Anesthetic-induced Burst Suppression EEG Activity Requires Glutamate-mediated Excitatory Synaptic Transmission

Many anesthetics evoke electroencephalogram (EEG) burst suppression activity in humans and animals during anesthesia, and the mechanisms underlying this activity remain unclear. The present study used a rat neocortical brain slice EEG preparation to investigate excitatory synaptic mechanisms underlying anesthetic-induced burst suppression activity. Excitatory synaptic mechanisms associated with burst suppression activity were probed using glutamate receptor antagonists (CNQX and APV), GABA receptor antagonists, and simultaneous whole cell patch clamp and microelectrode EEG recordings. Clinically relevant concentrations of thiopental (50–70 μM), propofol (5–10 μM) or isoflurane (0.7–2.1 vol%), 0.5–1.5 rat minimum alveolar concentration (MAC), 200–700 μM) evoked delta slow wave activity and burst suppression EEG patterns similar to in vivo responses. These effects on EEG signals were blocked by glutamate receptor antagonists CNQX (8.6 μM) or APV (50 μM). Depolarizing intracellular bursts (amplitude = 34.7 ± 4.5 mV, half width = 132 ± 60 ms) always accompanied EEG bursts, and hyperpolarization increased intracellular burst amplitudes. Barriages of glutamate-mediated excitatory events initiated EEG bursting activity. Glutamate-mediated excitatory postsynaptic currents were significantly depressed by higher anesthetic concentrations that depressed burst suppression EEG activity. A GABA_A agonist produced a similar EEG effect to the anesthetics. It appears that anesthetic effects at both glutamate and GABA synapses contribute to EEG patterns seen during anesthesia.

Keywords: EPSP, IPSC, membrane, neocortex, synapse, voltage clamp

Introduction

Thiopental, propofol and isoflurane produce burst suppression electroencephalogram (EEG) patterns which have been associated with surgical levels of anesthesia (Eger et al., 1971; Homer and Stanski, 1985; Ebrahim et al., 1994; Maclver et al., 1996a; Jantti et al., 1998; Hartikainen and Rorarius, 1999; Kazama et al., 1999; Mahon et al., 2001; Huotari et al., 2004). The neuronal circuitry and physiological mechanisms underlying EEG burst suppression activity remain not completely elucidated. Previous studies have indicated that anesthetic-induced burst suppression activity results from effects on intrinsic neocortical circuitry (Swank, 1949; Henry and Scoville, 1952; Steriade et al., 1994a), similar to epileptic-like bursts generated in undercut neocortex (Topolnik et al., 2003), and it has been hypothesized that local glutamate-mediated excitatory inputs trigger these neuronal bursts (Lukatch and Maclver, 1996; Hartikainen and Rorarius, 1999; Mahon et al., 2001).

Excitatory transmission displays different sensitivities to general anesthetics in individual brain regions. Clinically relevant anesthetic concentrations depress excitatory transmission in the olfactory cortex and hippocampus (Richards and White, 1975; Richards et al., 1975; Maclver et al., 1996b), but not the olfactory bulb (Nicoll, 1972). Glutamate transmission is clearly depressed by anesthetics in the neocortex (el-Behery and Pull, 1989; Berg-Johnsen and Langmoen, 1992; Larsen et al., 1994, 1998), but some excitatory synaptic drive within the neocortex remains during anesthesia (Gonzalez-Burgos and Barrionuevo, 2001; Valentine et al., 2004). If anesthetic-induced burst suppression activity is intrinsic to the neocortex and glutamate-mediated excitatory events trigger neuronal bursts, then significant levels of glutamatergic transmission should remain in the neocortex during anesthetic-induced burst suppression activity. However, if clinically relevant anesthetic concentrations strongly depress neocortical glutamatergic transmission and evoke bursting activity by directly activating intrinsic neuronal conductances (Hablitz and Johnston, 1981), then glutamate-mediated excitation may prove to be only a minor component of burst suppression activity. To discriminate between these possibilities, the present study used a neocortical EEG brain slice preparation (Lukatch and Maclver, 1996, 1997) to examine anesthetic effects on neocortical excitatory transmission during EEG slow wave, burst suppression and isoelectric activity.

Materials and Methods

Slice Preparation

Experiments were performed on brain slices isolated from juvenile male Sprague-Dawley rats (80–120 g) obtained from Simonsen Laboratories, Inc. (Gilroy, CA). Experimental protocols were approved by the Institutional Animal Care Committee at Stanford University and adhered to published guidelines of the NIH, Society for Neuroscience and American Physiological Society. Rats were anesthetized with diethyl ether and their brains were removed into cold (1–2°C) oxygenated artificial cerebrospinal fluid (ACSF). The ACSF had the following ionic composition (in mM): Na^+ 151.25; K^+ 3.5; Ca^{2+} 2.0; Mg^{2+} 2.0; Cl^− 130.5; HCO_3− 26; SO_4^{2−} 2.0; H_2PO_4^{−} 1.25; and glucose 10. Brains were sectioned in the coronal plane into 450 μm thick slices using a Vibratome (Vibraslice® Series 1000, Boston, MA). Prior to recording, slices were hemisected and placed on filter papers in a recovery chamber at the interface of a humidified carbogen (O_2/CO_2 95/5%) gas phase and ACSF liquid phase. Slices from both hemispheres were allowed at least 1 h to recover from the slicing procedure prior to submersion in ACSF in a recording chamber. The ACSF was saturated with carbogen gas and perfused at a rate of 2.0 ml/min, at room temperature (21–24°C). Rapid and accurate solution changes were made using a ValveBank® computerized perfusion system (AutoMate Scientific, Oakland, CA).

EEG Recording and Spectral Analysis

Control theta frequency EEG oscillations (4–8 Hz) were elicited in Oc2MM neocortex in the presence of carbocain (100 μM) and bicuculline (10 μM), as previously described (Lukatch and Maclver, 1996, 1997). Low resistance extracellular glass electrodes filled with ACSF recorded EEG signals in neocortical layers 2/3 (Fig. 1). In some experiments EEG signals were electrically evoked by stimulating (6 V,
500 μs, 0.033 Hz) the underlying white matter and/or deep layer 6. EEG signals were amplified by $10^4-100,000$ (model 210A, Brown-Lee Precision, San Jose, CA), filtered 1-30 Hz bandpass, 60 Hz notch (Cyberamp 380, Axon Instrument Co., Foster City, CA), digitized 512-2048 Hz (DataWave Technologies Corp., Longmont, CO) and stored on computer disk for further analysis. EEG spectral quantification was accomplished using fast Fourier transforms (FFTs) on 2.5 s epochs of data using DataWave software.

**EEG Bi-amplitude Discrimination Analysis**

Burst discharges usually measure at least twice the amplitude of theta oscillations in vivo (MacIver et al., 1996a) and in vitro (Lukatch and MacIver, 1996). This amplitude difference in EEG was used to quantify anesthetic-induced burst suppression activity. EEG activity was scored burst suppression activity when EEG signals crossed a preset amplitude threshold. In each experiment the amplitude threshold was set to 110% of maximum theta amplitudes observed during control conditions (Fig. 1C,D).

**Excitatory Postsynaptic Current (EPSC) Recording**

Whole cell (>1 GΩ seal) patch recording microelectrodes (4-8 MΩ) contained an internal solution composed of (in mM): K-gluconate, 100; EGTA, 10; MgCl₂, 5; HEPES free acid, 40; ATP, 0.3; and GTP, 0.3, pH 7.2, 280-290 mOsm. Whole cell recordings were obtained from layer 2/3 neurons in Oc2MM neocortex (Lukatch and MacIver, 1997). Signals were amplified ×50-1000 (Cyberamp 380, Axon Instrument Co.), low pass filtered <10 kHz (Axon Instrument Co.), digitized at 10 kHz (DataWave Technologies Corp.) and stored on computer disk for

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**Figure 1.** Data collection and analysis methodology. (A) Stimulating (STIM), whole cell and EEG recording electrode positions are depicted in a rat neocortical brain slice. (B) In the presence of carbachol and bicuculline, simultaneous EEG and whole cell recordings revealed intra- and extracellular theta frequency oscillations. (C) Bi-amplitude discrimination analysis was used to quantify burst suppression activity. Within each electrically stimulated data sweep (10 s) theta and burst events were detected (see methods). When oscillation peak negativity fell between theta thresholds (dotted line), a theta event was scored. Peak negatives falling between burst thresholds (solid line) were counted as burst events. Total counts for each data sweep provided a quantitative measure of EEG signals during control conditions (a), as well as during anesthetic-induced transition periods (b) and burst suppression (c). Initial events associated with electrical stimulation were not included in the analysis (gray box). (D) Theta and burst counts were plotted on separate histograms, with each bar representing one electrically stimulated data sweep. Bar heights indicate number of events observed during each data sweep (see Fig. 3). (E) EPSC analysis methodology. Unprocessed data was smoothed and inverted for frequency analysis. The first derivative (dV/dt) of this waveform was then calculated and a threshold detector (dashed line) was set at 2×RMS noise levels. Each positive slope crossing of the threshold detector was scored as one event. Note that in the smoothed and inverted waveform, each piggy-backed event (*) would be erroneously scored as only a single event. However, after taking the derivative, piggy-backed EPSCs were resolved as individual events. (F) For kinetic analysis a threshold detector (dashed line) was set such that only large amplitude EPSCs would be detected. Each detected event was extracted, and extracted EPSCs were averaged. Kinetic parameters measured from averaged responses were rise time (RT), third width (TW) and half width (HW). Decay time constants (Est. TAU) were estimated by subtracting rise times from one-third width times.
further analysis. Whole cell access resistances averaged 26.8 ± 10.4 MΩ, and ranged from 13 to 50 MΩ.

**EPSC Data Smoothing**

To enhance EPSC frequency data analysis, spontaneous activity was smoothed and inverted using DataWave software (Fig. 1F). The smoothing algorithm calculated moving averages and standard deviations for nine data points at a time. Data points outside of ±1 SD were eliminated and replaced by new points using linear interpolation for nine data points at a time. Resulting smoothed data provided a relatively noise-free signal for subsequent dV/dt analysis (below).

**EPSC Frequency Analysis**

One means of calculating EPSC frequency is to set a level detector and score each threshold crossing as one EPSC. However, new EPSCs often occur before previous EPSCs decay fully. This 'piggy-hacking' effect results in an underestimation of EPSC frequency because piggy-backed EPSCs cross the threshold detector only once, resulting in multiple, temporally coherent EPSCs being scored as a single event. To circumvent this problem, EPSC peaks were selectively enhanced by taking the derivative of each one second data sweep, as described previously (Cohen et al., 1992). The resulting waveform displayed sharp, large amplitude peaks which corresponded to individual EPSCs. These derivatized data were then analyzed by setting a threshold detector at approximately twice the signal noise (Fig. 1F). All peaks above this threshold were scored as individual events.

**EPSC Kinetic Analysis**

For each experimental condition the largest amplitude EPSCs were extracted by setting a threshold detector such that ~10% of all EPSCs crossed this threshold. Extracted EPSCs included 10 ms of pre-threshold crossing data and 100 ms of post-threshold crossing data. Following this initial automated extraction, extracted events were visually examined and all events containing more than one EPSC (i.e. piggy-backed events) were rejected. The remaining single EPSCs were averaged, and kinetic parameters were calculated from these averaged responses. Kinetic parameters measured were rise time, half width and one-third width (Fig. 1F). EPSC decay tau times were approximated by subtracting rise time from one-third width time.

**Pharmacological Agents**

Isoflurane was obtained from Abbott Laboratories (North Chicago, IL). Propofol was obtained from Zeneca Pharmaceuticals (Wilmslow, Cheshire, UK), thiopental and carbachol were obtained from Sigma (St Louis, MO). Bicuculline methiodide, 2-aminophosphonic acid (APV) and 6-cyano-7-nitroquinoxalin-2-3-dione (CNQX) were supplied by Research Biochemicals International (Natick, MA). All solutions were made up in spectrophotometric grade water (Omnisol) supplied by EM Science (Gibbstown, NJ). Chemicals for the ACSF and electrode solutions were reagent grade or better and obtained from J.T. Baker Inc. (Philadelphia, PA).

**Results**

**Anesthetics Altered EEG Activity**

Thiopental, propofol and isoflurane produced qualitatively similar effects on neocortical brain slice EEG activity; theta frequency oscillations (4−8 Hz) gave way to delta activity (1−4 Hz), followed by burst suppression and then isoelectric activity (Fig. 2). Fast Fourier transform (FFT) analysis effectively quantified theta and delta activity, but was inadequate for describing anesthetic-induced burst suppression activity due to the aperiodic nature of this activity. To determine anesthetic concentrations which reliably evoked burst suppression activity in brain slices, a bi-amplitude discrimination analysis was developed (see methods Fig. 1C,D).

The anesthetics investigated in the present study evoked burst suppression activity and abolished theta frequency oscillations, similar to their effects in vivo (Fig. 3) (Ogawa et al., 1992; Tomoda et al., 1993; Mahon et al., 2001; Huotari et al., 2004). Theta oscillations did not diminish in amplitude significantly during transitions from theta to burst suppression activity; rather, theta activity 'broke up' into fewer synchronized events (Fig. 1C ‘transition’), leading to a decrease in the total number of theta oscillations counted per data sweep. High anesthetic concentrations regularly forced EEG signals directly into isoelectric activity, with burst suppression only observed upon anesthetic washout (Fig. 3). Each anesthetic displayed a different kinetic profile, with isoflurane effects occurring most rapidly, followed by thiopental and then propofol.

Anesthetic effects on brain slice EEG activity were concentration dependent. Burst suppression activity was predominant at anesthetic concentrations over the following ranges: 50−70 µM thiopental, 5−10 µM propofol and 0.7−1.4 vol% (0.5−1.0 rat MAC, 200–400 µM; White et al., 1974) isoflurane (Fig. 3). Isoelectric activity prevailed at anesthetic concentrations ≥100 µM thiopental, 20 µM propofol and 2.1 vol% (~600 µM), isoflurane. Burst suppression activity was also produced by the GABA_A receptor agonist muscimol (10 µM), as previously reported (Lukatch and MacIver, 1996).

It was possible to force steady-state burst suppression EEG activity into an isoelectric state using glutamate receptor antagonists (Fig. 4A). Bursting activity was reversibly blocked by either APV (50 µM, n = 5), an NMDA receptor antagonist, or CNQX (8.6 µM, n = 3), an AMPA/kainate receptor antagonist.

**Whole Cell Neuronal Responses during Neocortical EEG Bursts**

Whole cell recordings from layer 2/3 and 5 neurons were used to examine cellular events underlying burst suppression activity. In the presence of any of the three anesthetics, EEG bursts ranged in amplitude from 150 to 600 µV, and typically occurred in clusters of 2−6 bursts, with inter-burst frequencies of ~0.3−0.8 Hz. Burst clusters occurred spontaneously every 15−40 s, and could also be evoked with electrical stimulation (6 V, 250 µs, 0.035 Hz). Burst events recorded in either thiopental, propofol or isoflurane appeared similar in nature (Fig. 4), with intracellular depolarizations always accompanying EEG bursts. EEG bursts, however, were not always associated with intracellular depolarizations, even when those depolarizations evoked neuronal discharge activity (Fig. 4B). The ability of individual neurons to trigger EEG bursts was investigated by using depolarizing current pulses to trigger action potentials during quiescent periods of EEG burst suppression activity. In eight neurons examined, repetitive neuronal discharge activity (>6 action potentials per depolarizing pulse, pulses of 0.02 Hz for 3 min) did not elicit or entrain EEG bursting activity.

Intracellular burst amplitudes, half widths and number of action potentials per burst were quantified in neurons current clamped at their resting potential (~62 to ~70 mV). Burst amplitudes were measured from baseline to the maximum depolarization underlying action potential discharges. Amplitudes ranged from 24.0 to 44.1 mV, and averaged 34.7 ± 4.5 mV [n = 60 bursts from five neurons exposed to either thiopental (70 µM, 2 cells) or isoflurane (0.7 vol%, 1 cell, 1.4 vol%, 2 cells)]. Intracellular burst half widths ranged from 70 to 360 ms, and averaged 132 ± 60 ms. Intracellular bursts typically evoked...
action potential discharges at resting membrane potentials, and the number of action potentials per burst depended on membrane potential (Fig. 4C). At rest, the number of action potentials associated with intracellular bursts ranged from one to eight spikes, and averaged 3.2 ± 1.1 action potentials per burst (n > 80 bursts in six cells). Hyperpolarizing neurons with injected current always led to large amplitude intracellular bursts with fewer action potentials.

Spontaneous bursting activity evoked by thiopental or isoflurane was frequently proceeded and followed by barrages of excitatory events (Fig. 5). These excitatory post synaptic potentials (EPSPs) tended to ramp up in amplitude and frequency immediately preceding the first burst in a burst cluster. Interestingly, the occurrence of enhanced excitatory events throughout the duration of the burst cluster was variable; inter-burst intervals could contain little to no excitatory events, or could be punctuated with intense volleys of large amplitude EPSPs. Voltage clamping neurons at various holding potentials showed that excitatory barrages increased in amplitude at progressively more negative membrane potentials (Fig. 5). Following barrage cessation individual excitatory post synaptic currents (EPSCs) could again be discriminated.

**Isoflurane Effects on Neocortical EPSCs**

Isoflurane effects on neocortical EPSC frequency, amplitude and kinetics were examined using whole cell voltage clamp recordings in neocortical layer 2/3 neurons. In these experiments, control conditions did not include a background of carbachol and bicuculline in the ACSF purfusate, since induced theta oscillations where not required. All neurons (n = 37) were voltage clamped at their resting membrane potential, which under control conditions ranged from -66 to -79 mV (mean ± SD = 70.8 ± 2.8 mV, n = 30). To confirm that excitatory events were glutamate-mediated, spontaneous neocortical EPSCs could be blocked with the glutamate receptor antagonists CNQX (8.6 µM) and APV (50 µM) (Fig. 6A, n = 5). A majority (>95%) of spontaneous EPSCs were insensitive to the sodium channel blocker TTX (Fig. 6B, n = 2), indicating that these EPSCs likely resulted from action potential-independent release of glutamate directly from nerve terminals (Hershkowitz et al., 1993; Cormier and Kelly, 1996).

Isoflurane depressed EPSC frequency and amplitude while having no effect on EPSC kinetics (Fig. 7). Under control conditions spontaneous EPSC frequency averaged 5.7 ± 3.3 Hz (n = 30), and ranged from 1.1 to 12.5 Hz. Over short time intervals (<2 min) it was common for EPSC frequency to vary significantly, with recurrent intermingling of quiescent periods (EPSC frequency < 1 Hz) and hyperactive activity (EPSC frequency > 10 Hz). In the presence of 1.4 and 2.8 vol% isoflurane, spontaneous EPSC frequency decreased to 2.4 ± 0.5 Hz (n = 6) and 2.0 ± 0.7 Hz (n = 5), respectively. Both of these values were significantly different from control EPSC frequencies [P < 0.05, analysis of variance (ANOVA)]. When matched to their same cell control frequency values, EPSC frequencies were depressed to 69 ± 25 and 52 ± 28% of their control values in the presence of 1.4 and 2.8 vol% isoflurane, respectively.

EPSC amplitudes under control conditions averaged 20.2 ± 8.6 pA (mean ± SD, n = 30), and were not significantly correlated with whole cell access resistances (R² = 0.132) or membrane holding potentials (R² = 0.090, linear regression) over the narrow voltage range examined in the present study. EPSC amplitudes diminished to 13.7 ± 3.7 (n = 5) and 10.7 ± 2.5 (n = 4) pA, in the presence of 1.4 and 2.8 vol% isoflurane, respectively. Significant depression of EPSC amplitudes was only observed at 2.8 vol% isoflurane (P < 0.05, ANOVA). When matched to their same cell control amplitude values, EPSC amplitudes were depressed to 81.0 ± 11.2 and 64.3 ± 13.1% of their control values in the presence of 1.4 and 2.8 vol% isoflurane, respectively.
These EPSC amplitude reductions occurred with no change in background noise levels or any significant effects on holding currents.

The depression of EPSC frequency was more than that which could be accounted for by the observed depression of amplitudes. When the level of the threshold detector was increased to 20 or 40%, to simulate the observed isoflurane-induced effects on EPSC amplitudes at the two concentrations, the resulting analysis showed only a small effect on frequency: 92% and 85% for each level. Similarly, when control EPSC recordings were attenuated by decreasing the gain to 80 or 65%, to simulate the isoflurane-induced depression, the resulting decrease in frequency was only to 94 and 86% of normal gain responses — not the lower ~70 and 50% observed experimentally. EPSC rise times and decay kinetics were unaffected by isoflurane. Under control conditions EPSC rise and decay times were variable (Fig. 7B), and uncorrelated with whole cell access resistances (rise, $R^2 = 0.004$; decay, $R^2 < 0.001$). Rise times ranged from 1.6 to 7.0 ms (mean $± SD = 4.6 ± 1.3$ ms, $n = 30$), and decay times ranged from 3.6 to 15.9 ms (8.2 $± 2.9$ ms). Within individual neurons EPSC rise times and decay times were poorly correlated ($R^2 = 0.184$), suggesting that channel kinetic variability contributed more to measured changes in EPSC kinetics than did dendritic filtering of EPSCs. In the presence of 1.4 and 2.8 vol% isoflurane, EPSC activation kinetics remained unchanged at 4.6 $± 0.6$ ms ($n = 6$), and 4.5 $± 1.1$ ms ($n = 4$), respectively (Fig. 7C). Decay times (control $\tau = 8.2 ± 2.9$ ms) also were not significantly altered by 1.4 vol% isoflurane ($\tau = 9.3 ± 2.1$ ms) or 2.8 vol% isoflurane ($\tau = 8.4 ± 4.3$ ms).

Neocortical EPSC frequency, amplitude and kinetics were examined in the presence of carbachol (100 $\mu$M) and bicuculline (10 $\mu$M), since these pharmacological agents were used to evoke and maintain EEG activity. Carbachol and bicuculline ($n = 3$) significantly ($P < 0.05$, ANOVA) increased EPSC frequencies (mean $± SD = 14.7 ± 0.9$ Hz) and amplitudes (32.7 $± 3.5$ pA) from control values (Fig. 7B,C), but had no effect on EPSC rise times (4.0 $± 1.1$ ms) or decay kinetics (8.7 $± 2.4$ ms). Similar to control conditions, isoflurane application (0.7–2.8 vol%) in the presence of carbachol and bicuculline had no effect current kinetics (Fig. 7B).

**Discussion**

The present study demonstrated that clinically relevant concentrations of propofol (5–10 $\mu$M), thiopental (50–70 $\mu$M) or isoflurane (0.7–2.1 vol%, 0.5–1.5 rat MAC) evoked a similar pattern of EEG slowing as seen in vivo (Henry and Scoville, 1952; Eger et al., 1971; Maclver et al., 1996a; Hartikainen and...
Rorarius, 1999; Huotari et al., 2004), including burst suppression EEG patterns in neocortical brain slices (Figs 1–3). Burst suppression activity required glutamate-mediated synaptic transmission, evinced by the ability of glutamate receptor antagonists CNQX and APV to reversibly force burst suppression to isoelectric activity. Whole cell current clamp recordings revealed large amplitude membrane potential depolarizations associated with EEG burst discharges (Fig. 4), further implicating glutamatergic transmission in burst genesis. In addition, excitatory synaptic event frequencies and amplitudes were increased during anesthetic-induced bursting activity (Fig. 5). Voltage clamp recordings demonstrated that excitatory current amplitudes increased with membrane potential hyperpolarization, also indicating that bursts were initiated by glutamate-mediated inward currents. Finally, anesthetic concentrations capable of evoking isoelectric EEG activity were found to significantly depress spontaneous EPSC amplitudes and frequencies (Fig. 7). Taken together, the above results suggest that burst suppression activity requires glutamate-mediated excitatory transmission, and that anesthetic-induced EPSC depression
appears to underlie the transition from burst suppression to isoelectric activity, as previously suggested (Lukatch and MacIver, 1996).

The ability to generate anesthetic-induced EEG burst suppression patterns in neocortical brain slices is consistent with previous in vivo studies which showed that undercut anesthetized neocortex supports EEG burst suppression activity (Swank, 1949; Henry and Scoville, 1952). Further evidence that anesthetic-induced neocortical bursts arise independent of ascending thalamic inputs comes from studies showing that cortical neuron discharge activity correlates better with EEG burst suppression patterns than does thalamic neuron discharge activity (Steriade et al., 1994a; Topolnik et al., 2003; see also Timofeev et al., 2000). In addition, corticocortical excitatory inputs were less depressed during burst suppression activity than were thalamocortical inputs (Steriade et al., 1994a). These results suggest that the mechanisms underlying neocortical burst suppression activity are intrinsic to neocortex and are consistent with models of persistent neuronal activity in cortex (Fellous and Sejnowski, 2003).

Previous studies in vivo have demonstrated that anesthetics hyperpolarize neocortical neurons during burst suppression activity (Steriade et al., 1994a). It has been suggested that hyperpolarization of EEG-generating neurons could contribute to burst suppression activity by removing membrane potential-dependent inactivation from low threshold voltage activated calcium and sodium channels, and by decreasing tonic cell discharge frequencies (Lukatch and MacIver, 1996). These conditions would favor a state where EEG-generating neurons become quiescent yet hyperexcitable, which in turn would lead to periods of suppressed EEG activity disrupted by large amplitude bursts in response to excitatory inputs or fast prepotentials (Crochet et al., 2004). Much of this remains speculative at this time. Clearly the mechanisms underlying periods of suppression likely involve activation of intrinsic inhibitory currents, especially GABA-gated chloride currents (Lukatch and MacIver, 1996), but could also involve potassium channels and also the generalized reduction of excitatory synaptic inputs observed in the present study.

Although previous studies have demonstrated that clinically relevant anesthetic concentrations depress excitatory transmission in various brain regions (Richards et al., 1975; Richards and White, 1975; el-Beheiry and Puil, 1989; Berg-Johnsen and Langmoen, 1992; Lukatch and MacIver, 1996; Maclver et al., 1996b), the present study demonstrated that glutamatergic transmission persisted in neocortex during anesthetic-induced burst suppression EEG activity. One way in which this was evident was that intracellular depolarizations always preceded EEG bursts. Interestingly, spontaneous intracellular bursts which elicited action potentials in individual neurons were not always associated with EEG bursts (Fig. 4). In fact, sustained depolarizing current pulse-induced action potential trains in eight neurons, from separate slices, did not evoke or entrain EEG bursts in the presence of appropriate anesthetic concentrations (see results). This finding suggests that although large neuronal populations participate in anesthetic-induced bursting activity, only a specific subset of neurons (none of which were encountered in the present study) is capable of initiating EEG bursts. Previous studies examining burst discharges in disinhibited hippocampal area CA3 cells have also demonstrated that only a subset of neurons were capable of entraining population bursts (Miles and Wong, 1983). Thus, certain neocortical cell types may act as EEG burst suppression pacemakers (Steriade and Amzica, 1994; Steriade et al., 1994b). In particular,
the 'fast-rhythmic-bursting' neurons described in neocortex would be likely candidates to play this role as pacemakers (Grenier et al., 2003).

Neocortical burst suppression pacemaker neurons may possess intrinsic membrane properties which render them susceptible to bursting activity in the presence of anesthetics, just as certain cell types have a greater predisposition to burst during epileptic activity (Connors, 1984; Steriade and Amzica, 1994). Once initiated by a burst pacemaker subpopulation, large neuronal populations appear to be rapidly recruited into EEG bursting activity by way of recurrent excitatory connections which have been previously described in neocortex (Deuchars et al., 1994). In support of this hypothesis, the present study showed that anesthetic-induced bursts were accompanied by barrages of excitatory synaptic currents with increased frequencies and amplitudes (Fig. 5). It should be pointed out that although burst-suppression EEG patterns could share similar biophysical mechanisms with other forms of hyperexcitability, they appear quite different from epileptic discharges seen both in vivo and in the brain slice model used for the present study. At progressively higher anesthetic concentrations in vivo, burst frequencies decrease while inter-burst EEG suppression times increase, until an isoelectric EEG signal dominates (Clark and Rosner, 1973; Tomoda et al., 1993; Maclver et al., 1996a; Huotari et al., 2004). In the present study, glutamate receptor antagonists forced transitions from burst suppression to isoelectric EEG activity (Fig. 4), suggesting that depressed glutamatergic transmission may contribute to this transition in vivo. The present study found that isoflurane concentrations which produced isoelectric EEG activity significantly depressed EPSC amplitudes and frequencies, while having no effect on EPSC kinetics (Fig. 7). Isoflurane-induced depression of spontaneous EPSC frequencies is consistent with previous studies which have shown that isoflurane depresses glutamatergic transmission in several cortical areas, apparently via a presynaptic mechanism (Berg-Johnsen and Langmoen, 1992; Larsen et al., 1994; Maclver et al., 1996b).

Concluding Remarks

Neocortical brain slices were capable of sustaining anesthetic-induced EEG burst suppression activity in the presence of clinically relevant thiopental, propofol or isoflurane concentrations. Robust glutamatergic transmission was evident during neocortical EEG burst suppression activity. Glutamate-mediated excitatory currents were significantly depressed at isoflurane concentrations which produced isoelectric EEG activity.
concentrations which blocked burst activity and evoked iso-electric EEG activity. Thus, anesthetic-induced neocortical burst suppression activity appear to involve sites of action which are intrinsic to neocortex, it requires intact glutamatergic transmission, and the transition from burst suppression to isoelectric EEG activity appears to result from an anesthetic-induced depression of glutamate-mediated excitatory synaptic transmission.

Notes
This research was supported by NIH GM054767 and USAF OSR/SCEE.
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