Doctor of Philosophy

Effect of anesthetics on neural activity and functional connectivity in the rat

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Effect of anesthetics on neural activity and functional connectivity in the rat

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ABSTRACT

Objective: The purpose of this study was to investigate the resting state neural activity in the rat cortex using bi-hemispheric laminar electrophysiological recordings of local field potential (LFP) as well as how varying types and doses of anesthetics (i.e., isoflurane and α-chloralose) influence the LFP activity and functional connectivity in comparison with the awake state.

Method: Adult male Sprague-Dawley rats were used for the electrophysiology experiment. The areas of skull and dura matter overlying the bilateral primary somatosensory cortices (S1) were removed for electrophysiological recordings. The electrophysiological recordings were performed in the forelimb region of the bilateral primary somatosensory cortices (S1fl) using two linear multi-electrode arrays. The extracellular recording signals were amplified and filtered between 0.1 and 500 Hz to record LFP. Recording of evoked response and resting state activity was conducted more than 10 min after anesthetic condition was changed. All data analyses for the LFP signal were conducted using a custom-written MATLAB code (The Mathworks; Natick, MA). The spectral power of the LFP signals at each channel was calculated by the Fast Fourier Transform algorithm in MATLAB for 5 minutes of resting state activity in each anesthetic condition. Principal Component Analaysis (PCA) was conducted to characterize a common signal component of

spontaneous activity in bilateral cortices.

Result: Slow wave in delta/theta bands increased as the dose of isoflurane increased regardless of the magnitude of increase, while the burst suppression pattern of activity emerged only when the isoflurane dose was high (1.5% or higher). Such burst-suppression might be associated with the neural inhibition/disinhibition mechanisms and could induce rapid habituation of sensory-evoked responses. On the other hand, the spontaneous LFP activity under anesthesia with α-chloralose was characterized by continuous large, slow oscillations. Under anesthesia with α-chloralose, sensory-evoked response intensified, resulting in more visible response in the ipsilateral somatosensory cortex than the awake state. Interhemispheric correlation of the resting state activity was lowest during the awake state, particularly between upper layers in bilateral cortices. α-chloralose tended to increase the interhemispheric correlation in a layer-specific manner, but isoflurane induced nonspecific global correlation.

Conclusion: The effects of different types and doses of anesthetics on the spontaneous neural activity should be taken into account when studying the resting state brain activity and functional connectivity of anesthetized animals.

Key Word: rs-fMRI, brain functional connectivity, electrophysiology, anesthetics effect.

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Abbreviations

Rs fMRI: resting state functional MRI

LFP: local field potential

BOLD: blood-oxygen-level-dependent

S1FL: primary somatosensory cortices

PCA: principal component analysis

PC1: 1st principal component

MUA: multi unit activity

CSD: current source density

ANOVA: analysis of variance

EcoG : Electrocorticography

INTRODUNTION

The brain works as a large network consisting of multiple functional modules and neuronal populations which are massively interconnected. Abundant spontaneous neural activity is observed in the brain across varying levels of consciousness, from which robust functional connectivity networks have been identified using various neuroimaging techniques. Blood-oxygen-level-dependent functional MRI (BOLD fMRI) is the most widely used neuroimaging technique in the study of resting state brain network particularly in human subjects due to its excellent spatial resolution and noninvasive nature. Spontaneous fluctuation in BOLD fMRI signal exhibited strong spatiotemporal correlation structures which successfully revealed distinct groups of functional networks, including somatomotor, visual, auditory, task-negative, hippocampal, language-related and attentional neural networks even in the resting state without any cognitive task or sensory input[24]. In addition, resting state network such as task-negative (or default-mode network) is robustly observed across various species and altered states of consciousness (e.g. sleep, anesthesia, etc.)[2, 3, 24]. However, BOLD fMRI reflects neural activity only in an indirect manner via a slow physiological process of neuro-vascular coupling, which lacks a fundamental understanding of neural communication in a biologically plausible time scale.

To study the neural basis of resting state BOLD fMRI signal, simultaneous microelectrode recording with resting state fMRI has been used. In human subjects, slow modulation of the firing rate and gamma LFP power were found to be bilaterally synchronized between auditory cortices, and interhemispheric correlation was also demonstrated in gamma ECoG power change in sensory cortices including visual system[13]. Leopold and his colleagues have demonstrated that spontaneous

BOLD fluctuations correlate with slow modulation of the spiking rate, MUA power and LFP power (gamma band and 2-15 Hz range) in the monkey visual cortex at rest [19, 21]. Delta oscillations in EEG recordings (0-4 Hz) were bilaterally synchronized in the primary somatosensory cortices of anesthetized rats, which supports the interhemispheric correlation of spontaneous BOLD fluctuations[11]. These studies on neural basis of resting state functional connectivity often involved altered states of consciousness such as sleep or anesthesia. Use of anesthetics is particularly common in animal fMRI studies but not much in human studies, which might limit parallel comparison of findings in the study of neural basis of resting state functional connectivity.

Use of anesthetics in resting state brain study raises several issues in interpretation of findings. First of all, the effect of anesthetics on spontaneous neural activity should be carefully considered for comparison with resting state study in wakeful or sleep states. It might be very helpful to identify the dose of anesthetics or sedation which can keep spontaneous brain activity pattern comparable to one in the wakeful state. In addition, many anesthetics affect cerebrovascular activity as well as neural activity; thus one cannot discriminate whether any difference in resting state fMRI findings comes from alteration in neural activity or a mere vascular effect of anesthetics. For example, isoflurane often introduces less specific global functional connectivity in resting BOLD fMRI in animals, and it is unclear whether such global correlation results from synchronized neural activity in the brain or from the vasodilation effect of isoflurane. In addition, the influences of various doses and types of anesthetics on resting state brain activity should be compared for accurate interpretation of findings in animal studies. Thus, direct electrophysiological measurement of spontaneous brain activity and functional connectivity are required

to confirm the effect of anesthetics on the resting state brain network.

Here we aimed to investigate spontaneous brain activity and functional connectivity in the rat in various states of consciousness using *in vivo* electrophysiological recording.We focused on interhemispheric functional connectivity in the bilateral somatosensory cortices in the rats which has been the most widely studied functional network in animal resting state fMRI studies in parallel with human findings. We compared resting state brain activity and functional connectivity in varying levels of consciousness using two commonly used anesthetics (isoflurane and α-chloralose) as well as in wakeful state. Here we directly examined how spontaneous neural activity and functional connectivity are modulated across altered states of consciousness in much higher spatio-temporal resolution along cortical depths.

METHODS

1. Animal

Adult male Sprague-Dawley rats (n = 8, 300-350 g) were used for the electrophysiology experiment. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) in Seoul Asan Medical Center. The rats were initially anesthetized with 2.0% isoflurane in medical air for 3 min, then maintained with 1.5% isoflurane during the surgical preparation. A polyethylene catheter (PE-50) was used to cannulate the right femoral artery and vein for anesthetic administration and blood gas analysis. Thereafter, the animals were tracheotomized and mechanically ventilated at rate of ~40 cycles/min. The areas of skull and dura matter overlying the bilateral primary somatosensory cortices (S1fl) were removed for electrophysiological recordings. Lidocaine of 2 mg was applied for reducing pain. The body temperature (37.0 °C) was maintained with a temperature-controlled heating pad placed under the rat's torso. The heart rate, arterial blood gas

and body temperature were monitored and carefully maintained at normal levels throughout the experiment. The duration of surgical preparation was up to 1 hour.

2. Electrophysiological recordings

The electrophysiological recordings were performed in the forelimb region of the bilateral primary somatosensory cortices (S1fl) using two linear multi-electrode arrays (See Figure 1A). The multi-electrode array has 23 contact points with a 100 μm separation between each contact, which spanned the entire depth of the cortex[5]. The electrode was intruded at the S1fl region (4.0 mm lateral and 0.5 mm anterior from the bregma) with the angle of 5 degrees to place the electrode perpendicular to the cortical layers. The extracellular recording signals were amplified and filtered between 0.1 and 500 Hz to record LFP. The LFP was recorded with a sampling rate of 1,000 Hz under the following conditions: (1) for 5 min during rest and (2) for 2 min during forelimb stimulation (~1.0 mA, 3 Hz, 12 pulses per train, duration of each pulse of 0.3 msec, inter-train interval of 6 sec).

The anesthetic condition was systematically modulated. At the beginning of the experiment, isoflurane was lowered to 1.0% for more than 10 minutes and then discontinued for recording for awake states. Pancuronium bromide, a muscle relaxant, was intravenously administered during awake state and light sedation (isoflurane < 1.0%) condition. Lidocaine was applied around the opening of skull to prevent pain and discomfort of the animal in awake and light sedation (0.5% isoflurane). Recording of evoked response and resting state activity was conducted more than 10 min after anesthetic condition was changed. After awake state recording, the dose of isoflurane was increased by 0.5% for each phase up to 2.0% where burst-suppression pattern of spontaneous activity was observed. Then, the dose of isoflurane was lowered to 1.0% and the recovery of neural activity was confirmed. After the isoflurane experiment, the anesthesia was switched continuous

intravenous infusion of α-chloralose. The dose of α-chloralose was initially 30 mg/kg·h and then increased to 60 mg/kg·h and higher. Electrophysiological recording was conducted after the switch period of more than 45 minutes for each step.

3. LFP data analysis

All data analyses for the LFP signal were conducted using a custom-written MATLAB code (The Mathworks; Natick, MA). The LFP signal was preprocessed using a band-pass filter between 0.5 and 100 Hz to remove low-frequency drifts and other noises. A band-stop filter between 59 and 61 Hz was applied to reject 60 Hz artifact. Bad channels of recording with large drift (> 20 mV) was corrected by interpolation of adjacent channels of recordings. The evoked responses were averaged at the onset of forelimb stimulation to exclude spontaneous background activity.

The spectral power of the LFP signals at each channel was calculated by the Fast Fourier Transform algorithm in MATLAB for 5 minutes of resting state activity in each anesthetic condition. We also calculated spectral power at the following frequency bands: delta (1~4 Hz), theta (4~8 Hz), alpha (8~13 Hz), beta (13~30 Hz) and gamma (30~100 Hz).

Zero-lag cross correlation was calculated between each channels of resting state recordings (within and between bilateral cortices). Coherence in specific frequency bands (delta to gamma bands as described above) was also estimated using Welsch's method. In addition, Principal Component Analysis (PCA) was conducted to characterize a common signal component of spontaneous activity in bilateral cortices.

RESULTS

1. Spontaneous activity

Examples of spontaneous LFP activity during different anesthesia conditions are shown in Figure 1. Spontaneous activity during awake state has the smallest amplitude, and has peak amplitude at the granular layer (depth of 700~900 um from cortical surface which corresponding to layer 4). As dose of isoflurane increases to 1.0%, slow waves enlarged in spontaneous activity, which resulted in an overall increase in the amplitude of spontaneous activity. With higher dose of isoflurane (1.5% or higher usually), burst-suppression pattern of spontaneous activity was observed. Spontaneous neural activity was suppressed for a few seconds, then large burst of activity followed. In anesthesia with α-chloralose, we did not observe clear burst-suppression pattern in spontaneous activity. Instead, continuous slow wave at delta band (2~3 Hz) that increased with higher dose of α-chloralose was observed. At high dose of α-chloralose, spontaneous slow wave activity became less frequent (shifting to lower frequency. See Fig 2A). Increased slow wave activity was profound in the lower layers of cerebral cortex (peak amplitude around cortical depth of ~1200 um) under anesthesia with isoflurane or α-chloralose.

We identified bursts and silent periods (suppression) in spontaneous LFP activity during isoflurane anesthesia (dose of 1.0%, 1.5% and 2.0%). The average duration of suppression period (inter-burst interval) increased with the dose of isoflurane: 703 ± 170 ms at isoflurane 1.0%, 992 ± 421 ms at isoflurane 1.5% and 2228 ± 1402 ms at isoflurane 2.0% (Mean ± S.D., respectively. See Figure 3A). Burst-suppression ratio (the ratio of total suppression period to the total recording period) also increased to 0.10 ± 0.16, 0.31 ± 0.28 and 0.63 ± 0.22 for isoflurane of 1.0%, 1.5% and 2.0% dose, respectively. As shown in Figure 3B, bursts consisted of both strong positive and negative polarization but suppression periods only involved weak and gradual positive polarization.

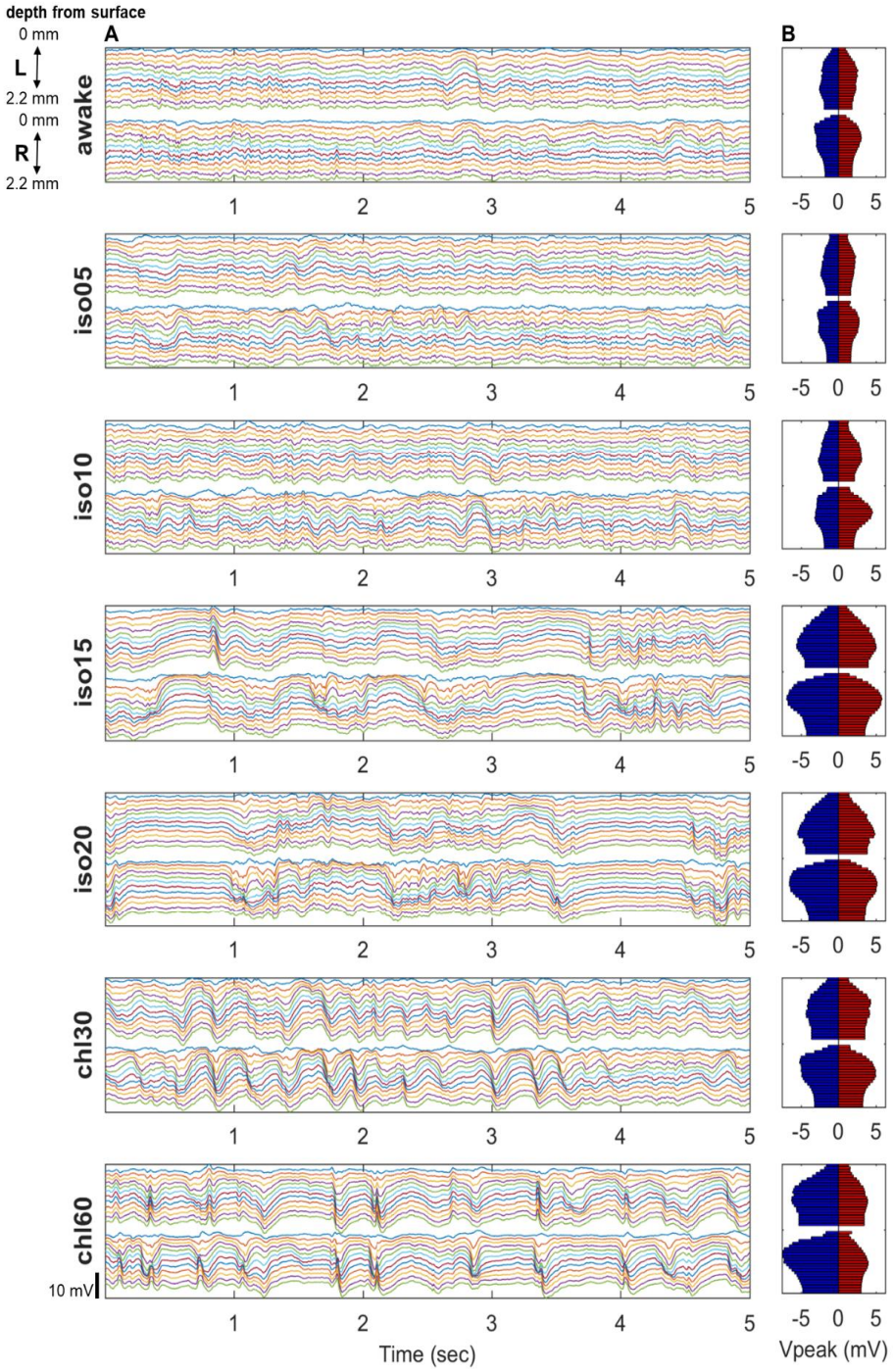


Figure 1. Examples of spontaneous LFP activity across different anesthesia conditions for left (L) and right (R) S1 across the laminar depth of 2.2 mm (from upper to lower). Burst-suppression pattern was observed under anesthesia with isoflurane (1.5% or higher) but not with α-chloralose. (Left) LFP recordings from every other channels in a pair of laminar electrodes are shown. (Right) Peak

amplitude in spontaneous LFP activity (1th and 99th percentiles in LFP time series). Results from a representative animal.

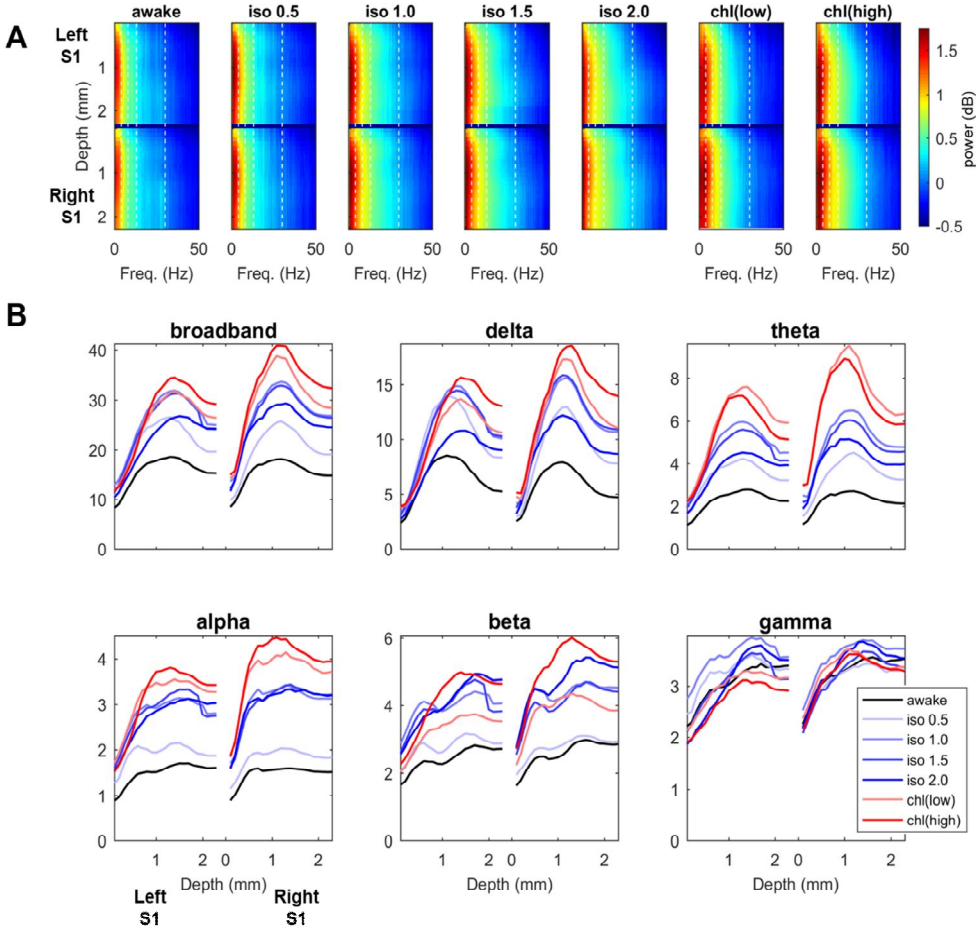


Figure 2. Spectral power of spontaneous LFP activity across different anesthesia conditions. (A) Spectral power distribution shown in a log scale. (B) Band power distribution along cortical depth. Slow oscillations such as delta and theta bands are primarily increased with anesthetics. Increase in delta and theta bands were focalized in the upper infragranular layer (900 um and deeper). Alpha and Beta band increase are more distributed along cortical depth. Gamma band power did not change across anesthetic conditions.

In spectral power, delta, theta, alpha and beta band powers were modulated by anesthetic conditions, but gamma band power did not change significantly (See Fig. 2). Slow wave activity (delta and theta band) was smallest in the awake state, and increased with isoflurane in dose-dependent manner, and decreased at isoflurane 2.0% due to extended suppression periods. Power spectrum exhibited a peak at delta band around 1~3 Hz, which tended to be slower as dose of isoflurane increased. α-chloralose also increased slow wave activity, and theta band power was particularly higher compared to isoflurane anesthesia. Slow wave activity reached its peak at cortical depth of 800~900 um (Layer 4) in awake state, but increase of slow wave with anesthetics (both isoflurane and α-chloralose) was profound at deeper layers, and moved the peak to cortical depth of 1200~1400 um. Alpha band power in awake state was more diffused across depth of 500 um or deeper (Layer 4~6). Isoflurane increased alpha band power in diffused distribution but more focal distribution was observed under α-chloralose anesthesia. Beta band power exhibited double peaks along cortical depth at the depth of 500 um (layer 4) and 1500 um (layer 6) in both awake and isoflurane anesthesia states, but high dose of α-chloralose resulted in increased beta activity peaking at depth of 1200 um. Gamma band power (30~100 Hz) showed gradual increase along cortical depth in awake state, and showed little change across varying anesthetic conditions.

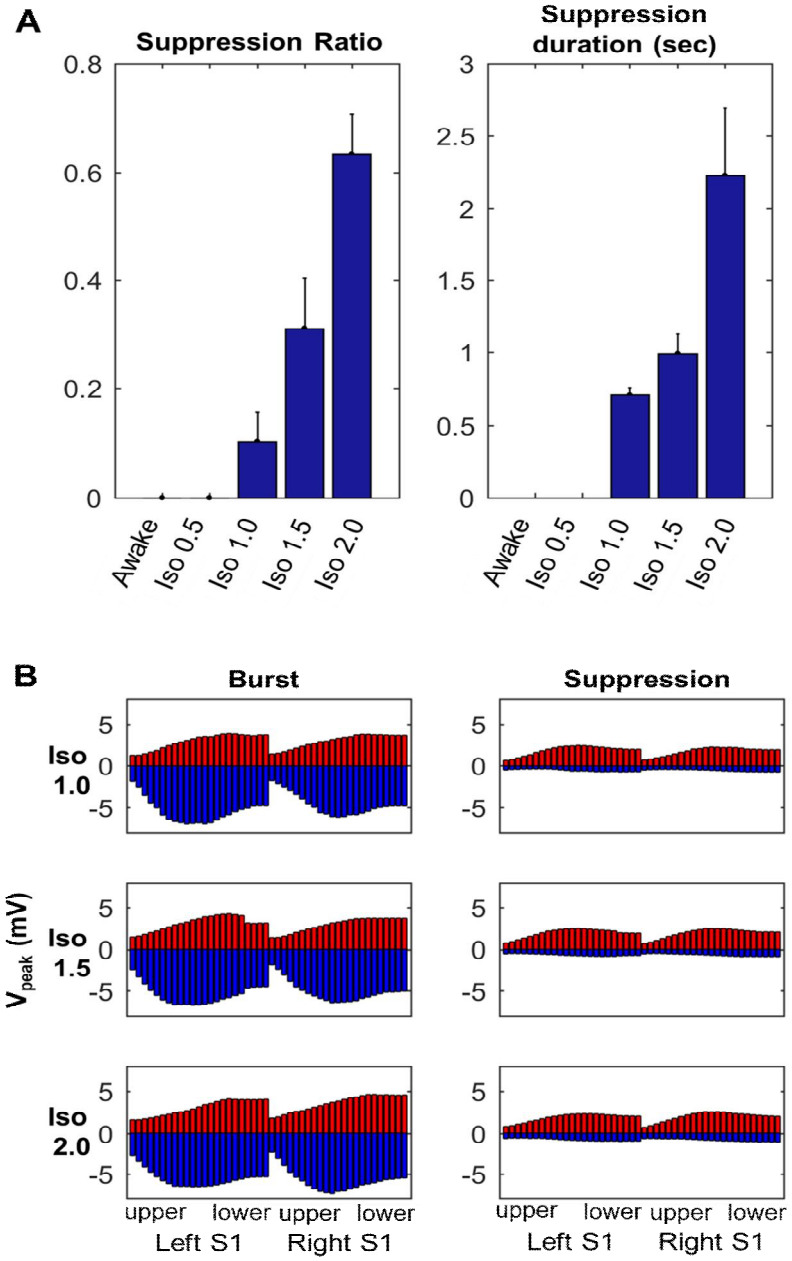


Figure 3. Burst-suppression characteristics in spontaneous activity under isoflurane anesthesia (dose of 1.0~2.0%). (A) Mean duration of suppression periods (in ms) and total ratio of suppression period in the recordings. Mean ± S.E.M. (B) Peak amplitude of spontaneous LFP activity (in mV) during bursts and suppression periods. Burst included both positive and negative polarization in large amplitude, but suppression period contained gradual positive polarization only.

In summary, spontaneous neural activity at awake state was distinct from

anesthetized states in spectral power distribution (weak delta/theta band oscillation) and the spatial profile along cortical depth profile. Both anesthesia increased slow waves in delta and theta bands with larger amplitude at deeper cortical layers. In addition, burst-suppression pattern of activity was characterized for deep anesthesia with isoflurane, but not in α-chloralose.

2. Evoked response

Electrical forepaw stimulation (1.0 mA, 3 Hz, duration of 0.3 ms) induced robust evoked responses in the contralateral S1fl region in awake state as shown in Fig. 4. Peak amplitude of sensory-evoked response in LFP recording was -6.68 ± 3.05 mV at cortical depth of 500 um in contralateral S1. Current source density (CSD) analysis revealed two primary current sources in contralateral S1: early current source at cortical depth of ~1000 um (the boundary between layer 4 and 5) and following large current source at cortical depth of ~400 um (layer 2/3). Multi unit activity (MUA) response was focalized at cortical depth of ~1000 um. Ipsilateral S1 exhibited much weaker response in accordance with our previous study: peak amplitude of -0.70 ± 0.45 mV at cortical depth of ~1000 um. Peak CSD response in ipsilateral S1 was less than 10% in amplitude compared to contralateral S1, and no distinguishable MUA response was observed.

Interestingly, α-chloralose significantly enhanced sensory-evoked response in bilateral S1 regardless of the dosage as shown in Fig. 4. In contralateral S1, peak LFP response was increased by 41% and 60% in average for low (30 mg/kg/hr) and high dose (60 mg/kg/hr) conditions, respectively ($p < 0.05$ for both). Peak CSD was also increased by 12% and 29%, and MUA response increased by 18% and 28%, respectively. Evoked response in the ipsilateral S1 was more drastically enhanced with α-chloralose: Peak LFP amplitude was increased from 0.70 mV to 1.57 mV and 1.98 mV for low and high dose of α-chloralose ($p < 0.01$ for both), and CSD response increased by 89% and 104% ($p < 0.05$ for both). However, ipsilateral MUA response was not significantly enhanced. In all cases, dose-dependent effect

(comparing low vs. high dose of α-chloralose) was insignificant. In conclusion, α-chloralose enlarged sensory-evoked response in LFP recordings, particularly stronger in ipsilateral S1 response.

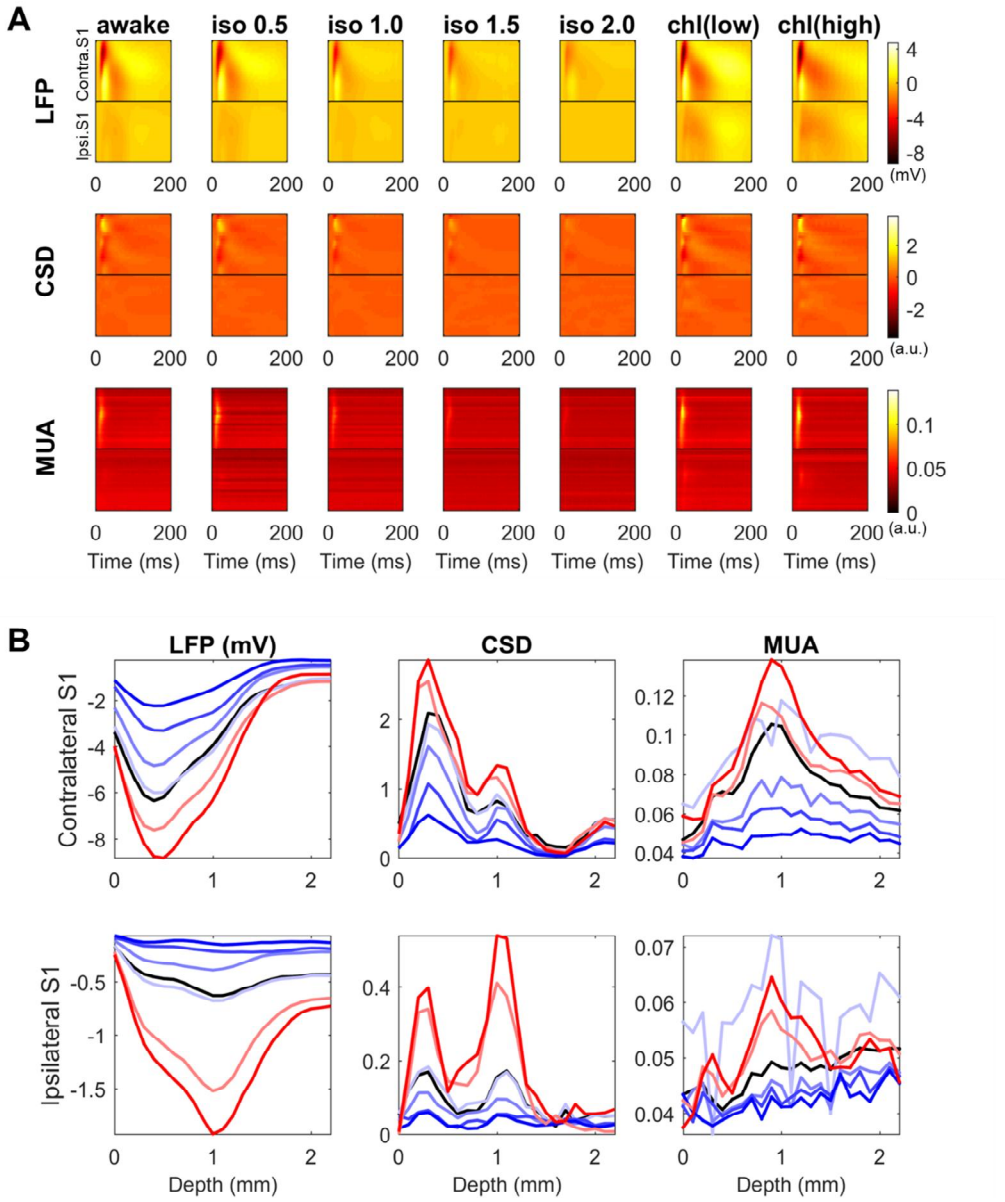


Figure 4. Evoked responses with electrical forepaw stimulation (1.0 mA, 0.3 us, 3 Hz). Isoflurane higher than 1.0% reduced the evoked response, but a-choloralose did not decrease cortical excitability.

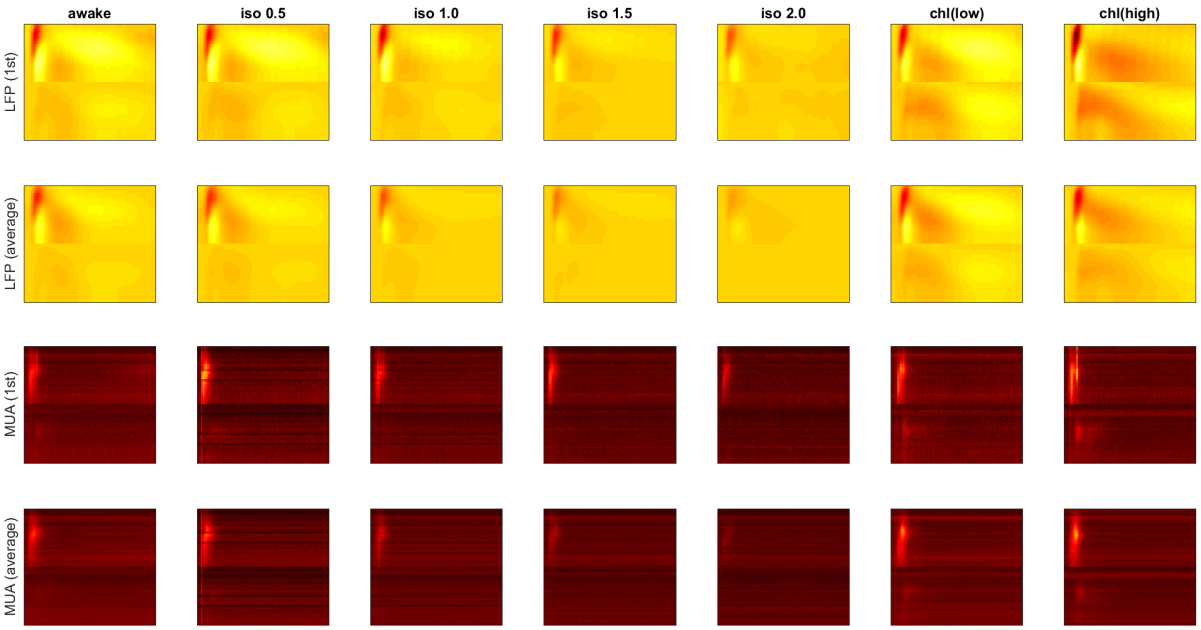
In contrast, isoflurane (dosage of 1.0% or higher) decreased evoked response in bilateral S1 in dose-dependent manner. Low dose (0.5%) of isoflurane did not significantly affect LFP, CSD or MUA response amplitude. However, peak amplitude in evoked LFP response was decreased to 75%, 52% and 42% of awake state in 1.0%, 1.5% and 2.0% dose of isoflurane, respectively. In comparison with awake state, peak CSD amplitude was also decreased to 66%, 44% and 33%, and MUA response was diminished to 77%, 69% and 53% for isoflurane dosage of 1.0%, 1.5% and 2.0%, respectively. One-way ANOVA revealed dose-dependent effect of isoflurane (across 1.0%, 1.5% and 2.0%) as well: $p = 0.0003$ for LFP, $p = 0.004$ for CSD, $p = 0.020$ for MUA).

When comparing evoked responses in a train of sensory stimuli (3 Hz, 4 sec duration), the first response tended to be stronger than the following responses (See Supplementary Fig. 1). The level of adaptation (defined as ratio between the amplitude of average response and the amplitude of the first response) was not significantly different across awake, isoflurane 0.5%, α-chloralose (30 mg), and α-chloralose (60 mg) conditions: 0.81 ± 0.14, 0.76 ± 0.14, 0.93 ± 0.14 and 0.72 ± 0.14, respectively (Mean ± S.D.). However, isoflurane of 1.0% or higher dose resulted in a drastic adaptation in sensory-evoked response, and the amplitude of the average response dropped to less than or equal to half of the amplitude of the first response: 0.49 ± 0.13, 0.48 ± 0.17, 0.41 ± 0.16 for isoflurane 1.0%, 1.5% and 2.0%, respectively. In other words, the initial response in each train of sensory stimuli was less affected with isoflurane anesthesia, but evoked response to consequent stimuli was rapidly habituated to about half of the amplitude of the initial response.

In summary, isoflurane and α-chloralose resulted in opposite effects in cortical excitability to sensory stimuli. High dose of isoflurane decreased sensory-evoked response, particularly with rapid neural habituation for repeated stimuli. α-chloralose did not diminish the evoked response, but rather intensified it (particularly stronger for ipsilateral S1). This direct effect of anesthetics on neural activity can explain some of variance in animal fMRI findings with electrical forepaw stimulation under

anesthesia (e.g. strong evoked fMRI response with α-chloralose, but weak evoked fMRI response with isoflurane).

Supplementary Figure 1. Neural adaptation in sensory-evoked responses in a train of forepaw stimulation. The first response in each train of stimuli was less affected by isoflurane anesthesia, but the following evoked responses were rapidly attenuated, resulting in the diminished average response.



3. Functional connectivity

We estimated functional connectivity within the unilateral somatosensory cortex and between the bilateral somatosensory cortices using zero-lag correlation as shown in Fig. 5. Awake state showed the weakest interhemispheric correlation between bilateral cortices, particularly in upper layers (depth of 900 um or less). Resting activity at the awake state was also less correlated between upper and lower layers within the same hemisphere, suggesting that neural populations in upper layers are relatively independent from lower layers. This upper layer activity seems to be

relatively independent between bilateral cortices as well, but lower layer activity had strong interhemispheric correlation.

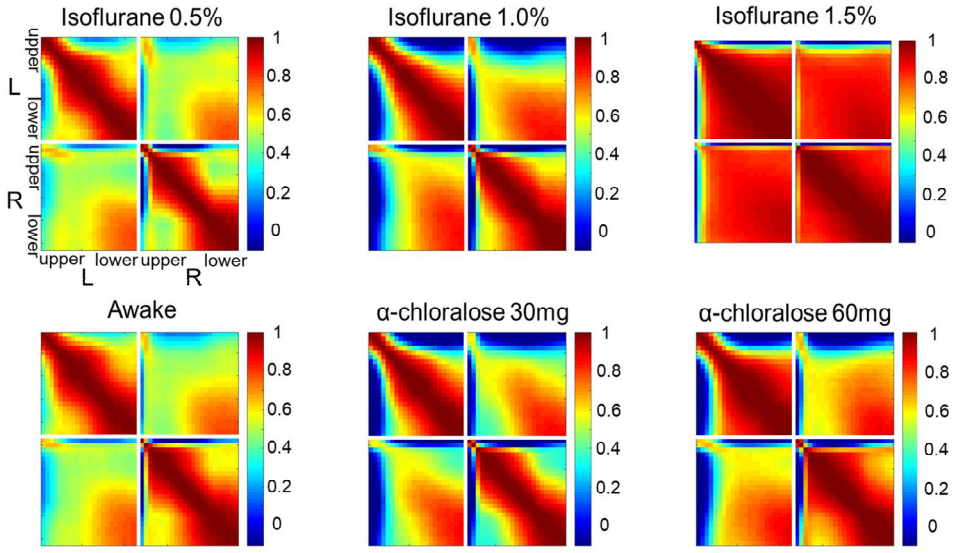


Figure 5. Interhemispheric correlation in spontaneous LFP activity across different anesthesia conditions.

Light sedation with isoflurane of 0.5% dose did not alter much of this pattern of intra/interhemispheric correlation. Isoflurane with dose of 1.0% or higher gradually introduced global correlation in bilateral cortices. Both within- and between-hemisphere correlations exhibited a more diffused pattern of correlation across cortical depth. Interhemispheric correlation was nonspecifically increased in all layers, and might be associated with the large slow wave observed in isoflurane anesthesia. In other words, nonspecific global activity seems to affect functional connectivity pattern in isoflurane anesthesia.

Using PCA, we estimated the contribution of global component in spontaneous activity as shown in Fig. 6. The first principal component (PC1) with the largest eigenvalue was assumed as a global signal component residing in bilateral cortices, and signal variance explained with this single global component was estimated. In awake state, 75 ± 6% (Mean ± S.D.) of spontaneous activity was explained by PC1.

Under anesthesia with isoflurane, the contribution of a single global component (i.e. PC1) was increased to 79 ± 9% at isoflurane 0.5%, 84 ± 7% at isoflurane 1.0%, 87 ± 5% at isoflurane 1.5% and 85 ± 7% at isoflurane 2.0%. The increased contribution of global component was significant at 1.0% or higher dose of isoflurane: $p = 0.0035$ for isoflurane 1.0%, $p = 0.0005$ for isoflurane 1.5% and $p = 0.0072\%$ for isoflurane 2.0%. Therefore, spontaneous activity in bilateral hemisphere was dominated with global oscillation under anesthesia with isoflurane. Under anesthesia with α-chloralose, global influence in resting neural activity was increased to 80 ± 4% (30 mg/kg.hr) and 80 ± 5% (60 mg/kg.hr): $p < 0.05$ and $p < 0.01$ compared to awake state, respectively. Such global oscillation is also likely to drive the spontaneous activity under anesthesia with α-chloralose (with distinct spectral power distribution as shown in Fig. 2).

Layer-specific correlation pattern was largely disrupted in anesthesia under isoflurane, but a moderate dose of α-chloralose (~30 mg/kg.hr) conserved layer-specific pattern in functional connectivity within- and between-hemisphere. The correlation patterns of upper and lower layers were still distinguishable. Interhemispheric correlation was increased compared to the awake states, with the difference in the layer-specific pattern observed in the previous study. Thus, slow wave activity in α-chloralose anesthesia increased interhemispheric correlation in the layer-specific organization which is distinct from nonspecific correlation observed in isoflurane. However, layer-specific correlation became diffused at the high dose (60 mg/kg.hr). Laminar complexity in spontaneous activity was also relatively maintained as shown in Fig. 6.

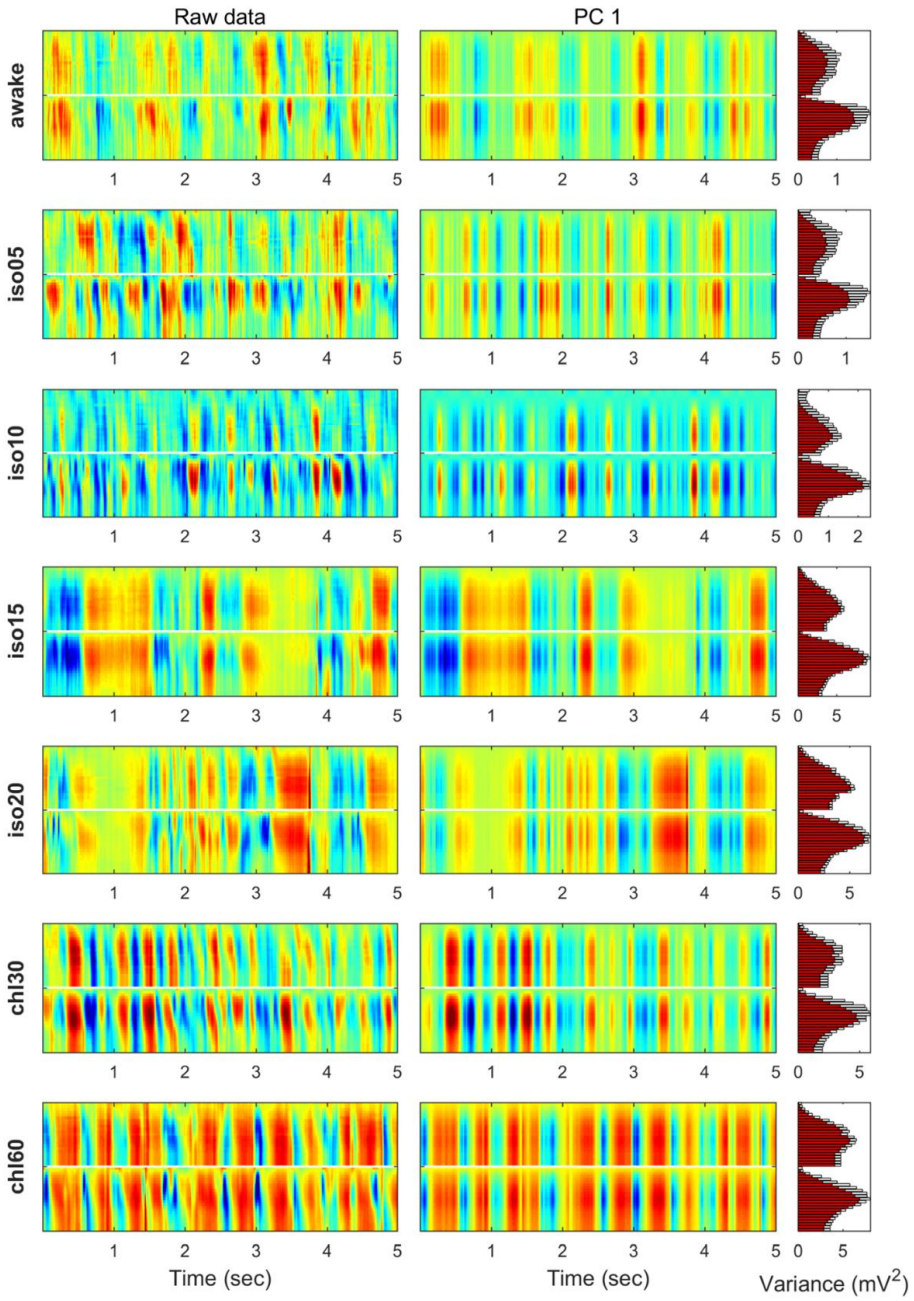


Figure 6. The amount of global activity (1st principal component, PC1) in the spontaneous neural activity across anesthesia conditions. Red bars in right panels show variance explained with the global activity of PC1. As dose of isoflurane increases, global oscillation in bilateral cortex becomes dominant in spontaneous activity. On the contrary, α-chloralose less affected contribution of global oscillation. Results from a representative animal.

We also estimated coherence within- and between-hemisphere in specific frequency bands (delta: 1~4 Hz, theta: 4~8 Hz, alpha: 8~13 Hz, beta: 13~30 Hz, gamma: 30~100 Hz) as shown in Fig 7. Within each hemisphere, slow waves such as delta and theta bands were more widely correlated across cortical depth, but faster waves such as alpha, beta and gamma band showed a distinctive boundary at ~1000 um (the upper boundary of layer 5) in awake state. Thus, population activity at fast oscillation might be distinct across upper and lower layers within the cortical column. Interhemispheric correlation tended to be stronger in slow waves and weaker in fast waves. For example, very weak correlation (and no layer-specificity) was observed in beta and gamma bands across varying anesthetic conditions. In contrast, interhemispheric coherence was robust for delta, theta, alpha bands, and was also modulated by anesthetic conditions. Nonspecific global correlation under isoflurane anesthesia was profound at delta band, but theta and alpha bands mostly preserved the layer-specific pattern observed in awake state. Low dose of α-chloralose (30 mg/kg.hr) strengthened interhemispheric coherence at delta, theta and alpha bands compared to awake state, but these interhemispheric coherences diminished with a high dose of α-chloralose (60 mg/kg.hr), particularly for alpha band. In summary, slow oscillation tends to show higher coherence within and between hemispheres and modulated by anesthetics (primarily for delta bands). Faster oscillations such as gamma band seems to be more localized within and between hemispheres and less contributing to the interhemispheric functional connectivity.

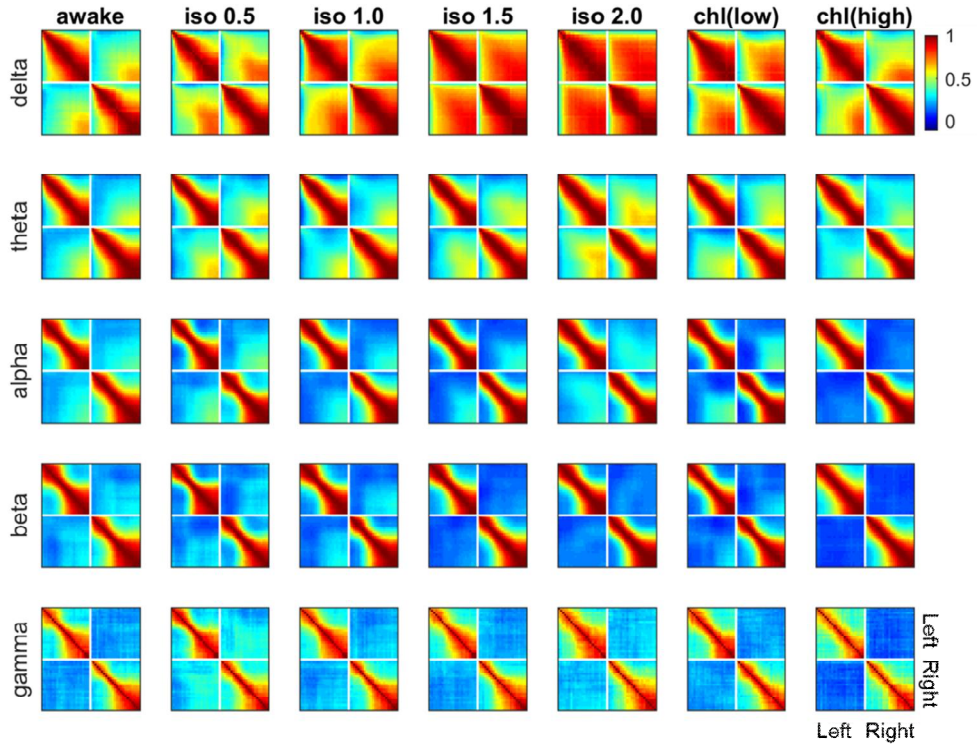


Figure 7. Band coherence in spontaneous neural activity across varying anesthesia conditions.

DISCUSSION

Effect of anesthetics on neural activity

The present study was designed to explore possible electrophysiological links between the sensory-evoked response and resting state spontaneous activity in a popular rat model by perturbing the brain using two commonly used anesthetics, isoflurance and α-chloralose. Although some inhibitive mechanisms are known to be shared by both anesthetics at the molecular and receptor levels (e.g., $GAVA_A$) [7, 8] , a clear understanding of the neurophysiological alterations is still work in progress, and the effects of anesthesia on the complex brain activities remained to be unveiled. In this work, two-site electrophysiological recordings at the bilateral cortices were acquired using a pair of laminar electrodes gathering

signals throughout the whole cortical thickness to describe and quantify the anesthetic effects on the well-known sensory evoked response and FC within and between these regions. As results, both anesthetics largely affected the electrophysiological activities of the evoked responses to electrical forelimb stimuli. Interestingly, isoflurane decreased the evoked S1fl response amplitude in a dose-dependent manner whereas α-chloralose significantly increased the evoked response in comparison to that acquired under isoflurane anesthesia. The spatiotemporal characteristics of spontaneous neuroelectric activities and resultant FC were also differently affected by either anesthetic. Such changes under anesthesia raise a challenge whether the variable evoked-response and/or FC strengths under anesthesia provide genuine understanding and representation of the intrinsic behavior and/or integrity of neuronal networks.

Upon comparing two anesthetics in other previous reports, α-chloralose has been preferred over isoflurane in the animal fMRI studies of sensory-evoked responses, due to the fact that α-chloralose delivers well-defined, consistent fMRI responses with a reliable SNR whereas isoflurane often fails to produce robust activations or requires much stronger stimulation [5, 12]. Although the isoflurane results are similarly comparable between fMRI and electrophysiology, the matching conclusion of the reduced evoked activity between two approaches is probably confounded with a potent vasodilation effect of isoflurane (vs. slight vasoconstriction caused by alpha-chloralose), which disrupts the normal neurovascular coupling, further affecting the sensory-evoked fMRI responses [14]. Such possibility requires the elucidation of underlying electrophysiological events to avoid misinterpretation of the results from other indirect measures of neuronal events. On the other hand, the FC analyses based on spontaneous activities in both previous resting state fMRI and electrophysiology studies also demonstrated a distinctive difference between two anesthetics. A number of investigators in both fMRI and electrophysiological studies similarly reported non-specific correlation pattern by isoflurane while α-chloralose were shown to preserve the connectivity between the established brain areas [11, 26]. Although such differences have been documented, a comprehensive understanding of such anesthetic effects on spontaneous FC in relation to the changes in the evoked response characteristics has not been available. The current study explored this gap by providing the quantitative description of the spatiotemporal alteration in

electrophysiology.

Neural basis of interhemispheric functional connectivity

Synchronization in spontaneous neural activity in bilateral cerebral cortex was observed with varied laminar organization patterns across distinct anesthesia conditions. Awake and light sedation states (isoflurane 0.5%) abundant with fast band activity showed relatively lower interhemispheric correlations, but robust interhemispheric synchronization was clearly observed in the infragranular layers. Interhemispheric correlation in neural activity was modulated by the differing dosages and types of anesthetics. Increases in the interhemispheric correlation and laminar organization were observed in anesthesia under α-chloralose, but high dose of isoflurane resulted in a non-specific synchronization of neural activity along the whole cortical layers. It might imply that burst-suppression pattern of activity occurs globally along cortical layers between bilateral cerebral cortices. In accordance with this notion, we found that the global activity component (PC1) took more portion of neural activity recording in high dose of isoflurane conditions. This global neural modulation might override the original fine laminar organization in interhemispheric functional connectivity. In contrast, α-chloralose anesthesia preserved complexity and layer-specific patterns in spontaneous neural activity; it is therefore likely to be a more suitable anesthetic agent for studying functional connectivity in the brain.

Among different frequency bands, slow waves such as delta and theta bands exhibited similar modulations in interhemispheric coherence patterns across different doses and types of anesthetics. Delta and theta band oscillations were major components in spontaneous neural activity, particularly in anesthetized states. In contrast, faster waves in beta and gamma bands changed less in their amplitude across different doses and types of anesthetics, and tended to be more localized within and between hemispheres. In the previous study with monkeys, coherence in

slower frequency bands was found to be higher between distant recording sites (within-hemisphere) than in faster bands[6]. High coherence in slower frequency bands may be a general principle expanded to inter-hemispheric connectivity as well.

Implications in resting state fMRI study

There are several studies on the neural basis of resting state fMRI signal in animal brains, but most of them focus on very slow modulation of LFP band power such as band-limited power[9, 11, 15, 16, 17, 19, 21]. The first study of rats under anesthesia with α-chloralose[11] revealed correlated modulation of delta band power as a neural basis of interhemispheric functional connectivity between bilateral somatosensory cortices, but later studies in monkeys and rats reported modulation primarily in gamma band or broadband LFP. Interhemispheric synchronization in gamma band power modulation was also found in human intracortical recordings[13].

However, our present findings caution these findings in band power modulation, particularly in animals anesthetized with isoflurane. High doses of isoflurane have been well-known to induce a burst-suppression pattern of activity as shown in the present study and others[9, 15, 16]. Occasional bursts of neural activity can induce abrupt increase of LFP power across the broadband, and it was postulated as the neural origin of slow band power modulation and resting state BOLD fMRI fluctuation[9, 16]. Such burst-suppression type of neural activity is only observed with a specific dose and type of anesthetic, thus cannot explain the neural basis of functional connectivity and resting state fMRI in awake states. In addition, anesthetized states introduced nonspecific correlation across cortical depths within and between hemispheres, particularly in slow frequency bands such as delta wave. A resting fMRI study in rats with varying dose of isoflurane (1.0~1.8%) reported such non-specific global correlation of BOLD fMRI signals[10], suggesting the influence of isoflurane anesthesia on global brain activity and functional connectivity. In contrast, light sedation with isoflurane (~0.5%) was reported to have less influence on gross

functional connectivity patterns in resting state fMRI signal in rats, which was in accordance with our findings[23].

The present study showed layer-specific correlation in raw LFP recordings and band coherence, shedding light on synchronization of bilateral cortical activity in a much faster scale (in milisecond to sub-second). Many previous studies on the neural basis of resting state fMRI focused on slow band power modulation in gamma band or spiking activity, with the exception of one rodent study using α-chloralose[11]. However, neural communication in faster time scales is more biologically plausible and can provide complementary information on functional connections in the brain. For example, α-chloralose anesthesia enhanced both sensory-evoked responses in the ipsilateral S1 and intehemipsheric correlation in resting brain activity (particularly in delta and theta coherence), implying a possibility that both share the same neural connectivity and mechanism. For a better understanding of the physiological role of 'resting state functional connectivity' in the brain, one should incorporate direct measurement of neural activity with extensive knowledge on underlying neurophysiology. The present study can provide the fundamental basis for resting state functional connectivity or functional neuroimaging study in animal models, particularly ones using anesthetic agents.

CONCLUSION

This study explored and compared the altered electrophysiological characteristics of these approaches to provide better understanding of functional activation and resting state FC in animal models, particularly ones under the influence of anesthetic agents. The findings strongly encourage that the direct measurement of baseline neural activity and understanding of underlying neurophysiology should precede the determination of FC and sensory information processing efficiency.

The effects of different types and doses of anesthetics on the spontaneous neural activity should be taken in account when studying resting state brain activity and functional connectivity in anesthetized animals.

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국문요약

목적: 이 연구에서는 양반구 층상에서의 LFP 전기 생리학적 기록을 이용하여 쥐의 피질에서 휴식 상태에서의 신경 활성을 확인하고자 한다. 뿐만 아니라 마취제의 종류와 용량의 변화가 깨어있는 상태와 비교하여 LFP 활성과 기능적 연결성에 영향을 미치는지 알아보고자 한다.

방법: 수컷 SD 쥐를 사용하였고, 전기 생리학적 기록을 위해 두개골과 양측 일차체성감각 피질 바로 위의 경막을 제거하였다. 전기 생리학적 기록은 2 개의 가느다란 멀티 전극 전지판을 이용하여 양측 일차체성감각 피질의 앞다리 영역에서 수행하였다. 세포 외 기록 신호는 0.1 ~500 Hz 사이의 LFP 기록을 증폭 및 여과시켰다. 유발 반응과 휴식기 전위의 기록은 마취 상태가 변화된 후 10 분 이상 수행하였다. LFP 신호의 모든 데이터 분석은 매틀랩 코드를 이용하여 수행하였다. 각각의 채널에서 LFP 신호의 스펙트럼 출력 밀도는 각 마취 상태에서의 휴식기 활성 상태 5 분 동안 매틀랩의 Fast Fourier Transform algorithm 에 의해 측정되었다. 주성분 분석은 양측 피질에서 자발적 전위의 공통 신호 성분 특징을 위해 실행하였다.

결과: 델타파/세타파에서 완서파는 증가의 강도에 상관없이 이소플루레인의 농도에 따라 증가하였고, 반면에 전위의 파열억제패턴은 이소플루레인 농도가 1.5% 이상일 때만 나타났다. 파열억제패턴은 신경 억제/탈억제 메커니즘과 관련있는 것으로 보이며, 감각유발반응의 빠른 습관화 작용을 유도하는 것으로 보인다.
반면에, 알파-클로랄로즈 마취 하에서의 자발적인 LFP 전위(활성)는 계속적인 크고 느린 진동으로 특징 지어진다. 알파-클로랄로즈 마취 하에서 강화된 감각유발반응은 깨어있는 상태보다 동측의 체성감각피질에서의 더 많은 가시응답을 보였다. 휴식기 전위의 양반구 상관성은 깨어있는 상태 동안 특히 양측 피질의 위층 사이에서 가장 낮았다. 알파-클로랄로즈는 층 특이적 방식으로 양반구 연관성이 증가하는 경향을 보였으나, 이소플루레인은 비특이적인 포괄적 연관성이 감소하는 경향을 보였다.

결론: 휴식기 상태의 뇌 활성과 마취된 동물의 기능적 연결성 연구를 할 때 자발적인 신경 활성에 대한 다양한 유형의 마취제의 효과를 고려해야 한다..

중심단어: 휴식기 기능적 자기 공명 영상, 뇌기능적 연결성, 전기 생리학, 마취제 효과