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Methylphenidate Actively Induces Emergence from General Anesthesia

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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 we tested the hypothesis that methylphenidate (an inhibitor of dopamine and norepinephrine transporters) induces emergence from isoflurane anesthesia.

Methods—Using adult rats we tested the effect of methylphenidate IV on time to emergence from isoflurane anesthesia. We 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 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. Droperidol IV 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 compared to with methylphenidate was 200 [155, 331] s

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(median, [95% confidence interval]). During continuous inhalation of isoflurane, methylphenidate induced return of righting in a dose-dependent manner, induced a shift in electroencephalogram power from delta to theta, and induced an increase in minute ventilation. Administration of droperidol (0.5 mg/kg IV) prior to methylphenidate (5 mg/kg IV) largely inhibited methylphenidate-induced emergence behavior, electroencephalogram changes, and changes in minute ventilation.

Conclusions—Methylphenidate actively induces emergence from isoflurane anesthesia by increasing arousal and respiratory drive, possibly through activation of dopaminergic and adrenergic arousal circuits. Our findings suggest that methylphenidate may be clinically useful as an agent to reverse general anesthetic-induced unconsciousness and respiratory depression at the end of surgery.

Introduction

General anesthesia is a reversible coma, actively induced and maintained by administering intravenous 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 pharmacologically reversed when no longer desired (*e.g.*, muscle relaxants, opioids, benzodiazepines, and anticoagulants), this is not the case for general anesthetic-induced loss of consciousness.

At present, there is no agent available to actively induce emergence from general anesthesia. This is largely due to our limited knowledge of the molecular mechanisms of general anesthetic actions, hampering 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] While cholinergic,[3,4] orexinergic,[5] and histaminergic[6] arousal pathways have been implicated in emergence, the roles of other arousal pathways are yet unknown.

Methylphenidate is widely prescribed 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 also been reported to increase prefrontal cortex histamine levels 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 present study was conducted to test the hypothesis that methylphenidate induces emergence from isoflurane 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 anesthesia. We also performed spectral analysis of electroencephalogram recordings to assess changes induced by methylphenidate during continuous isoflurane 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 grams 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 2-3% isoflurane in oxygen prior to placement of a lateral tail vein intravenous 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 intravenous 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 liters. Initially the chamber was primed with isoflurane at a fresh gas flow rate of 2-3 liters/min, and then the rate was lowered to 1-2 liters/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 of normal saline, and sterile filtered immediately prior to administration. Droperidol was diluted in normal saline to a final volume of 0.5 ml prior to administration. The intravenous tubing (approximate volume 0.6 ml) was always flushed with 2 ml of 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,[5,6,10] the inhaled concentration of isoflurane was fixed at 1.5% (~1 minimum alveolar concentration). After 40 min, rats received either normal saline or methylphenidate (5 mg/kg IV). Isoflurane was continued for five additional minutes, 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*.[4,11]

After the final 40-min equilibration period, normal saline (2 ml) was administered and the rectal temperature probe was removed. Five minutes later, methylphenidate was administered. To establish a dose-response relationship, we administered three different doses of methylphenidate (0.05 mg/kg, 0.5 mg/kg, or 5 mg/kg IV) 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 surgically implanted at least 7 days prior to recording. General anesthesia was induced with xylazine (5-10 mg/kg intraperitoneal) and ketamine (50-100 mg/kg intraperitoneal), and supplemented with isoflurane (1-2%). A microdrill was used to make four holes at the following stereotactic coordinates: A0L0, A6L3, A6L-3, and A10L2 relative to the lambda.[12] 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, as well as on postoperative days 1 and 2.

The potential difference between electrodes A0L0 and A6L3, or between electrodes A0L0 and A6L-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 was filtered between 0.3-100 Hz. No line filter was used. The sampling rate was 512 Hz.

After baseline recordings were taken for 10 min while awake, rats 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 above, we found that the righting attempts produced too many motion artifacts. Therefore we performed the electroencephalogram experiments 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 a minimum isoflurane exposure of 40 min, normal saline was administered and the temperature probe was removed. Five minutes later, methylphenidate was administered.

Spectral analysis was performed using Matlab 7.11 (Mathworks, Natick, MA) and the Chronux software (Cold Spring Harbor, NY).[13] 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.[14] To determine the difference between two spectra, a two-sample Kolmogorov-Smirnov test[15] was performed on the spectral power as a function of frequency computed from the 30 windows in the

premethylphenidate 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 equilibration in the chamber for 30 min, normal saline or droperidol (0.5 mg/kg IV) was administered 5 min prior to methylphenidate (5 mg/kg IV). Because plethysmography recordings are very sensitive to motion artifacts, we used a higher isoflurane dose (1.5% or ~1 minimum alveolar concentration), since 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 sec, acquired at 100 Hz, and analyzed in four second 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 *et al.*[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 catheter placement. 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 four-second epochs. A premethylphenidate arterial blood sample was drawn following at least 30 min of equilibration in 1.5% isoflurane, and a postmethylphenidate sample was drawn 15 min after methylphenidate administration. Samples were promptly analyzed using CG4+ cartridges in a Vetscan iStat 1 (Abaxis, Union City, CA) blood gas analyzer.

Statistical analysis of the effect 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 where possible, results are reported in terms of 95% confidence intervals based on *z*-tests, *t*-tests or Mann-Whitney tests. We used a Bayesian Monte Carlo procedure to compute Bayesian 95% (credibility) confidence 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 of the conjugacy between the prior and the likelihood 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 on group compared to 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% confidence intervals and 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 Bonferonni correction to compare spectra in animals before and after receiving methylphenidate. We considered a result to be statistically significant based on the 95% confidence intervals comparing two groups if zero was not in the interval, based on hypothesis tests if the *p*-values were less than 0.05 or in the case of the Bayesian analyses, if the relevant posterior probability was greater than 0.95.

Statistical analysis of the effect 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 standard error 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 (AR(p)) with a nonzero mean to the data before and after the administration of methylphenidate. Because these models have a nonzero mean, we devised an efficient cyclic descent parameter estimation algorithm.[18] Within the cyclic descent algorithm we used the least-squares algorithm to estimate the AR 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 the order of the AR (p) model to be different for each segment. We chose the best order p of the AR model on each segment 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 standard error of the mean were used to compute the 95% confidence interval for the difference between the physiological 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 emergence for animals that received normal saline was 280 s (n=12), *versus* 91 s (n = 12) for animals that received methylphenidate (5 mg/kg IV). The median difference in time to emergence between these two groups was 200 s with a 95% confidence interval computed using the Mann-Whitney test of [155, 331] s. This median difference was statistically significant ($p < 0.0001$, two-sided Mann-Whitney test).

Methylphenidate induces emergence during continuous inhalation of isoflurane

To test the hypothesis that methylphenidate increases arousal, we performed the following experiments during 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 intravenous 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, 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 very 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 six rats that received 0.05 mg/kg. The Bayesian 95% confidence interval 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% confidence interval 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 was 0.999 indicating that the two differences are highly significant.

As shown in figure 2C, the median time to righting after methylphenidate 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% confidence interval 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 droperidol (0.5 mg/kg IV) 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, 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 occasionally observed. None of these animals had return of righting, compared to the 11 of 12 animals that had return of righting after receiving normal saline prior to the same dose of methylphenidate (fig. 2D). The 95% Bayesian confidence interval 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 was 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, administration of methylphenidate (5 mg/kg IV) induced a prompt shift within 30 s back 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 droperidol (0.5 mg/kg IV) 5 min prior to methylphenidate (5 mg/kg IV). 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. During the awake state (fig. 4A), electroencephalogram power was mainly in the theta frequency range (4-8 Hz). However, continuous inhalation of 1.0% isoflurane (fig. 4B) caused a large increase in delta power (<4 Hz). Although intravenous injection of normal saline produced no appreciable change in the power spectrum, administration of methylphenidate (5 mg/kg IV) produced a prompt shift in power from delta to theta. When droperidol (0.5 mg/kg IV) was administered instead of normal saline, however, 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 0.05 significance level the two-sided Kolmogorov-Smirnov test with Bonferonni correction rejects the null hypothesis at all frequencies except those marked with white squares. In four rats that received normal saline (fig. 5A), methylphenidate (5 mg/kg IV) induced a rapid shift in peak power from delta to theta, and the difference in power before and after methylphenidate was statistically significant at most frequencies between 0-10 Hz (two-sided Kolmogorov-Smirnov test, $p < 0.05$). However, as shown in figure 5B, four rats that received droperidol (0.5 mg/kg IV) prior to methylphenidate only had 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 1, 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 droperidol (0.5 mg/kg IV) was administered instead of normal saline five minutes prior to methylphenidate (5 mg/kg IV), 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 to 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, while 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 anesthesia there were statistically significant changes in arterial pH and p_aCO_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 $VA_{post}/VA_{pre} = (p_aCO_2)_{pre}/(p_aCO_2)_{post}$, where “pre” and “post” denote premethylphenidate and postmethylphenidate, respectively. The slight increase in P_aO_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 to 20 mmHg) while 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 anesthesia by increasing arousal. In addition, our plethysmography and blood gas experiments revealed that methylphenidate also increases minute ventilation, which increases the rate of anesthetic elimination from the brain.[20] Emergence from isoflurane 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 utilize 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 previously reported for loss of righting in uninstrumented mice using a rotating anesthetizing chamber (0.81% isoflurane, with a 95% confidence interval between 0.78% and 0.84%).[5] The stimulation provided by the temperature probe and the IV catheter in our rats was likely comparable to 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 very similar experimental conditions as 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 and colleagues[3] showed that intraventricular administration of the cholinesterase inhibitor neostigmine to rats during isoflurane 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 and colleagues[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 subjects,[24] 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 bispectral index, which are neurophysiological correlates of increased arousal.

Orexinergic arousal pathways have also 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.[26,27] Mesa and colleagues[28] reported that a patient suffering from narcolepsy underwent three different operations between 1979 and 1995, and required 8-10 h to emerge from general anesthesia each time. Kelz and colleagues[5] demonstrated in mice that both genetic and pharmacologic ablation of orexinergic signaling delays time to righting after discontinuation of isoflurane or sevoflurane general anesthesia. Interestingly, the inhaled anesthetic concentration required to produce loss of righting was unchanged in orexin-ablated mice compared to wild-type, indicating that orexinergic neurons are not involved in the mechanisms underlying induction of general anesthesia. In a separate study by Zecharia *et al.*,[29] 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[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 may also play a role in emergence from general anesthesia.

Decades ago, the clinical utility of methylphenidate as an analeptic, *i.e.*, a central nervous system stimulant, was explored in psychiatry and anesthesiology.[30] However, at that time, its mechanism of action was unknown. Psychiatrists reported using the drug to promote arousal in patients suffering from overdoses of antipsychotic medications,[31] and to facilitate psychiatric interviewing.[32] Studies conducted in the perioperative period suggested that methylphenidate promoted faster recovery after general anesthesia.[33,34] However, the only placebo-controlled, double-blinded study reported no difference in recovery time.[35] Prior to 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 present findings suggest that the widespread use of droperidol may have confounded the results of some of the earlier clinical studies of methylphenidate. On the other hand, because

methylphenidate is now widely prescribed to treat attention deficit hyperactivity disorder, there are recent reports of increased anesthetic requirements in patients who take methylphenidate.[36,37] These reports are consistent with our finding that methylphenidate antagonizes isoflurane anesthesia.

Methylphenidate is known to inhibit dopamine and norepinephrine transporters with similar affinities (K_i = 250 nM and 150 nM, respectively).[7] Dopaminergic neurons in the ventral tegmental area and substantia nigra pars compacta promote arousal, and play an important role in cognition and reward through projections to the thalamus, basal forebrain, nucleus accumbens, cortex, lateral dorsal tegmentum, and locus ceruleus.[38] A third cluster of wake-active, dopaminergic neurons has been recently identified in the ventral periaqueductal gray area.[39] There is recent evidence that enhancement of dopaminergic neurotransmission increases ventilatory drive in cats,[40-42] 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,[2] and their inhibition is important in the sedative actions of propofol[43] and dexmedetomidine.[44] In addition, arousal-promoting histaminergic neurons arising from the tuberomammillary nucleus may also play a role in the actions of methylphenidate,[8] although the mechanisms underlying this pathway have yet to be clearly defined. Therefore 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 of droperidol has no effect on isoflurane potency in rats,[45] and that the EC_{50} for loss of righting in mice is 40 mg/kg,[46] 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.[47,48] Therefore our results with droperidol are consistent with the notion that dopaminergic and noradrenergic neurotransmission play important roles in methylphenidate-induced emergence. Further studies will be necessary 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 poorly understood, it has not been possible to design antagonists of general anesthetics. However, our results suggest that methylphenidate actively induces emergence from isoflurane 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 two decades of use in the treatment of Attention Deficit Hyperactivity Disorder.[7] Our findings suggest that intravenous 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.

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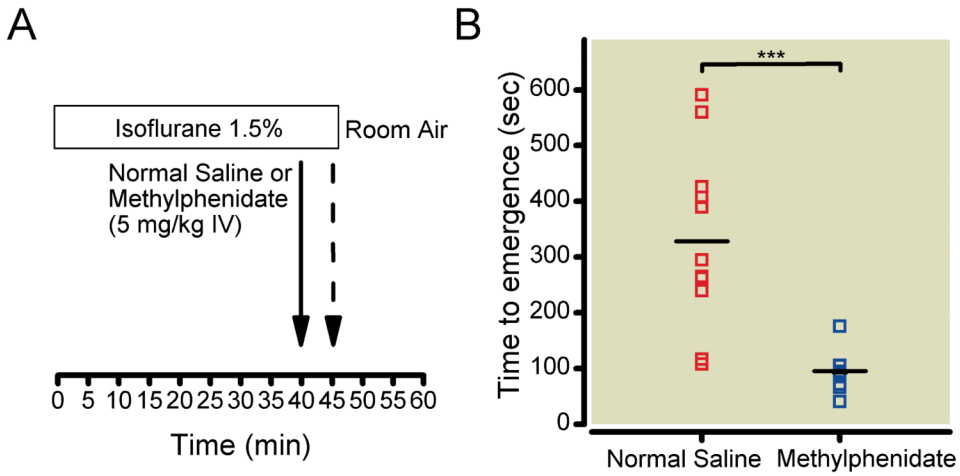

Fig. 1.

Methylphenidate decreases time to emergence from isoflurane anesthesia. (A) Rats inhaled isoflurane (1.5%) for a total of 45 min, and received normal saline or methylphenidate (5 mg/kg IV, solid arrow) 5 min before removal from the anesthetizing chamber (dashed arrow). Time to emergence was defined as the time from termination of isoflurane to return of righting (*i.e.*, all four paws touching the floor). (B) Scatter plot of time to emergence for rats that received normal saline *versus* methylphenidate (5 mg/kg IV). The line represents the median. *** $P < 0.0001$.

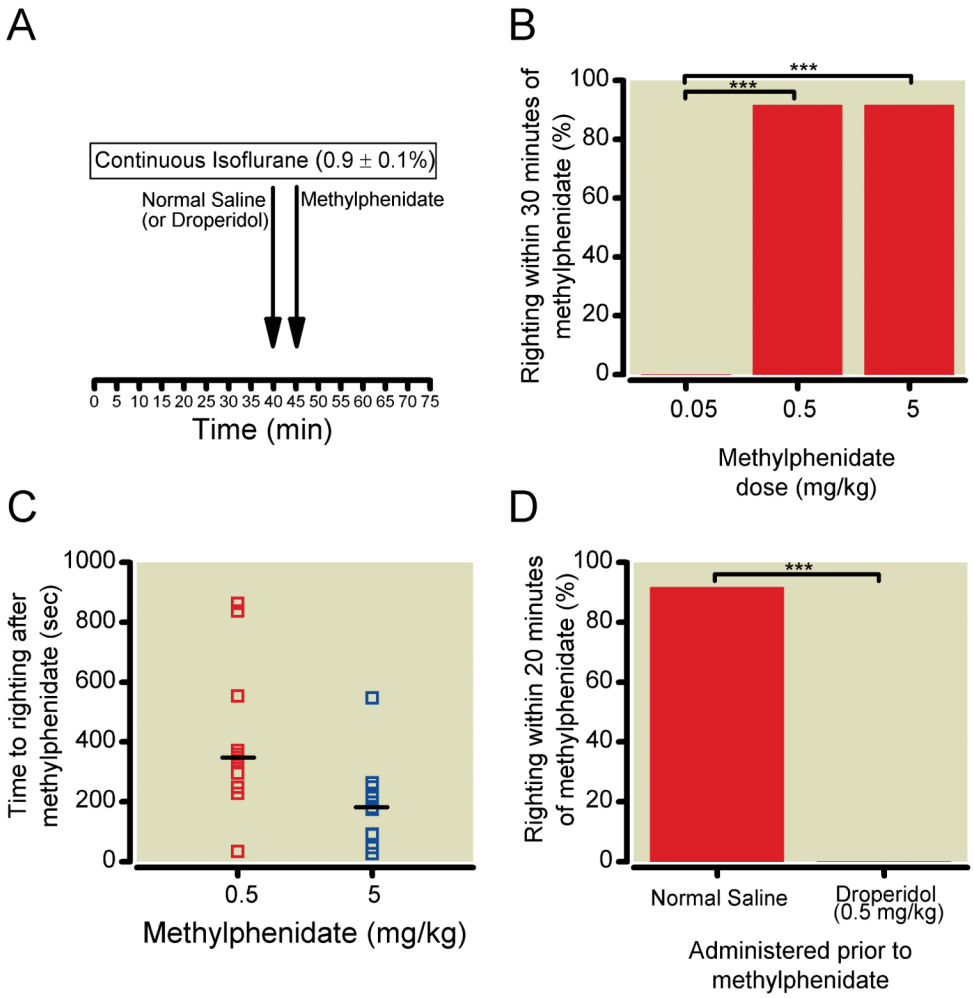

Fig. 2.

Methylphenidate induces emergence during continuous inhalation of isoflurane. (A) Rats inhaled isoflurane at a dose sufficient to maintain loss of righting for a total of 40 min, and received normal saline. Five minutes later, methylphenidate was administered IV. Isoflurane was continued at the same dose until return of righting occurred or 30 min elapsed. (B) Dose-dependence of methylphenidate-induced emergence. (C) Scatter plot of time to righting for rats that received 0.5 *versus* 5 mg/kg IV of methylphenidate. The line represents the median. (D) After pretreatment with droperidol (0.5 mg/kg IV) instead of normal saline, high-dose methylphenidate (5 mg/kg IV) did not induce return of righting in any of the six animals tested. *** posterior probability > 0.95, * $P < 0.05$.

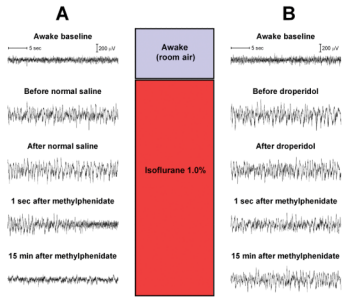

Fig. 3.

Methylphenidate-induced electroencephalogram changes during continuous inhalation of isoflurane are inhibited by droperidol. (A) Thirty-second epochs of electroencephalogram recordings from a single rat show the change from an active, theta-dominant pattern during the awake state to the delta-dominant pattern during inhalation of isoflurane (1.0%). The latter pattern is unchanged after the administration of normal saline. Administration of methylphenidate (5 mg/kg IV) induced a prompt shift in the electroencephalogram back to an active theta-dominant pattern similar to that observed during the awake state. This pattern persisted for more than 15 min. (B) Thirty-second epochs of raw electroencephalogram recordings from a different animal than (A) show the same patterns during the awake and anesthetized states. Administration of droperidol (0.5 mg/kg IV) induced no appreciable change in the electroencephalogram pattern. However, when methylphenidate (5 mg/kg IV) was administered 5 min after droperidol, the electroencephalogram did not return to the active, theta-dominant pattern observed during the awake state. Rather, the delta-dominant pattern persisted.

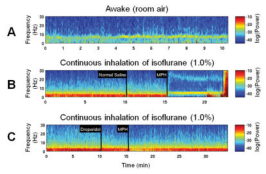

Fig. 4.

Spectral analysis of electroencephalogram data reveals a shift in power induced by methylphenidate that is inhibited by droperidol. Warm colors (*e.g.*, red) represent higher power at a given frequency, while cool colors (*e.g.*, blue) represent lower power. (A) A representative spectrogram computed from a rat in the awake state shows predominance of theta power (4-8 Hz). (B) A representative spectrogram computed from a rat inhaling isoflurane (1.0%) shows predominance of delta power (<4 Hz) before and after administration of normal saline. However, administration of methylphenidate (5 mg/kg IV) promptly induced a shift in power to an active theta-dominant pattern similar to that observed during the awake state. This animal began to move vigorously approximately 5 min after methylphenidate administration, generating significant motion artifacts. Therefore the experiment was promptly terminated. (C) A representative spectrogram computed from a rat that received droperidol (0.5 mg/kg IV) instead of normal saline shows that similar to the rat in (B), delta power is dominant during inhalation of isoflurane (1.0%), before and after administration of droperidol. However, after administration of droperidol, methylphenidate (5 mg/kg IV) did not induce a shift in electroencephalogram to the theta-dominant pattern characteristic of the awake state. In addition, this animal showed no purposeful movement after methylphenidate administration.

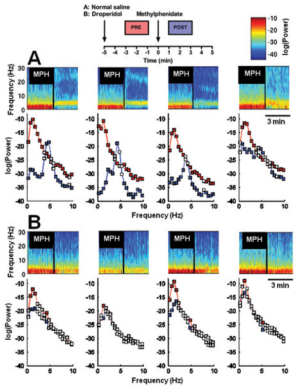

Fig. 5.

Electroencephalogram power spectra and spectrograms computed for each of four animals reveal a shift in peak power from delta to theta after administration of methylphenidate that is inhibited by droperidol. The top panel shows the two-minute windows used to compute power spectra before methylphenidate administration (red, “PRE”), and after methylphenidate administration (blue, “POST”). (A) Spectrograms and power spectra computed from animals that received normal saline prior to methylphenidate (MPH). Power spectra show results of the Kolmogorov-Smirnov test for the 2-min periods before and after methylphenidate administration. At a 0.05 significance level (with Bonferonni correction) the Kolmogorov-Smirnov test rejects the null hypothesis at all frequencies except those marked with white squares. Statistically significant changes occurred at most frequencies between 0-10 Hz. (The fourth animal moved during the time window used for the analysis, and therefore motion artifact may account for the persistent high delta power observed after methylphenidate administration in this animal.) (B) Spectrograms and power spectra computed from animals that received droperidol prior to methylphenidate (MPH). After droperidol, methylphenidate only induced statistically significant decreases in delta power.

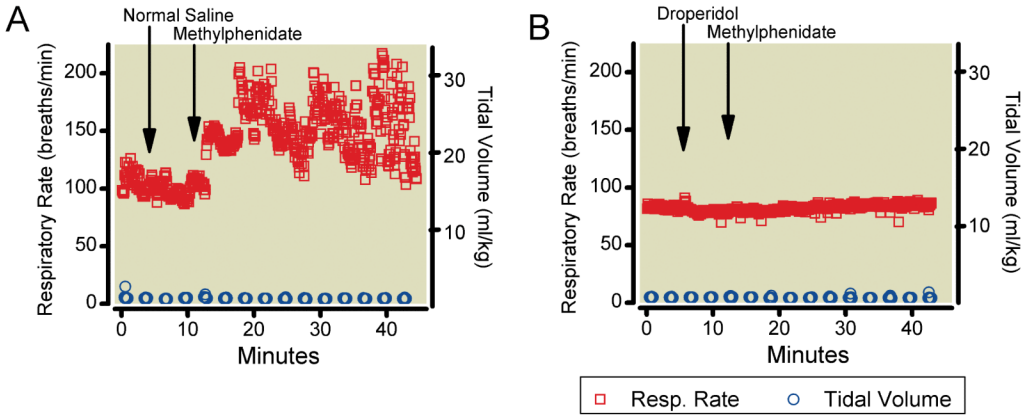

Fig. 6.

Methylphenidate induces an increase in respiratory rate that is inhibited by droperidol. (A) Time series of respiratory rate (filled circles) and tidal volume (open squares) recorded from one animal during inhalation of isoflurane (1.5%). Normal saline and methylphenidate (5 mg/kg IV) were administered at the indicated times. Methylphenidate induced a prompt and sustained increase in respiratory rate from 103 to 154 breaths/min ($p < 10^{-16}$), while tidal volume remained essentially unchanged. (B) When a different animal was pretreated with droperidol (0.5 mg/kg IV) instead of normal saline, methylphenidate induced little change in respiratory rate or tidal volume.

Table 1

Respiratory Rate (Breaths/Min) in Individual Animals Pretreated with Normal Saline during Isoflurane General Anesthesia.

Animal	**1**	**2**	**3**	**4**
RR before methylphenidate (mean, [95%CI])	83.4 [81.5,85.4]	84.3 [83.5,85.2]	103.5 [98.4,108.5]	97.8 [96.3,99.3]
RR after 5 mg/kg IV methylphenidate (mean, [95%CI])	112.6 [109.5,115.7]	94.2 [93.4,95.0]	153.8 [150.5,157.2]	116.7 [114.0,119.4]
Change in mean RR (mean, [95%CI])	+29.2 [+25.5,+32.9]	+9.8 [+8.7,+11.0]	+50.36 [+44.3,+56.4]	+18.9 [+15.8,+21.9]
z-statistic	15.9	16.6	16.7	12.3
***p*-value**	$<10^{-16}$	$<10^{-16}$	$<10^{-16}$	$<10^{-16}$

CI = confidence interval; RR = respiratory rate.

Table 2

Respiratory Rate (Breaths/Min) in Individual Animals Pretreated with Droperidol (0.5 mg/kg IV) during Isoflurane General Anesthesia.

Animal	**1**	**2**	**3**	**4**
RR before methylphenidate (mean, [95%CI])	81.9 [80.7,83.0]	75.8 [75.2,76.4]	80.5 [79.8,81.2]	70.6 [69.2,72.0]
RR after 5 mg/kg IV methylphenidate (mean, [95%CI])	86.3 [85.8,86.8]	78.0 [77.1,78.9]	84.1 [83.4,84.9]	75.5 [74.9,76.1]
Change in mean RR (mean, [95%CI])	+4.4 [+3.2,+5.7]	+2.2 [+1.1,+3.3]	+3.6 [+2.6,+4.6]	+4.9 [+3.3,+6.4]
z-statistic	7.0	4.1	7.0	6.3
***p*-value**	<0.0001	<0.0001	<0.0001	<0.0001

CI = confidence interval; RR = respiratory rate.

Table 3

Arterial Blood Gas Analysis during Isoflurane General Anesthesia (n = 6).

	pH	p_aCO_2 (mmHg)	p_aO_2 (mmHg)
Before methylphenidate (mean, [95%CI])	7.45 [7.41.7.50]	43 [40,47]	226 [200,252]
After 5 mg/kg IV methylphenidate (mean, [95%CI])	7.51 [7.46,7.55]	35 [32,38]	241 [211,270]
***p*-value (paired t test)**	0.004	0.0001	0.144

CI = confidence interval.

Table 4

MAP (mmHg) in Individual Animals during Isoflurane General Anesthesia.

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

CI = confidence interval; MAP = mean arterial pressure.

Table 5

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

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

CI = confidence interval; HR = heart rate.