

Activation of D1 Dopamine Receptors Induces Emergence from Isoflurane General Anesthesia

Norman E. Taylor, M.D., Ph.D.,* Jessica J. Chemali, B.E.,† Emery N. Brown, M.D., Ph.D.,‡ Ken Solt, M.D.§

ABSTRACT

Background: A recent study showed that methylphenidate induces emergence from isoflurane anesthesia. Methylphenidate inhibits dopamine and norepinephrine reuptake transporters. The objective of this study was to test the hypothesis that selective dopamine receptor activation induces emergence from isoflurane anesthesia.

Methods: In adult rats, we tested the effects of chloro-APB (D1 agonist) and quinpirole (D2 agonist) on time to emergence from isoflurane general anesthesia. We then performed a dose–response study to test for chloro-APB–induced restoration of righting during continuous isoflurane anesthesia. SCH-23390 (D1 antagonist) was used to confirm that the effects induced by chloro-APB are specifically mediated by D1 receptors. In a separate group of animals, spectral analysis was performed on surface

* Clinical Fellow, Department of Anaesthesia, Harvard Medical School, Boston, Massachusetts, and Resident, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts. † Research Assistant, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital. ‡ Anesthetist, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital; Warren M. Zapol Professor, Department of Anaesthesia, Harvard Medical School; Professor of Computational Neuroscience, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts; Professor of Health Sciences and Technology, Institute for Medical Engineering and Science, Massachusetts Institute of Technology. § Assistant Professor, Department of Anaesthesia, Harvard Medical School; Assistant Anesthetist, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital; and Research Affiliate, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology.

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Address correspondence to Dr. Solt: Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, 55 Fruit Street, GRB-444, Boston, Massachusetts 02114. ksolt@partners.org. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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What We Already Know about This Topic

- Methylphenidate, which inhibits dopamine and norepinephrine reuptake transporters, induced emergence from isoflurane anesthesia in previous studies, but whether selective dopamine receptor activation could also have a similar effect is not known

What This Article Tells Us That Is New

- Activation of D1 receptors by chloro-APB decreased time to emergence from isoflurane anesthesia and produced behavioral and neurophysiologic evidence of arousal during continuous isoflurane anesthesia, consistent with a D1-mediated arousal response that promotes emergence from isoflurane anesthesia

electroencephalogram recordings to assess neurophysiologic changes induced by chloro-APB and quinpirole during isoflurane general anesthesia.

Results: Chloro-APB decreased median time to emergence from 330 to 50 s. The median difference in time to emergence between the saline control group ($n = 6$) and the chloro-APB group ($n = 6$) was 222 s (95% CI: 77–534 s, Mann–Whitney test). This difference was statistically significant ($P = 0.0082$). During continuous isoflurane anesthesia, chloro-APB dose-dependently restored righting ($n = 6$) and decreased electroencephalogram δ power ($n = 4$). These effects were inhibited by pretreatment with SCH-23390. Quinpirole did not restore righting ($n = 6$) and had no significant effect on the electroencephalogram ($n = 4$) during continuous isoflurane anesthesia.

Conclusions: Activation of D1 receptors by chloro-APB decreases time to emergence from isoflurane anesthesia and produces behavioral and neurophysiologic evidence of arousal during continuous isoflurane anesthesia. These findings suggest that selective activation of a D1 receptor–mediated arousal mechanism is sufficient to induce emergence from isoflurane general anesthesia.

THE discovery that anesthetic-induced immobility is mediated primarily in the spinal cord^{1–3} has led to a growing interest in studying anesthetic mechanisms at the

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level of neural circuits and systems.^{4,5} Recent studies suggest that the process of emergence from general anesthesia is distinct from the process of induction,⁶ and, in particular, the roles of ascending arousal pathways in emergence from general anesthesia are becoming recognized.^{4,5,7} Cholinergic,^{8–10} noradrenergic,¹¹ histaminergic,^{12,13} and orexinergic^{14,15} arousal pathways have been implicated in emergence from general anesthesia, but the role of dopamine remains unclear.

It is widely accepted that dopamine plays an important role in behavioral arousal.^{16–18} Electrolytic lesions of dopaminergic neurons have been shown to induce a coma-like state,¹⁹ and mice with selective loss of dopamine in the brain appear hypoactive and apathetic.²⁰ Dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) send projections to key arousal-promoting brain regions, including the dorsal raphe, locus ceruleus, pedunculo-pontine and laterodorsal tegmental areas, basal forebrain, and the perifornical area of the lateral hypothalamus, and, in turn, these arousal-promoting centers also send inputs to the VTA and SNc.¹⁶ The existence of these projections alone suggests that dopamine is intimately involved in regulating arousal.

Recent studies show that methylphenidate induces emergence from general anesthesia with isoflurane²¹ and propofol.²² However, methylphenidate is known to inhibit both dopamine and norepinephrine reuptake transporters with similar affinities ($K_i = 250$ nM and 150 nM, respectively),²³ and both are known to promote arousal. The current study was performed in adult rats to test the hypothesis that selective activation of dopaminergic neurotransmission is sufficient to induce emergence from isoflurane general anesthesia. First, we tested the effects of the specific D1 and D2 dopamine receptor agonists chloro-APB and quinpirole, respectively, on time to emergence from a standardized isoflurane general anesthetic. We then tested the behavioral effects of chloro-APB and quinpirole during continuous isoflurane general anesthesia. In a separate group of rats with preimplanted electrodes, we recorded the electroencephalogram during isoflurane general anesthesia and performed spectral analysis to compare the recordings taken before and after dopamine agonist administration.

Materials and Methods

Animal Care and Use

All studies were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (Boston, Massachusetts), which serves as our Institutional Animal Care and Use Committee. Ten male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used for this study. The age range was approximately 3–6 months, and the weight range was 322–565 g. The same six rats were used in random order for all behavioral experiments, whereas a separate group of four rats were used for all electroencephalogram recordings. Each animal was provided with at least 3 days of rest between experiments. Animals were kept on a standard day–night cycle (lights on at 7:00 AM and off at 7:00 PM), and all experiments

were performed during the day. Because rats are nocturnal animals, all experiments were conducted during the “night” phase of the rat sleep–wake cycle.

Anesthetizing Protocol

After inducing general anesthesia with isoflurane (2–3%) in oxygen, a 24-gauge intravenous catheter was placed in a lateral tail vein, a rectal temperature probe was inserted, and the animal was placed in a cylindrical acrylic anesthetizing chamber, as previously described.²¹ A heating pad was placed under the chamber to maintain rectal temperature between 36.5°C and 37.4°C. Gas was continuously sampled from the distal portion of the chamber, 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 was purchased from Henry Schein (Melville, NY). The D1 receptor agonist chloro-APB (6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide), the D2 receptor agonist quinpirole (trans-(–)-(4aR)-4, 4a, 5, 6, 7, 8, 8a, 9-Octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline monohydrochloride), and the D1 receptor antagonist SCH-23390 (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) were purchased from Sigma–Aldrich (St. Louis, MO). All drugs were dissolved in normal saline to a final volume of 0.5 ml and administered intravenously *via* the lateral tail vein catheter. Chloro-APB solutions were sonicated in an ultrasound water bath to facilitate dissolution. The IV tubing (approximate volume 0.6 ml) was always flushed with 2 ml of normal saline after drug administration to ensure complete delivery.

Time to Emergence After a Standardized Isoflurane General Anesthetic

The inhaled concentration of isoflurane was fixed at 1.5% (approximately 1 median alveolar concentration). After 40 min, rats received normal saline IV, chloro-APB (3 mg/kg IV), or quinpirole (5 mg/kg IV). Isoflurane was continued for 5 additional minutes, after which 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).

Emergence During Continuous Isoflurane General Anesthesia

The isoflurane concentration was held at a dose that produced loss of righting with no purposeful movement for 40 consecutive minutes, as described previously.²¹ Purposeful movements were defined as any movements other than random muscle twitches, such as lifting of the head, opening

of the eyes, twisting of the torso, kicking, clawing, chewing, licking, or grooming. The intravenous catheter was then flushed with 2 ml of normal saline. Five minutes after this injection, quinpirole (5 mg/kg IV) or chloro-APB was administered. To establish a dose–response relationship, three different doses of chloro-APB (0.03 mg/kg, 0.3 mg/kg, or 3 mg/kg IV) were administered on different days. In a separate experiment, the D1 receptor antagonist SCH-23390 (0.2 mg/kg IV) was administered instead of the normal saline control, followed 5 min later by chloro-APB (3 mg/kg IV). The same six rats were used for each experimental condition, in random order, with at least 3 days of rest between experiments. After intravenous drug administration, 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, as detailed previously.^{21,22} Briefly, rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) under isoflurane general anesthesia. A microdrill (Patterson Dental Supply Inc., Wilmington, MA) was used to make four holes at the following stereotaxic coordinates: A0L0, A6L3, A6L-3, and A10L2 relative to the lambda.²⁴ An electrode with mounting screw and socket (Plastics One, Roanoke, VA) was screwed into each hole, and the sockets were inserted in a pedestal (Plastics One) before being fixed permanently with dental acrylic cement.

On the day of an experiment, the potential difference between electrodes A0L0 and A6L3 (right somatosensory cortex) or between electrodes A0L0 and A6L-3 (left somatosensory cortex) was recorded based on which signal gave less motion artifact. The signal was referenced to A10L2 and recorded using a QP511 Quad AC Amplifier System (Grass Instruments, West Warwick, RI) and a USB-6009 14-bit data acquisition board (National Instruments, Austin, TX). The sampling rate was 512 Hz, and no line filter was used. Data were filtered between 0.3 Hz and 50 Hz. Baseline recordings were taken for 10 min in the awake state before any drugs were administered. The rats were then anesthetized with isoflurane and placed in the anesthetizing chamber in the prone position with the isoflurane dose fixed at 1.0%. After a minimum isoflurane exposure of 40 min, normal saline or SCH-23390 (0.2 mg/kg IV) was administered and the temperature probe was removed. Five minutes later, either chloro-APB (3 mg/kg IV) or quinpirole (5 mg/kg IV) was administered, and isoflurane anesthesia was continued at the same dose for an additional 30 min.

Spectral analysis was performed using Matlab 7.11 (Mathworks, Natick, MA) and the Chronux software (Cold Spring Harbor, NY), as previously described.^{21,22} Briefly, spectrograms were calculated using sliding windows of 2-s duration stepped through 0.05 s. The resulting spectral estimates have a bandwidth of 1.5 Hz. Mean power spectra were compared

before and after quinpirole or chloro-APB administration using the Kolmogorov–Smirnov test. 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 preagonist and postagonist periods. We used a Bonferroni correction to adjust the significance level for multiple hypothesis testing.

Statistical Analysis of the Effects of Chloro-APB and Quinpirole on Emergence Times, Return of Righting Responses, and Spectrograms

Prism 5.04 (Graphpad Software, San Diego, CA) and Matlab R2010b (Mathworks) 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) to assess the effect of the different agonists on return of righting during continuous isoflurane general anesthesia, as described previously.^{21,22} The posterior densities for the differences in the proportion of animals that had return of righting were 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 in each animal group. To provide a conservative check on the assessments made by the 95% CI, the nonparametric tests were also used to assess statistical significance. The Mann–Whitney test was used to test the hypothesis that chloro-APB hastens time to emergence from isoflurane general anesthesia. We used the two-sided Kolmogorov–Smirnov test with a Bonferroni correction to compare spectra in animals before and after agonist administration.

To characterize the righting propensity as a function of dose of chloro-APB and to conduct between-group comparisons of differences in righting propensity, we analyzed the data using a Bayesian logistic regression model with an uninformative prior density. In the Bayesian analysis, we used a Monte Carlo procedure to compute Bayesian 95% credibility (confidence) intervals for $P_k - P_1$. This is the difference in the righting propensities between the group that received dose *k*, and the group that received normal saline. We define P_k as the righting propensity in the group that received dose *k* of chloro-APB and P_1 as the righting propensity in the group that received normal saline. We denote $k = 2$ as the 0.03 mg dose, $k = 3$ as the 0.3 mg dose, and $k = 4$ as the 3 mg dose. Unlike CI computed using frequentist methods, the Bayesian 95% CI can be interpreted as having probability 0.95 that the value of $P_k - P_1$ lies between the lower and upper limits of the interval based on the data in the current sample.²⁵ We applied this type of Monte Carlo algorithm in our previous studies using methylphenidate to induce active emergence from isoflurane and propofol general anesthesia.^{21,22} The details of this Bayesian analysis are summarized in the appendix.

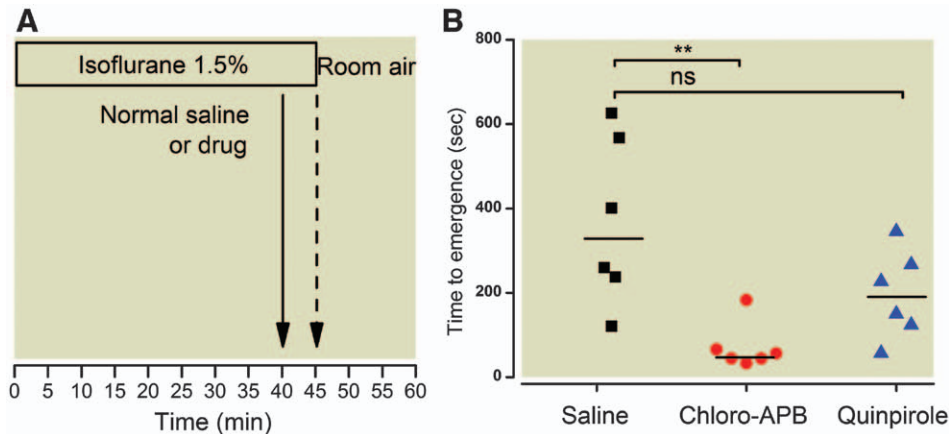


Fig. 1. The D1 receptor agonist chloro-APB decreases time to emergence from isoflurane anesthesia. *A*, Rats inhaled isoflurane (1.5%) and received normal saline, chloro-APB (3 mg/kg IV), or the D2 receptor agonist quinpirole (5 mg/kg IV) after 40 min (solid arrow). Five minutes later, the animals were removed from the anesthetizing chamber to return of righting (*i.e.*, all four paws touching the floor). 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, chloro-APB, and quinpirole ($n = 6$ each). The lines represent the medians. ** $P < 0.01$.

We considered a result to be statistically significant based on the 95% CI if the interval does not contain zero. In this case, we have P value less than 0.05. In the case of the Bayesian analyses, the result is statistically significant if the relevant posterior probability was greater than 0.95.

Results

Chloro-APB Reduces Time to Emergence from Isoflurane General Anesthesia

The protocol for this experiment is summarized in figure 1A. As shown in figure 1B, administration of chloro-APB (3 mg/kg IV) caused a significant reduction in time to emergence from isoflurane general anesthesia. The median time to emergence for animals that received normal saline was 330 s (95% CI: 160–577 s, $n = 6$) versus 50 s (95% CI: 12–130 s, $n = 6$) for animals that received chloro-APB. The median difference in time to emergence between these two groups was 222 s (95% CI: 77–534 s, Mann–Whitney test). This median difference was statistically significant ($P = 0.0082$).

The median time to emergence for rats that received quinpirole (5 mg/kg IV) was 189 s (95% CI: 85–305 s, $n = 6$). The median difference in time to emergence between the normal saline control group and the quinpirole group was 155 s (95% CI: –30 to 417 s, Mann–Whitney test). This median difference was not statistically significant ($P = 0.17$). We report that the quinpirole result was not significant based on a sample of six animals per group, assuming a power of 0.8 and a type I error of 0.05, for an anticipated average difference of 199 s with an SD of 172 s, based on our previous results with methylphenidate.²¹ That is, we designed the current study to detect emergence time effects that were at least as large as those we observed previously with methylphenidate.

Chloro-APB Induces Emergence during Continuous Inhalation of Isoflurane

As illustrated in figure 2A, these experiments were conducted during continuous inhalation of isoflurane. The minimum concentration of isoflurane sufficient to maintain loss of righting was established for each rat, and this dose was continuously delivered to the chamber throughout the experiment. The average final dose of isoflurane was the same for all six experimental groups ($1.0 \pm 0.1\%$, mean \pm SD), with no statistically significant difference between groups.

As shown in figure 2B, the rats in the control group that received only normal saline ($n = 6$) did not exhibit an arousal response, and none had restoration of righting. However, six of six rats that received the D1 agonist chloro-APB (0.03 mg/kg IV) exhibited purposeful movements (defined as any movement other than a muscle twitch, such as lifting of the head, opening of the eyes, twisting of the torso, kicking, clawing, chewing, licking, or grooming) within 5 min of drug administration, and three of six rats had restoration of righting within 30 min, despite continuous inhalation of isoflurane at the same dose.

Chloro-APB restored the righting reflex in a dose-dependent fashion. Restoration of righting occurred in four of six and five of six rats at chloro-APB doses of 0.3 mg/kg and 3 mg/kg, respectively. Although some rats failed to right themselves within 30 min of drug administration, all doses of chloro-APB produced an arousal response, despite continuous isoflurane anesthesia. At the highest dose of 3 mg/kg, chloro-APB induced purposeful movements within 30 s in six of six rats. The Bayesian 95% CIs for the difference in the propensities to have restoration of righting between rats in the chloro-APB groups (0.03, 0.3 and 3 mg/kg) versus the normal saline group were 0.089–0.448, 0.236–0.913, and 0.349–0.987 respectively. The posterior probability that the difference was greater than 0 exceeded

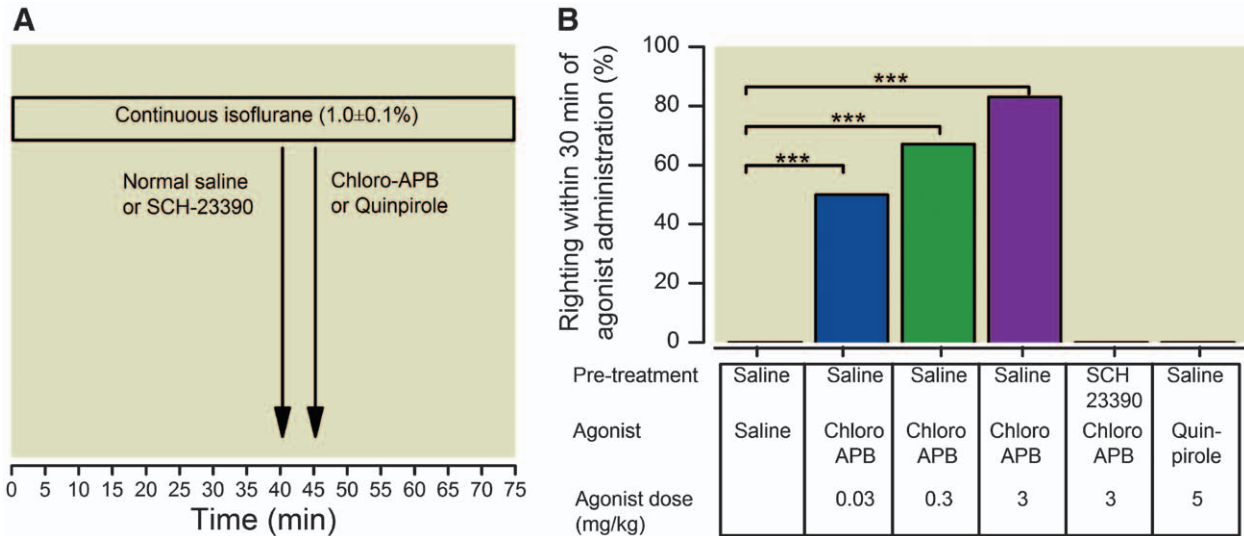


Fig. 2. Chloro-APB induces emergence during continuous isoflurane general anesthesia. **A**, Rats inhaled isoflurane at a dose sufficient to maintain loss of righting for a total of 40 min and received normal saline or the D1 receptor antagonist SCH-23390. Five minutes later, chloro-APB or quinpirole was administered IV. Isoflurane was continued at the same dose until return of righting occurred or 30 min elapsed. **B**, For each drug regimen, the percentage of rats that had restoration of righting within 30 min of dopamine agonist administration is shown ($n = 6$ each). Normal saline alone elicited no arousal response. Rats that received normal saline followed by chloro-APB exhibited restoration of righting in a dose-dependent manner. Pretreatment with the D1 receptor antagonist SCH-23390 (0.2 mg/kg IV) instead of normal saline inhibited restoration of righting by the highest dose of chloro-APB (3 mg/kg IV). Rats that received normal saline followed by the D2 receptor agonist quinpirole did not exhibit restoration of righting. *** Posterior probability greater than 0.99.

0.99 for each comparison, indicating that each was statistically significant.

In rats that received the D1 receptor antagonist SCH-23390 (0.2 mg/kg IV) 5 min before chloro-APB, the highest dose of chloro-APB (3 mg/kg IV) failed to restore the righting reflex. These animals exhibited some sluggish limb movements immediately after the administration of chloro-APB, but showed no other signs of arousal. The Bayesian 95% CI between rats that received normal saline *versus* SCH-23390 was 0.21–0.91, with a posterior probability of 0.998, indicating that the difference was statistically significant. The D2 receptor agonist quinpirole (5 mg/kg IV) failed to elicit an arousal response during continuous isoflurane general anesthesia ($n = 6$).

SCH-23390 Inhibits Chloro-APB-induced Electroencephalogram Changes during Continuous Inhalation of Isoflurane

Electroencephalogram data were recorded from rats with preimplanted extradural skull electrodes. These experiments were performed in the prone position to minimize motion artifacts associated with righting attempts. Spectrograms were computed from the continuous electroencephalogram data to analyze changes in electroencephalogram power over time. Typical results from individual rats are shown in figure 3. In awake rats (fig. 3A), electroencephalogram power was mainly in the θ frequency range (4–8 Hz). Continuous inhalation of 1.0% isoflurane (fig. 3B–D) caused a large increase in δ power (0–4 Hz). Injection of normal

saline produced no arousal response and no appreciable change in the spectrogram, but administration of the D1 agonist chloro-APB (3 mg/kg IV) induced behavioral signs of arousal (*i.e.*, purposeful movements such as kicking, clawing) in four of four rats, as well as a prompt decrease in δ power on the electroencephalogram (fig. 3B). Pretreatment with the D1 antagonist SCH-23390 (0.2 mg/kg IV) inhibited the chloro-APB-induced arousal response and decrease in electroencephalogram δ power (fig. 3C). The D2 agonist quinpirole (5 mg/kg IV) failed to induce behavioral arousal or electroencephalogram changes during continuous isoflurane general anesthesia (fig. 3D).

Figure 4 shows power spectra computed from four different rats during continuous isoflurane general anesthesia. Figure 4A illustrates the 2-min “pre-drug” (blue) and “post-drug” periods used for analysis. Because the time to onset of the arousal response was somewhat variable, the “post-drug” period began 5 min after drug administration. At any given frequency, statistically significant differences between power spectra are depicted in color, whereas the white boxes show differences that do not reach statistical significance. As shown in figure 4B, treatment with chloro-APB (3 mg/kg IV) induced a statistically significant decrease in power at most frequencies under 15 Hz for four of four rats ($P < 0.05$). Decrease in power was more pronounced at δ and α (8–12 Hz) frequencies. As shown in figure 4C, rats that received SCH-23390 (0.2 mg/kg IV) before chloro-APB had only small changes in δ power, with no statistically significant changes at most frequencies between 0 Hz and 30 Hz.

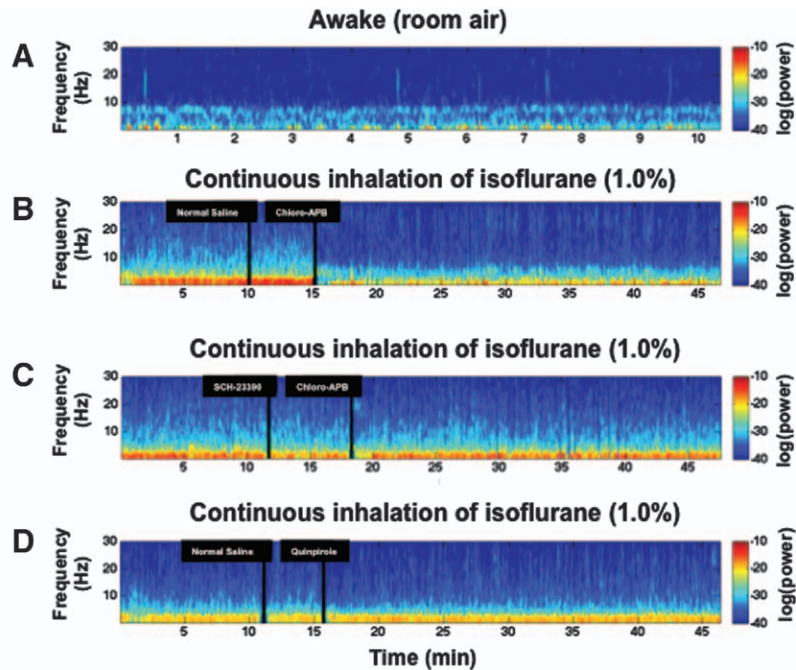


Fig. 3. Spectral analysis of electroencephalogram data reveals a decrease in δ power induced by chloro-APB that is inhibited by SCH-23399. Warm colors (e.g., red) represent higher power at a given frequency, whereas cool colors (e.g., blue) represent lower power. *A*, A representative spectrogram computed from a rat in the awake state shows predominance of θ power (4–8 Hz). *B*, A representative spectrogram computed from a rat inhaling isoflurane (1.0%) shows predominance of δ power (<4 Hz) before and after administration of normal saline. However, administration of chloro-APB (3 mg/kg IV) promptly induced a decrease in δ power. *C*, A representative spectrogram computed from a rat that received the D1 receptor antagonist SCH-23399 (0.2 mg/kg IV) instead of normal saline shows that similar to the rat in *B*, δ power is dominant during inhalation of isoflurane (1.0%), before and after administration of SCH-23399. However, after the administration of SCH-23399, chloro-APB (3 mg/kg IV) failed to induce a decrease in δ power. *D*, A representative spectrogram computed from a rat that received normal saline followed by the D2 receptor agonist quinpirole (5 mg/kg IV) shows that quinpirole did not induce a decrease in δ power.

Similarly, administration of quinpirole (5 mg/kg IV) induced only minor, insignificant changes in electroencephalogram power at frequencies under 30 Hz (fig. 4D).

Discussion

The current study was conducted to test the hypothesis that specific dopamine receptor agonists induce emergence from isoflurane anesthesia. The results show that the D1 agonist chloro-APB decreases time to emergence after isoflurane general anesthesia and induces emergence during continuous inhalation of isoflurane. In addition, chloro-APB induces changes in electroencephalogram power consistent with arousal. The chloro-APB-induced arousal response is inhibited by the D1 antagonist SCH-23399, strongly suggesting that the arousal effect is specifically mediated by D1 receptors. In contrast, the D2 agonist quinpirole failed to induce emergence from isoflurane general anesthesia.

In a previous study, it was shown that methylphenidate, an inhibitor of dopamine and norepinephrine reuptake transporters, induces emergence from isoflurane general anesthesia.²¹ It was also shown that droperidol inhibits the arousal-promoting actions of methylphenidate, but because droperidol inhibits both dopaminergic and adrenergic

receptors, the specific neurotransmitter systems responsible for the effects of methylphenidate were not clear. The current results show that activation of D1 receptors alone is sufficient to induce emergence from general anesthesia with isoflurane. It has been reported that chloro-APB and other D1 agonists are respiratory stimulants that reverse opioid-induced respiratory depression,²⁶ but not analgesia.²⁷ Therefore, it is likely that chloro-APB produced a large reduction in time to emergence from isoflurane general anesthesia (fig. 1) by a combination of increased minute ventilation and increased arousal, similar to methylphenidate.²¹

Qualitatively, the behavioral arousal response induced by chloro-APB was not as pronounced as the response observed previously with methylphenidate. That is, chloro-APB generally induced less vigorous movements compared to methylphenidate. The electroencephalogram changes were also less pronounced: while methylphenidate and chloro-APB both induced a decrease in δ power, methylphenidate increased θ power,²¹ whereas chloro-APB did not. Rather, chloro-APB induced a decrease in power at most frequencies under 30 Hz. Taken together, these results suggest that chloro-APB induces an attenuated arousal response compared to methylphenidate. This is likely because methylphenidate activates both dopaminergic

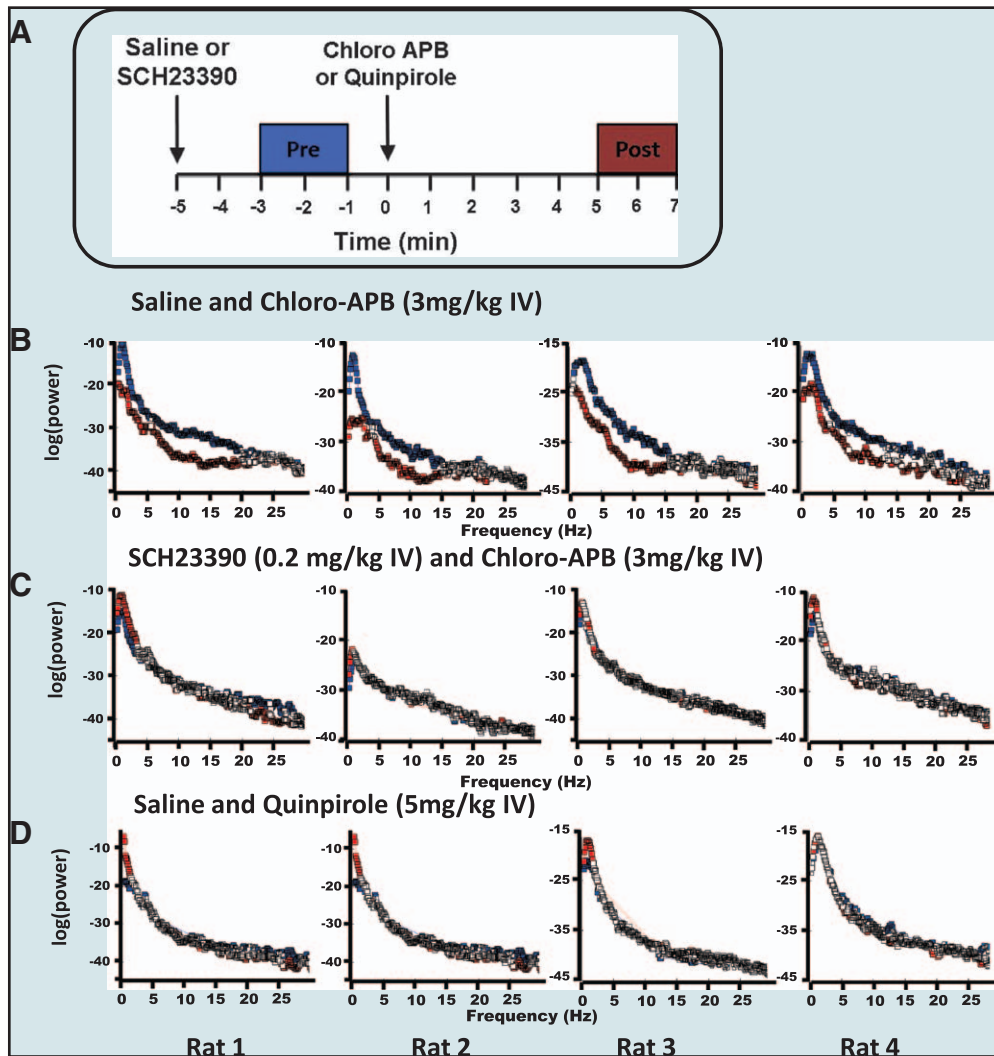


Fig. 4. Electroencephalogram power spectra computed for each of four animals during continuous isoflurane general anesthesia. *A*, The 2-min windows used to compute power spectra before dopamine agonist administration (*blue*, “PRE”) and after administration (*red*, “POST”). *B*, Power spectra computed from animals that received normal saline before chloro-APB, showing results of the Kolmogorov–Smirnov test for the 2-min periods before and after chloro-APB administration. At a 0.05 significance level (with Bonferroni correction), the Kolmogorov–Smirnov test rejects the null hypothesis at all frequencies, except those marked with *white squares*. Statistically significant decreases in power occurred at most frequencies under 15 Hz. *C*, Power spectra computed from animals that received the D1 receptor antagonist SCH-23390 (0.2 mg/kg IV) before chloro-APB (3 mg/kg IV). After SCH-23390, chloro-APB failed to induce statistically significant changes in the power spectrum. *D*, Power spectra computed from animals that received normal saline followed by quinpirole (5 mg/kg IV). Quinpirole induced no statistically significant changes in the power spectrum.

and adrenergic neurotransmission, whereas chloro-APB acts selectively at D1 receptors.

We selected chloro-APB and quinpirole because they are widely used in animal studies as specific dopamine receptor agonists, and they are readily available. Although there is evidence that quinpirole may also interact with receptors other than D2 receptors,²⁸ it was ineffective at inducing emergence from isoflurane anesthesia. Therefore, we conclude that D2 receptors (and any other putative molecular targets of quinpirole) are unlikely to play an important role in emergence from general anesthesia. However, because we only tested a

single, high dose of quinpirole, it is unlikely but still possible that lower doses may have produced an arousal response.

There are five types of G-protein–coupled dopamine receptors, broadly divided into two classes: D1-like and D2-like.²⁹ The D1 subfamily includes the D1 and D5 receptors, which are postsynaptic and mediate excitation by activating G-proteins that stimulate cyclic adenosine monophosphate synthesis. Stimulation of the D1 receptor depolarizes neurons ascending to the thalamus, the lateral hypothalamus, and the basal forebrain and neurons descending to the dorsal raphe nucleus and the locus ceruleus.¹⁶ Some of these neuronal groups project to the

nonspecific thalamocortical system, whereas others project through the ventral extrathalamic pathway, both of which stimulate cortical activation.³⁰ A variety of specific D1 agonists have been shown to increase wakefulness and spontaneous grooming, while reducing both rapid eye movement and nonrapid eye movement sleep.^{31,32} It was previously reported that administration of a D1 receptor agonist, SKF-38393, decreases time to emergence from pentobarbital anesthesia in rabbits³³ and rats.³⁴ Taken together with our finding that chloro-APB induces restoration of righting and electroencephalogram changes consistent with arousal during continuous isoflurane general anesthesia, it is likely that a D1 receptor-mediated arousal mechanism plays an important role in emergence from general anesthesia.

The D2-like class of dopamine receptors (which includes D2, D3, and D4 receptors) mediate inhibition *via* activation of G-proteins that inhibit cyclic adenosine monophosphate synthesis, suppress calcium currents, and activate potassium currents.¹⁶ Unlike D1 receptors, D2 receptors are found both pre- and postsynaptically and have a more complicated effect on arousal. Biphasic, dose-dependent effects on sleep have been observed,³⁵ with decreased wakefulness occurring at low doses of quinpirole (0.015 mg/kg) and increased wakefulness with concomitant reductions in rapid eye movement and nonrapid eye movement sleep occurring at higher doses (1 mg/kg).³⁶ It has been hypothesized that the effects at low doses of D2 agonists are mediated through activation of the presynaptic autoreceptors, whereas the increase in wakefulness after larger doses depends on activation of the postsynaptic D2 receptors.¹⁶ In the current study, large doses of quinpirole (5 mg/kg IV) produced no appreciable arousal effect in rats anesthetized with isoflurane. In previous studies using rabbits and rats anesthetized with pentobarbital, the same dose of quinpirole failed to reduce time to emergence,³³ which is consistent with our results. Although D2 agonists have been shown to affect the sleep-wake cycle, the arousal-promoting effects may be too weak to induce arousal during general anesthesia.

The VTA and SNc are the two major sources of dopamine in the brain.¹⁶ The nigrostriatal pathway that projects from SNc to the striatum is a component of the basal ganglia that is crucial for movement control,³⁷ and loss of SNc neurons leads to Parkinson disease. There are two main pathways that arise from the VTA: the mesolimbic pathway and the mesocortical pathway. The mesolimbic pathway that projects to the nucleus accumbens, amygdala, and hippocampus plays a key role in processing reward, motivation, emotion, and reinforcement.³⁸ The mesocortical pathway, which has a major projection to the prefrontal cortex, complements the function of the mesolimbic pathway and aids in cognition.³⁹

Dopaminergic neurons in the VTA and the SNc innervate brain areas involved in sleep regulation, including the serotonergic cells of the dorsal raphe nucleus, the noradrenergic

cells of the locus ceruleus, the cholinergic cells of the pedunculo-pontine and laterodorsal tegmental nuclei, and the basal forebrain and neurons that modulate behavioral states in the thalamus.¹⁶ In turn, these areas, as well as orexin-containing neurons located in the lateral hypothalamus, have reciprocal inputs to the VTA and SNc. Electrolytic lesions of the VTA and SNc in cats have been reported to induce a “total lack of behavioral arousal,”¹⁹ and selective loss of dopamine in mice causes marked hypoactivity.²⁰ All of these data support the idea that dopaminergic neurons in the VTA or SNc are important for behavioral arousal.

However, the specific role of dopamine in arousal remains controversial, because dopaminergic neurons in the VTA and SNc do not significantly change their mean firing rates during the sleep-wake cycle,^{40,41} and neurotoxic lesions of the ventral midbrain (which includes the VTA and SNc) have been reported to cause contradictory insomnia and hyperactivity.⁴² Recently, a group of wake-active dopaminergic neurons was identified in the ventral periaqueductal gray area, and it has been suggested that these cells may be important for regulating arousal.⁴³ However, further characterization of these neurons has not been reported. More studies are needed to determine which population of dopaminergic neurons is responsible for the arousal effects that lead to active emergence from general anesthesia.

In summary, the results of the current study demonstrate that activation of D1 dopamine receptors is sufficient to induce emergence from isoflurane general anesthesia, whereas activation of D2 receptors does not produce an arousal response under identical experimental conditions. These findings suggest that methylphenidate-induced arousal during general anesthesia is mediated, at least in part, by activation of D1 receptors. D1 receptors may be a rational target for the development of novel therapeutic agents that induce emergence from general anesthesia.

Appendix

Bayesian Logistic Regression

To define our Bayesian logistic regression model, we note that our experiment used the same six rats at each dose level. However, the dose levels were studied in random order, and the experiments within a given rat were separated by 3 days. Therefore, a reasonable assumption is that the responses are independent. Let x_k be the k^{th} dose level of chloro-APB. These are $x_1 = 0$, $x_2 = 0.03$, $x_3 = 0.3$ and $x_4 = 3$ mg, where the zero dose is normal saline. Let $z_k = \log(x_k)$ where for $x_1 = 0$ we take $x_1 = 0.003$ to avoid problems with an undefined number. Let n_k be the number of righting response observed from the six animals at the k^{th} dose level. Assume the logistic regression model⁴⁴

$$\log p_k(1 - p_k)^{-1} = \theta_1 + \theta_2 z_k \quad (1)$$

It follows that the joint density of the data $n = (n_1, n_2, n_3, n_4)$ or equivalently the likelihood of $\theta = (\theta_1, \theta_2)$ is

$$f(n | \theta, z) = \prod_{k=1}^4 p_k^{n_k} (1 - p_k)^{6 - n_k} \quad (2)$$

where $z = (z_1, z_2, z_3, z_4)$. If we assume $f(\theta)$, a prior density for θ , then by Bayes' rule the posterior density of θ is

$$f(\theta | n, z) = \frac{f(\theta)f(n | \theta, z)}{f(n | z)} \quad (3)$$

where $f(n | z) = \int f(\theta)f(n | \theta, z)d\theta$ is the normalizing constant. We approximate the posterior density $f(\theta | n, z)$ as the Gaussian density whose mean is $\hat{\theta}$, the maximum likelihood estimate of θ , and whose covariance matrix is $I(\hat{\theta})^{-1}$, the inverse of the observed Fisher information matrix.²⁵

This approximation of the posterior density is equivalent to computing the Gaussian approximation to the posterior under the assumption that the prior density of θ is uninformative.²⁵ We estimated $\hat{\theta}$ using the Matlab logistic regression procedure.

Bayesian Monte Carlo Analysis

Given the approximate posterior density $f(\theta | n, z)$, we compute the posterior densities $f(p_k - p_1 | n, z)$ for $k = 2, 3, 4$, by using the following Monte Carlo algorithm:

Draw θ from $f(\theta | n, z)$.

Compute $p_k = [1 + \exp(\theta_1 + \theta_2 z_k)]^{-1} \exp(\theta_1 + \theta_2 z_k)$ and $p_1 = [1 + \exp(\theta_1 + \theta_2 z_1)]^{-1} \exp(\theta_1 + \theta_2 z_1)$.

Compute $p_k - p_1$.

Do 1 to 3 10,000 times.

The histogram of the 10,000 $p_k - p_1$ values is a Monte Carlo approximation to the posterior density $f(p_k - p_1 | n, z)$. The lower and upper limits of the 95% Bayesian credibility (confidence) interval are the 250th smallest value and the 9,750th smallest value in the Monte Carlo sample, respectively. We compute the probability that $p_k > p_1$ as $\Pr(p_k > p_1 | n, z) \doteq \ell / 10,000$, where ℓ is the number of times that $p_k > p_1$ in the Monte Carlo sample.^{21,22}

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Tonalgia, “the Most Satisfactory Local Anesthetic”



Alarmed by the rising frequency of reported anesthetic accidents in the 1890s, American dentists began exploring ways to avoid using nitrous oxide, ether, or chloroform on their patients. Among a crowd of proprietary local anesthetics marketed to these professionals was Tonalgia. The floral trade card (above) touted Tonalgia as “the most satisfactory local anesthetic ever used.” Tonalgia’s active ingredient was a uniquely vasoconstricting local anesthetic ... named cocaine. (Copyright © the American Society of Anesthesiologists, Inc.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.