Paradoxical Emergence

Administration of Subanesthetic Ketamine during Isoflurane Anesthesia Induces Burst Suppression but Accelerates Recovery

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ABSTRACT

Background: Promoting arousal by manipulating certain brain regions and/or neurotransmitters has been a recent research focus, with the goal of trying to improve recovery from general anesthesia. The current study tested the hypothesis that a single subanesthetic dose of ketamine during isoflurane anesthesia would increase cholinergic tone in the prefrontal cortex and accelerate recovery.

Methods: Adult male rats were implanted with electroencephalography electrodes (frontal, parietal, and occipital cortex) and a microdialysis guide cannula targeted for the prefrontal cortex. After establishing general anesthesia with isoflurane, animals were randomly assigned to receive a saline control or ketamine injection. When isoflurane was discontinued nearly 90 min after drug or saline administration, recovery from anesthesia was measured by experimenters and blinded observers. During the entire experiment, electrophysiologic signals were recorded and acetylcholine was quantified by high-performance liquid chromatography with electrochemical detection.

Results: A single dose of subanesthetic ketamine caused an initial 125% increase in burst suppression ratio (last isoflurane sample: 37.48 ± 24.11% *vs.* isoflurane after ketamine injection: 84.36 ± 8.95%; $P < 0.0001$), but also a significant 44% reduction in emergence time (saline: 877 ± 335 s *vs.* ketamine: 494 ± 108 s; $P = 0.0005$; n = 10 per treatment). Furthermore, ketamine caused a significant 317% increase in cortical acetylcholine release (mean after ketamine injection: 0.18 ± 0.16 pmol *vs.* ketamine recovery: 0.75 ± 0.41 pmol; $P = 0.0002$) after isoflurane anesthesia was discontinued.

Conclusions: Administration of subanesthetic doses of ketamine during isoflurane anesthesia increases anesthetic depth but—paradoxically—accelerates the recovery of consciousness, possibly through cholinergic mechanisms. **(Anesthesiology 2017; 126:482-94)**

THERE has been a recent focus on the neurobiology of emergence from anesthesia, including attempts to reverse anesthetic effects or accelerate recovery through arousal-promoting interventions.[1–8] In terms of reversing anesthetic effects, manipulations of the thalamus,[1,2] the cholinergic system,[9] and the dopaminergic system[5] have predominated. Nicotine infused in central median thalamus has been shown to reverse the effects of sevoflurane,[1] while infusing an antibody to a voltage-gated potassium channel in the same region reverses both sevoflurane and desflurane anesthesia.[2] Increasing cholinergic tone through acetylcholinesterase inhibitors has been shown to induce electroencephalographic effects related to arousal and has reversed both sevoflurane- and propofol-induced unconsciousness in humans.[10,11] Most recently, there has been a focus on dopaminergic modulation through the administration of methylphenidate,[3,12,13] dopamine agonists,[4] or direct electrical stimulation of the ventral tegmental area[5]; Solt and colleagues have demonstrated that these pharmacologic and nonpharmacologic interventions induce

What We Already Know about This Topic

- Cholinergic activation can facilitate arousal under general anesthesia. Ketamine has several neural effects that may contribute to arousal, including increasing cholinergic tone and high-frequency cortical activity. The authors hypothesized that administration of subanesthetic ketamine during isoflurane anesthesia would accelerate emergence.

What This Article Tells Us That Is New

- In a rat model of isoflurane anesthesia, a single dose of ketamine markedly increased the burst suppression ratio but, paradoxically, decreased emergence time compared to saline. Ketamine enhanced cholinergic tone and high-frequency cortical activity during recovery, which might be the mechanism by which it facilitates emergence. These findings are unique because ketamine both deepens anesthesia and accelerates recovery.

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reanimation in animals exposed to continuous propofol or isoflurane. In terms of accelerating recovery after anesthetic discontinuation, caffeine has been recently shown to reduce the time to emergence from both propofol and isoflurane.[14]

What has not been explored is the modulation of an anesthetic state to more closely resemble natural sleep. In humans, slow-wave sleep predominates earlier in the night, with increasing frequency and bout length of rapid eye movement sleep that builds to a crescendo as emergence from sleep approaches.[15,16] Since rapid eye movement sleep is characterized by increased cortical activity and cholinergic tone,[16,17] it has been proposed that it prepares the brain for wakefulness.[15,18] This has also been suggested to be the case during ontogeny, as the proportion of time spent in rapid eye movement sleep is highest during the third trimester; the ontogenetic theory suggests that the activation patterns *in utero* prepare the brain for sensory input at birth.[15,19–21]

Ketamine anesthesia shares certain traits with rapid eye movement sleep, including high-frequency cortical activity, high cholinergic tone in the cortex, and dream states.[22–24] We hypothesized that administration of subanesthetic ketamine during isoflurane anesthesia would increase cholinergic tone in prefrontal cortex, increase high-frequency cortical activity, and accelerate emergence once isoflurane was discontinued.

Materials and Methods

Animals

All experiments were approved by the University of Michigan Committee on Use and Care of Animals (Ann Arbor, Michigan) and were conducted in accordance with The Public Health Service Policy on Humane Use and Care of Laboratory Animals (National Institutes of Health Publication 80-23). Adult (2- to 3-month-old), male Sprague–Dawley rats (n = 20; Harlan/Envigo, USA) were housed in identical chambers under a 12-h:12-h light:dark cycle with continuous access to food and water. Animals were randomly divided into ketamine or saline groups.

Surgical Procedures

At least 1 week before the experiment, animals underwent head cap surgery. General anesthesia was induced with 4% isoflurane (Piramal Critical Care, Inc., USA), which was measured continuously by spectrometry (Cardiocap/5; Datex-Ohmeda, USA). Once anesthetized, animals were placed in a Kopf Model 962 stereotaxic frame (David Kopf Instruments, USA) with a Kopf model 920 rat adapter and rat anesthesia mask (Kopf Model 906). Isoflurane delivery was then reduced to 2.5%. During the entire surgery, vital signs were taken every 15 min and a heating pad (Far Infrared Warming Pad; Kent Scientific Corp., USA) was used to keep the body temperature consistent (± 0.6°C). Rats received a single dose of the nonsteroidal antiinflammatory drug carprofen (5 mg/kg, subcutaneous; Rimadyl; Zoetis, Inc., USA). Craniotomy was then performed, and a CMA/11 guide cannula (CMA Microdialysis; Harvard Apparatus, USA) was implanted above the prefrontal cortex at 3 mm anterior to bregma, 0.5 mm lateral to the midline, and 3.2 mm ventral to the skull surface.[25] The guide cannula was placed either on the left or on the right side of the brain to ensure that any observed effect was not due to brain laterality. In addition, six electrodes to record electroencephalogram made out of stainless steel wire (A-M Systems, Inc., USA) were placed at the following stereotaxic coordinates: one frontal, opposite side of the guide cannula, at 3.0 mm anterior to bregma and 2.5 mm lateral to the midline; two parietal at 4 mm posterior to bregma and 2.5 mm lateral to midline on both sides; two occipital at 8 mm posterior to bregma and 2.5 mm lateral to midline on both sides; and one on the nasal commissure as the reference electrode. In addition, rats received two electromyography electrodes placed into the neck muscle to evaluate muscle movement during emergence. All electroencephalogram electrodes were plugged into a plastic multichannel electrode pedestal (8K00022980IF and 8K00000363DC; Plastics One, USA). Electromyography electrodes were plugged into a second pedestal. Guide cannula and electrode arrays were fixed to the skull using dental acrylic (Stoelting Co., USA). Animals were then allowed to recover from surgery for 7 days and were thereafter conditioned to being housed in a Plexiglas recording chamber (Raturn; Bioanalytic Systems, Inc., USA).

Electroencephalographic Data Acquisition

Electroencephalogram signals from 10 saline- and 10 ketamine-treated animals were amplified (5,000×) and filtered (0.1 to 300 Hz; Grass Amplifier Model 15 LT; 15A54 Quad Amplifier, USA). Data were digitized and recorded at a sampling rate of 1,000 Hz using AcqKnowledge software (V4.1, MP150; Biopac Systems, Inc., USA).

Segmentation and Quantification of Electroencephalographic Burst and Suppression

The raw electroencephalogram signals were exported into MATLAB (version 2015b; MathWorks, Inc., USA) and downsampled to 250 Hz for further analysis. The spectral analysis was performed with fast Fourier transform to provide information on frequency content of bursts and artifacts in the electroencephalogram signals. The data were then transformed in three steps: (1) signals were bandpass filtered between 5 and 30 Hz through a four-order Butterworth filter using a zero-phase forward and reverse algorithm; (2) the Hilbert transform of the bandpass signal was used to calculate the instantaneous amplitude to approximate the high-frequency power,[26] which was further smoothed with a moving average filter of 200 ms; (3) a threshold calculated

from the manually labeled suppression periods (mean plus 3 or 4 SD based on visual inspection) was applied to the transformed signal to yield a binary series of burst and suppression states for each rat. In this study, the minimum length of burst and suppression periods were set to 0.5 s, and burst suppression ratio was calculated as percentage of time spent in suppression of each 1-min binary series. Electroencephalogram epochs were analyzed from the beginning through the end of the 12.5-min duration of acetylcholine sample collection to ensure parallel information for acetylcholine sampling and electroencephalogram recording. To validate our results, we applied two alternative methods (variance-based method[27] and nonlinear energy operator–based method[28]) to replace the amplitude approach in step 2. In the first alternative method, the SD of the bandpassed signal was computed for each 200-ms interval with a 150-ms overlap; in the second method, the energy of the bandpassed signal was calculated by the nonlinear energy operator and smoothed using a 200-ms moving average. Since the three different types of analysis did not show any differences, we report only the first analysis for this study. Further, the detection methods were applied to different channels of electroencephalogram signals (monopolar: frontal, left/right parietal, left/right occipital; bipolar: left–right parietal, left–right occipital, left parietal–left occipital, and right parietal–right occipital), and the channel was selected to be analyzed based on visual inspection. Frontal channel was selected in 18 of 20 rats, and left-parietal channel was used in the other two rats. No significant heterogeneity across the five recorded cortical sites was found. The frequency bandwidths in step 1 were varied: 5 to 30, 5 to 50, and 3 to 125 Hz,[26] and the difference of less than 8 Hz and greater than 47 Hz.[28] We found that 5 to 30 Hz and 5 to 50 Hz analyses yielded similar results, which were superior to the other two cases in almost all rats (in agreement with visual inspection). Furthermore, different values were tested for the threshold of mean + c × SD in step 3: c = 3 to 7 and the one in best agreement with visual inspection was selected; c = 3 for 8 and c = 4 for 12 of 20 rats.

Power Spectral Analysis

The power spectrogram was calculated based on discrete Fourier transform with 2-s segment size and 1 s overlapping for each frequency bin (0.5 to 250 Hz with 0.5-Hz bin size; *spectrogram.m* in MATLAB signal processing toolbox [MathWorks, Inc.]). For each rat, the absolute power was calculated in each channel and the spectrogram from the same channel used in the raw signal and expressed in a log scale.

To relate the electroencephalogram power with changes of acetylcholine level during the whole experiment, the spectrogram was estimated in nonoverlapped 10-s bins using the Welch method in order to achieve a better estimation of spectral density function. Furthermore, normalized power was calculated as the fraction of a specific frequency power in the total power over all frequency bands and averaged across all electroencephalogram channels; the averaged power values were calculated for the following frequency bands: delta (0.5 to 4 Hz), theta (4 to 10 Hz), alpha (10 to 15 Hz), beta (15 to 25 Hz), low gamma (25 to 55 Hz), medium gamma (65 to 125 Hz), high gamma (125 to 175 Hz), and ultra-high gamma (185 to 250 Hz) at each studied sample, with the mean and SD values across all rats receiving either ketamine or saline.

Quantification of Acetylcholine Release in the Prefrontal Cortex

Acetylcholine release (pmol/12.5 min) in the prefrontal cortex was measured from nine saline-treated and eight ketamine-treated animals using high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems, Inc., USA, and Showa Denko America, Inc., USA). Chromatograms were digitized and quantified using LC Real Time Analysis Program (Showa Denko America, Inc., USA) in reference to a seven-point standard curve ranging from 0.05 to 1.0 pmol. CMA/11 microdialysis probes (CMA Microdialysis; Harvard Apparatus) were perfused continuously with Ringer's solution (147 mM NaCl, 2.4 mM $CaCl_2$, 4.0 mM KCl, 10 μM neostigmine; pH 6.0 ± 0.2). Salts for Ringer's solution were purchased from Sigma-Aldrich (USA). Flow rate was held constant at 2.0 μl/min using a CMA/400 syringe pump (CMA Microdialysis; Harvard Apparatus). The microdialysis probe had a cuprophane membrane of 1-mm length, 0.24-mm diameter, and a molecular cutoff of 6,000 Da. Microdialysis samples were collected every 12.5 min (25 μl/sample) for subsequent quantification of acetylcholine release from the prefrontal cortex. To ensure that changes seen in acetylcholine release were not due to an artifact of intraexperimental variations in probe membrane function, *in vitro* probe recovery levels were compared between pre- and postexperimental measurements.

Experimental Design

Figure 1A illustrates the experimental design. On the day of the experiment, a microdialysis probe was inserted through the guide cannula into the prefrontal cortex, and animals were connected to the electroencephalogram recording system and placed into a modified Raturn (fig. 1B). The modification allows the Raturn to be sealed such that inhaled anesthetics can be administered while the animal behaves freely. During the entire experiment, acetylcholine samples as well as vital signs were collected every 12.5 min with simultaneous electroencephalogram signal acquisition. During the time period spanning the first three acetylcholine sample collection points (37.5 min), animals were kept awake with gentle handling. This ensures that all animals started the experiment in the same baseline state. Experiments started at approximately 12:00 PM (± 1 h). After the baseline wake phase, the Raturn was sealed and filled with isoflurane in high-flow oxygen (10

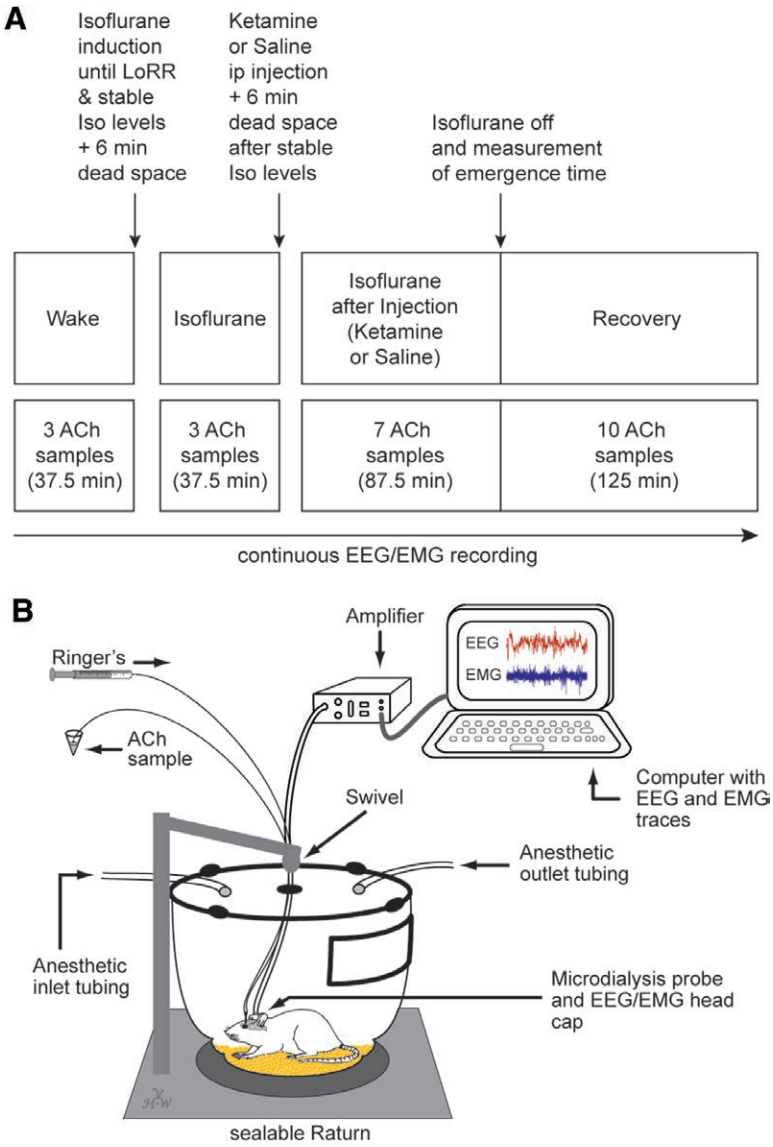

Fig. 1. Study design. (*A*) Schematic timeline illustrating the procedure for each animal in this study. Text above the *boxes* describes the manipulations performed during the experiment. The *upper row of boxes* describes the different states the animal is in, while the *lower row of boxes* illustrates the timeline and number of acetylcholine (ACh) samples collected during the different time points. Throughout the entire experiment, electroencephalography (EEG) and electromyography (EMG) were recorded. (*B*) Schematic shows a modified Raturn in which the animal can freely behave while EEG/EMG data are recorded and microdialysis sampling is performed during various levels of arousal. ip = intraperitoneal; Iso = isoflurane; LoRR = loss of righting response (surrogate for unconsciousness).

l/min) until inlet and outlet of the Raturn were reading 2.5% isoflurane for 2 min. After anesthetic induction, as defined by the loss of righting reflex, the animal was placed on its back and a temperature probe was inserted rectally through a door in the Raturn. Isoflurane levels were then kept at 1.5% throughout the rest of the experiment (note that 1.4% is the minimum alveolar concentration for isoflurane in rodents).[29] After a dead space collection period of 6 min (the time it takes the sample to go through the probe and tubing), three prefrontal cortex acetylcholine samples were collected during isoflurane anesthesia. After the third sample, the Raturn door was opened and 25 mg/kg ketamine or an equivalent volume of saline was injected into the intraperitoneal space of the animal. This is significantly below anesthetic dosing levels, since 150 mg/kg (intraperitoneal) is required to induce loss of righting reflex in rats.[24] After stable isoflurane levels and 6 min of dead space, seven samples were collected during isoflurane-after-ketamine or isoflurane-after-saline injection. After the 87.5 min of sample collection, isoflurane was discontinued and the time it took for the animal to right itself (the surrogate for emergence time) was measured while 10 acetylcholine samples during this recovery period were collected. Emergence time was determined by two independent observers, at least one of which was blinded to the experimental condition.

Histologic Analysis

Three to seven days after the experiment, rats were deeply anesthetized and decapitated. Brains were removed, frozen, and sectioned coronally at a thickness of 40 μm. Serial prefrontal cortex sections were slide-mounted, dried, fixed with paraformaldehyde vapor at 80°C, and stained with cresyl violet. Tissue sections were compared to a rat brain atlas[25] to localize microdialysis sites.

Statistical Analysis

All data were evaluated with the input from the Center for Statistical Consultation and Research at the University of Michigan (Ann Arbor, Michigan). Sample size was estimated as n = 10 per treatment group based on previous experiments with similar design.[24] In addition, after initial experiments, sample size was statistically calculated for 80% power with a two-tailed comparison. Burst suppression ratio was analyzed using a two-way ANOVA with Tukey multiple comparisons *post hoc* test. Changes in electroencephalogram power (for each frequency band), breathing rate, and core body temperature were analyzed using a two-way ANOVA with Sidak multiple comparisons test. Emergence time from anesthesia was analyzed using a Mann–Whitney U test. Drug effect on acetylcholine release was analyzed using a two-way ANOVA with Tukey multiple comparisons *post hoc* test for mean value comparisons and a two-way ANOVA with Bonferroni correction for timeline comparisons. Statistical analysis was performed using SAS v9.2 (SAS Institute, Inc., USA) and Prism v7.0 programs (GraphPad Software, Inc., USA). $P < 0.05$ was considered statistically significant.

Results

Histology

Serial coronal tissue sections of each brain were used to identify the stereotaxic coordinates of every microdialysis site relative to a rat brain atlas.[25] Due to broken microdialysis probes, acetylcholine release could not be measured in three animals, two from the ketamine and one from the saline group. The histologic results described in this article are from 17 rats in which microdialysis sites were localized within the prefrontal cortex (fig. 2).

Effect of Ketamine on Electroencephalographic Burst Suppression

Burst suppression, a sign of deep anesthesia, was seen more frequently and with longer phases in ketamine-treated animals. Figure 3, A and B, shows representative electroencephalogram traces and power spectrograms for saline and ketamine, respectively, emphasizing the transition into burst suppression after ketamine was given. The traces show the timeline from the last isoflurane sample during the actual injection phase to the first two isoflurane samples after injection. Burst suppression ratio was significantly increased after injection of ketamine (fig. 3C). Two-way ANOVA revealed a significant difference between saline and ketamine, $F(1,54) = 47.78$, $P < 0.0001$; time points (last isoflurane-only sample *vs.* first and second isoflurane after injection [saline *vs.* ketamine] sample), $F(2,54) = 5.08$, $P = 0.0095$; and interaction, $F(2,54) = 12.63$; $P < 0.0001$. Tukey multiple comparisons *post hoc* test demonstrated significant differences between the last isoflurane-only sample and the first isoflurane-after-ketamine sample ($P < 0.0001$) causing a significant 125% increase in burst suppression ratio. A significant 107% increase in burst suppression ratio was seen comparing the last isoflurane sample with the second isoflurane-after-ketamine sample ($P = 0.0005$). Comparisons between the last isoflurane-only sample and the first or second isoflurane-after-saline samples did not show any differences. The first and second isoflurane-after-ketamine samples showed a significant increase in burst suppression ratio when compared to isoflurane-after-saline samples 1 and 2 ($P < 0.0001$ for both comparisons).

Effect of Ketamine on Emergence Time

Emergence time was defined as the time between the discontinuation of isoflurane anesthesia and the return of righting reflex. A second person who was blinded to the treatment reanalyzed and verified the wake-up time independently through video and electroencephalogram/electromyography recordings. Emergence time did not vary significantly ($P = 0.9861$) between the two persons analyzing the data and reported time values differed 14 ± 15 s, on average. Giving a subanesthetic dose of ketamine (25 mg/kg) during isoflurane anesthesia significantly ($P = 0.0005$) decreased emergence time by 44% from 877 ± 335 s for saline-treated to 494 ± 108 s for ketamine-treated animals (fig. 4).

Effect of Ketamine on Electroencephalographic Power during Recovery

To evaluate whether ketamine caused changes during the recovery phase, electroencephalogram power was analyzed using a two-way ANOVA followed by Sidak multiple comparison test for each frequency band (fig. 5, A–H). Table 1 summarizes the statistical results. Ketamine treatment mainly affected electroencephalogram power of higher frequencies (low gamma, medium gamma, and high gamma) causing a significant increase ($P < 0.0001$) for each of the three frequencies during the beginning of the recovery phase after isoflurane was discontinued (fig. 5, F–H).

Effect of Ketamine on Breathing Rate and Body Temperature

To verify that the decrease in emergence time was not caused by an increase in respiration, we analyzed the breathing rate, which was measured every 12.5 min throughout the entire experiment. Using a two-way ANOVA, no significant difference in breathing rate between saline- and ketamine-treated animals was found ($P = 0.1672$; fig. 6A).

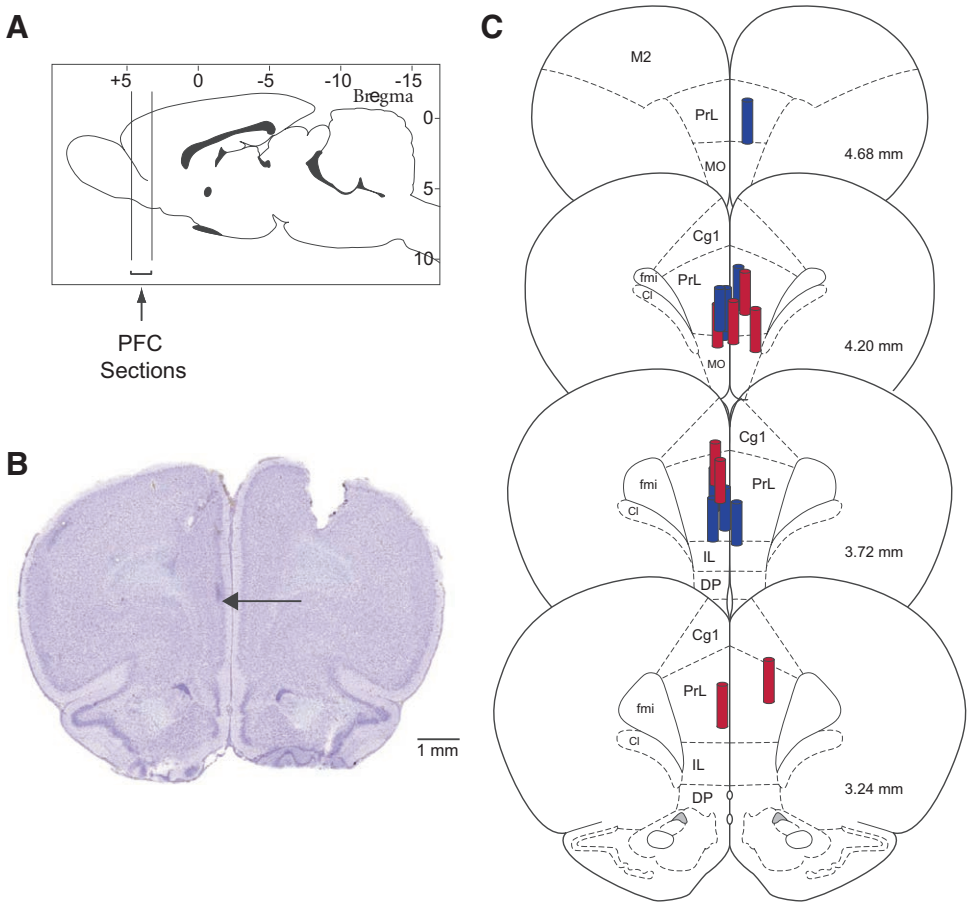

Fig. 2. Histologic confirmation of dialysis sites within the prefrontal cortex. (*A*) *Vertical lines* on a sagittal diagram of a rat brain[25] depict the anterior-to-posterior range of the microdialysis sites within the prefrontal cortex. (*B*) A cresyl violet–stained coronal brain section shows a typical microdialysis site within the prefrontal cortex. The *arrow* marks the most ventral position of the microdialysis membrane. (*C*) The coronal plates were modified from a rat brain atlas[25] to illustrate the location of the microdialysis sites within the prefrontal cortex. The size of the dialysis membrane is indicated by the cylinder drawn to scale, relative to the brain. Microdialysis probes of ketamine-treated animals are in *red* and those of saline-treated animals are in *blue*. Cg1 = cingulate cortex area 1; Cl = caudal interstitial nucleus of the medial longitudinal fasciculus; DP = dorsal peduncular cortex; fmi = forceps minor of the corpus callosum; IL = infralimbic cortex; M2 = secondary motor cortex; MO = medial orbital cortex; PFC = prefrontal cortex; PrL = prelimbic cortex.

In addition, body temperature was measured during general anesthesia to evaluate the effects of ketamine. After the animal lost consciousness, a rectal temperature probe was inserted and measurements of body core temperature were taken every 12.5 min (at each acetylcholine sampling) until the animal emerged from anesthesia. A two-way ANOVA showed no significant difference between saline- and ketamine-treated animals in core body temperature ($P = 0.8292$; fig. 6B).

Effect of Ketamine on Acetylcholine Release

A two-way ANOVA revealed a significant difference in acetylcholine release from the prefrontal cortex across the various time points (wake, isoflurane, isoflurane after injection, and recovery; $F(3,60) = 16.85$, $P < 0.0001$) and in the time points and treatment (saline *vs.* ketamine) interaction, $F(3,60) = 2.884$, $P = 0.0430$ (fig. 7A). Tukey multiple comparisons test demonstrates that acetylcholine release from the prefrontal cortex is significantly decreased (around 70%) when animals are under isoflurane anesthesia (P [saline] = 0.0095, P [ketamine] = 0.0139). Neither saline nor ketamine injection changed the suppressed acetylcholine values during isoflurane anesthesia, which remained significantly lower in comparison to the wake phase (P [saline] = 0.0095), P [ketamine] = 0.0365). After isoflurane was discontinued, ketamine caused a significant ($P < 0.0002$) 317% increase in acetylcholine release during the recovery phase compared with exposure to isoflurane. During this recovery phase, ketamine induced a significant ($P < 0.0283$) 103% increase in acetylcholine release when compared with animals treated with saline. Overall, ketamine increased acetylcholine release by 34% during the recovery phase when compared with the wake phase, while animals treated with saline showed a 35% decrease in acetylcholine release during recovery compared with the wake phase.

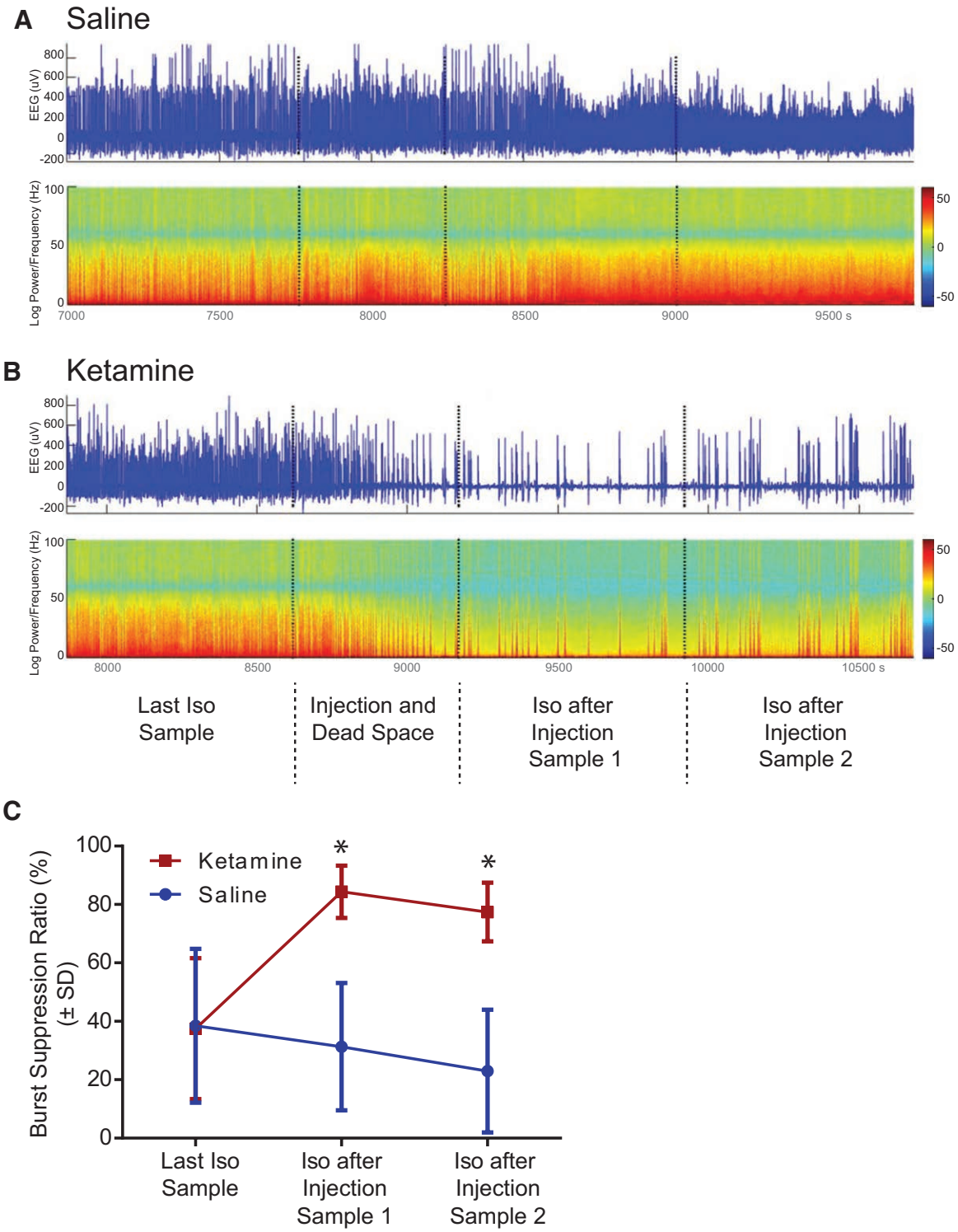

Fig. 3. Effect of ketamine injection on electroencephalogram burst suppression ratio. (*A* and *B*) Representative electroencephalographic (EEG) traces (*upper*) and power spectrogram (*lower*) during the transition phase from the last isoflurane (Iso)-only sample up to the second Iso-after-injection sample for saline- (*A*) and ketamine-treated (*B*) animals. The EEG traces and spectrograms show four different phases divided by the *vertical dotted lines*. *Color bar* indicates normalized power in log scale in decibel (dB). (*C*) Rats treated with ketamine showed a significant 125% increase (*) in burst suppression ratio during Iso-after-injection sample 1 and a 107% increase (*) during Iso-after-injection sample 2 when compared to the last Iso-only sample in the ketamine group (*red* data points). In addition, ketamine-treated animals showed a significant enhancement in burst suppression ratio compared to saline-treated animals (*red vs. blue* data points). Graph shows data as burst suppression ratio in percent ± SD.

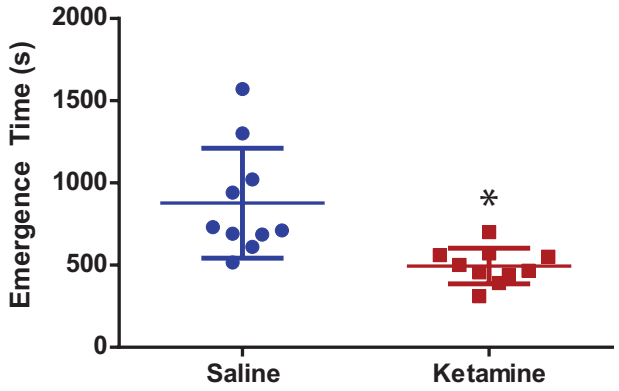

Fig. 4. Emergence time. Animals receiving ketamine showed a significant 44% decrease in the time required to emerge from isoflurane anesthesia after it was discontinued. *Blue* = saline, *red* = ketamine; statistical significance with *$P < 0.05$.

A timeline analysis (fig. 7B) for acetylcholine release using a two-way ANOVA revealed significant differences in treatment, saline *versus* ketamine, $F(1, 345) = 43.08$, $P < 0.0001$; in the time points, $F(22, 345) = 12.42$, $P < 0.0001$; and in the interaction between drug treatment and time points, $F(22, 345) = 3.192$, $P < 0.0001$. Bonferroni multiple comparisons *post hoc* test reveals significant increases in acetylcholine release in the ketamine-treated group during recovery samples 1 ($P = 0.0222$), 2 ($P < 0.0001$), 3 ($P = 0.0009$), 4 ($P = 0.0333$), and 5 ($P = 0.01$), which represent the first 62.5 min after discontinuing isoflurane anesthesia.

Discussion

The data presented in this study demonstrate that a single intraperitoneal injection of subanesthetic ketamine during isoflurane anesthesia causes (1) a significant increase in burst suppression ratio; (2) a significant reduction in emergence time once isoflurane was discontinued; (3) significant increases in high-frequency cortical activation during recovery from anesthesia; and (4) a significant increase in acetylcholine release from the prefrontal cortex during the recovery period. These findings are unique because we would expect an adjunct drug that modulates levels of consciousness to either deepen a general anesthetic state or facilitate recovery. By contrast, subanesthetic ketamine both deepens the anesthetic state and facilitates recovery. This *paradoxical emergence* also challenges the intuition that increased time spent in burst suppression necessarily results in prolonged recovery.

Based on known molecular mechanisms of ketamine and the observed microdialysis results, we suggest that effects on glutamatergic and cholinergic systems might, in part, explain the findings. Ketamine and isoflurane had a combined effect (either additive or synergistic) on the observed depth of anesthesia, as evidenced by the increased burst suppression ratio during the isoflurane-after-ketamine phase. Similar effects were described previously with the addition of ketamine to propofol administration.[30,31] This phenomenon is potentially attributable to ketamine's molecular actions as an antagonist of the glutamatergic *N*-methyl-D-aspartate receptor.[32–35] In support of this, a similar effect was described in a study by Scheller *et al.*,[36] in which the noncompetitive *N*-methyl-D-aspartate receptor antagonist MK-801 was given to isoflurane-anesthetized rabbits, resulting in electroencephalogram burst suppression.

Furthermore, isoflurane was shown to have a dominant and suppressive effect on cholinergic neurons, as evidenced by the continuous suppression of acetylcholine levels in the prefrontal cortex despite the administration of ketamine, which is well known to activate subcortical arousal centers[37] and increase cortical cholinergic tone when given alone.[24,38] The discontinuation of isoflurane allowed (to adopt a metaphor from genetics) the *derepression* of ketamine's effects, resulting in increased cholinergic tone during recovery and potentially accelerating emergence.

More than 300 million patients worldwide undergo major surgery each year, the majority with general anesthesia.[39] Although general anesthetics are powerful and effective tools to reversibly modulate levels of consciousness, prolonged or abnormal recovery from general anesthesia can have significant consequences.[40–42] Although anesthesiologists have pharmacologic tools to antagonize specific drugs acting at specific receptors (*e.g.*, benzodiazepines),[43] only recently has research focused on reversing anesthetic effects by promoting arousal systems.[1–6,8,12] This has been typically accomplished by pharmacologic manipulation using catecholamine reuptake inhibitors,[3,12] dopamine agonists,[4] central nervous system stimulants,[13] and nicotine,[1,3,4,6,12] or by electrical stimulation of specific brain regions such as the ventral tegmental area[5] or parabrachial nucleus.[8] In many of these studies, resumption of righting response (as a surrogate for emergence) was restored while the animal was still exposed to general anesthetics and a direct arousal-promoting effect was observed. The current study is unique and advances the field because it revealed that the addition of a second anesthetic drug could accelerate the recovery from the first after enhancing its effect (*i.e.*, deepening the anesthetic state). We specifically chose ketamine because it shares some traits with rapid eye movement sleep, including cortical activation, increased cholinergic tone, and dream states.[16,17,23,24,38]

Several studies indicate that acetylcholine is critical for wakefulness and cognitive function[17,44–46] and that the cholinergic arousal system is involved in the emergence from general anesthesia. Alkire *et al.*[1] showed that injection of nicotine into the central medial thalamus induced resumption of righting during sevoflurane anesthesia. In humans, physostigmine, a cholinesterase inhibitor, can reverse loss of consciousness from sevoflurane[11] and propofol[10] anesthesia. Hudetz *et al.*[9] evaluated intraventricular administration of the acetylcholinesterase inhibitor neostigmine and found

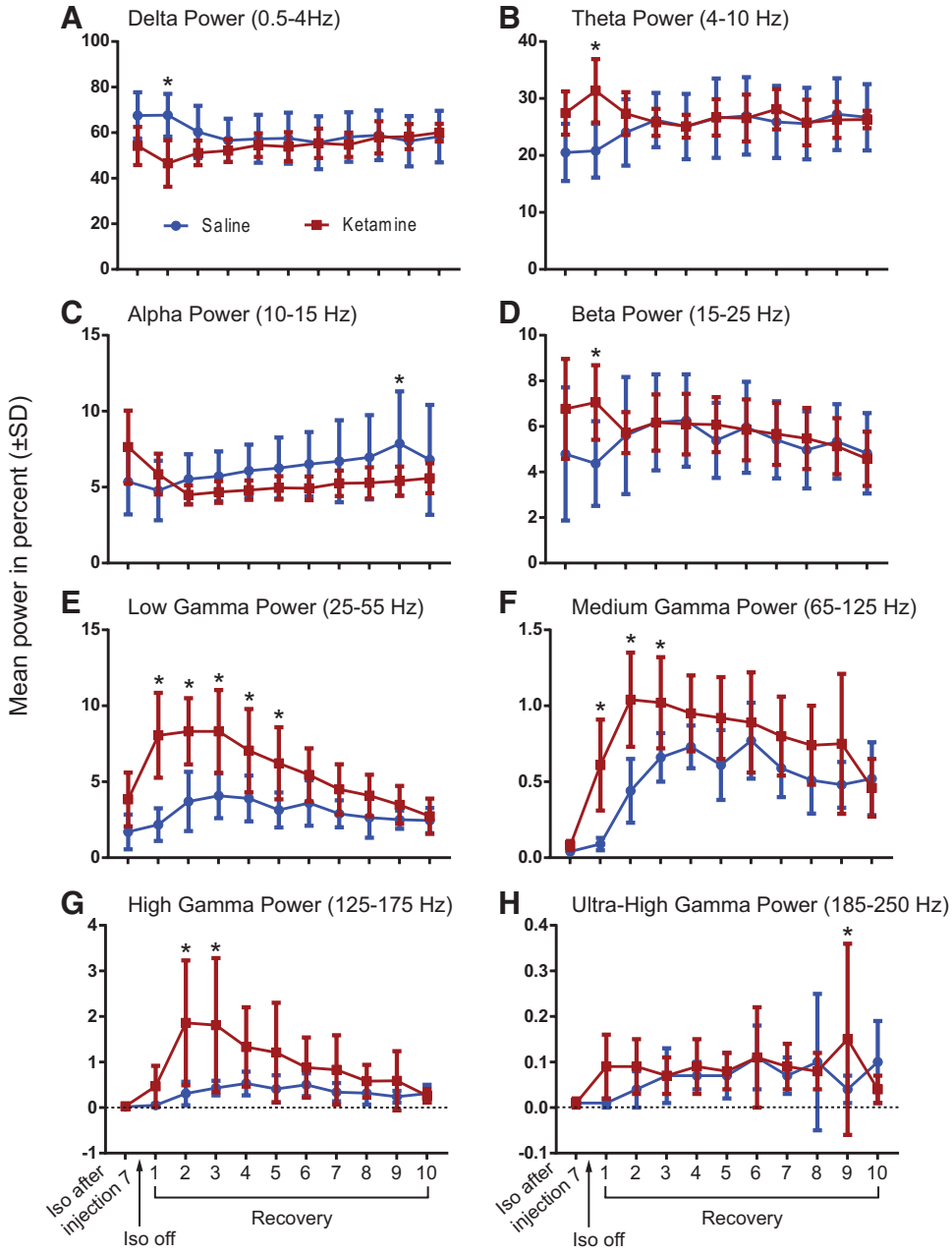

Fig. 5. Electroencephalography during recovery. Electroencephalographic spectral analysis shows the power for all frequency bands throughout the 10 recovery samples. The averaged power values were calculated for the following frequency bands: (*A*) delta (0.5 to 4 Hz), (*B*) theta (4 to 10 Hz), (*C*) alpha (10 to 15 Hz), (*D*) beta (15 to 25 Hz), (*E*) low gamma (25 to 55 Hz), (*F*) medium gamma (65 to 125 Hz), (*G*) high gamma (125 to 175 Hz), and (*H*) ultra-high gamma (185 to 250 Hz) with the mean and SD values across all the rats injected with either ketamine or saline. *Blue line* shows data from saline-treated animals; *red line* shows data from ketamine-treated animals. Statistical significance with $^*P < 0.05$. Iso = isoflurane.

an increase in cross-approximate entropy of the electroencephalogram and signs of arousal. This supports ketamine's observed effect on cortical cholinergic tone during the recovery phase as a plausible mediator of accelerated recovery.

Ketamine appears to affect *neural inertia*, defined by Friedman *et al.*[47] as a barrier to behavioral state transitions. Unlike past studies of neural inertia, ketamine appears to have dynamic effects depending on the neurochemical milieu of the brain. When ketamine is added to isoflurane anesthesia, it increases neural inertia, as evidenced by an increase in burst suppression ratio and a reduced variability in electroencephalographic state. However, ketamine reduces neural inertia after discontinuation of isoflurane, lowering resistance to the transition into wakefulness and narrowing the variability in emergence time. As such, ketamine might be a unique tool to probe new dimensions of the concept of neural inertia.

Table 1. Statistical Analysis for Electroencephalogram Power Frequencies during Recovery

	Delta (0.5–4 Hz)		Theta (4–10 Hz)		Alpha (10–15 Hz)		Beta (15–25 Hz)	
	F(DFn, DFd)	*P* Value	F(DFn, DFd)	*P* Value	F(DFn, DFd)	*P* Value	F(DFn, DFd)	*P* Value
Treatment	F(1,180) = 11.42	0.0009*	F(1,180) = 4.188	0.0422*	F (1, 180) = 20.95	<0.0001*	F (1, 180) = 2.329	0.1288
Time point	F(9,180) = 0.5377	0.8457	F(9,180) = 0.2997	0.9741	F (9, 180) = 1.504	0.1495	F (9, 180) = 1.566	0.1285
Interaction	F(9,180) = 2.961	0.0026*	F(9,180) = 2.481	0.0108*	F (9, 180) = 1.189	0.3047	F (9, 180) = 1.43	0.1782
Sidak multiple comparisons test	Saline *vs.* ketamine		Saline *vs.* ketamine		Saline *vs.* ketamine	*P* Value	Saline *vs.* ketamine	
	Recovery 1	< 0.0001*	Recovery 1	< 0.0001*	Recovery 1	0.8837	Recovery 1	0.0033*
	Recovery 2	0.1898	Recovery 2	0.7652	Recovery 2	0.9060	Recovery 2	> 0.9999
	Recovery 3	0.9459	Recovery 3	> 0.9999	Recovery 3	0.9060	Recovery 3	> 0.9999
	Recovery 4	0.9986	Recovery 4	> 0.9999	Recovery 4	0.7420	Recovery 4	> 0.9999
	Recovery 5	0.9874	Recovery 5	> 0.9999	Recovery 5	0.7061	Recovery 5	0.9859
	Recovery 6	> 0.9999	Recovery 6	> 0.9999	Recovery 6	0.4399	Recovery 6	> 0.9999
	Recovery 7	0.9932	Recovery 7	0.9761	Recovery 7	0.5824	Recovery 7	> 0.9999
	Recovery 8	> 0.9999	Recovery 8	> 0.9999	Recovery 8	0.3532	Recovery 8	0.9989
	Recovery 9	> 0.9999	Recovery 9	> 0.9999	Recovery 9	0.0319*	Recovery 9	> 0.9999
	Recovery 10	> 0.9999	Recovery 10	> 0.9999	Recovery 10	0.7925	Recovery 10	> 0.9999
	Low Gamma (25–55 Hz)		Medium Gamma (65–125 Hz)		High Gamma (125–175 Hz)		Ultrahigh Gamma (185–250 Hz)	
Two-way ANOVA	F(DFn, DFd)	*P* Value	F(DFn, DFd)	*P* Value	F(DFn, DFd)	*P* Value	F(DFn, DFd)	*P* Value
Treatment	F(1, 180) = 122.3	< 0.0001*	F(1, 180) = 60.65	< 0.0001*	F(1, 180) = 49.38	< 0.0001*	F(1, 180) = 3.58	0.0601*
Time point	F(9, 180) = 10.61	< 0.0001*	F(9, 180) = 8.266	< 0.0001*	F(9, 180) = 4.846	< 0.0001*	F(9, 180) = 0.9208	0.5083
Interaction	F(9, 180) = 5.305	< 0.0001*	F(9, 180) = 2.787	0.0044*	F(9, 180) = 3.06	0.0020*	F(9, 180) = 1.938	0.0492*
Sidak multiple comparisons test	Saline *vs.* ketamine		Saline *vs.* ketamine		Saline *vs.* ketamine		Saline *vs.* ketamine	
	Recovery 1	< 0.0001*	Recovery 1	< 0.0001*	Recovery 1	0.7918	Recovery 1	0.2143
	Recovery 2	< 0.0001*	Recovery 2	< 0.0001*	Recovery 2	< 0.0001*	Recovery 2	0.8167
	Recovery 3	< 0.0001*	Recovery 3	0.0167*	Recovery 3	< 0.0001*	Recovery 3	> 0.9999
	Recovery 4	0.0007*	Recovery 4	0.4190	Recovery 4	0.0575	Recovery 4	0.9998
	Recovery 5	0.0010*	Recovery 5	0.0645	Recovery 5	0.0575	Recovery 5	> 0.9999
	Recovery 6	0.1676	Recovery 6	0.9671	Recovery 6	0.8743	Recovery 6	> 0.9999
	Recovery 7	0.3230	Recovery 7	0.4864	Recovery 7	0.6089	Recovery 7	0.9998
	Recovery 8	0.4702	Recovery 8	0.3560	Recovery 8	0.9896	Recovery 8	0.9998
	Recovery 9	0.9088	Recovery 9	0.1643	Recovery 9	0.9212	Recovery 9	0.0200*
	Recovery 10	> 0.9999	Recovery 10	0.9999	Recovery 10	> 0.9999	Recovery 10	0.6067

Two-way ANOVA with Sidak multiple comparisons test.

*$P < 0.05$ considered significantly different.

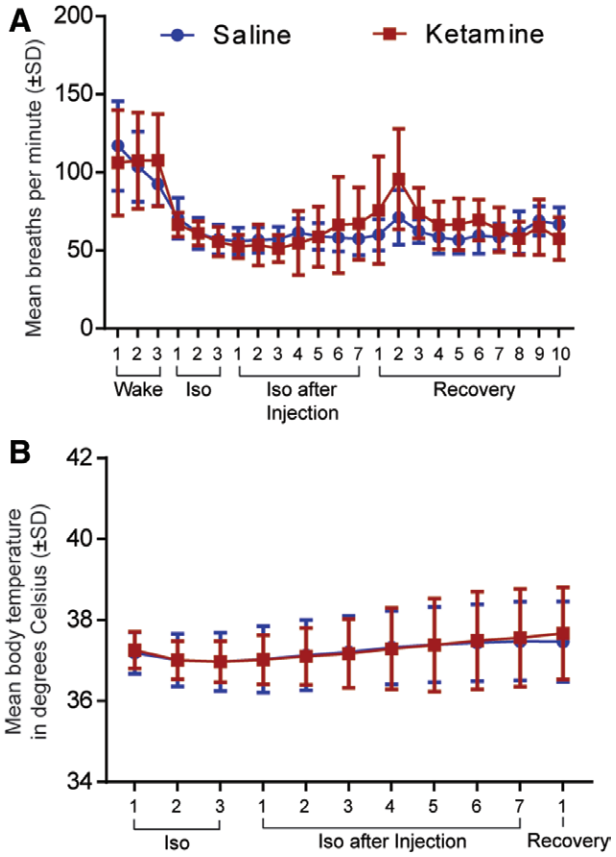

Fig. 6. Breathing rate and core body temperature are not affected by ketamine treatment. (*A*) There is no difference in breathing rate (min ± SD) between saline- (*blue*) and ketamine-treated (*red*) animals. (*B*) Body temperature (°C; ±SD) does not vary between saline- and ketamine-injected animals. Iso = isoflurane.

Previous data have shown that subanesthetic injections of ketamine in rats[48,49] and humans[50] increase electroencephalogram power in the gamma bandwidth. In this study, the electroencephalogram power increase in various gamma frequencies did not occur directly after injection of ketamine, but nearly 90 min later when isoflurane was discontinued. This effect suggests that the excitatory properties of ketamine when given alone[24] are blunted by the effect of isoflurane.[31] Interestingly, higher frequency gamma activation appears to be increased in ketamine-treated animals slightly before the animal emerges from anesthesia, while in saline-treated animals increase in gamma activity is not only less pronounced, but appears directly at the time of emergence (fig. 5; Supplemental Digital Content 1, Figure, http://links.lww.com/ALN/B364, and Supplemental Digital Content 2, Figure, http://links.lww.com/ALN/B365, showing normalized power spectrogram of all saline- and ketamine-treated animals). These data suggest that an increase in higher frequency cortical activity contributes to arousal, which is consistent with our recent findings.[51] The fact that breathing rate and core body temperature did not differ between saline- and ketamine-treated animals indicates that the reduction in emergence time is not due to pharmacokinetics (*i.e.*, increased isoflurane clearance with increased respiration) or the known sympathomimetic effects of ketamine.[52,53]

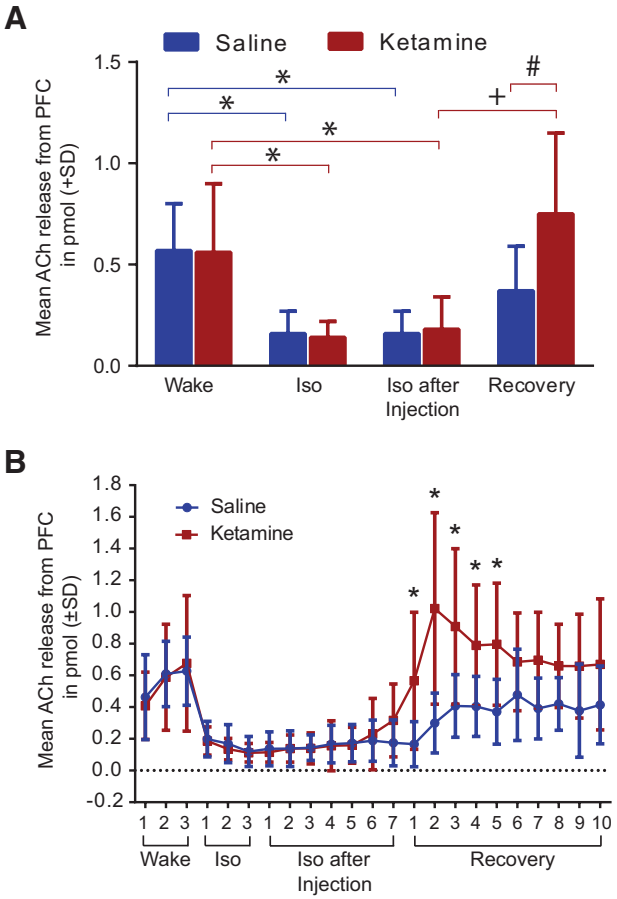

Fig. 7. Mean acetylcholine (ACh) release in the prefrontal cortex (PFC) during saline and ketamine injection. (*A*) No difference was seen between the two treatment groups during the wake phase. ACh release in the PFC was significantly decreased by isoflurane (Iso) administration (*). Injection of ketamine during Iso anesthesia did not result in any change in ACh levels compared to saline. However, after Iso was discontinued, ACh significantly increased (+) in ketamine-treated animals (n = 8) during recovery phase while animals in the saline group showed no significant difference. Compared to the saline group (n = 9), animals injected with ketamine showed a significant increase in ACh release during the recovery phase (#). Data are shown as mean ACh release (pmol) from PFC (+SD). (*B*) ACh release timeline in the PFC. No difference was seen between saline and ketamine injection during the wake, Iso, and Iso-after-injection phases. However, ketamine-treated animals showed a significant increase in ACh release within the PFC for the first 62.5 min during the recovery phase after discontinuation of Iso. Data are shown as mean ACh release from the PFC (± SD). *Blue line* = saline treatment; *red line* = ketamine treatment; statistical significance *,+,#$P < 0.05$.

Limitations

Isoflurane and ketamine have complex pharmacologic and neural effects; the nature of their interactions at the circuit and behavioral level remain to be clarified. Analyzing only the effect of the cholinergic system on emergence from general anesthesia is a limitation of this study, since multiple neurotransmitter systems are involved in the recovery process.[4,9] Furthermore, the prefrontal cortex is not the only brain region included in the cholinergic arousal system. Therefore, further assessment of whether acetylcholine in the prefrontal cortex plays a causal role in emergence time is needed, as well as a multimodal analysis evaluating the interactions between different neurotransmitters and brain regions. It will also be necessary to verify that ketamine actually improves postanesthetic cognition and not merely reanimation. The time point at which ketamine was injected was arbitrary. Further studies are needed to investigate how the results of this study are affected by changes in the time at which ketamine is administered. Finally, the clinical relevance of this phenomenon is unknown. Additional studies with surgical patients in the routine perioperative period (which often includes multiple psychoactive drugs and pain) are necessary.

Conclusion

The administration of subanesthetic ketamine enhances the effects of isoflurane as an anesthetic but accelerates emergence, possibly through cholinergic mechanisms and high-frequency activity in the cortex. This *paradoxical emergence* has compelling implications at both the clinical and the neuroscientific levels. Further work is required to assess the effects of these drug interactions on neural circuits and the relevance for improved perioperative care.

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Competing Interests

The authors declare no competing interests.

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