesthesia,[15,16] whereas others suggest limited efficacy.[17,18]

This study was performed to test the hypothesis that physostigmine induces reanimation from isoflurane anesthesia in adult rats. By using the same experimental protocols used previously in our laboratory, these experiments allowed us to perform a direct comparison to our previous results with methylphenidate.[9] We also performed additional experiments to compare the effects of physostigmine and methylphenidate at a high dose of isoflurane that induced burst suppression on the electroencephalogram (EEG).

METHODS

Ethics Statement

All studies were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee and were performed in strict accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Invasive procedures and experiments were always performed under general anesthesia, and appropriate analgesia was provided after surgery. All efforts were made to minimize animal suffering.

Animal Care and Use

In our previous study using the same experimental methods with methylphenidate,[9] the SD for time to emergence was 153 seconds for the normal saline group and 32 seconds for the methylphenidate group. We made the initial prediction that physostigmine would decrease the SD for time to emergence similar to methylphenidate, but to a lesser extent. Therefore, for our power calculation, we estimated the SD of the physostigmine group to be 80 seconds. To detect an effect size of 90 seconds with a type I error of 0.05 and power of 0.8, we calculated that a sample size of 9 rats in each group would be required.

Twenty-four male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used for this study. One group of 9 rats was used in a random order for all behavioral experiments without glycopyrrolate, and a second group of 9 rats was used for all behavioral experiments with glycopyrrolate. In addition, a third group of 6 rats was used in a random order for all experiments involving EEG recordings. Each animal was provided with at least 3 days of rest between experiments. Animals were kept on a standard day–night cycle (lights on at 7:00 AM and off at 7:00 PM), and all experiments were performed during the day.

Anesthetizing Protocol

After inducing general anesthesia with isoflurane (2%–3%) in oxygen, a 24-gauge IV catheter was placed in a lateral tail vein, a rectal temperature probe was inserted, and the animal was placed in a cylindrical acrylic anesthetizing chamber as previously described.[9] A heating pad was placed under the chamber to maintain rectal temperature between 36.5°C and 37.4°C. Gas was sampled from the distal end of the chamber, and isoflurane, oxygen, and carbon dioxide concentrations were continuously monitored with a calibrated Ohmeda 5250 anesthetic agent analyzer (GE Healthcare, Waukesha, WI).

Preparation and Delivery of Drugs

Isoflurane, physostigmine, and glycopyrrolate were obtained from Henry Schein (Melville, NY), Akorn (Lake Forest, IL), and American Regent (Shirley, NY), respectively. Methylphenidate was purchased from Sigma-Aldrich (St. Louis, MO), dissolved in normal saline to a final volume of 0.5 mL, and sterile filtered before IV administration through the lateral tail vein catheter. The IV tubing (approximate volume, 0.6 mL) was always flushed with 2 mL of normal saline after drug administration to ensure complete drug delivery.

Time to Emergence After a Standardized Isoflurane General Anesthetic

After placement of the lateral tail vein catheter, the inhaled concentration of isoflurane was fixed at 1.5%, and normothermia was maintained with a warming pad as described previously.[9] After 40 minutes, rats (n = 9) received normal saline or physostigmine IV (0.1 or 0.2 mg/kg). The same 9 rats were used for all 3 conditions, in a random order, with at least 3 days of rest between experiments. Isoflurane was continued for 5 additional minutes, after which the rat was taken from the chamber and the temperature probe removed. The animal was placed supine on a warming pad and inspired room air. Time to emergence was defined as the time from termination of isoflurane to return of righting (prone position with all 4 paws touching the floor). To test whether the peripheral muscarinic side effects of physostigmine affected time to emergence, additional experiments were conducted with the same anesthesia protocol in a separate group of rats (n = 9) that received normal saline or physostigmine (0.2 or 0.4 mg/kg IV) with glycopyrrolate (0.2 mg per 1 mg physostigmine, e.g., 0.04 mg/kg glycopyrrolate for 0.2 mg/kg physostigmine). The same 9 rats were used for all 3 conditions, in a random order, with at least 3 days of rest between experiments.

Test for Reanimation During Continuous Isoflurane General Anesthesia

The isoflurane concentration was held at a dose that produced loss of righting with no purposeful movement for 40 consecutive minutes, as described previously.[9] The IV catheter was then flushed with 2 mL of normal saline to ensure patency of the IV and to confirm that the injection of normal saline did not induce an arousal response. Five minutes after this control injection, physostigmine alone (0.2 mg/kg) or physostigmine (0.4 mg/kg) with glycopyrrolate (0.08 mg/kg) was administered (n = 9 each). After IV drug administration, each animal continued to inhale the same dose of isoflurane for 20 minutes.

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Electroencephalogram Electrode Placement and Recording

Extradural EEG electrodes were implanted surgically at least 7 days before recording, as detailed previously.[9,10] On the day of an experiment, the potential difference between electrodes A0L0 and A6L3 (right somatosensory cortex) or between electrodes A0L0 and A6L3 (left somatosensory cortex) was recorded based on which signal gave less motion artifact. The signal was referenced to A10L2 and recorded using a QP511 Quad AC Amplifier System (Grass Instruments, West Warwick, RI) and a USB-6009 14-bit data acquisition board (National Instruments, Austin, TX). The sampling rate was 512 Hz, and no line filter was used. The EEG signal was filtered between 0.3 and 50 Hz.

Rats were anesthetized with isoflurane and placed in the chamber, with the inhaled isoflurane concentration fixed at 1.0% or 2.0%. The EEG was recorded continuously, and the prone position was used in all EEG experiments to minimize the motion artifacts.[9] After a minimum isoflurane exposure of 40 minutes, normal saline was administered IV as a control injection, and 5 minutes later, physostigmine (0.2 mg/kg IV) or methylphenidate (5 mg/kg IV) was administered. Isoflurane anesthesia was continued at the same dose for an additional 20 minutes.

Spectral Analysis of the Electroencephalogram

Multitaper methods from the Chronux toolboxes in MATLAB 8.3 (The MathWorks, Inc., MA) were used to construct time-frequency domain spectrograms and perform frequency domain spectral analysis.[19,20] Spectrograms were constructed from EEG data using a 3-second window with a step size of 0.25 seconds. The spectrogram frequency band was from 0 to 35 Hz, the half-bandwidth was 1 Hz, and 5 tapers were used in its calculation.

Power spectral densities were computed from 2-minute time windows predrug and postdrug administration. For all experiments, the predrug window was 150 to 30 seconds before injection, and the postdrug window was 30 to 150 seconds after injection. Group power spectra were created using multitaper methods and the time-frequency toggle bootstrap.[21] The time-frequency toggle bootstrap was used to obtain independent and identically distributed time-domain samples from the EEG. This allowed us to construct confidence intervals using a percentile bootstrap. The power spectral density within each bootstrapped EEG sample was constructed by calculating the spectral density for the first 2 seconds of bootstrapped EEG sample and then by stepping 2 seconds until the end of the time window. Each power spectral density had a frequency range of 0 to 35 Hz (141 discrete frequencies) and a half-bandwidth of 1 Hz, and it was constructed using 3 tapers. For EEG recordings that had periods of burst suppression, the suppression component was omitted from the spectral analysis. To account for uncertainty in group spectral densities, 95% confidence intervals were constructed around the mean using the percentile bootstrap.[22]

Classification of Electroencephalographic Bursts and Suppressions

Bursts and suppressions were classified for EEG experiments where animals continuously inhaled 2.0% isoflurane. EEGs were detrended by convoluting the EEG with a Gaussian function and then subtracting the convolution from the original signal. The EEG was transformed to the energy domain using the discrete version of the nonlinear energy operator.[23] The energy domain was additionally convoluted with a Gaussian function for smoothing, and then a manual threshold was set to separate high-energy bursts from the lower energy suppressions. EEG values that fell above the threshold were classified as bursts, and values that fell below the threshold were classified as suppressions.

Calculation of the Burst Suppression Probability

EEG data that were classified as bursts were given a value of 0, and data that were classified as suppressions were given a value of 1. This binary time series was used by the burst suppression probability (BSP) algorithm to find the probability of burst suppression, with 0 indicating no probability of suppression and 1 indicating an isoelectric EEG. Unlike the burst suppression ratio, the BSP gives the instantaneous probability of burst suppression, as well as a corresponding measure of uncertainty in the form of confidence intervals on a 1-second timescale.[24,25]

Statistical Analysis

Prism 5.04 (Graphpad Software, San Diego, CA) or MATLAB 8.4 was used for statistical analysis, and, when possible, results are reported in terms of 95% confidence intervals based on the bootstrap analysis. Confidence intervals constructed using the percentile bootstrap were used to test whether physostigmine accelerates emergence from isoflurane anesthesia.

A Bayesian Monte Carlo procedure was used to compute Bayesian 95% credibility intervals (CIs). The CIs were used to determine the efficacy of methylphenidate or physostigmine to restore righting, as previously described.[9] We computed the posterior probability that the propensity to restore righting in the methylphenidate group was greater than in the physostigmine group. We considered the difference to be statistically significant if the posterior probability was >0.95.

The effects of methylphenidate and physostigmine during isoflurane-induced burst suppression were analyzed by comparing the BSP before and after drug injection. We compared "predrug" time points (from 5 minutes before injection to the time of injection) with "postdrug" time points (from 2 minutes after injection to 7 minutes after injection). We did not use time points during the first 2 minutes after drug administration to allow the drugs sufficient time to take effect. Comparisons were made using a Bayesian Monte Carlo procedure. Ten thousand time points were randomly chosen from the "predrug" and "postdrug" time periods. The joint distribution of the state process was estimated for these 10,000 pairs, and the difference between the state processes was found. We then calculated 95% confidence intervals for these 10,000 differences. If the overall confidence bounds were negative, this was considered a statistically significant decrease in BSP. Conversely, if the overall bounds were positive, this was considered to be a statistically significant increase in BSP. If the bounds were both positive and negative, it was concluded that there was no statistically significant difference in BSP.

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RESULTS

Physostigmine Increases Time to Emergence from Isoflurane General Anesthesia

Figure 1A illustrates the experimental protocol used to test for time to emergence from isoflurane general anesthesia. Time to emergence was defined as the time from termination of general anesthesia until return of righting. As shown in Figure 1B (n = 9), rats that received normal saline had a mean time to emergence of 278 seconds (95% CI, 192–383 seconds). Rats that received 0.1 mg/kg physostigmine had a small increase in mean time to emergence to 328 seconds (95% CI, 261 to 397 seconds), but this increase was not statistically significant (mean difference, 50 seconds; 95% CI, –75 to 161 seconds). However, rats that received 0.2 mg/kg physostigmine had a mean time to emergence of 533 seconds (95% CI, 436–633 seconds), and the difference between this group and the normal saline group was statistically significant (mean difference, 255 seconds; 95% CI, 114–384 seconds).

To test whether the peripheral cholinergic effects of physostigmine were affecting emergence time, in a separate group of rats (n = 9), the antimuscarinic agent glycopyrrolate was coadministered with physostigmine. As shown in Figure 1C, in this group, the mean time to emergence after normal saline (control) was 181 seconds (95% CI, 133–240 seconds). Rats that received physostigmine (0.2 mg/kg IV) with glycopyrrolate (0.04 mg/kg IV) had an increase in mean time to emergence of 382 seconds (95% CI, 302–458 seconds), and this increase was statistically significant (mean difference, 201 seconds; 95% CI, 109–293 seconds). Rats that received an even higher dose of physostigmine (0.4 mg/kg) with glycopyrrolate (0.08 mg/kg) had a mean time to emergence of 360 seconds (95% CI, 280–444 seconds), and the difference between this group and the normal saline group was also statistically significant (mean difference, 179 seconds; 95% CI, 84–276 seconds).

Physostigmine Does Not Induce Reanimation During Continuous Isoflurane Anesthesia

The continuous dose of isoflurane required to maintain loss of righting (0.9% ± 0.1%, mean ± SD) was the same as in our previous study.[9] None of the rats had return of righting during the 20-minute observation period after the administration of physostigmine alone (0.2 mg/kg IV, n = 9). Although 1 animal had occasional head and limb movements after physostigmine administration, 8 of 9 animals did not exhibit any behavioral signs of arousal. Righting attempts, grooming, or escape behaviors that were commonly observed with methylphenidate in our previous study[9] were not observed with physostigmine.

To determine whether physostigmine did not restore righting due to insufficient dosing or peripheral cholinergic effects, we used a separate group of rats (n = 9) under the same anesthetizing conditions, but coadministered high doses of physostigmine (0.2 mg/kg or 0.4 mg/kg IV) with glycopyrrolate (0.2 mg per 1 mg physostigmine). At both doses, 0 of 9 rats had return of righting within 20 minutes of physostigmine/glycopyrrolate coadministration. In the animals that received the highest dose of physostigmine (0.4 mg/kg IV) with glycopyrrolate (0.08 mg/kg IV), increased muscle twitching was observed, but righting was not restored in any of the animals.

Physostigmine Decreases Electroencephalogram Power During Continuous Inhalation of 1.0% Isoflurane

A representative EEG recorded during continuous inhalation of 1.0% isoflurane (Fig. 2A) shows that the administration of physostigmine (0.2 mg/kg IV) decreased the overall amplitude of the signal. A spectrogram computed from the same animal (Fig. 2B) shows that physostigmine decreased δ (<4 Hz) power and increased θ (4–8 Hz) power. The combined power spectra computed from all 6 animals (Fig. 2C) show that physostigmine increased θ power, decreased δ power and α (8–12 Hz) power, and shifted peak power from δ to θ. The nonoverlapping 95% confidence intervals in the power spectra indicate that these changes in EEG power were statistically significant.

Physostigmine Decreases BSP During Continuous Inhalation of 2.0% Isoflurane

A representative EEG recorded during continuous inhalation of 2.0% isoflurane (Fig. 3A) shows that the administration of physostigmine (0.2 mg/kg IV) changed the burst

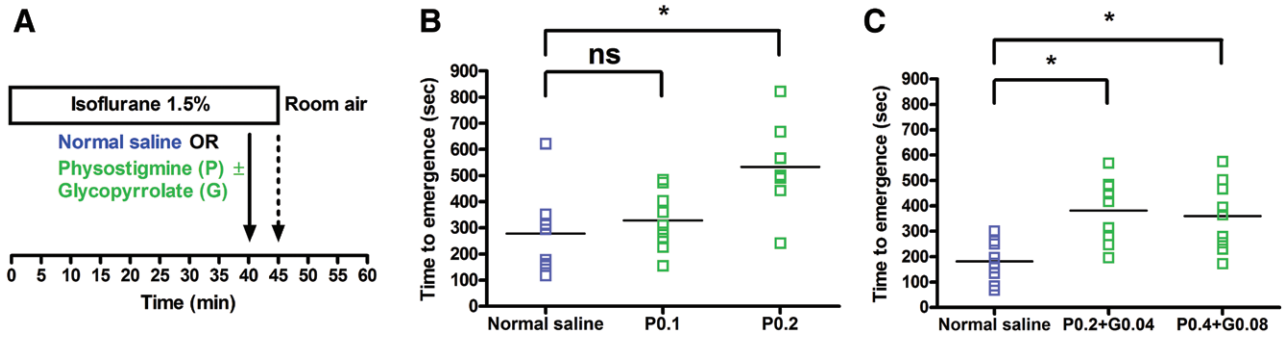


Figure 1. Physostigmine increases time to emergence from isoflurane general anesthesia. (A) Rats inhaled 1.5% isoflurane in oxygen for 40 minutes and then received normal saline or physostigmine ± glycopyrrolate IV (solid arrow). After 5 additional minutes of isoflurane anesthesia, rats were removed from the anesthetizing chamber (dashed arrow), and emergence was defined as return of righting. (B) Scatter plots showing emergence times (n = 9). P0.1 = physostigmine 0.1 mg/kg; P0.2 = physostigmine 0.2 mg/kg. For each data set, the horizontal line represents the mean. (C) Scatter plots showing emergence times for a separate cohort of rats (n = 9). P0.2 + G0.04 = physostigmine 0.2 mg/kg and glycopyrrolate 0.04 mg/kg; P0.4+G0.08 = physostigmine 0.4 mg/kg and glycopyrrolate 0.08 mg/kg. *Significant difference between groups with 95% confidence interval. ns = not significant.

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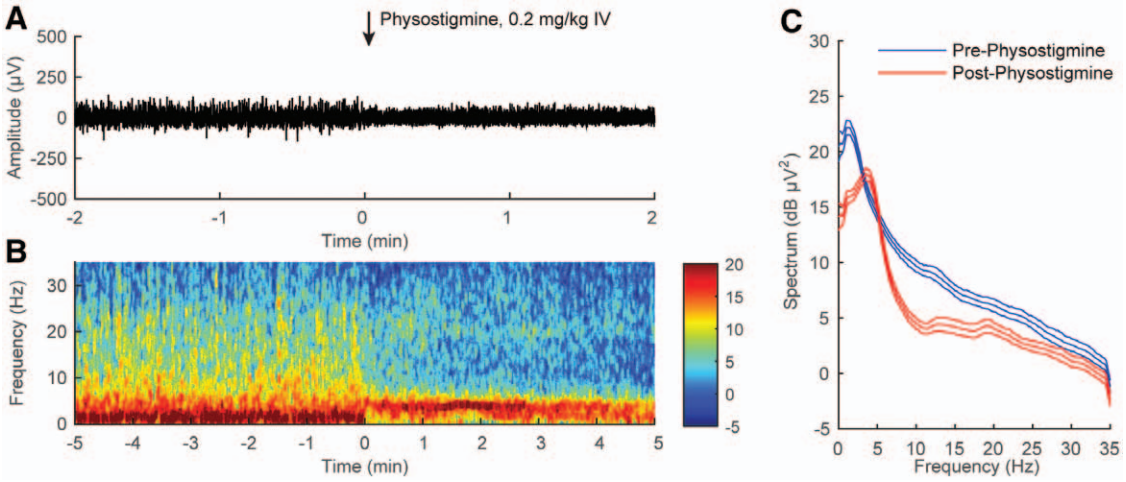


Figure 2. Physostigmine increases θ power during continuous inhalation of 1.0% isoflurane. (A) A representative electroencephalogram recorded from a rat inhaling 1.0% isoflurane, with time = 0 indicating the start of physostigmine (0.2 mg/kg IV) injection. There was a decrease in overall amplitude shortly after drug administration. (B) A spectrogram computed from the same animal in (A), with warm colors (e.g., red) indicating high power and cool colors (e.g., blue) indicating low power at any given frequency. Physostigmine (0.2 mg/kg IV) was administered at time = 0. A rapid shift in peak power from δ to θ occurred after drug injection. (C) The prephysostigmine power spectral density (computed from a 2-minute window of −150 to −30 seconds before drug injection) and the postphysostigmine power spectral density (computed from a 2-minute window of 30 to 150 seconds after drug injection) and their 95% confidence intervals (with Bonferroni correction) were constructed around the mean spectra across all rats (n = 6). Physostigmine increased θ power, decreased δ and α power, and shifted peak power from δ to θ.

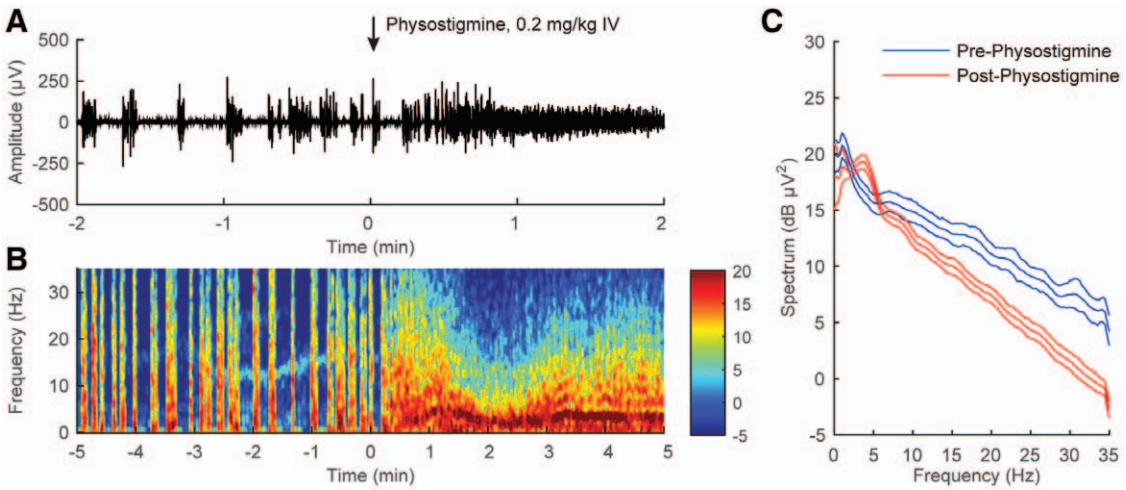


Figure 3. Physostigmine reverses burst suppression during continuous inhalation of 2.0% isoflurane. (A) A representative electroencephalogram recorded from a rat inhaling 2.0% isoflurane, with time = 0 indicating the beginning of physostigmine (0.2 mg/kg IV) injection. The electroencephalogram changed from a burst suppression pattern to a new rhythm without isoelectric (suppression) periods after physostigmine administration. (B) A spectrogram computed from the same animal in panel A shows that physostigmine increased δ, θ, and α power. (C) Power spectral densities computed from burst periods 2 minutes before physostigmine and 2 minutes after physostigmine, and their 95% confidence intervals (with Bonferroni correction), were constructed around the mean spectra across all rats (n = 6). Power at higher frequencies was reduced after the administration of physostigmine.

suppression pattern to a new rhythm without isoelectric (suppression) periods. A spectrogram computed from the same animal in Fig. 3B shows that the new rhythm after physostigmine administration had high δ, θ, and α power. The combined power spectra computed from burst periods in all 6 animals (Fig. 3C) shows that power at higher frequencies was significantly decreased after physostigmine administration.

Figure 4 shows the computed BSP for each of the 6 rats before and after physostigmine administration. The administration of physostigmine (0.2 mg/kg IV) induced a large decrease in BSP in 6 of 6 animals, and in 3 of 6 rats, the BSP became 0 (i.e., suppression periods were no longer present). The Bayesian Monte Carlo comparison between 10,000 randomly chosen time points before and after physostigmine administration yielded a statistically significant decrease in BSP for 6 of 6 rats.

Methylphenidate Does Not Decrease BSP During Continuous Inhalation of 2.0% Isoflurane

Figure 5A shows a representative EEG recorded from a rat that received methylphenidate during continuous inhalation

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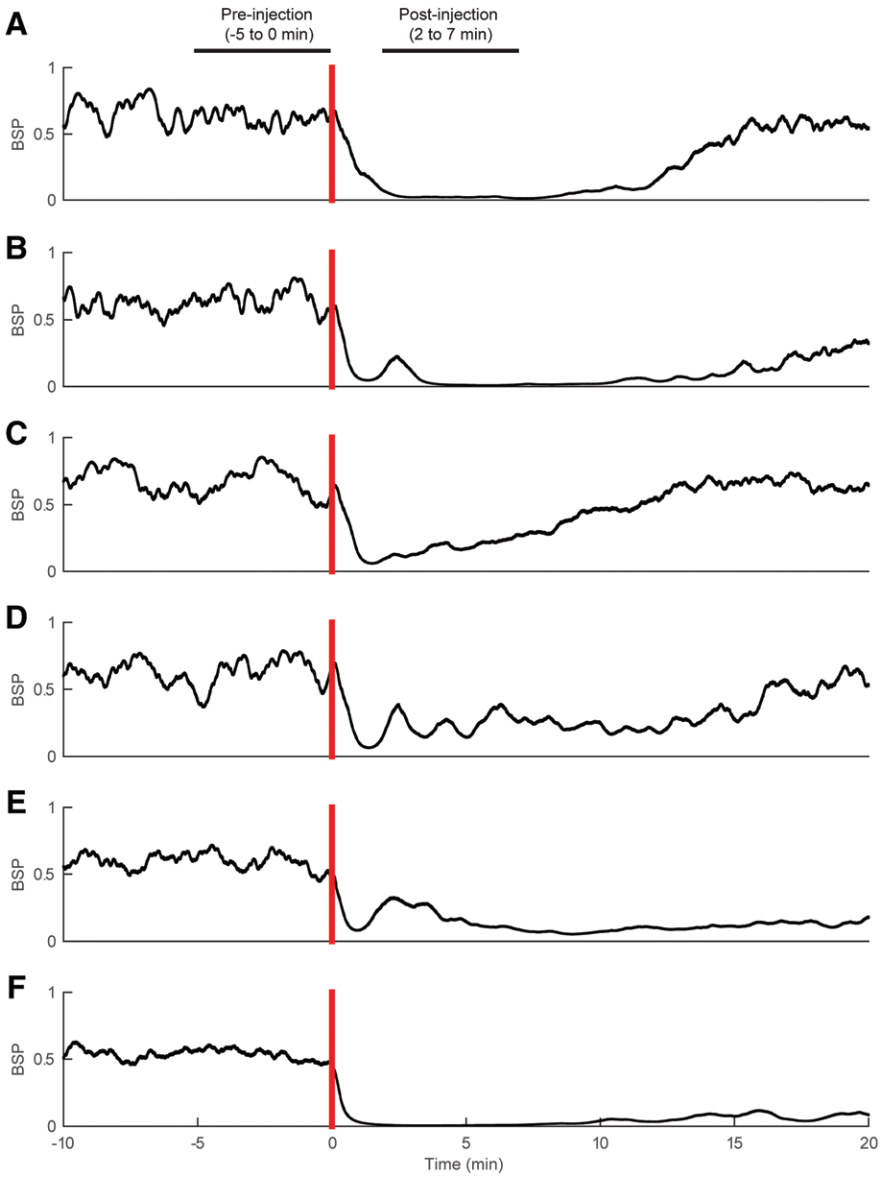


Figure 4. Physostigmine decreases burst suppression probability (BSP) during continuous inhalation of 2.0% isoflurane. (A–F) The BSP computed for each of 6 rats during continuous inhalation of 2.0% isoflurane, from 10 minutes before physostigmine (0.2 mg/kg IV) to 20 minutes after physostigmine. All 6 rats had large reductions in BSP after physostigmine administration. When comparing the "predrug" and "postdrug" periods shown in the figure (indicated by the horizontal bars), physostigmine induced statistically significant reductions in BSP in 6 of 6 rats.

of 2.0% isoflurane. Unlike physostigmine, methylphenidate did not induce an appreciable change in the EEG pattern, which remained in burst suppression. The spectrogram computed from the same animal (Fig. 5B) demonstrates that methylphenidate did not induce obvious changes, and the combined analysis for all 6 rats (Fig. 5C) shows that methylphenidate did not induce significant changes in the power spectrum.

The BSP computed for each of the 6 rats (Fig. 6) shows that, unlike physostigmine, methylphenidate induced relatively small (if any) changes in BSP. The Bayesian Monte Carlo comparison between 10,000 randomly chosen time points before and after methylphenidate administration yielded no statistically significant difference in BSP for 6 of 6 rats.

DISCUSSION

In this study, we employed the same experimental protocols used previously in our laboratory to provide a direct comparison between the arousal effects of physostigmine and methylphenidate during isoflurane general anesthesia in rats. Unlike methylphenidate, physostigmine did not decrease time to emergence from isoflurane general anesthesia, and at doses ≥0.2 mg/kg (alone or coadministered with glycopyrrolate), physostigmine increased time to emergence. In addition, physostigmine did not restore righting during continuous isoflurane anesthesia, regardless of whether glycopyrrolate was coadministered.

As in our previous studies,[1,9,10,12] we only used the righting reflex as our behavioral end point and did not employ a formal arousal scoring system to document more subtle signs of arousal during continuous isoflurane anesthesia. This is because there is currently no standard arousal scoring system in the literature, although several groups have devised such systems in an effort to refine measures of the anesthetized state.[8,26–28] Furthermore, because physostigmine can produce significant effects at the neuromuscular junction, it is difficult to distinguish whether minor peripheral movements

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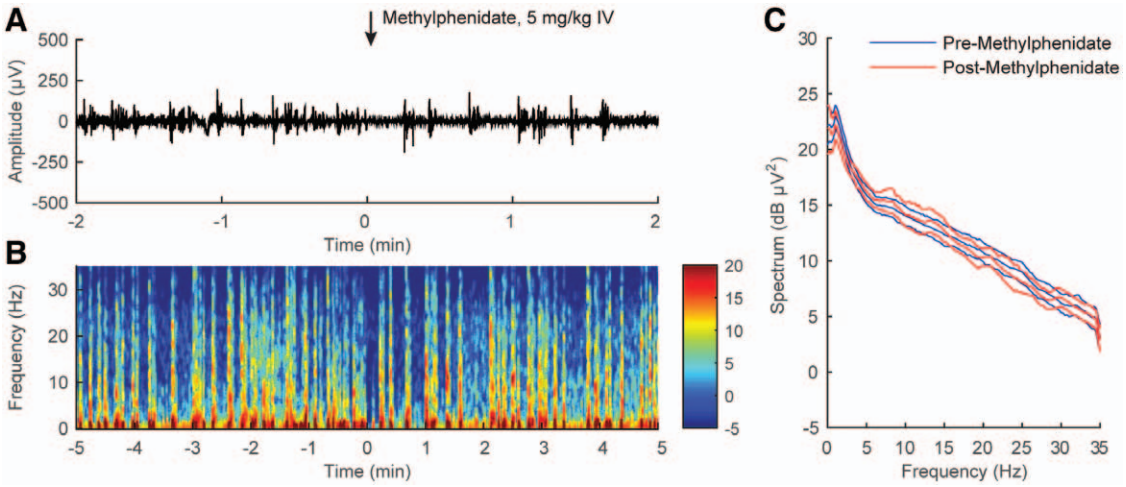


Figure 5. Methylphenidate does not induce significant electroencephalogram changes during continuous inhalation of 2.0% isoflurane. (A) A representative electroencephalogram recorded from a rat inhaling 2.0% isoflurane, with time = 0 indicating the start of methylphenidate (5 mg/kg IV) injection. The electroencephalogram remained in a burst suppression pattern after methylphenidate was administered. (B) A spectrogram computed from the same animal shows that methylphenidate did not induce significant changes in spectral power. (C) Power spectral densities computed from burst periods 2 minutes before methylphenidate and 2 minutes after methylphenidate, and their 95% confidence intervals (with Bonferroni correction), were constructed around the mean spectra across all rats (n = 6). Methylphenidate did not induce significant changes in the power spectrum.

in the absence of righting represent physostigmine-induced muscle twitches or a true change in arousal state. Although the highest dose of physostigmine (0.4 mg/kg) coadministered with glycopyrrolate (0.08 mg/kg) caused more muscle twitching than lower doses of physostigmine (0.1–0.2 mg/kg), these movements were distinct from the purposeful movements (e.g., grooming, clawing, and righting attempts) observed with methylphenidate,[9] a D1 agonist,[12] and VTA stimulation[1] during isoflurane anesthesia.

The coadministration of glycopyrrolate with physostigmine did not decrease time to emergence when compared with saline controls and did not cause restoration of righting during continuous isoflurane anesthesia. These findings suggest that the peripheral muscarinic side effects of physostigmine are not the reason for the lack of reanimation observed with physostigmine. The present results are consistent with the report by Reed et al.[28] who found that the combination of physostigmine and glycopyrrolate (at the same doses used in this study) does not restore righting in rats during continuous propofol anesthesia. Because glycopyrrolate does not cross the blood–brain barrier,[14] the presence or absence of glycopyrrolate should not affect the EEG results. Therefore, we did not perform additional EEG experiments with glycopyrrolate.

However, it is likely that physostigmine produced some degree of muscle weakness because of high acetylcholine levels at the neuromuscular junction. Eikermann et al.[29] previously reported that neostigmine dose dependently causes a decrease in electromyography activity in the genioglossus and diaphragm in isoflurane-anesthetized rats. Physostigmine and neostigmine are cholinesterase inhibitors that have similar peripheral effects, so it is reasonable to assume that physostigmine caused some degree of muscle weakness in our study as well, which may have contributed to the prolonged emergence times.

Several studies have suggested that physostigmine may be clinically useful as an arousal promoting agent to reverse the effects of general anesthesia. In dogs, the administration of physostigmine during halothane anesthesia produced a rapid change in EEG to a low-amplitude pattern similar to the awake state, although no behavioral evidence of arousal was noted.[30] In surgical patients, physostigmine reduced postoperative somnolence after halothane anesthesia.[31]

Hudetz et al.[32] reported that the direct administration of neostigmine into the cerebral ventricles of rats during isoflurane anesthesia produced an increase in cross-approximate entropy of the EEG and elicited behavioral signs of arousal, such as spontaneous limb movements and orofacial exploratory movements. Alkire et al.[6] reported that the microinjection of nicotine in the central medial thalamus (CMT) restored righting during continuous sevoflurane inhalation, providing evidence that cholinergic pathways that activate the thalamus induce arousal during general anesthesia. The latter group also showed that microinfusion of an antibody to a voltage-gated potassium channel in the CMT produces similar behaviors,[33] suggesting that the CMT plays an important role in modulating arousal. These studies suggest that the route of administration is important for drugs that stimulate central cholinergic neurotransmission. Because peripheral cholinergic neurotransmission is involved in neuromuscular transmission as well as the parasympathetic nervous system, physostigmine can produce profound peripheral side effects that can be avoided when the drugs are administered in the cerebral ventricles.

In studies involving human volunteers, physostigmine reversed propofol-induced unconsciousness in 9 of 11 subjects[15] and reversed sevoflurane-induced unconsciousness in 5 of 8 subjects.[34] Both of these studies reported that the administration of physostigmine produced significant increases in the auditory steady-state response and bispectral index. In a follow-up study by the same research group,[35] 8 of 11 subjects regained consciousness during propofol anesthesia with physostigmine administration, and positron emission tomography was used to demonstrate that increases in regional cerebral blood flow to the thalamus and precuneus correlated with physostigmine-induced arousal.

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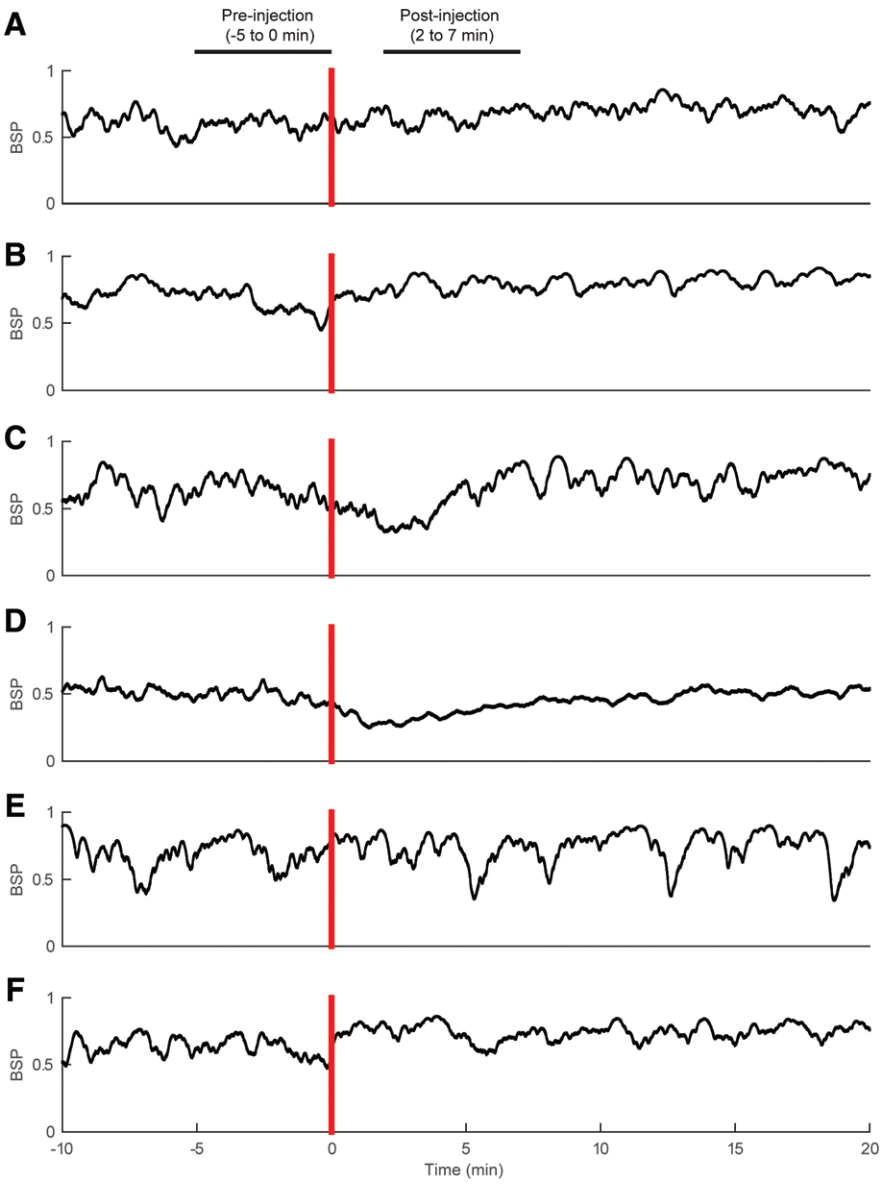


Figure 6. Methylphenidate does not induce significant changes in burst suppression probability (BSP) during continuous inhalation of 2.0% isoflurane. (A–F) The BSP computed for each of 6 rats during continuous inhalation of 2.0% isoflurane, from 10 minutes before methylphenidate (5 mg/kg IV) to 20 minutes after methylphenidate administration. When comparing the "predrug" and "postdrug" periods shown in the figure (indicated by the horizontal bars), methylphenidate did not induce statistically significant changes in BSP in any of the rats.

However, several studies have suggested that physostigmine does not antagonize the effects of general anesthesia. In dogs, physostigmine transiently increased halothane minimum alveolar concentration but then produced a dose-dependent decrease in minimum alveolar concentration,[36] and the authors concluded that physostigmine would not be effective for treating postoperative somnolence after halothane anesthesia. In patients undergoing breast surgery, Paraskeva et al.[37,38] found that physostigmine does not accelerate time to recovery from sevoflurane anesthesia and does not change the bispectral index, even when sevoflurane is used as the sole anesthetic.[17] Röhm et al.[18] found that physostigmine does not accelerate emergence after desflurane anesthesia, and Zvosec et al.[39] reported that physostigmine is not effective for treating γ-hydroxybutyrate intoxication.

During continuous inhalation of 1.0% isoflurane, we found that physostigmine induced a shift in peak EEG power from δ to θ, similar to the results of our previous study with methylphenidate.[9] However, unlike methylphenidate, physostigmine failed to restore righting during isoflurane anesthesia and did not evoke a significant behavioral arousal response. Our findings suggest that both drugs induce neurophysiologic changes that lead to cortical activation during isoflurane anesthesia.

Although it is possible that the observed lack of behavioral arousal with physostigmine may have been because of muscle weakness, as noted earlier, several groups have reported similar dissociations between neurophysiologic and behavioral evidence of arousal. Luo and Leung[7] reported that the microinjection of histamine into the nucleus basalis magnocellularis of rats during deep isoflurane anesthesia caused a reduction in the burst suppression ratio, which did not correlate directly with behavioral evidence of arousal. Our group recently found that atomoxetine, a selective inhibitor of the norepinephrine transporter, induces a shift in spectral power from δ to θ during isoflurane anesthesia but does not elicit signs of behavioral arousal.[11] Conversely, McCarren et al.[27] recently reported that dexmedetomidine microinjected into the ventrolateral preoptic area during isoflurane anesthesia induces behavioral arousal

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but does not elicit spectral changes in the EEG. Similarly, we reported that the D1 agonist 6-Chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine induces behavioral arousal including return of righting during continuous isoflurane anesthesia in rats, but only produces relatively small changes in EEG spectral power.[12]

Interestingly, physostigmine consistently induced a large, statistically significant decrease in BSP during inhalation of 2.0% isoflurane, whereas methylphenidate did not. Burst suppression is an EEG pattern indicating a state of markedly decreased metabolic activity,[40] and the present results suggest that brain metabolic activity is significantly increased when physostigmine is administered during isoflurane anesthesia, whereas methylphenidate has little to no effect in this regard. These differences may be because of the distinct characteristics of cholinergic and monoaminergic arousal circuits in the brain. Future studies with intracranial recordings and circuit-specific manipulations are needed to shed more light on these mechanisms.

In conclusion, the results of this study suggest that methylphenidate is more likely than physostigmine to promote active emergence from general anesthesia. However, physostigmine induces significant EEG changes during burst suppression that are consistent with neurophysiologic antagonism of isoflurane anesthesia. Taken together, the current findings and the results of our previous studies suggest that cholinergic and monoaminergic stimulation produce distinct arousal states during isoflurane general anesthesia.

DISCLOSURES

Name: Jonathan D. Kenny.
Contribution: This author helped conduct the study, analyze the data, and write the manuscript.
Attestation: Jonathan D. Kenny has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Conflicts of Interest: Jonathan D. Kenny declares no conflicts of interest.
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Conflicts of Interest: Jessica J. Chemali has filed a patent application for the use of IV methylphenidate to reverse general anesthesia in surgical patients.
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Conflicts of Interest: Ken Solt has filed a patent application for the use of IV methylphenidate to reverse general anesthesia in surgical patients.
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