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Volatile Anesthetics Enhance Flash-induced γ Oscillations in Rat Visual Cortex

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Background: **The authors sought to understand neural correlates of anesthetic-induced unconsciousness. Cortical γ oscillations have been associated with neural processes supporting conscious perception, but the effect of general anesthesia on these oscillations is controversial. In this study, the authors examined three volatile anesthetics, halothane, isoflurane, and desflurane, and compared their effects on flash-induced γ oscillations in terms of equivalent concentrations producing the loss of righting reflex (1 minimum alveolar concentration for the loss of righting [MAC_{LR}]).**

Methods: **Light flashes were presented every 5 s for 5 min, and event-related potentials were recorded from primary visual cortex of 15 rats with a chronically implanted bipolar electrode at increasing anesthetic concentrations (0–2.4 MAC_{LR}). Early cortical response was obtained by averaging poststimulus (0–100 ms) potentials filtered at 20–60 Hz across 60 trials. Late (100–1,000 ms) γ power was calculated using multitaper power spectral technique. Wavelet decomposition was used to determine spectral and temporal distributions of γ power.**

Results: **The authors found that (1) halothane, isoflurane, and desflurane enhanced the flash-evoked early cortical response in a concentration-dependent manner; (2) the effective concentration for this enhancement was the lowest for isoflurane, intermediate for halothane, and the highest for desflurane when compared at equal fractions of the concentration that led to a loss of righting; (3) the power of flash-induced late (> 100 ms) γ oscillations was augmented at intermediate concentrations of all three anesthetic agents; and (4) flash-induced γ power was not reduced below waking baseline even in deep anesthesia.**

Conclusions: **These findings suggest that a reduction in flash-induced γ oscillations in rat visual cortex is not a unitary correlate of anesthetic-induced unconsciousness.**

HOW general anesthetic agents produce unconsciousness remains a mystery, despite decades of scientific research into their cellular and molecular actions. Our limited understanding of what phenomenal consciousness really is, how it may arise from neurobiologic events, and how to objectively assess its presence or absence has hampered the elucidation of anesthetic mechanisms.

Cortical γ (20–60 Hz) oscillations in the electroencephalogram, local field potentials, and unit activities have been implicated in cognition,[1] attention,[2] arousal,[3] memory,[4] perception,[5] and consciousness.[6] A reduction in γ oscillations has been suggested as a neural correlate of the anesthetic-induced unconsciousness.[7–11] Investigations of the effects of general anesthetics on the resting electroencephalogram,[12,13] middle latency auditory evoked potentials,[14–17] and auditory steady state responses[18–20] have contributed to this contention.

At variance with the above results, several experimental studies in humans and animals have found that the amplitude of cortical and hippocampal high-frequency β or γ oscillations was preserved or even enhanced during sedation with midazolam[21] or during urethane, ether, isoflurane, or halothane anesthesia.[22–25] A possible reason for this discrepancy is that many of the previous studies did not systematically investigate the concentration-dependent effect of the anesthetic agents. Also, the effects of different agents have not been compared using the same experimental protocol at graded, steady state concentrations.

In this study, we compared the effects of halothane, isoflurane, and desflurane on flash-induced γ oscillations in the rat visual cortex. These anesthetic agents are well known to have important differences in potency and region-specific effects.[26–28] Finding a common neurophysiologic effect of different agents has the potential to reveal a unitary component of anesthetic action producing unconsciousness.[12]

The clinically effective concentrations of halothane, isoflurane, and desflurane are different for different endpoints, such as analgesia, atonia, amnesia, and hypnosis. Because we were interested in neural activities related to the loss of consciousness, we compared the effects of these agents at equivalent concentrations at which they presumably produce unconsciousness. The loss of righting reflex (LORR) has been used widely as a standard behavioral index of unconsciousness in the rat.[11,29–33] Therefore, the critical concentration of each agent that produced the LORR was used to define the agent's equivalent concentration. We demonstrate that the three anesthetics augmented the amplitude of flash-induced γ oscillations at concentrations associated with the LORR. This finding casts doubt on the view of diminished γ power as a neural correlate of unconsciousness.

Materials and Methods

The experimental procedures and protocols used in this investigation were reviewed and approved by the

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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*[34] and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.[35] Every effort was made to minimize the number of animals used and their suffering.

Electrode Implantation

Fifteen adult male Sprague-Dawley rats were kept on a reversed light–dark cycle in a dedicated room of the Biomedical Resource Center for 5–10 days before electrode implantation. On the day of the aseptic surgery, the rats (250–300 g) were anesthetized using isoflurane (Abbot Laboratories, Chicago, IL) in an anesthesia box (28.8 × 14.4 × 12.0 mm). The animal's head was secured in a rat stereotaxic apparatus (model 900; Kopf Instruments, Tujunga, CA), and a gas anesthesia adaptor (Stoelting Co., Wood Dale, IL) was placed over the snout to continue anesthesia at 1.5% isoflurane. The animals were breathing spontaneously. Body temperature was monitored with a rectal probe and maintained at 37°C *via* a water-circulating heating pad. The dorsal surface of the head was prepared for sterile surgery with iodine spray. Steam-sterilized instruments and drapes were used during surgery. Sterile 1% xylocaine was injected under the skin, and a midline incision was made. The skin was laterally reflected, the exposed cranium was gently scraped of connective tissue, and any bleeding was cauterized.

For recording of intracortical field potentials, a concentric, bipolar semimicro electrode (contacts separation of 0.5 mm; SNEX-100X; Rhodes Medical Instruments, Inc., Woodland Hills, CA) was stereotaxically implanted in the primary visual cortex at coordinates 7 mm posterior, 2–3 mm lateral, and 2–2.3 mm vertical relative to the bregma[36] and was secured to the cranium with cold-cure resin. A stainless steel machine screw in the caudal cranium was used as a ground electrode. Two additional skull screws were placed to anchor the skullcap to the cranium, and the assembly was embedded in resin. An antibiotic (10 mg/kg intramuscular enrofloxacin) and pain medication (0.02–0.05 mg/kg subcutaneous Buprenex [Reckitt Benckiser Health Care, Ltd., Hall, United Kingdom]) were administered. Analgesic injections of Buprenex (0.02–0.05 mg/kg subcutaneously twice daily) continued for 3 days, and the antibiotic injections of enrofloxacin (10 mg/kg intramuscular twice daily) were administered for 14 days. The animal was kept in the reversed dark–light cycle room for 7–10 days and observed for any infection or other complications.

Event-related Potential Experiment

Each rat was assigned to event-related potential (ERP) experiments under each of the three anesthetic agents administered on different days. One week of recovery was allowed before repeating the experiment with a different agent. On the day of the experiment, the animal was placed in a cylindrical plastic restrainer of 6 cm in diameter (Harvard rodent restrainer, model AH-52-0292; Harvard Apparatus, Holliston, MA) stationed inside a rectangular, transparent, plastic anesthesia box. While awake, the animal had limited movement of its head and limbs but was not able to crawl out of the cylinder. The animal's rectal body temperature was maintained at 37°C with a thermostat-controlled, water-circulated heating pad. The rat was positioned in the apparatus under inhalational anesthesia, vaporized into a mixture of 30% oxygen and 70% nitrogen gas (flow rate of 5.3 l/min), with the same agent that was chosen for that day's experiment. The animal was breathing spontaneously. When all connections were in place, the anesthetic was turned off, and a 1-h equilibration period was allowed for the animal to regain consciousness. The animal was already fully awake in less than 10 min after the anesthetic administration was terminated, as judged by its attempts to crawl out of the restrainer.

After 1 h of equilibration time, 5 min of spontaneous field potentials were recorded, followed by recording of the ERP to flash stimulation. Subsequently, the anesthetic concentration was increased from 0 to 2.0% in increments of 0.1–0.2% for halothane or isoflurane, or from 0 to 9.0% in increments of 1% for desflurane. The anesthetic concentration was monitored through a sampling line connected to the anesthesia box using a gas analyzer (POET II; Criticare Systems, Inc., Waukesha, WI). Recording of spontaneous field potentials and ERP was repeated at each increased concentration after a 20-min equilibration. Because halothane is known to have a longer equilibration time than either isoflurane or desflurane, the equilibration time of 20 min was chosen to achieve the steady state level of anesthesia that is sufficient for halothane and, as such, also sufficient for the two other agents. Gentle knocking on the side of the anesthesia box was performed to control the animal's level of arousal before testing.

For flash stimulation, a light guide, connected to a stroboscopic light source (EG & G Electro-Optics, Salem, MA) housed in a soundproof box, was directed at the front of the anesthesia box, 18 cm from the rat's face and centered to achieve binocular stimulation. The stimulation consisted of 60 discrete flashes repeated every 5 s in a darkened room for a total period of 5 min. The interstimulus interval of 5 s was chosen to ensure that the stimulus-related activity dissipated at least several seconds before the onset of the next stimulus.

The signals were amplified at a gain of 10,000, analog band-pass filtered at 1–250 Hz, analog notch filtered at 60 Hz (second-order filter, rejection at 60 Hz of −40 dB), and digitally sampled at 500 Hz (WINDAQ Data Acquisition Software; DATAQ Instruments, Akron, OH).

A few animals did not survive the ERP experiments with all three agents. From the total of 15 rats, 14 were tested with halothane, 11 of which were also tested with

isoflurane, and 8 of which were also tested with desflurane. One animal was tested with isoflurane and desflurane but not halothane.

Righting Reflex Experiment

One week after the ERP experiment, the animal was tested for LORR as a function of anesthetic concentration. As in the electrophysiologic experiment, the animal was placed in the anesthesia box, but without the restrainer. The same experimental protocol was applied to incrementing the anesthetic concentration as in the ERP experiment. The righting reflex was tested by tilting the anesthesia box sideways by 30° to roll the animal to its side. The righting reflex was marked as present when the animal made a purposeful attempt to right itself. Note that every time the animal made an attempt to right itself, it succeeded. Spontaneous head movement or random limb movement in the absence of righting was not taken as an indication of righting.

In addition to righting, the presence of whisker, corneal, tail, and foot pinch responses was tested in a few animals to obtain a more complete characterization of the rats' behavioral responses. The response to whisker stimulation was assessed by gently stroking the whiskers on one side of the rat's face with a cotton-tipped applicator and was marked as present if the animal turned its head toward the stimulus. The corneal response was assessed by gently touching the cornea of one eye with a cotton-tipped applicator, and was marked as present if the rat blinked immediately after the stimulation. Finally, the response to tail or foot pinch was assessed by gently pinching the tail or foot of the rat with a hemostat and was marked as present if the animal withdrew its foot or tail in response to stimulation. Because only a few animals were used in these tests, no quantitative or statistical analyses on whisker, corneal, tail, or foot pinch data were performed. Only the data from the righting reflex experiment were used in the analysis.

As mentioned above, some animals did not survive the ERP experiments with all three agents. A few additional rats died after the ERP experiments were completed but before the righting studies were initiated. For that reason, only 5 rats from the ERP experiments were tested for the loss of righting with halothane and isoflurane, 4 of which were also tested with desflurane. To assemble a statistically appropriate group of animals, additional rats were included in the righting protocol. Thus, 9 additional rats were tested with halothane, 7 of which were also tested with isoflurane. Three additional animals were tested with desflurane only. Hence, the total number of animals tested for the loss of righting was 14 for halothane, 12 for isoflurane, and 7 for desflurane.

The inhaled percent anesthetic concentration at which the righting reflex was abolished was determined by averaging the concentration at which the righting reflex was still preserved and the concentration at which it was lost. We defined this value as one minimum alveolar concentration for the loss of righting (1 MAC_{LR}). For all subsequent analyses, the percent inhaled concentrations were expressed as a fraction of 1 MAC_{LR}. This definition was introduced in remote analogy of the traditional minimum alveolar concentration (MAC) used to define the critical concentration of inhalational anesthetics associated with an absence of a response to nociceptive stimulation 50% of the time (E_{50}).

Data Analysis

Single-trial ERPs comprising 1-s poststimulus periods were extracted from the record in each experiment for every anesthetic level using a threshold peak-detection algorithm. These data were normalized to the SD of the concatenated 1-s-long prestimulus activity in the waking state. To examine the effect of anesthetic agents on γ oscillations, single-trial ERP data were band-pass filtered at 20–60 Hz with a bidirectional Butterworth digital filter (n = 2). The use of the bidirectional filter ensures that the original phase information of the ERP is preserved. All consecutive analyses were performed on the γ-filtered data.

The ERP data in each experiment were first examined on a trial-to-trial basis using the visualization tool "ERP image."[37–39] To quantify the temporal and frequency-dependent characteristics of the ERP between 0 and 1,000 ms after stimulus, the single-trial ERP data were wavelet transformed using complex Morlet wavelets.[40] The wavelet decomposition was chosen over more traditional short-time fast Fourier transform–based time-frequency methods because it offers variable time and frequency resolutions and allows a reliable detection of rapid transient changes in signal amplitude at higher frequencies. Because the duration of the wavelet is shorter for higher-frequency bands, this method provides a better compromise between temporal and spectral resolutions and has been found to be particularly useful in the analysis of stimulus-induced γ oscillations.[40] It also offers improved performance compared with narrow-band filtering because it is designed to minimize side-lobe energy and reduce spectral leakage.

As described in detail elsewhere,[40] the wavelet transformation of the single-trial ERP data involved convolving the signal with complex Morlet wavelets with central frequency ranging from 20 to 60 Hz in increments of 1 Hz. Each Morlet wavelet is characterized by a Gaussian envelope in both time and frequency domains around its central frequency. Hence, the SD of the envelope in the time domain determines the temporal resolution of the wavelet, and the SD of the envelope in the frequency domain determines the frequency bandwidth of the wavelet around its central frequency. For the central frequencies of interest, the temporal resolution and spectral bandwidth of the wavelet ranged from 55.7 ms

and ±2.8 Hz at 20 Hz to 18.5 ms and ±8.6 Hz at 60 Hz, respectively.

To calculate band power as a function of time for every trial, the wavelet power was averaged within spectral windows of 20–30, 31–40, 41–50, and 51–60 Hz. The averaging is acceptable because it minimizes the time-frequency overlap in 10-Hz-wide spectral windows resulting from continuous wavelet decomposition. The ERP image tool was then used to display the single-trial wavelet power in each spectral window. As is shown in the Results section, these wavelet plots suggested the need to separately examine the early (0–100 ms) and the late (100–1,000 ms) ERP components, termed the *early cortical response* (ECR) and *late γ oscillations*, respectively.

The ECR was calculated by averaging the ERP data in each experiment across multiple trials. Because ECR has little phase variability from trial to trial, the averaging was acceptable for this analysis. The amplitude of ECR was determined as the difference from the most positive (maximum) to the most negative (minimum) peak between 0 and 100 ms.

The power of the late (100–1,000 ms) γ oscillations was estimated from the single-trial ERP data using the Thomson multitaper power spectral estimation technique, a short-segment fast Fourier transform analysis that uses orthogonal windows. This technique was chosen because it offers superior performance in the analysis of short temporal data segments with a high degree of nonstationarity.[41,42] Due to trial-to-trial phase variation of the late γ oscillations, an averaging of original ERP data were not a desirable approach in this case. Instead, γ-band power was calculated by first averaging the power spectrum within the range of 20–60 Hz in each trial and then averaging across all trials to obtain one datum per anesthetic concentration in each experiment. Thus, these data reflect γ power of both stimulus-locked and nonstimulus locked events. All calculations were done with MATLAB 6.0 (MathWorks Inc., Natick, MA).

Statistical Analysis

To minimize experiment-to-experiment variance, all ECR amplitude and late γ power data were normalized to their respective means obtained by averaging across all anesthetic concentrations in each experiment. Because it was difficult to obtain exactly the same anesthetic concentration in each experiment, the data were averaged within selected anesthetic ranges of 0.4–0.75, 0.8–1.25, 1.3–1.75, and 1.8–2.40 MAC_{LR} in addition to the waking state. This procedure ensured that at least one datum was included in each range and allowed the statistical comparison among different anesthetic levels. To test for a significant effect of anesthetic concentration on ECR and late γ oscillations, the general linear model analysis of variance was used with the anesthetic concentration as a fixed factor and the rat as a random variable. The Bonferroni comparison was used to test for a significant difference in observations at different anesthetic concentrations from waking control. Statistical analyses were conducted using MINITAB (Minitab Inc., State College, PA).

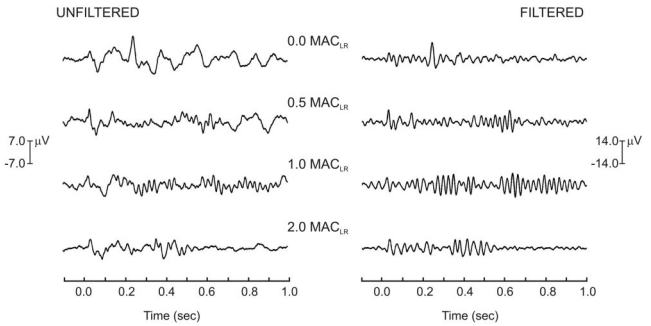

Fig. 1. Example of flash-induced unfiltered and γ-filtered (20–60 Hz) event-related potential recorded in the striate cortex of the same rat in response to a single flash at selected halothane concentrations. Note the different scales for unfiltered and filtered event-related potential due to filtering amplitude enhancement. One MAC_{LR} is the minimum alveolar concentration at which the righting reflex is lost. Each trace represents 100 ms of prestimulus and 1,000 ms of poststimulus activity. At 0.0 MAC_{LR}, typical low-frequency (1–4 Hz) afterdischarge with superimposed high-frequency (30–40 Hz) γ oscillations was present after the flash. The filtered version of the event-related potential recorded clearly reveals the presence of γ oscillations between 0 and 1,000 ms after stimulus. Halothane disrupted the afterdischarge at 0.5 MAC_{LR} and augmented γ oscillations at 1.0 MAC_{LR}. At 2.0 MAC_{LR}, γ oscillations between 600 and 1,000 ms were suppressed but were still strongly present between 0 and 500 ms after stimulus.

Results

Righting Reflex

In animals anesthetized with halothane, the LORR occurred between 0.7 and 1.0% (1 MAC_{LR} = 0.90 ± 0.1%; n = 14) concentration. In animals anesthetized with isoflurane, the righting reflex was also lost between 0.7 and 1.0% (1 MAC_{LR} = 0.84 ± 0.1%; n = 12) concentration. In animals anesthetized with desflurane, the LORR occurred between 3.5 and 5.0% (1 MAC_{LR} = 4.26 ± 0.5%; n = 9) concentration. In a few animals tested, the whisker reflex was lost in the same concentration range as the righting reflex for all three anesthetic agents. The responses to corneal stimulation, tail, and foot pinch were preserved past the LORR.

Anesthetic Effect on Single-trial ERP

Figure 1 shows a typical example of the unfiltered and γ-filtered versions of the same ERP to a single flash at four selected values of MAC_{LR} for halothane from one animal. At 0.0 MAC_{LR} (waking), typical low-frequency (1–4 Hz) afterdischarge with superimposed relatively low-amplitude γ (30–40 Hz) oscillations was present after the flash. The presence of γ oscillations over the 1,000-ms poststimulus period is better revealed by

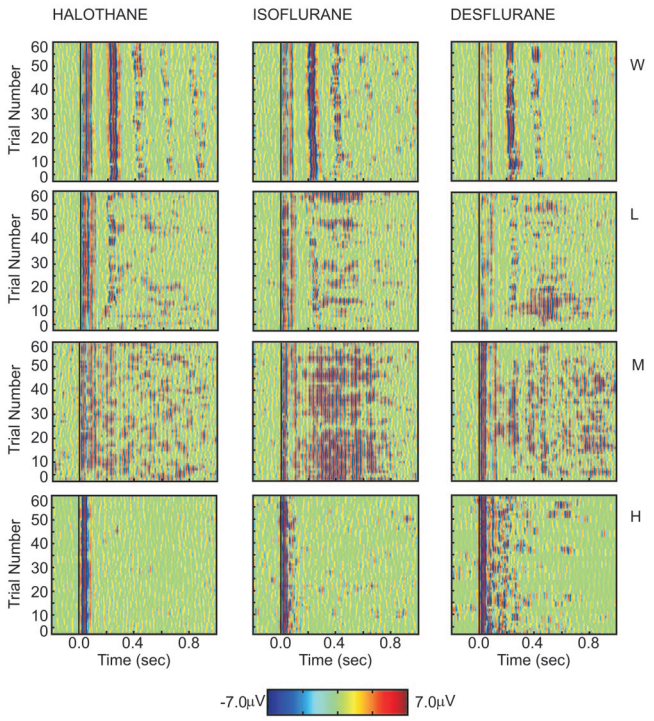

Fig. 2. Examples of flash-induced, γ-filtered (20–60 Hz) event-related potential images from data recorded in the striate cortex of the same rat at selected halothane, isoflurane, and desflurane concentrations (W: 0.0 MAC_{LR}, L: 0.5 MAC_{LR}, M: 1.0 MAC_{LR}, and H: 2.0 MAC_{LR}). One MAC_{LR} is the minimum alveolar concentration at which the righting reflex is lost. Each horizontal trace represents 200 ms of prestimulus and 1,000 ms of poststimulus activity; potential variations are color coded. In the waking state (W), bursts of γ oscillations were present after each flash and repeated at approximately 200-ms intervals with decaying amplitude over 1,000 ms after stimulus. Anesthetic agents at low (L) concentration disrupted the periodicity of γ bursts and transformed them into a more continuous activity spanning most of the 100- to 1,000-ms poststimulus period at medium (M) concentration. At high (H) concentration, late γ oscillations were diminished, but the early components of the evoked response, confined to 0–100 ms, were still present.

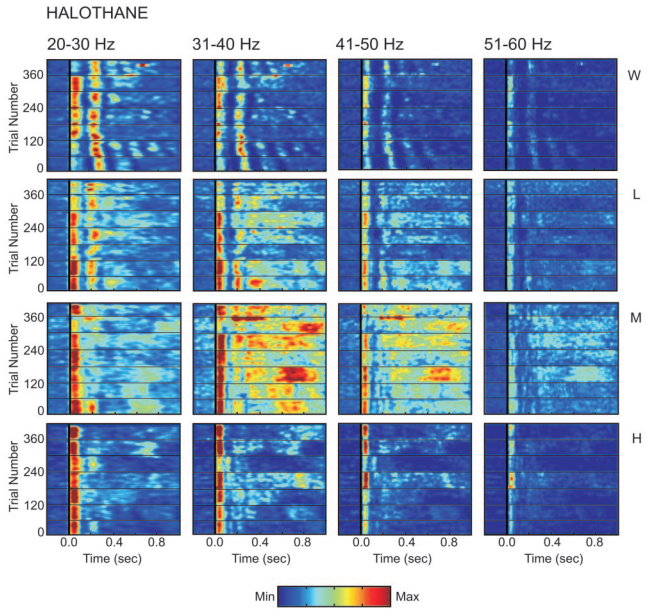

Fig. 3. Examples of wavelet transforms of the γ-filtered single-trial event-related potentials from seven experiments at different halothane levels, selected from four concentration ranges (W: 0.0 MAC_{LR}, L: 0.4–0.6 MAC_{LR}, M: 1.0–1.25 MAC_{LR}, and H: 2.1–2.5 MAC_{LR}). One MAC_{LR} is the minimum alveolar concentration at which the righting reflex is lost. Each horizontal trace represents wavelet power calculated from a single data trial consisting of 200 ms of prestimulus and 1,000 ms of poststimulus activity. To minimize frequency overlap resulting from continuous wavelet decomposition, the wavelet power was averaged within 10-Hz-wide spectral windows of 20–30, 31–40, 41–50, and 51–60 Hz in each trial. To construct each image, 60 single-trial wavelet power traces from seven experiments were stacked to arrive at 420 trials. *Horizontal black lines* are drawn to separate the trials from separate experiments. The same amplitude scale is used to display all images. The spectral power of the early response (0–100 ms) was the highest at low γ frequencies (20–30 Hz) at all concentrations. In contrast, late γ power was highest in the 31- to 50-Hz window at low (L) and medium (M) concentrations. Halothane augmented late γ oscillations in the frequency range of 31–50 Hz and at medium concentrations, which included the concentration at which the righting reflex was abolished. The augmented activity exhibited high temporal variations from trial to trial and even more from animal to animal.

the filtered version of the ERP. Halothane disrupted the afterdischarge at low concentration (0.5 MAC_{LR}) and augmented γ oscillations at intermediate concentration (1.0 MAC_{LR}). At 2.0 MAC_{LR}, γ oscillations between 600 and 1,000 ms were suppressed but were still strongly present between 0 and 500 ms after stimulus.

Figure 2 shows typical examples of γ-filtered single-trial ERP data from one animal at four selected values of MAC_{LR} for halothane, isoflurane, and desflurane. At 0.0 MAC_{LR}, bursts of γ oscillations were present after each flash. The γ bursts repeated at approximately 200-ms intervals with decaying amplitude over the 1,000-ms poststimulus period. Anesthetic agents disrupted the periodicity of γ bursts at low concentration (0.5 MAC_{LR}) and transformed the bursts into a more continuous oscillations spanning most of the 100- to 1,000-ms poststimulus period at intermediate concentration (1 MAC_{LR}). At 2.0 MAC_{LR}, late γ oscillations were diminished, but the early components of the cortical evoked response, confined to 0–100 ms, were still present. These effects were similar with all three agents and suggested that the anesthetics, in general, had distinct effects on the ECR and late γ oscillations.

Figures 3 shows further examples of the single-trial ERP in the form of wavelet transforms from seven experiments at different halothane levels selected from four concentration ranges. To minimize the frequency overlap resulting from continuous wavelet decomposition, the wavelet power was averaged within 10-Hz-wide frequency ranges. The figure shows that spectral components of the early response (0–100 ms) were not depressed by halothane even at high concentrations (2.1–2.5 MAC_{LR}). The spectral power of the response was the highest in the low γ frequency range (20–30 Hz) at all anesthetic concentrations. In variance with its

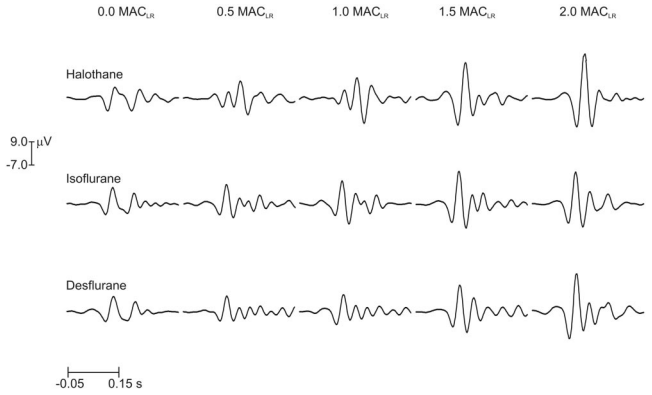

Fig. 4. Early cortical evoked response in the waking state and at four selected concentrations of halothane, isoflurane, and desflurane from the same animal as an example. Each trace represents the γ-filtered event-related potential between 0 and 150 ms averaged across 60 trials. Note that all three anesthetic agents augmented, rather than reduced, the maximum peak-to-peak amplitude of the early cortical response in a concentration-dependent manner. One MAC$_{LR}$ is the minimum alveolar concentration at which the righting reflex is lost.

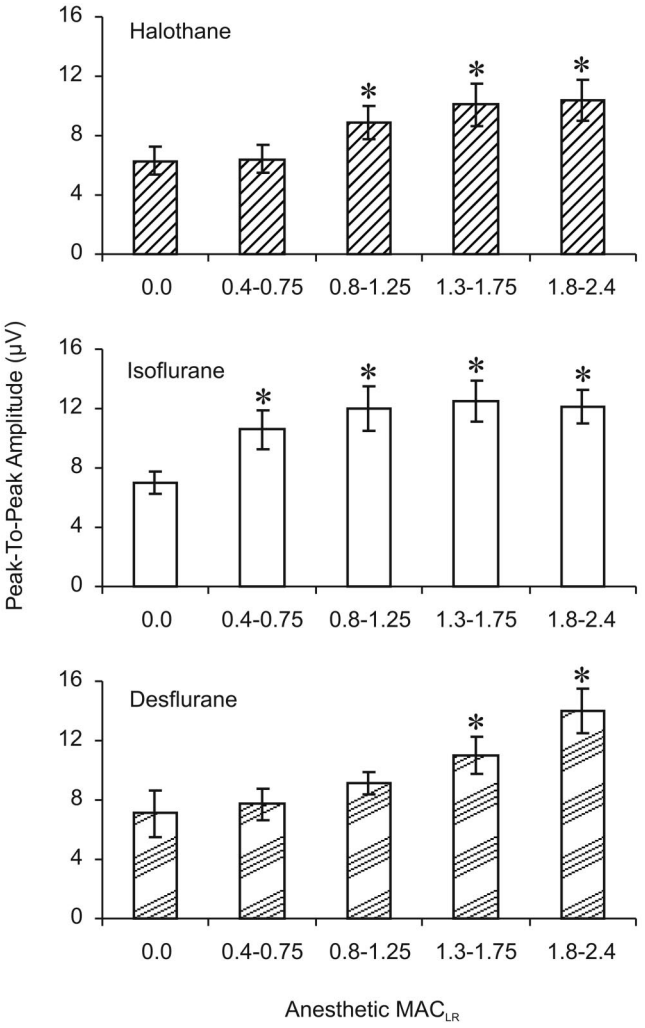

Fig. 5. Concentration-dependent effect of halothane, isoflurane, and desflurane on the amplitude of the flash-induced early cortical evoked response. The amplitude of the response was calculated as the difference between the most positive (maximum) and the most negative (minimum) peaks within 0 and 100 ms of the γ-filtered event-related potential in each trial. *Bars* represent averages from all animals. Each agent enhanced, rather than reduced, the amplitude of the early cortical response in a concentration-dependent manner. * Significant difference ($P \leq 0.05$) from waking control. When compared at equivalent low concentrations, isoflurane was the most effective of the three agents, because it significantly enhanced the response at 0.4–0.75 MAC$_{LR}$, whereas desflurane was the least effective, because it produced a significant increase in the early cortical response only at 1.3–1.75 MAC$_{LR}$. One MAC$_{LR}$ is the minimum alveolar concentration at which the righting reflex is lost. At 1.8–2.4 MAC$_{LR}$, all three agents significantly augmented the early cortical response relative to waking baseline. The final augmentation at this level was not significantly different among the agents.

effect on the early response, halothane augmented late γ oscillations in the 100–1,000 ms window. This effect was the most pronounced in the 30- to 50-Hz range and at medium concentrations (1.0–1.25 MAC$_{LR}$), which included the concentration associated with the LORR. As the figure shows, the augmented activity was variable from trial to trial, and even more from animal to animal, but this did not alter the general trend of γ augmentation within the 1,000-ms poststimulus period. The wavelet transform images obtained for two other anesthetic agents showed patterns similar to those shown here.

Anesthetic Effect on ECR

Figure 4 shows a typical example of the ECR in the waking state and at four selected anesthetic concentrations of the three agents from the same animal. Although individual components of the response varied as a function of anesthetic concentration, all three agents augmented rather than reduced the maximum peak-to-peak amplitude of the ECR. This effect was the most pronounced at highest anesthetic concentrations studied (1.8–2.4 MAC$_{LR}$).

Figure 5 compares the group average effects of the anesthetics on ECR from all experiments. Clearly, each agent enhanced, rather than reduced, the amplitude of ECR in a concentration-dependent manner. There were some differences in the magnitude of their effect at low equivalent concentrations. Thus, isoflurane significantly ($P \leq 0.05$) augmented ECR already at 0.4–0.75 MAC$_{LR}$, whereas desflurane produced a significant increase ($P \leq 0.05$) only at 1.3–1.75 MAC$_{LR}$. The effect of halothane was intermediate relative to the other two agents. Despite this difference, all three agents significantly ($P \leq 0.05$) augmented ECR at high concentrations (1.8–2.4 MAC$_{LR}$). This enhancement relative to waking baseline was 1.7-fold with halothane or isoflurane and 2-fold with desflurane (no significant difference).

Anesthetic Effect on Late γ Oscillations

Figure 6 summarizes the group average effects of the anesthetics on late γ power in the 100- to 1,000-ms window from all animals. All three agents significantly

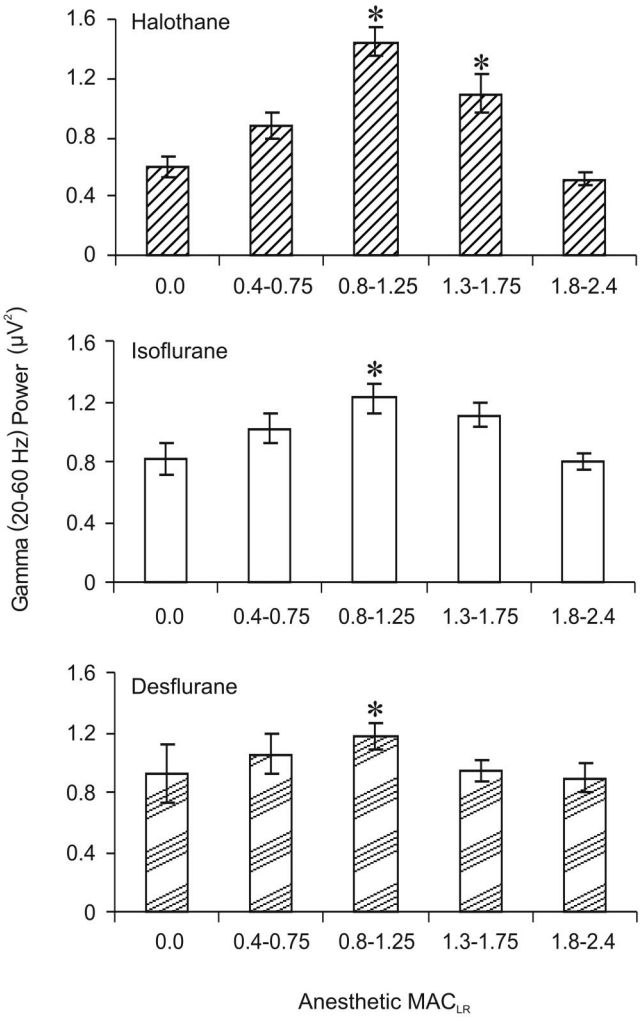

Fig. 6. Concentration-dependent effect of halothane, isoflurane, and desflurane on flash-induced late (100–1,000 ms) γ power in the rat visual cortex. Gamma (20–60 Hz) power was calculated from single-trial analysis. Each *bar* represents a group average of γ power from all animals. * Significant difference from waking baseline at $P \leq 0.05$ level. The following main observations can be made: (1) all three agents significantly augmented late γ power at 0.8–1.25 minimum alveolar concentration for the loss of righting (MAC_{LR}), which includes the concentration at which the righting reflex was lost; and (2) the augmentation of late γ power was reversed at higher anesthetic concentrations and was not significantly different from waking baseline at 1.8–2.4 MAC_{LR} for any of the three agents.

($P \leq 0.05$) augmented late γ power at intermediate anesthetic concentrations (0.8–1.25 MAC_{LR}), which included the concentration at which the righting reflex was abolished. This enhancement of late γ power relative to waking baseline was 2.5-fold with halothane, 1.5-fold with isoflurane, and 1.3-fold with desflurane (not significantly different). The augmentation of late γ power was gradually reversed at higher anesthetic concentrations and was not significantly different from the waking baseline in deeply anesthetized states for any of the three agents.

Discussion

The major findings of this study are (1) halothane, isoflurane, and desflurane enhanced the flash-evoked ECR in a concentration-dependent manner; (2) when the effects of the three agents were compared at equal fractions of MAC_{LR}, the effective concentration for an increase in ECR was the lowest for isoflurane, intermediate for halothane, and the highest for desflurane; (3) the power of flash-induced late (> 100 ms) γ oscillations was augmented at intermediate concentrations near MAC_{LR} for all three anesthetic agents; and (4) flash-induced γ power was not reduced below waking baseline even in deep anesthesia. These findings suggest that a reduction in flash-induced γ oscillations in rat visual cortex is not a unitary correlate of the anesthetic-induced unconsciousness.

Methodologic Considerations

In this study, the gross movement of the animals was limited by a body restraint. The potential confounding effect of the restraint is recognized. However, we believe that the restraint did not influence the conclusions of our study for several reasons. First, the rats showed no behavioral signs of discomfort in the waking or lightly anesthetized states in either anesthetic group. During testing, the animals seemed comfortable and calm. They rarely made an attempt to free themselves from the restrainer but seemed to accept their condition, sometimes engaging in chewing, sniffing, and whisking as seen during normal exploratory behavior. Second, when we compared flash-induced cortical responses between waking restrained and freely moving conditions, no difference was found. Therefore, we believe that the restraint did not produce undue arousal and did not influence the result. Although occasional head and orofacial movements were present, it is unlikely that motion artifacts would have contaminated the results because the cortical potentials were recorded differentially, with bipolar electrodes that sample local field activity of small cortical region, and are unaffected by far-field potential sources, such as muscle electrical activity.

Loss of Righting as a Measure of Unconsciousness

Traditionally, the potencies of inhalational anesthetic agents are compared in terms of the MAC, which designates the threshold at which the response to nociceptive stimulation (surgical cut or tail pinch) is absent 50% of the time. Because in this study we were interested in the agents' hypnotic effects, instead of MAC we used MAC_{LR}. Although there is no objective measure for the loss of consciousness in animals, in addition to our lack of understanding of the nature of animal consciousness, the LORR is thought to be a suitable behavioral index of pharmacologically induced unconsciousness in the rat and has been used extensively as such.[11,29–31,33,43,44]

This practice is based on the observation that the rat loses its righting reflex at approximately the same MAC fraction at which humans do not respond to verbal commands—the generally accepted index of human unconsciousness.

It is important to emphasize that a comparison of different anesthetic agents' effective concentrations at equivalent fractions of their MAC and their MAC_{LR} can lead to different results. Although MAC is tied to the anesthetic-induced loss of nociceptive response, which is mediated in the major part by spinal mechanisms,[45] MAC_{LR} reflects the hypnotic effect of anesthetics that depends on a cortical or thalamocortical process.[12,46] Also, volatile anesthetics likely exert their antinociceptive effects principally through *N*-methyl-D-aspartate receptors,[47] whereas their hypnotic effects are linked to action at γ-aminobutyric acid type A ($GABA_A$)[48] or cholinergic[27] receptors. Because of the difference in mechanisms, the potencies of various anesthetic agents may compare differently depending on which particular scale, *i.e.*, MAC or MAC_{LR}, is used. For example, in this study, we found that rats lost their righting reflex at the same percent inhaled concentrations of halothane and isoflurane. This observation may be surprising because the percent inhaled concentrations corresponding to 1 MAC of these agents have been reported different.[49] However, there are other data that support this finding. For example, isoflurane is known to be more potent than halothane in suppressing the response to verbal and tactile commands when compared at equivalent MAC in human volunteers. Specifically, 1 MAC halothane (1.1%) is necessary to produce unresponsiveness,[50] whereas 0.5 MAC isoflurane (0.8%) is sufficient to achieve the same endpoint.[51] The percent inhaled concentrations of halothane and isoflurane required for the suppression of these responses were more similar than their MAC values. We also demonstrated previously that halothane and isoflurane are equipotent in affecting the interhemispheric synchronization of frontal electroencephalogram and ablating the righting reflex when measured in percent concentration but not in terms of MAC.[52] Thus, agent-specific depression of cortical function is measured more accurately by fractions of MAC_{LR} than that of MAC.

Anesthetic Effect on the Early Cortical Response

Our findings will be compared to those in humans and as well as to those in similar rat experiments. In humans, the average early cortical evoked response is routinely measured using scalp electrodes and is normally referred to as the *middle latency evoked potential*. Because the rat ECR is measured in the same poststimulus time window (0–100 ms) as the middle latency evoked potential, a limited comparison with the human evoked potential is possible. The middle latency evoked potential shows an oscillatory pattern in the γ frequency band[17,20,53] that coincides with the γ band we examined in this study. In general, volatile anesthetic agents are known to decrease the amplitude and increase the latency of middle latency auditory evoked response,[15,54–57] a finding that lead to the development of a clinical anesthetic depth monitor.[58] Middle latency visual evoked potentials have also been found suppressed by anesthetics,[59] although they are more variable[60,61] than the auditory evoked response.

In contrast to the findings just described, we showed that the three volatile anesthetics, halothane, isoflurane, and desflurane, all augmented ECR relative to waking. This somewhat surprising finding is not without support from previous experimental studies. For example, Santarelli *et al.*[44] showed that the auditory evoked response was increased in some rats during isoflurane anesthesia. Rabe *et al.*[62] found that halothane at intermediate concentrations (0.25–1.0%) augmented both auditory or visual evoked responses in the rat. We found that volatile anesthetic agents augmented ECR at all concentrations up to 2% of halothane and isoflurane and up to 9% of desflurane.

The difference between rat and human study results is probably not due to a difference in sensory modality because, in humans, both auditory and visual middle latency responses were shown to be depressed by the anesthetic agents. Although there is an obvious difference in species, we find it unlikely that the mechanism of anesthesia would fundamentally differ among the mammalian species. The electroencephalographic effects of anesthetic agents are similar in all these species. Furthermore, because ECR was augmented by at least three volatile anesthetic agents, it is unlikely that agent specificity would provide an explanation.

More plausibly, the difference in the early cortical evoked response between human and some of the animal studies may be due to a difference in the type of electrodes and the neuronal activity they are suited to record. Namely, scalp electroencephalographic electrodes used in human studies record synchronized cortical electrical potentials from an estimated cortical volume of approximately 10 cm^3 per electrode. These potentials reflect not only sensory-specific neuronal activity but also activity outside of modality-specific cortex. For example, the traditional middle latency auditory evoked response recorded between the ear and the neocortex of humans may also reflect long-range corticocortical as well as thalamocortical synchronization of sensory evoked activity. In contrast, small bipolar electrodes used in our study to record from the visual cortex sample local field potentials from a tissue volume of approximately 0.01 cm^3 that reflect synchronized activity of a relatively small group of neurons. Because the anatomy of local neuronal circuits is different from that of large-scale corticocortical networks, anesthetic agents may exert a different effect on long-range and local

synchronization of sensory-stimulus related activity. For example, it is conceivable that anesthetic agents may suppress long-range synchronization while augmenting local synchronization. The anesthetic effect on sensory activity of long-range corticocortical networks would be more apparent in the human scalp recordings, whereas the local circuit effects would be revealed by the local intracortical recordings such as those done in the current study.

Although all three anesthetic agents augmented ECR at high enough concentration, there was some difference in the agents' potencies at low concentrations: Isoflurane augmented ECR at concentrations at which the other two agents did not, and desflurane augmented ECR at higher concentrations than either halothane or isoflurane. In fact, isoflurane is known to be more effective than halothane in depressing the activity of single neurons,[63] the electroencephalogram,[57] auditory evoked potentials,[57] cerebral metabolism, and blood flow.[28] Why desflurane was the least effective of the three agents is less clear. Desflurane is believed to suppress the central nervous system to a degree similar to that produced by isoflurane.[64–67]

Anesthesia and the Late γ Oscillations

In addition to the flash-induced early cortical response, which represents a brief stimulus-locked γ oscillatory potential, a delayed increase in the power of γ oscillations was observed and termed *late γ oscillations*. The phase of late γ oscillations relative to the flash is more dispersed than that of the early cortical response and is therefore not detectable by signal averaging, only by single-trial analysis. To distinguish this activity from the average evoked potential, some authors use the term *stimulus-induced* as opposed to *stimulus-evoked activity*.[40]

As with the ECR, we were surprised to find that volatile anesthetics significantly augmented, rather than reduced, the flash-induced late γ oscillations. In fact, γ power was not reduced below waking baseline even in surgical anesthesia. Unlike their differing effects on ECR, the three agents were similarly effective in augmenting late γ power near 1 MAC_{LR}. As discussed in the previous section, the effect of anesthetics on scalp-recorded and intracortically recorded potentials may be different. Therefore, direct extrapolation of the current findings to human electroencephalographic events is not appropriate. However, it may be argued that local field potentials recorded with bipolar intracortical electrodes would give a more accurate representation of the effect of anesthetic agents on stimulus-induced synaptic events than do macropotentials obtained with scalp electrodes. Therefore, our data provide a more accurate insight into the local neuronal mechanism of anesthetic action with respect to visual sensory processes. In this sense, our findings suggest that volatile anesthetics do not suppress the generation of stimulus-induced γ oscillations in visual cortex, as it may have been concluded from human studies. Instead, anesthetics may suppress interregional or long-range synchronization of neuronal activity in the γ frequency range.[12]

Although the average γ power was significantly augmented by all three anesthetics, the individual response potentials varied from trial to trial, as well as from animal to animal. This variation was clearly shown by the wavelet decomposition revealing both the temporal and spectral distributions of flash-induced γ power. Trial-to-trial variations in the stimulus response may reflect spontaneous variations in the state of arousal and in cortical neuronal excitability.[68] To examine this possibility, future experiments involving longer ERP recording times at steady state anesthetic levels may be of interest to perform.

Neural Correlates of Anesthetic-induced Unconsciousness

The important question that we face is whether the event of loss of consciousness or, in the current experiment, more correctly the LORR, can be related to an anesthetic-induced change in either the early or the late visual evoked cortical γ response. Such a change could be viewed as a neural correlate of unconsciousness.[69] For the latter, one would hope to find a relatively abrupt change in the evoked response that would occur consistently at an anesthetic concentration that first produces the behavioral sign of unconsciousness and that would be invariant with respect to the administered anesthetic agent. Because the three volatile agents differed in their effects on ECR near 1 MAC_{LR}, ECR does not seem to satisfy the above requirements to be a unitary correlate of unconsciousness. Moreover, the ECR increased, rather than decreased, in anesthesia, making it even less plausible as a correlate of unconsciousness.

The significance of late stimulus-induced γ oscillations in cognitive, sensory-perceptual, and voluntary motor functions has been repeatedly demonstrated.[1,3–6,70–78] This implies the hypothesis that anesthetic-induced unconsciousness may be associated with the suppression of stimulus-induced late γ oscillations, rather than that of the early, stimulus-locked response. However, we found that the power of flash-induced late γ oscillations was augmented by the three volatile anesthetic agents at concentrations near MAC_{LR}. In fact, γ power was not reduced below waking baseline even in deep anesthesia. Therefore, we conclude that anesthetic-induced unconsciousness cannot be ascribed to a reduction in power of either the early, stimulus-evoked or late, stimulus-induced γ oscillations in rat visual cortex.

As already mentioned, this negative finding does not refute the potential importance of an anesthetic depression of interregional or long-range γ synchronization as an underlying mechanism of unconsciousness. A disrup-

tion of both corticocortical and thalamocortical connectivity may be associated with surgical anesthesia.[7,46] Furthermore, anesthetic-induced changes in the temporal pattern of γ oscillations, rather than the changes in γ power, may be important. We observed that in waking and shallow anesthetic states, the flash produced bursts of γ oscillations, which were transformed into more-or-less continuous activity at intermediate anesthetic concentrations. This transformation may reflect a change in interregional synchronization of γ oscillations underlying neuronal events potentially associated with unconsciousness. It is also possible that the increased synchronization of local synaptic activity, revealed as an enhancement of local γ power, may disrupt the more subtle spatiotemporal patterns of local γ oscillations associated with information processing and consciousness. Testing of this hypothesis would require multichannel recordings of the neuronal ensemble activity at various anesthetic levels; such experiments are in progress in our laboratory.

Cellular Mechanism of γ Enhancement by Anesthetic Agents

The cellular mechanism of the anesthetic enhancement of early and late γ oscillations is unknown. Volatile anesthetics are known to potentiate neurotransmission at $GABA_A$ receptors by enhancing receptor affinity and prolonging the postsynaptic hyperpolarization current.[48] It has been indicated that volatile anesthetics also inhibit α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–type glutamate receptors and, at high concentrations, potentiate kainate receptors.[79] Buzsaki *et al.*[80,81] proposed that $GABA_A$ synaptic transmission in a network of fast-spiking interneurons in the hippocampus may play a role in the generation of synchronized γ oscillations. Other investigators[82–84] extended this hypothesis to cortical networks. This then raises the possibility that halothane, isoflurane, and desflurane may augment γ oscillations through an enhancement of $GABA_A$ transmission in the cortical interneuron network.

Why the increase in γ power is reversed at higher anesthetic concentrations is less clear. It is possible that the anesthetic modulation of $GABA_A$ receptor activity is nonlinear, such that too much inhibition counteracts the oscillations or that the anesthetic depression of excitatory transmission at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors overcomes the $GABA_A$-mediated enhancement of γ oscillations at higher concentrations. Another explanation may be that the depression of γ power is due to the potentiation of kainate receptors.[79] Future experiments using means for selective receptor modulation may help to test some of these hypotheses.

In conclusion, our findings suggest that the anesthetic-induced unconsciousness, referenced by the LORR, is associated with an increase rather than a decrease in flash-induced intracortical potentials in the γ frequency band. Both the stimulus-locked early cortical and phase-dispersed late γ oscillations were enhanced at concentrations producing unconsciousness and were not reduced below the waking baseline even in deep anesthesia. These results suggest that neither of these two parameters is a unitary correlate of the anesthetic-induced unconsciousness.

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