Neuroscience 123 (2004) 269–278

HALOTHANE AUGMENTS EVENT-RELATED γ OSCILLATIONS IN RAT VISUAL CORTEX

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Abstract—Cortical γ oscillations have been associated with neural processes supporting cognition and the state of consciousness but the effect of general anesthesia on γ oscillations is controversial. Here we studied the concentration-dependent effect of halothane on γ (20–60 Hz) power of event-related potentials (ERP) in rat primary visual cortex. ERP to light flashes repeated at 5-s intervals was recorded with chronically implanted, bipolar, intracortical electrodes at selected steady-state halothane concentrations between 0 and 2%. γ-Band power was calculated for 0–1000, 0–300 and 300–1000 ms poststimulus periods and corresponding prestimulus (PS) periods. Multitaper power spectral analysis was used to estimate γ power from both single-trial and average ERP in order to differentiate between phase-locked (evoked) and non-phase-locked (induced) γ activities. Significant PS γ power was present at all halothane concentrations. Flash elicited an increase in γ power that lasted up to 1 s poststimulus at all halothane concentrations. Halothane at intermediate concentrations (0.5–1.2%) augmented both PS and ERP γ power two to four times relative to the waking baseline. γ Power was not different between waking and deeply anesthetized (2%) levels. γ Power reached maximum, as predicted by a Gaussian fit of power-concentration data, at halothane concentration (0.86%) similar to the concentration (0.73%) that abolished the righting reflex, a behavioral index of loss of consciousness. Evoked, i.e. stimulus-locked, γ power was present during the first 300 ms poststimulus but not later, and was approximately 50% of single-trial ERP γ power. Single-trial γ power was present also at 300–1000 ms poststimulus, reflecting ERP not phase-locked to the stimulus.

In summary, these observations suggest that (1) γ activity is present in states ranging from waking to deep halothane anesthesia, (2) halothane does not prevent the transfer of visual input to striate cortex even at surgical plane of anesthesia, and (3) anesthetic-induced loss of consciousness, as reflected by the loss of righting reflex, is not correlated with a reduction in γ power. Variance with other studies may be due to an underestimation of γ power by ERP signal averaging as compared with single-trial analysis.

Key words: anesthesia, consciousness, EEG, evoked potential, righting, synchrony.

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Abbreviations: EEG, electroencephalogram; ERP, event-related potential; FP, field potential; LOC, loss of consciousness; PS, prestimulus.

Identification of the neuronal targets and mechanisms of the hypnotic action of general anesthetics has been a major challenge to anesthesia research. Despite decades of studies of anesthetic pharmacology and anesthetic-receptor molecular interaction, a mechanistic model of anesthetic ablation of consciousness has not emerged. This is arguably due in part, to our inability to objectively assess consciousness and to our lack of general understanding of its neurophysiological correlates.

Much effort has been devoted to assess the adequacy or "depth" of the anesthetic state, focusing mainly on the measurement of either resting or sensory-evoked cortical electrical activity. The general effect of most anesthetics on the electroencephalogram (EEG) is thought to be synchronization, characterized by a shift of the EEG spectrum to low-frequency, high-amplitude components. Numerous indices to quantify these EEG changes have been introduced such as, the power spectral edge, median and zero-crossing frequencies, bispectral index, etc. Some of these are now used in clinical instruments designed to monitor anesthetic depth (Gugino et al., 2001; Tempe, 2001). The hypnotic action of anesthetics has also been characterized through their effect on sensory evoked potentials. General anesthetics typically decrease the amplitude and increase the latency of early cortical evoked potential components (Jones and Aggarwal, 2001). An anesthetic depth monitor measuring changes in the cortical auditory evoked potentials has recently been introduced in clinical practice (Struys et al., 2002). However, many of these monitoring techniques have been found to be anesthetic agent-specific or not uniquely related to agent effect or clinical use (Jones and Aggarwal, 2001; Tempe, 2001). Furthermore, current instrumentation lacks the sound neurophysiological basis for the assumption that it specifically assesses the state of consciousness.

Recently, there has been growing interest in the role of spontaneous and evoked γ (20–60 Hz) EEG oscillations in conscious cognition. γ Oscillations have been associated with behavioral arousal (Munk et al., 1996; Maloney et al., 1997; Herculano-Houzel et al., 1999; Fetz et al., 2000), selective attention (Bouyer et al., 1980; Tiitinen et al., 1993; Muller et al., 2000; Steinmetz et al., 2000; Fries et al., 2001), short-term memory (Tallon-Baudry et al., 1999b), preparation for motor acts (Pfurtscheller et al., 1994; Donoghue et al., 1998; Raichle et al., 2001), and formation of perceptual representations (Keil et al., 1999; Srinivasan et al., 1999; Tallon-Baudry et al., 1999a).

Studies that investigated the effects of general anesthetics on γ oscillations have, however, yielded conflicting results. For example, John et al. (2001) demonstrated in a

0306-4522/04$30.00+0.00
doi:10.1016/j.neuroscience.2003.09.014

large patient population a significant reduction in EEG γ power during surgical anesthesia. Likewise, a suppression of γ activity during surgical anesthesia was demonstrated by Uchida et al. (2000). In contrast, Veselis' group (Feshchenko et al., 1997) found an increase in β (or low γ) power at the point of loss of consciousness. Likewise, in rats, Vanderwolf (2000) demonstrated that the amplitude of γ activity was often greater during surgical anesthesia than in the waking state. Kral et al. (1999) found that light anesthesia produced a peak in EEG spectrum around 30 Hz in rats.

General anesthetics have been shown to suppress middle latency auditory evoked potentials that appear to oscillate at γ frequency (Schwender et al., 1994; Dutton et al., 1999; Kochs et al., 2001). They have also been shown to reduce the 40-Hz auditory steady-state response (Schwender et al., 1994; Gilron et al., 1998; Plourde et al., 1998; Bonhomme et al., 2000; Kochs et al., 2001); this effect has been related to anesthetic-induced unconsciousness. Similar observations were made with respect to the visual evoked response (Sloan, 1997). Visual evoked response studies in the rat are, however, contradictory (Yeoman et al., 1979; Rabe et al., 1980).

The abovementioned studies were limited in that they focused on the first 100 ms of the evoked response only. The anesthetic effect on late oscillatory events has not been investigated. Furthermore, since sensory evoked potentials were routinely extracted from the background EEG by signal averaging, the results were limited to stimulus-locked (evoked) γ events. Non-stimulus-locked (induced) γ oscillations, which are characterized by trial-to-trial latency jitter and possibly later onset (>300 ms) (Bertrand and Tallon-Baudry, 2000), were not revealed in these studies, since they were eliminated by signal averaging. It is this induced γ activity, however, that has been suggested to underlie feature binding and conscious perception and thus is worthy of further investigation with regard to anesthetic action (Eckhorn et al., 1988; Muller et al., 1996).

Despite previous observations of γ EEG in rats in anesthetized states (Buzsaki et al., 1983; Kral et al., 1999; Vanderwolf, 2000), a systematic, concentration-dependent study of volatile anesthetic effect on γ oscillations in the rat, with or without visual stimulation, has not been performed. The objective of this study was to systematically assess the concentration-dependent effect of volatile anesthetic halothane on spontaneous and visual flash-induced γ oscillations up to 1 s poststimulus in the rat primary visual cortex. To account for both evoked and induced γ activity, we applied both averaging and single-trial analyses (Jung et al., 2001). In addition, we used the loss of righting reflex in the rat as a behavioral index of the loss of consciousness (LOC) (Devor and Zalkind, 2001; Ma et al., 2002; Flood et al., 2002; Gustafsson et al., 1996; Kissin et al., 1983; Yang et al., 1992). We demonstrate that halothane has pronounced concentration-dependent augmenting effect on both spontaneous and flash-induced γ activity in rat visual cortex contrary to the common view in the clinical literature.

EXPERIMENTAL PROCEDURES

The experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee. 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, D.C., 1996). All efforts were made to minimize the number of animals used and their suffering.

Electrode implantation

Five adult male Sprague–Dawley rats were kept on reversed light/dark cycle in dedicated rooms of the Animal Resource Center for 2 weeks prior to physiological experiments. On the day of the aseptic surgery, the rats (250–300 g) were anesthetized using isoflurane (Abbot Laboratories, Chicago, IL, USA) in an anesthesia box. The animal's head was secured in a rat stereotaxic apparatus (Model 900; Kopf Instruments, Tujunga, CA, USA) and a gas anesthesia adaptor (Stoelting Co., Wood Dale, IL, USA) was placed over the snout to continue anesthesia at 1.5% isoflurane. Body temperature was maintained at 37 °C via a water-circulating heating pad. The dorsal surface of the head was prepared for sterile surgery with betadine. 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 and the exposed cranium was gently scraped of connective tissue and any bleeding cauterized.

For recording of intracortical field potentials, a concentric, bipolar semi-micro electrode (SNEX-100X; Rhodes Medical Instruments, Inc., Summerland, CA, USA) 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 bregma (Paxinos and Watson, 1998) 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 screws anchored the skullcap to the cranium and the assembly was embedded in resin. An antibiotic (10 mg/kg Enrofloxancin i.m.) and pain medication (0.02–0.05 mg/kg Buprenex s.c.) were administered. Analgesic injections of Buprenex (0.02–0.05 mg/kg s.c. twice daily) continued for 3 days and the antibiotic injections of Enrofloxancin (10 mg/kg i.m. twice daily) were administered for 14 days. The animal was observed for 7–10 days for any infection or other complications.

Experimental protocol

Following 7–10 days of recovery, the animal was placed in the cylindrical plastic restrainer of 6 cm diameter (Harvard rodent restrainer; Model AH-52-0292; Harvard Apparatus, Holliston, MA, USA), stationed inside a rectangular, transparent, plexiglass anesthesia box. While awake, the animal had limited movement of its head and limbs, but could not crawl out of the cylinder. The animal's body temperature was maintained at 37 °C with a thermostat-controlled, water-circulated heating pad. The rat was positioned in the apparatus under halothane anesthesia, vaporized into a mixture of 30% O_2 and 70% N_2, and when all connections were in place, the anesthetic was turned off. The animal was breathing spontaneously. Following 1 h of equilibration time, halothane concentration was raised in 12–13 increments from 0 to 2.0%. 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, USA). Ten-minute equilibration time was allowed at each halothane setting prior to the recording of field potentials (FP). A light guide, connected to a stroboscopic light source (EG & G Electro-Optics, MA, USA) 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. At each halothane concentration following a gentle knocking on the side of anesthesia box, the FP was recorded in response to visual stimulation. The visual 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 stimulus-related activity would dissipate at least several seconds before the next stimulus onset. The FP was 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 50 and 60 Hz of 0 and −40 dB, respectively), and digitally sampled at 500 Hz (WINDAQ Data Acquisition Software; DATAQ Instruments, Akron, OH, USA). The experiment was repeated one to two times in each animal, with an interval of 7–10 days separating the experiments.

In six additional Sprague–Dawley rats the loss of righting reflex as a function of halothane concentration was tested. The loss of righting reflex was used as a behavioral index of the LOC in the rat. The same experimental protocol was applied as in the electrophysiological experiments. 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. Spontaneous head movement or random limb movement during tilt were not taken as an indication of righting.

Data analysis

FP segments comprising 1-s prestimulus (PS) and 1-s poststimulus (ERP) periods were extracted from the record using a threshold peak-detection algorithm developed in our laboratory. Inspection of the records showed that stimulus-related activity dissipated after 1 s poststimulus.

To examine spectral distribution of single-trial ERP power, poststimulus data were low-pass filtered at 60 Hz with a bi-directional Chebychev Type I digital filter ($N=8$, ripple$=0.01$; MATLAB 6.0; MathWorks Inc., Natick, MA, USA). The power spectral estimates were obtained using Thomson's Multitaper Power Spectral Analysis (MATLAB 6.0). This technique was chosen because it offers superior performance for the analysis of short data segments with a high degree of nonstationarity (Bronez, 1992).

To examine the dependence of γ power on halothane concentration, single-trial PS and ERP data were band-pass filtered at 20–60 Hz with the same Chebychev filter. We used the visualization tool "ERP image" (Jung et al., 2001) to inspect single-trial PS and ERP activity in each experiment. γ Power for PS and ERP data were then estimated using the Multitaper technique. ERP power was determined separately for two poststimulus time intervals of 0–300 ms and 300–1000 ms. Corresponding PS power was determined from PS data of the same duration. γ Power obtained at each halothane concentration was first averaged across the spectrum (20–60 Hz) for both PS and ERP. γ Band power was then averaged for all single-trial segments to obtain one datum per anesthetic concentration in each experiment for both PS and ERP.

To compare single-trial power with power from time-averaged ERP signals, band-pass filtered single-trial ERP's were averaged before power estimation. γ Band power was then calculated using the Multitaper technique as before, for two poststimulus time intervals of 0–300 ms and 300–1000 ms. γ Band power was then averaged across the spectrum (20–60 Hz) to obtain one datum per anesthetic concentration in each experiment for both PS and ERP.

Statistical analysis

Noting that the effect of anesthetic concentration on γ power was nonlinear, the data were approximated with various nonlinear functions. Fourth-order Gaussian function provided the best fit and was used to estimate peak power halothane concentration for each data set. To minimize experiment-to-experiment variance, γ-band power was normalized to the mean across all concentrations in each experiment prior to curve fitting. To test for a significant effect of visual stimulation on γ power, the analysis of covariance was used with the anesthetic concentration as a covariate, pre- and poststimulus condition as a fixed factor and the experiment as a random variable. The same technique was used to compare single-trial and average γ powers and to compare power data from different time windows. Each comparison was performed separately for both 0–300 ms and 300–1000 ms intervals. Curve fitting was performed using the software Sigma Plot (SPSS Inc., Chicago, IL, USA) Statistical analyses were carried out using MINITAB (Minitab Inc., State College, PA, USA).

RESULTS

Righting reflex

Six animals that were tested for the loss of righting reflex as a function of halothane concentration demonstrated consistent results. The righting reflex of all rats was lost between 0.7% and 0.8% halothane. Namely, in four animals the loss of righting reflex occurred at 0.7% halothane; in the other two rats the righting reflex was lost at 0.8%.

Effect of halothane on ERP power spectrum

Fig. 1 shows average power spectral estimates of 1-s long single-trial ERP data at seven halothane concentrations. It is apparent that various frequency bands were differentially affected by the anesthetic. Thus, 20–60 Hz power was elevated at intermediate halothane concentrations of 0.5–1.2% relative to either low or high halothane levels. In contrast, 1–20 Hz power was mostly reduced with increasing halothane concentration, although a reversal of this trend was apparent in the 1–5 Hz range at the highest halothane levels. The initial reduction in 1–10 Hz power with increasing anesthetic concentration was likely due to a suppression of the flash-induced afterdischarge that typically follows flash stimulation as illustrated in Fig. 1. The reversal in 1–5 Hz power at 1.4 and 1.5% halothane was consistent with the development of Δ waves in the background EEG.

Effect of halothane on γ power

Typical single-trial γ-filtered PS and ERP data at four selected halothane concentrations are shown in the form of "ERP images" in Fig. 2. Starting with 0% halothane, a consistent oscillation in ERP was apparent following each flash. Note that the activity spanned at least 600 ms poststimulus, well beyond the range of cortical middle latency evoked potentials that occur within the first 100 ms. The effect of halothane appeared to be an enhancement of amplitude, shown most clearly at halothane concentration near 0.8%. Although significant activity was present past 300 ms, the phase became more variable trial to trial. The effect of phase dispersion is illustrated by the averaged waveforms displayed under each ERP image, which predict smaller oscillation amplitude. At 1.5% halothane, when most of the ERP activity was reduced, the middle latency evoked components near 40–60 ms were still present at unchanged amplitude. Note that PS γ oscillations are vis-

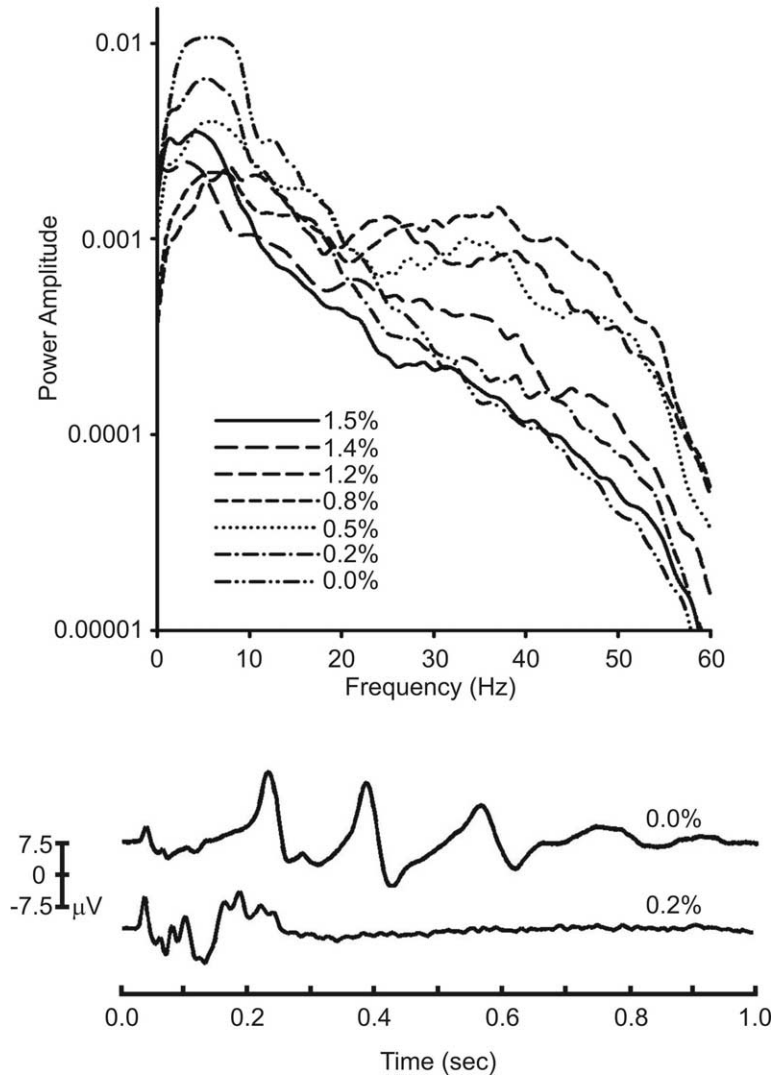

Fig. 1. Concentration-dependent effect of halothane on flash-evoked ERP in rat primary visual cortex. Top panel shows power spectral amplitude estimates of 1-s long single-trial ERP data at seven selected halothane concentrations. Displayed spectra represent average power from eight experiments. Various frequency bands are differentially affected by halothane: 20–60 Hz (γ) power is increased at halothane concentrations 0.5–1.2% while power in the 1–20 Hz range is reduced. The latter effect is probably related to the early depression of flash-induced afterdischarge potentials; see bottom panel for an example. Flash-induced afterdischarge with a dominant frequency of approximately 4 Hz is evident at 0% halothane but is diminished at 0.2% halothane.

ible at all four halothane concentrations and seem particularly strong at 0.8%.

To determine the poststimulus period when evoked activity is the most expressed, averaged ERP signals from all experiments were examined as a function of halothane concentrations. Fig. 3 shows that the average ERP that represents phase-locked or evoked γ activity was essentially limited to the first 300 ms poststimulus. The non-phase-locked or induced γ activity, readily apparent in the single-trial ERPs of Fig. 1 past 300 ms, was masked here by signal averaging. Likewise, PS γ oscillations were hidden by signal averaging at all seven halothane levels. To account for the induced ERP components, single-trial analysis was carried out in two temporal windows of 0–300 ms and 300–1000 ms. The latter window contains almost exclusively induced activity.

Fig. 4 compares PS, single-trial ERP and average ERP γ power at various halothane concentrations separately, for two time windows. Three main observations can be made. First, halothane exerted a similar nonlinear effect on all three γ activities, i.e. γ power was increased at intermediate concentrations of 0.5–1.2%. γ Power was not different between waking and deeply anesthetized states ($\geq$1.5%). Second, single-trial ERP γ power was significantly ($P<0.001$) greater than either average ERP or single-trial PS power. Both of these observations apply to both 0–300 and 300–1000 ms windows. Third, when comparing power obtained from the two temporal windows, single-trial ERP power was significantly ($P<0.001$) higher

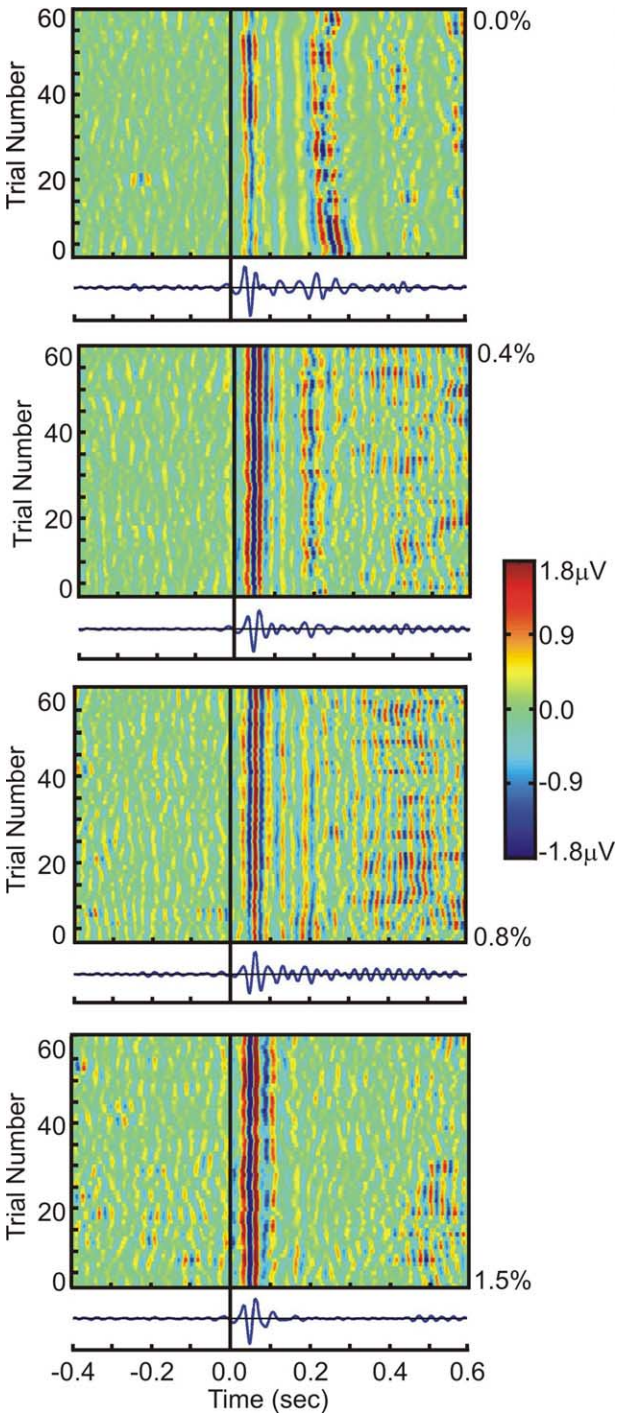

Fig. 2. Flash-induced, γ (20–60 Hz)-filtered ERP images from data recorded in rat striate cortex at four selected halothane concentrations. Each horizontal trace represents 1 s of data; potential variations are color-coded. Flash is applied at 0 s. Average single-trial PS and ERP activity is shown below each image for comparison. Flash produces prolonged ERP oscillations in the γ frequency range in waking and halothane anesthesia but the amplitude and pattern of oscillations vary with the anesthetic concentration. Increasing halothane concentration augments both PS and poststimulus (ERP) γ activity. Late ERP amplitude appears highest at 0.8% halothane. γ Activity reflected by the average PS and ERP signals is small compared with those suggested by the image display due to the phase dispersion of the oscillations.

during the first 300 ms poststimulus than during the following period whereas average ERP power was diminished between 300 and 1000 ms. The latter observation is consistent with the notion that non-phase-locked (induced) γ activity as revealed by the single-trial analysis, is prolonged compared with the phase-locked (evoked) activity revealed by ERP averaging. PS power, representing spontaneous EEG activity, was the same in the two time windows.

As observed, the anesthetic dependence of ERP and PS γ powers was similar. The halothane concentration at which maximum enhancement of γ power occurred was obtained from Gaussian curve fits of the three data sets as shown in Fig. 4. The halothane concentration at peak γ power was the same, 0.86%, for single-trial PS, ERP, and average ERP suggesting that halothane had a similar effect on induced, evoked and spontaneous γ activities, at least, during the first 300 ms poststimulus period.

The effect of flash on γ power was also estimated from the Gaussian curve fit. In the 0–300 ms time window, at 0.86% halothane producing maximum γ power, stimulus enhanced single-trial γ power by approximately four-fold. This enhancement was smaller, about two-fold, in the subsequent 300–1000 ms interval. Peak ERP γ power from single-trial analysis was two times higher than average ERP γ power suggesting that evoked and induced γ activities contributed nearly equally to γ power following flash stimulation.

Finally, the effect of halothane on 20–60 Hz γ power was compared with that of 60–100 Hz (high γ) power at various halothane concentrations. The same filtering and power estimation techniques as in the case of 20–60 Hz γ power were implemented to determine the 60–100 Hz power. This analysis revealed that the 60–100 Hz power was negligible at all halothane concentrations compared with the 20–60 Hz power. For example, the peak 20–60 Hz power in the 0–300 ms interval was approximately 20 times greater than the 60–100 Hz power suggesting that the frequency around 60 Hz was an appropriate cutoff for the present analysis.

DISCUSSION

In this study, we examined the concentration-dependent effects of halothane on γ oscillations in the rat visual cortex before and after single flash stimuli. We demonstrated that both pre- and poststimulus γ activity was enhanced at intermediate halothane concentrations but was not different from its waking baseline and surgically anesthetized levels. We also showed that during the first 300 ms poststimulus, approximately half of the γ power was phase-locked to the flash while the other half was not. Only non-phase-locked (induced) γ activity was present between 300 and 1000 ms poststimulus. This result shows that single flash stimuli induce γ oscillations that last up to 1 s and can only be revealed by single-trial analysis. The findings also imply that LOC that likely occurs at approximately 0.7–0.8% halothane concentration does not corre-

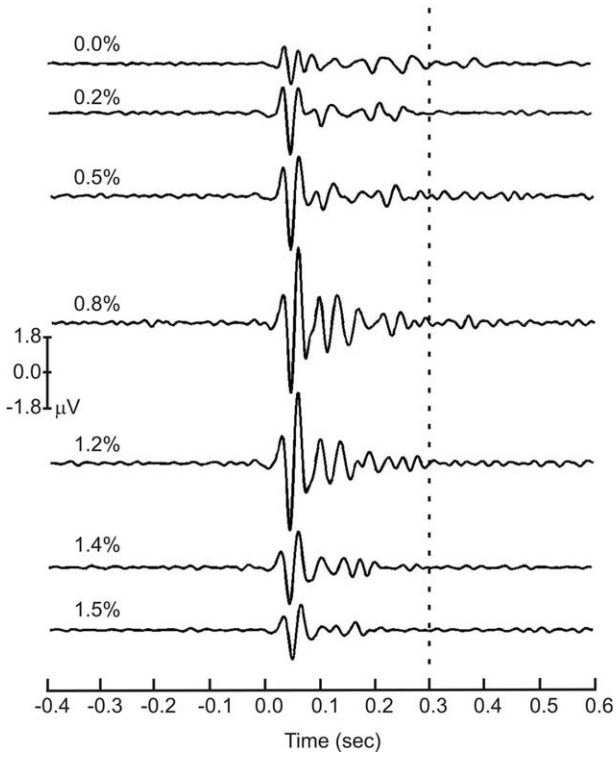

Fig. 3. Flash-induced average ERP filtered to γ (20–60 Hz) at seven selected halothane concentrations. Flash is applied at 0 ms. Graphs represent average data from several experiments. Halothane at intermediate concentrations amplifies the average ERP. The damped oscillatory response to flash is limited to approximately 300 ms poststimulus duration at all anesthetic concentrations.

late with a reduction in either spontaneous or stimulus-related γ activity.

General anesthetics and spontaneous γ oscillations

γ Oscillations of the EEG have been associated with conscious cognitive functions (Bouyer et al., 1980; Tiitinen et al., 1993; Pfurtscheller et al., 1994; Munk et al., 1996; Maloney et al., 1997; Donoghue et al., 1998; Herculano-Houzel et al., 1999; Keil et al., 1999; Srinivasan et al., 1999; Tallon-Baudry et al., 1999a; Fetz et al., 2000; Muller et al., 2000; Steinmetz et al., 2000; Fries et al., 2001). As a result, a reduction in γ oscillations has been suggested as a possible indicator of anesthetic-induced hypnosis and even LOC (Schwender et al., 1997; John et al., 2001; Sleigh et al., 2001; Uchida et al., 2000). Recently, John et al. (2001) reported a global decrease in EEG γ power in patients anesthetized with various agents at surgical levels of anesthesia (absent response to painful stimuli) and suggested a correlation between the reduction in γ power and LOC. However, γ power was augmented at lower anesthetic concentrations associated with anesthesia induction, which was likely closer to the threshold of LOC than the anesthetic level at which γ reduction was reported. In our study, the LOC was marked by the loss of righting reflex, which occurs at shallower anesthetic concentrations than the loss of response to painful stimuli. Similarly, an augmentation of high β (or low γ) activity was demonstrated by Veselis' group (Feshchenko et al., 1997) with various intravenous agents. Recently, Gugino et al. (2001) showed that light sedation with propofol or sevoflurane was also accompanied by an increase in 12.5–25 Hz β power. Thus, the difference in conclusions attained by John et al. (2001) versus ourselves and others (Feshchenko et al., 1997) is likely due to a comparison of different anesthetic states rather than an actual difference in γ behavior with anesthetic administration.

Several previous studies in rats also revealed enhanced γ oscillations during anesthesia. Vanderwolf (2000) showed that the amplitude of spontaneous γ EEG during anesthesia with urethane, isoflurane or ether, was often greater than in the normal waking state. Increased γ EEG activity during urethane anesthesia has also been demonstrated in the rat hippocampus by Buzsaki et al. (1983). Kral et al. (1999) observed a peak in the EEG spectrum near 30 Hz in rats lightly anesthetized with isoflurane. A limitation of these studies was, however, that they did not examine the effect of anesthetics at multiple steady-state levels.

Using multiple steady-state controlled concentrations of the anesthetic, we now show that halothane in moderate concentrations augments intracortical γ oscillations in agreement with previous animal studies. Furthermore, γ power is not reduced below the waking baseline even at surgical level of anesthesia associated with absent response to pain. Since LOC certainly occurs at shallower than surgical anesthesia, these find-

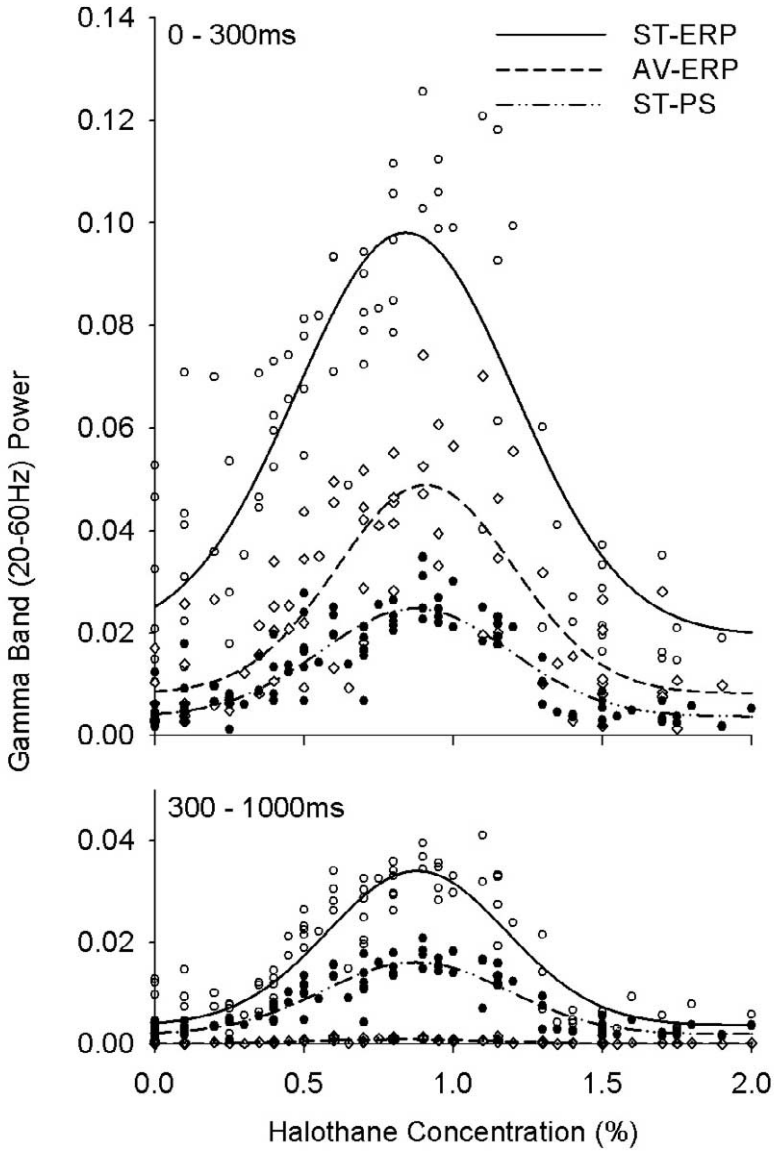

Fig. 4. The concentration-dependent effect of halothane on PS and flash-induced poststimulus γ power in rat visual cortex. Poststimulus γ power data shown are from both single-trial (ST-ERP) and average (AV-ERP) data, representing total and stimulus-locked ERP, respectively. PS power is from single-trial analysis (ST-PS). Results obtained for two peri-stimulus time windows, 0–300 ms and 300–1000 ms are shown in the top and bottom panels. Curves show fitted fourth-order Gaussian functions. The following main observations are made. (1) Halothane augments both pre- and poststimulus γ powers; the maximal effect is predicted at 0.86% concentration. (2) Flash stimulus augments γ power essentially independent of the applied halothane concentration (Single-trial vs. PS, both panels). (3) γ Power obtained from single-trial ERP is higher than from average ERP, indicating partial phase dispersion of stimulus-induced γ activity. Phase-locked power is 50% of total during 0–300 ms poststimulus (top panel) and essentially zero past 300 ms (bottom panel).

ings suggest that γ power is not reduced as the transition to the unconscious state takes place. Although there is no objective measure of LOC in animals, the loss of righting reflex is thought to be the best currently available behavioral index of LOC in the rat and has been used extensively as such (Devor and Zalkind, 2001; Ma et al., 2002; Flood et al., 2002; Gustafsson et al., 1996; Kissin et al., 1983; Yang et al., 1992). The loss of righting reflex in rat and the loss of response to verbal commands in human-the generally accepted human index of LOC, occur at approximately half of their respective threshold concentrations necessary to prevent movement in response to a painful stimulus. Thus, the latter threshold concentration reflecting the loss of nociceptive response does not correspond to the transition from wakefulness to unconsciousness (Kissin et al., 1983; Antognini and Carstens, 2002). If the loss of righting reflex in the rat correctly reflects the LOC, the LOC would in fact coincide with maximum γ EEG power.

It is possible that the functional dependence of γ power on anesthetic concentration would be slightly different with different anesthetic agents. For example, Vanderwolf

(2000) studied the effects of isoflurane anesthesia and observed bursts of resting γ activity during deep surgical levels with amplitudes significantly higher than during waking. Isoflurane is known to produce burst suppression of the EEG at concentrations approaching 1.7% but burst suppression does not occur with halothane used in our experiments. Despite likely agent differences, a common implication of these studies is that the conscious state does not correlate with γ power itself.

Since our observations of the PS γ activity were based on the assumption that it is representative of the spontaneous γ EEG, we felt it necessary to examine if the two are in fact similar. A comparison of PS and spontaneous γ power as a function of anesthetic concentration revealed that PS γ power was very similar to spontaneous γ power (peak PS γ power was only 1.4 times greater than peak spontaneous γ power), and hence can be treated as such.

General anesthetics and stimulus-related γ oscillations

Although spontaneous γ activity itself may not correlate with the conscious state, it is possible that sensory stimulus-related γ activity reflects it more closely. In the present experiments, we examined stimulus-related γ activity to flash, a simple sensory stimulus. We found that even this simple stimulus significantly and transiently enhanced γ activity in the rat visual cortex. However, halothane did not attenuate but instead augmented, at intermediate concentrations, the stimulus' γ effect. This result further supports the notion that the magnitude of γ power alone, whether spontaneous or stimulus-related, is not a correlate of the conscious state.

The results of experimental studies in rats are incoherent. Dutton et al. (2000) demonstrated that isoflurane, desflurane, and nitrous oxide depressed middle latency auditory evoked potentials in a concentration-dependent manner. Yeoman et al. (1979) showed that 0–100 ms components of both auditory and visual evoked responses were depressed at moderate levels of enflurane but were augmented at higher concentrations. In contrast, Rabe et al. (1980) observed that auditory and visual middle latency potentials were enhanced by halothane in the concentration range of 0.25–1.0% but were depressed at 2.0% with no change in latency at corresponding anesthetic concentrations. We note that all these results pertain to averaged evoked potentials.

Our results are most comparable with those of Rabe et al. (1980) in that they predict an enhancement of stimulus-related γ activity at intermediate halothane concentrations. The reason for the difference with other human and rat studies is unclear. One technical difference from previous studies is that we did not specifically measure the amplitude or latency of specific components of the evoked potential but calculated the power of γ activity over prolonged time intervals up to 1 s poststimulus. However, an inspection of average evoked potential components within the first 100 ms reveals a similar dependence of these middle-latency components on halothane concentration to that of γ power.

We extended the previous evoked potential studies by performing both average and single-trial analyses and thus differentiated between the evoked and induced γ powers. With single-trial analysis we were able to show that even a simple flash stimulus induces a prolonged γ oscillatory response up to 1-s duration; the signal averaging obscured this response past 300 ms. This is important because the late induced γ activity has been associated with cognitive-perceptual functions (Tallon-Baudry et al., 1996) and may plausibly be sensitive to anesthetic action. The effect of anesthesia on this late induced γ activity has not been systematically investigated. Nevertheless, as shown here, the effect of halothane on evoked and induced γ oscillations was qualitatively similar; thus, a specific effect of anesthesia on either type of γ oscillation could not be isolated at this time. Whether or not evoked and induced γ activities are generated by the same mechanism is unclear. Tallon-Baudry et al. (1997) concluded that the two types of γ oscillation in humans are of different cortical origins. However, the similar dependence of the two γ types on halothane concentration demonstrated in this study suggests that the two may be mechanistically related and may differ in their phase relation to the stimulus.

The qualitatively similar effects of halothane concentration on PS and poststimulus γ activities suggest that the effect of the anesthetic on γ power is independent of the visual stimulus. Furthermore, the stimulus appears to exert an effect essentially independent of the anesthetic state, as its effect on γ power is present at all halothane concentrations. Thus, if stimulus-related γ power in the visual cortex in fact indicated sensory information processing, these results would suggest that even surgical plane of halothane anesthesia would not prevent the transfer of visual input to striate cortex. How visual processing at higher cortical levels ultimately leading to sensory perception may be affected by the anesthetic is not apparent from the present observations. Nevertheless, these data suggest it is unlikely that visual information transfer is simply blocked by the anesthetic at the thalamic level (Alkire et al., 2000). An effect on higher cortical integration should probably be taken into account to explain anesthetic-induced loss of conscious perception.

Anesthetic effects and γ activity

The reason for the observed biphasic character of the concentration-dependent effect of halothane on γ power is unknown. One may speculate that it may be related to a dose-dependent interaction of the anesthetic with various receptor systems. All volatile anesthetics including halothane potentiate neurotransmission at $GABA_A$ receptors by enhancing receptor affinity and prolonging the postsynaptic hyperpolarization current (Krasowski and Harrison, 1999). They are also known to inhibit AMPA receptors. Wang and Buzsaki (1996) and Buzsaki (2001) proposed that $GABA_A$ synaptic transmission in a network of fast-spiking hippocampal interneurons may play an important role in the generation of synchronized γ oscillations. Since halothane enhances $GABA_A$ transmission, it may augment γ oscillations through such an effect on the cortical inter-

neuron network. Why the increase in γ power is reversed at higher halothane concentration is less clear. It may be that the GABA is itself nonlinear, such that too much inhibition itself interferes with the oscillations or that the anesthetic depression of excitatory transmission at AMPA receptors begins to counteract the GABA enhancement of γ activity at higher halothane concentrations. Future experiments employing means for selective receptor modulation may help to test these hypotheses.

Acknowledgements—This publication is based on work supported by grants from the National Institute of Health, GM-56398, and MH-51358 and from the National Science Foundation, BES-0002945. Special thanks to Senior Engineer Richard Rys for the design and construction of electronic equipment.

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(Accepted 3 September 2003)