Differential Effects of Isoflurane on High-frequency and Low-frequency γ Oscillations in the Cerebral Cortex and Hippocampus in Freely Moving Rats

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ABSTRACT

Background: Cortical γ oscillations are thought to play a role in conscious cognitive functions. Suppression of 40-Hz γ activity was implicated in the loss of consciousness during general anesthesia. However, several experimental studies found that γ oscillations were preserved in anesthesia. The authors investigated the concentration-dependent effect of isoflurane on spontaneous γ oscillations in two frequency bands and three distinct brain regions in the rat.

Methods: Adult Sprague-Dawley rats were chronically implanted with epidural and coaxial depth electrodes to record cortical field potentials in frontal cortex, visual cortex, and hippocampus in waking and at steady-state isoflurane concentrations of 0.4, 0.8, and 1.2%. The γ power was calculated for the frequency bands 30–50 and 70–140 Hz. Temporal variation and interregional synchrony of γ activity were analyzed using wavelet transform. Loss of consciousness was indexed by the loss of righting reflex.

Results: Rats lost their righting reflex at 0.8 ± 0.1% isoflurane. High-frequency γ power was decreased by isoflurane in a concentration-dependent manner ($P < 0.001$, 50% decrease at 0.8% isoflurane) in all brain regions. Low-frequency γ power was unaffected by isoflurane. The duration and interregional synchrony of high-frequency γ bursts was also reduced ($P1 < 0.001$, 40% decrease at 0.8% isoflurane).

Conclusions: Distinction between high- and low-frequency γ bands is important when evaluating the effect of general

What We Already Know about This Topic

- The effect of anesthetics on γ oscillations as an electrophysiologic measure of anesthetic effect has been controversial

What This Article Tells Us That Is New

- Isoflurane reduced the power of high-frequency, but not low-frequency, γ oscillations in the cerebral cortex and hippocampus of freely behaving rats
- This suggests an association between reduced high-power γ oscillations with diminishing consciousness during general anesthesia

anesthetics on brain electrical activity. Spontaneous 40-Hz γ power does not indicate the state of consciousness. The attenuation and interregional desynchronization of high-frequency γ oscillations appear to correlate with the loss of consciousness.

HOW general anesthetics influence thalamocortical γ oscillations is of interest to understand the neural correlates of anesthetic-induced unconsciousness. However, the effect of anesthetics on γ oscillations has been controversial. Unlike human investigations,[1–3] most experimental studies found that both cortical and hippocampal γ oscillations were preserved or even augmented under general anesthesia,[4–9] thus questioning a presumed unitary correlation between γ activity and consciousness. A notable exception is the study by Ma *et al.*[10] that found some correlation between hippocampal γ oscillations and consciousness in rats.

Factors that may have contributed to the discrepancy between human and animal studies include the technique of electroencephalogram recording, the recorded cortical region, the method of spectral analysis, the type and depth of anesthesia, and a possible difference in the nature of γ oscillations in various species. In particular, scalp-recorded γ activity in humans is extremely attenuated by the skull and largely consists of electromyographic signals. Another possible factor is that in clinical anesthesia the concentration-dependent effect of the anesthetic agents is rarely studied. As a consequence, γ oscillations are usually compared between the conscious state and the surgical plane of anesthesia. This comparison fails to reveal whether a critical change in γ activity occurs precisely at the time of loss of consciousness. Experimental studies lend themselves to an

investigation of the effect of anesthetics at graded, steady-state concentrations.

In our previous studies,[7,8] the effect of volatile agents on γ power was examined in the presence of intermittent visual stimulation. Sensory stimulation may produce cortical arousal even during anesthesia,[11] which may be responsible for the observed enhancement of γ oscillations with anesthesia. Therefore, in the current work, we reexamined the effect of isoflurane anesthesia on spontaneous cortical γ oscillations in the absence of stimulation. In addition, we extended our study to the high-frequency γ range (70–140 Hz), which has rarely been studied before. To further extend the study, we measured γ oscillations in both frontal and visual cortex and in hippocampus. As we will show, isoflurane exerted a differential effect on low- and high-frequency γ activity in all three regions, suggesting that a distinction between various γ frequency bands is indeed important. Isoflurane reduced the power of high-frequency γ oscillations only, suggesting that the latter may be associated with diminishing consciousness during general anesthesia.

Materials and Methods

The experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee (Medical College of Wisconsin, Milwaukee, Wisconsin). All procedures conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996). Every effort was made to minimize the number of animals used and their suffering.

Electrode Implantation

Seven adult male Sprague-Dawley rats (250–310 g) were kept on a reversed light-dark cycle for 5–10 days before surgery. Recording electrodes were chronically implanted with 1.5–2% isoflurane anesthesia with spontaneous breathing under aseptic conditions. Sterile, 0.5% bupivacaine was applied subcutaneously for local anesthesia. For cortical potentials, stainless steel screw electrodes were placed through burr holes bihemispherically in the frontal cortex (3.2 mm anterior, 2.5 mm lateral, from bregma) and in primary visual cortex (7 mm posterior, 2.5 mm lateral, from bregma). The contact surfaces were epidural. In both regions, the potentials were recorded in bipolar fashion, between the homologous electrodes in the two hemispheres. For hippocampal recording, concentric bipolar semimicroelectrodes (SNEX-100X, Rhodes Medical Instruments Inc., Woodland Hills, CA) were positioned stereotaxically so that their contacts straddled the CA1 region (tip contact at 4 mm posterior, 3 mm lateral, −3 mm vertical, from bregma, contact separation 0.5 mm). The accuracy of the electrode placement was histologically verified in two animals. Lead wires from the electrodes were terminated in a six-channel molded plastic electrode

pedestal (Plastics One Inc., Roanoke, VA). The assembly was secured to the cranium with cold-cure dental resin and Cerebond skull adhesive (Leica Microsystems, Bannockburn, IL). The analgesic carprofen (5 mg/kg subcutaneously once daily) and the antibiotic enrofloxacin (10 mg/kg subcutaneously once daily) were administered after surgery for 2 and 7 days, respectively.

Experimental Protocol

Rats were tested in a transparent plastic anesthesia chamber without any physical constraint to their movement. During anesthesia, body temperature was monitored with a rectal probe and maintained at 37°C with a thermostat-controlled, electric heating pad (CWE, Inc., Ardmore, PA). Isoflurane was vaporized into a mixture of 30% oxygen and 70% nitrogen at a gas flow rate of 5 l/min. Field potentials were first recorded in the waking condition, then after incrementally increasing the isoflurane concentration to 0.4%, 0.8%, and 1.2%. Isoflurane concentration in the anesthesia chamber was monitored using a POET II gas analyzer (Criticare Systems Inc., Waukesha, WI). An equilibration time of 20 min was allowed before field potentials were recorded for 10 min at each anesthesia concentration. Before recording the field potentials, the righting reflex of the animals was tested by tilting the anesthesia box by 30 degrees to roll the animal to its side. The righting reflex was scored current when the animal made a purposeful attempt to right itself. Spontaneous movement of the snout, head, or limb without postural changes was scored as absent righting.

Data Analysis and Statistics

Electrode signals were amplified at a gain of 10,000, analog band-pass-filtered at 1–500 Hz, analog notch-filtered at 60 Hz (second order filter, rejection at 60 Hz of −40 dB), and digitally sampled at 2,500 Hz using the WINDAQ Data Acquisition Software (DATAQ Instruments, Akron, OH). For subsequent spectral analysis, the data were downsampled to 500 Hz. In all experiments, power spectra were calculated from a 3- to 6-min segment of data free from motion artifacts. Large artifacts in the electroencephalogram were only seen during gross motor movement; we were looking for a recorded period free from such events. Power spectra were calculated using the Welch spectral estimation method with a 250-point window and 90% overlap that yielded a reasonably smooth spectrum. The γ band powers were obtained from the spectra by averaging signal power in the 30- to 50-Hz and 70- to 140-Hz bands. To quantify the temporal pattern of γ oscillations, time-frequency power was calculated with the complex Morlet wavelet transform from a series of 3-min data segments. By thresholding the γ power in each frequency band, the duration of γ bursts for the two bands was determined at all anesthetic concentrations. The median of γ power distribution in the control state was chosen as a uniform threshold for all four states. Intraburst power was calculated as the median of all values for each γ
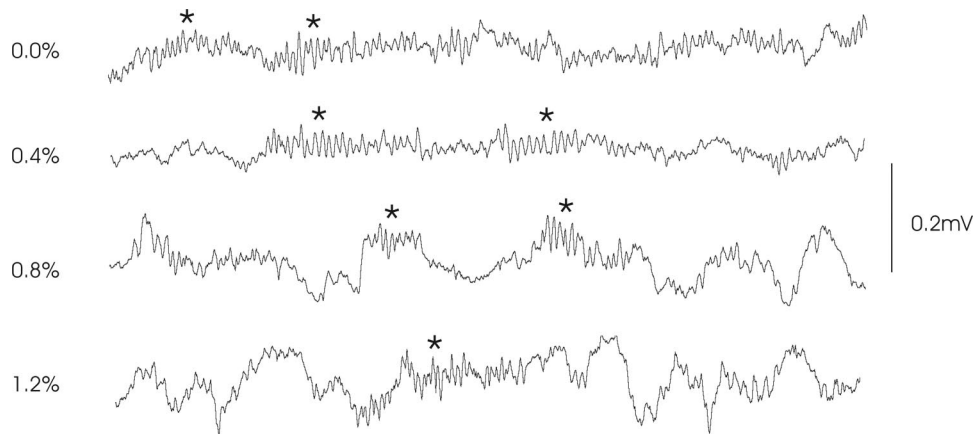
Fig. 1. Examples of γ oscillations in the frontal cortex of one rat at four concentrations of isoflurane. All traces are original recordings, sampled at 2.5 kHz after 1–500 Hz analog band-pass filtering, and subsequently downsampled to 500 Hz. All traces are 3 s long. The γ bursts are marked with asterisks.

band, brain region, and anesthetic state. To examine the temporal correlation or synchrony of γ bursts among the three brain regions, the binary time series representing the γ-active states were used to calculate three correlation coefficients for the three pairs of regions.

To test the effect of isoflurane on γ power, we first performed an omnibus analysis of variance (ANOVA) with brain region, γ band and isoflurane concentration as repeated measures, and the subject (rat) as random variable; γ power was the dependent variable. When the interaction term was significant, the component effects were examined. In particular, the isoflurane effects on high- and low-frequency γ powers were tested using linear and quadratic contrasts. A similar ANOVA model and follow-up tests were used for the effects of isoflurane on γ burst duration, burst power, and burst synchrony. Cohen's f^2 was calculated as statistical effect size for both main effects and interactions. Spectral analyses were performed using MATLAB version 7.5.0 (MathWorks Inc., Natick, MA) and statistical analysis was performed using NCSS 2007 (NCSS, Kaysville, UT). All figures indicate means and standard deviations.

Results

We found that γ oscillations were current during waking and at all examined isoflurane concentrations. As a general observation, γ activity was rarely continuous but more often intermittent, lasting at most for a few seconds. Bursts of γ oscillations near 40 Hz could be well recognized in the raw signal. At higher isoflurane concentrations, γ oscillations were often seen riding on slower waves, such as δ. A few examples are illustrated in figure 1. The intermittency of γ oscillations was also evident in the signals' time-frequency representation obtained by wavelet transform. The examples in figure 2 illustrate γ bursts near 40 Hz as well as at higher frequencies in the waking condition.

To determine the effect of isoflurane on γ oscillations, band powers for low-frequency (30–50 Hz) and high-fre-

quency (70–140 Hz) γ were calculated from the power spectrum in each brain region. Using ANOVA, we obtained a significant main effect of isoflurane concentration ($P < 0.001$, $f^2 = 0.80$) and a significant interaction between isoflurane concentration and the γ band ($P < 0.001$, $f^2 = 0.26$). Follow-up tests indicated a significant decrease in high-frequency γ power ($P < 0.001$, linear trend, $f^2 = 1.16$), but no change in low-frequency γ power ($P = 0.69$, $f^2 = 0.01$), at increasing isoflurane concentration. At 0.8% isoflurane, at which the rats lost their righting reflex, high-frequency γ power was reduced by approximately 50%. There was no difference in γ power among the three brain regions ($P = 0.88$, $f^2 = 0.01$), and there was no significant interaction between the region and isoflurane concentration ($P = 0.14$, $f^2 = 0.01$), indicating that isoflurane's effect was not region-specific. Figure 3 illustrates the power spectra at different isoflurane concentrations in three brain regions from one experiment as an example, and figure 4 presents the summary of data from all experiments.

Next, we wished to determine whether isoflurane altered the temporal structure of γ oscillations with respect to their intermittent nature. To this end, episodes of low-frequency and high-frequency γ activity were identified in the wavelet transform by a suitable thresholding of the corresponding band-power and then measuring the duration and power of the γ bursts. Using the same ANOVA model as before, we found that the γ burst duration was significantly influenced by isoflurane ($P < 0.001$), and that this effect depended on the γ frequency band ($P < 0.05$). In particular, isoflurane shortened the bursts in the high-frequency γ band ($P < 0.001$, linear trend, $f^2 = 0.52$). There was, on average, a 40% decrease in the duration of high-frequency γ bursts at 0.8% isoflurane. In contrast, the duration of low-frequency γ bursts changed in a biphasic manner: it increased at 0.4%, which was reversed at 0.8% and 1.2% ($P < 0.001$, quadratic trend). There was no change in the intraburst power ($P = 0.97$, $f^2 = 0.00$); only the burst duration varied. These results
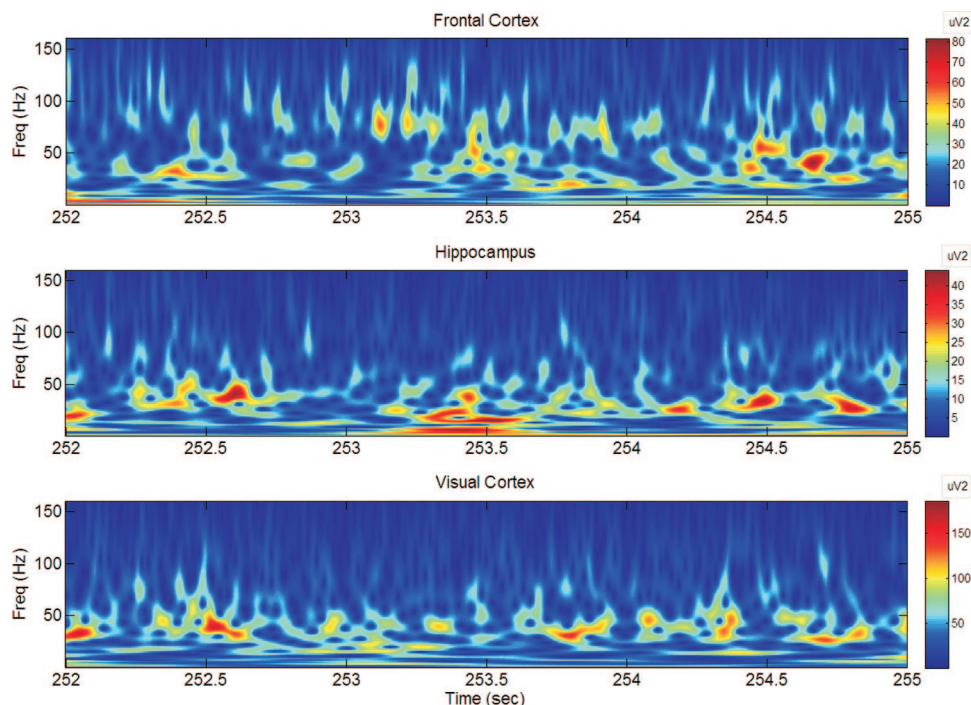
Fig. 2. Time-frequency power as calculated by the complex Morlet wavelet transform in three brain regions of a waking rat. Note the high temporal dynamics of γ power in all cases.

are consistent with the overall decrease in time-averaged high-frequency γ power and the preservation of low-frequency γ power.

Finally, we examined whether the γ bursts were temporally correlated among the three brain regions and if their synchrony was altered by isoflurane (fig. 5). To this end, the binary time series representing the γ-active states were used to calculate three correlation coefficients among the three brain regions, and the effect of isoflurane on these coeffi-

cients was tested with ANOVA with the same independent variables as before, with the exception that now pairs of regions took the place of individual regions. We found a substantial synchrony of the γ burst periods in the waking condition as indicated by the pairwise correlation coefficients that ranged from 0.50 to 0.59 in the three pairs of regions. When isoflurane was increased, all correlation coefficients decreased in the high-frequency γ band ($P < 0.001$, linear trend, $f^2 = 0.80$), but not in the low-frequency γ band ($P =$
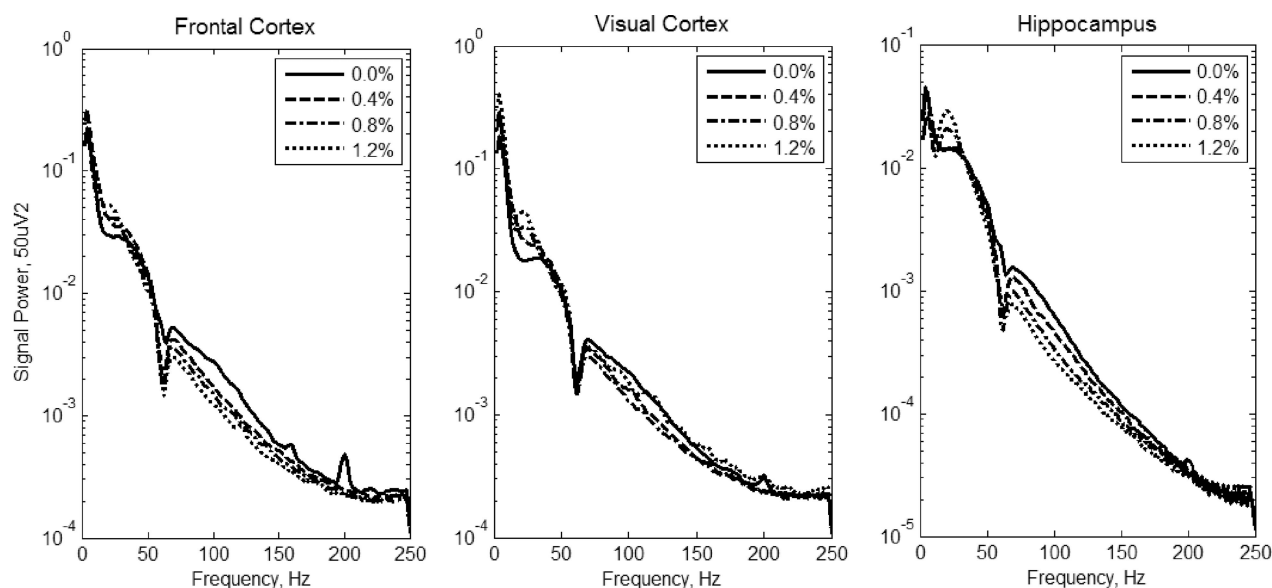

Fig. 3. Power spectra in the three different brain regions and at four levels of consciousness in one rat. Power spectra were obtained with the Welch spectral estimation method using 250 points at 500-Hz sampling frequency and 90% window overlap. Note the progressive decrease in high-frequency (70–140 Hz) γ power with an increasing concentration of isoflurane.
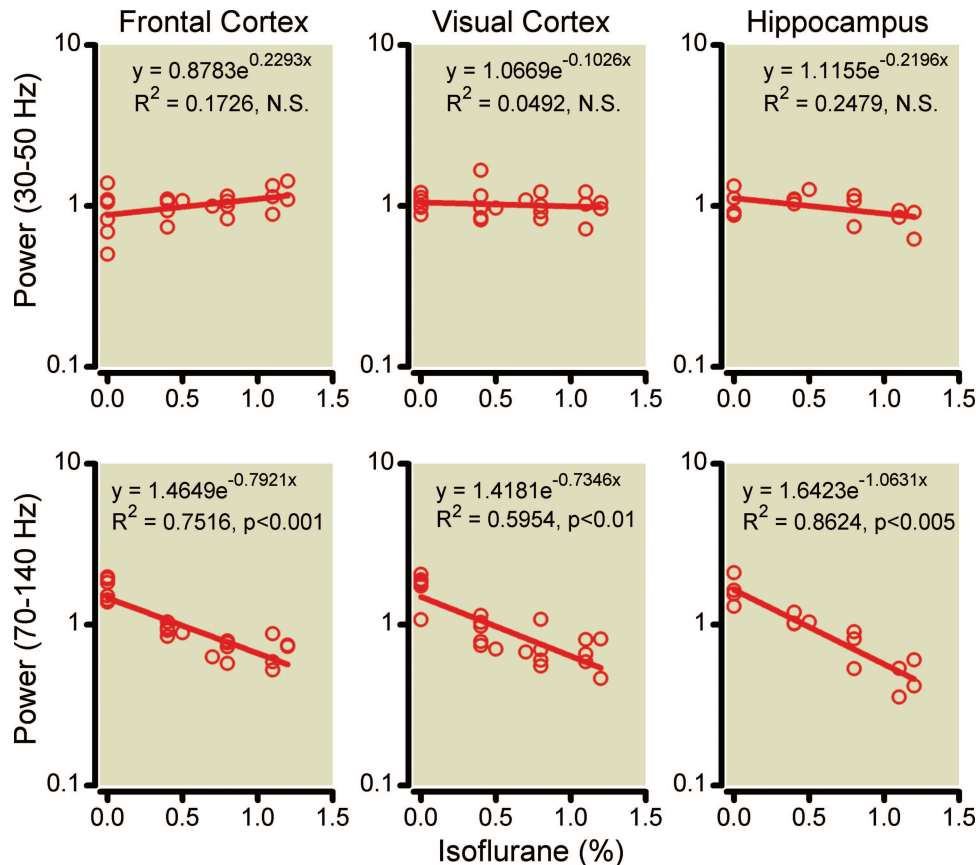
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Fig. 4. Concentration-dependent effect of isoflurane on cortical and hippocampal γ power from all experiments. In each of the three brain regions, isoflurane suppressed high-frequency (70–140 Hz) γ power but it did not significantly alter low-frequency (30–50 Hz) γ power.

0.43, $f^2 = 0.01$). A 40% decrease in high-frequency γ correlation occurred at 0.8% isoflurane. There was no difference in isoflurane's effect among the three pairs of regions ($P = 0.95$, $f^2 = 0.01$).

Discussion

The current results support and extend recent indications that isoflurane, at moderate hypnotic concentrations, does not suppress cortical γ oscillation at the prototypical 40-Hz frequency. It is this 40-Hz γ oscillation that has been previously associated with various aspects of conscious information processing.[12–17] Kulli and Koch[18] suggested early on that the absence of 40-Hz oscillations may indicate a loss of consciousness during general anesthesia. Numerous studies have addressed the effect of anesthetic agents on γ oscillations in both humans and animals.[1–3,6,9,10,19,20] Nevertheless, the answer to this question has remained controversial, especially in animal studies. The γ oscillations are more difficult to measure with scalp electrodes in humans than with epidural or intracortical electrodes in animals. Therefore, the γ-frequency signals recorded in animals may, in general, be considered more reliable. Our previous studies in rats suggested that stimulus-induced γ oscillations were augmented by various volatile agents compared with the waking condition.[7,8] The current results extend this finding to spontaneous 40-Hz γ oscillations, and support our previous proposition that 40-Hz γ power cannot serve as a unitary indicator or neural correlate of unconsciousness during general anesthesia.

In contrast to 40-Hz γ activity, high-frequency γ power activity in the 70–140 Hz range was clearly suppressed by isoflurane in a concentration-dependent manner. This is a new finding, although similar observations were previously made in the rat hippocampus.[10] The functional significance of high-frequency cortical γ oscillations for general anesthesia is currently unclear. They do play significant roles in information processing in the hippocampus,[21] and they have been observed in the cerebral cortex as well.[22]

The signal power of the electroencephalogram in the high-frequency γ range is quite low, and is often thought to carry electromyographic artifacts. However, we believe that any electromyographic contamination to our data was unlikely. We had ample opportunity to observe our rats during various waking behaviors as well as during rest, and thus were able to discern the electroencephalographic signals from those arising from such artifacts. Moreover, our recordings in the hippocampus were obtained with deep bipolar electrodes that are virtually free from electromyographic artifacts, and the data collected from this region lead to the same conclusion as that from data recorded from the cortex. High-fre-
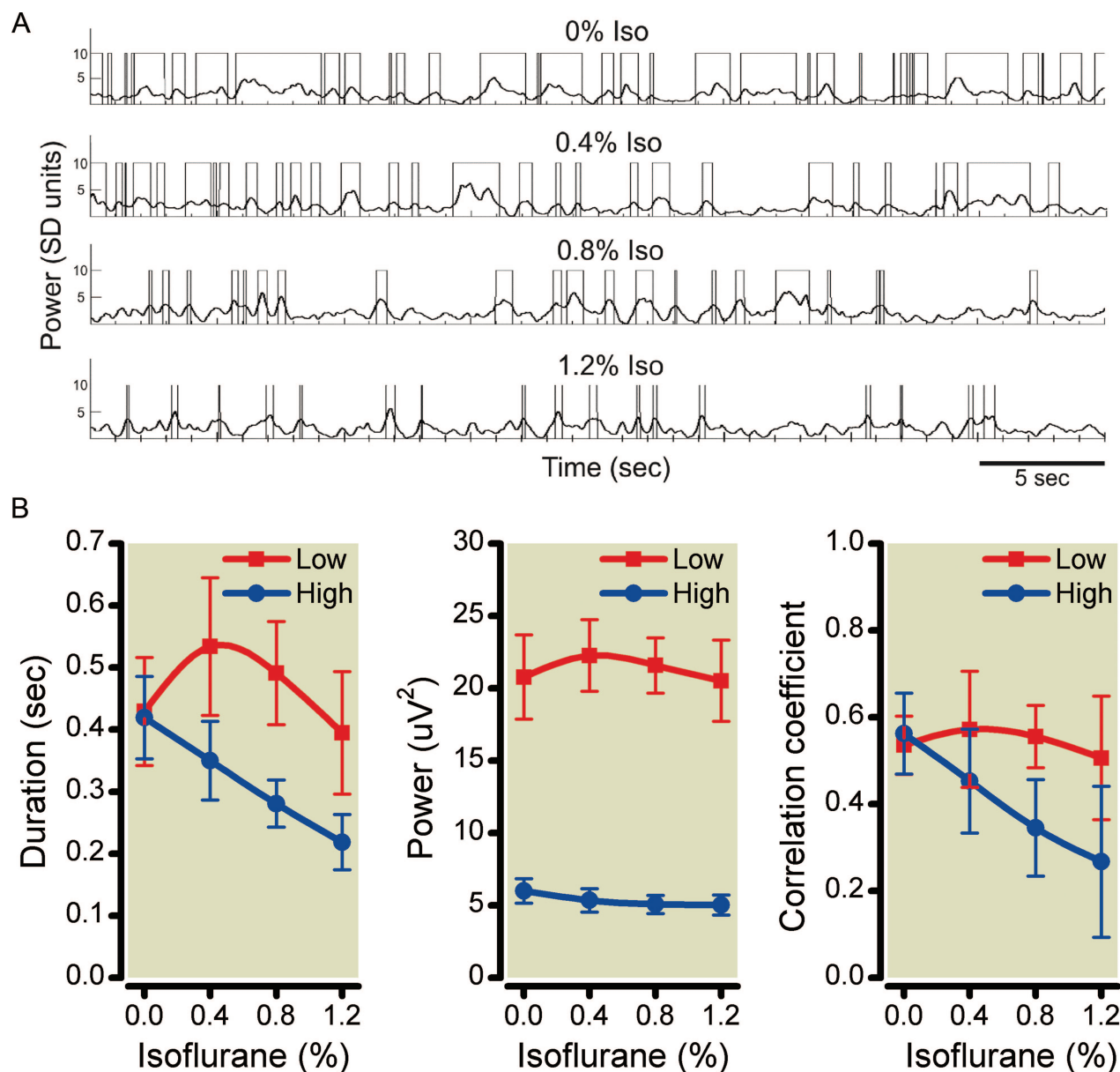
Fig. 5. The effect of isoflurane (Iso) on the properties of γ bursts. (*A*) Example of thresholding of high-frequency γ bursts at median value after 1-Hz low-pass filtering; data are from frontal cortex. SD = standard deviation. (*B*) Summary of data from all experiments for γ burst duration, intraburst power, and interregional burst synchrony in four states of consciousness in the low-frequency (30–50 Hz) and high-frequency (70–140 Hz) γ band. Isoflurane produced significant decreases in high-frequency γ burst duration and synchrony (linear trend, $P < 0.001$).

quency γ waves were difficult to visualize in the original electroencephalogram (unless filtering was applied); however, the obtained power spectra gave a clear indication of the change in activity of these waves. Thus, we are confident that the observed suppression of high-frequency γ power is a real electroencephalographic effect.

Recently, Silva *et al.*[23] explored the effect of isoflurane on thalamic and somatosensory cortical field potentials and various derived electroencephalographic indices. They found that 50- to 100-Hz γ power was significantly decreased at 0.8% isoflurane compared with the waking baseline; however, a concentration-dependent effect of isoflurane on γ

power was present in their study. The potentially important differences that may account for the deviation from our results are that our experiments were performed on freely moving rats, the electroencephalogram was recorded in different brain regions, and a slightly higher γ frequency range (70–140 Hz) was studied.

In our study, the decrease in high-frequency γ power was accompanied by a gradual shortening of the duration of γ bursts, whereas the intraburst γ power was unchanged. This suggests that the overall reduction in high-frequency γ power at increasing concentrations of isoflurane was related, predominantly, to a gradual shortening of the transient γ bursts.

Although the mechanism of this change is unclear, the temporal structure of γ activity may be important for coding neural information and/or conscious information processing.[21] In particular, the increasingly bursty character of γ activity during deepening anesthesia may reflect shorter windows of effective information processing.

Moreover, we found that as the γ bursts became shorter, they became less correlated among the three recorded brain regions. These changes suggest a spatial disorganization of γ activity and possibly reduced capacity for information integration across the brain – a hallmark for loss of consciousness during general anesthesia.[24] It has been shown that only consciously perceived stimuli synchronize γ oscillations among distant brain regions.[25] In addition, John *et al.*[2] found that the critical change in the electroencephalogram that correlated with the loss and return of consciousness in patients was the reduction and subsequent restoration of long-distance γ coherence. We demonstrated that volatile anesthetics attenuated stimulus-induced increase in long-range γ coherence between frontal cortex and visual cortex, whereas local γ coherence remained unchanged.[26] All of these studies were limited to the traditional low-frequency γ frequency range. Although we did not measure γ phase coherence, only γ burst synchrony, the results are qualitatively consistent with the former findings.

We should note that high-frequency γ power, burst duration, and burst synchrony were reduced but not completely eliminated by isoflurane. Whether the observed degree of reduction is sufficient to explain a loss of consciousness remains an open question. Given the view that consciousness itself is a graded phenomenon,[24,27] a plausible interpretation of the findings is that a graded reduction in high-frequency γ power translates to a graded reduction in consciousness. Investigators who consider consciousness as an all-or-none phenomenon prefer to seek a threshold or state transition effect as a neuronal correlate of unconsciousness.[28,29] Nevertheless, the two views may not be mutually exclusive; the transition between consciousness and unconsciousness may occur relatively abruptly because of system instability, giving the impression of a binary transition at a macroscopic behavioral level, yet transitioning through a series of graded neural microstates.

In conclusion, the current results confirm and extend our previous findings regarding the effects of volatile anesthetics on γ oscillations. The power of the prototypical 40-Hz γ oscillations is not decreased by isoflurane, although a biphasic change in γ burst duration is observed. High-frequency γ oscillations are attenuated by isoflurane in a concentration-dependent manner owing to a shortening of the γ bursts, and may thus be more closely related to diminishing consciousness during anesthesia.

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ANESTHESIOLOGY REFLECTIONS

An "Olefiant Gas" Two-Dollar Receipt

According to this two-dollar receipt (*left*), one method for generating "The Olefiant Gas" was patented on the Ides of March in 1858. (The most successful anesthetic associated with this "oil-making" gas was ethylene, which would be popularized 70 yr later.) This printed note cautioned that the "holder of this binds himself under a penalty not to divulge the secret of this Receipt" for $2. The "Directions" for "The Olefiant Gas" read: "2 Quarts of Alcohol. / 1 Pint of Camphene. / 2 Ounces of Alum. / 1 Teaspoonful of Curcuma Liquid. / Mix, let stand 12 hours, then Use." Scrawled on the reverse of this receipt (*right*) is the secret key to actually using this prescription: "Put this Powder in one quart of alcohol or fluid then use one Teaspoonful to the other three Ingredients." (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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