ABSTRACT

Background: Although accumulating evidence suggests that arousal pathways in the brain play important roles in emergence from general anesthesia, the roles of monoaminergic arousal circuits are unclear. In this study, the authors tested the hypothesis that methylphenidate (an inhibitor of dopamine and norepinephrine transporters) induces emergence from isoflurane general anesthesia.

Methods: Using adult rats, the authors tested the effect of intravenous methylphenidate on time to emergence from isoflurane general anesthesia. They then performed experiments to test separately for methylphenidate-induced changes in arousal and changes in minute ventilation. A dose–response study was performed to test for methylphenidate-induced restoration of righting during continuous isoflurane general anesthesia. Surface electroencephalogram recordings were performed to observe neurophysiological changes. Plethysmography recordings and arterial blood gas analysis were performed to assess methylphenidate-induced changes in respiratory function. Intravenous droperidol was administered to test for inhibition of methylphenidate’s actions.

Results: Methylphenidate decreased median time to emergence from 280 to 91 s. The median difference in time to emergence without methylphenidate compared with administration of methylphenidate was 200 [155–331] s (median, [95% CI]). During continuous inhalation of isoflurane, methylphenidate induced return of righting in a dose-dependent manner, induced a shift in electroencephalogram power from delta (less than 4 Hz) to theta (4–8 Hz), and induced an increase in minute ventilation. Administration of intravenous droperidol (0.5 mg/kg) before intravenous methylphenidate (5 mg/kg) largely inhibited methylphenidate-induced emergence behavior, electroencephalogram changes, and changes in minute ventilation.

Conclusions: Methylphenidate actively induces emergence from isoflurane general anesthesia by increasing arousal and respiratory drive, possibly through activation of dopaminergic and adrenergic arousal circuits. The authors’ findings suggest that methylphenidate may be useful clinically as an agent to facilitate emergence from general anesthesia.

What We Already Know about This Topic

• Recent evidence suggests that mechanisms involved in emergence from general anesthesia are amenable to selective pharmacological manipulation
• Methylphenidate is active in a number of neurotransmitter systems that are involved in arousal pathways

What This Article Tells Us That Is New

• Methylphenidate reduced time to emergence from isoflurane anesthesia and produced electroencephalographic signs of arousal in rats
• This effect might be used clinically to facilitate emergence from anesthesia
Methylphenidate Induces Emergence from Anesthesia

agent to reverse general anesthetic-induced unconsciousness and respiratory depression at the end of surgery.

GENERAL anesthesia is a reversible coma, actively induced and maintained by administering IV and inhalational drugs.1 In contrast, emergence from general anesthesia has been treated as a passive process whereby anesthetic drugs are merely discontinued at the end of surgery and no drugs are administered to actively reverse their effects on the brain and central nervous system. The timing of emergence can be unpredictable because many factors, including the nature and duration of the surgery and the age, physical condition, and body habitus of the patient, can greatly affect the pharmacokinetics and pharmacodynamics of general anesthetics. Although the actions of many drugs used in anesthesiology are reversed pharmacologically when no longer desired (e.g., muscle relaxants, opioids, benzodiazepines, and anticoagulants), this is not the case for general anesthetic-induced loss of consciousness.

Currently, there is no agent available to actively induce emergence from general anesthesia. This is largely because of our limited knowledge of the molecular mechanisms of general anesthetic actions, which hampers the development of drugs that antagonize the actions of general anesthetics. However, accumulating evidence suggests that ascending arousal pathways in the brain can play important roles in emergence from general anesthesia.2 Although cholinergic,3,4 orexinergic,5 and histaminergic6 arousal pathways have been implicated in emergence, the roles of other arousal pathways remain unknown.

Methylphenidate is prescribed widely for the treatment of attention deficit hyperactivity disorder in children and adults and acts primarily by inhibiting the dopamine and norepinephrine reuptake transporters,7 thereby increasing dopaminergic and adrenergic neurotransmission. Recently, methylphenidate has been reported to increase prefrontal cortex histamine concentrations in rats.8 Dopamine, norepinephrine, and histamine are monoamine neurotransmitters that promote arousal through pathways emanating from nuclei in the pons, midbrain, and hypothalamus.2,9 Therefore, the current study was conducted to test the hypothesis that methylphenidate induces emergence from isoflurane general anesthesia.

We first tested whether methylphenidate affects time to emergence from a standardized general anesthetic with isoflurane. We then investigated two possible mechanisms by which methylphenidate may act: (1) increased arousal or (2) increased minute ventilation. To test for increased arousal, we performed experiments to assess whether methylphenidate induces restoration of righting under continuous isoflurane general anesthesia. We also performed spectral analysis of electroencephalogram recordings to assess changes induced by methylphenidate during continuous isoflurane general anesthesia. To test for increased minute ventilation, we obtained plethysmography and arterial blood gas data to analyze changes in respiratory status induced by methylphenidate.

Materials and Methods

Animal Care and Use

Animal studies were approved by the Subcommittee on Research Animal Care, Massachusetts General Hospital, Boston, Massachusetts. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 351–565 g were used. For the experiments to determine time to emergence, as well as the methylphenidate dose–response studies under continuous isoflurane general anesthesia, experiments were performed using the same 12 rats in random order. Separate groups of animals were used for the electroencephalogram (4 rats), plethysmography (4 rats), and blood gas studies (6 rats). In animals that underwent multiple experiments, 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

Rats were anesthetized in an induction chamber with isoflurane (2–3%) in oxygen before placement of a lateral tail vein IV catheter (24 gauge, 19 mm). A rectal temperature probe was inserted, and the animal was placed in a cylindrical acrylic anesthetizing chamber. The chamber was custom-built and equipped with ports for anesthetic gas delivery, sampling, and scavenging, as well as IV drug administration. A heating pad was placed under the chamber to keep the animal warm, and the body temperature was kept between 36.5°C and 37.4°C.

The volume of the chamber was 4.6 l. Initially, the chamber was primed with isoflurane at a fresh gas flow rate of 2–3 l/min, and then the rate was decreased to 1–2 l/min. The carrier gas was oxygen. Gas was continuously sampled from the distal portion of the chamber (opposite from the fresh gas inlet), and isoflurane, oxygen, and carbon dioxide concentrations in the chamber were monitored using a calibrated Ohmeda 5250 anesthetic agent analyzer (GE Healthcare, Waukesha, WI).

Preparation and Delivery of Drugs

Isoflurane, methylphenidate hydrochloride, and droperidol were purchased from Henry Schein (Melville, NY), Sigma–Aldrich (St. Louis, MO), and American Regent (Shirley, NY), respectively. Normal saline, methylphenidate, and droperidol were always administered intravenously. Methylphenidate was weighed, dissolved in 0.5 ml normal saline, and filtered in a sterile manner immediately before administration. Droperidol was diluted in normal saline to a final volume of 0.5 ml before administration. The IV tubing (approximate volume 0.6 ml) was always flushed with 2 ml normal saline after methylphenidate or droperidol to ensure complete delivery of drug.
**Time to Emergence after a Standardized Isoflurane General Anesthetic**

To test the hypothesis that methylphenidate decreases time to emergence from a standardized isoflurane anesthetic, an endpoint that has been used in several recent studies of anesthetic emergence, the inhaled concentration of isoflurane was fixed at 1.5% (~1 minimum alveolar concentration). After 40 min, rats received either normal saline or IV methylphenidate (5 mg/kg). Isoflurane was continued for 5 additional min, then the rat was taken out of the chamber, and the temperature probe was 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 (i.e., all four paws touching the floor).

**Administration of Methylphenidate during Continuous Isoflurane General Anesthesia**

The rat was positioned supine in the anesthetizing chamber, and the inhaled concentration of isoflurane was initially fixed at 2.0% for 20 min, then reduced to 0.8% over 15–20 min, and then maintained at 0.8% for 40 min. If the rat made any purposeful movement, the isoflurane concentration was increased by 0.1% and maintained for another 40 min. This process was repeated until the final dose of isoflurane sufficient to maintain loss of righting reflex was established, and this dose was administered throughout the remainder of the experiment. This protocol was based on previously published methods described by Alkire et al.

After the final 40-min equilibration period, normal saline (2 ml) was administered, and the rectal temperature probe was removed. Methylphenidate was administered 5 min later. To establish a dose–response relationship, we administered three different doses of IV methylphenidate (0.05 mg/kg, 0.5 mg/kg, or 5 mg/kg) on different days. After administration of methylphenidate, each animal continued to inhale the same dose of isoflurane for 30 min or until restoration of righting occurred.

**Electroencephalogram Electrode Placement, Recording, and Spectral Analysis**

Extradural electroencephalogram electrodes were implanted surgically at least 7 days before recording. General anesthesia was induced with intraperitoneal xylazine (5–10 mg/kg) and intraperitoneal ketamine (50–100 mg/kg), and supplemented with isoflurane (1–2%). A microdrill was used to make four holes at the following stereotactic coordinates: A0L0, A6L3, A6 l-3, and A10L2 relative to the lambda. Polytetrafluoroethylene-coated, 200-μm diameter stainless steel electrode wires (A-M Systems, Sequim, WA) were inserted and secured with small stainless steel screws and permanently fixed with dental acrylic cement. Carprofen (5 mg/kg subcutaneous) was administered for analgesia on the day of surgery and on postoperative days 1 and 2.

The potential difference between electrodes A0L0 and A6L3 or between electrodes A0L0 and A6 l-3 (whichever gave less motion artifact) 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). Data were filtered between 0.3 and 100 hertz (Hz). No line filter was used. The sampling rate was 512 Hz.

Baseline recordings were taken for 10 min while the rats were awake; rats then were anesthetized with isoflurane and placed in the anesthetizing chamber. Although we initially attempted to perform the electroencephalogram experiments simultaneously with the behavioral experiments described, we found that the righting attempts produced too many motion artifacts. Thus, we performed the electroencephalogram experiments with the rats in the prone position with the isoflurane dose fixed at 1.0%. These modifications allowed us to minimize electroencephalogram motion artifacts without restraining the animals. After rats had a minimum isoflurane exposure of 40 min, we administered normal saline and removed the temperature probe. Five minutes later, we administered methylphenidate.

Spectral analysis was performed using Matlab 7.11 (Mathworks, Natick, MA) and the Chronux software (Cold Spring Harbor, NY). Spectrograms were calculated using sliding windows of 2-s duration stepped through 0.05 s. For each window, multitaper spectrum estimation was performed using five tapers. The resulting spectral estimates have a bandwidth of ± 1.5 Hz. Mean power spectra were compared before and after methylphenidate administration using Kolmogorov-Smirnov tests. To determine the difference between two spectra, a two-sample Kolmogorov-Smirnov test was performed on the spectral power as a function of frequency computed from the 30 windows in the pre- and postmethylphenidate periods. We used a Bonferroni correction to adjust the significance level for multiple hypothesis testing.

**Plethysmography**

Rats were placed in a custom-built plethysmography chamber, and the isoflurane concentration in the chamber was maintained at 1.5%. After the rats underwent equilibration in the chamber for 30 min, normal saline or IV droperidol (0.5 mg/kg) was administered 5 min before IV methylphenidate (5 mg/kg). Because plethysmography recordings are sensitive to motion artifacts, we used a higher isoflurane dose (1.5% or ~1 minimum alveolar concentration) because animals at this dose of isoflurane did not exhibit purposeful movements after methylphenidate was administered.

A differential pressure transducer and demodulator (Models CD15 and MP45–14–871; Validyne Engineering, Northridge, CA) were used to convert the chamber pressure to an analog signal. The signal was high-pass filtered at 15 s, acquired at 100 Hz, and analyzed in 4-s epochs using a USB-6009 data acquisition board (National Instruments) and LabView Software (version 8.5 for Macintosh, National Instruments). Chamber carbon dioxide levels were maintained at or less.
than 0.5% in the open-flow configuration. A heating pad was used to warm the rat from beneath. Chamber air temperature and relative humidity were measured with a thermometer-hygrometer (Fisher Scientific, Pittsburgh, PA) and used to estimate tidal volumes during intermittent chamber closure using methods described by Drorbaugh and Fenn.16

**Arterial Blood Gas and Hemodynamic Recordings**

Rats with femoral artery catheters (Charles River Laboratories) were placed in the anesthetizing chamber after lateral tail vein IV catheters were placed. The isoflurane dose was kept constant at 1.5%. Mean arterial blood pressure and heart rate were measured using a pressure transducer (TruWave, Edwards Life Sciences, Irvine, CA) interfaced with a custom-built amplifier (AD620 operational amplifier; Jameco Electronics, Belmont, CA). The signal was digitized at 1,000 Hz using a USB-6009 data acquisition board (National Instruments) and analyzed in 4-s epochs. A premethylphenidate arterial blood sample was drawn after at least 30 min of equilibration in isoflurane (1.5%), and a postmethylphenidate sample was drawn 15 min after methylphenidate administration. Samples were analyzed promptly using CG4+ cartridges in a Vetscan iStat 1 (Abaxis, Union City, CA) blood gas analyzer.

**Statistical Analysis of the Effects of Methylphenidate on Emergence Times, Return of Righting Responses, and Spectrograms**

Prism 4.03 (Graphpad Software, San Diego, CA) and Matlab (Mathworks, Natick, MA) were used for statistical analysis, and when possible, results are reported in terms of 95% CI based on Z-tests, t tests, or Mann–Whitney tests. We used a Bayesian Monte Carlo procedure to compute Bayesian 95% CI (credibility intervals) to assess the effect of methylphenidate dose on return of righting during continuous isoflurane general anesthesia.17 For this computation, we assumed a binomial model as the sampling density or likelihood function for the propensity of animals in a given group to have return of the righting response. We took as the prior density for each group the uniform density on the interval (0, 1) because it is uninformative. Because this uniform density is a conjugate prior density for the beta density, that is, this prior density is proportional to the likelihood function of the binomial model, the posterior density for each group is a beta density.17 The posterior densities for the differences in the proportion of animals that had return of righting were then computed by using standard Matlab simulation procedures. Instead of P values for the Bayesian analyses, we computed the posterior probability that the propensity to right was greater in one group than in the other.

A one-way ANOVA was used to assess whether there were significant differences among the final isoflurane doses of animals that received the three different doses of methylphenidate. To provide a conservative check on the assessments made by the 95% CI, the parametric tests and nonparametric tests were also used to assess statistical significance. The Mann–Whitney test was used to test the hypothesis that methylphenidate hastens time to emergence from isoflurane general anesthesia and to test the dose dependence of methylphenidate on time to righting during continuous isoflurane general anesthesia. A paired t test was used to test the hypothesis that methylphenidate produces a respiratory alkalosis during isoflurane general anesthesia. We used the two-sided Kolmogorov-Smirnov test with a Bonferroni correction to compare spectra in animals before and after methylphenidate administration. We considered a result to be statistically significant based on the 95% CIs comparing two groups if zero was not in the interval, based on hypothesis tests if the P values were <0.05 or in the case of the Bayesian analyses, if the relevant posterior probability was greater than 0.95.

**Statistical Analysis of the Effects of Methylphenidate on Respiratory Rate, Mean Arterial Blood Pressure, and Heart Rate**

To estimate the effect of methylphenidate on respiratory and cardiovascular variables, we performed within-animal analyses because we had sufficient samples to estimate the mean of each variable and its SE before and after drug administration for each animal. To do so, we performed time-series modeling of these measurements to take account of their serial dependence and thereby compute appropriate estimates of variance for within-animal, two-sample Z-tests. That is, we fit different autoregressive models of order p with a nonzero mean to the data before and after the administration of methylphenidate. Because these models have nonzero means, we devised an approximate maximum likelihood parameter estimation algorithm using cyclic descent.18 Within the cyclic descent algorithm, we used the least-squares algorithm to estimate the autoregressive parameters and conditional maximum likelihood estimation to compute the mean and variance parameters.14 The cyclic descent algorithm iterated between the least-squares and the conditional maximum likelihood procedures until convergence was achieved.

We allowed p, the order of the autoregressive model, to be different for each segment. We chose the model order on each segment by using Akaike’s Information Criterion.14 We computed the approximate standard errors of the parameters by estimating the parameter covariance matrix as the inverse of the observed Fisher information matrix.19 By design, these standard errors of the parameters take account of the serial dependence in the data. The estimated mean and SEM were used to compute the 95% CI for the difference between the physiologic variable before and after methylphenidate based on a Z-statistic.

**Results**

**Methylphenidate Hastens Time to Emergence from a Standardized Isoflurane Anesthetic**

Figure 1A provides a schematic of the protocol for this experiment. As shown in figure 1B, the median time to emer-
Methylphenidate Induces Emergence during Continuous Inhalation of Isoflurane

To test the hypothesis that methylphenidate increases arousal, we performed the following experiments on rats undergoing continuous inhalation of isoflurane. Because isoflurane was not discontinued, any emergence mechanism involving accelerated isoflurane excretion would not be possible. At the start of these experiments (fig. 2A), the minimum concentration of inhaled isoflurane sufficient to maintain loss of righting was established for each rat (see Materials and Methods for details), and this dose was continuously delivered to the chamber throughout the experiment. The final dose of isoflurane was 0.9% ± 0.1% (mean ± SD). After equilibration, none of the animals exhibited purposeful movement in response to IV injection of normal saline or removal of the temperature probe, indicating that mild stimulation did not produce arousal at this depth of anesthesia.

Five minutes after normal saline was administered, methylphenidate was administered. At the maximum dose of 5 mg/kg, purposeful movements (e.g., lifting of the head, opening of the eyes, twisting of the torso, kicking, clawing, chewing, licking, and grooming) were observed within 30 s for all 12 rats, despite continuous inhalation of isoflurane at the same, fixed dose. All of the rats remained active and continued to move about in the chamber after righting. Per our animal protocol, we concluded the experiment after the righting reflex was restored.

As shown in figure 2B, return of righting occurred in 11 of 12 rats after administration of methylphenidate at this dose. Return of righting also occurred in 11 of 12 rats after administration of a 10-fold lower dose (0.5 mg/kg IV), but there were no signs of arousal in any of the 6 rats that received 0.05 mg/kg. The Bayesian 95% CI for the difference in the propensities to have return of righting between rats in the 5 mg/kg methylphenidate group and those in the 0.05 mg/kg methylphenidate group was 0.39–0.94. The Bayesian 95% CI for the difference in the propensities to have return of righting between rats in the 0.5 mg/kg methylphenidate group and those in the 0.05 mg/kg methylphenidate group was 0.40–0.94. For both comparisons, the posterior probability of the difference being greater than zero was 0.999, indicating that both differences are highly significant.

As shown in figure 2C, the median time to righting after methylphenidate administration during continuous inhalation of isoflurane was 181 s for rats that received 5 mg/kg and 348 s for rats that received 0.5 mg/kg. The median difference in time to righting during continuous inhalation of isoflurane between these two groups was 173 s, with a 95% CI computed using the Mann–Whitney test of 50–332 s. This median difference was statistically significant (P < 0.0001, two-sided Mann–Whitney test).

As shown in figure 2D, the median time to righting after methylphenidate administration during continuous inhalation of isoflurane was 181 s for rats that received 5 mg/kg and 348 s for rats that received 0.5 mg/kg. The median difference in time to righting during continuous inhalation of isoflurane between these two groups was 173 s, with a 95% CI computed using the Mann–Whitney test of 50–332 s. This median difference was statistically significant (P = 0.01, two-sided Mann–Whitney test). There was no statistically
significant difference in the final isoflurane dose among the animals that received the three different doses of methylphenidate ($P = 0.3$, F test for one-way ANOVA).

**Droperidol Inhibits Methylphenidate-induced Emergence Behavior**

In a group of animals ($n = 6$) continuously inhaling isoflurane at a dose sufficient to maintain loss of righting as above, the protocol illustrated in figure 2A was repeated with the exception that IV droperidol (0.5 mg/kg) was administered in place of normal saline. None of the animals exhibited purposeful movement in response to the administration of droperidol or subsequent removal of the temperature probe. Five minutes after droperidol administration, the highest dose of methylphenidate used in this study (5 mg/kg) was administered. These animals generally exhibited no purposeful movement after methylphenidate administration, although some sluggish limb movements were observed occasionally. None of these animals had return of righting, compared with the 11 of 12 animals that had return of righting after receiving normal saline before the same dose of methylphenidate (fig. 2D). The 95% Bayesian CI for the difference in righting propensity between these two conditions is 0.39 – 0.94. The posterior probability that the propensity to right for those that received saline was greater than that for those that received droperidol was 0.999, indicating a highly significant difference.

**Droperidol Inhibits Methylphenidate-induced Electroencephalogram Changes during Continuous Inhalation of Isoflurane**

Electroencephalogram data were recorded from rats with preimplanted extradural skull electrodes ($n = 4$). Results from an individual rat are shown in figure 3A. In the awake state before the administration of any drugs, animals showed an active-high-frequency, low-amplitude electroencephalogram pattern, which changed to a low-frequency, high-amplitude pattern during continuous inhalation of isoflurane (1.0%). Although the electroencephalogram pattern did not change after injection of normal saline or removal of the
temperature probe, the administration of IV methylphenidate (5 mg/kg) induced a prompt shift within 30 s to an active high-frequency, low-amplitude pattern similar to that observed during the awake state. This change persisted for more than 15 min. Figure 3B shows 30-s epochs of raw electroencephalogram recordings from a single animal that received IV droperidol (0.5 mg/kg) 5 min before IV methylphenidate (5 mg/kg) administration. After droperidol administration, methylphenidate did not induce electroencephalogram changes consistent with arousal.

To assess changes in electroencephalogram power over time, spectrograms were computed from the continuous electroencephalogram data recorded from each animal. Typical results from individual rats are shown in figure 4. When the rats were in an awake state (fig. 4A), electroencephalogram power was mainly in the theta frequency range (4–8 Hz). However, continuous inhalation of isoflurane (1.0%) (fig. 4B) caused a large increase in delta power (less than 4 Hz). Although IV injection of normal saline produced no appreciable change in the power spectrum, the administration of IV methylphenidate (5 mg/kg) produced a prompt shift in power from delta to theta. However, when IV droperidol (0.5 mg/kg) was administered instead of normal saline, methylphenidate failed to induce these electroencephalogram changes (fig. 4C).

Figure 5 shows spectrograms and power spectra with the results of the Kolmogorov-Smirnov test computed from 2-min time windows before and after methylphenidate administration. At a significance level of 0.05, the two-sided Kolmogorov-Smirnov test with Bonferroni correction rejects the null hypothesis at all frequencies except those marked with white squares. In four rats that received normal saline (fig. 5A), IV methylphenidate (5 mg/kg) induced a rapid shift in peak power from delta to theta, and the difference in power before and after methylphenidate administration was statistically significant at most frequencies between 0 and 10 Hz (two-sided Kolmogorov-Smirnov test, \( P < 0.05 \)). However, as shown in figure 5B, four rats that received IV droperidol (0.5 mg/kg) before methylphenidate administration had only small, statistically significant decreases in delta power (two-sided Kolmogorov-Smirnov test, \( P < 0.05 \)), and the shift in peak power from delta to theta was absent.

**Methylphenidate Induces an Increase in Minute Ventilation That Is Inhibited by Droperidol**

As demonstrated in the representative result shown in figure 6A, methylphenidate (5 mg/kg) induced a substantial increase in respiratory rate during continuous inhalation of isoflurane (1.5%). At this dose of isoflurane, purposeful movements consistent with arousal were not
induced by methylphenidate. Within-animal analysis demonstrated that methylphenidate induced a statistically significant increase in respiratory rate for each animal that ranged from 10 to 51 breaths/min (table 2, two-sided Z-test within-animal corrected for serial correlation, all \( P < 10^{-16} \)). Although two of the four rats had statistically significant changes in tidal volume, the changes were small and inconsistent.

As demonstrated in the typical result shown in figure 6B, when IV droperidol (0.5 mg/kg) was administered instead of normal saline 5 min before IV methylphenidate (5 mg/kg), there was only a negligible increase in respiratory rate. Within-animal analysis revealed that methylphenidate produced a statistically significant increase in respiratory rate in each of these animals that ranged from 2 to 4 breaths/min (table 2, two-sided Z-test within-animal corrected for serial correlation, all \( P < 0.0001 \)). However, those increases were appreciably smaller than the 10–51 breaths/min increases observed in the animals that were pretreated with normal saline. Although all four rats had statistically significant changes in tidal volume, the changes were small (4–17%) and inconsistent (one had an increase, whereas the other three had decreases).

**Methylphenidate Induces a Significant Respiratory Alkalosis and Small Hemodynamic Changes during Isoflurane General Anesthesia**

As shown in table 3, during continuous isoflurane general anesthesia there were statistically significant changes in arterial pH and PaCO\(_2\) after the administration of methylphenidate. Assuming no change in baseline metabolism, the calculated increase in alveolar ventilation (VA) was 24 ± 6% using the relationship:

\[
VA_{\text{post}}/VA_{\text{pre}} = (P_{CO2})_{\text{pre}}/(P_{CO2})_{\text{post}}
\]

where “pre” and “post” denote premethylphenidate and postmethylphenidate, respectively. The slight increase in PaO\(_2\) after methylphenidate was not statistically significant (two-sided paired \( t \) test, \( P = 0.14 \)).

Within-animal analyses showed that four animals had statistically significant increases in mean arterial blood pressure (3–20 mmHg), whereas two had no significant change (table 4). Four animals had insignificant increases in heart rate, and two had small but statistically significant increases (6 and 15 beats/min) in heart rate (table 5).

**Discussion**

In this study, we found that methylphenidate actively induces emergence from isoflurane general anesthesia by increasing arousal. In addition, our plethysmography and blood gas experiments revealed that methylphenidate increases minute ventilation, which increases the rate of anesthetic elimination from the brain.\(^20\) Emergence from isoflurane general anesthesia is dose-dependent,\(^21\) therefore methylphenidate-induced ventilatory stimulation likely contributes to accelerating time to emergence.

Our protocol for testing loss of righting reflex did not use a rotating anesthetizing chamber, and the average dose of isoflurane required to maintain loss of righting in our study was 0.9%. This dose was slightly higher than the dose reported previously for loss of righting in uninstrumented mice using a rotating anesthetizing chamber (isoflurane [0.81%], with a 95% CI between 0.78% and 0.84%).\(^1\) The stimula-
tion provided by the temperature probe and the IV catheter in our rats was likely comparable with the stimulation provided by the rotating anesthetizing chamber in uninstrumented mice.

Electroencephalogram and plethysmography studies were performed separately from behavioral experiments with some modifications in the experimental protocols designed to minimize motion artifacts. Electroencephalogram studies performed under experimental conditions similar to that of the behavioral studies demonstrated a consistent shift from delta to theta power within 30 s of methylphenidate administration. These results agree with a previous study that found methylphenidate induces a theta rhythm in rats anesthetized with chloral hydrate.22 The plethysmography experiments performed at a higher dose of isoflurane (1.5%, or approximately 1 minimum alveolar concentration) demonstrated increases in respiratory rate and minute ventilation. It is reasonable to conclude that these changes are similar to the changes that would have been observed in animals that regained the righting reflex in the behavioral studies.

Cholinergic arousal pathways have been studied most extensively in the context of emergence from general anesthesia. Hudetz et al.3 showed that intraventricular administration of the cholinesterase inhibitor neostigmine to rats during isoflurane general anesthesia produced an increase in cross-approximate entropy of the electroencephalogram and elicited behavioral signs of arousal, such as spontaneous limb movements and orofacial explorative movements. Alkire et al.4 showed that injection of nicotine into the central medial thalamus induced return of righting during continuous inhalation of sevoflurane, providing evidence for cholinergic pathways that activate the thalamus inducing arousal from general anesthesia. In patients, physostigmine has been reported to reduce postoperative somnolence after halothane general anesthesia.23 In studies involving human volunteers, physostigmine reversed propofol-induced loss of consciousness in 9 of 11 subjects24 and reversed sevoflurane-induced loss of consciousness in 5 of 8 subjects.25 Both of these studies reported that administration of physostigmine produced significant increases in auditory steady-state response and

### Table 1. Respiratory Rate (breaths/min) in Individual Animals Pretreated with Normal Saline during Isoflurane General Anesthesia

<table>
<thead>
<tr>
<th>Animal</th>
<th>RR before methylphenidate (mean, [95% CI])</th>
<th>RR after 5 mg/kg IV methylphenidate (mean, [95% CI])</th>
<th>Change in mean RR (mean, [95% CI])</th>
<th>Z-statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.4 [81.5–85.4]</td>
<td>112.6 [109.5–115.7]</td>
<td>+29.2 [25.5 to +32.9]</td>
<td>15.9</td>
<td>&lt;10⁻¹⁶</td>
</tr>
<tr>
<td>2</td>
<td>84.3 [83.5–85.2]</td>
<td>94.2 [93.4–95.0]</td>
<td>+9.8 [8.7 to +11.0]</td>
<td>16.6</td>
<td>&lt;10⁻¹⁶</td>
</tr>
<tr>
<td>3</td>
<td>103.5 [98.4–108.5]</td>
<td>153.8 [150.5–157.2]</td>
<td>+50.36 [44.3 to +56.4]</td>
<td>16.7</td>
<td>&lt;10⁻¹⁶</td>
</tr>
<tr>
<td>4</td>
<td>97.8 [96.3–99.3]</td>
<td>116.7 [114.0–119.4]</td>
<td>+18.9 [15.8 to +21.9]</td>
<td>12.3</td>
<td>&lt;10⁻¹⁶</td>
</tr>
</tbody>
</table>

CI = confidence interval; IV = intravenous; RR = respiratory rate.
Orexinergic arousal pathways have been shown to play an important role in emergence from general anesthesia. Orexins are arousal-promoting peptide neurotransmitters released by neurons in the perifornical area of the hypothalamus, and abnormal orexinergic signaling leads to narcolepsy.\textsuperscript{26,27} Mesa et al.\textsuperscript{28} reported that a patient with narcolepsy underwent three different operations between 1979 and 1995 and required 8–10 h to emerge from general anesthesia each time. Kelz et al.\textsuperscript{29} demonstrated in mice that genetic and pharmacologic ablation of orexinergic signaling delays time to righting after discontinuation of isoflurane or sevoflurane general anesthesia. The inhaled anesthetic concentration required to produce loss of righting was unchanged in orexin-ablated mice compared with wild-type mice, indicating that orexinergic neurons are not involved in the mechanisms underlying induction of general anesthesia. In a separate study by Zecharia et al.,\textsuperscript{30} intraventricular administration of orexin-A was shown to reduce time to righting after propofol and dexmedetomidine administration.

Although there are several monoaminergic arousal pathways, they have been less well studied in the context of emergence from general anesthesia. Luo and Leung\textsuperscript{6} recently reported that injection of histamine into the basal forebrain of rats decreased time to righting after isoflurane general anesthesia, increased respiratory rate, and shifted electroencephalogram activity from a burst suppression pattern ("deep" general anesthesia) to a \( \delta \) pattern ("lighter" general anesthesia). Their results suggest that enhanced histaminergic neurotransmission in the basal forebrain also may play a role in emergence from general anesthesia.

Decades ago, the clinical utility of methylphenidate as an analeptic (\textit{i.e.}, a central nervous system stimulant) was explored in psychiatry and anesthesiology.\textsuperscript{30} However, at that time, its mechanism of action was unknown. Psychiatrists reported using the drug to promote arousal in patients experiencing overdose of antipsychotic medications\textsuperscript{31} and to facilitate psychiatric interviewing.\textsuperscript{32} Studies conducted in the perioperative period suggested that methylphenidate promoted faster recovery after general anesthesia.\textsuperscript{33,34} However, the only placebo-controlled, double-blind study reported no difference in recovery time.\textsuperscript{35} Before the United States Food and Drug Administration’s black box warning on droperidol in 2001, the drug was widely used in anesthesiology practices as a sedative and antiemetic. Our current findings suggest that the widespread use of droperidol may have confounded the results of some of the previous clinical studies of methylphenidate. However, because methylphenidate is widely prescribed to treat attention deficit hyperactivity disorder, there are recent reports of increased anesthetic requirements in patients who take methylphenidate.\textsuperscript{36,37} These reports are consistent with our finding that methylphenidate antagonizes isoflurane general anesthesia.

Methylphenidate is known to inhibit dopamine and norepinephrine transporters with similar affinities (\( K_i = 250 \text{ nM} \) and 150 nM, respectively).\textsuperscript{7} Dopaminergic neurons in the ventral tegmental area and substantia nigra pars compacta promote arousal and play an important role

### Table 2. Respiratory Rate (breaths/min) in Individual Animals Pretreated with IV Droperidol (0.5 mg/kg) during Isoflurane General Anesthesia

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR before methylphenidate (mean, [95% CI])</td>
<td>81.9 [80.7–83.0]</td>
<td>75.8 [75.2–76.4]</td>
<td>80.5 [79.8–81.2]</td>
<td>70.6 [69.2–72.0]</td>
</tr>
<tr>
<td>RR after 5 mg/kg IV methylphenidate (mean, [95% CI])</td>
<td>86.3 [85.8–86.8]</td>
<td>78.0 [77.1–78.9]</td>
<td>84.1 [83.4–84.9]</td>
<td>75.5 [74.9–76.1]</td>
</tr>
<tr>
<td>Change in mean RR (mean, [95% CI])</td>
<td>+4.4 [3.2 to +5.7]</td>
<td>+2.2 [1.1 to +3.3]</td>
<td>+3.6 [2.6 to +4.6]</td>
<td>+4.9 [3.3 to +6.4]</td>
</tr>
<tr>
<td>Z-statistic</td>
<td>7.0</td>
<td>4.1</td>
<td>7.0</td>
<td>6.3</td>
</tr>
<tr>
<td>( P ) value (paired ( t ) test)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

CI = confidence interval; IV = intravenous; RR = respiratory rate.

### Table 3. Arterial Blood Gas Analysis during Isoflurane General Anesthesia (\( n = 6 \))

<table>
<thead>
<tr>
<th></th>
<th>pH (mean, [95% CI])</th>
<th>( \text{Paco}_2 ) (mmHg)</th>
<th>( \text{Pao}_2 ) (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before methylphenidate (mean, [95% CI])</td>
<td>7.45 [7.41–7.50]</td>
<td>43 [40–47]</td>
<td>226 [200–252]</td>
</tr>
<tr>
<td>After 5 mg/kg IV methylphenidate (mean, [95% CI])</td>
<td>7.51 [7.46–7.55]</td>
<td>35 [32–38]</td>
<td>241 [211–270]</td>
</tr>
<tr>
<td>( P ) value (paired ( t ) test)</td>
<td>0.004</td>
<td>0.0001</td>
<td>0.144</td>
</tr>
</tbody>
</table>

CI = confidence interval; IV = intravenous.
in cognition and reward through projections to the thalamus, basal forebrain, nucleus accumbens, cortex, lateral dorsal tegmentum, and locus ceruleus. A third cluster of wake-active, dopaminergic neurons was identified recently in the ventral periaqueductal gray area. There is recent evidence that enhancement of dopaminergic neurotransmission increases ventilatory drive in cats, which suggests that a dopaminergic mechanism may also play a role in the methylphenidate-induced increase in alveolar ventilation.

Noradrenergic neurons in the locus ceruleus promote arousal through projections to the thalamus, basal forebrain, preoptic areas, and cortex, and their inhibition is important in the sedative actions of propofol and dexmedetomidine. In addition, arousal-promoting histaminergic neurons arising from the tuberomammillary nucleus may play a role in the actions of methylphenidate, although the mechanisms underlying this pathway have not been clearly defined. Thus, methylphenidate likely induces emergence by enhancing arousal-promoting monoaminergic (i.e., dopaminergic, noradrenergic, and possibly histaminergic) neurotransmission.

We found that administration of droperidol inhibits both the arousal-promoting effects and the increase in alveolar ventilation induced by methylphenidate during isoflurane general anesthesia. It has been reported that 3 mg/kg droperidol has no effect on isoflurane potency in rats, and that the EC_{50} for loss of righting in mice is 40 mg/kg, suggesting that our relatively modest rodent dose of 0.5 mg/kg had little impact on the anesthetic state of the animals in our study. This conjecture is further supported by the lack of electroencephalogram changes observed in our animals after droperidol administration. Droperidol is known primarily as an antagonist at D2 dopamine receptors, but it has also been shown to inhibit α1-adrenergic receptors. Therefore our results with droperidol are consistent with the notion that dopaminergic and noradrenergic neurotransmission play important roles in methylphenidate-induced emergence. Additional studies will be needed to elucidate which arousal pathways are responsible for the specific actions of methylphenidate, although it is likely that the simultaneous activation of multiple monoaminergic arousal pathways contributes to its efficacy.

Because the molecular mechanisms underlying the actions of general anesthetics are only now becoming more clearly understood, it has not been possible to design antagonists of general anesthetics. However, our results suggest that methylphenidate actively induces emergence from isoflurane general anesthesia by stimulating monoaminergic arousal pathways. These results demonstrate a novel approach for identifying clinically useful antagonists of general anesthetics at the level of neural circuits. Methylphenidate has a well-established safety record in children and adults, through its more than 2 decades of use in the treatment of attention deficit hyperactivity disorder. Our findings suggest that IV methylphenidate could be used in adult and pediatric patients at the conclusion of surgery to reverse general anesthetic-induced unconsciousness and aid in the recovery of cognitive function.

Table 4. Mean Arterial Pressure (mmHg) in Individual Animals during Isoflurane General Anesthesia

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP before methylphenidate (mean, [95% CI])</td>
<td>96.6 [96.3–96.9]</td>
<td>82.6 [81.7–83.5]</td>
<td>88.1 [87.7–88.4]</td>
<td>86.7 [86.1–87.2]</td>
<td>100.2 [99.7–100.8]</td>
<td>83.4 [82.7–84.1]</td>
</tr>
<tr>
<td>MAP after 5 mg/kg IV methylphenidate (mean, [95% CI])</td>
<td>117.1 [116.1–118.0]</td>
<td>90.9 [90.0–91.7]</td>
<td>91.3 [91.0–91.5]</td>
<td>85.2 [85.0–85.5]</td>
<td>100.7 [100.3–101.2]</td>
<td>90.8 [89.4–92.2]</td>
</tr>
<tr>
<td>Change in MAP (mean, [95% CI])</td>
<td>+20.5 [+19.5 to +21.5]</td>
<td>-8.3 [-7.1 to -9.5]</td>
<td>+3.2 [+2.8 to +3.7]</td>
<td>-1.4 [-2.0 to -0.8]</td>
<td>+0.5 [-0.2 to +1.2]</td>
<td>+7.4 [+5.8 to +9.0]</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;10^{-16}</td>
<td>&lt;10^{-16}</td>
<td>&lt;10^{-16}</td>
<td>1</td>
<td>0.07</td>
<td>&lt;10^{-16}</td>
</tr>
</tbody>
</table>

CI = confidence interval; IV = intravenous; MAP = mean arterial pressure.

Table 5. Heart Rate (beats/min) in Individual Animals during Isoflurane General Anesthesia

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR before methylphenidate (mean, [95% CI])</td>
<td>401.0 [400.4–401.7]</td>
<td>390.5 [389.8–391.2]</td>
<td>351.7 [351.2–352.1]</td>
<td>359.3 [357.9–360.6]</td>
<td>357.6 [357.1–358.2]</td>
<td>361.3 [360.4–362.1]</td>
</tr>
<tr>
<td>HR after 5 mg/kg IV methylphenidate (mean, [95% CI])</td>
<td>386.8 [385.3–388.4]</td>
<td>383.3 [382.3–384.4]</td>
<td>365.5 [365.2–365.9]</td>
<td>360.0 [358.7–361.3]</td>
<td>364.0 [363.0–365.0]</td>
<td>358.6 [357.8–359.5]</td>
</tr>
<tr>
<td>Change in HR (mean, [95% CI])</td>
<td>-14.2 [-15.9 to -12.5]</td>
<td>-7.2 [-8.4 to -5.9]</td>
<td>+13.9 [+13.3 to +14.5]</td>
<td>+0.72 [-1.19 to +2.64]</td>
<td>+6.4 [+5.3 to +7.5]</td>
<td>-2.6 [-3.8 to -1.4]</td>
</tr>
<tr>
<td>P value</td>
<td>1</td>
<td>&lt;10^{-16}</td>
<td>0.2236</td>
<td>&lt;10^{-16}</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

CI = confidence interval; HR = heart rate; IV = intravenous.
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ANESTHESIOLOGY REFLECTIONS

Haynes’ Arabian Balsam

One of America’s more unusual “analgesic” remedies, Haynes’ Arabian Balsam (above) was “entered according to act of Congress, in the year 1850, by A. Haynes, M.D.” Whether consumed in small doses internally or larger volumes externally as a liniment, the Balsam was confirmed by federal chemists to be nothing more than “a mixture of cottonseed oil, turpentine and oil of cumin.” In 1916, the company was fined $20 after pleading no contest in court to fraudulently advertising its Balsam as a remedy for blindness, croup, deafness, diphtheria, erysipelas, piles, and rheumatism. So, given its name, was Haynes’ Arabian Balsam originally a Saudi salve? Absolutely not. Rather, consider how Hayes’ analgesic was orally dosed thrice daily for treating common colds: 1) a half teaspoon for men or 2) 1 fluid ounce for horses, or at least for Arabian horses. (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

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