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Anesthesia differentially modulates spontaneous network dynamics by cortical area and layer

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Sellers KK, Bennett DV, Hutt A, Fröhlich F. Anesthesia differentially modulates spontaneous network dynamics by cortical area and layer. *J Neurophysiol* 110: 2739–2751, 2013. First published September 18, 2013; doi:10.1152/jn.00404.2013.—Anesthesia is widely used in medicine and research to achieve altered states of consciousness and cognition. Whereas changes to macroscopic cortical activity patterns by anesthesia measured at the spatial resolution of electroencephalography have been widely studied, modulation of mesoscopic and microscopic network dynamics by anesthesia remain poorly understood. To address this gap in knowledge, we recorded spontaneous mesoscopic (local field potential) and microscopic (multiunit activity) network dynamics in primary visual cortex (V1) and prefrontal cortex (PFC) of awake and isoflurane anesthetized ferrets (*Mustela putoris furo*). This approach allowed for examination of activity as a function of cortical area, cortical layer, and anesthetic depth with much higher spatial and temporal resolution than in previous studies. We hypothesized that a primary sensory area and an association cortical area would exhibit different patterns of network modulation by anesthesia due to their different functional roles. Indeed, we found effects specific to cortical area and cortical layer. V1 exhibited minimal changes in rhythmic structure with anesthesia but differential modulation of input layer IV. In contrast, anesthesia profoundly altered spectral power in PFC, with more uniform modulation across cortical layers. Our results demonstrate that anesthesia modulates spontaneous cortical activity in an area- and layer-specific manner. These finding provide the basis for *1*) refining anesthesia monitoring algorithms, *2*) reevaluating the large number of systems neuroscience studies performed in anesthetized animals, and *3*) increasing our understanding of differential dynamics across cortical layers and areas.

anesthesia; laminar structure; LFP; network dynamics; resting state

ANESTHESIA IS ROUTINELY USED in both human patients for invasive procedures and systems neuroscience for electrophysiological and imaging studies of brain activity. Yet, there is a gap in knowledge between the well-characterized molecular targets of anesthetic agents throughout the central nervous system (Alkire et al. 2008) and the effects on overall behavioral state such as loss of consciousness (Brown et al. 2011). Specifically, little is known about how anesthesia modulates brain activity at the network level to achieve profound alterations in arousal and cognition. Bridging this gap by elucidating the network-level effects of anesthesia will *1*) aid in the development of more refined anesthesia monitoring techniques to reduce the number of anesthesia-related adverse side effects, *2*) instigate the reinterpretation of decades of work on systems neuroscience conducted in anesthetized animals, and *3*) provide fundamental insight into cortical network dynamics across cortical layers and areas.

Traditionally, anesthesia has been assumed to suppress brain activity (Friedman et al. 2010; Steyn-Ross et al. 2004), yet recent studies have revealed that anesthetic agents may rather modulate the dynamics of large-scale neuronal networks (Cimenser et al. 2011; Lewis et al. 2012; McCarthy et al. 2012). At the macroscopic level, anesthesia alters the electroencephalogram (EEG) by shifting oscillatory activity from high-frequency, low-amplitude patterns to low-frequency, high-amplitude activity (Voss and Sleigh 2007). Recent analysis strategies to quantify the modulation of network dynamics have revealed that anesthesia may disrupt integration of information across brain regions through decreasing long-range coherence (Imas et al. 2005, 2006; John and Prichep 2005) and reduce cortical information capacity by shrinking the repertoire of distinct activity patterns (Alkire et al. 2008).

In support of such sophisticated modulation of network dynamics by anesthesia, studies using functional magnetic resonance imaging (fMRI) in awake and anesthetized primates have found profoundly altered stimulus-evoked responses and functional connectivity induced by anesthesia (Liu et al. 2013a). Despite only indirect coupling between blood oxygenation dynamics measured by fMRI and electrical brain activity (Logothetis and Wandell 2004; Magri et al. 2012), fMRI has provided important indications of the complexity of the network-level effects of anesthesia. Resting-state fMRI (rs-fMRI) in humans has demonstrated reduced functional connectivity during anesthesia compared with the awake state, which scaled with depth of anesthesia (Peltier et al. 2005). Also, a growing body of evidence suggests that anesthesia does not affect all cortical areas similarly (Bonhomme et al. 2012; Heinke and Koelsch 2005; Heinke and Schwarzbauer 2001). In particular, rs-fMRI findings indicate that propofol-induced loss of consciousness correlates with decreased corticocortical and thalamocortical connectivity in frontoparietal networks, whereas connectivity is preserved in sensory cortices (Boveroux et al. 2010). Positron emission tomography data (White and Alkire 2003) in humans has also demonstrated that anesthesia-induced

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loss of consciousness by isoflurane or halothane is accompanied by decreased corticocortical and thalamocortical connectivity. However, this remains an area of debate, because computational models investigating propofol anesthesia suggest that there is increased functional coupling between the thalamus and cortex (Ching et al. 2010).

Yet, the changes in mesoscopic and microscopic network dynamics caused by anesthesia as a function of cortical area, cortical layer, and anesthetic depth remain poorly understood. To address this gap in knowledge, in the present study we examined how anesthesia modulates spontaneous network activity in a primary sensory area (primary visual cortex, V1) and a higher-order association area (prefrontal cortex, PFC) by electrophysiological recordings of local field potential (LFP, mesoscopic network activity) and multiunit spiking activity (MU, microscopic network activity) in awake and anesthetized ferrets. We used isoflurane at three concentrations (0.5%, 0.75%, and 1.0%, each with continuous and equal xylazine administration for maintaining adequate sedation) because isoflurane is a commonly employed anesthetic in neuroscience. We hypothesized that a primary sensory cortical area (V1) and an association cortical area (PFC) would exhibit differential modulation of network dynamics in response to anesthesia due to their different functional roles. Indeed, we found that the effects of anesthesia on these two cortical areas were vastly different. In V1, modulation induced by anesthesia in input layer IV (granular layer) differed from modulation of activity in supragranular and infragranular layers. In contrast, in PFC, anesthesia altered network dynamics and induced highly rhythmic activity patterns with fewer differences across cortical layers. To our knowledge, this is the first study that comprehensively examines the dose-dependent effects of an anesthetic on two different cortical areas across layers with such high temporal resolution.

MATERIALS AND METHODS

Surgery. Adolescent female ferrets (*Mustela putoris furo*, 15–20 wk old) were used in this study. All experiments were conducted in animals that had not reached sexual maturity to avoid possible estrous-dependent changes in physiology. This intermediate model species was chosen because of key similarities with primates; in particular, ferrets have a gyrencephalic cortex, a highly developed visual system, and cortical association areas such as PFC. Aseptic surgical procedures were used to prepare animals for multichannel electrophysiological recordings in V1 and PFC. Animals received an initial intramuscular injection of ketamine (30 mg/kg) and xylazine (1–2 mg/kg). The method of anesthesia maintenance used during surgery depended on the specific experimental preparation (see below). Animal physiology (electrocardiogram, pulse oxygen level, end-tidal CO_2 for a subset of animals, and rectal body temperature) was continuously measured. End-tidal CO_2 was between 30 and 50 mmHg (Kohn 1997). Animals were warmed with a water heating blanket to maintain a rectal temperature of 38.0–39.0°C. The animal's eyes were protected with paralube for the duration of surgery.

Surgical procedures consisted of an initial midline incision of the scalp, retraction of the soft tissue, and a circular craniotomy located over V1 (~3 mm anterior to lambda and 9 mm lateral to midline) and/or PFC (~5 mm anterior to bregma and 2 mm lateral to the midline). The potential for brain swelling was reduced with a preventative injection of furosemide (1 mg/kg im). Dura was removed, and the brain was covered with warm, sterile 4% agar. Probe location in V1 was verified by eliciting visually evoked potentials and mapping receptive fields, whereas insertion location in PFC was confirmed by histology to be in the anterior sigmoid gyrus (Duque and McCormick 2010) (recording probe dipped in DiI before insertion; Invitrogen, Grand Island, NY). A stainless steel head post was implanted with bone screws and dental cement. All procedures were approved by the University of North Carolina-Chapel Hill Institutional Animal Care and Use Committee (UNC-CH IACUC) and exceed guidelines set forth by the National Institutes of Health and U.S. Department of Agriculture.

Experiments in anesthetized animals. Most recordings in anesthetized animals ("anesthetized recordings," female ferrets) were conducted immediately following surgery. For these experiments, animals were intubated and artificially respirated (10–11 ml, 50 beats/min, 100% medical-grade oxygen), and isoflurane was used to maintain deep anesthesia during surgery. These animals were continuously administered an intravenous infusion via the cephalic vein (4.25 ml/h 5% dextrose lactated Ringer, 0.015 ml/h xylazine during surgery with the addition of 0.079 ml/h vecuronium bromide during recordings). Paralysis by vecuronium bromide was used to enhance the stability of electrophysiological recordings. Surgical procedures are outlined above.

Anesthetized recordings were conducted during resting state (dark room, no stimuli) under varying concentrations of isoflurane anesthesia (Iso, 0.5%, 0.75%, 1.0%). Continuous intravenous administration of xylazine guaranteed the complete absence of withdrawal response to toe pinch for all concentrations of isoflurane used in this study. The use of xylazine as an additional anesthetic was mandated by UNC-CH IACUC requirements. Unless otherwise stated, "anesthesia" subsequently refers to this paradigm of isoflurane and xylazine administration. The temporal order of anesthetic concentration was randomized across animals. Twenty minutes elapsed after anesthetic concentration were changed before a new recording was started, exceeding the ~4–7 min required to reach new baseline neural activity for our experimental setup. The animal's eyes were moistened with saline before each recording, and animal vital signs were monitored throughout the recordings.

Two linear 16-channel silicon probes (100-μm contact site spacing along the z-axis; Neuronexus, Ann Arbor, MI) were used in cases of dual craniotomies. Animals were head-fixed during these recordings. A silver chloride wire tucked between the skull and soft tissue and held in place with 4% agar in saline was used as the reference for both linear probes. Each probe was slowly advanced into cortex with a micromanipulator (Narishige, Tokyo, Japan); correct depth was determined by small deflections of the LFP at superficial electrode recording sites and larger deflections of the LFP at deeper electrode recording sites. Unfiltered signals were first amplified with MPA8I head stages with gain 10 (Multichannel Systems, Reutlingen, Germany) and then further amplified with gain 500 (model 3500; A-M Systems, Carlsborg, WA), digitized at 20 kHz (Power 1401; Cambridge Electronic Design, Cambridge, UK), and digitally stored using Spike2 software (Cambridge Electronic Design). In this study, all three concentrations of isoflurane anesthesia corresponded to a lack of behavioral responses. Burst suppression was not present in any recording; rather, we found rhythmic occurrence of UP (active phase) and DOWN states (quiet phase); the DOWN states were relatively short, typically at most 1–1.5 s long, and therefore did not last the tens of seconds typically seen during the suppression period of burst suppression patterns. Infrared videography (Handycam HDR-cx560v; Sony, Tokyo, Japan) of the animal was conducted.

Experiments in awake animals. Before surgery in animals that were recorded from while awake ("awake recordings"), animals were trained to be calmly restrained for up to 2 h. Female ferrets were used for awake recordings because their growth had plateaued, and they were therefore more suited for chronic implants compared with males. All animals were spayed in case they were kept until the age of sexual maturity; all animals in this study were used before reaching the age of sexual maturity. Deep anesthesia was maintained for the duration of the surgery with intramuscular injections of ketamine (30 mg/kg) and

xylazine (1–2 mg/kg) approximately every 40 min. Surgical procedures are outlined above. Additionally, the base of a custom-fabricated cylindrical chamber with a removable cap (material: Ultem 1000) was secured to the skull with bone screws and dental cement to allow subsequent access to the craniotomy for recordings. On completion of these surgical procedures, the incision was closed with sutures and treated with antibiotic cream. Yohimbine (0.25–0.5 mg/kg im) was then administered to reverse anesthesia. The animal was kept warm with a heating blanket and observed during recovery from anesthesia. Meloxicam (0.2 mg/kg im) and enrofloxacin (0.5 mg/kg im) were administered to prevent infection and to minimize postsurgical discomfort.

Awake recordings during resting state (dark room, no stimuli) began after animals had fully recovered from surgery (at least 5 days). Each recording session was a brief period (typically <2 h) during which the animal was restrained and head-fixed. Multichannel electrophysiological data were recorded with acutely inserted linear 32-channel silicon probes (50-μm contact site spacing along the z-axis; Neuronexus). In these electrodes, the reference was located on the same shank (0.5 mm above the top recording site) and was positioned in the 4% agar in saline above the brain. Infrared videography used to monitor whisking and minor movements, together with the fact that the animal's eyes remained open, established the absence of sleep during these recording sessions. A subset of animals that had been used for awake recordings (both V1 and PFC craniotomy locations) was also used for anesthetized recordings to minimize the number of animals used in this study. At the conclusion of the study, all animals were humanely killed with an overdose of pentobarbital sodium and immediately perfused with 4% formaldehyde in 0.1 M phosphate-buffered saline for subsequent histological verification of recording locations.

Data analysis and statistical methods. Recorded broadband signals were processed offline with custom-written scripts in MATLAB (The MathWorks, Natick, MA). Continuous recordings were segmented into nonoverlapping 5-s trials. A subset of these trials was manually excluded because of motion artifacts in the LFP signal (defined as extreme values in the raw trace). If not stated otherwise, figures represent medians across recordings sessions, recording sites, and trials (62 recording sessions; total trials in V1: awake = 3,612, 0.5% Iso = 729, 0.75% Iso = 2,557, 1.0% Iso = 2,860; total trials in PFC: awake = 6,327, 0.5% Iso = 2,298, 0.75% Iso = 2,722, 1.0% Iso = 3,394). If not stated otherwise, values are presented as medians ± SE. Time-dependent frequency content was determined by convolution of raw extracellular voltage signals with a family of Morlet wavelets (0.5–40 Hz, step width 0.5 Hz) with normalized amplitude, providing an optimal trade-off between time and frequency uncertainty (Goupillaud et al. 1984). Total power in each frequency band (delta = 0.5–4 Hz, theta = 4–8 Hz, alpha = 8–12 Hz, beta = 12–20 Hz, gamma = 20–40 Hz) was calculated by taking the median value across all trials. When present results are compared with those of prior studies, it is important to consider that there is no consensus about the frequency range used for each frequency band, particularly in differentiating the beta and gamma frequency bands. For readability, we did not divide beta into "beta 1" and "beta 2" but attributed frequencies often assigned to "beta 2" (23–30 Hz) to the gamma band. Early seminal work looking at fast oscillations that increased during alertness and during sensory processing examined frequency ranges from 20 to 40 Hz (Steriade et al. 1991) in cats, another carnivore intermediate model species. Spectra are first presented averaged across all recording electrodes and subsequently shown by cortical layer; all spectra are shown on a logarithmic scale. Bootstrapping with 100 iterations of resampling, a distribution-independent method, was used to calculate standard errors when parametric models were a poor fit for the data. Cross-correlation was determined as peaks of the cross-correlogram computed with the MATLAB xcorr function; the trial-shuffled control correlation was subtracted to exclude changes in correlation peak caused by changes in signal amplitude across awake and anesthetized recordings. High-pass-filtered data (4th-order Butterworth filter, 300-Hz cutoff) were subjected to a threshold of −3 SD for detection of action potentials (multiunit activity). To quantify the correspondence between mesoscopic LFP oscillatory structure and microscopic MU activity, spike-field coherence (SFC) was calculated. Spike-triggered averages from 1-s segments of LFP around each spike were obtained. Multitaper spectral estimates were used to determine spectra of the spike-triggered averages (MATLAB pmtm function with time-bandwidth product of 3.5). SFC values are the ratio of spike-triggered average spectra to the average of spectra calculated from each LFP segment (Fries 2009). The choice of frequency analysis for SFC was motivated by existing literature to enable comparisons with the present findings. Ten-second nonoverlapping trials were used to determine SFC to provide longer data windows (trials in V1: awake = 1,715, 0.5% Iso = 360, 0.75% Iso = 1,115, 1.0% Iso = 1,423; trials in PFC: awake = 2,288, 0.5% Iso = 1,137, 0.75% Iso = 1,187, 1.0% Iso = 1,628). The nonparametric Kruskal-Wallis test was implemented using the MATLAB function kruskalwallis to determine if samples from awake animals and animals administered different concentrations of anesthesia came from the same distribution. One-way ANOVA with Tukey's honestly significant difference criterion was used to correct for multiple comparisons.

To verify electrode location in V1, receptive fields were determined by presenting the animal with a series of gray screens with one square of a 19-10 grid colored white or black for 40 ms. Each square was shown for 30 repeats (order randomized) with 160 ms between each stimulus. Evoked MU spiking for each grid location was determined by calculating the number of spikes elicited between 60 and 140 ms after presentation of each stimulus and then subtracting the number of spikes that occurred in the 80 ms preceding presentation of the stimulus. Histology procedures consisted of cutting 100-μm coronal sections of fixed tissue using a Vibratome. Cresyl violet was used for Nissl staining. Stained sections and sections with DiI tracks from the recording electrodes were imaged using an Olympus BX51 microscope.

RESULTS

To elucidate the effects of anesthesia on mesoscopic and microscopic cortical network dynamics, we performed electrophysiological recordings of spontaneous activity in absence of sensory stimulation. We studied network dynamics in awake ($n = 6$) and anesthetized ferrets ($n = 5$). In both conditions, the eyes of the animals were open (Fig. 1*A*, infrared image of right eye of an awake and anesthetized ferret). To test our hypothesis that anesthesia differentially modulates dynamics in different cortical areas, we compared the effects of isoflurane anesthesia in a sensory cortical area, primary visual cortex (Fig. 1*B*, V1, central vision, lateral gyrus), and a higher-order association cortical area, prefrontal cortex (Fig. 1*B*, PFC, anterior sigmoid gyrus). We used linear depth probes to simultaneously record LFP and MU activity from all cortical layers (Fig. 1*C*) to determine if different elements of the cortical microcircuit were equally sensitive to modulation by anesthesia. Three concentrations of isoflurane anesthetic (0.5%, 0.75%, and 1.0% Iso) were used to assess differences in network activity between the awake and the anesthetized states (62 recording sessions total). In agreement with our hypothesis, we found that anesthesia had fundamentally different effects on V1 and PFC.

Anesthesia increased spectral power in PFC but altered distribution of power in V1. We found that anesthesia had differential effects on the oscillation structure of network activity in V1 and PFC. Relative to activity in the awake animal, the LFP in V1 exhibited changes in rhythmic structure

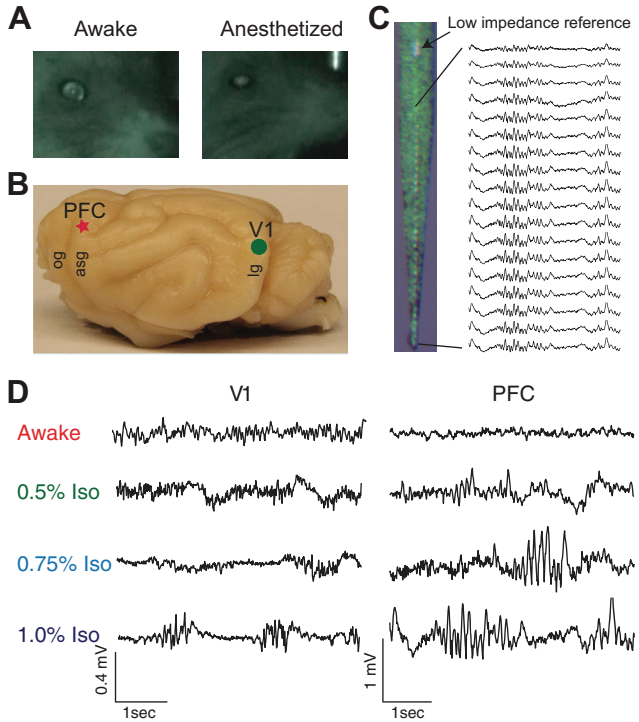

Fig. 1. Extracellular local field potential (LFP) recordings were conducted in ferret primary visual cortex (V1) and prefrontal cortex (PFC) to study the effects of anesthesia on spontaneous network activity. *A*: infrared images of an awake (*left*) and anesthetized ferret (*right*) show eyes were open in both cases during electrophysiological recordings. *B*: recording probe locations in the left hemisphere of ferret cortex. PFC: asg, anterior sigmoid gyrus (2 mm from midline) posterior to presylvian sulcus og, orbital gyrus. V1: lg, lateral gyrus (9 mm from midline, central vision). *C*: linear probes with 16 and 32 channels were used to simultaneously record from all cortical layers. In 32-channel probes (shown), the low-impedance reference was 0.5 mm above the most superficial electrode site. *D*: LFP traces from V1 (*left*) and PFC (*right*) of awake and anesthetized (0.5%, 0.75%, and 1.0% isoflurane, Iso) animals. LFP structure in V1 was altered by anesthesia, and LFP amplitude and rhythmicity were strongly increased by anesthesia in PFC. Note different scales for V1 and PFC.

with increased depth of anesthesia (Fig. 1*D*, *left*). The LFP measured in PFC in the awake animal exhibited only minimal rhythmic structure but showed prominent slow rhythms during anesthesia (Fig. 1*D*, *right*). Furthermore, V1 exhibited frequency-specific modulation of global power with varying depths of anesthesia (Fig. 2*A*, *left*, dotted lines indicate ±2 SD). In awake animals, the LFP in V1 exhibited a spectral peak at ~18 Hz (Fig. 2*A*, *left*, trace *inset*). With 0.5% and 0.75% Iso, the V1 LFP spectral peak occurred at a lower frequency. This spectral peak in V1 of animals anesthetized with 0.5% and 0.75% Iso was similar to the spindle frequency peak that appeared in anesthetized recordings in PFC (discussed below). With 1.0% isoflurane anesthesia, there was no longer a pronounced peak in V1 spectral power. In contrast, PFC in the awake animal did not exhibit a clear spectral peak. With anesthesia, the entire spectrum shifted to higher power (Fig. 2*A*, *right*, dotted lines indicate ±2 SD) and a peak in the spindle frequency at ~10 Hz emerged (Fig. 2*A*, *right*, trace *inset*). To further probe these marked differences in frequency structure, we calculated total power in each frequency band traditionally associated with distinct cognitive and behavioral functions (Wang 2010) (delta = 0.5–4 Hz, theta = 4–8 Hz, alpha = 8–12 Hz, beta = 12–20 Hz, gamma = 20–40 Hz). Relative to awake animals, anesthesia modestly modulated total power in V1 for each frequency band (Fig. 2*B*, *left*; see Table 1 for all values). The most pronounced change was the suppression of power in the beta band with anesthesia (beta power: awake = 8.05 ± 0.14, 0.5% Iso = 5.66 ± 0.07, 0.75% Iso = 5.22 ± 0.04, 1.0% Iso = 4.90 ± 0.04; Kruskal-Wallis test between all anesthesia concentrations significant to $P < 0.001$), which corresponds to the loss of the 18-Hz peak in the spectrum (Fig. 2*A*, *left*). In contrast, in PFC, anesthesia dramatically increased power in all frequency bands, most profoundly in the delta range (Fig. 2*B*, *right*, delta power: awake = 4.94 ± 0.23, 0.5% Iso = 26.19 ± 0.66, 0.75% Iso = 31.01 ± 0.63, 1.0% Iso = 52.19 ± 0.32; see Table 2 for all values). This increase in slow rhythmic power reflects the increase in cortical slow oscillations commonly associated with anesthesia (Steriade et al. 1993).

Given the two different modulation profiles for V1 and PFC, we next examined how anesthesia affected the relative contributions of the different frequency bands to the overall LFP signal. We computed the power in each frequency band as a percentage of total power and again found two very different effect profiles of anesthesia. In V1, the distribution of power in the different frequency bands was mostly resilient to anesthesia. The limited changes to the power distribution included both increases and decreases in relative contribution when awake animals were compared with animals anesthetized with different isoflurane concentrations (Fig. 3*A*). Again, in clear contrast to V1, the relative distribution of power in PFC shifted from the gamma to the delta band. We found an almost doubled contribution of delta oscillations to the overall spectrum when comparing awake to deeply anesthetized (1.0% Iso) animals [Fig. 3*B*, delta (black): awake = 22%, 0.5% Iso = 31%, 0.75% = 32%, 1.0% Iso = 43%]. Concomitantly, the relative contribution of the gamma band shrank to less than half [Fig. 3*B*, gamma (yellow): awake = 30%, 0.5% Iso = 19%, 0.75% = 19%, 1.0% Iso = 14%]. Interestingly, the intermediate frequency bands (theta, alpha, and beta) failed to show such a pronounced redistribution of relative power with anesthesia. In summary, these analyses demonstrate that, in agreement with our hypothesis, network dynamics in V1 were quite resilient to anesthesia, whereas PFC exhibited profound alterations in rhythmic structure in presence of anesthetics.

Laminar effects of anesthesia. Given the distinct functional roles of different cortical layers, we next examined if the changes in spectral power with anesthesia were uniform across cortical depth. We found that the prominent peak at ~18 Hz in V1 of the awake animal was almost exclusively localized to deep (infragranular) layers (Fig. 4*A*, *left*, red box). The spectral peaks seen at slightly lower frequencies with intermediate concentrations of anesthesia (Fig. 2*A*, 0.5% and 0.75% Iso) were also predominantly in infragranular layers (Fig. 4, *B* and *C*, *left*, red box). The highest concentration of anesthesia (1.0% Iso) abolished this intermediate frequency peak in the deep layers (Fig. 4*D*, *left*). In contrast, compared with spectral power in awake animals in PFC (Fig. 4*A*, *right*), spectral power in PFC was greatly increased across all cortical layers with 0.5% (Fig. 4*B*, *right*), 0.75% (Fig. 4*C*, *right*), and 1.0% Iso (Fig. 4*D*, *right*). A local peak around 10 Hz is evident in layer IV and infragranular layers of PFC in anesthetized animals, corresponding to the peak in spindle frequencies (Fig. 2*A*, *right*). Cortical layers likely mediate sophisticated information processing, in which individual layers play different roles in the

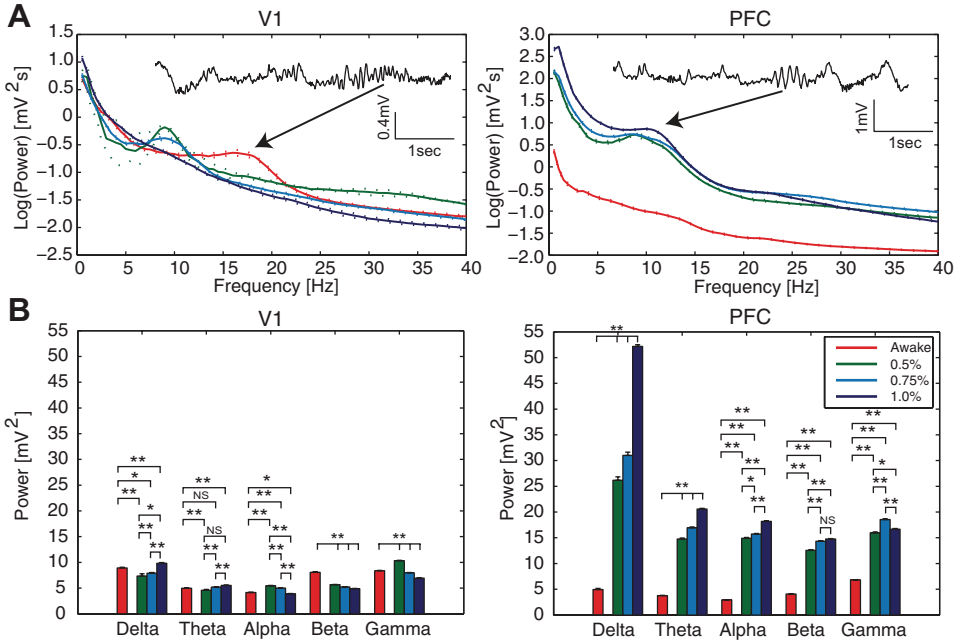

Fig. 2. Anesthesia modulated LFP in a frequency-specific manner in V1 but induced broadband enhancement of LFP power in PFC. *A*, *left*: time-averaged LFP spectra (0.5–40 Hz) in V1 demonstrate modulation of power as a function of anesthesia (LFP trace *inset*: ~18 Hz activity in the awake animal). The spectral peak was at lower frequencies for anesthesia concentrations of 0.5% and 0.75% Iso and was abolished by 1.0% Iso anesthesia. *Right*, dramatic increase in LFP spectral power by anesthesia in PFC. Anesthesia induced a spectral peak in the spindle frequency, ~10 Hz (LFP trace *inset*: 1.0% Iso). Dotted lines indicate ±2 SD calculated by bootstrap. *B*: total power in each frequency band. *Left*, anesthesia modulated power specific to each frequency band in V1. *Right*, in PFC, anesthesia mediated broadband power increase, with the most prominent effect in the delta range. Error bars indicate 1 SE. $*P < 0.05$, power in frequency bands was significantly different. $**P < 0.001$, power in frequency bands was significantly different across awake and anesthetized animals. NS, nonsignificant.

overall functioning of cortical microcircuits. We therefore examined if anesthesia impaired these distinct processing roles by increasing the correlation between the activity in different layers. To this end, we calculated the average of exhaustive pairwise cross-correlations of electrodes (shuffle controlled). Overall, LFP signals in V1 exhibited lower cross-correlation than those in PFC (Fig. 4*E*, note different scales). In further agreement with our hypothesis that modulation by anesthesia varies by cortical areas, correlation increased in PFC but decreased in V1 in anesthetized animals compared with awake animals (cross-correlation V1: awake = 0.0012 ± <0.0001, 0.5% Iso = 0.0005 ± 0.0002, 0.75% Iso = 0.0008 ± 0001, 1.0% Iso = 0.0011 ± 0001, Kruskal-Wallis test between all anesthesia concentrations significant to $P < 0.05$, except awake and 0.5% Iso are nonsignificant; cross-correlation PFC: awake = 0.0004 ± 0.0003, 0.5% Iso = 0.0121 ± 0.0012, 0.75% Iso = 0.0149 ± 0.0015, 1.0% Iso = 0.0316 ± 0.0009, Kruskal-Wallis test between all anesthesia concentrations significant to $P < 0.001$). Thus we also found a selective increase in correlated processing across layers with anesthesia in PFC.

Cortical area- and layer-specific alterations to microscopic network dynamics with anesthesia. Thus far, we have presented key differences in the mesoscopic network structure of V1 and PFC induced by anesthesia based on LFP recordings. We next asked if microscopic dynamics in these cortical circuits, mediated by action potential firing, were similarly modulated. In looking at simultaneous LFP and MU traces, increased coordination is evident between mesoscopic and microscopic processes with anesthesia, particularly in PFC. Both V1 and PFC in the awake animal exhibited MU firing that was not time-locked to any gross structures of the LFP (Fig. 5*A*, *top*, V1; Fig. 5*B*, *top*, PFC). With anesthesia, MU activity in V1 and PFC became more rhythmic (Fig. 5*A*, *bottom*, V1; Fig. 5*B*, *bottom*, PFC). The slow rhythm in both LFP and MU activity was generated by alternating epochs of MU firing and quiescence; this activity structure corresponds to the slow oscillation (UP and DOWN states) that represents a hallmark of anesthesia (shaded boxes in Fig. 5 highlight DOWN states). Taking into account cortical depth, we quantified these changes to the mesoscopic and microscopic network activity by probing for region-specific effects of anesthesia on MU firing rates and on the temporal relationship between LFP and MU (SFC).

In agreement with our hypothesis that V1 and PFC would also exhibit differential modulation of microscopic dynamics, we found area-specific changes in MU firing rate. Averaged across cortical layers, anesthesia did not significantly modulate firing rate in V1 or PFC (Fig. 6*A*, V1 median firing rate: awake = 24.6 ± 2.87, 0.5% Iso = 24 ± 6.40, 0.75% = 23.6 ± 0.65, 1.0% Iso = 23.9 ± 0.49, Kruskal-Wallis test between all anesthesia concentrations nonsignificant at $P = 0.05$; PFC median firing rate: awake = 23.9 ± 0.68, 0.5% Iso = 24.8 ± 1.54, 0.75% = 25.7 ± 1.08, 1.0% Iso = 24.2 ± 0.37, Kruskal-Wallis test between all anesthesia concentrations nonsignificant at $P = 0.05$). Given these nonsignificant changes in

Table 1. *Total spectral power by frequency band in V1*

	Total Spectral Power, mV^2				
	Delta	Theta	Alpha	Beta	Gamma
Awake	8.93 ± 0.127	4.99 ± 0.083	4.14 ± 0.061	8.06 ± 0.138	8.38 ± 0.082
0.5% Iso	7.35 ± 0.446	4.60 ± 0.164	5.44 ± 0.0801	5.66 ± 0.068	10.33 ± 0.080
0.75% Iso	7.89 ± 0.148	5.20 ± 0.063	5.00 ± 0.052	5.22 ± 0.039	7.97 ± 0.055
1.0% Iso	9.82 ± 0.149	5.52 ± 0.069	3.91 ± 0.055	4.90 ± 0.044	6.92 ± 0.076

Values are medians ± SE. Iso, isoflurane; V1, primary visual cortex.

Table 2. *Total spectral power by frequency band in PFC*

	Total Spectral Power, mV^2				
	Delta	Theta	Alpha	Beta	Gamma
Awake	4.94 ± 0.235	3.77 ± 0.052	2.94 ± 0.027	4.08 ± 0.027	6.82 ± 0.033
0.5% Iso	26.19 ± 0.664	14.76 ± 0.197	14.91 ± 0.146	12.56 ± 0.123	15.95 ± 0.190
0.75% Iso	31.01 ± 0.629	16.93 ± 0.167	15.72 ± 0.120	14.31 ± 0.095	18.53 ± 0.167
1.0% Iso	52.19 ± 0.315	20.59 ± 0.123	18.19 ± 0.111	14.77 ± 0.084	16.68 ± 0.138

Values are medians ± SE. PFC, prefrontal cortex.

MU firing rates when averaged across cortical depth, we next examined if modulation of MU activity depended on cortical depth, since the firing of neurons in different layers likely perform distinct tasks. Indeed, we found depth-dependent response profiles in V1 and in PFC. In V1, moderate concentrations of anesthesia (0.5% and 0.75% Iso) increased MU firing specifically in input layer IV with a concomitant reduction of firing in the other layers (Fig. 6*B*, *left*). In PFC, MU spiking decreased exclusively in supragranular layers for intermediate concentrations of anesthesia (0.5% and 0.75% Iso) (Fig. 6*B*, *right*). Together, these data show that specifically layer IV was susceptible to changes in firing rate induced by anesthesia in V1 but not in PFC. This unique, differential alteration to the activity of the input layer in a primary sensory cortex points toward modulation by anesthesia, based on the functional role of specific cortical layers.

Anesthesia induced targeted increases in spike-field coherence. Having established that anesthesia had area- and layer-specific effects on both mesoscopic and microscopic network dynamics independently, we next asked how anesthesia altered the relationship between mesoscopic LFP network dynamics and microscopic MU firing. SFC was used to measure preferential firing of action potentials as a function of LFP phase. For a given frequency, higher values indicate that MU firing was more tightly coupled to the LFP phase. In awake animals, both V1 and PFC exhibited low SFC across cortical layers (Fig. 7*A*). With all concentrations of anesthesia, spiking in V1 was more tightly coupled to the phase of the LFP at all frequencies in supragranular and infragranular layers (Fig. 7, *B–D*, *left*). The strongest increase in coupling was found in the slowest and fastest frequencies. Notably, increase in SFC exhibited layer dependence and remained minimal in input layer IV for all concentrations of anesthesia. Anesthesia in PFC resulted in increased SFC in superficial layers at higher frequencies and across layers at low frequencies (Fig. 7, *B–D*, *right*). In PFC, the strength of SFC increased with deepening anesthesia from 0.5% to 1.0% Iso. In summary, MU spiking was mostly independent of LFP phase in the awake animal. In the anesthetized animal, MU spiking was modulated by LFP phase in a cortical area- and layer-specific manner. These results further confirm that understanding the effects of anesthesia on cortex requires not only recordings across cortical layers but also analysis strategies that bridge the micro- and mesoscopic scale.

DISCUSSION

Although the molecular targets of anesthetics have been well characterized, changes in mesoscopic and microscopic network dynamics caused by anesthesia are not well understood. In the present study, we utilized in vivo electrophysiological recordings to investigate these alterations as a function of cortical area, cortical layer, and anesthetic depth. We developed this anesthesia model in ferrets, which have a gyrencephalic cortex similar to that of humans, to increase the translational relevance of this study. In agreement with our hypothesis, we found that anesthesia induced profoundly different modulation of both mesoscopic and microscopic network activity in a primary sensory cortical area (V1) and an association cortical area (PFC). The present study provides a dramatic improvement in spatial resolution of network dynamics compared with previous work using EEG. The use of laminar probes allowed for measurement of modulation by anesthesia with layer specificity. We found that layer IV in V1 was mostly resistant to spectral changes induced by anesthesia, whereas infragranular layers exhibited pronounced modulation of these mesoscopic dynamics. Contrastingly, PFC demonstrated dramatically increased LFP power with anesthesia across cortical layers. Modulation of microscopic dynamics by anesthesia also exhibited specificity by cortical area and layer. MU spiking was preferentially increased in V1 in input layer IV, whereas spiking in PFC decreased at the most superficial electrodes at lower concentrations of anesthesia. We also found that layer IV in V1 was spared from increases in SFC induced by anesthesia; in PFC, superficial layers and slow frequencies exhibited increased SFC with deepening anesthesia.

Modulation of spectral power. Activity in different frequency bands has been correlated with a broad range of cognitive and behavioral states (Wang 2010). The targeted modulation of specific frequency bands by anesthesia likely reflects changes in underlying network dynamics, which may

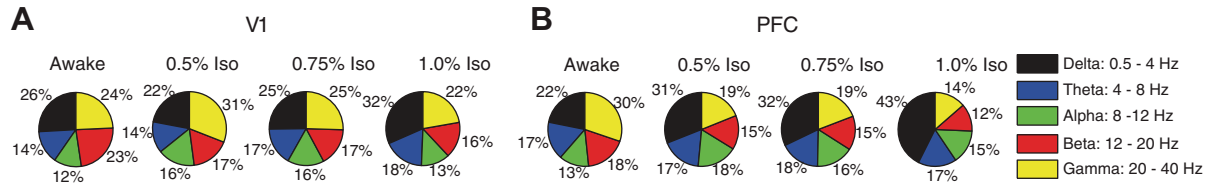

Fig. 3. Distribution of oscillatory power across frequency bands was differentially affected by depth of anesthesia in V1 and PFC. *A*: power in each frequency band as a percentage of total power. Distribution of power across frequency bands in V1 was mostly resistant to change by anesthesia. The modest differences between awake and anesthetized animals varied by frequency band and exhibited no apparent monotonic relationship. *B*: in PFC, distribution of spectral power shifted from gamma (yellow) to delta (black) frequency bands with increasing depth of anesthesia.

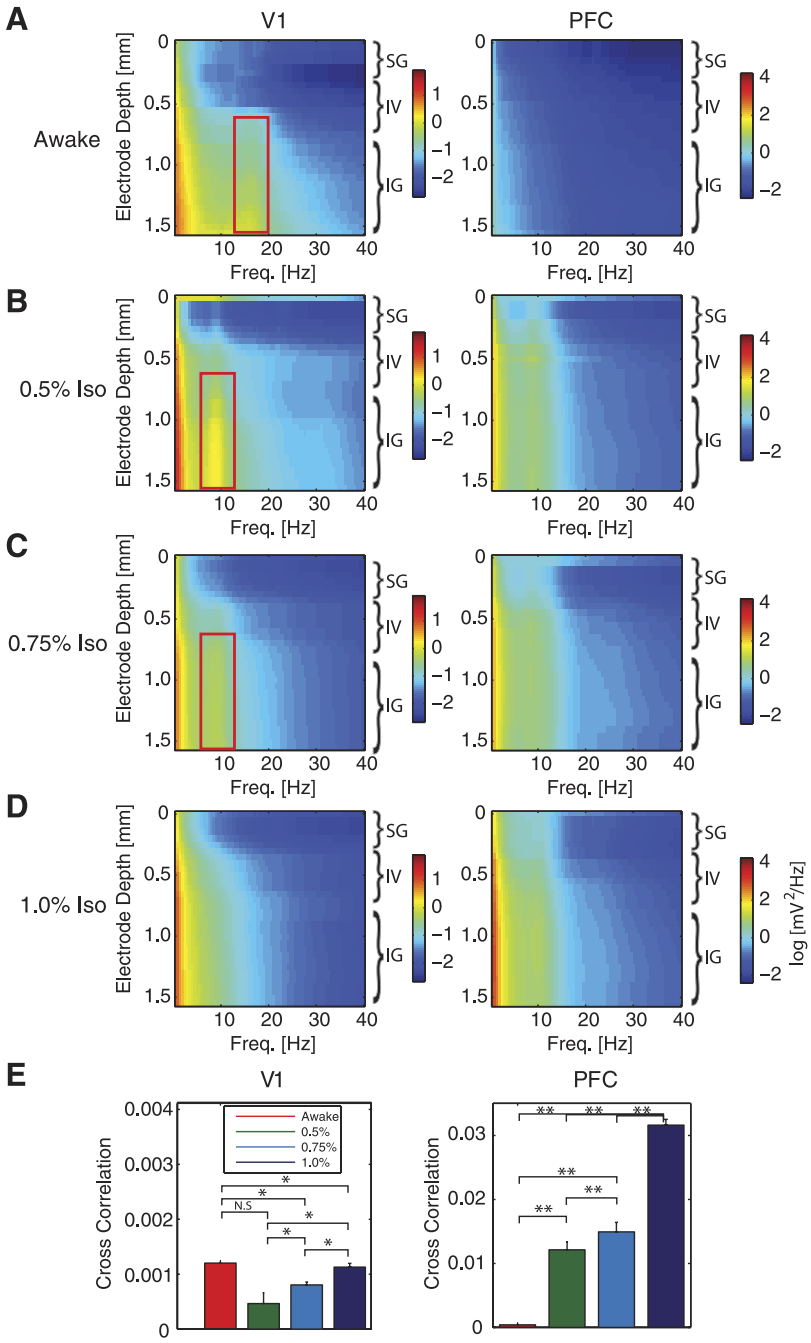

Fig. 4. Activity across cortical layers maintained independence with anesthesia in V1 but became strongly correlated in PFC. *A*, *left*: in V1 of awake animals, oscillatory power was stronger at deeper cortical layers. Red box highlights the infragranular localization of the ~18-Hz oscillatory peak found exclusively in the awake animal. *Right*, activity in PFC of the awake animal did not exhibit any intermediate frequency spectral peak. Note different color scales for V1 and PFC. SG, supragranular layers; IV, layer IV; IG, infragranular layers. *B*, *left*: in V1 of animals anesthetized with 0.5% Iso, the intermediate frequency peak appeared at a lower frequency (~10 Hz) as highlighted by the red box. This oscillation was localized in IG. *Right*, 0.5% Iso increased oscillatory power across layers in PFC relative to power in the awake animal. *C*, *left*: in V1 of animals anesthetized with 0.75% Iso, the intermediate frequency peak (red box) appeared at ~10 Hz. This oscillation was localized in IG layers. *Right*, compared with power in the awake animals, 0.75% Iso increased oscillatory power across layers in PFC. *D*, *left*: 1.0% Iso abolished the intermediate frequency peak in V1. *Right*, in PFC, 1.0% Iso increased spectral power across cortical layers compared with power in awake animals. An increase in low-frequency power is evident relative to corresponding power in animals anesthetized with 0.5% and 0.75% Iso. *E*: correlation of activity across cortical layers was much higher in PFC. *Left*, in V1, anesthesia decreased the cross-correlation of LFP activity across cortical layers. *Right*, increasing concentrations of anesthesia increased cross-correlation of LFP activity across cortical layers in PFC. Error bars indicate 1 SE. $*P < 0.05$; $**P < 0.001$, Kruskal-Wallis test indicates significant difference between anesthesia concentrations. Note different scales for V1 and PFC. Freq., frequency.

lead to the dramatic behavioral effects caused by anesthesia in animals and humans. We found that the distribution of power across frequency bands in V1 in the awake animal exhibited little change compared with the data from anesthetized animals. V1 exhibited alterations in spectral power in specific frequency bands. The beta frequency peak in V1 of awake animals, which localized to deeper layers, is in agreement with reports of sub-gamma power in deeper layers of V1 in awake macaque monkeys (Maier et al. 2010). Our results demonstrate that isoflurane anesthesia abolished this beta peak. Given that the beta range has been implicated as a carrier for visual attention (Wrobel 2000), changes to this rhythm may underlie changes in visual processing as well as altered integration with other brain regions. In contrast, the modulation profile in PFC was characterized by broad increases in power and two-fold changes in the relative presence of different cortical oscillations. A comparison of mesoscopic and microscopic network dynamics in our animal model with previous findings in humans revealed some similar modulatory effects of anesthesia. A frontal shift of EEG power during anesthesia, anteriorization, is commonly observed in animals (Tinker et al. 1977) and humans (Feshchenko et al. 2004; Gugino et al. 2001). Previous reports in humans found increases in delta, theta, and alpha power in frontal areas with deepening administration of propofol and sevoflurane (gamma frequencies were not investigated; Feshchenko et al. 2004; Gugino et al. 2001). Our PFC recordings in anesthetized animals exhibited dramatic increases in power across all frequency bands. Our anesthetized recordings

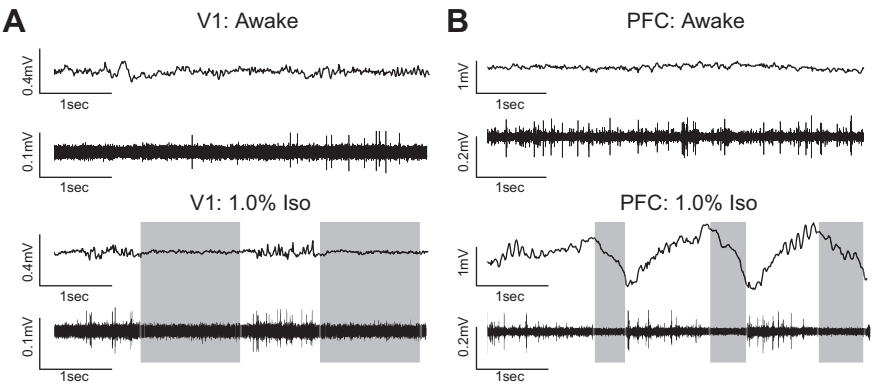

Fig. 5. Paired LFP and multiunit (MU) spiking traces. *A*: matching LFP and high-pass filtered traces of MU spiking activity from representative trials for V1. In the anesthetized animal (*bottom*), MU firing became more rhythmic and correlated with structure of the LFP. Shaded boxes indicate DOWN states. *B*: same representation as in *A*, for PFC. Anesthesia strongly increased rhythmic structure of the LFP and coordinated MU firing. Shaded boxes indicate DOWN states. Note different scales for V1 and PFC.

also exhibited a PFC spectral peak in the alpha range as seen in anesthetized humans (Purdon et al. 2013). Additionally, both V1 and PFC showed a shift in power toward lower frequencies with deep anesthesia, in accordance with a long history of human studies (Faulconer 1952; Gibbs et al. 1937). Use of high-density EEG in humans anesthetized with propofol has demonstrated increased delta and alpha activity in frontal electrodes sites (Cimenser et al. 2011). In agreement, our results demonstrate increased total power as well as increased relative power for both of these frequency bands in PFC. Cimenser et al. (2011) also reported decreased alpha and increased delta activity at occipital sites. In our study, we found the same modulation pattern, but only for the deepest anesthesia concentration of 1% Iso. More prominently, our data showed a strong suppression of oscillations in the beta band in V1 for all concentrations of anesthesia. Visual attention has been associated with beta band activity; therefore, the reduction in beta band power may be correlated with the suppression of attentional processing during anesthesia. The differences in both overall and relative spectral power modulation in V1 and PFC indicate differential effects of anesthesia in these two cortical areas. Moreover, we found that the prominent modulation of the beta band in V1 was mostly localized to deeper cortical layers. This suggests that examination at the spatial resolution of individual layers is necessary to fully understand modulation of network activity.

Modulation according to distinct functional roles across laminar structure and cortical area. By organizing with respect to laminae, neuronal networks exhibit differential patterns of spontaneous and evoked firing by layer (de Kock et al. 2007; Sakata and Harris 2009; Wallace and Palmer 2008). It has been unknown if and how anesthesia differentially modulates network activity in V1 and PFC based on laminar structure. Because of the complex feedforward and feedback projections across laminae (Douglas and Martin 2004), differential modulation of activity across cortical layers by anesthesia could significantly alter processing of sensory information. Intriguingly, our results show that the effects of anesthesia are not consistent across cortical layers. In supragranular layers, SFC increased across frequencies in anesthetized V1. Layer IV in V1 exhibited different modulation compared with supragranular and infragranular layers, with minimal SFC in layer IV during anesthesia. In infragranular layers of V1, SFC increased with anesthesia across all frequencies, with the most prominent effect in the delta frequency range and at fast frequencies. Interestingly, the modulation of SFC by anesthesia in V1 exhibited a different laminar profile compared with the increase in oscillatory power with deepening cortical depth. This indicates that increased SFC was not driven by stronger oscillatory power. The beta spectral peak seen in V1 of awake animals localized to infragranular layers. With 0.5% and 0.75% Iso in V1, the beta peak was not present, but a spectral peak in the alpha frequency range appeared in infragranular layers. With 1.0% Iso in V1, no mid-frequency spectral peak was evident. In PFC, spectral power increased across all cortical layers with 0.5%, 0.75%, and 1.0% Iso. Supragranular layers in PFC exhibited increased SFC at faster frequencies. In layer IV and infragranular layers of PFC in anesthetized animals, a local peak around 10 Hz developed (corresponding to the spindle frequencies) and SFC increased at slow frequencies. In PFC, both spectral power and SFC increased in layer IV and infragranular layers at low frequencies. However, increased SFC across frequencies in supragranular layers did not correspond with change in the spectral power.

The primary input to V1 layer IV comes from the lateral geniculate nucleus (LGN) (Hubel and Wiesel 1972). Anesthesia has been shown to decrease levels of spontaneous activity in the LGN and decrease the firing rates of LGN neurons responding to visual stimuli (Alitto et al. 2011). Although overall spiking rates in V1 were not significantly altered by anesthesia, layer IV exhibited increased firing with a concomitant decrease in firing rate in other layers during 0.5% and 0.75% Iso. This seemingly paradoxical increase of activity with anesthesia has been previously examined by modeling propofol anesthesia and might be caused by antisynchrony of interneuron activity mediated by the M-current (McCarthy et al. 2008). Alterations in the balance of excitatory and inhibitory drive between awake and anesthetized animals (Haider et al. 2013) could underlie the differences observed in cortical network dynamics.

Our results also demonstrate that the laminar effects of anesthesia are specific to cortical area; modulation of firing rate was similar across layers in PFC, with alterations only in the most superficial layers. Activity across electrodes was highly correlated during anesthesia in PFC. Therefore, it appears that modulation of network dynamics varies on the basis of not only cortical layer but also cortical area. A possible explanation for this specificity stems from differences in the structural and functional architecture of these cortical areas. Layer IV in ferret V1 is highly granulated, whereas granulation of layer IV in PFC is rather poor (Duque and McCormick 2010), indicating there are variations in the cellular composition. PFC is involved in mediating higher cognitive functions (Fuster 2008; Jacobsen 1936) and is critical for top-down feedback to opti-

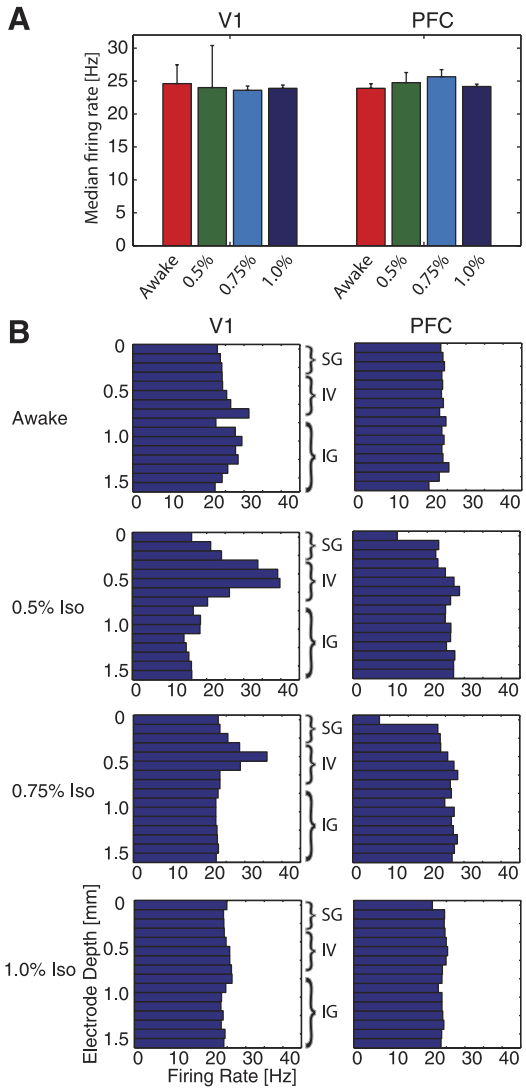

Fig. 6. Anesthesia altered MU firing rate and spiking across cortical layers differently in V1 and PFC. *A*: median spontaneous MU firing rates exhibited nonsignificant difference with anesthesia in V1 and PFC. Error bars indicate 1 SE. Kruskal-Wallis tests indicated no significant differences between anesthesia concentrations within each cortical area at $P = 0.05$. *B*: MU firing as a function of cortical depth. *Left*, in V1, intermediate concentrations of anesthesia (0.5% and 0.75% Iso) increased MU firing specifically in layer IV with concomitant decreases in spiking rate in SG and IG layers. *Right*, MU firing rates decreased with 0.5% and 0.75% Iso exclusively in SG layers. A concentration of 1.0% Iso did not exhibit alteration to spiking across cortical layers in PFC.

mize processing of behaviorally relevant sensory information (Buschman and Miller 2007; Fritz et al. 2010; Gregoriou et al. 2009). Necessary for these functions, layer IV in ferret PFC receives many afferents from the mediodorsal nuclei of the thalamus (Duque and McCormick 2010). Various anesthetics have been shown to decrease thalamic activity (Andrada et al. 2012) and thalamocortical connectivity (Hudetz 2012). At first glance, reduced thalamic activity is seemingly at odds with increased MU firing in layer IV of V1. However, layer IV in V1 receives not only feedforward excitation from the thalamus but also feedforward inhibition (from layer IV inhibitory interneurons driven by the thalamus) (Miller 2003). Therefore, decreased thalamic activity may reduce feedforward inhibition, resulting in similar MU spiking rates between awake and anesthetized animals. Elucidating whether anesthetics act directly on cortical areas or if alterations in activity are mediated by thalamocortical connectivity is an area of active research (Boly et al. 2012; Kim et al. 2012). Furthermore, the propagation of cortical activity across layers may differ between sensory processing and spontaneous activity (Sakata and Harris 2009). Taken together, these results demonstrate that the effects of anesthesia vary by cortical area and cortical layer. Interestingly, the laminar profiles of these effects vary for microscopic spiking activity, mesoscopic spectral power changes, and SFC when the coherence between these levels of network activity is examined.

Improvements in spatial and temporal resolution. Work conducted using EEG and fMRI has provided valuable insight into macroscopic activity changes induced by anesthesia. However, these recording modalities have inherent limitations in spatial and temporal resolution (Babiloni et al. 2009). Furthermore, fMRI is not a direct measure of neural activity, but rather depends on fluctuations in the blood oxygenation level-dependent (BOLD) signal (Brown et al. 2007), which change with

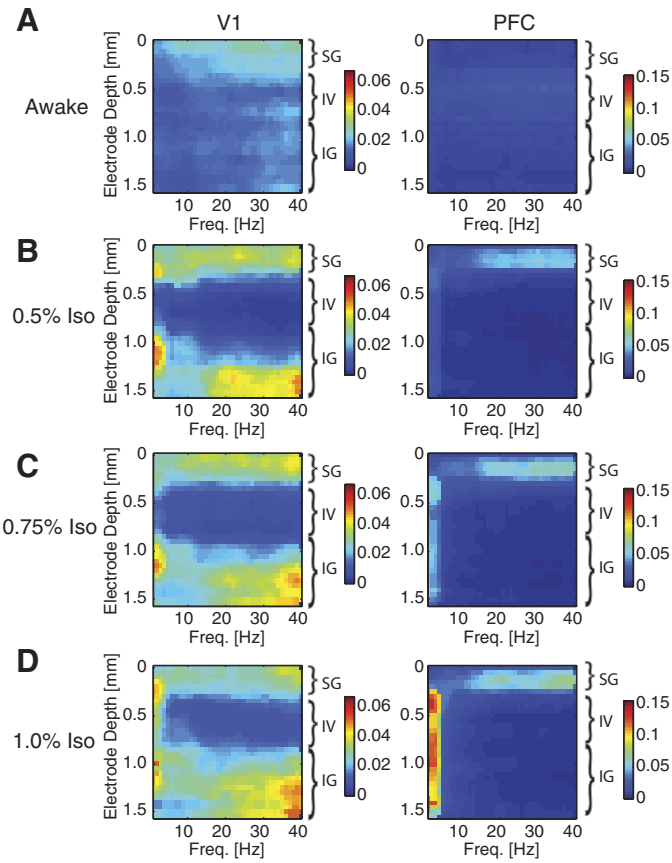

Fig. 7. Anesthesia induced frequency-, layer-, and cortical area-specific increases in spike-field coherence (SFC). *A*: SFC was minimal in awake recordings in both V1 (*left*) and PFC (*right*). Note different color scales for V1 and PFC. *B*: SFC increased with 0.5% Iso. V1 (*left*) exhibited increased SFC in SG and IG layers. Input layer IV was resistant to increased SFC induced by anesthesia. PFC (*right*) exhibited increased SFC primarily in SG at faster frequencies. *C*: with 0.75% Iso, SFC increased in V1 (*left*) in SG and IG layers, whereas PFC (*right*) exhibited increased SFC at SG layer and across layers at slow frequencies. *D*: SFC was strongest with 1.0% Iso. SFC was strongest in V1 (*left*) with 1.0% Iso; layer IV remained resistant to increased SFC induced by anesthesia. In PFC (*right*), 1.0% Iso induced strong SFC at slow frequencies across layers and at SG layers.

neuronal energy demands. This neurovascular coupling is directly dependent on blood flow and blood volume (Kannurpatti et al. 2008). Particularly relevant for the current study, anesthesia has been shown to alter blood flow to the brain, thereby disrupting normal neurovascular coupling. Isoflurane is known to induce vasodilation of cerebral arteries in a dose-dependent manner (Flynn et al. 1992) and increase relative blood flow to subcortical regions (Hansen et al. 1988; Reinstrup et al. 1995). Studies in animals (Disbrow et al. 2000) and humans (Antognini et al. 1997) have reported decreased BOLD activation with increasing concentrations of anesthetics, including isoflurane. However, these results are difficult to interpret given the unknown contributions of alterations in neurovascular coupling versus true modulation of neural activity. Additionally, evidence suggests that the fMRI activity-electrophysiology relationship varies across cortical areas because of differences in neurovascular coupling, particularly in sensory cortices compared with association cortical areas (Ojemann et al. 2013). Even with improvements in neuroimaging acquisition and analysis strategies (Alkire 2008; He et al. 2011), these techniques are still indirect measures of neural activity, with limited spatial and temporal resolution. Our electrophysiological recordings provide direct measures of neural activity with excellent spatial and temporal resolution in comparison. Furthermore, our approach does not suffer from potential confounds caused by alterations in blood supply to cortical areas.

Comparison of different anesthetic agents. There are a large number of different anesthetic agents, which act through a variety of molecular mechanisms. Isoflurane is an inhalant anesthetic that potentiates GABA acting on $GABA_A$ receptors, exerting effects in cortical and subcortical areas (Harrison et al. 1993). However, the potentiation achieved by isoflurane is typically half that caused by propofol (see below), indicating that isoflurane also acts at other molecular targets (Franks 2006). These targets may include glycine receptors (Harrison et al. 1993) as well as two-pore-domain potassium channels (Patel et al. 1999). At the mesoscopic network level, the power of spontaneous gamma oscillations have been found to be unchanged by increasing concentrations of isoflurane anesthesia in rats (Hudetz et al. 2011). Similarly, we found only small total power changes in the gamma frequency band in V1, but more prominent alterations in the power of gamma frequencies relative to total power. At the microscopic network level, administration of isoflurane in rat somatosensory cortex has been shown to reduce spontaneous action potential firing (Hentschke et al. 2005). Xylazine, an agonist for α2-adrenoceptors, which are found widely across cortical layers (Hedler et al. 1981; Nicholas et al. 1993), was also used in this study. Activation of α2-adrenoceptors has been shown to increase cortical activity in prefrontal cortex by acting on the H-channels (Wang et al. 2007). Taken together, these studies may explain why we did not find differences in MU firing rate between awake and anesthetized animals in PFC. $GABA_A$-mediated decreases in firing rate could have been compensated for by increased cortical activity driven by α2-adrenoceptors in PFC. Previous studies testing the application of α2-adrenergic agonists in rat visual cortex support the role for α2-adrenoceptors in the modulation of sensory inputs to the visual cortex through increasing single-to-noise ratio in visually driven cells (when delivered in low concentrations) and decreasing firing rates of visually driven and non-visually driven cells (when administered at high concentrations) (Kolta et al. 1987). It remains to be fully elucidated how the interaction of isoflurane and α2-adrenoceptors affect spontaneous MU spiking activity in visual cortex. In comparing our findings to other studies using isoflurane anesthesia, it is important to remember that xylazine was continuously administered to all anesthetized animals. This contributed to an anesthetic drug-sparing effect, in which lower concentrations of isoflurane were required to maintain complete sedation (Doherty et al. 2007; Soares et al. 2004). Therefore, anesthesia at 0.5%, 0.75%, and 1.0% Iso in this study corresponded to a lack of behavioral responses and induced UP and DOWN states but did not lead to burst suppression. Burst suppression activity is characterized by periods of high-voltage activity that are interspersed quasi-periodically with long (10–20 s) periods of isoelectric activity; importantly, burst suppression activity is not rhythmic (Ching et al. 2012). The anesthetic paradigm used in this study did not induce burst suppression because DOWN states remained relatively short (1–1.5 s at the longest) and alternated rhythmically with UP states. However, it should be noted that there is no firmly agreed upon delineation between UP and DOWN state activity and burst suppression.

Propofol is another commonly used anesthetic, delivered intravenously, which acts primarily at $GABA_A$ receptors to potentiate inhibitory current flow. The specific subunit composition of the $GABA_A$ receptor appears to modulate the effect of propofol (Franks 2006). Cats anesthetized with propofol exhibit a spectral peak around 12 Hz and decreased spontaneous single-unit firing of occipital cortical neurons (Andrada et al. 2012). Loss of consciousness (LOC) induced by propofol in humans is marked by frontal alpha oscillations as measured by EEG (Purdon et al. 2013). Our results show a similar spectral peak in the alpha band in PFC, despite the use of different anesthetics.

Theories of cortical disintegration and reduced encoding capacity. There are two related leading postulates of the mechanism underlying behavioral alternations during anesthesia. The first is characterized by disruption of cortical integration (Alkire et al. 2008; Hudetz 2006). Anesthetics may disrupt cortical integration by acting on structures that facilitate long-range corticocortical interactions (Mashour 2004). Sensory neuronal processing may still occur, in a form either identical to or altered from the awake state (Plourde et al. 2006), but conscious sensation is abolished because of impaired transmission or an inability of other brain regions to receive or interpret the information. The thalamus is likely involved in this information processing pathway; isoflurane has been shown to attenuate the output of somatosensory signals from an area of the rat thalamus, whereas its input is only marginally affected (Detsch et al. 1999). Comparison of connectivity as measured by fMRI in awake marmoset and during propofol anesthesia has shown that propofol decreases thalamocortical connectivity (Liu et al. 2013b). Humans anesthetized with sevoflurane show a dose-dependent reduction of synchronized neural activity during resting state (temporally correlated slow fluctuations between functionally related areas) as determined by fMRI scans and connectivity maps calculated from seed regions in motor areas (Peltier et al. 2005). LOC induced by propofol anesthesia in humans has been associated with the functional isolation of cortical regions but the preservation of connectivity within local networks as measured by electrocorticography

(ECoG) and implanted temporal cortex microelectrode arrays (Cimenser et al. 2011; Lewis et al. 2012; McCarthy et al. 2012). Although not the same as anesthesia, sleep exhibits many similar behavioral effects, namely, the loss of consciousness. Indeed, lack of consciousness during sleep has been related to a breakdown in effective cortical connectivity, or the ability of a set of neuronal groups to causally affect the firing of other neuronal groups (Massimini et al. 2005, 2009). Our results contribute to this postulated mechanism of anesthesia by providing evidence of profoundly different effects of anesthesia on a primary sensory area and an association cortical area. V1 exhibited minimal alterations in spectral power compared with PFC, which displayed massive differences in anesthetized spectra relative to the awake animal.

In the second proposed mechanism of anesthesia, a disruption in the repertoire of cortical activity patterns reduces the brain's ability to encode information (Alkire et al. 2008). Our correlation results support this hypothesis. Correlation of activity across electrodes was much higher with anesthesia in PFC but reduced in V1. The ability to maintain sensory responses during anesthesia may be linked with less correlated activity across cortical layers in this primary sensory area. In contrast, increased correlation across cortical layers in PFC may be related to the corresponding lack of consciousness or higher-order cognitive functions.

The focus of the present study was on elucidating the cortical area- and layer-specific effects of anesthesia when steady-state brain dynamics in awake and anesthetized animals are compared. We chose to look at spontaneous cortical dynamics because of the emerging functional role of spontaneous activity in cortex (Berkes et al. 2011; Fox and Raichle 2007; Han et al. 2008). Together with recent studies on the loss of consciousness induced by anesthesia (Alkire et al. 2000; Massimini et al. 2012; Purdon et al. 2013), the insights into the effects of anesthesia on mesoscopic and microscopic network dynamics with high resolution provide an emerging picture of anesthesia as a complex and sophisticated modulator of cortical network activity. The present results provide a starting point for developing computational models to further understand the complex interaction between mesoscopic and microscopic network activity. The present work clearly demonstrates that such models should incorporate laminar profiles of activity and be tailored to specific cortical areas to accurately represent the functioning of cortical networks.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.K.S., D.V.B., and F.F. conception and design of research; K.K.S., D.V.B., and F.F. performed experiments; K.K.S. and F.F. analyzed data; K.K.S., A.H., and F.F. interpreted results of experiments; K.K.S. prepared figures; K.K.S., A.H., and F.F. drafted manuscript; K.K.S., D.V.B., A.H., and F.F. edited and revised manuscript; K.K.S., D.V.B., A.H., and F.F. approved final version of manuscript.

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